ChemComm

Accepted Manuscript

View Article Online
DOI: 10.1039/C8CC06759G

Journal Name

COMMUNICATION

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Biomimetic synthesis of 2-substituted *N*-heterocycle alkaloids by one-pot hydrolysis, transamination and decarboxylative Mannich reaction

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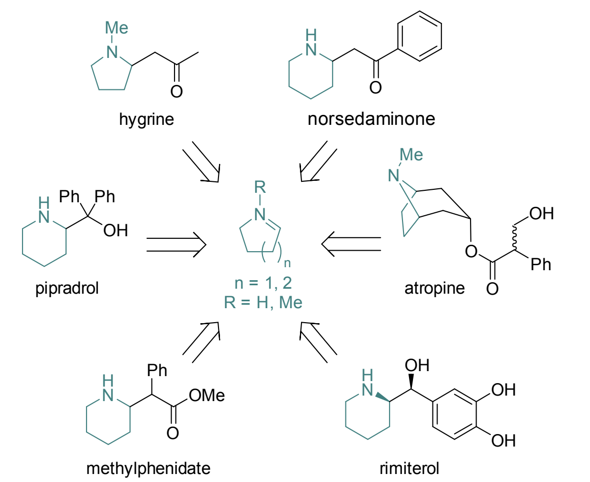
Heterocycles based on piperidine and pyrrolidine are key moieties in natural products and pharmaceutically active molecules. A novel multi-enzymatic approach based on the combination of a lipase with an α,ω-diamine transaminase is reported, opening up the synthesis, isolation and characterisation of a broad range of 2-substituted *N*-heterocycle alkaloids.

Nitrogen-containing heterocyclic compounds (alkaloids) represent a diverse range of natural products found as secondary metabolites in various kingdoms of life, notably in approximately 20% of all plant species.[1] Alkaloids often possess potent biological activities which make them highly attractive targets for the development of pharmaceutical drugs such as antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine) and antibacterial (e.g., chelerythrine) agents. Other alkaloids have shown to act as stimulants (e.g., caffeine, nicotine), narcotics (e.g., cocaine, morphine) and as poisons (e.g., coniine). As a result, many have found usage in traditional medicine, as well as in modern drug development as essential building blocks and pharmacophores for medicinal chemistry. In a recent survey of U.S. FDA approved drugs, 59% contained at least one nitrogen heterocycle.[2]

Pyrrolidine and piperidine type alkaloids, in particular, are abundant in Nature, and are derived from lysine and the non-proteinogenic amino acid ornithine, respectively. The biosynthetic pathway involves initial decarboxylation of these amino acid precursors followed by oxidative deamination to form reactive cyclic intermediates (Scheme 1). Subsequent Mannich-type nucleophilic addition provides the 2-substituted pyrrolidine/piperidine carbon frameworks which are the basis of a diverse range of alkaloids containing 5-membered rings, such as tropanes and pyrrolizidines, and 6-membered rings, such as quinolizidine and lycopodium alkaloids.[3] The frequent occurrence of these structural ring motifs in a large number of pharmaceutical products (such as antidepressants,



Scheme 1. Representative natural products and APIs based on 2-substituted pyrrolidine/piperidine alkaloids derived from the cyclic imines of lysine or ornithine.

psychostimulants, adrenergic and cycloplegic drugs)[2,4] make these compounds attractive targets for total synthesis (Scheme 1).

These compounds and their analogues have been the subject of many synthetic studies, most often based on Mannich-type reactions, both with and without the presence of an organocatalyst (Scheme 2). One reported method involves a one-pot three-component Mannich reaction and cyclisation, mediated by an excess of L-proline, although no stereoselectivity was observed.[5] Similarly, direct oxidative coupling of tertiary amines with non-activated ketones has been described with catalytic L-proline in combination with vanadium acetate; however, this reaction suffers from a limited substrate scope and afforded only modest isolated yields.[6] Another common synthetic approach uses the Henry-Nef protocol involving the generation of nitroalkenes followed by acid hydrolysis; this method requires several synthetic steps and multiple chromatographic separations leading to a poor average overall yield (~40%) of the alkaloid, in *N*-protected

