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Biocatalytic One-Carbon Ring Expansion of Aziridines to Azetidines via a Highly Enantioselective [1,2]-Stevens Rearrangement

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Abstract

We report enantioselective one-carbon ring expansion of aziridines to make azetidines as a new-to-nature activity of engineered ‘carbene transferase’ enzymes. A laboratory-evolved variant of cytochrome P450_{BM3}, P411-AzetS, not only exerts unparalleled stereocontrol (99:1 er) over a [1,2]-Stevens rearrangement, but also overrides the inherent reactivity of aziridinium ylides, cheletropic extrusion of olefins, to perform a [1,2]-Stevens rearrangement. By controlling the fate of the highly reactive aziridinium ylide intermediates, these evolvable biocatalysts promote a transformation which cannot currently be performed using other catalyst classes.

Graphical Abstract

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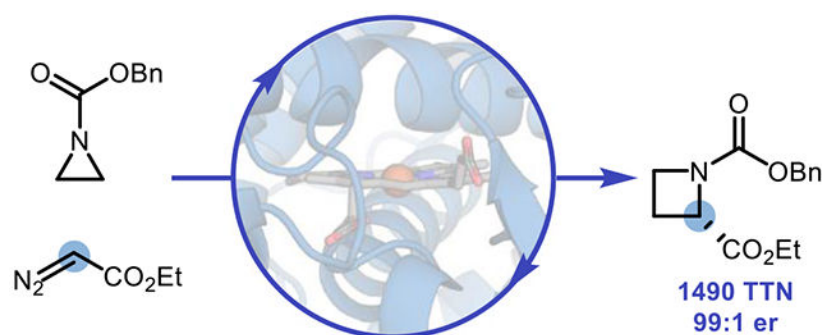
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General information and protocols, detailed description of experimental methods, characterization data for all compounds, NMR spectra of all products, and chiral GC traces of all products. (SI_1)

Full nucleotide and amino-acid sequences for all reported enzyme variants. (SI_2)

The authors declare no competing financial interest.



Highly stereoselective rearrangement of aziridinium ylides

Ring-size manipulation has emerged as a powerful strategy to convert readily available cyclic structures into ring-expanded or ring-contracted compounds that are more difficult to synthesize using conventional means.¹ In particular, “cut and sew” strategies relying on transition-metal catalyzed oxidative addition across C–C bonds are useful approaches for insertion of carbon monoxide or two-carbon fragments such as olefins and alkynes to effect one- or two-carbon ring expansions, respectively.² For nitrogen-containing heterocycles, one possible strategy for ring expansion is to induce a [1,2]-Stevens rearrangement by formation of an ammonium ylide, resulting in one-carbon ring expansion.³ Pioneering works by Hata, West, and Couty demonstrated this approach for 4- to 5-membered ring expansions, wherein treatment of an azetidine with a diazo compound in the presence of a copper catalyst provided facile access to the corresponding pyrrolidine.⁴ Conceptually, carbene transfer followed by an intramolecular [1,2]-Stevens rearrangement complements “cut and sew” reactions for non-carbonylative, one-carbon homologation of nitrogen-containing compounds. Given the prevalence of nitrogen heterocycles across numerous sectors of the chemical industry, especially pharmaceuticals,⁵ extending these methodologies to other saturated *N*-heterocycles would represent a new approach for the synthesis of important chiral amine building blocks.

