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VIP Asymmetric Cyclopropanation Very Important Paper

Biocatalytic Strategy for the Highly Stereoselective Synthesis of Fluorinated Cyclopropanes

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Abstract: Fluorinated cyclopropanes are highly desired pharmacophores in drug discovery owing to the rigid nature of the cyclopropane ring and the beneficial effects of C–F bonds on the pharmacokinetic properties, cell permeability, and metabolic stability of drug molecules. Herein a biocatalytic strategy for the stereoselective synthesis of mono-fluorinated and gem-difluoro cyclopropanes is reported though the use of engineered myoglobin-based catalysts. In particular, this system allows for a broad range of gem-difluoro alkenes to be cyclopropanated in the presence of diazoacetonitrile with excellent diastereo and enantiocontrol (up to 99:1 d.r. and 99 % e.e.), thereby enabling a transformation not currently accessible with chemocatalytic methods. The synthetic utility of the present approach is further exemplified through the gram-scale synthesis of a key gem-difluorinated cyclopropane intermediate useful for the preparation of fluorinated bioactive molecules.

Introduction

Cyclopropanes are privileged structural motifs that are found in a wide array of important natural products and bioactive molecules.[1] The distinctive conformational and electronic properties of cyclopropanes make them valuable structural motifs in medicinal chemistry.[2] On the other hand, fluorinated substituents are ubiquitous in pharmaceutical drugs, with over 20 % of drugs on the market containing one or more fluorine atoms.[3] The strategic introduction of fluorine into bioactive molecules have the potential to dramatically changes their pharmacokinetic profile, hydrogen bonding abilities, pKa value, lipophilicity, and/or cell permeability without introducing steric alterations.[3–4] In this context, the fusion of the unique attributes of cyclopropanes with the favorable properties of fluorine substituents makes fluorinated cyclopropanes a particularly attractive pharmacophore for medicinal chemistry and drug discovery. Notable examples of biologically active molecules containing fluorinated cyclopropanes include Zosuquidar, an antineoplastic drug in clinical trials for the treatment of acute myeloid leukemia,[5] the fluoroquinolone antibiotic Sitafloxacion;[6] and the cyclopropanecarboxamide A, a lysophosphatidic acid receptor 2 antagonist (Figure 1).[7] There is a thus a high interest in accessing enantioenriched fluorocyclopropanes.

Current strategies to access enantioenriched fluorocyclopropanes included the kinetic resolution of racemic difluorocyclopropyl esters,[8] Michael-type addition elimination cyclization in combination with chiral auxiliaries,[9] or the asymmetric hydrogenation of fluorocyclopropenes.[10] An alternative and more direct route to the preparation of these compounds is through the cyclopropanation of fluorinated olefins through metal-catalyzed carbene transfer reactions (Scheme 1).

Despite major advances in metal-catalyzed olefin cyclopropanations,[11] the asymmetric cyclopropanation of fluorinated olefins has however represented a major challenge.[12] While Haufe and co-workers reported the cyclopropanation of α-fluorinated styrene with ethyl diazoacetates using copper catalyst and chiral bis(oxazoline) ligand (Scheme 1),[13] this method exhibited only modest

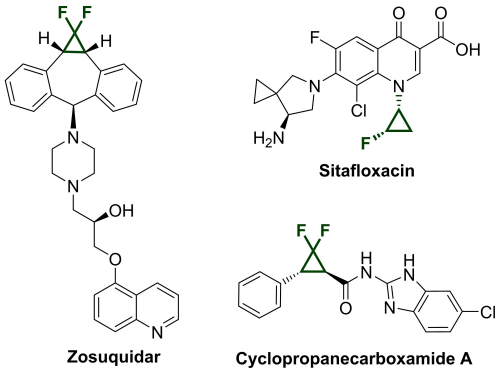

Figure 1. Representative drug molecules containing mono- and gem-difluoro cyclopropane moieties.

