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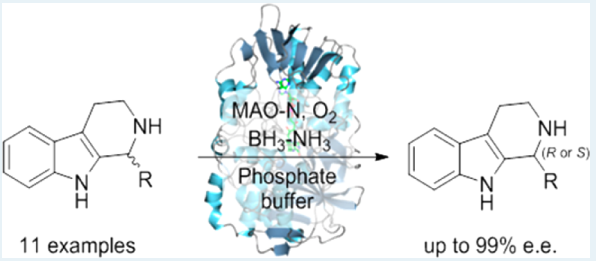
Monoamine Oxidase (MAO-N) Catalyzed Deracemization of Tetrahydro-β-carbolines: Substrate Dependent Switch in Enantioselectivity

Diego Ghislieri, Deborah Houghton, Anthony P. Green, Simon C. Willies, and Nicholas J. Turner*

School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester, M1 7DN, U.K.

Supporting Information

ABSTRACT: The tetrahydro-β-carboline (THBC) ring system is an important structural motif found in a large number of bioactive alkaloid natural products. Herein we report a broadly applicable method for the synthesis of enantiomerically pure β-carbolines via a deracemization procedure employing the D9 and D11 variants of monoamine oxidase from *Aspergillus niger* (MAO-N) in combination with a nonselective chemical reducing agent. Biotransformations were performed on a preparative scale, leading to the synthesis of optically enriched products in excellent enantiomeric excess (e.e.; up to 99%) and isolated yield (up to 93%). Interestingly, a switch in enantioselectivity associated with the MAO-N variants is observed as the nature of the C-1 substituent of the THBC is varied. Molecular modeling provided an explanation for this observation and highlighted key active site residues which were modified, resulting in an increase in (*R*)-selectivity associated with the enzyme. These results provide insight into the factors which influence the selectivity of the MAO-N variants, and may offer a platform for future directed evolution projects aimed toward the challenge of engineering (*R*)-selective amine oxidase biocatalysts.



KEYWORDS: *β-carbolines, biocatalysis, chiral amine, monoamine oxidase, deracemization*

INTRODUCTION

Tetrahydro-β-carbolines (THBCs) are an important family of bioactive alkaloids that are widespread in nature. Important examples include the antileishmanii compound harmicine **1**, the antihypertensive drugs reserpine **2** and ajmalicine **3**, and the stimulant yohimbine **4** (Figure 1). The complex molecular architectures and the wealth of biological activities associated with the THBCs have attracted significant attention from the synthetic community, and numerous pioneering studies describing the total synthesis of these structures have been reported.[1−4] The development of methods for the asymmetric synthesis of THBCs continues to represent an important synthetic challenge and although a number of approaches have been described previously, including the use of chiral auxiliaries,[5,6] transition metal catalysts,[7−9] organocatalysts,[10,11] and more recently biocatalysts,[12−14] the development of broadly applicable, alternative strategies remains of great interest.

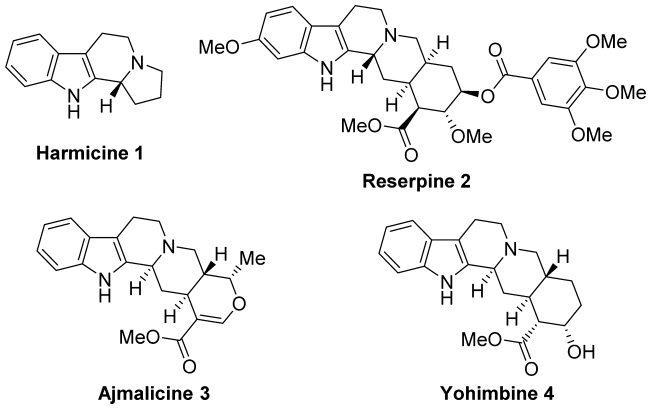


Figure 1. Representative examples of alkaloids containing the β-carboline skeleton.

We have previously described a chemoenzymatic method for the deracemization of chiral amines to generate the enantiomerically pure isomer via a redox catalytic cycle (Figure 2).[15] Variants of monoamine oxidase from *Aspergillus niger* (MAO-N) have been developed in our laboratory, via directed evolution approaches, which catalyze the enantioselective oxidation of chiral amines with broad structural features. When coupled with a nonselective chemical reducing agent (e.g., ammonia-borane), these engineered enzymes have been shown to mediate the deracemization of chiral primary, secondary, and tertiary amines.[15−17]

