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Synthesis of 2,5-disubstituted pyrrolidine alkaloids *via* a one-pot cascade using transaminase and reductive aminase biocatalysts

Bruna Z. Costa,[a,b], James L. Galman,[a], Iustina Slabu,[a] Scott P. France,[a] Anita J. Marsaioli[b] and Nicholas J. Turner.[a]*

Abstract: A multi-enzymatic cascade process involving transaminases (TA) and reductive aminases (RedAms) to produce enantiomerically pure 2,5-disubstituted pyrrolidine alkaloids from their respective 1,4-diketones is reported. Several TAs were screened and the best results for diketone monoamination were obtained with an *R*-selective TA from *Mycobacterium chlorophenicum* and with an *S*-selective TA from *Bacillus megaterium*. Pyrroline reduction was best performed by a reductive aminase from *Ajellomyces dermatitidis* (*Ad*RedAm). Finally, a biocatalytic one-pot cascade was implemented using the aforementioned enzymes and a variety of 2-methyl-5-alkylpyrrolidines were produced with high (>99%) conversion, diastereomeric and enantiomeric excess values.

Introduction

2,5-Disubstituted pyrroline and pyrrolidine ring systems (Figure 1) are important structural motifs for many alkaloids commonly found in plants, insects and animals.[1] Specifically, myrmicine ants[2] and poison frogs[3] are natural sources of a vast structural diversity of 2,5-disubstituted pyrrolines and pyrrolidines. In addition, several biological activities have been attributed to these compounds including antimicrobial, insecticidal and repellent properties.[4]

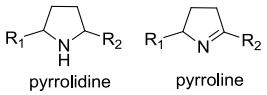
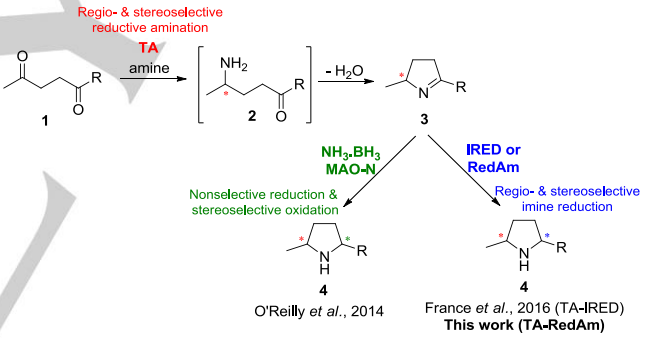


Figure 1. 2,5-Disubstituted pyrroline and pyrrolidine alkaloids.

From this perspective, assessing the structure-bioactivity relationship of 2,5-disubstituted pyrrolines and pyrrolidines alkaloids is essential for a full understanding and complete characterisation of their biological function. However, due to the scarcity of abundant natural material, further biological studies rely on total synthesis as an indispensable tool to obtain these compounds in an enantiopure form. The asymmetric syntheses of pyrrolidine alkaloids have been mainly performed using chiral chemical catalysts;[5] though enzymatic processes have gained popularity as the required reaction conditions are milder, greener and more cost effective.[6]

The proposed biosynthetic route to solenopsins[7], the most abundant alkaloid class found in the *Solenopsis* ant venom, inspired a multi-enzymatic cascade process to the synthesis of 2,5-disubstututed pyrrolidines (Scheme 1). This cascade involves the transamination of the less hindered carbonyl group of a diketone (**1**), catalysed by a stereoselective transaminase (*R*- or *S*-TA), producing the enantiopure aminoketone **2**, which spontaneously cyclizes affording the corresponding imine **3**. Further reduction, using a stereoselective reagent or (bio)catalyst, leads to the desired product (**4**) with controlled stereochemistry.



Scheme 1. Multi-enzymatic cascade reactions to synthesize pyrrolidines (**4**) from diketones (**1**). TA: transaminase, IRED: imine reductase, MAO-N: monoamine oxidase.