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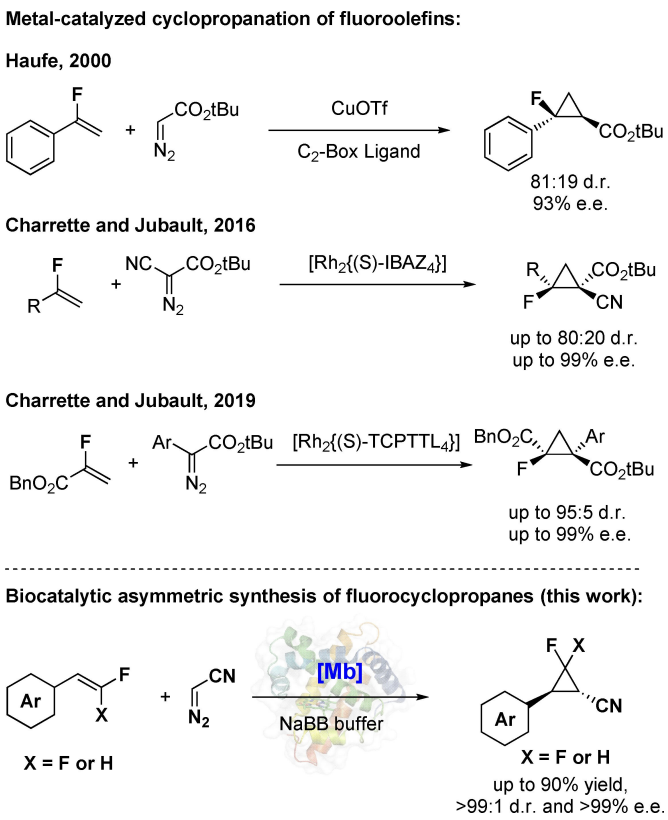


Scheme 1. Contemporary strategies for the asymmetric synthesis of fluorocyclopropanes via metal-catalyzed carbene transfer.

stereoselectivity and its scope was not demonstrated beyond α-fluorostyrenes. More recently, Charette and Jubault reported success in the asymmetric cyclopropanation of activated olefins[14] and α-fluoroacrylates using rhodium-based catalysts (Scheme 1).[15] In contrast, the asymmetric cyclopropanation of geminal difluoro olefins as well as that of β-fluorostyrenes have remained elusive. Difluorocyclopropanes have been obtained through intramolecular reactions[16] or through the cyclopropanation of olefins with difluorocarbenes,[17] but the synthesis of optically active fluorinated cyclopropanes using this methods has not been possible.[18]

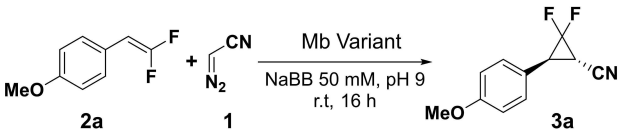
Recently, biocatalysis has emerged as a promising avenue for the realization of cyclopropanation reactions.[19] In particular, our group and others have shown the ability of engineered heme-containing proteins and other metalloenzymes to catalyze these reactions with high activity and stereoselectivity.[20] More recently, these enzymatic reactions were shown to overcome reactivity limitations associated with metal-mediated carbene transfer catalysts, providing an avenue for new and challenging transformations.[20c–e,l] Recognizing the limitations in methodologies for the synthesis of optically active fluorocyclopropanes as summarized above, we report herein the development of an efficient biocatalytic strategy for the stereoselective synthesis of mono- and gem-difluoro cyclopropanes via hemoprotein-catalyzed functionalization of fluorinated olefins in the presence of diazoacetonitrile (DAN) as carbene donor reagent.

