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Regio- and Stereoselective ω-Transaminase/MAO-N Cascade for the Synthesis of Chiral 2,5-Disubstituted Pyrrolidines

*Elaine O'Reilly, Cesar Iglesias, Diego Ghislieri, Jennifer Hopwood, James L. Galman, Richard C. Lloyd and Nicholas. J. Turner**

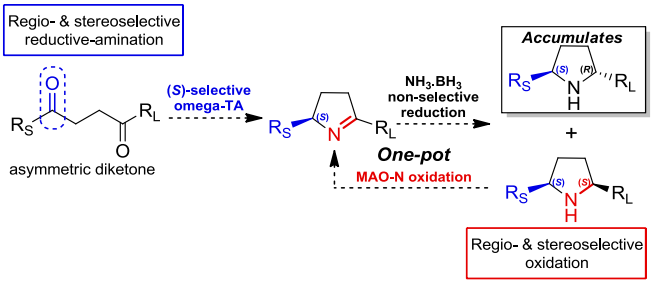
Abstract

Biocatalytic approaches to the synthesis of optically pure chiral amines starting from simple achiral building blocks are highly desirable as such motifs are present in a wide variety of important natural products and pharmaceutical drugs. Herein we report a novel one-pot ω-transaminase/monoamine oxidase cascade for the synthesis of chiral 2,5-disubstituted pyrrolidines. Biotransformations proceed with excellent enantio- (>94% *ee*) and diastereoselectivity (>98% *ee*) and can be performed on a preparative scale. This methodology exploits the complementary regio- and stereoselectivity displayed by both enzymes which ensures that the stereogenic centre established by the transaminase is not affected by the monoamine oxidase and highlights the potential of this multi-enzyme cascade for the efficient synthesis of chiral building blocks.

The exquisite chemo-, regio- and stereoselectivity displayed by enzymes has led to their widespread application as catalysts for stereocontrolled organic synthesis.[1] These properties, coupled with their ability to catalyse reactions under similar conditions, has enabled the development of elegant multi-enzyme cascade processes in which the product formed by the action of the first enzyme becomes the starting material for the subsequent biotransformation.[2] Such tandem processes alleviate the need for protecting group manipulations and intermediate purification steps, providing cost-effective routes to target molecules.

Among the most synthetically useful biocatalysts for the synthesis of chiral amines are the ω-transaminase (TA) family and variants of monoamine oxidase from *Aspergillus niger* (MAO-N).[3] TAs are capable of mediating the reductive amination of pro-chiral ketones, providing the corresponding chiral amines.[3a-e] MAO-N catalyses the oxygen-dependent conversion of amines to imines and is typically selective for the (*S*)-enantiomer.[3f-l] Variants of MAO-N have been exploited for the deracemization of primary, secondary and tertiary amines with diverse structural motifs.[3a,3f-j] The development of several chemo-enzymatic routes[3c,4] to industrially important target molecules employing these two enzyme classes is testament to the advances in protein engineering[1a,5] which have resulted in the development of biocatalysts with the desired substrate scope, selectivity and stability.

2,5-disubstituted pyrrolidines are important scaffolds in pharmaceutical drugs[6] and natural products[7] and considerable efforts have been devoted to developing asymmetric routes to both *cis*- and *trans*-disubstituted derivatives resulting in moderate to good stereoselectivity.[8] The lack of stereofacial bias induced by the pre-exisiting C-2-stereocentre means that obtaining the *trans*-diastereomers *via* reduction of the corresponding imine in high *de* is not straightforward. Our approach to their synthesis (Scheme 1) features a highly selective TA mediated reductive amination of an achiral 1,4-diketone to generate an optically active pyrroline followed by diastereoselective chemo-enzymatic conversion to the corresponding pyrrolidine by the use of MAO-N/$NH_3.BH_3$.



Scheme 1. A chemo-enzymatic approach for the synthesis of 2,5-disubstituted pyrrolidines employing an ω-transaminase (TA) and monoamine oxidase (MAO-N).

