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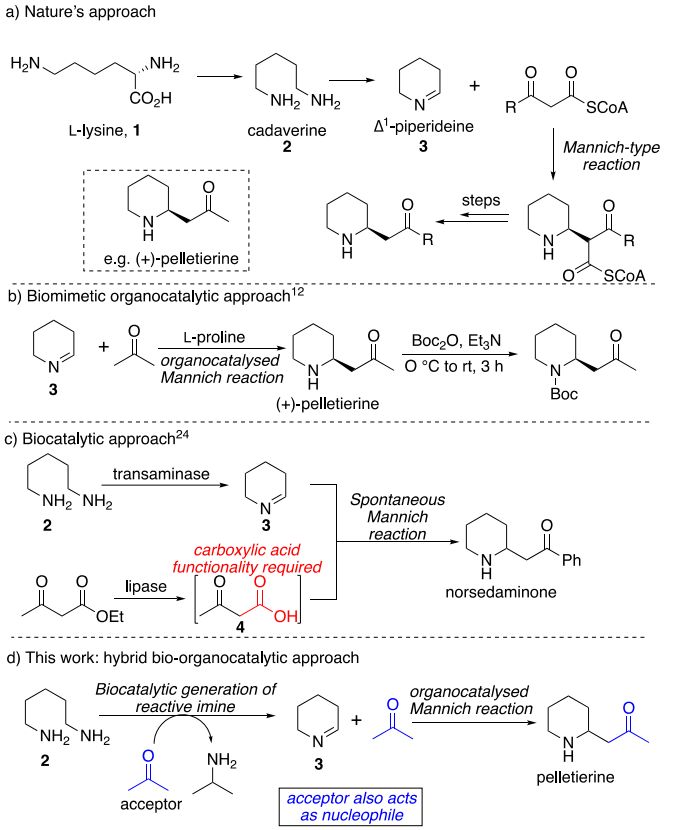
Combining bio- and organocatalysis for the synthesis of piperidine alkaloids.

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There is continued interest in developing cascade processes for the synthesis of key chiral building blocks and bioactive natural products (or analogues). Here, we report a hybrid bio-organocatalytic cascade for the synthesis of a small panel of 2-substituted piperidines, relying on a transaminase to generate a key reactive intermediate for the complexity building Mannich reaction.

The Mannich reaction plays an important role in the biosynthesis of many alkaloids, including pyrrolidines and piperidines that are derived from ornithine and lysine respectively.[1,2] The natural pathway involves initial amino acid decarboxylation, followed by oxidative deamination to form reactive cyclic imines (Scheme 1a). A subsequent Mannich-type reaction with a suitable nucleophile leads to the formation of a wide range of nitrogen-containing heterocyclic alkaloids, whose structures are prevalent in Nature[2] (Figure 1).

The first reports of the application of organocatalysts appeared in the literature two decades ago[3] involving enamine[4] and iminium[5] catalysis, and the area developed rapidly in subsequent years; including a seminal report of a proline catalysed Mannich reaction.[6] The propensity of the Mannich reaction to generate alkaloid pharmacophores has led to its extensive investigation in total synthesis, and notably, many of these synthetic approaches rely on the use of organocatalysts.[7-13] Monaco *et al*.[12] investigated the biomimetic organocatalytic asymmetric synthesis of 2-substituted alkaloids using a variety of catalysts and solvent systems (Scheme 1b). The highest yields were reported with L-proline as the catalyst, which functions to activate the carbonyl through the generation of an intermediate enamine.[14] This readily available organocatalyst has also been



Scheme 1 Natural and synthetic strategies for the synthesis of 2-substituted piperidine alkaloids.

successful at mediating Mannich reactions between dihydro-ß-carbolines,[10] thiazines and oxazines,[15] among others. Pyrrolidines and piperidines have been targets for numerous (chemo)enzymatic methodology in recent years,[16-23] due to the high levels of selectivity achievable using enzymes.

Recently, the natural biosynthetic pathway responsible for the synthesis of these N-heterocycles (Scheme 1a) has inspired the development of a biocatalytic cascade approach for their

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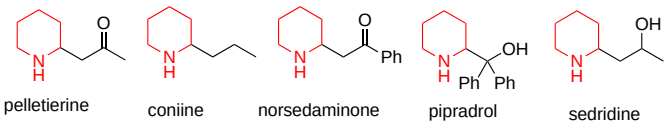


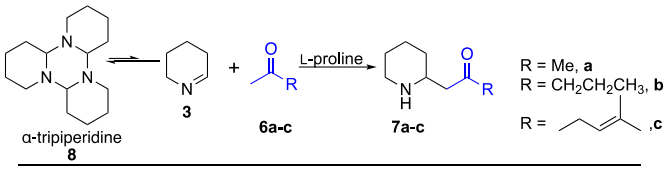
Figure 1 A selection of naturally occurring 2-substituted piperidine alkaloids.