Results and Discussion

The challenge associated with the synthesis of gem-difluorocyclopropanes via cyclopropanation of gem-difluoro-olefins is apparent from by the lack of reactivity of these substrates in the presence of organometallic catalysts commonly adopted in carbene transfer reactions (SI Table S1). This phenomenon can be, at least in part, attributed to the highly electrodeficient nature of these olefins which make them unreactive toward electrophilic metallocarbenes typically engaged in these transformations. Using myoglobin (Mb) as carbene transfer biocatalyst, we previously observed that olefin cyclopropanation reactions in the presence of diazoacetonitrile (DAN) grant a notably broader substrate scope compared to analogous reactions in the presence of diazoesters (e.g., ethyl diazoacetate (EDA)), including poorly reactive substrates such as aliphatic or 1,2-disubstituted olefins.[20d] We surmise this broadened scope stems from enhanced (electrophilic) reactivity of the DAN-carbene intermediate vs. EDA-derived carbene intermediate involved in these reactions,[21] as a combined result of the stronger electronwithdrawing effect and lower steric demands of the nitrile (–CN) vs. ester group (–CO_2Et). These considerations prompted us to test the reactivity of a panel of Mb variants toward catalyzing the cyclopropanation of β,β′-difluoro-styrene **2a** in the presence of diazoacetonitrile (DAN). Similar to hemin, wild-type sperm whale Mb and various engineered Mb variants, including Mb(H64V,V68A) (a.k.a. Mb*), a highly active cyclopropanation biocatalyst in the presence of DAN and other diazo reagents,[20a,d,f,l] showed no activity toward formation of the desired *gem*-difluorocyclopropane product **3a** (Table 1, entry 1 and entry 5). In contrast, two Mb variants containing larger space-creating mutations at position 64 and 68, namely Mb(H64A,V68G) and Mb(H64V,V68G) showed promising activity for the desired transformation, affording **3a** in 10 % and 14 % yield, respectively (Table 1, entries 5–6). Importantly, these variants also showed excellent diastereoselectivity (>99:1 d.r.) and enantioselectivity (>99:1 and 97:3 e.r.) in the transformation. Insightfully, the single-site variants Mb(H64V) and Mb(V68G) (Table 1, entry 3–4) showed no activity, indicating that the two active site mutations have a synergistic effect on reactivity.

Based on these findings, a library of Mb variants incorporating the H64V/A and V68G mutations in combination with other active site mutations at position L29, F43, and I107 were prepared and screened in the model reaction (SI Figure S1). However, most of these variants show a total loss of activity or exhibited parent-like activity with no improvement in yield and/or enantioselectivity (SI Table S2). Next, we extended our mutagenesis studies to position 69, i.e., a second-sphere residue which is proximal to the hot-spot position 68 but not in direct proximity to the heme cofactor. Interestingly, introduction of mutations Leu→Ala or Val mutations at this second-sphere site

Table 1: Activity and selectivity of Mb and variants in the cyclopropanation of difluorinated styrene (**2a**) with diazoacetonitrile (DAN) to give **3a**.



2a **1** **3a**

Entry	Catalyst	Yield (%)[a]	d.r.	e.r.[b]
1	Hemin	0	n.d.	n.d.
2	Mb Wt	0	n.d.	n.d.
3	Mb(H64V)	0	n.d.	n.d.
4	Mb(V68G)	0	n.d.	n.d.
5	Mb*(H64V,V68A)	0	n.d.	n.d.
6	Mb(H64A,V68G)	10	>99:1	>99:1
7	Mb(H64V,V68G)	14	>99:1	97:3
8	Mb(H64A,V68G,L69A)	18	>99:1	>99:1
9	Mb(H64A,V68G,L69V)	26	>99:1	>99:1
10	Mb(H64A,V68G,L69V)[c]	45	>99:1	>99:1
11	Mb(H64V,V68G,L69A)	21	>99:1	94:6
12	Mb(H64V,V68G,L69V)	54	>99:1	97:3
13	Mb(H64V,V68G,L69V)[c]	73	>99:1	97:3
14	Mb(H64V,V68G,L69V)[d]	86	>99:1	97:3

