P450-catalyzed asymmetric cyclopropanation of electron-deficient olefins under aerobic conditions

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COMMUNICATION

P450-catalyzed asymmetric cyclopropanation of electron-deficient olefins under aerobic conditions

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A variant of P450 from *Bacillus megaterium* five mutations away from wild type is a highly active catalyst for cyclopropanation of a variety of acrylamide and acrylate olefins with ethyl diazoacetate (EDA). The very high rate of reaction enabled by histidine ligation allowed the reaction to be conducted under aerobic conditions. The promiscuity of this catalyst for a variety of substrates containing amides has enabled synthesis of a small library of precursors to levomilnacipran derivatives.

Introduction

Expanding the range of synthetic transformations catalyzed by biocatalysts will increase their usage by the synthetic community and aid in the discovery of new biologically active molecules.[1] Enantioselective cyclopropanation is a highly sought after transformation as it can be used to construct multiple stereocenters in one step and synthesize key components of biologically relevant targets. While many catalysts for cyclopropanation using transition metals have been developed,[2] the cost and difficulty of these processes have limited their use on scale in industry. As an alternative to these methods, our laboratory has developed a biocatalytic method for cyclopropanation of styrenes in the presence of diazo compounds using engineered variants of cytochrome P450 from *Bacillus megaterium* (P450-BM3).[3] The reaction takes place in water at ambient temperature and the catalyst can carry out tens of thousands of catalytic turnovers. Additionally, we found that mutation of the proximal cysteine ligand in P450-BM3 to serine (C400S) led to an increase in the Fe^{III}-Fe^{II} redox potential by 140 mV, thereby allowing reduction of the Fe^{III} resting state to the catalytically-active Fe^{II} species under physiological conditions (Figure 1).[4] This key finding afforded us the ability to conduct these reactions using whole cells expressing the enzyme, an important advantage of our system because it does not need exogenous reductant or purified protein.

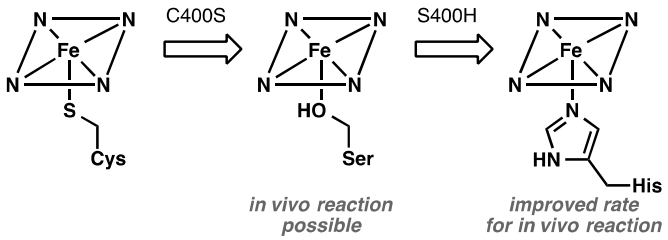
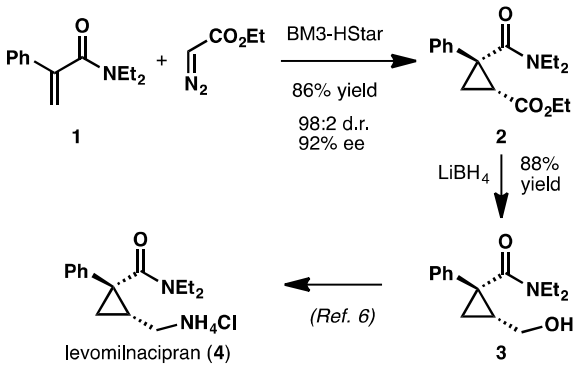


Figure 1. Evolution of P450-BM3 for cyclopropanation activity by mutation of the axial ligand at position 400.

More recently, we discovered that mutation of the cysteine at position 400 of P450-BM3 to histidine led to a further dramatic increase in the rate of cyclopropanation of olefins.[5] Through iterative site-saturation mutagenesis, we engineered a P450-BM3 variant, BM3-HStar (T268A-C400H-L437W-V78M-L181V) five mutations away from wild type P450-BM3, which catalyzed cyclopropanation of acrylamide **1** in greater than 92% yield with 92% enantioselectivity and 2:98 diastereoselectivity (Scheme 1). Conversion of cyclopropane **2** to alcohol **3** constituted a formal synthesis of levomilnacipran,[6] the psychoactive enantiomer of milnacipran and a selective serotonin and norepinephrine reuptake inhibitor recently approved by the US Food and Drug Administration.[7]