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Electronic Supplementary Information (ESI) available: Experimental section, characterisation data, copies of the NMR spectra. See DOI: 10.1039/x0xx00000x

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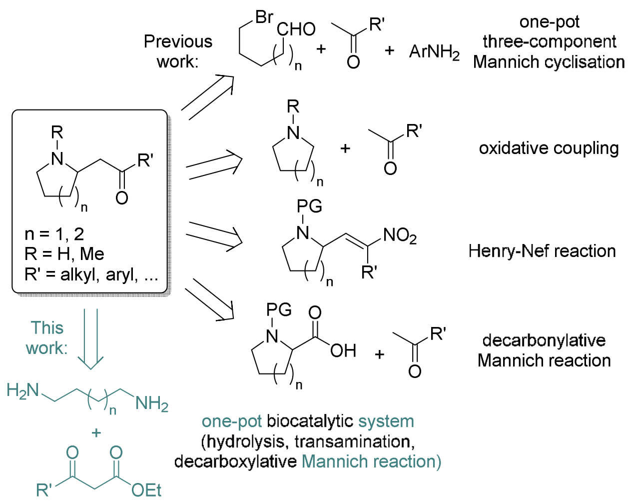
DOI: 10.1039/C8CC06759G



Scheme 2. Strategies for the synthesis of 2-substituted pyrrolidine/piperidine alkaloids.

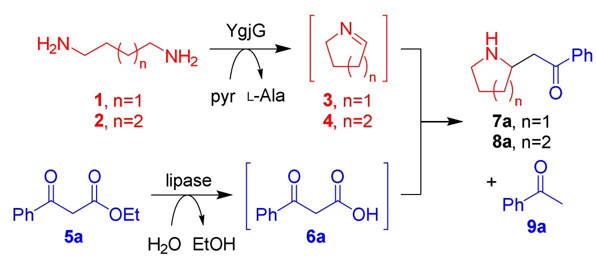
form.[7] Finally, a recent strategy involved a decarbonylative Mannich reaction that requires the conversion of protected prolines to the corresponding acid chlorides, which undergo a decarbonylative C-C bond formation reaction with methyl ketones, providing a direct access to various *N*-protected pyrrolidine alkaloids.[8]

An alternative approach for the synthesis of substituted piperidines and pyrrolidines relies on the use of engineered biocatalysts. The expanding toolbox of enzymes available for the synthesis of chiral amines has allowed the development of new synthetic routes for asymmetric amine synthesis based on ω-transaminases,[9] reductive aminases,[10] ammonia lyases,[11] as well as deracemisation and dynamic kinetic resolution processes (e.g., with monoamine oxidases[12] or hydrolases[13]). Biocatalytic strategies for the synthesis of these classes of alkaloids rely on mimicking the fundamental lysine and ornithine catabolism pathway. The first step involves the conversion of 1,4-diaminobutane (putrescine, **1**) or 1,5-diaminopentane (cadaverine, **2**) to cyclic imines 1-pyrroline **3** and 1-piperideine **4**, respectively. These reactive intermediates are highly susceptible to nucleophilic attack, and the Mannich addition of 3-ketoacids followed by decarboxylation would provide a variety of 2-substituted pyrrolidine/piperidine analogues. Recently, diastereomerically pure 2,6-disubstituted piperidines have been synthesised via an ω-transaminase mediated aza-Michael reaction, in a one-pot reaction starting from complex diketone precursors.[14] Herein, we describe an alternative approach towards the production of pyrrolidine/piperidine alkaloids that mimics the natural biosynthetic pathway, by assembling an enzyme cascade consisting of a transaminase and a lipase, employing biogenic diamines and readily available 3-ketoesters as substrates.