Initially we examined the ω-TA mediated selective mono-amination of commercially available 1,4-diketone **1a**, which bears a small methyl and a large phenyl substituent (Scheme 2). The first example of the asymmetric bioamination of 1,5-diketones was recently reported with excellent regio- and stereoselectivity achieved.[9] Commercially available (*S*)-selective ATA113 was found to be highly regioselective, mediating the reductive amination of **1a** exclusively on the methyl ketone at a substrate concentration of 25 mM, using L-alanine as the amine donor. The resulting 1,4-amino ketone (*S*)-**2a** subsequently underwent spontaneous cyclization providing (*S*)-**3a** in high yield (91%) and excellent *ee* (>99%). The lactate dehydrogenase (LDH)/glucose dehydrogenase (GDH) system was used to drive the equilibrium towards product and recycle the NAD^+ cofactor (see supporting information for detailed scheme). (*R*)-selective ATA117 also catalysed the regio-

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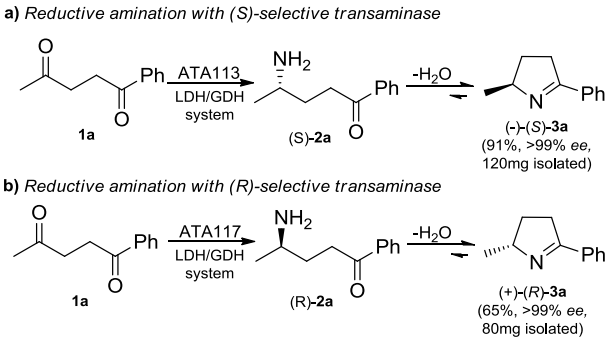
and stereoselective mono-amination of **1a**, affording pyrroline (*R*)-**3a** in 65% yield and > 99% *ee*.



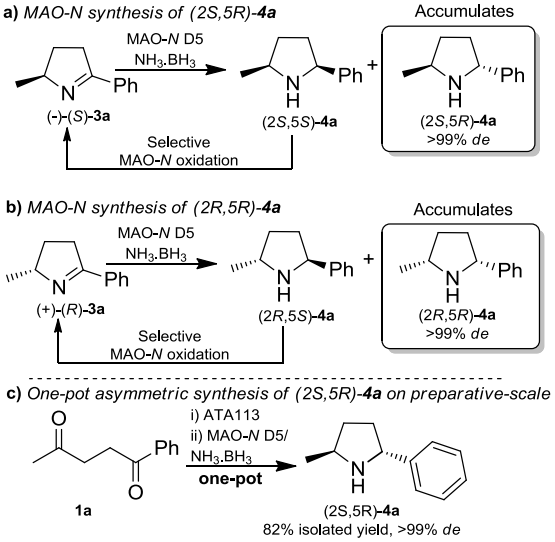
Scheme 2. Preparative-scale (25mM **1a**) reductive amination of diketone **1a** mediated by (*S*)-selective ATA113 and (*R*)-selective ATA117, followed by spontaneous cyclisation.

Having established an effective means of accessing optically pure pyrrolines on a preparative scale, we subsequently explored a route for the diastereoselective synthesis of 2,5-disubstituted pyrrolidine **4a** starting from **3a** (Scheme 3). 2,6-disubstituted piperidines have been prepared *via* a chemo-enzymatic route employing an ω-transaminase followed by diastereoselective hydrogenation using Pd/C.[9] However, the same strategy is not applicable to the diastereoselective synthesis of 2,5-disubstituted pyrrolidines due to poor diastereostereoselectivity in the reduction step. We envisaged using MAO-N variants in combination with $NH_3.BH_3$ for the asymmetric synthesis of **4a**. Two MAO-N variants (D5 and D9) were selected based on their known activity and excellent selectivity towards structurally related amine frameworks, including pyrrolidines and piperidines.[3h,3k] Our strategy relies upon MAO-N variants displaying complete regio- and stereoselectivity to avoid stereorandomisation of the C-2-stereocentre generated by the (*S*)-selective ω-TA. Imine **3a** is in equilibrium with the open chain amino ketone (*S*)-**2a** and hence optimization of the MAO-N/$NH_3.BH_3$ oxidation/reduction cycle was necessary in order to prevent the formation of undesired amino alcohol as a side product**.** Ketone reduction was minimised by lowering the concentration of MAO-N biocatalyst during the reaction while maintaining a high concentration of $NH_3.BH_3$. Under these conditions rapid reduction of pyrroline **3a** occurred ensuring that a minimal concentration of the amino ketone was present during the biotransformation.

Treatment of (*S*)-**3a** with $NH_3.BH_3$ afforded **4a** initially as a mixture of diastereoisomers with a slight excess of the (2*S*,5*S*)-isomer (*de* ~10%) (Scheme 3a). Both MAO-N variants mediated the oxidation of (2*S*,5*S*)-**4a** diastereoisomer exclusively and displayed complete regioselectivity for the more bulky phenyl side of the pyrrolidine. Following successive rounds of selective oxidation with the MAO-N D5 variant and non-selective reduction with $NH_3.BH_3$, (2*S*,5*R*)-**4a** was isolated in greater than 99% *de*. Despite a bias for the formation of the *cis*-diastereoisomer upon reduction with $NH_3.BH_3$, the combination with MAO-N yielded solely the *trans*-reduction product, (2*S*,5*R*)-**4a**. The complementary regioselectivity displayed by the ω-TA and MAO-N variants circumvents epimerisation of the C-2-(*S*)-centre and provides a method for accessing optically pure 2,5-pyrrolidines.