Despite their promising properties,⁶ azetidines are underrepresented relative to closely related nitrogen-containing heterocycles: this is due to a lack of robust synthetic methods to access these species,^{7–8} especially using asymmetric catalysis.^{9–10} Application of a ring-expansion strategy for the asymmetric, one-carbon homologation of readily prepared aziridines via carbene insertion would be an attractive new entry toward the enantioselective synthesis of azetidines (Figure 1). However, this approach comes with two major selectivity challenges. The first is the innate reactivity of the intermediate aziridinium ylides, which undergo highly favorable cheletropic extrusion of olefins in many contexts.¹¹ Schomaker and others have demonstrated that these reactive intermediates can be harnessed in [2,3]-Stevens rearrangements and other ring-opening reactions.¹² However, we are unaware of any examples of a one-carbon ring expansion of aziridines through a [1,2]-Stevens rearrangement strategy. Secondly, the diradical mechanism of the [1,2]-Stevens rearrangement¹³ has made it a challenging reaction class for asymmetric catalysis: few asymmetric variations have been reported.¹⁴ Enantiopure quaternary ammonium salts can undergo [1,2]-Stevens rearrangements with *N*-to-*C* chirality transfer;¹⁵ however, escape of

the radical pair from the solvent cage is often competitive with radical recombination,¹⁶ and erosion of enantiopurity is often observed. General strategies for stereocontrol over these rearrangements are an unmet challenge facing the field of asymmetric catalysis.

The joint selectivity challenges presented by the asymmetric one-carbon ring expansion of aziridines into azetidines requires a potential catalyst not only to select for the [1,2]-Stevens rearrangement in preference to cheletropic extrusion of olefins, but also to exert enantiocontrol over potential radical intermediates. Nature utilizes ring-size manipulation in the biosynthesis of natural products, with common strategies for biocatalytic ring expansion including oxidative ring expansions¹⁷ and carbocation rearrangements.¹⁸ Furthermore, enzymes derived from cytochrome P450_{BM3}, such as cytochromes P411, and other hemoproteins have emerged as powerful catalysts for carbene transfer reactions,¹⁹ and formation of strained rings such as cyclopropanes and cyclopropenes with excellent stereoselectivities has been reported.²⁰ The most common reactions of enzymatic iron carbenoid intermediates are additions across π -systems^{19–20} or X–H bond insertions;^{21–22} biocatalytic C–N bond insertion through Stevens rearrangements of any kind have yet to be reported. We envisioned that a carbene transfer enzyme could potentially achieve the requisite chemo- and stereoselection necessary to perform this challenging reaction (Figure 1).

We initiated our studies by screening a panel of hemoproteins for the model reaction of benzyl aziridine-1-carboxylate **1** with ethyl diazoacetate (EDA) as a carbene precursor to provide enantioenriched azetidine **2** (Table 1) in suspensions of *Escherichia coli* (*E. coli*) whole cells. We were delighted to find that a variant of P411_{BM3}-CIS²³ with the additional mutations P248T, I263G, and L437F (“Parent F2”), provided the product with 3.6% yield, 73 total turnover numbers (TTNs), and 90:10 er favoring the (*S*)-enantiomer (Entry 1). Parent F2 is derived from hemoproteins originally engineered for the cyclopropanation of heteroatom-substituted olefins²⁴ and is 17 mutations away from its wild-type progenitor, cytochrome P450_{BM3} from *Bacillus megaterium*, which natively catalyzes the oxidation of long-chain fatty acids.²⁵ Control experiments revealed that hemin is unable to catalyze this reaction (see SI for details). Further control reactions indicated that the observed formation of the ring-opened hydrolysis product of **1** is not an enzyme-dependent process. No other aziridine-derived byproducts (e.g., cheletropic extrusion products¹¹, carbene insertion into the benzylic C–H^{21c}, or α -amino C–H bonds of the substrate^{21e}) were identified, including a second ring expansion to form the corresponding pyrrolidine.⁴ Further experiments demonstrated that neither **2** nor the unsubstituted benzyl azetidine-1-carboxylate underwent ring expansion under the disclosed conditions. Chemoselectivity for aziridine ring expansion over azetidine ring expansion in this system can be attributed to the increased pyramidalization at nitrogen observed for acylaziridines and related compounds, which increases their *N*-nucleophilicity relative to less strained amides.²⁶