In previous studies we investigated class III α,ω-diamine transaminase (α,ω-DTA) genes *spuC* (part of the polyamine uptake and utilization pathway in *Pseudomonas putida*) and *ygjG* (the complementary gene in *Escherichia coli*), which both utilise linear diamines but differ in their acceptor substrates.[15,16] The different substrate specificities indicate distinct evolution histories with possible common degradation pathways between these microorganisms.[17] In this study, we focused on YgjG, which exhibits a strict preference for linear diamines, with α-ketoglutarate (or pyruvate) as acceptor. The *ygjG* gene from *E.coli* K-12 was overexpressed in *E. coli* BL21 (DE3) and, in order to provide an easy to use biocatalyst for preparative chemistry, the cell-free lysate was employed instead of the purified enzyme. Preliminary experiments were performed using diamine **2** in the presence of pyruvate as the amino acceptor to generate the reactive imine intermediate **4**, followed by a Mannich-type addition with benzoylacetic acid **6a** to access the alkaloid norsedaminone **8a**. A one-pot tandem enzyme cascade was envisaged, avoiding the sequential addition of reagents because of the tendency for the reactive imine intermediates to polymerize under the reaction conditions.[15] However, attempts at performing this cascade using YgjG and **6a** (Table 1, entry 1) were unsuccessful, affording unsatisfactory amounts of the alkaloid product, due to the spontaneous loss of the carboxyl moiety via decarboxylation.[18,19]

To overcome this problem, we decided to generate labile **6a** *in situ* from its commercially available ethyl ester **5a**, via the addition of a lipase. Previous reports have described the transaminase mediated asymmetric amination of both ester **5a** and acid **6a**, which would lead to undesirable side products in our cascade.[19,20] However, YgjG showed no activity towards **5a**

Table 1. Optimisation of the lipase-transaminase cascade.[a]



Entry	Substrates (ratio)	Lipase	Product distribution [%][b]		
			7a-8a	9a	6a
1	**2**+**6a** (1 : 1)	none	5	92	3
2	**2**+**5a** (1 : 1)	PPL	29	29	42
3[c]	**2**+**5a** (1 : 1)	PSL	15	29	55
4	**2**+**5a** (1 : 1)	CALB	59	15	26
5[c]	**2**+**5a** (1 : 1)	F-AP15	11	30	59
6	**2**+**5a** (1 : 1)	TLL	22	29	49
7	**2**+**5a** (1 : 1)	CALB[d]	79	12	9
8	**2**+**5a** (1.5 : 1)	CALB[d]	89	8	3
9	**2**+**5a** (1 : 1.5)	CALB[d]	90	8	2
10	**1**+**5a** (1.5 : 1)	CALB[d]	98	1	1

a: Expt. cond.: 5.0-7.5 mM substrates, 7.5 mM pyruvate, 1 mM PLP, 0.5% v/v DMSO, 200 μL/mL lysate, 2 mg/mL lipase, 50 mM Tris-HCl buffer, pH 9.0, 30°C, 250 rpm, 18 h. b: Measured by reverse-phase HPLC. c: <5% of **5a** was left unreacted. d: 5 mg/mL lipase concentration.

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DOI: 10.1039/C8CC06759G

and **6a**, likely due to the small size and greater hydrophobicity of the active site entrance compared to other class III transaminases.[21] Several commercially available lipases known to have broad substrate tolerance (see ESI for the full list) were tested with an equimolar mixture of amine and ketoester substrates (Table 1, entries 2-6). The product distribution of the lipase reactions gave modest conversions to the piperidine alkaloid **8a** whilst still yielding undesirable acetophenone side product **9a** resulting from the partial decarboxylation of **6a**. Of the commercial enzymes tested, the immobilised CALB (Novozyme 435) gave the highest product conversion (entry 4, 59%). Encouraged by these preliminary results, we increased the lipase concentration and a higher percentage of **8a** was attained (entry 7). Further increases in lipase concentration had a slightly negative influence on the production of **8a** (data not shown), and this observation could result from a higher rate of hydrolysis. Increasing the concentration of either **2** or **5a** gave a similar increase of conversion to **8a** (entries 8-9). Thus, given the higher cost of **5a** compared to **2**, we decided to use an excess of diamine in all subsequent experiments. Under these optimised conditions, we also tested natural diamine substrate **1** to ensure this reaction was not limited to **2**, and the system furnished pyrrolidine alkaloid **7a**, with near perfect conversion (entry 10, 98%).