The first example describing the enzymatic monoamination of a diketone followed by spontaneous cyclisation was reported by Simon *et al.*[8] The piperideines obtained thereby were chemically hydrogenated or reduced affording the respective *cis*- and *trans*-piperidines.[8,9] O'Reilly and co-workers,[10] subsequently, described a biocatalytic cascade using TAs and monoamine oxidases (MAO-N) to synthesize 2,5-disubstituted pyrrolidines from 1,4-diketones (Scheme 1, red-green route). Despite the one-pot enzymatic cascade approach, the developed system still depends on a chemical step ($NH_3.BH_3$ non-selective reduction). An alternative *in vitro* multi-enzymatic cascade process was then sought using imine reductases (IRED), the natural biocatalysts for imine reduction. Therefore, in 2016, France *et al.*[11] developed the first biocatalytic cascade involving TAs and IREDs for the synthesis of disubstituted pyrrolidine and piperidines scaffolds (Scheme 1, red-blue route). Inspired by the success of the aforementioned processes, herein we report the application of a TA-RedAm cascade to produce

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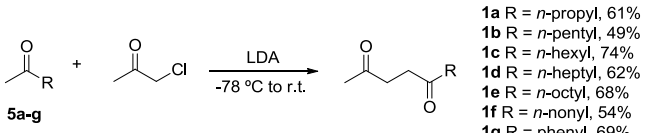
2,5-dialkyl pyrrolidines from the respective 1,4-diketones. Previous reports only evaluated substrates bearing a small methyl group and a large phenyl group as substituents and, this present work focuses on compounds bearing at least one small or medium-size alkyl chain (3- to 9-carbons long) substituent. These substrates, to our knowledge, have not previously been investigated and were chosen based on their potential to afford the pyrrolidine alkaloids commonly found in nature. In addition, we also aim to contribute to the development of transaminase and reductive aminase-catalysed processes applied to the production of value-added compounds.

Results and Discussion

Seven 1,4-diketones substrates (**1a-g**) bearing alkyl chain substituents varying from 3- to 9-carbons long were chemically synthesised (Scheme 2). This route[12] involved lower cost reagents, making it more attractive when compared to other synthetic alternatives.



Scheme 2. Synthetic route to 1,4-diketones **1a-g**.

Aiming for the production of enantiomerically pure 2-methyl-5-alkyl pyrrolines, we investigated a total of twelve *R*- and *S*-transaminases from previously published work,[13] and also novel transaminases cloned and expressed from our *in*-house culture collection (See Supporting Information). DL-alanine was used as amino donor and the lactate dehydrogenase (LDH)/glucose dehydrogenase (GDH) system was applied to shift the equilibrium towards product formation and ensure full reaction conversion.[14]

Most of the evaluated WT TAs gave high conversion and enantiomeric excess (*ee*) values (Table 1). Substrates **1a-d,g** were fully converted into their respective enantiopure pyrrolines (*ee* >99%) when using *R*-TA from *Mycobacterium chlorophenicum* *Mc*TA and *S*-TA *Bacillus megaterium* *Bm*TA[13b,e]. We also tested our highest expression variants *Bm*TA-S119G and *Vf*TA-N118G, which in comparison to their wild type enzymes had improved activity towards known keto acceptors acetophenone and benzylacetone; however, it was revealed not to be as active towards these substrates. Elongation of the diketone alkyl chain decreased the enzymatic conversion, which is presumably related to the substrate solubility, a major drawback when performing reactions in aqueous media.[15] Higher co-solvent (DMSO) percentages (5-20%) and temperatures (30 and 37 ºC) were considered for reactions involving substrates **1e-f** and enzymes *Mc*TA and *Bm*TA-WT, improving conversion rates up to >95% within 24 h (see Supplementary Information). Furthermore, TAs have the ability to distinguish between keto groups in a diketone substrate with exquisite selectivity.[8,10] In this current work, all TAs showed excellent regioselectivity (>99%) and only the sterically less demanding ketone moiety was aminated, with no detectable imine product generated from the amination of the bulky ketone substituent. Consequently, considering both reaction conversion and stereoselectivity results, transaminases *Mc*TA and *Bm*TA were selected for further experiments involving the coupling with imine reductases (IREDs) in a one-pot cascade format biotransformation.

In nature, the asymmetric reduction of imines to the corresponding amines is catalysed by imine reductases (IREDs). These enzymes are NADPH-dependent oxidoreductases and are related to several biosynthetic pathways including the one affording ant venom alkaloids.[7,16] Recently IREDs have been extensively applied to reduce a wide range of prochiral cyclic imines generating chiral monosubstituted piperidines and pyrrolidines, often with high conversion and enantiomeric excess.[17]

