A Transaminase Triggered Aza-Michael Approach for the Enantioselective Synthesis of Piperidine Scaffolds

James Ryan,† Mindagus Siauciulis,‡ Andrew Gomm,† Beatriz Maciá,*,† Elaine O'Reilly,‡*,‡ Vittorio Caprio*‡

†Faculty of Science & Engineering, Division of Chemistry & Environmental Science, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, United Kingdom
‡School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

Supporting Information Placeholder

ABSTRACT: The expanding "toolbox" of biocatalysts opens new opportunities to redesign synthetic strategies to target molecules by incorporating a key enzymatic step into the synthesis. Herein, we describe a general biocatalytic approach for the enantioselective preparation of 2,6-disubstituted piperidines starting from easily accessible pro-chiral ketoenones. The strategy represents a new biocatalytic disconnection, which relies on an ω-TA-mediated aza-Michael reaction. Significantly, we show that the reversible enzymatic process can power the shuttling of amine functionality across a molecular framework, providing access to the desired aza-Michael products. The intramolecular aza-Michael reaction (IMAMR) is a powerful method for the preparation of simple and architecturally complex nitrogen heterocycles and alkaloid skeleta.† An ideal strategy for the synthesis of such heterocycles and alkaloids is a tandem reductive amination/IMAMR sequence (Figure 1), allowing direct, one-pot conversion of readily available prochiral ketoenones 2 to stereodefined, highly functionalized cyclic products 1. However, the approach is dependent upon amination conditions where there is i) no reduction of the double bond, ii) no amination of the enone carbonyl, iii) stereoselective amination of the desired ketone and iv) no amination of the pendent piperidine ketone. Owing to these demands, the strategy outlined in Figure 1 is currently beyond traditional chemical synthesis and IMAM strategies are characterised by step-wise introduction of N/O-functionality with a consequent reliance on protecting group manipulations.‡

Biocatalysis allows us to reevaluate synthetic strategies and enables disconnections that are not possible using traditional chemical synthesis or catalysis.§ ω-Transaminases (ω-TA) enzymes are emerging as extremely important catalysts for the synthesis of optically pure chiral amines starting from readily available prochiral ketoenones.|| Despite the challenges associated with the use of ω-TAs, including the necessity for high equivalents of sacrificial amine donor, the application of an (R)-selective ω-TA variant for the industrial-scale synthesis of the anti-diabetic drug, Sitagliptin, highlights their enormous synthetic potential.|| These enzymes rely on the cofactor pyridoxyl-5'-phosphate (PLP) to mediate the amination of ketones,§ with no requirement for reducing agents, and therefore have the potential to be applied effectively for the synthesis of a broad range of piperidines following the strategy outlined in Figure 1. While previous studies have shown that excellent regioselectivity can be achieved in the conversion of sterically demanding 1,4- and 1,5-diketones bearing one bulky group,§ there is no literature precedence for such selectivity on substrates with two accessible ketones.

Figure 1. An attractive retrosynthesis for the preparation of heterocycles and alkaloids starting from ketoenones.

Here we describe a new biocatalytic disconnection for the regio- and stereoselective synthesis of a range of 2,6-disubstituted piperidines exploiting a key biocatalytic transamination followed by a spontaneous IMAMR. Furthermore, for substrates where high regioselectivity is not expected, we specifically exploit the reversible nature of the biocatalytic amination process to ensure that the amine functionality is ultimately installed at the desired position in a strategy that would not be possible using a classical reductive amination.

Two commercially available ω-TA biocatalysts from Codexis, which have complementary selectivity, were chosen to evaluate the methodology on a small panel of diketones 3a-e. These diketones are readily available via oxidative cleavage of 1-methylcyclopentene followed by reaction with a suitable
phosphorus ylid (see ESI). Complete regioselectivity in the amination step of ketones 3a-d was anticipated from previous literature. As expected, both the (S)- and (R)-selective ω-TA enzymes mediated the transamination reaction exclusively on the methyl ketone in >99% ee (Table 1). Following transamination, a spontaneous IMAMR occurs, providing the 2,6-disubstituted piperidines as a mixture of diastereoisomers. Conveniently, epimerization readily occurred upon standing in MeOH, presumably via a retro-aza-Michael reaction, providing products 4a-d in >99% de. A particularly important aspect of this transformation is the requirement for only 2 equivalents of the low-cost isopropylamine donor in the absence of in-situ by-product removal strategies, owing to the powerful driving force of the 1,4-addition reaction. Firstly, this gave us confidence that the reversible amination strategy could be successfully exploited for the conversion of substrates with two accessible ketones. Additionally, employing these conditions does not lead to any undesired amination of the product pendent ketone. The aza-Michael reaction also drives amination of bulkier ketones and a reversal in the selectivity previously observed during transamination of 1,4,1,5-dicarboxyls. Thus, ethylketone 3e provides piperidine 4e, albeit with reduced yield/ee, using the alternate (S)-selective ATA256.