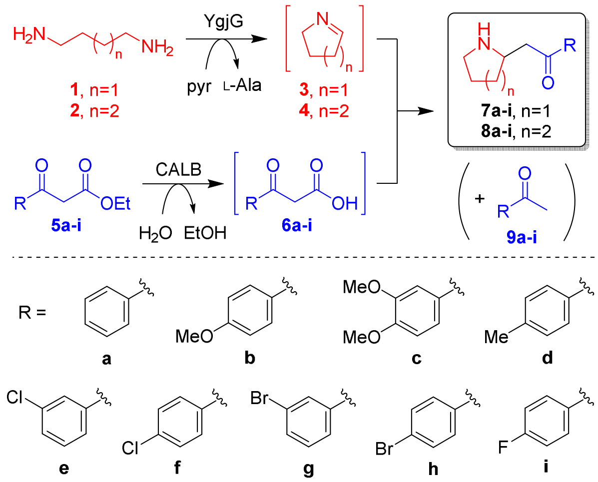
Building on the successful results obtained with Mannich-type addition processes under optimised conditions, the reaction was tested with both diamines (**1-2**) and a panel of aromatic 3-ketoesters (**5a-i**), to assess the general scope of the reaction. The results (Table 2) revealed that both **1** and **2** could react with a variety of ketoesters bearing electron-donating substituents (**5b-d**) or halogens (**5e-i**) at various positions on the aromatic ring, yielding the corresponding 2-substituted pyrrolidines and piperidines **7-8a-i** with moderate to excellent conversions (54-99%). Most notably, we demonstrated the facile and efficient synthesis of the natural product ruspolinone **7c** and its piperidine analogue **8c** (71 and 69% isolated yields, respectively).

In order to demonstrate the preparative synthesis of products accessible via this method, we performed large-scale reactions with selected aromatic ketoesters from the previous panel (**5a-c**) and we also expanded the substrate scope to aliphatic ketoesters (**5j-m**). Substrate concentrations were increased (25 mM ketoester, 30 mM diamine) to facilitate isolation and purification of the products (Scheme 3). Following a simple work-up procedure involving quenching of the reaction mixture with aqueous HCl, the aromatic derivatives could be isolated easily in good overall yields (60-81%), while for the smaller and more hydrophilic aliphatic derivatives yields were lower (50-78%) due to the higher solubility of the targeted compounds.[‡] These preparative scale reactions demonstrate the effectiveness of our one-pot system to provide access to piperidine/pyrrolidine alkaloid scaffolds for synthetic applications.

All compounds were isolated as racemates as expected, based on the known propensity of 2-substituted piperidine/pyrrolidine alkaloids to racemise readily at basic pH, with hygrine and pelletierine reported to racemise via a retro aza-Michael reaction.[22] Attempts to deracemise some of these products using the well-established monoamine oxidase (MAO-N) or 6-hydroxy-D-nicotine oxidase (HDNO) deracemisation protocols proved to be unsuccessful, with only compounds **8a**-**c** generating low ee values (<10%, data not shown).

Lastly, we considered *N*-methylputrescine **10** as an alternative substrate for the transaminase step. In Nature, **10** is oxidatively deaminated by methylputrescine oxidase[23] to generate a highly reactive pyrrolinium cation, a central metabolic precursor belonging to tropane and pyrrolidine alkaloids biosynthesis. To our delight, α,ω-DTA YgjG readily

Table 2. One-pot biocatalytic cascade synthesis of piperidine/pyrrolidine alkaloids **7-8a-i** from diamines **1-2** and aromatic ketoacids **5a-i**.[a]



Diamine	Ketoester	Product distribution [%][b]			
		7a-i	8a-i	9a-i	6a-i
1	**5a**	98	–	1	1
	5b	99	–	1	0
	5c	99	–	1	0
	5d	84	–	8	9
	5e	88	–	9	3
	5f	88	–	8	4
	5g	80	–	14	5
	5h	87	–	5	8
	5i	99	–	1	0
2	**5a**	–	89	8	3
	5b	–	89	5	6
	5c	–	95	3	2
	5d	–	64	15	21
	5e	–	54	44	2
	5f	–	65	33	2
	5g	–	55	43	1
	5h	–	73	27	0
	5i	–	89	9	2

a: Expt. cond.: 7.5 mM diamine, 5.0 mM ketoester, 7.5 mM pyruvate, 1 mM PLP, 0.5% v/v DMSO, 200 µL/mL lysate, 5 mg/mL CALB, 50 mM Tris-HCl buffer, pH 9.0, 30°C, 250 rpm, 18 h. b: Measured by reverse-phase HPLC.