While there have only been a few reported examples of cyclopropanation of electron-deficient olefins with transition metal catalysts, these published reports showed broad generality on a variety of olefins and tolerance to electron-neutral substituents of varying sizes.[8] In contrast, enzymes often require strong substrate binding for catalysis and thus are highly specific for a particular molecule. For instance, P450-BM3 only undergoes the requisite spin shift for molecular oxygen activation in the presence of a strongly bound substrate like palmitic acid.[9] While this exquisite

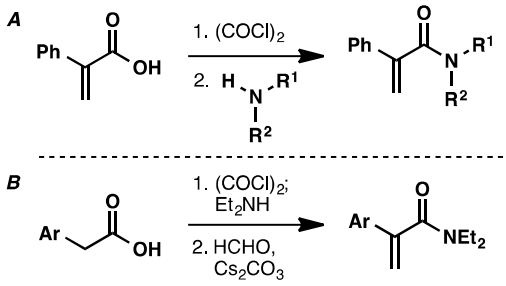
selectivity can be advantageous in some cases, it is also a significant synthetic limitation because each evolved enzyme can only be used for sterically similar substrates. However, as P450-BM3 contains a large hydrophobic binding pocket and strong substrate binding is not required for unnatural cyclopropanation activity, we hypothesized that our BM3-Hstar variant may be a more general catalyst for a variety of acrylate and acrylamide substrates. Furthermore, previous SAR studies of milnacipran analogs have shown that many molecules within this family are active against monoamine transporters.[10] Thus, we were interested in employing the BM3-Hstar biocatalyst for enantioselective synthesis of levomilnacipran analogs.



Scheme 1. Biocatalytic cyclopropanation of acrylamide **1** *en route* to formal synthesis of levomilnacipran.

Results and Discussion

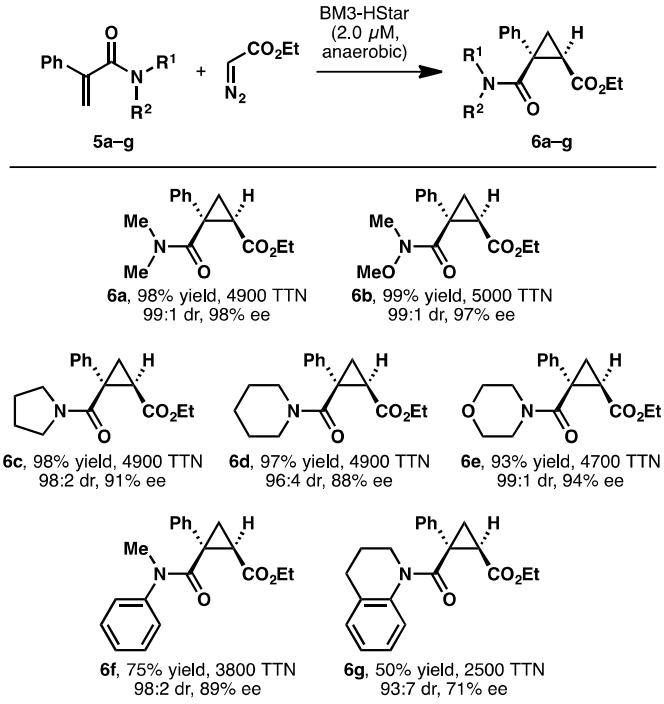
To examine the generality of cyclopropanation by BM3-Hstar, a library of acrylamides was synthesized. The substituent on the amide moiety was varied by conducting Schotten-Baumann reactions on atropic acid with the appropriate amines (Scheme 2A). In parallel, a range of phenylacetic acid derivatives was converted to the corresponding diethyl carboxamide, followed by condensation with paraformaldehyde to arrive at the appropriate acrylamides (Scheme 2B). Variation on both the amide and the aryl moieties would allow us to probe the steric and electronic restriction the enzyme scaffold places on the cyclopropanation reaction.



Scheme 2. Preparation of acrylamide library from the appropriate carboxylic acid containing variation at a) the amide moiety or b) the aryl group.

When we combined a variety of small- to medium-sized acrylamides with EDA in the presence of *Escherichia coli* cells expressing BM3-Hstar, we observed formation of the desired cyclopropanes in more than 90% yield with excellent diastereoselectivity and enantioselectivity (Table 1). Notably, Weinreb amide **6b**, a valuable intermediate for further transformations,[11] could be synthesized in excellent yield and selectivity. Unsymmetrical amides such as **5f** could also be cyclopropanated in good yields. Previous biological studies with a racemic sample of tetrahydroquinolinyl analog of milnacipran have shown that it is very active and highly selective for inhibition of epinephrine and serotonin monoamine transporters.[12] A precursor to this potent molecule, **6g**, can be prepared in 50% yield and good selectivity with BM3-Hstar. While the yield of this reaction is lower than what was observed for smaller amide substrates, our method provides a facile method for rapidly accessing enantioenriched **6g**.