preparation *via* a Mannich-type reaction, relying on the *in- situ* generation of both the reactive cyclic imine and nucleophilic carbonyl partner (Scheme 1c).[24] While this approach enables access to a variety of 2-substituted N-heterocyclic alkaloids, the authors note the requirement for the carboxylic acid functionality on the nucleophile (**4**) to observe any significant Mannich product, and this limits the scope of the methodology. Such biocatalytic cascade processes for the synthesis of high-value pharmaceutical drugs and natural products (or analogues) continue to receive considerable attention.[25] They are often operationally simple and negate the need for the isolation and purification of intermediates. However, there is also a growing interest in the development of hybrid cascades, employing combinations of compatible bio-, organo-, and transition metal catalysis, where the unique advantages of each catalyst can be harnessed.[26-28]

Here, we describe a hybrid bio-organocatalytic approach for the synthesis of 2-substituted piperidines, relying on transaminase-mediated *in situ* generation of Δ^1-piperideine (**3**), followed by a proline-catalysed Mannich reaction with a small panel of aliphatic ketones (Scheme 1d). Our methodology takes inspiration from the natural biosynthesis (Scheme 1a), the organocatalysed (Scheme 1b) and biocatalysed (Scheme 1c) approach for the preparation of these important alkaloids and exploits the advantages of each strategy. The approach relies on the use of a single transaminase (TA) enzyme to generate a reactive intermediate, which undergoes a Mannich-type reaction in the presence of proline to form the target. Another elegant aspect of this cascade is the dual role of the ketone, which acts as a transaminase acceptor substrate and nucleophile in the cascade.

Established methods for proline mediated Mannich reactions are typically performed in organic solvents. For this reason, initial efforts focused on determining whether the proline catalysed Mannich reaction between Δ^1-piperideine (**3**) and ketones **6a**-**c** were compatible with the necessary aqueous conditions. Δ^1-Piperideine (**3**) was synthesised as previously reported.[12] Like other cyclic imines, **3** exists in equilibrium in solution as a complex diastereomeric mixture of trimeric (major) and monomeric forms. α-Tripiperidine **8** was allowed to react with ketones **6a**-**c** under typical biotransformation conditions (Table 1). While high pH is not necessary for the Mannich reaction, pH 10 was selected, after an initial screen showed that basic conditions are required for an efficient biotransformation when using cadaverine (data not shown). This is consistent with previous studies from our own laboratory.[29] When no proline was employed, only low conversion to **7a** and **7b** were observed (Table 1, entries 1/2). A

Table 1 Optimisation of L-proline-catalysed Mannich reaction with ketones **6a**-**c**.



Entry	Ketone	L-proline (mM)	Conv. (%)	Product
1	**6a**	0	13	**7a**
2	**6b**	0	11	**7b**
3	**6c**	0	0	**7c**
4	**6a**	2	19	**7a**
5	**6a**	10	29	**7a**
6	**6a**	20	40	**7a**
7	**6a**	50	58	**7a**
8	**6a**	70	65	**7a**
9	**6a**	100	85	**7a**
10	**6b**	100	28	**7b**
11	**6c**	100	11	**7c**

Conditions: α-tripiperidine **8** (3.33 mM; giving 10 mM of Δ^1-piperideine **3** in solution), ketone (200 mM, 20 equiv.), L-proline (0, 2, 10, 20, 50, 70 and 100 mM (0, 0.2, 1, 2, 5, 7 and 10 equiv. respectively)) , HEPES (100 mM, pH 10), DMSO (10% v/v), 37 °C, 200 rpm, 48 hours. Final reaction volume is 1mL. Conversion was measured by GC-FID and values represent the mean of least three replicates.

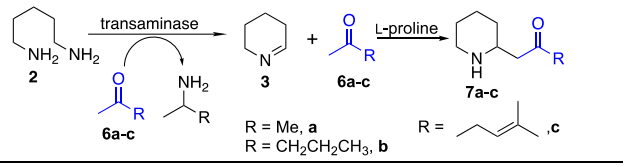
significant increase in conversion was possible with the addition of 100 mM L-proline, leading to 85% conversion to **7a** (Table 1, entry 9). Increasing the chain length of the ketone nucleophile resulted in a decrease in conversion (Table 1, entries 10/11), a trend previously observed to some degree by Monaco *et al*.[12] While there is no clear explanation for the lower conversions achieved with longer chain nucleophiles **6b** and **6c**, it may be down to competing enamine formation with the unsymmetrical ketones and we have not investigated the reasons for this in any detail. A broader panel of nucleophile partners may be needed to explore this observation further. A recently reported biocatalytic strategy targeting similar N-heterocycles relied on the use of 3-ketoesters (Scheme 1c),[24] and, unlike this study, does not allow the inclusion of readily available ketones in the cascade.

The reaction was then extended to include the biocatalytic formation of Δ^1-piperideine **3** from cadaverine **2** (Table 2). The transformations were performed at 24 and 48 hours (see Table S1 for full details, including 24-hour reactions), with higher conversions observed after 48 hours. Reactions performed at 50 °C (entries 10/12/14) using engineered ATA256, led to slightly lower conversions, suggesting that Δ^1-piperideine formation is more favourable at 37 °C. Overall, the conversion to the alkaloid products achieved using the cascade approach are consistent with those observed in the absence of the TA (Table 1), confirming that the TA step is not hindering the reaction progress.