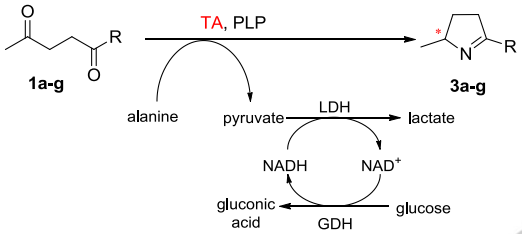
At the outset of our study, we chose the racemic 2,5-disubstituted pyrroline **3c** as a standard substrate to investigate the reduction potential of three known IREDs: *R*-IRED from *Streptomyces sp.* GF3587[18], *S*-IRED from *Streptomyces sp.* GF3546[19] and *Ao*IRED from *Amycolatopsis orientalis*.[20] Biotransformations were initially performed in a whole-cell system as it promotes *in vivo* cofactor regeneration by only supplementing the reaction medium with glucose.

Surprisingly, none of the evaluated IREDs afforded conversion values greater than 40% (see Supporting Information). In order to improve the enzyme performance, several reaction conditions were evaluated: expression media (LB medium plus IPTG *versus* auto-induction medium); pre-treatment of cells with ethanol 10% v/v (to improve the cell permeability to organic substrates); substrate feeding in 1, 2 or 3 steps (to avoid any possible substrate inhibition); different reaction temperatures (25, 30, 37 ºC), pH (6-9) and co-solvents (DMSO or DMF); and finally the use of purified enzymes with a cofactor regeneration system ($NADP^+$/GDH/glucose). Unfortunately, no significant increase in the reaction conversions was observed.

Our next approach was to test a novel subclass of imine reductases known as reductive aminases (RedAms) recently highlighted for their newly discovered reductive amination activity. These enzymes were reported by Aleku and co-workers[21] for the reductive coupling of a broad range of carbonyl compounds and a variety of primary and secondary amines.

In the present work, two RedAms were applied for the reduction of the racemic pyrroline standard **3c** using whole-cell or purified enzyme systems (see Supporting Information). *Aspergillus oryzae* *Asp*RedAm gave a positive result only when using the purified enzyme, which can be related to its low expression level in *E. coli* cells. Conversely, *Ajellomyces dermatitidis* *Ad*RedAm afforded better results in both systems and >95% conversion was achieved after 24 h using the purified enzyme. Furthermore, both diastereoisomers of pyrrolidine **4c** were produced in comparable yields showing that the pre-existing stereogenic centre does not have a major effect on the stereoselectivity of the imine reduction. These results demonstrate that RedAms can complement and expand the substrate scope of the IRED family. Furthermore, it is possible that the nature of RedAm catalysis, which requires the concurrent binding of two substrates in 'reductive amination' mode, confers to these enzymes a greater tolerance for more demanding substrates in 'imine reduction' mode.

Table 1. TA-mediated conversion of 1,4-diketones **1a-g** into 2,5-disubstituted pyrrolines **3a-g**.



TAs	Diketones													
	1a		1b		1c		1d		1e		1f		1g	
	Conv %	*ee* %	Conv %	*ee* %	Conv %	*ee* %	Conv %	*ee* %	Conv %	*ee* %	Conv %	*ee* %	Conv %	*ee* %
ATA-117	29	>99	85	>99	96	>99	94	>99	77	>99	75	>99	90	>99
*Mc*TA	>99	>99	>99	>99	>99	>99	98	>99	71	>99	55	>99	>99	>99
*Pt*TA	>99	88	>99	90	>99	84	99	84	90	88	5	82	>99	94
*Ps*TA	>99	92	>99	96	>99	90	>99	88	93	92	7	88	>99	96
*Bm*TA-WT	>99	>99	>99	>99	>99	>99	97	>99	85	>99	70	>99	>99	>99
*Bm*TA S119G	97	>99	>99	>99	93	>99	77	>99	13	>99	21	>99	89	>99
*Cv*TA	>99	48	>99	60	>99	46	>99	34	99	34	7	42	>99	58
Pc spuC	>99	87	>99	90	>99	86	>99	86	>99	88	5	78	>99	94
Pf spuC	>99	88	>99	90	>99	87	>99	85	99	88	6	86	>99	94
ATA-117-Rd-11	92	14	91	14	81	14	75	34	34	52	40	50	93	40
*Vf*TA-WT	>99	78	>99	74	>99	56	>99	64	95	64	61	60	>99	78
*Vf*TA N118G	>99	84	90	78	>99	66	94	70	1	74	2	68	92	76