Table 1. Scope of acrylamides with variation on the amide moiety.[a,b,c]



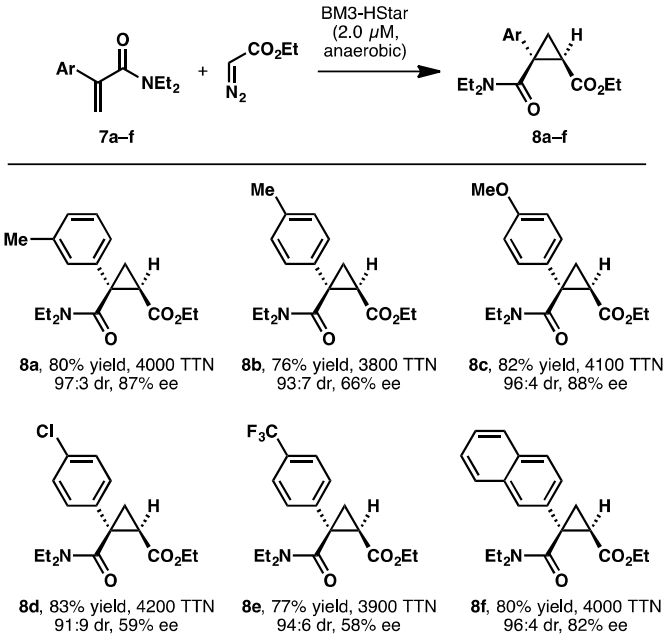
[a] Reactions were carried out with whole cells expressing BM3-HStar (2.0 μM), glucose (250 mM, 50 μM), acrylamide (10 μM), EDA (10 μM) in M9-N buffer (500 μL) at room temperature. [b]Yields and d.r. were determined by gas chromatography of the crude reaction mixture calibrated for the appropriate products using 0.4 μmol of 2-phenylethanol as internal standard. Enantioselectivity was determined by chiral super-critical fluid chromatography (SFC). [c]Relative and absolute configurations were assigned based on analogy to **2**.

Moving to the aryl group of the acrylamide, we found that both sterically- and electronically-demanding aryl substituents are well-tolerated (Table 2). Acrylamides containing electron-rich aryl groups (**7a-c**) provided the corresponding cyclopropane products in good to high yield and great stereoselectivity and even substrates containing *p*-Cl or *p*-CF_3 electron withdrawing substituents (**7d** and **7e**, respectively) were readily cyclopropanated with BM3-Hstar, albeit with lower yields. Additionally, increasing the size of the aryl group to naphthyl did not diminish the yield of the reaction.

BM3-Hstar is surprisingly insensitive to the size and shape of the acrylamide, considering that substrates like **5a** and **5g** differ by

seven carbons and even those with rigid substituents like naphthyl (**7f**) react readily within the protein. Interestingly, the diastereo- and enantioselectivity of the cyclopropanation remained consistent for all of the substrates examined, suggesting that the enzyme facilitates the approach of the olefin to the putative iron carbenoid generated at the P450-heme prosthetic group in the same orientation for all of the substrates examined. However, changing the acrylamide to the analogous acrylate (Scheme 3), despite having little effect on the yield, led to diminished diastereoselectivity and enantioselectivity of the reaction. This result suggests that the amide group is important for stereocontrol in cyclopropanation with BM3-Hstar and that our evolved protein may be tuned for this particular functional group.

Table 2. Scope of acrylamides with variation on the aryl ring.[a,b,c]



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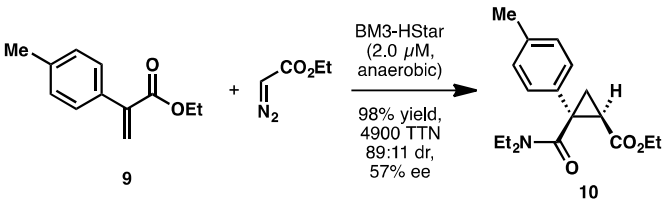
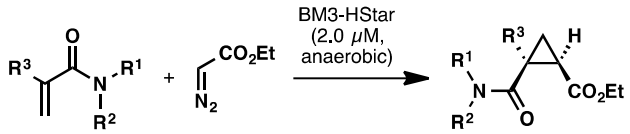


Scheme 3. Reaction of acrylate **9** with EDA catalyzed by BM3-HStar.