Encouraged by this promising initial activity and high enantioselectivity, we chose Parent F2 as a starting point for directed evolution to improve enzyme performance using iterative site-saturation mutagenesis (SSM) of residues located in the heme domain (Entries 2–11), screening for improved azetidine yield by gas chromatography. Sites were selected for mutagenesis based on success in previous directed evolution campaigns of P450_{BM3} as

well as prior knowledge of residues responsible for substrate binding and catalysis in the heme domain of this protein scaffold.^{17a} Ten beneficial mutations were identified during this campaign, resulting in a more efficient ‘azetidine synthase’ (P411-AzetS) with a net improvement of 16-fold in TTN and improved enantioselection (99:1 er). With P411-AzetS in hand, we next examined the impact of varying the reaction conditions on the product yield (Entries 12–17). Notably, increasing the scale from 4 μ mol to 100 μ mol resulted in an increase in the reaction yield. When the concentrations of **1** and EDA were doubled to 20 mM and 30 mM, respectively, a decrease in reaction yield was observed (although TTN increased). The ring expansion reaction also proceeded in clarified cell lysate, albeit with decreased yields when compared to analogous reactions performed with whole-cell suspensions. Lastly, decreasing the reaction temperature from 22 to 4 °C did not have a meaningful impact on the reaction yields when run in whole-cell suspensions.

Next, we sought to examine the substrate scope of this reaction and whether or not the new selectivities we observed could be extended to other substrates (Scheme 1). When this reaction was run at 0.5-mmol scale, azetidine **2** could be formed in 75% yield, 1490 TTN, 67% isolated yield, and 99:1 er. Other aromatic groups could be used in lieu of a phenyl group with uniformly high enantioselection observed in all cases. Notably, a thiophene-bearing aziridine could undergo chemoselective ring expansion to azetidine **3** with no observed cyclopropanation byproducts. This selectivity is notable not only because thiophenes are known to react with EDA-derived metal carbenoids under mild conditions,²⁷ but also because Parent F2 was originally engineered to perform cyclopropanation of heteroatom-substituted olefins.²⁴ Fluorine substituents were also tolerated on the arene ring at the *para*, *meta*, and *ortho* positions to furnish fluorinated products **4–6**. In addition to EDA, other diazoacetate compounds could participate in one-carbon ring expansion with at least 99:1 er (**7–8**). When methyl diazoacetate was used as the carbene precursor to yield **9**, a notable decrease in er (81:19) was observed. One hypothesis for this decrease in enantiopurity is that the smaller aliphatic chain allows for greater conformational freedom of the iron porphyrin carbene intermediate or the putative diradical intermediate. This explanation is consistent with prior work on enzyme-mediated carbene transfer reactions using perfluoroalkyl-stabilized diazo compounds as carbene precursors, where the substrate chain length has a profound influence on the absolute stereochemical configuration of the reaction product.^{21c} The reaction could also be scaled up from 0.5-mmol scale to 10-mmol scale to furnish **2** in 1220 TTN, 61% yield, and 99:1 er with an isolated yield of 1.44 g (55% isolated yield), demonstrating that gram-scale production of enantioenriched azetidines is viable using this platform and that extension of this activity could be a powerful tool for the asymmetric synthesis of chiral heterocycles.

The current P411-AzetS lineage performs poorly with other substrate classes. Aziridine substrates with substituents on the carbon backbone of the ring were unable to undergo ring expansion due to their pronounced capacity for ring opening by hydrolysis relative to unsubstituted aziridine rings. This limitation also prevented *N*-alkyl or *N*-aryl aziridines from serving as viable substrates. Other classes of nitrogen protecting groups (e.g., amides and sulfonamides) demonstrated poor activity; one explanation is that the decreased *N*-nucleophilicity of these species hinders their ability to form aziridinium ylides. Finally,

other carbamate-protecting groups (e.g., -Boc, -Alloc, and -CO₂Me) did not form the desired products, suggesting that the arene may be necessary for proper substrate binding with this lineage of enzymes. With respect to the diazo coupling partner, diazoacetates were uniquely effective: when other diazo coupling partners were subjected to the reaction conditions, only unreacted diazo starting materials or dimerization products were recovered. Efforts to expand the observed, unprecedented reactivity and selectivity to the synthesis of other classes of azetidines are ongoing.