Reactions have thus far been performed with 10 mM cadaverine, meaning the maximum concentration of product is also 10 mM. Additionally, 20 eq. of ketone was employed, leading to poor atom economy. It is worth noting that at least 2

eq. of the ketone partner are required, as one equivalent acts as the amine acceptor in the initial biotransformation and the second as the nucleophile in the subsequent Mannich reaction (Scheme 1d). Increasing the concentration of cadaverine from 10 mM to 50 mM (Table 2, entry 6), while maintaining the ketone concentration at 200 mM, had little effect on the overall conversion to **7a**, while a further increase to 100 mM did result in a significant drop in conversion (entry 4). Although this is unsurprising, as there is only 1eq of ketone available for the TA and Mannich steps, our conditions are very efficient when compared to previous reports, which typically utilise multiple equivalents of the nucleophile.[10,12] Ketones **6b**-**c** were also tested under these conditions (entries 11-14) and conversions were considerably lower.

Table 2 Analytical scale bio-organocatalytic cascade for the synthesis of pyrrolidines **7a-c**, using 200 mM of ketone **6a-c** and altering the concentrations of diamine, ketone and proline catalyst.

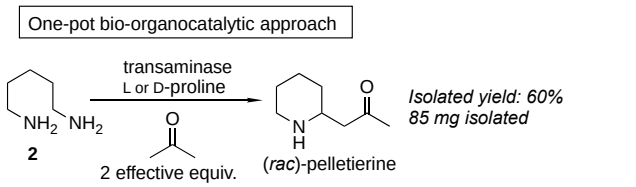


Entry	Ketone	**Conc. 3** (mM)	Ketone equiv.	L-Pro equiv.	Temp. °C	Conv. %
1	**6a**	10	20[a]	0	37	11
2	**6a**	50	4[a]	0	37	4
3	**6a**	10	20[a]	1	37	29
4	**6a**	100	2[a]	1	37	37
5	**6a**	10	20[a]	2	37	46
6	**6a**	50	4[a]	2	37	65
7	**6a**	10	20[a]	5	37	66
8	**6a**	10	20[a]	7	37	68
9	**6a**	10	20[a]	10	37	75
10	**6a**	10	20[a]	10	50	71
11	**6b**	10	20[a]	10	37	30
12	**6b**	10	20[a]	10	50	23
13	**6c**	10	20[a]	10	37	12
14	**6c**	10	20[a]	10	50	10

Conditions: Cadaverine (10, 50 or 100 mM), ketone (200 mM), L-proline (0, 10, 20, 50, 70 and 100 mM (0, 1, 2, 5, 7 and 10 equiv. respectively)), ATA256 (5 mg/mL), HEPES (100 mM, pH 10), PLP (1 mM), DMSO (10 % v/v), 37 °C, 200 rpm, 48 hours. [a]200 mM of ketone was used. Final reaction volume is 1mL. Conversion was measured by GC-FID and values represent the mean of least three replicates.

Finally, we show that our methodology can be carried out on a preparative scale, using just 2 effective equivalents (4 eq. in total) of the ketone. Both L- and D-proline afforded comparable conversions (57% and 60% respectively) and enabled the isolation of up to 85 mg of the natural product pelletierine as a racemate. Despite the use of the chiral organocatalysts, these 2-substituted piperidines are known to readily racemise.[12]

Scheme 2 Preparative scale synthesis of (*rac*)-**7a** using ATA256 in combination with L- or D-proline. Reaction conditions: cadaverine **2** (50 mM), acetone (200 mM, 2 effective Mannich equivalents), L- or D-proline (100 mM, 2 equiv./200 mol %), ATA-256 (5 mg/mL), HEPES buffer (100 mM, PLP (1 mM)), DMSO (10 % *v/v*). Conversion was measured by GC-FID and *ee* determined by HPLC.



In conclusion, our methodology aims to expand the scope of hybrid bio-organocatalytic cascade processes for the synthesis of valuable targets. The benefits of employing transaminases to convert prochiral carbonyls to the corresponding chiral amines or amino acids is well established. However, we have a continued interest in expanding the utility of these enzymes, by exploiting their ability to generate reactive intermediates *in situ* that undergo further chemistry and enable significant complexity generation. Despite organocatalysis being a well-established field, the area continues to expand[30] and will present new opportunities for the development of hybrid catalytic methodology. The approach presented here enables access to the natural product pelletierine, using a one-pot chemoenzymatic cascade, where the ketone partner acts as both an amine acceptor and nucleophile, and demonstrates the synthetic utility of designing hybrid cascade processes. We also propose that this methodology could be adapted to take place *in vivo*, within a cell that supplies the amine donor, ATA and coenzyme,[31] and will explore this whole-cell system soon.

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