Reaction conditions: 5 mM olefin, 20 mM diazoacetonitrile, Mb expressing *E. coli* ($OD_{600}=20$) in sodium borate buffer (NaBB) 50 mM (pH 9.0), 500 µL scale, room temperature, 16 h, anaerobic. [a] Yield as determined by GC using 1,3 benzodiaxole as internal standard. [b] As determined by chiral HPLC. [c] Mb expressing *E. coli* ($OD_{600}=40$).) [d] DAN added portion-wise over 1-hour period (reaction time 16 h)

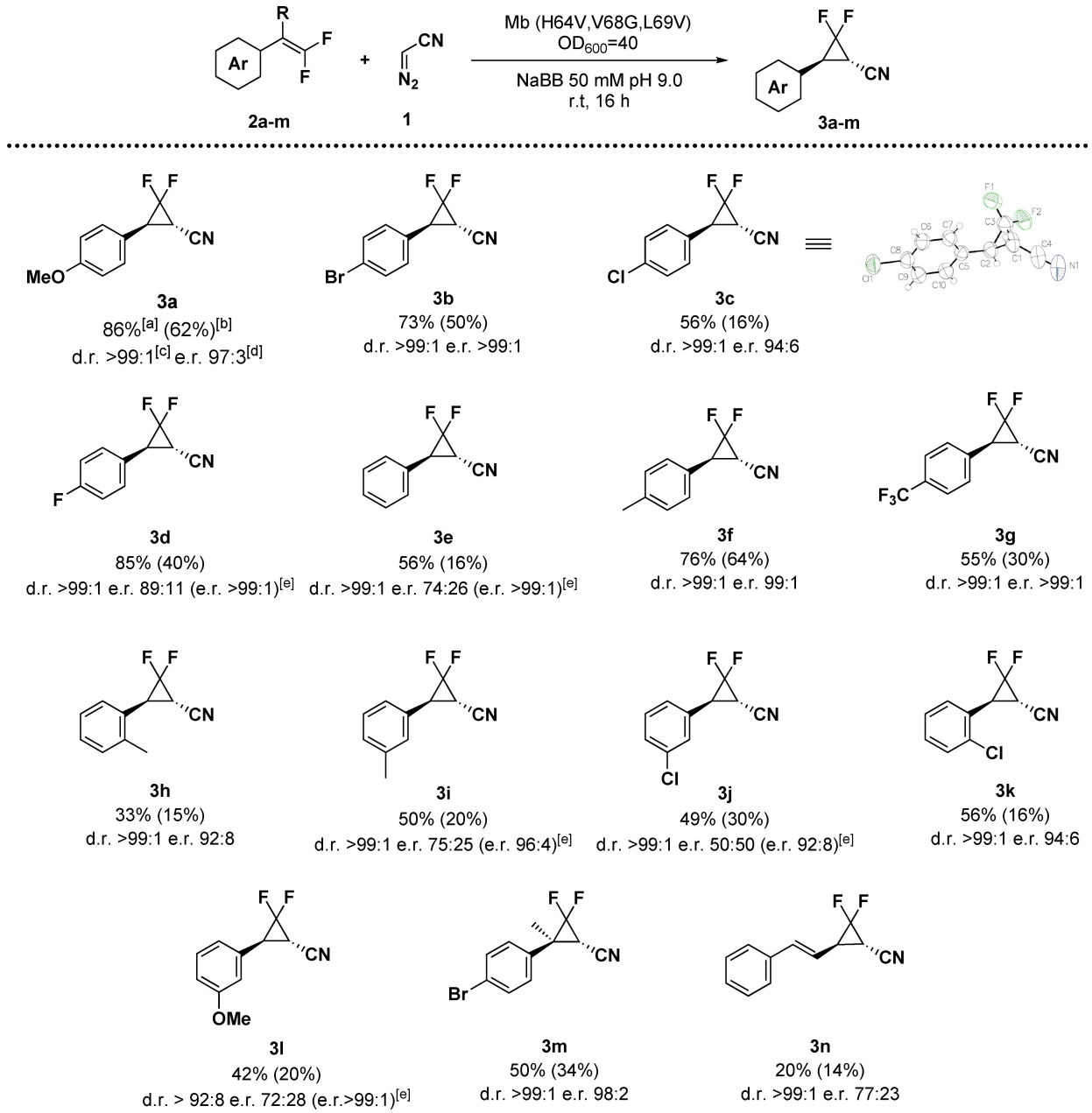
resulted in two- to four-fold increase in activity (21–54 % vs. 10–14 %, Table 1 entries 8–12) compared to the parent variants. Using the most active variant, Mb-(H64 V,V68G,L69 V), the yield of the enzymatic reaction could be further improved upon optimization of the catalyst loading ($OD_{600}=40$ vs. 20; Table 1, entry 13 vs. 12; see also Supporting Information Table S3) and through portion-wise addition of the diazoacetonitrile reagent. Under these optimized conditions, the Mb(H64 V,V68G,L69 V) catalyst is able to afford the desired gem-difluoro-cyclopropane **3a** in 86 % yield and high diastereo- and enantioselectivity (> 99:1 dr and 97:3 er; Table 1, Entry 14). Notably, this represents a first example of successful intermolecular cyclopropanation of gem-difluoro-olefins.