Reaction conditions: substrate (5 mM), DMSO (1%), TA (2 mg/mL), GDH (1 mg/mL), LDH (0.5 mg/mL), PLP (1 mM), DL-alanine (500 mM), NAD^+ (1.5 mM), glucose (100 mM), Tris buffer (100 mM, pH 8.0), 30 ºC, 250 rpm, 24 h. *R*-selective TAs: ATA-117 (Codexis), *Mycobacterium chlorophenicum Mc*TA and ATA-117-Rd11 (Codexis). *S*-selective TAs: *Pseudomonas fluorescens Pt*TA, *Pseudomonas stutzeri Ps*TA, *Bacillus megaterium Bm*TA-WT, *Bm*TA S119G, *Chromobacterium violaceum Cv*TA, *Pseudomonas chlororaphis Pc* spuC, *Pseudomonas fluorescens Pf* spuC, *Vibrio fluvialis Vf*TA-WT, *Vf*TA N118G.

Having established separately the viability of both amination and reduction steps, we turned our attention to the implementation of an efficient enzymatic cascade to produce 2-methyl-5-alkylpyrrolidines. *Mc*TA and *Bm*TA-WT were selected, respectively, as *R*- and *S*-selective transaminases along with *Ad*RedAm as an *R*-selective imine reductase.

A previous report regarding the synthesis of 2-methyl-5-phenylpyrrolidine *via* a similar cascade[11], used IRED whole-cells as biocatalysts. We opted for using only purified enzymes, as the previous single-step biotransformations gave full conversions in these conditions. Most importantly, preliminary experiments indicate no overall effect of *Ad*RedAm on the first biocatalytic step. Therefore, further one-pot cascade biotransformations were performed adding all reagents and enzymes at the start of the reaction, avoiding a two-step one-pot procedure.

Using the aforementioned reaction setup, high conversion, *d.r.* and *ee* values were obtained for the majority of the substrates (Table 2). Conversion of diketones **1a-e** into pyrrolidines **4a-e** reached >95% after 24 h. Diketone **1f**, the less soluble substrate bearing a nonyl group substituent, was poorly converted in the first cascade step. On the other hand, diketone **1g** was completely converted by both TAs, but the reduction step showed a considerably lower efficiency. As previously reported by Aleku[21], substrates bearing a phenyl group do not seem to be favoured by RedAms, which can be account for the lower conversion results.

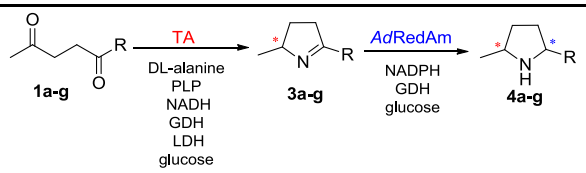
*Mc*TA and *Bm*TA produced respectively *R*- and *S*-single enantiomers of the pyrrolines **3a-g**, which were subsequently reduced by the *R*-selective *Ad*RedAm, affording both *cis*- and *trans*-pyrrolidine diastereoisomers (Table 2). *Bm*TA-*Ad*RedAm cascades produced exclusively *cis*-diastereoisomers, as predicted by the enantiomeric preferences of the enzymes. Although the expected *trans*-pyrrolidines were the main product from the *Mc*TA-*Ad*RedAm cascade, *cis*-pyrrolidines were also obtained. These results showed that the selectivity of *Ad*RedAm was influenced by the pre-existing *R*-stereogenic center, which is presumably related to a higher thermodynamic stability of 2,5-disubstituted *cis*-pyrrolidines when compared to the respective *trans*-diastereoisomers. In addition, when considering the *Mc*TA-*Ad*RedAm cascade, loss of diastereoselectivity seems to be intensified by alkyl chain elongation.

Based on the enantiomeric preferences of the evaluated enzymes, the absolute configuration of the 2,5-disubstituted pyrrolidines was assigned as *cis*-(2*S*,5*R*) and *trans*-(2*R*,5*R*).

Finally, preparative-scale reactions (50 mL) using the medium-size chain diketones **1b** and **1d** as substrates and in the same conditions previous optimised for the analytical-scale reactions were carried out to demonstrate the synthetic applicability of the one-pot cascade (Table 3). The results demonstrate that this

cascade is promising and can be applied in a large-scale format, affording enantiomeric pure compounds that are synthetically challenging to prepare via chemical routes.

Table 2. One-pot cascade for conversion of diketones **1a-g** into pyrrolidines **4a-g**.