Having developed efficient individual biocatalytic routes for the synthesis of optically pure pyrroline **3a** and target chiral 2,5-pyrrolidine **4a**, we next sought to combine the ω-TA and MAO-N biocatalysts in a one-pot cascade (Scheme 3c). Diketone **1a** was exposed to ATA 113 followed by MAO-N and $NH_3.BH_3$, providing the target (2*S*,5*R*)-**4a** in 82% yield and > 99% *de*.

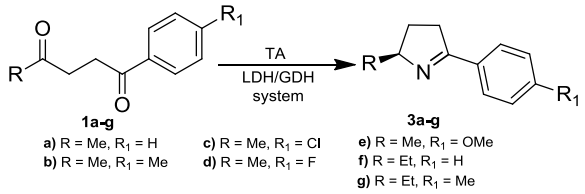
To allow access to the (2*R*,5*R*)-**4a** diastereoisomer, the enantiomer (*R*)-**3a**, derived from the use of ATA117, was treated with the $NH_3.BH_3$/MAO-N combination (Scheme 3b). Following non-selective reduction to give a mixture of (2*R*,5*S*)-**4a** and (2*R*,5*R*)-**4a**, both MAO-N variants mediated the oxidation of the (2*R*,5*S*)-isomer selectively, providing (2*R*,5*R*)-**4a** exclusively after successive rounds of oxidation/reduction. The stereochemistry at C-2 has minimal effect on the activity of the MAO-N enzyme and no effect on the stereoselectivity, with the target (2*R*,5*R*)-**3a** isolated in >99% *de*.



Scheme 3. a & b) MAO-*N* D5 mediated analytical-scale synthesis of (2*S*,5*R*)- and (2*R*,5*R*)-**4a**; **c)** One-pot TA/MAO-N cascade for the preparative-scale asymmetric synthesis of (2*S*,5*R*)-**4a**. Reduction of the starting diketone by $NH_3.BH_3$ prevented the addition of all of the reagents concurrently.

The generality of the TA/MAO-N cascade process was investigated by examining a series of diketones **1b-g** using ATA113 as well as a novel transaminase (*pf*-ATA) from *Pseudogulbenkiania ferrooxidans*[10] (Table 1). *Pf*-ATA shares 95% sequence identity with the transaminase from *Chromobacterium violaceum* (*cv*-ATA) (ATCC 12472).[11] ATA113 mediated the reductive amination of diketones **1b-g** which spontaneously cyclised yielding pyrrolines **3b-g** as the sole regioisomers in excellent conversion and high *ee* values. Unsurprisingly, replacement of the small methyl for a larger ethyl substituent resulted in a slightly reduced *ee* (entry 11 & 13). The biotransformations performed with *Pf*-ATA proceeded with reduced selectivity, with *ee* values lower than those achieved with ATA113. Interestingly, replacement of the methyl substituent by ethyl resulted in a switch in stereoselectivity, providing (*R*)-**3f-g** as the predominant enantiomers (entries 12 & 14, see supporting information for absolute configuration and *ee* determination). We also compared the selectivity observed with *pf*-ATA to related *cv*-ATA against diketones **1a** and **1d-e** and noted comparable conversion and selectivity (see supporting information). (*R*)-selective ATA117 also mediated the reductive amination of diketones **1a** and **1d-e** in >99 % conversion and *ee* (see supporting information).

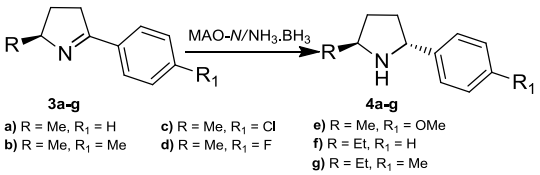
Table 1. TA mediated reductive amination of **1a-g**



Entry	Substrate	ω-TA	Conv. [%]	*ee* [%]
1	**1a**	ATA113	> 99	> 99 (*S*)
2	**1a**	*P. ferrooxidans*	> 99	75 (*S*)
3	**1b**	ATA113	> 99	> 99 (*S*)
4	**1b**	*P. ferrooxidans*	> 99	> 78 (*S*)
5	**1c**	ATA113	> 99	> 99 (*S*)
6	**1c**	*P. ferrooxidans*	> 99	68 (*S*)
7	**1d**	ATA113	> 99	> 99 (*S*)
8	**1d**	*P. ferrooxidans*	> 99	76 (*S*)
9	**1e**	ATA113	> 99	> 99 (*S*)
10	**1e**	*P. ferrooxidans*	> 99	78 (*S*)
11	**1f**	ATA113	60	96 (*S*)
12	**1f**	*P. ferrooxidans*	> 99	76 (*R*)
13	**1g**	ATA113	> 99	94 (*S*)
14	**1g**	*P. ferrooxidans*	75	46 (*R*)