Next, we explored the scope of this methodology using gem-difluoro-styrene derivatives containing different electron-donating or electron-withdrawing groups at the *ortho, meta*, and *para* positions (Scheme 2). *Para* substituted substrates were all efficiently processed by the Mb-(H64V,V68G,L69V) enzyme, leading to the corresponding gem-difluoro-cyclopropanes **3a**–**3g** in good to high yields (55–90 %) and with excellent diastereoselectivity (up to >99:1 d.r.) and enantioselectivity (up to >99:1 e.r.) (Scheme 2). Both electron-donating groups (e.g., **3f**) and strong electrowithdrawing groups (e.g., **3g**) were well tolerated by the biocatalyst. X-ray crystallographic analysis of **3c** (CCDC 2352509[22]) permitted elucidation of the 1*S*,2*S* configuration of the cyclopropane products, which mirrors that of Mb(H64V,V68A)-catalyzed cyclopropanation with EDA and DAN.[20d,f] Interestingly, the enzyme's enantioselectivity across the series of *para*-substituted styrenyl substrates was found to correlate with the size of the *para* substituent (Br (99 % e.e.) ≈ Me (98 % e.e.) > OMe (94 % e.e.) ≈ Cl (92 % e.e.) > F (78 % e.e.) > H (48 % e.e.)), regardless of their electronic effects (Br ≈ Me and OMe ≈ Cl).

Styrene derivatives bearing *meta* and *ortho* substituents were also transformed by the enzyme to the corresponding gem-difluoro-cyclopropanes (**3h**–**3l**), albeit with generally lower yields compared to the *para* substituted counterparts. While moderate enantioselectivity was observed for the *meta*-substituted products (e.g. **3i** and **3l**), the ortho-substituted cyclopropane products were obtained in high to excellent enantiomeric excess. Similarly, alpha substitution on the olefin was well accepted by the enzyme which afforded cyclopropane **3n** in 50 % yield and high enantioselectivity (e.r. 98:2). In addition, using the present strategy, diene **2n** could also be successfully converted to the desired cyclopropane **3n**, albeit in modest yield.

In view of the modest enantioselectivity of Mb-(H64V,V68G,L69V) for the *meta*-substituted substrates, we sought to address this limitation using a mechanism-based rational design approach. As mentioned above, the 1*S*,2*S* enantioselectivity of Mb(H64A,V68G,L69V) in this reaction mirrors that of Mb(H64V,V68A)-catalyzed cyclopropanation of styrene with EDA,[20d,f] implying a similar arrangement of the reactive carbene and styrene substrate in the active site of the enzyme. Based on the previously established stereochemical model for the latter reaction,[21] the styrene ring in the present transformation is expected to be positioned in proximity to the 'distal histidine' position 64 of the enzyme. Accordingly, a Val→Ala mutation was introduced at this site to better accommodate *meta* substituents in the aryl ring of the olefin. Gratifyingly, the resulting variant, Mb(H64A,V68G,L69V), showed high enantioselectivity (i.e., 92:8 to >99:1 e.r.) not only across the target substrates **3i**, **3j** and **3l** (Scheme 2), but also across the entire substrate set of substrates (SI Figure S2), thus nicely complementing the scope of Mb-(H64V,V68G,L69V).

