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Highly Stereoselective Synthesis of Fused Cyclopropane-γ-Lactams via Biocatalytic Iron-Catalyzed Intramolecular Cyclopropanation

Xinkun Ren,[†] Ajay L. Chandgude,[†] and Rudi Fasan*

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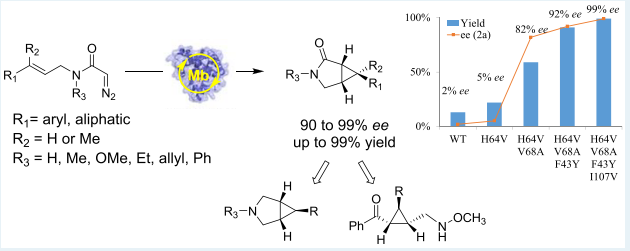
ABSTRACT: We report the development of an iron-based biocatalytic strategy for the asymmetric synthesis of fused cyclopropane-γ-lactams, which are key structural motifs found in synthetic drugs and bioactive natural products. Using a combination of mutational landscape and iterative site-saturation mutagenesis, sperm whale myoglobin was evolved into a biocatalyst capable of promoting the cyclization of a diverse range of allyl diazoacetamide substrates into the corresponding bicyclic lactams in high yields and with high enantioselectivity (up to 99% ee). These biocatalytic transformations can be performed in whole cells and could be leveraged to enable the efficient (chemo)enzymatic construction of chiral cyclopropane-γ-lactams as well as β-cyclopropyl amines and cyclopropane-fused pyrrolidines, as valuable building blocks and synthons for medicinal chemistry and natural product synthesis.



KEYWORDS: *myoglobin, carbene transfer, cyclopropanation, protein engineering, lactams*

Cyclopropanes fused to lactam and pyrrolidine units constitute key pharmacophores in many pharmaceuticals, such as cyproximide,[1] boceprevir,[2] amitifadine,[3] and trovafloxacin[4] (Figure 1). These structural motifs are also found in a

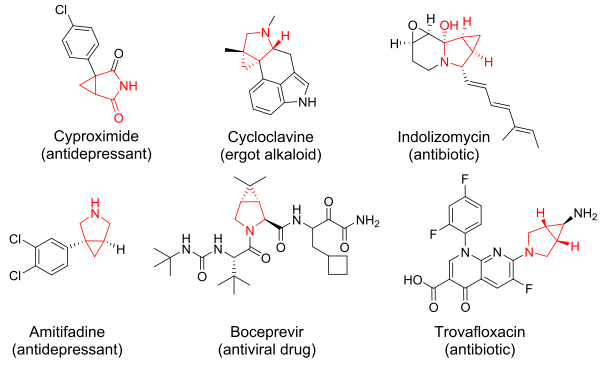


Figure 1. Drugs and bioactive natural products containing cyclopropyl-fused lactam and pyrrolidine moieties

number of biologically active natural compounds,[5,6] including the ergot alkaloid cycloclavine[7] and the antibiotic rachelmycin and indolizomycin.[8] In nature, these motifs are generated by action of SAM-dependent enzymes via SAM-mediated methyl transfer to an olefinic group of the substrate, followed by cyclization of the resulting carbocationic intermediate (e.g., indolizomycin).[5] As revealed by the biosynthesis of cycloclavine, an alternative biosynthetic strategy involves the participation of a nonheme iron-/α-keto-glutarate-dependent oxidase to catalyze the formation of a bicyclic dihydropyrrole core which is then reduced by a imine reductase to give a cyclopropyl-pyrrolidine.[9] In this case, formation of the cyclopropane ring is believed to proceed via an hydroxylation (or halogenation) step followed by enamine-mediated intramolecular S_N2 or, more plausibly, via a ring-closing radical mechanism (Scheme 1a).[9−11] Unfortunately, given the high substrate specificity of these biosynthetic enzymes, the scope of these enzymatic transformations beyond the native substrates remains very limited.