TA	Diketone	Conv. to 3a-g (%)	Conv. to 4a-g (%)	*d.r.* (*cis:trans*)	*ee* (%)
*Mc*TA	1a[a]	>99	95	6:94	>99
	1b[a]	>99	>99	12:88	>99
	1c[a]	>99	>99	7:93	>99
	1d[a]	98	97	12:88	>99
	1e[b]	>99	93	25:75	>99
	1f[b]	73	92	16:84	>99
	1g[a]	>99	20	38:62	>99
*Bm*TA	1a[a]	>99	97	97:3	>99
	1b[a]	>99	>99	>99:1	>99
	1c[a]	>99	>99	>99:1	>99
	1d[a]	93	>99	>99:1	>99
	1e[b]	92	96	>99:1	>99
	1f[b]	55	95	>99:1	>99
	1g[a]	>99	66	97:3	>99

Reaction conditions: diketone (5 mM), TA (1 or 2 mg/mL), *Ad*RedAm (0.5 or 1 mg/mL), GDH (1 mg/mL), LDH (0.5 mg/mL), DL-alanine (500 mM), glucose (100 mM), NAD^+ (1.5 mM), $NADP^+$ (0.5 mM), PLP (1 mM) in Tris buffer (100 mM, pH 8.0), 30 ºC, 250 rpm, 24 h.
Optimised conditions:
[a] TA (1 mg/mL), *Ad*RedAm (0.5 mg/mL), 1% DMSO as co-solvent.
[b] TA (2 mg/mL), *Ad*RedAm (1 mg/mL), 5% DMSO as co-solvent.

Table 3. Preparative-scale synthesis of pyrrolidines **4b** and **4d** applying the TA-RedAm cascade.

Substrate	Cascade	Product	Isolated yield (%)	*d.r.* (*cis:trans*)	*ee* (%)
1b	*Mc*TA + *Ad*RedAm	*trans*-(2*R*,5*R*)-**4b**	69	14:86	>99
	*Bm*TA + *Ad*RedAm	*cis*-(2*S*,5*R*)-**4b**	77	>99:1	>99
1d	*Mc*TA + *Ad*RedAm	*trans*-(2*R*,5*R*)-**4d**	68	15:85	>99
	*Bm*TA + *Ad*RedAm	*cis*-(2S,5*R*)-**4d**	69	>99:1	>99

Reaction conditions: diketone (5 mM), TA (1 mg/mL), *Ad*RedAm (0.5 mg/mL), GDH (1 mg/mL), LDH (0.5 mg/mL), DL-alanine (500 mM), glucose (100 mM), NAD^+ (1.5 mM), $NADP^+$ (0.5 mM), PLP (1 mM) in Tris buffer (100 mM, pH 8.0), 30 ºC, 250 rpm, 24 h.

Conclusions

In summary, an one-pot enzymatic cascade using TAs and RedAms has been successfully implemented for the regio- and stereoselective synthesis of a panel of 2-methyl-5-alkylpyrrolidines from the corresponding 1,4-diketones. Both biocatalytic reactions were previously evaluated in a single-step reaction format. Transaminases showed excellent (>99%) regioselectivity for the monoamination of the sterically less demanding methyl ketone moiety while *Ad*RedAm catalysed the reduction of the afforded 2,5-disubstituted pyrroline. The compatibility of both purified enzymes (TAs and *Ad*RedAm) allowed the development of the reported one-pot cascade eliminating the necessity of costly intermediate purification steps. In addition, the enantiopure pyrroline and pyrrolidine derivatives obtained herein are analogous to the ones found in the Myrmicine ant venom and in the skin extract of Dendrobates poison frogs and can be further evaluated to assess their biological function.

Experimental Section

General

GC analyses were performed on an Agilent 6850 GC (Agilent, Santa Clara, CA, USA) coupled with a flame ionization detector (FID), equipped with a fused silica capillary column HP-1 (30 m x 0.32 mm x 0.25 µm, Agilent) for general purposes or CP-Chirasil-DEX CB (25 m x 0.25 mm x 0.25 µm, Agilent) for enantiomeric discrimination. GC-MS analyses were performed on an Agilent 7890B chromatograph coupled to an Agilent 5977B mass spectrometer with an electron ionization source (EI) operating at 70 eV and equipped with a fused silica capillary column HP-1ms (30 m x 0.25 mm x 0.25 µm).