The efficiency of the MAO-N/NH_3.BH_3 step with the isolated pyrrolines **3b-g** was next examined (Table 2). In general, the D9 variant showed higher selectivity and employing either the D5 or D9 MAO-N variants allowed access to all Me/Ar and Et/Ar substituted pyrrolidines in excellent *de*. We have also extended the one-pot TA/MAO-N cascade for the synthesis of (2*S*,5*R*)-**4b**, (2*S*,5*R*)-**4d** and (2*S*,5*R*)-**4e** in > 99% conversion and > 99% *de* starting from the corresponding diketones (Table 3), demonstrating the generality of this one-pot approach.

Table 2. MAO/NH_3.BH_3 mediated asymmetric synthesis of (2S,5R)-**4a-g**



Entry	Substrate	MAO-N variant	*de* [%] (2*S*,5*R*)
1	**3a**	D5	> 99
2	**3a**	D9	96
3	**3b**	D5	88
4	**3b**	D9	98
5	**3c**	D5	> 99
6	**3c**	D9	> 99
7	**3d**	D5	> 99
8	**3d**	D9	> 99
9	**3e**	D5	68
10	**3e**	D9	> 99
11	**3f**	D5	64
12	**3f**	D9	> 99
13	**3g**	D5	56
14	**3g**	D9	96

Table 3. ATA113/MAO-N one-pot cascade for the synthesis of (2*S*,5*R*)-**4a**, (2*S*,5*R*)-**4b**, and (2*S*,5*R*)-**4d-e**

Ketone	ω-TA	MAO-N	Conv. [%]	*de* [%]
1a[‡]	ATA113	D5	> 99	> 99 (2*S*,5*R*)-**4a**
1b[‡‡]	ATA113	D9	> 99	> 99 (2*S*,5*R*)-**4b**
1d[‡‡]	ATA113	D9	> 99	> 99 (2*S*,5*R*)-**4d**
1e[‡‡]	ATA113	D9	> 99	> 99 (2*S*,5*R*)-**4e**

[‡]25mM substrate concentration; [‡‡]5mM substrate concentration.

In summary, the combination of two complementary biocatalysts has been demonstrated by a novel one-pot, chemo-enzymatic cascade for the synthesis of a panel of 2,5-disubstituted pyrrolidines from the corresponding 1,4-diketones. The ω-TA is highly selective for the sterically less demanding methyl ketone while the monoamine oxidase MAO-N shows an overwhelming preference for the more bulky portion of the corresponding pyrrolidine. The compatibility of both biocatalysts means that the reaction can be performed in one-pot, without the need for costly intermediate purification steps. The chemo-enzymatic approach exploits four distinct biocatalytic operations and takes advantage of the complementary regioselectivity displayed by the ω-TA and MAO variants to establish two stereogenic centres. All biocatalysts described herein are commercially available[12] and hence readily accessible for practical application.

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[12] a) MAO-N Screening Kits: http://www.discovery-bc.co.uk/monoamineoxidase.php (2013); b) Transaminases: http://www.codexis.com

Layout 2:

((Catch Phrase))

Elaine O'Reilly, Cesar Iglesias, Diego Ghislieri, Jennifer Hopwood, James L. Galman, Richard C. Lloyd and Nicholas J. Turner*

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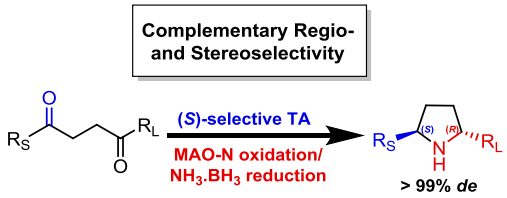
Regio- and Stereoselective ω-Transaminase/MAO-N Cascade for the Synthesis of Chiral 2,5-Disubstituted Pyrrolidines



A novel ω-transaminase (ω-TA)/monoamine oxidase (MAO-N) cascade process for the synthesis of chiral 2,5-disubstituted pyrrolidines is reported. The methodology exploits the complementary regio- and stereoselectivity displayed by both enzymes which ensures that the stereogenic centre established by the TA reaction is not affected by the MAO-N catalysed step and highlights the application of these biocatalysts for the asymmetric synthesis of chiral pyrrolidines.