Toward introducing a biocatalytic strategy to access valuable cyclopropane-fused lactam scaffolds, we envisioned the possibility to promote the cyclization of *N*-allyl-diazoacetamides via an intramolecular cyclopropanation reaction catalyzed by an engineered myoglobin. In recent years, engineered myoglobins[12−16] along with engineered P450s[17−20] and artificial metalloenzymes[21−28] have emerged as promising biocatalysts for catalyzing intermolecular olefin cyclopropanations via carbene transfer processes. More recently, the scope of myoglobin-based carbene transferases could be extended to the cyclization of diazoacetates, thus demonstrating the possibility to execute sterically demanding intramolecular cyclopropanation reactions within the "active site" of this hemoprotein.[29] Building upon this progress, we envisioned the opportunity to exploit the myoglobin scaffold

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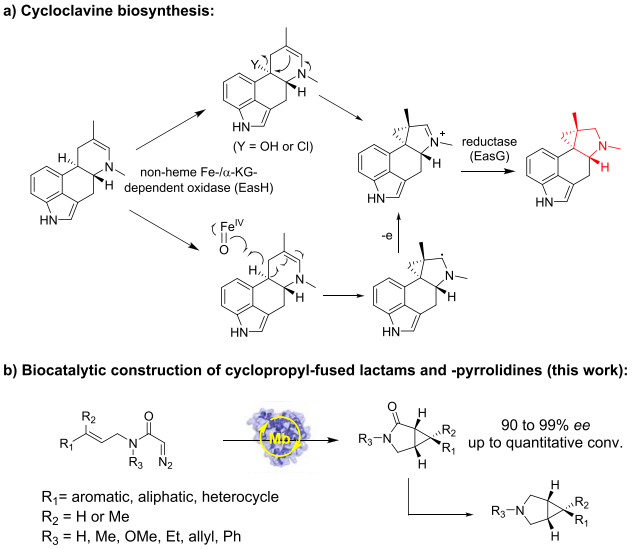
Scheme 1. Biosynthetic vs (Chemo)Biocatalytic Synthesis of Cyclopropyl-Fused Lactam/Pyrrolidine Scaffolds

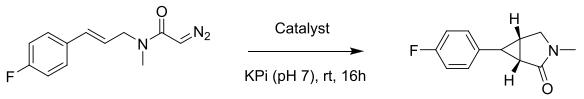


for directing the asymmetric synthesis of fused cyclopropane-γ-lactams starting from readily accessible diazoacetamide substrates. Synthetic methods to execute this transformation have been notably scarce and limited to the use of rare and expensive metals (Rh, Ru).[30−33] Furthermore, achieving broad substrate tolerance and/or high levels of enantioselectivity using these protocols has been notoriously challenged by competing side-reactions and the occurrence of *trans/cis* isomerism across the amide bond of diazoacetamides.[31] Alternative methods to access chiral γ-lactams via Pd-catalyzed cyclopropane functionalization have also been reported.[34] Complementing these chemocatalytic approaches, we describe herein the development of an iron-based biocatalytic strategy for the highly enantioselective construction of fused cyclopropane-γ-lactams via the intramolecular cyclopropanation of allyl diazoacetamides (Scheme 1b). This approach offers an efficient and sustainable route to afford highly enantioenriched bicyclic lactams of prominent value for medicinal chemistry and/or natural product synthesis.

In initial studies, we tested wild-type sperm whale myoglobin (Mb) along with a panel of other hemoproteins (e.g., $P450_{BM3}$, catalase, cytochrome *c*) for their ability to catalyze the intramolecular cyclopropanation of (*E*)-2-diazo-*N*-(3-(4-fluorophenyl)allyl)-*N*-methylacetamide (**1a**) to give **2a** (Table 1). However, all of these proteins showed poor to no detectable activity (0−13% conversion) and very low enantioselectivity (1−9% ee) in this reaction (Table S1). We also tested Mb(H64V,I107S), a Mb variant previously optimized for the intramolecular cyclopropanation of allyl α-diazoacetates.[29] This biocatalyst showed improved activity for formation of **2a** compared to Mb (32% vs 13% conv.) but significantly reduced enantioselectivity (45% ee; Table 1, entry 2) compared with the ester counterpart (99% ee),[29] highlighting the need to optimize the Mb scaffold for the transformation targeted here. This requirement can be attributed to the inherently different steric demands and conformational properties of the allyl diazoacetamide substrates compared to allyl diazoacetates as a result of the N-substituted amide vs ester bond present in these molecules.