Material

Commercially available reagents were used throughout without further purification. All reagents and solvents were purchased from Sigma-Aldrich or Alfa Aesar. Commercially available enzymes ATA117, LDH-103 and GDH (CDX-9012) were purchased from Codexis in the form of lyophilised extracts. TAs, IREds and RedAms were expressed in *E. coli* BL21(DE3) cells (Invitrogen), using ampicillin or kanamycin as antibiotic, and the proteins were purified by Ni^{2+}-affinity chromatography.

Biotransformations

All analytical biotransformations were performed in a 500 µL-scale in 2 mL vials. Reaction mixtures were incubated at 250 rpm and 30 ºC for 24 h, quenched by adding NaOH solution (50 µL, 5 mol/L) and extracted with *tert*-butyl methyl ether (500 µL). Organic layers were dried over $MgSO_4$ anhydrous and analysed by GC-FID for enzymatic conversion and enantiomeric excess determination.

Diketones bioamination

Reactions were performed using diketone substrate (**1a-g**, 5 mM), transaminase (2 mg/mL), pyridoxal phosphate (PLP, 1 mmol/L), glucose dehydrogenase (GDH, 1 mg/mL), lactate dehydrogenase (LDH, 0.5 mg/mL), NAD^+ (1.5 mM), glucose (100 mM) and alanine (500 mM) in Tris buffer (100 mmol/L, pH 8).

Cascade reactions

Reactions were performed using the diketone substrate (**1a-g**, 5 mM), *Mc*TA or *Bm*TA (1 or 2 mg/mL), *Ad*RedAm (0.5 or 1 mg/mL), glucose dehydrogenase (GDH, 1 mg/mL), lactate dehydrogenase (LDH, 0.5 mg/mL), pyridoxal phosphate (PLP, 1 mmol/L), NAD^+ (1.5 mM), $NADP^+$ (0.5 mM), glucose (100 mM) and alanine (500 mM) in Tris buffer (100 mM, pH 8).

Preparative Scale Biotransformations

Pyrrolidines **3b** and **3d** were synthesised *via* the TA-*Ad*RedAm cascade. Preparative scale reactions were performed in a 50 mL-scale in 250 mL flasks containing the diketone substrate (**1b** or **1d**, 50 mg), *Mc*TA or *Bm*TA (1 mg/mL), *Ad*RedAm (0.5 mg/mL), glucose dehydrogenase (GDH, 1 mg/mL), lactate dehydrogenase (LDH, 0.5 mg/mL), pyridoxal phosphate (PLP, 1 mmol/L), NAD^+ (1.5 mM), $NADP^+$ (0.5 mM), glucose (100 mM) and alanine (500 mM) in Tris buffer (100 mmol/L, pH 8). The reaction mixtures were stirred at 250 rpm and 30 ºC. After completion, the reactions were quenched by adjusting the pH to 12 with 5 M NaOH solution and extracting with *tert*-butyl methyl ether or diethyl ether (3 x 50 mL). The organic layers were dried over $MgSO_4$ anhydrous and the solvent was removed under reduced pressure to afford the desired pyrrolidines without any further purification step.

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Keywords: pyrrolidine alkaloids • transaminase • imine reductase • reductive aminase.

[1] T. Aniszewski, in *Alkaloids – Secrets Life*, Elsevier, **2007**, pp. 61–139.

[2] a) J. Chen, C. L. Cantrell, D. Oi, M. J. Grodowitz, *Toxicon* **2016**, *122*, 127–132. b) R. M. M. Adams, T. H. Jones, J. T. Longino, R. G. Weatherford, U. G. Mueller, *J. Chem. Ecol.* **2015**, *41*, 373–385. c) A. M. A. Mashaly, A. M. Ahmed, M. S. Al-Khalifa, T. M. Nunes, E. D. Morgan, *Biochem. Syst. Ecol.* **2010**, *38*, 875–879. d)T. H. Jones, A. N. Andersen, J. C. Kenny, *Chem. Biodivers.* **2009**, *6*, 1034–1041. e) T. H. Jones, H. L. Voegtle, H. M. Miras, R. G. Weatherford, T. F. Spande, H. M. Garraffo, J. W. Daly, D. W. Davidson, R. R. Snelling, *J. Nat. Prod.* **2007**, *70*, 160–168. f) T. H. Jones, V. E. Zottig, H. G. Robertson, R. R. Snelling, *J. Chem. Ecol.* **2003**, *29*, 2721–2727. g) T. H. Jones, M. S. Blum, P. Escoubas, T. M. M. Ali, *J. Nat. Prod.* **1989**, *52*, 779–784.