In our previous report, we hypothesized that the much improved rate of cyclopropanation of acrylamide **1** catalyzed by BM3-HStar would out-compete inhibition by molecular oxygen, thereby allowing the cyclopropanation reaction to be performed under aerobic conditions. To test the generality of this behavior, cyclopropanation of **5a–g**, **7a–f**, and **9** was performed under ambient atmosphere without degassed buffer or glucose. A comparison of the reaction for each substrate under aerobic versus anaerobic conditions is presented in Table 3. We observed that reactions with substrates **5a–5g** showed minimal loss in yield when conducted under aerobic conditions. However, the effect of oxygen inhibition became more appreciable when the substituents on the aryl ring were varied. In particular, electron-withdrawing substituents and the naphthyl group gave the most significant drop in yield when the reaction was run under aerobic conditions. Presumably, the more electron-deficient nature of the olefin and the increase in steric bulk (in the case of naphthyl substrate **7f**) led to a slower rate of cyclopropanation and as a result, inhibition by atmospheric oxygen is competitive.

Table 3. Comparison of anaerobic and aerobic reaction with BM3-HStar.



Product	Anaerobic Yield (%)	Aerobic Yield (%)	Aerobic yield/anaerobic yield
6a	98	98	1.0
6b	99	98	0.99
6c	98	98	1.0
6d	97	95	0.98
6e	93	92	0.99
6f	75	75	1.0
6g	50	45	0.90
8a	80	71	0.89
8b	76	68	0.89
8c	82	72	0.88
8d	83	61	0.73
8e	77	62	0.81
8f	80	54	0.68
10	98	95	0.97

Conclusions

In summary, we demonstrated that P450-catalyzed cyclopropanation can be used for rapid synthesis of a variety of enantioenriched precursors to levomilnacipran derivatives and that the engineered enzyme BM3-Hstar is a relatively general catalyst for this transformation. Our findings showed that this catalyst tolerates substrates with substituents on both the amide and the aryl components. In all the cases examined, the diastereoselectivity of the reaction is excellent and the enantioselectivity ranges from moderate to high, implying that the protein still plays an important role in orienting substrates that it was not specifically evolved for. In addition, we have demonstrated that this biocatalytic cyclopropanation of electron-deficient alkenes could be conducted under aerobic conditions, without loss of yield for many of the cases examined. Our findings suggest that the activity of P450-BM3 variants for unnatural modes of reactivity may be more promiscuous than for natural monooxygenation.

Acknowledgements

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Notes and references

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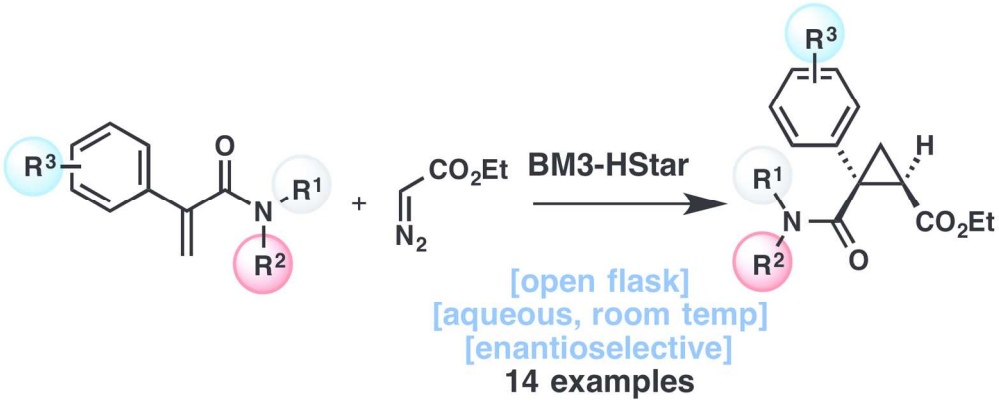

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