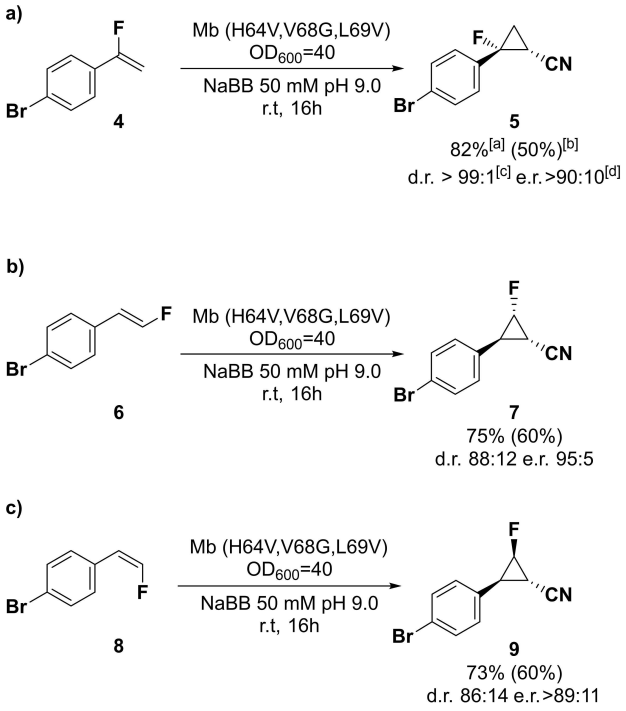
Having established the scope of the methodology for the enantioselective synthesis of gem-difluoro cyclopropanes, we sought to explore its application for the asymmetric cyclopropanation of monofluorinated olefins, which have also remained elusive (in the case of beta-fluoro-styrenes) or largely underdeveloped in the case of alpha-fluoro-styrene.[13a] Gratifyingly, Mb(H64V,V68G,L69V)-catalyzed reaction with α-fluoro styrene **4** produced the desired monofluorinated cyclopropane **5** in good yield (82 %) and good enantioselectivity (e.r. 90:10) (Scheme 3, entry a). In addition, both trans-β-fluoro-styrene derivative **6** and cis-β-fluoro-styrene derivative **8** could be converted to the desired cyclopropane products **7** and **9**, respectively, in high yields



Scheme 2. Reaction conditions: 5 mM olefin, 20 mM diazoacetonitrile, Mb (H64V,V68G,L69V) expressing *E. coli* ($OD_{600}=40$) in sodium borate buffer 50 mM pH 9.0, room temperature, 16 h, anaerobic conditions. [a] Yield as determined by fluorine NMR using trifluorotoluene as internal standard. [b] Isolated yield on preparative scale 0.025 mM. [c] Diastereomeric ratio (d.r.) as determined by fluorine NMR and chiral HPLC. [d] Enantiomeric ratio (e.r.) as determined by chiral HPLC. [e] e.r. obtained with variant H64A,V68G,L69V, yields for this variant can be found on Figure S2.

(73–75 %) and good to high enantiomeric excess (up to 95:5 e.r.) (Scheme 3, Entries b–c). X-ray crystallography of compound **7** (CCDC 2352508[22]) confirmed the absolute configuration of this compound as the expected isomer (1*S*,2*S*,3*S*) (See Supporting Information Figure S4).