A hypothetical mechanism for the one-carbon ring expansion of aziridines is shown in Figure 2. The reaction of a hemoprotein with a suitable carbene precursor forms an electrophilic iron carbenoid intermediate, which could be trapped by a sufficiently nucleophilic aziridine. Ammonium ylides are commonly proposed as intermediates in hemoprotein-catalyzed N–H insertion reactions,²² and Schomaker has reported numerous examples where carbamate-protected aziridines react with metal-carbenoid electrophiles to form aziridinium ylides.^{11,12c–f} At the present time, it is not clear whether this intermediate would exist as a “free” or metal-bound ylide, although computational analysis of enzymatic N–H insertion reactions suggests that ammonium ylide intermediates react after dissociation from the iron center.^{22f} Finally, the aziridinium ylide could undergo the desired [1,2]-Stevens rearrangement preferentially over cheletropic extrusion of ethylene, liberating the desired product and regenerating the hemoprotein. We envisioned that the active site of an enzyme could mimic solvent caging effects, which are known to exert selectivity over radical recombination in [1,2]-Stevens rearrangements, to achieve asymmetric induction during ring expansion.^{15–16} Such effects may also explain why free hemin is unable to catalyze the reaction in the absence of the specific confinement provided by the active sites of this enzyme lineage. Additionally, hemoproteins demonstrate high stereoselectivity in radical reactions, both in their native activity²⁸ as well as in new-to-nature activity cultivated through protein engineering,²⁹ lending further support to this hypothesis.

In summary, we have demonstrated unprecedented hemoprotein-catalyzed [1,2]-Stevens rearrangement in the context of a one-carbon ring expansion of aziridines to azetidines. This system not only represents a rare example of a highly enantioselective [1,2]-Stevens rearrangement of ammonium ylides, but also exhibits unprecedented selectivity for the [1,2]-Stevens rearrangement of aziridinium ylides over cheletropic extrusion of ethylene. We are optimistic that observed selectivities can be extended to other types of [1,2]-Stevens rearrangements, providing the grounds for future work in this area toward the synthesis of enantioenriched heterocycles and other chiral amines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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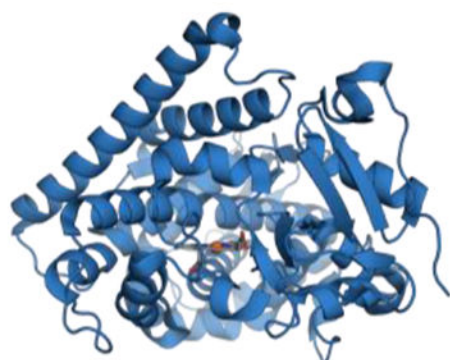
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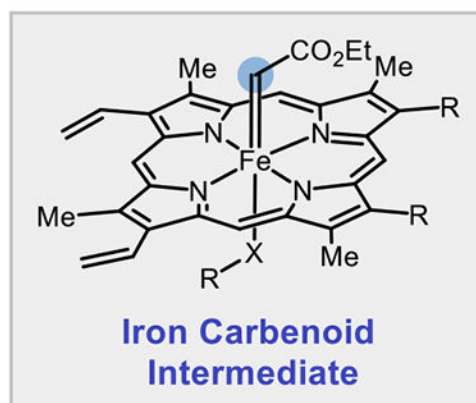
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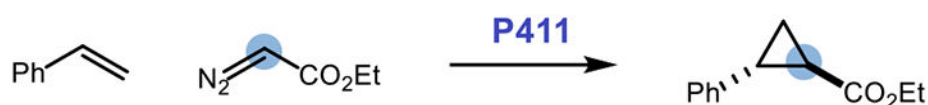


