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

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Supporting Information

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.[1]

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

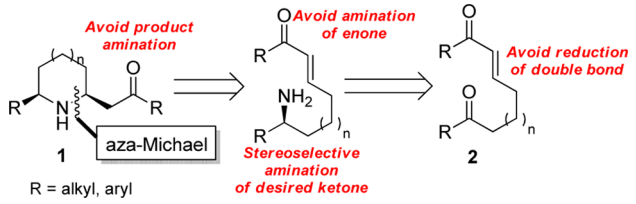

Figure 1. Attractive retrosynthesis for the preparation of heterocycles and alkaloids starting from ketoenones.

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 characterized by stepwise introduction of N/O-functionality with a consequent reliance on protecting group manipulations.[2]

Biocatalysis allows us to re-evaluate synthetic strategies and enables disconnections that are not possible using traditional chemical synthesis or catalysis.[3] ω-Transaminase (ω-TA) enzymes are emerging as extremely important catalysts for the synthesis of optically pure chiral amines starting from readily available prochiral ketones.[4] 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 antidiabetic drug, Sitagliptin, highlights their enormous synthetic potential.[4a] These enzymes rely on the cofactor pyridoxyl-5′-phosphate (PLP) to mediate the amination of ketones,[5] 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. Although 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,[6] there is no literature precedence for such selectivity on substrates with two accessible ketones.

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 dicarbonyls **3a−d**. These compounds 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.[6] 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[7] as a mixture of diastereoisomers.

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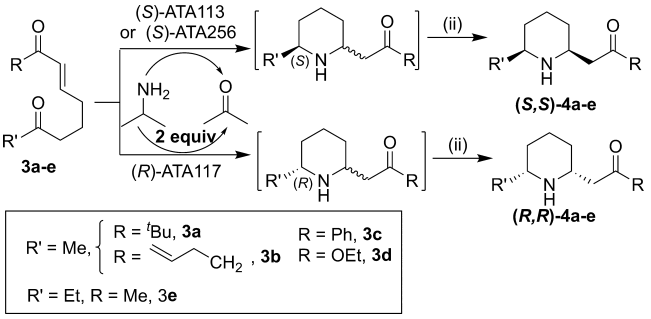
Table 1. ω-TA-Mediated Transamination/IMAMR Cascade of Ketoenones 3a−e[a]



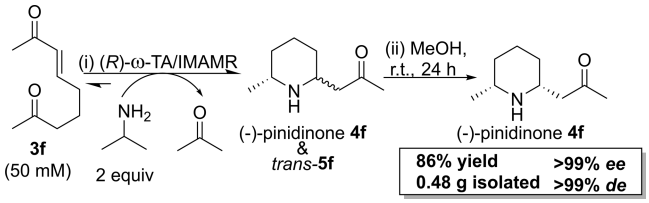
Substrate	ω-TA	Conv (%)[b]	ee (%)[c]	de (%)[d]	Yield (%)[e]
3a	ATA113	>99[f]	>99	>99[g]	78 (*S,S*)[h]
3a	ATA117	>99[f]	>99	>99[g]	88 (*R,R*)[h]
3b	ATA113	>99	>99	>99	92 (*S,S*)[h]
3b[i]	ATA117	>99[f]	>99	>99	90 (*R,R*)[h]
3c	ATA113	>99	>99	>99	76 (*S,S*)[h]
3c[i]	ATA117	50[f]	>99	>99	44 (*R,R*)[h]
3d	ATA113	>99	>99	>99[j]	72 (*S,S*)[h]
3d	ATA117	>99	>99	>99[j]	70 (*R,R*)[h]
3e[i,k]	ATA256	>99[f]	70[l]	>99	50 (*S,S*)[h]

[a]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. [b]Conversion determined by ^{1}H NMR after 24 h. [c]ee determined by chiral GC or HPLC (see ESI). [d]de determined by NMR after the epimerization step. [e]Isolated yield after flash chromatography. [f]Conversion after 48 h. [g]Epimerization was carried out at 65 °C for 24 h. [h]Configuration assigned by analogy with **4f** and in agreement with NOESY experiments (see ESI). [i]4 equiv of isopropylamine were used. [j]Epimerization was carried out in EtOH at 80 °C for 24 h. [k]Reaction carried out at 50 °C. [l]See ref 8.

Conveniently, epimerization readily occurred upon standing in MeOH, presumably via a retro-aza-Michael reaction,[8] providing products **4a−d** in >99% de. A particularly important aspect of this transformation is the requirement for only 2 equiv of the low-cost isopropylamine donor in the absence of in situ byproduct removal strategies, owing to the powerful driving force of the 1,4-addition reaction. Significantly, 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 with a reversal in the selectivity previously observed during transamination of 1,4/1,5-dicarbonyls.[6] Thus, ethylketoenone **3e** provides piperidine **4e**, albeit with reduced yield/ee, using the alternate (*S*)-selective ATA256.[8]

In light of this, it was envisaged that the same methodology could be employed to access the naturally occurring defense alkaloid (−)-pinidinone **4f**[9,10] 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 although 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 ATA117

Scheme 1. Preparative Scale Conversion of 3f to 4f (−)-Pinidinone Using (*R*)-Selective ATA117



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

Table 2. Results from Biotransformations with 3f Employing ATA113 and ATA117[a]

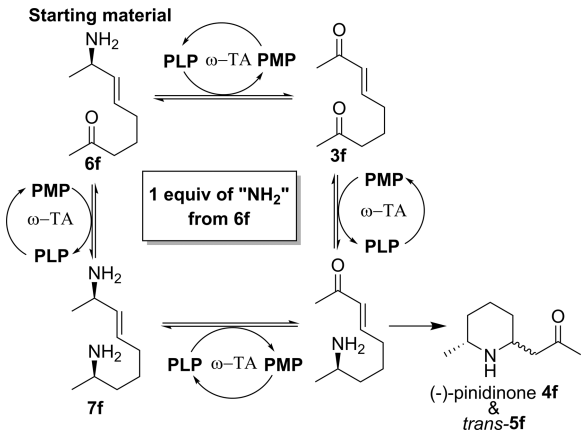
Substrate	ω-TA	Conv (%)[b]	ee (%)[c]	de (%)[d]	Yield (%)[e]
3f	ATA113	>99[f]	>99	>99	91 (*S,S*)[g]
3f	ATA117	>99[f]	>99	>99	90 (*R,R*)[g]

[a−e]See Table 1 footnotes. [f]This transformation could also be carried out using 1.1 equiv of isopropylamine (55 mM) with identical conversion. [g]Absolute configuration determined by correlation with known compounds (see ESI).

were obtained with (*S*)-selective ATA113. We have also demonstrated that 1.1 equiv 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 equiv of isopropylamine (Scheme 1).

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

Scheme 2. Proposed Mechanism for the Formation of (−)-Pinidinone 4f and *trans*-5f from the Single Amine Equivalent 6f



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

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thermodynamically stable ketone, which readily undergoes an IMAMR. Although 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 biocatalytic aza-Michael strategy for the enantioselective conversion of pro-chiral ketoenones 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 is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b07024.

Details of compound preparation, characterization and NMR/GC/HPLC spectra (PDF)

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Notes

The authors declare no competing financial interest.

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