To develop a more efficient and selective biocatalyst for the present reaction, we screened an in-house mutability landscape library of Mb variants based on the distal histidine variant H64V in which the shape of the heme pocket was systematically varied by substituting each of the active site amino acid residues (i.e., Leu 29, Phe43, Val68, Ile107) to any of the other 19 amino acids (Table S2).[13] Encouragingly, this strategy led to the identification of Mb(H64V,V68A) and Mb(H64V,V68G) as promising catalysts for converting **1a** to the bicyclic product **2a**, exhibiting significantly improved efficiency (57−59% yield) and enantioselectivity (81−82% ee; Table 1, entries 3−4) compared with Mb or Mb(H64V,I107S). The (1*R*,5*S*,6*S*)-configuration of **2a** was assigned on the basis of crystallographic analysis of **2b** and **2k** (Figure S4−S5; Tables S9−S10). Mb(H64V,V68A) was then subjected to site-saturation mutagenesis of the yet unaltered active site residues Phe43, Ile107, and Leu29, followed by screening of the resulting libraries in whole cells using **1a** as the substrate. The most promising "hits" were validated by in vitro experiments with purified protein prior to the next round of directed evolution. Gratifyingly, progressive improvement of both the activity and stereoselectivity of the enzyme for the synthesis of **2a** was achieved through

Table 1. Intramolecular Cyclopropanation of Allyl-α-diazoacetamide 1a with Mb and Variants Thereof[a]



1a **2a**

entry	catalyst	OD_{600}	yield[b]	TON	ee
1	Mb	-	13%	15	2%
2	Mb (H64V,I107S)	-	32%	40	45%
3	Mb (H64V,V68A)	-	59%	75	82%
4	Mb (H64V,V68G)	-	57%	70	81%
5	Mb (F43Y,H64V,V68A,I107V)	-	>99%	125	99%
6[c]	Mb (F43Y,H64V,V68A,I107V)	-	94%	235	98%
7[c]	Mb (F43Y,H64V,V68A,I107V)	40	>99% (90%)[e]	240	>99%
8[d]	Mb (F43Y,H64V,V68A,I107V)	40	82%	200	>99%

[a]Reaction conditions: 2.5 mM (*E*)-2-diazo-*N*-(3-(4-fluorophenyl)allyl)-*N*-methylacetamide (**1a**), 20 μM Mb variant (or C41(DE3) *E. coli* cells at indicated OD_{600}) in KPi buffer (50 mM, pH 7), 10 mM $Na_2S_2O_4$ (protein only), r.t., 16 h in anaerobic chamber. [b]GC yield based on the calibration curves with authentic standards. [c]With 5 mM **1a**. [d]Reaction time: 15 min. [e]isolated yield.

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optimization of position 43 and 107 after two rounds of mutagenesis and library screening (Figure 2, Table S3).

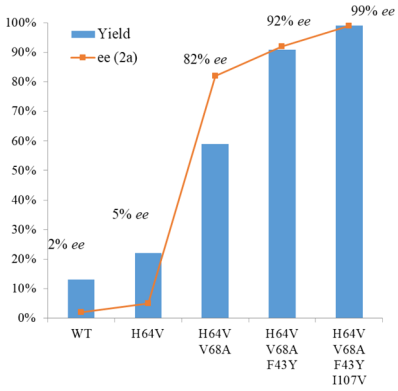


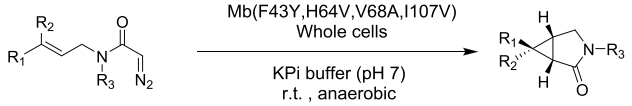
Figure 2. Overview of biocatalyst evolution process.

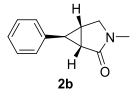
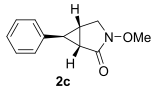
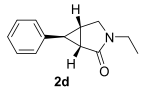
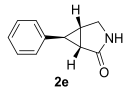
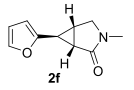
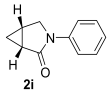
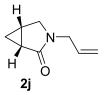
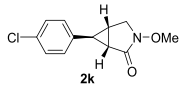
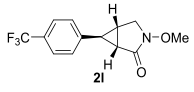
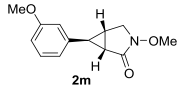
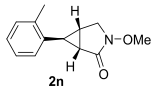
Ultimately, this process led to the development of Mb(F43Y,H64V,V68A,I107V) as an optimal biocatalyst for this reaction, enabling the quantitative conversion of **1a** into **2a** with 99% ee (Table 1, entry 5). Interestingly, the large majority of the beneficial mutations accumulated in this Mb variant entail an expansion of the distal heme pocket (i.e., His64 → Val; Val68 → Ala/Gly, Ile107 → Val) compared with our previously reported Mb-based catalysts for intermolecular olefin cyclopropanation with EDA (Mb(H64V,V68A))[12] or intramolecular cyclopropanation of allyl diazoesters (Mb(H64V,I107S)),[29] which is consistent with the increased steric demands associated with the cyclization of the *N*-substituted diazoacetamide substrate.