Cytochrome P411



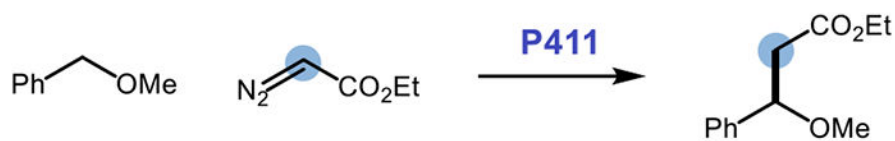
Established Reactivity of Iron Carbenoids

1. Addition to π -systems: 2 C-C Bonds Formed



Access to cyclopropanes, cyclopropenes, and bicyclobutanes

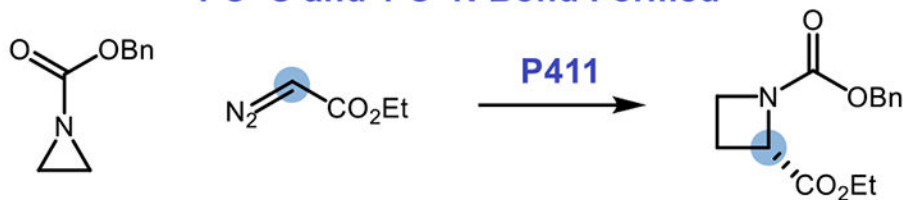
2. Insertion into X-H Bonds: 1 C-H and 1 C-X Bond Formed



Insertion into C-H, Si-H, B-H, S-H, and N-H bonds

New Reactivity: Insertion into C-N Bonds

1 C-C and 1 C-N Bond Formed



Ring size manipulation of aziridines to azetidines

Figure 1:
Classification of enzyme-mediated carbene transfer reactions for various bond disconnections.