[3] J. W. Daly, H. M. Garraffo, T. F. Spande, in *Alkaloids Chem. Biol. Perspect.* (Ed.: S.W. Pelletier), Elsevier, Oxford, **1999**, pp. 1–161.

[4] a) A. Touchard, S. R. Aili, E. G. P. Fox, P. Escoubas, J. Orivel, G. M. Nicholson, A. Dejean, *Toxins (Basel).* **2016**, *8*, 1–28. b) C. Macfoy, D. Danosus, R. Sandit, T. H. Jones, H. M. Garraffo, T. F. Spande, J. W. Daly, *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **2005**, *60*, 932–937. c) M. S. Blum, *J. Toxicol. Toxin Rev.* **1992**, *11*, 115–164. d) J. W. Daly, Y. Nishizawa, M. W. Edwards, J. A. Waters, R. S. Aronstam, *Neurochem. Res.* **1991**, *16*, 489–500. e) D. Bacos, J. J. Basselier, J. P. Celerler, C. Lange, E. Marx, G. Lhommet, P. Escoubas, M. Lemaire, J. L. Clement, *Tetrahedron Lett.* **1988**, *29*, 3061–3064. f) T. H. Jones, M. S. Blum, A. N. Andersen, H. M. Fales, P. Escoubas, *J. Chem. Ecol.* **1988**, *14*, 35–45.

[5] a) S. J. Pérez, M. A. Purino, D. A. Cruz, J. M. López-Soria, R. M. Carballo, M. A. Ramírez, I. Fernández, V. S. Martín, J. I. Padrón, *Chem. - A Eur. J.* **2016**, *22*, 15529–15535. b) H.-Q. Deng, X.-Y. Qian, Y.-X. Li, J.-F. Zheng, L. Xie, P.-Q. Huang, *Org. Chem. Front.* **2014**, *1*, 258–266. c) Z. Amara, J. Caron, D. Joseph, *Nat. Prod. Rep.* **2013**, *30*, 1211–1225. d) S. C. Söderman, A. L. Schwan, *Org. Lett.* **2013**, *15*, 4434–4437. e) H. Shu, A. R. Noble, S. Zhang, L. Miao, M. L. Trudell, *Tetrahedron* **2010**, *66*, 4428–4433. f) S. Zhang, L. Xu, L. Miao, H. Shu, M. L. Trudell, *J. Org. Chem.* **2007**, *72*, 3133–3136. g) F. A. Davis, M. Song, A. Augustine, *J. Org. Chem.* **2006**, *71*, 2779–2786. h) P. Hammann, in *Org. Synth. Highlights II* (Ed.: H. Waldman), VCH Verlagsgesellschafi MbH, Weinheim, **1995**, pp. 323–334.

[6] a) R. A. Sheldon, J. M. Woodley, *Chem. Rev.* **2018**, *118*, 801–838. b) M. Hönig, P. Sondermann, N. J. Turner, E. M. Carreira, *Angew. Chemie - Int. Ed.* **2017**, *56*, 8942–8973. c) R. A. Sheldon, P. C. Pereira, *Chem. Soc. Rev.* **2017**, *46*, 2678–2691. d) R. O. M. A. de Souza, L. S. M. Miranda, U. T. Bornscheuer, *Chem. - A Eur. J.* **2017**, *23*, 12040–12063.

[7] S. Leclercq, J. Braekman, D. Daloze, *Naturwissenschaften* **1996**, *83*, 222–225.

[8] R. C. Simon, B. Grischek, F. Zepeck, A. Steinreiber, F. Belaj, W. Kroutil, *Angew. Chemie - Int. Ed.* **2012**, *51*, 6713–6716.

[9] a) R. C. Simon, F. Zepeck, W. Kroutil, *Chem. Eur. J.* **2013**, *19*, 2859–2865. b) R. C. Simon, C. S. Fuchs, H. Lechner, F. Zepeck, W. Kroutil, *Eur. J. Org. Chem.* **2013**, 3397–3402.

[10] E. O'Reilly, C. Iglesias, D. Ghislieri, J. Hopwood, J. L. Galman, R. C. Lloyd, N. J. Turner, *Angew. Chemie - Int. Ed.* **2014**, *53*, 2447–2450.

[11] S. P. France, S. Hussain, A. M. Hill, L. J. Hepworth, R. M. Howard, K. R. Mulholland, S. L. Flitsch, N. J. Turner, *ACS Catal.* **2016**, *6*, 3753–3759.