The cyclopropane products described in Schemes 2 and 3 were isolated from whole cell reactions using purified DAN generated via diazotization of aminoacetonitrile. Although the volatility of these substrates somewhat affects the isolated yields compared to analytical yields, the synthetic value of these transformations is highlighted by the lack of reactivity of organometallic catalysts (SI Table S1) and the need for long synthetic sequences (4 steps) for generating racemic standards of these compounds (SI Figure S6 and S7). Further enhancing the synthetic accessibility of the present method, we also established that the use of purified DAN reagent is not necessary and that in situ generated DAN can be telescoped into the whole cell biotransformation without the need of any specialized glassware/reaction setup[20d] and with no impact on the enzyme's activity and

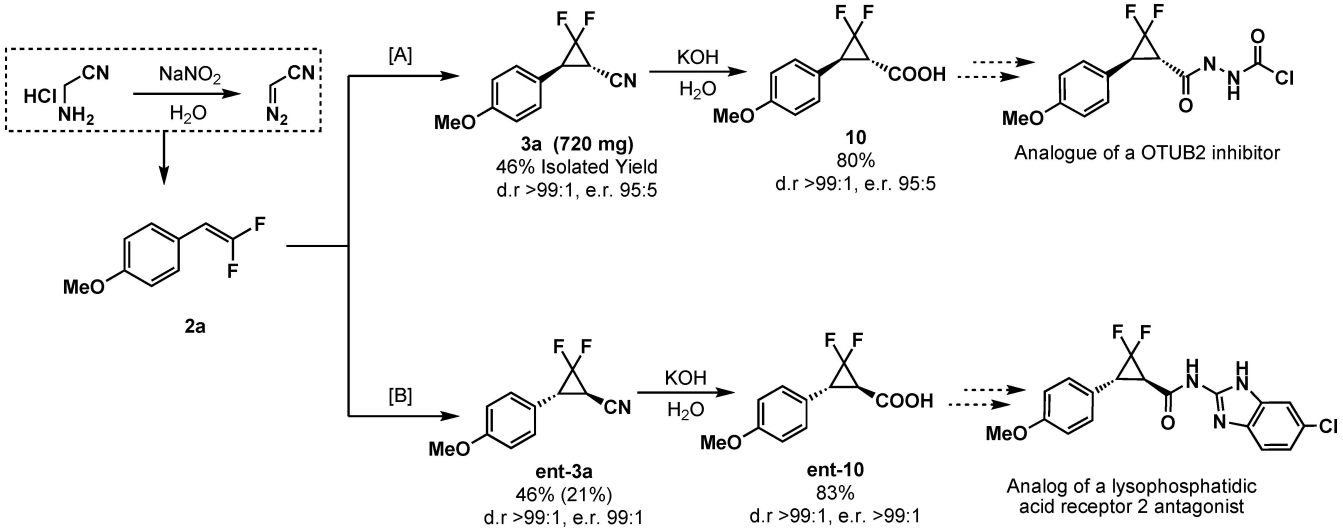


Scheme 3. Biocatalytic cyclopropanation of monofluoro styrene derivatives. Reaction conditions: 5 mM olefin, 20 mM diazoacetonitrile, Mb (H64V,V68G, L69V) expressing *E. coli* ($OD_{600}=40$) in Sodium Borate Buffer 50 mM pH 9.0, room temperature, 16 h. [a] Yield as determined by fluorine NMR using trifluorotoluene as internal standard. [b] Isolated yield on preparative scale 0.025 mM. [c] Diastereomeric ratio (d.r.) as determined by fluorine NMR and chiral HPLC [d] Enantiomeric ratio (e.r.) as determined by chiral HPLC.

stereoselectivity. Using this protocol, a 7.5 mmol-scale reaction was carried out that resulted in the isolation of 720 mg of the desired geminal difluoro cyclopropane **3a** in high diastereo- and enantiomeric excess and 46 % isolated yield, thus demonstrating the scalability of the approach. Finally, using a different Mb variant (L29V,F33A,H64T,V68L), the same transformation could be carried out with opposite enantioselectivity, producing the 1*R*,2*R*-configured gem-difluoro-cyclopropane product **ent-3a** in 46 % yield and with excellent diastereo and enantioselectivity (>99:1 d.r. and >99:1 e.r.; Scheme 4B). These results thus demonstrate the feasibility of performing these transformations with enantiodivergent selectivity.