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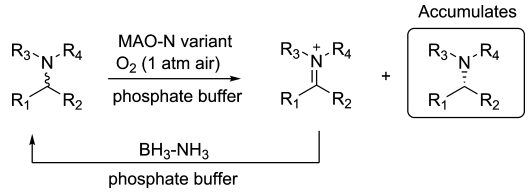
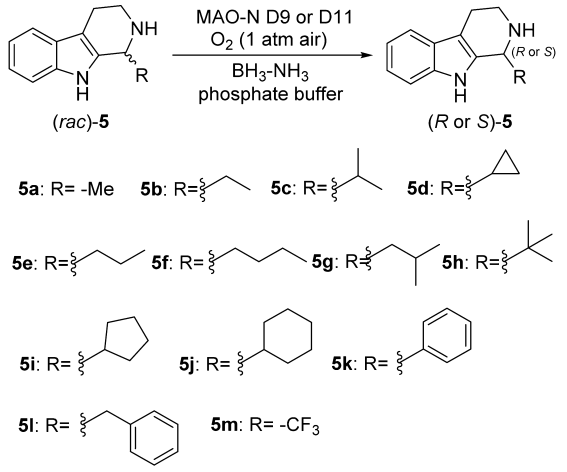


Figure 2. Generic catalytic cycle for the deracemization of chiral amines using a combination of an enantioselective amine oxidase with a nonselective chemical reducing agent.

Recently we reported the application of the D9 variant of MAO-N in the deracemization of eleagnine, leptaflorine, and harmicine, three alkaloid natural products belonging to the *β*-carboline subclass.[18] In this study the D9 variant was found to be selective for oxidation of the (*S*)-enantiomers of these substrates. We now describe the application of MAO-N variants for the enantioselective oxidation of a series of 1-substituted *β*-carbolines, thus providing a broadly applicable strategy for the synthesis of these structures in optically pure form. Significantly, a switch in enantioselectivity is observed as the nature of the C1 substituent is varied. The origins of this inversion in selectivity are explored, and the results provide a previously unreported insight into the factors which influence the enantioselectivity associated with the MAO-N variants.

A panel of aliphatic and aromatic 1-substituted racemic THBCs was synthesized from tryptamine hydrochloride and the required aldehyde via a Pictet–Spengler reaction according to a previously reported procedure[13] (see Supporting Information). The racemic *β*-carbolines were then subjected to a deracemization protocol using *Escherichia coli* whole cells expressing two different MAO-N variants (D9 and D11) to compare the activity and enantioselectivity (Figure 3). The MAO-N D11 variant was recently developed for the oxidation of structures containing the benzhydrylamine template, and differs from the D9 variant by a single point mutation (H430G) which results in an increase in the volume of the small pocket within the active site.[18] Biotransformations were carried out at 15 mM concentration in 1 M phosphate buffer (pH = 7.8) using 4 equiv of BH_3–NH_3 as the reducing agent.

The results are shown in Figure 3 and demonstrate that *β*-carbolines **5a** and **5d**–**k** can be deracemized using either the D9 or D11 variants (or both), providing the optically enriched products in good to excellent enantiomeric excess (e.e.). The racemic 1-methyl substituted THBC **5a** was converted to the corresponding (*R*)-enantiomer in >99% e.e. with both the D9 and the D11 variant. Replacing the methyl group **5a** with the bulkier ethyl **5b** and *iso*-propyl **5c** substituents resulted in a reduction in e.e. of the products, even after prolonged reaction times (>72 h). Biotransformations performed on the single enantiomers (*S*)-**5b** and (*R*)-**5b** in the absence of reducing agent show that the MAO-N variants mediate the oxidation of each enantiomer at comparable rates (see Supporting Information), providing an explanation for the low e.e. associated with this substrate. Increasing the size and lipophilicity of the C1 substituent led to an increase in e.e. of the deracemized products (substrates **5e**–**5k**). The MAO-N D11 variant is a particularly effective biocatalyst for the deracemization of those substrates which possess sterically demanding C1 substituents, while the D9 variant was shown to be more effective in the deracemization of the cyclopropyl derivative **5d**. Presumably the increase in active site volume associated with the D11 variant (cf. MAO-N D9) resulting



Entry	Time (h)	MAO-N D9 e.e. (%)	MAO-N D11 e.e. (%)
5a	24	>99 (*R*)	99 (*R*)
5b	84	36 (*R*)	25 (*R*)
5c	72	40 (*S*)	33 (*S*)
5d	48	86 (n.d.)	35 (n.d.)
5e	48	99 (*S*)	>99 (*S*)
5f	48	96 (*S*)	99 (*S*)
5g	48	58 (*S*)	>99 (*S*)
5h	48	62 (*S*)	96 (*S*)
5i	48	85 (*S*)	96 (*S*)
5j	48	80 (*S*)	97 (*S*)
5k	72	30 (*S*)	92 (*S*)
5l	48	-	-
5m	48	-	-