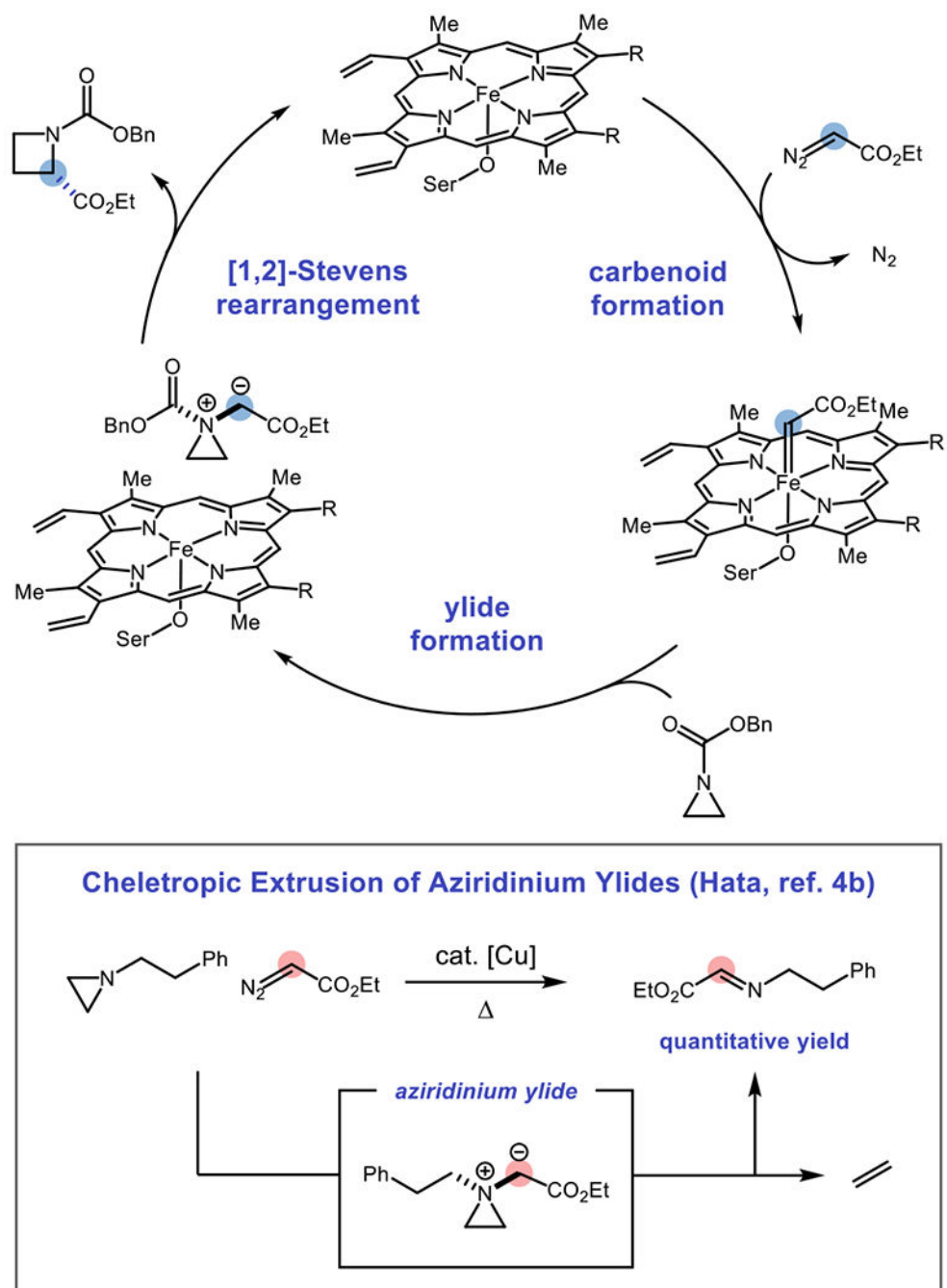
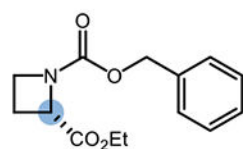
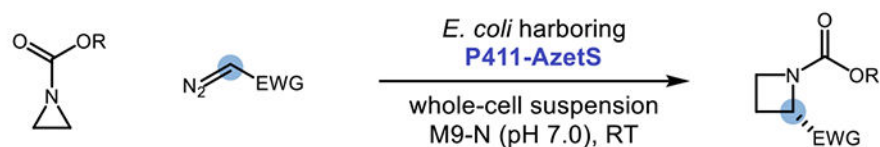
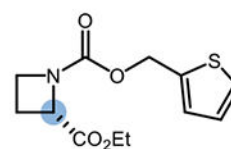


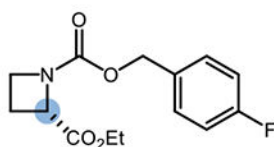
Figure 2: Possible catalytic cycle for one-carbon ring expansion of aziridines to furnish chiral azetidines, with cheletropic extrusion of ethylene as a possible side reaction. The ammonium ylide may also remain iron-bound.