An added advantage of the present method stems from the versatile nature of the nitrile group, which is readily amenable to further functionalization for chemical diversification purposes as shown previously.[20d] For example, facile hydrolysis of the nitrile group in **3a** and **ent-3a** yielded the carboxylic acids **10** and **ent-10** with no loss in enantiopurity, these compounds furnishing key precursors for the synthesis of gem-difluoro analogs of a covalent OTUB2 inhibitor[23] and lysophosphatidic acid receptor 2 antagonist,[7] respectively (Scheme 4).

In summary, we report a first example of intermolecular asymmetric cyclopropanation of gem-difluoro olefins to afford gem-difluoro-cyclopropanes, which are highly desirable building blocks in medicinal chemistry (Figure 1). This transformation was made possible using engineered myoglobin-based carbene transferases, and specifically Mb (H64V,V68G,L69V) and Mb (H64A,V68G,L69V), and diazoacetonitrile as carbene donor reagent. Using this methodology, a broad range of vinylarene substrates were converted to the corresponding gem-difluoro-cyclopropanes in good yields (up to 88 %) and with high to excellent stereoselectivity (>99:1 d.r. and >99:1 e.r.). In addition, this methodology is scalable and can be extended to the transformation of α- and β-monofluorinated olefins as shown in



Scheme 4. Synthetic utility of the biocatalytic enantioselective cyclopropanation.[A] Reaction conditions = on a 7.5 mmol scale, 5 mM olefin, 20 mM diazoacetonitrile, Mb (H64V,V68G,L69V)) expressing *E. coli* ($OD_{600}=17$) in Sodium Borate Buffer 50 mM pH 9.0, room temperature, 36 h. [B] Reaction conditions = on a 0.25 mM scale, 5 mM olefin, 20 mM diazoacetonitrile, Mb(L29V,F33A,H64T,V68L) expressing *E. coli* ($OD_{600}=40$) in Sodium Borate Buffer 50 mM pH 9.0, RT, 16 h

the preparation of the fluorocyclopropanes **5**, **7**, and **9**. The possibility of achieving enantiocomplementarity in this transformation was also demonstrated. This strategy provides a direct and efficient route to highly valuable, and currently hard to access or inaccessible, fluorinated cyclopropane building blocks for medicinal chemistry applications as well as a streamlined path to the preparation of monofluorinated or gem-difluoro analogs of existing bioactive molecules.

Supporting Information

The data that support the findings of this study are available in the supplementary material of this article.

The authors have cited additional references within the Supporting Information.[20d,24,25]

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Asymmetric cyclopropanation · Biocatalysis · Carbene Transfer · Fluorinated cyclopropanes · Myoglobin

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Communication

Asymmetric Cyclopropanation

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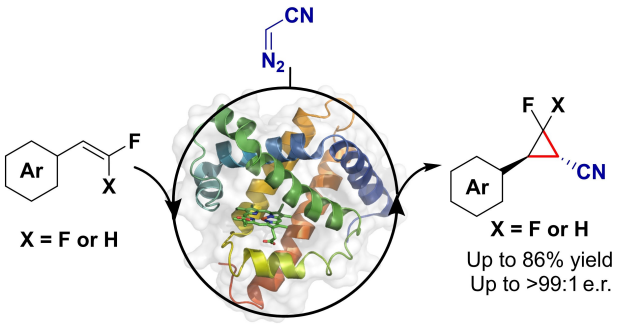
Biocatalytic Strategy for the Highly Stereoselective Synthesis of Fluorinated Cyclopropanes



A first enantioselective method for the cyclopropanation of difluorinated olefins is presented. Using engineered myoglobin-based catalysts in combination with diazoacetonitrile as the carbene precursor, the present method enables the production of different fluorinated cyclopropanes with high yields and high enantioselectivity.