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

David C. Miller,† Ravi G. Lal,† Luca A. Marchetti, †§ and Frances H. Arnold*†

† Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125
§ Present Address: Department of Biosystems Science and Engineering, ETH Zürich, 4058 Basel, Switzerland

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 P450BM3, 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.

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. 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. 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. 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. 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, 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, azetidines are underrepresented relative to closely related nitrogen-containing heterocycles: this is due to a lack of robust synthetic methods to access these species, especially using asymmetric catalysis. 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 towards 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.

Figure 1: Classification of enzyme-mediated carbene transfer reactions for various bond disconnections.
Schomaker and others have demonstrated that these reactive intermediates can be harnessed in [2,3]-Stevens rearrangements and other ring-opening reactions. 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 rearrangement has made it a challenging reaction class for asymmetric catalysis: few asymmetric variations have been reported. Enantiopure quaternary ammonium salts can undergo [1,2]-Stevens rearrangements with N-to-C chirality transfer; however, escape of the radical pair from the solvent cage is often competitive with radical recombination, 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 chelotropic 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 one-carbon ring expansion including oxidative ring expansions and carbocation rearrangements. Furthermore, enzymes derived from cytochrome P450BM3, such as cytochromes P411, and other hemoproteins have emerged as powerful catalysts for carbene transfer reactions, and formation of strained rings such as cyclopropanes and cyclopropanes with excellent stereoselectivities has been reported. The most common reactions of enzymatic iron-carbenoid intermediates are additions across π-systems or X–H bond insertions; 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 stereoselectivity 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 P411BM3-CIS with the additional mutations P248T, I263G, and L437F (“Parent F2”), provided the product with 3.7% yield, 73 total turnover numbers (TTNs), and 90:10 er favoring the (S)-enantiomer (Entry 1). Parent F2 is derived from hemoproteins originally for the cyclopropanation of heteroatrom-substituted olefins and is 17 mutations away from its type progenitor, cytochrome P450BM3 from Bacillus megaterium, which natively catalyzes the oxidation of long-chain fatty acids. Control experiments revealed that heme 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., chelotropic extrusion products, carbene insertion into the benzylic C–H or α-N–H bonds of the substrate) were identified, including a second ring expansion to form the corresponding pyrrolidine. 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.

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

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

Reactions were performed on the designated scale and run for 16 h with 10 mM of EDA, 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 (e.r.) of the product was determined by chiral GC.

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 P450BM3 as well as prior knowledge of residues responsible for substrate binding and catalysis in the heme domain of this protein scaffold. 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 μmol to 100 μmol 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. 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 enantioselectivity 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 carbeneoids under mild conditions, but also because Parent F2 was originally engineered to perform cyclopropanation of heteroatom-substituted olefins. 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.

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-allyl 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 arené 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-carbeneoid intermediate, which could be trapped by a sufficiently nucleophilic aziridine. Ammonium ylides are commonly proposed as intermediates in hemoprotein-catalyzed N–H insertion reactions, and Schoemaker has reported numerous examples where carbamate-protected aziridines react with metal-carbeneoid electrophiles to form aziridinium ylides. At the present time, it is not clear whether this intermediate would exist as a “free” or metal-bound ylide. Finally, the aziridinium ylide could undergo the desired [1,2]-Stevens rearrangement preferentially over chelotropic 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 redocking in [1,2]-Stevens rearrangements, to achieve asymmetric induction during ring expansion.15-16 Hemoproteins demonstrate high stereoselectivity in radical reactions, both in their native reactivity as well as in new-to-nature activity cultivated through protein engineering, 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.

ASSOCIATED CONTENT

The Supporting Information is available free of charge at: Supporting Information Placeholder

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.

REFERENCES


AUTHOR INFORMATION

Corresponding Author

Frances H. Arnold – Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, United States; orcid.org/0000-0002-4027-364X; Email: frances@cheme.caltech.edu

Authors

David C. Miller – Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, United States; orcid.org/0000-0002-4560-8824

Ravi G. Lal – Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, United States; orcid.org/0000-0001-6943-4147

Luca A. Marchetti – Department of BioSystems Science and Engineering, ETH Zürich, 4058 Basel, Switzerland; orcid.org/0000-0002-6100-4465

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Research was sponsored by the U.S. Army Research Office and accomplished under contracts W911NF-19-D-0001 and W911NF-19-2-0026 for the Institute for Collaborative Biotechnologies. D.C.M. was supported by a Ruth Kirschstein NIH Postdoctoral Fellowship (F32GM128247). The authors wish to thank Dr. Sabine Brinkmann-Chen, Nathaniel Goldberg, and Dr. Nicholas Porter for assistance preparing the manuscript.


For selected examples, see: (a) Malik, S.; Nadir, U.K. A Facile Synthesis of 1-Arenesulfonylazetidines through Reaction of 1-Arenesulfonylaziridines with Dimethylsulfonium Methyli

(10) For synthetic sequences leading to enantioenriched aziri-
dines, see: (a) Kapoor, R.; Chawla, R.; Singh, S.; Yadav, L.D.S. Organo-
catalyzed Asymmetric Synthesis of 1,2,4-Trisubstituted Azeti-
dines by Reduction of Cyclopropane of Azidomethyl Adducts of Enones. *Synlett*, **2012**, *13*, 1321. (b) Hanessian, S.; Bernstein, N.; Yang, R.Y.; Maguire, R. Asymmetric synthesis of L-azetidine-2-carboxylic acid and 3-substituted congeners—conformationally constrained an-
ospecific Cross-Coupling Reactions of Nitrogen-Containing Stereocen-


(12) (a) Clark, J.S.; Hodgson, P.B.; Goldsmith, M.D.; Blake, A.J.; Cooke, P.A.; Street, L.J. Rearrangement of ammonium ylides produced by intramolecular reaction of catalytically generated metal carbena-


(28) Ortiz de Montellano, P.R. Hydrocarbon Hydroxylation by Cytochrome P450 Enzymes. Chem. Rev. 2010, 110, 932.

Authors are required to submit a graphic entry for the Table of Contents (TOC) that, in conjunction with the manuscript title, should give the reader a representative idea of one of the following: A key structure, reaction, equation, concept, or theorem, etc., that is discussed in the manuscript. Consult the journal’s Instructions for Authors for TOC graphic specifications.

Highly stereoselective rearrangement of aziridinium ylides