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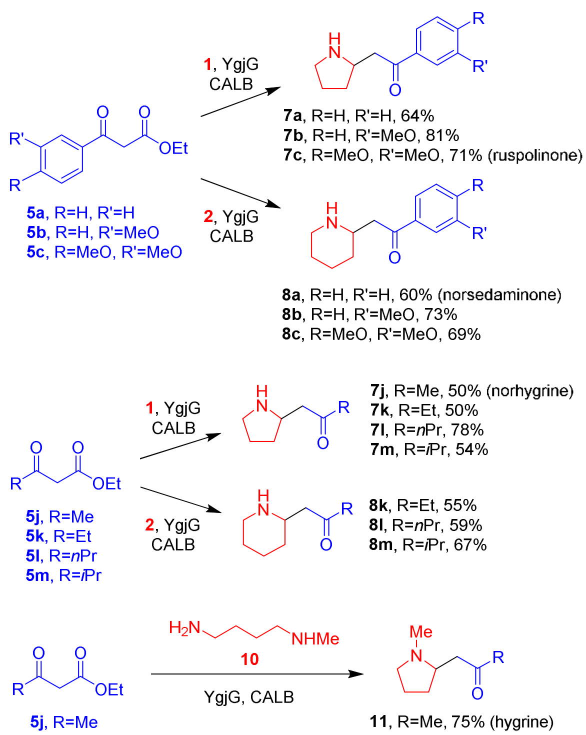
Scheme 3. Preparative scale one-pot biocatalytic synthesis of selected piperidine/pyrrolidine alkaloids (with isolated yields).



catalysed the deamination of **10**, expanding the substrate scope to *N*-methylalkaloids. As a representative example, hygrine **11** was obtained under the same conditions, in 75% isolated yield (Scheme 3).

In summary, we have designed and tested a novel multienzymatic protocol for the synthesis of 2-substituted and *N*-substituted piperidine/pyrrolidine alkaloids, in a one-pot fashion, under mild conditions and starting from commercially available substrates. The products isolated and characterised are natural alkaloids or analogs, many of which have been shown to possess a wide range of biological activities (e.g., anticoagulant,[24] antimicrobial against pathogenic yeasts,[25] anthelmintic against parasitic worms[26]).

Acknowledgements

N.J.T. acknowledges the ERC for the award of an Advanced Grant. J.L.G. acknowledges the support of the BIOINTENSE project, financed through the European Union 7th Framework Programme (grant agreement no. 312148). I.S. acknowledges a CASE award from BBSRC and Dr. Reddy's (grant code BB/K013076/1).

Conflicts of interest

The authors declare no conflict of interest.

Notes and references

‡ Unexpectedly, pelletierine **8j** could not be obtained in sufficient purity, in spite of multiple attempts to isolate it. This is likely due to known degradation (see for instance: E. C. Carlson, L. K. Rathbone, H. Yang, N. D. Collett and R. G. Carter, *J. Org. Chem.*, 2008, **73**, 5155) and high volatility, leading to significant loss of product.