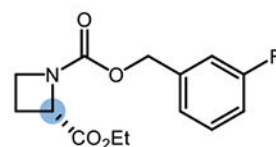
75% (67%) yield,
1490 TTN, 99:1 er



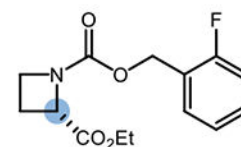
38% (38%) yield,
764 TTN, 99:1 er



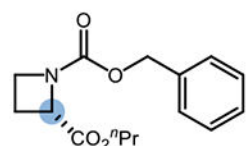
31% (27%) yield,
626 TTN, 99:1 er



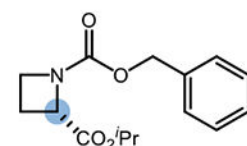
34% (29%) yield,
681 TTN, 99:1 er



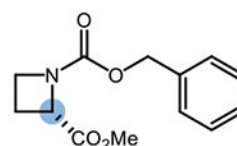
41% (36%) yield,
811 TTN, 99:1 er



18% (17%) yield,
363 TTN, 99:1 er

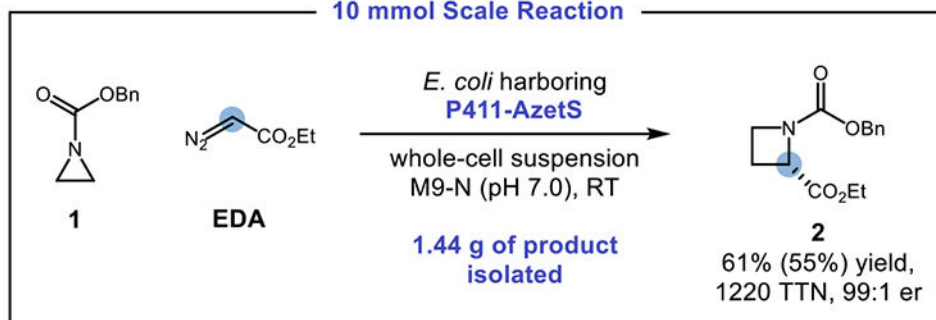


28% (28%) yield,
564 TTN, >99:1 er



20% (18%) yield,
402 TTN, 81:19 er

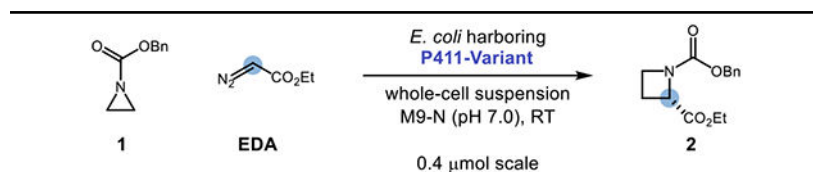
10 mmol Scale Reaction



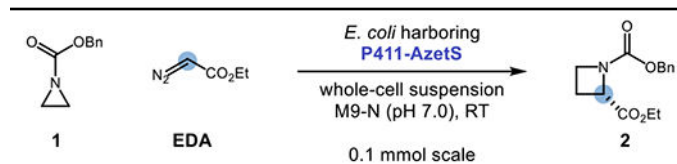
Scheme 1: Substrate Scope^a

^a Reactions were performed on 0.5-mmol scale unless otherwise specified. Analytical yields and TTN were determined by GC-FID. Yields for isolated and purified material are designated in parentheses. The er was determined by Chiral GC. For 0.5-mmol scale reactions, all numbers reported represent the average of two trials. For the 10-mmol scale reaction, the reported numbers represent one run.

Table 1:

Lineage and Reaction Optimization^a

Entry	Variant	Mutations Relative to Prior Generation	TTN	Yield (%)	e.r.
1	Parent F2	None	73	3.6	90:10
2	F2.1	G263Y	70	3.5	75:25
3	F2.2	T327V	126	6.3	56:44
4	F2.3	A330T	193	9.6	59:41
5	F2.4	H266P	394	19.7	62:38
6	F2.5	M177Q	699	34.9	94:6
7	F2.6	T436G	945	47.3	93:7
8	F2.7	L233F	997	49.8	94:6
9	F2.8	T149M	1040	52.0	99:1
10	F2.9	R47Q	1190	59.7	99:1
11	P411-AzetS	M118K	1200	59.9	99:1



Entry	Change from Conditions Above	TTN	Yield (%)	e.r.
12	None	1580	79.1	99:1
13	20 mM [1]; 30 mM [EDA]	2200	55.0	99:1
14	Lysate	1090	54.4	99:1
15	Lysate; 20 mM [1]; 30 mM [EDA]	1570	39.3	99:1
16	4 °C	1610	80.2	99:1
17	Lysate; 4 °C	1380	68.7	99:1

^aReactions were performed on the designated scale and run for 16 h with 10 mM of **1**, 15 mM of EDA, and 5 μM of protein. TTN and yields were determined via GC analysis of crude reaction mixtures relative to an internal standard and represent the average of three experiments. The enantiomeric ratio (er) of the product was determined by chiral GC.