Figure 3. MAO-N (D9 and D11) mediated deracemization of *β*-carbolines **5a**–**m**. Biotransformation conditions: [S] = 15 mM; [BH_3–NH_3] = 60 mM; wet cells =200 mg/mL; 1 M potassium phosphate buffer (pH 7,8); 37 °C; 250 rpm; pH = 7.8. Reactions were carried out on a 4.5 *μ*mol scale, e.e. values were determined by chiral HPLC.

from the H430G mutation facilitates the binding of the sterically demanding substrates. Unfortunately, neither of the MAO-N variants displayed activity toward the benzyl and $-CF_3$ substituted THBCs (**5l** and **5m** respectively). To demonstrate the applicability of these MAO-N variants in preparative scale reactions, the deracemization of substrates **5e**–**g** and **5k** was performed on a 0.4 mmol scale, and the enantiomerically enriched products were isolated in good yields (>85%) and e.e. (99% for **5e**–**g**, 90% for **5k**).

Determination of the absolute configuration of the amines derived from the deracemization process established that both D9 and D11 variants exhibited (*S*)-selectivity for **5a**–**b** and (*R*)-selectivity for **5c**–**k** highlighting a switch in enantiopreference which is maintained in both variants. In an attempt to gain insight into the molecular basis underlying this change in enantioselectivity we decided to examine the different binding modes of the substrates in the MAO-N active site. Substrates (*S*)-**5a**, (*S*)-**5b**, (*R*)-**5b**, and (*R*)-**5g** were docked into the active site of the MAO-N D11 variant (PDB id: 3zdn)[18] (Figure 4) using Discovery Studio (Accelrys Software Inc.). The (*S*)-enantiomers of **5a** and **5b** adopt a putative binding mode in

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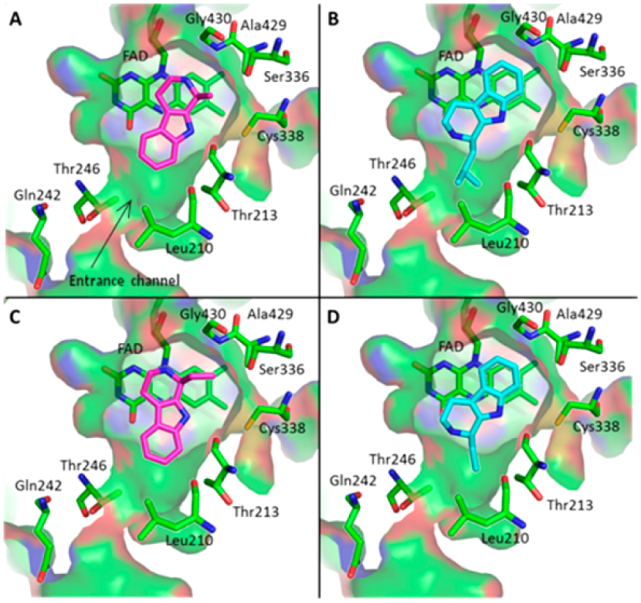

Figure 4. Docking of (*S*)-**5a** (A), (*R*)-**5g** (B), (*S*)-**5b** (C), and (*R*)-**5b** (D) into the active site of MAO-N D11, highlighting the alternative binding modes associated with the (*R*)- and (*S*)-enantiomers.

which the C1 substituent is buried within the active site and points away from the entrance channel. Attempts to dock (*S*)-**5g** failed to provide a reasonable productive binding for this substrate, presumably because of unfavorable steric interactions between the bulky C1 substituent and active site residues. This situation is consistent with the experimental observations which show that the selectivity of the MAO-N variants toward the (*S*)-enantiomers decreases as a function of increasing substrate size. The docking results show that the (*R*)-enantiomers of **5b** and **5g** adopt an alternative binding mode in which the C1 substituent points toward the active site entrance channel. However, closer inspection of the docked structures suggests that the THBC is not positioned sufficiently far into the active site to represent a productive binding mode because of steric clashes in the region of Ala429. We propose that an increase in the steric interactions between the C1 substituent and the amino acid residues around the entrance channel would effectively force the THBC structure further into the active site, thus enhancing the probability of accessing a catalytically productive binding mode. This provides a plausible explanation for the enhanced (*R*)-selectivity associated with the MAO-N variants as the size of the C1 substituent is increased. Alternatively, modification of the amino-acid residues around the active-site entrance channel to increase the steric interactions with the C1 THBC substituents should lead to an enhanced selectivity toward the (*R*)-enantiomers and would provide further evidence in favor of our proposed explanation.