Table 1. ω-TA-mediated transamination/IMAMR cascade of ketoenones 3a-e.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ω-TA</th>
<th>Conv (%)b</th>
<th>ee (%)c</th>
<th>de (%)d</th>
<th>Yield (%)e</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>ATA13</td>
<td>&gt;99f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>58 (S,S)h</td>
</tr>
<tr>
<td>3a</td>
<td>ATA17</td>
<td>&gt;99f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>88 (R,R)h</td>
</tr>
<tr>
<td>3b</td>
<td>ATA13</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>92 (S,S)h</td>
</tr>
<tr>
<td>3b</td>
<td>ATA17</td>
<td>&gt;99f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>90 (R,R)h</td>
</tr>
<tr>
<td>3c</td>
<td>ATA13</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>76 (S,S)h</td>
</tr>
<tr>
<td>3c</td>
<td>ATA17</td>
<td>50f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>44 (R,R)h</td>
</tr>
<tr>
<td>3d</td>
<td>ATA13</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>72 (S,S)h</td>
</tr>
<tr>
<td>3d</td>
<td>ATA17</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>70 (R,R)h</td>
</tr>
<tr>
<td>3e</td>
<td>ATA256</td>
<td>&gt;99f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>90 (S,S)h</td>
</tr>
</tbody>
</table>

* Reaction conditions: (i) ω-TA (5 mg/mL), substrate (50 mM), isopropylamine (100 mM), pyridoxyl-5-phosphate (PLP, 2 mM), HEPES buffer (100 mM, pH 7.5), 30 °C, 150 RPM, 24 h; (ii) MeOH, r.t., 24 h. 4 Conversion determined by 1H-NMR after 24 h. 4 ee determined by chiral GC or HPLC (see ESI). 4 de determined by NMR after the epimerization step. 4 Isolated yield after flash chromatography. Conversion after 48 h. 4 Epimerization was carried out in EtOH at 50 °C for 24 h. 4 Configuration assigned by analogy with 3f and in agreement with NOESY experiments (see ESI). 4 4 equivalents of isopropylamine were used. 4 Epimerization was carried out in EtOH at 80 °C for 24 h. 4 Reaction carried out at 50 °C. 4 See reference 8.

In light of this, it was envisaged that the same methodology could be employed to access the naturally occurring defense alkaloid (−)-pinidinone 4f from the corresponding dimethyl ketoenone 3f (Scheme 1). An additional level of complexity is associated with this diketone as the ω-TA is not expected to show any regioselectivity in the amination step. We reasoned that while two amine products would initially be formed resulting from amination of the methyl ketone and enone, the reversible nature of the biocatalytic amination coupled with the spontaneous 1,4-addition would drive the shuttling of the undesired amine to allow exclusive isolation of (−)-pinidinone 4f and trans-5f. As expected, incubation of 3f with ATA17 afforded a mixture of diastereoisomers 4f and 5f with >99% conversion and >99% ee, which was easily epimerized to (−)-pinidinone 4f with >99% de (Table 2). Comparable results were obtained with (S)-selective ATA113. We have also demonstrated that 1.1 equivalents of the amine donor were sufficient to achieve >99% conversion (Table 2, footnote f). The synthetic utility of our methodology is showcased by the ease of upscaling, allowing access to 0.48 g of (−)-pinidinone employing only 2 eq of isopropylamine (Scheme 1).

Table 2. Results from biotransformations with 3f employing ATA113 and ATA117.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ω-TA</th>
<th>Conv (%)b</th>
<th>ee (%)c</th>
<th>de (%)d</th>
<th>Yield (%)e</th>
</tr>
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<tbody>
<tr>
<td>3f</td>
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<td>&gt;99f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>91 (S,S)h</td>
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<tr>
<td>3f</td>
<td>ATA17</td>
<td>&gt;99f</td>
<td>&gt;99</td>
<td>&gt;99g</td>
<td>90 (R,R)h</td>
</tr>
</tbody>
</table>

* See Table 1 footnotes. 4 This transformation could also be carried out using 1.1 eq. of isopropylamine (55 mM) with identical conversion. 4 Absolute configuration determined by correlation with known compounds (see ESI).

Scheme 1. Preparative scale conversion of 3f to 4f (−)-pinidinone using (R)-selective ATA117.

To support our hypothesis that the amine functionality can be shuttled across the molecular framework, amino ketone 6f was synthesized in 5 steps (see ESI) and exposed to ATA117 in the absence of any additional amine donor or acceptor (Scheme 2). After 24 h, complete consumption of 6f was observed along with the formation of a mixture of (−)-pinidinone 4f and trans-5f. To our knowledge, this is the first example of an ω-TA reaction that does not require a separate donor and acceptor. The enzyme bound PLP forms pyridoxamine phosphate (PMP) using 6f as the amine donor and generates diketone 3f. The amine functionality is then shuttled to the more thermodynamically stable ketone, which readily undergoes an IMAMR. While bis-amine 7f was not observed during the course of the reaction, it is likely that it is an intermediate. The efficiency of this conversion is striking, as the single amine equivalent available in the reaction has come from the starting material 6f.
In conclusion, we have developed an extremely efficient biocatalyticaza-Michael strategy for the enantioselective conversion of pro-chiral ketonones to 2,6-disubstituted piperidines, with excellent conversion and isolated yield. Our approach reveals that coupling a reversible ω-TA reaction with a strong thermodynamic driving force allows the amine functionality to be shuttled across a molecular framework to form the desired product. This work significantly expands the scope of ω-TA methodology in total synthesis and we are currently exploring the utility of this dynamic chemistry for the synthesis of more complex alkaloid scaffolds.

ASSOCIATED CONTENT

Supporting Information
The supporting information contains details of compound preparation, characterization and NMR/GC/HPLC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors
*v.caprio@mmu.ac.uk
*elaine.oireilly@nottingham.ac.uk
b.macia-ruiz@mmu.ac.uk

Notes
The authors declare no competing financial interests.

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REFERENCES


(8) (R,R)-NHE was prepared using (R)-selective ATA025 in 2% yield (see SI for further details) for ee determination purposes. No optimization on the TA enzyme selection with substrate 3e has been carried out.


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