Further investigations with Mb(F43Y,H64A,V68A,I107V) demonstrated its compatibility with whole-cell reactions (Table S4) as well as its tolerance to higher substrate loadings (i.e., 5 mM), without noticeable loss in product conversion (94%) and enantioselectivity (98% ee; Table 1, entry 6). Under these conditions, the reaction reaches >80% conversion within 15 min (entry 8) and full conversion in less than 3 h (Figure S2). A whole-cell reaction with Mb(F43Y,H64V,V68A,I107V)-expressing *E. coli* cells was then performed at a 0.2 mmol scale resulting in the isolation of 36.9 mg of enantiopure **2a** (>99% ee) in 90% isolated yield (entry 7), thus demonstrating the scalability of this biocatalytic method. Additional experiments showed that the Mb variant supports up to 440−450 total turnovers under catalyst-limited conditions (Table S4, entries 7 and 12) with an initial product formation rate of 27 turnovers min^{-1}.

To examine its substrate scope, Mb(F43Y,H64V,V68A,I107V) was challenged with a diverse panel of allyl α-diazoacetamide derivatives in whole-cell reactions on a semipreparative (0.2 mmol) scale (Table 2). To our delight, substrates carrying different N-substitutions such as methyl (**1b**), methoxy (**1c**), and ethyl (**1d**) groups were all efficiently processed to afford the desired bicyclic products **2b−2d** in high to quantitative yields (90−99%) and with excellent enantioselectivity (>99% ee). Allyl diazoacetamides with "unprotected" secondary amide group such as **1e** have represented notoriously difficult substrates for transition metal-catalyzed cyclopropanation because of catalyst poisoning via amide coordination to the metal and/or competition by carbene insertion into the amide N−H bond.[30−33] In stark contrast, **1e** could be processed by the Mb-

Table 2. Substrate Scope for Mb(F43Y,H64V,V68A,I107V)-Catalyzed Cyclization of Allyl α-Diazoacetamides[a]



Entry	Product	Yield[b]	e.e.
1	 2b	93 % (82 %)	>99%
2	 2c	99 % (89 %)	>99%
3	 2d	90 % (82 %)	>99%
4	 2e	31 % (23 %)	>99%
5	 2f	82 % (70 %)	94%
6[c]	 2g	54 % (45 %)	90%
7[c]	 2h	99 % (71 %)	94%
8[c]	 2i	99 % (83 %)	99%
9[c]	 2j	57 % (43 %)	93%
10	 2k	75 % (69 %)	>99%
11	 2l	71 % (67 %)	90%
12	 2m	99 % (90 %)	>99%
13	 2n	99 % (81 %)	>99%

[a]Reaction conditions: 5 mM allyl α-diazoacetamide, Mb(F43Y,H64V,V68A,I107V)-expressing *E. coli*. (OD_{600} = 40) in KPi buffer (50 mM, pH 7), 40 mL-scale, r.t., 16 h. [b]Product conversion as

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Table 2. continued

determined by GC. Yields of isolated products are reported in brackets. Errors are within 10%. [c]Using Mb(H64V,V68G)-expressing *E. coli.* (OD_{600} = 40).

(F43Y,H64V,V68A,I107V) catalyst to yield the corresponding bicyclic lactam **2e** with excellent enantioselectivity (>99% ee) (Table 2, entry 4). Heterocycle-containing **1f** was also efficiently cyclized to afford **2f** in 94% ee.