Biotransformations were conducted with the MAO-N D10 variant[18] which differs from MAO-N D9 and MAO-N D11 by four point mutations of residues in the active site entrance channel (L210F, T213L, Q242M, and T246M). These modifications have the effect of increasing steric obstruction around the active site entrance channel. With the MAO-N D10 variant, the switch in enantioselectivity was found to occur with a substituent as small as an ethyl group (**5b**), showing relatively good selectivity toward the (*R*)-enantiomer, whereas both D9 and D11 showed poor enantioselectivity with a preference for the (*S*)-enantiomer (Table 1). In addition, with slightly larger

Table 1. MAO-N (D10 and D11) Mediated Deracemization of *β*-Carbolines 5a−5d[a]

entry	time (h)	MAO-N D10 e.e. (%)	MAO-N D11 e.e. (%)
5a	24	96 (*R*)	99 (*R*)
5b	48	49 (*S*)	12 (*R*)
5c	48	94 (*S*)	22 (*S*)
5e	48	99 (*S*)	>99 (*S*)

[a]Biotransformation conditions: [S] = 15 mM; [BH_3−NH_3] = 60 mM; wet cells = 200 mg/mL; 1 M potassium phosphate buffer (pH 7.8); 37 °C; 250 rpm; pH = 7.8. Reactions were carried out on a 4.5 μmol scale, e.e. values were determined by chiral HPLC.

substituents such as *iso*-propyl (**5c**), MAO-N D10 shows much higher (*R*)-enantioselectivity (94% e.e.) compared to the D9 and D11 variants which were both (*R*)-selective but displayed only low enantioselectivity (40 and 33% e.e. respectively). Modeling of (*R*)-**5c** into the D10 and D11 active sites reveals that the substrate is positioned closer to the flavin adenine dinucleotide (FAD) cofactor in MAO-N D10 (THBC-N2−FAD-N5 distance 3.56 Å) compared with a distance of 3.90 Å in the MAO-N D11, thus providing an explanation for the observed enhanced (*R*)-selectivity associated with the D10 variant (Figure 5). These results demonstrate that residues in

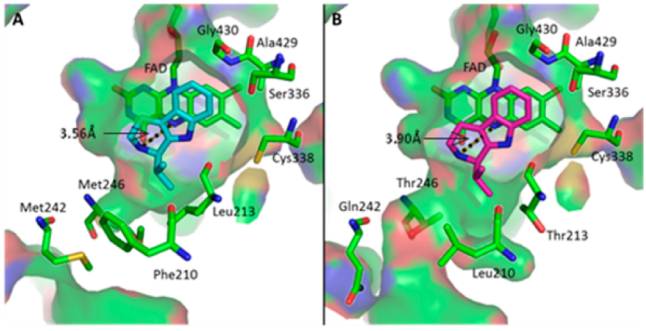

Figure 5. Docking of (*R*)-**5c** in the active site of MAO-N D10 (A) and MAO-N D11 (B). The THBC-N2−FAD-N5 distance is reduced in the D10 variant (cf MAO-N D11) as a result of increased steric obstruction around the active site channel.

the active site channel have an important effect upon the enantioselectivity of MAO-N variants toward THBC substrates. It is anticipated that further engineering of these and other residues around the channel could serve to further optimize the selectivity of MAO-N toward THBCs and alternative classes of substrates.

In conclusion, we have shown that chemo-enzymatic deracemization reactions, using a combination of monoamine oxidase from *A. niger* (MAO-N) and a nonselective chemical reducing agent, can be successfully applied to a range of different 1-substituted THBCs **5a−k**. Interestingly, a switch in enantioselectivity is observed as the nature of the C-1 substituent is varied. The results of extensive substrate screening and docking simulations provide valuable insights into the factors which influence the selectivity of MAO-N variants, and offer a platform for future directed evolution projects aimed toward the significant challenge of engineering enantio-complementary amine oxidase enzymes.

EXPERIMENTAL SECTION

MAO-N Mediated Preparative Deracemization: General Procedure. In a 50 mL Falcon tube, the required amine

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hydrochloride salt (0.4 mmol) and BH_3–NH_3 (1.6 mmol) were dissolved in potassium phosphate buffer (25 mL, 1 M, pH = 7.8). The pH of the solution was adjusted to 7.8 by addition of NaOH. Cell pellet *from E. coli* cultures (4 g) containing MAO-N D11 was added to the solution. The tube was placed in a shaking incubator and shaken at 37 °C and 250 rpm. Reactions were monitored by chiral phase HPLC. Upon completion of the reaction (typically after 48 h), aqueous NaOH (200 μL, 10 M) and CH_2Cl_2 (25 mL) were added. The layers were separated by centrifugation (4000 rpm, 5 min.), and the aqueous phase was further extracted with CH_2Cl_2 (20 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Nicholas.turner@manchester.ac.uk.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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