1 J. Ziegler and P. J. Facchini, Annu. *Rev. Plant Biol.*, 2008, **59**, 735–769.
2 E. Vitaku, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2014, **57**, 10257.
3 P. M. Dewick, in *Medicinal Natural Products*, John Wiley & Sons Ltd., Chichester, UK, 2013.
4 R. Vardanyan, *Classes of Piperidine-Based Drugs* (ch. 10), in: Piperidine-Based Drug Discovery, Elsevier, 2018.
5 S. Chacko and R. Ramapanicker, *Tetrahedron Lett.*, 2015, **56**, 2023.
6 A. Sud, D. Sureshkumar and M. Klussmann, *Chem. Commun.*, 2009, **7345**, 3169.
7 C. Bhat and S. G. Tilve, *Tetrahedron Lett.*, 2011, **52**, 6566.
8 Y.-C. Shih, P.-H. Tsai, C.-C. Hsu, C.-W. Chang, Y. Jhong, Y.-C. Chen and T.-C. Chien, *J. Org. Chem.*, 2015, **80**, 6669.
9 I. Slabu, J. L. Galman, R. C. Lloyd and N. J. Turner, *ACS Catal.*, 2017, **7**, 8263.
10 G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan and N. J. Turner, *Nat. Chem.*, 2017, **9**, 961.
11 F. Parmeggiani, N. J. Weise, S. T. Ahmed and N. J. Turner, *Chem. Rev.*, 2018, **118**, 73.
12 D. Ghislieri, A. P. Green, M. Pontini, S. C. Willies, I. Rowles, A. Frank, G. Grogan and N. J. Turner, *J. Am. Chem. Soc.*, 2013, **135**, 10863.
13 J. Paetzold and J. E. Bäckvall, *J. Am. Chem. Soc.*, 2005, **127**, 17620.
14 E. O'Reilly, C. Iglesias, D. Ghislieri, J. Hopwood, J. L. Galman, R. C. Lloyd and N. J. Turner, *Angew. Chem. Int. Ed.*, 2014, **53**, 2447.
15 I. Slabu, J. L. Galman, N. J. Weise, R. C. Lloyd and N. J. Turner, *ChemCatChem*, 2016, **8**, 1038.
16 J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd and N. J. Turner, *Green Chem.*, 2017, **19**, 361.
17 L. a Nahum, S. Goswami and M. H. Serres, *Physiol. Genomics*, 2009, **38**, 250.
18 J. Kim, D. Kyung, H. Yun, B. K. Cho, J. H. Seo, M. Cha and B. G. Kim, *Appl. Environ. Microbiol.*, 2007, **73**, 1772.
19 S. Mathew, S. Jeong, T. Chung, S. Lee and H. Yun, *Biotechnol. J.*, 2016, **11**, 185.
20 O. Buß, M. Voss, A. Delavault, P. Gorenflo, C. Syldatk, U. Bornscheuer and J. Rudat, *Molecules*, 2018, **23**, 1211.
21 H. J. Cha, J. Jeong, C. Rojviriya and Y. Kim, *PLoS One*, 2014, **9**, e113212.
22 M. R. Monaco, P. Renzi, D. M. Scarpino Schietroma and M. Bella, *Org. Lett.*, 2011, **13**, 4546.
23 H. D. Boswell, B. Dräger, W. R. McLauchlan, A. Portsteffen, D. J. Robins, R. J. Robins and N. J. Walton, *Phytochemistry*, 1999, **52**, 871.
24 J. M. Grisar, G. P. Claxton, K. T. Stewart, R. D. MacKenzie and T. Kariya, *J. Med. Chem.*, 1976, **19**, 1195.
25 A. Al-Shamma, S. D. Drake, L. E. Guagliardi, L. A. Mitscher and J. K. Swayze, *Phytochemistry*, 1982, **21**, 485.
26 L. H. Yan, F. Dagorn, E. Gravel, B. Séon-Méniel and E. Poupon, *Tetrahedron*, 2012, **68**, 6276.

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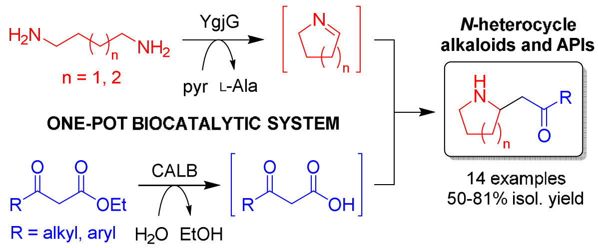

A novel multi-enzymatic approach enabled the facile synthesis of a broad range of biologically active 2-substituted piperidine and pyrrolidine alkaloids.