[12] C. R. Jones, G. Dan Pantos, A. J. Morrison, M. D. Smith, *Angew. Chemie - Int. Ed.* **2009**, *48*, 7391–7394.

[13] a) J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd, N. J. Turner, *Green Chem.* **2017**, *19*, 361–366. b) N. Van Oosterwijk, S. Willies, J. Hekelaar, A. C. Terwisscha Van Scheltinga, N. J. Turner, B. W. Dijkstra, *Biochemistry* **2016**, *55*, 4422–4431. c) C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, et al., *Science* **2010**, *329*, 305–309. d) D. Koszelewski, M. Goritzer, D. Clay, B. Seisser, W. Kroutil, *ChemCatChem* **2010**, *2*, 73-77. e) R. L. Hanson, B. L. Davis, Y. Chen, S. L. Goldberg, W. L. Parker, T. P. Tully, M. A. Montana, R. N. Patel, *Adv. Synth. Catal*. **2008**, *350*, 1367–1375. f) U. Kaulmann, K. Smithies, M. E. B. Smith, H. C. Hailes, J. M. Ward, *Enzyme Microb. Technol.* **2007**, *41*, 628–637. g) J.-S. Shin, H. Yun, J.-W. Jang, I. Park, B.-G. Kim, *Appl. Microbiol. Biotechnol.* **2003**, *61*, 463–471.

[14] D. Koszelewski, I. Lavandera, D. Clay, D. Rozzell, W. Kroutil, *Adv. Synth. Catal.* **2008**, *350*, 2761–2766.

[15] a) S. Wang, X. Meng, H. Zhou, Y. Liu, F. Secundo, Y. Liu, *Catalysts* **2016**, *6*, 32. b) A. M. Klibanov, *Nature* **2001**, *409*, 241–246.

[16] J. H. Schrittwieser, S. Velikogne, W. Kroutil, *Adv. Synth. Catal.* **2015**, *357*, 1655–1685.

[17] a) M. Lenz, N. Borlinghaus, L. Weinmann, B. M. Nestl, *World J. Microbiol. Biotechnol.* **2017**, *33*, DOI 10.1007/s11274-017-2365-8. b) J. Mangas-Sanchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan, N. J. Turner, *Curr. Opin. Chem. Biol.* **2017**, *37*, 19–25. c) G. Grogan, N. J. Turner, *Chem. Eur. J* **2016**, *22*, 1900–1907.

[18] a) S. Hussain, F. Leipold, H. Man, E. Wells, S. P. France, K. R. Mulholland, G. Grogan, N. J. Turner, *ChemCatChem* **2015**, *7*, 579–583. b) K. Mitsukura, M. Suzuki, S. Shinoda, T. Kuramoto, T. Yoshida, T. Nagasawa, *Biosci. Biotechnol. Biochem.* **2011**, *75*, 1778–1782.

[19] a) F. Leipold, S. Hussain, D. Ghislieri, N. J. Turner, *ChemCatChem* **2013**, *5*, 3505–3508. b) K. Mitsukura, T. Kuramoto, T. Yoshida, N.

Kimoto, H. Yamamoto, T. Nagasawa, *Appl Microbiol Biotechnol* **2013**, *97*, 8079–8086.

[20] G. A. Aleku, H. Man, S. P. France, F. Leipold, S. Hussain, L. Toca-Gonzalez, R. Marchington, S. Hart, J. P. Turkenburg, G. Grogan, et al., *ACS Catal.* **2016**, *6*, 3880–3889.

[21] G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan, N. J. Turner, *Nat. Chem.* **2017**, *9*, 961–969.

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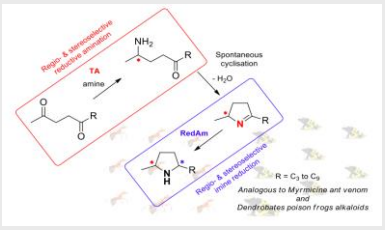
Entry for the Table of Contents

FULL PAPER

An one-pot enzymatic cascade using transaminase and reductive aminase biocatalysts has been successfully implemented for the regio- and stereoselective synthesis of a panel of 2-methyl-5-alkylpyrrolidines from the corresponding 1,4-diketones.



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Page No. – Page No.

Synthesis of 2,5-disubstituted pyrrolidine alkaloids *via* a one-pot cascade using transaminase and reductive aminase biocatalysts