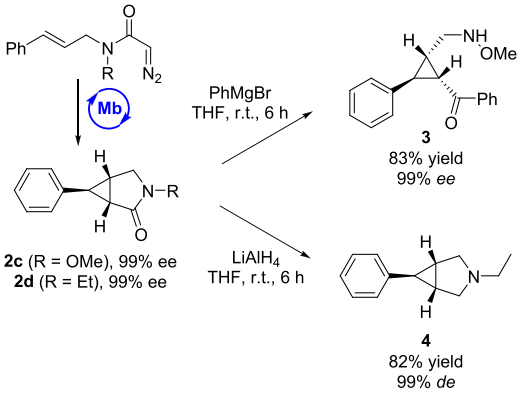
Encouraged by these results, we extended our studies to diazoacetamides containing unactivated olefinic groups such as **1g−1j**. While Mb(F43Y,H64V,V68A,I107V) was found capable of accepting all these compounds (23%−99% conv.), it showed reduced enantioselectivity for formation of **2h−2j** compared with the aryl-containing **2a** and **2b−2e** (28−90% ee vs 90−99% ee; Table S5). Importantly, the desired bicyclic products **2h−2j** could be obtained with a high degree of enantioselectivity (90−99% ee) in good to quantitative yields (54−99%) using Mb(H64V,V68G), an earlier Mb variant identified during the catalyst evolution process (Figure 2, Table S3). From these results, it can be evinced that positions Phe43 and Ile107 are critical for high stereoinduction in the presence of large (i.e., Ar) vs small (-H, -Me) substituents connected to the olefinic group. The results with the *N*-phenyl- and *N*-allyl substituted diazoacetamides **2i** and **2j**, respectively, further demonstrated the broad tolerance of this Mb-based biocatalytic method to substitutions at the level of the amide group.

Finally, the tolerance of Mb(F43Y,H64V,V68A,I107V) toward substitutions of the phenyl group was assessed using **1k−1n**. Notably, *para*, *meta*, and *ortho* substitutions on this group were equally well-tolerated by the biocatalyst resulting in the formation of the cyclopropyl-γ-lactams **2k−2n** in good to quantitative yields (71−99%) and with good to excellent enantioselectivity (90−99% ee; entries 10−13). The results with the *ortho*-substituted **2n** highlighted the tolerance of this catalyst to steric hindrance in close proximity to the olefinic bond. Notably, both Mb variants exhibit a consistent (1*R*,5*S*,6*S*) stereoselectivity across this panel of substrates, as determined on the basis of X-ray crystallography (Figure S4−S5) and comparative chiral SFC/GC analyses.

In addition to enable the asymmetric synthesis of cyclopropyl-γ-lactam core structures for medicinal chemistry (Figure 1), we envisioned the present method could offer a convenient tool for the chemoenzymatic synthesis of valuable β-cyclopropyl amines and cyclopropyl-fused pyrrolidines, the latter representing key pharmacophores in various drugs and bioactive natural products (Figure 1). Illustrating this point, arylation of enantiopure **2c** obtained via Mb(F43Y,H64V,V68A,I107V)-catalyzed cyclization afforded the trisubstituted cyclopropane **3** in 83% isolated yield and 99% ee (Scheme 2). Furthermore, reduction of enantiopure **2d** in the presence of $LiAlH_4$ gave the bicyclic pyrrolidine **4** in 82% isolated yield as a single diastereomer (Scheme 2).

In summary, we have reported the development of a highly enantioselective, biocatalytic strategy for the asymmetric construction of bicyclic cyclopropane-γ-lactams from *trans*-allyl diazoacetamide derivatives. These biocatalytic transformations can be performed in whole cells and can be applied to gain stereoselective access to key synthons for medicinal chemistry and natural product synthesis. On the basis of these results, we foresee that engineered myoglobins can be leveraged to develop iron-based biocatalytic strategies for other types of challenging intramolecular carbene transfer reactions in the future.

Scheme 2. Chemoenzymatic Synthesis of β-Cyclopropyl Amines and Cyclopropyl-Fused Pyrrolidines



■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscatal.9b05383.

Experimental procedures, additional figures and tables, compound characterization data, NMR spectra, chiral GC/SFC chromatograms (PDF)

crystallographic data for **2b** (CIF)

crystallographic data for **2k** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

Rudi Fasan − *Department of Chemistry, University of Rochester, Rochester, New York 14627, United States;* orcid.org/0000-0003-4636-9578; Email: rfasan@ur.rochester.edu

Authors

Xinkun Ren − *Department of Chemistry, University of Rochester, Rochester, New York 14627, United States;* orcid.org/0000-0001-6645-9074

Ajay L. Chandgude − *Department of Chemistry, University of Rochester, Rochester, New York 14627, United States;* orcid.org/0000-0001-8236-9626

Complete contact information is available at: https://pubs.acs.org/10.1021/acscatal.9b05383

Author Contributions

[†]X.R and A.L.C. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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