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## A Chemo-Enzymatic Route to Enantiomerically Pure Cyclic Tertiary Amines

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Enantiomerically pure tertiary amines are commonly used in organic synthesis as chiral auxiliaries/chiral bases<sup>1</sup> and catalysts for asymmetric synthesis.<sup>2</sup> In addition, chiral tertiary amines are valuable intermediates for the synthesis of pharmaceuticals and agrochemicals.<sup>3</sup> However, in contrast to primary and secondary amines, there are relatively few methods reported for the preparation of enantiomerically pure tertiary amines. Current approaches include classical resolution of the corresponding racemates,<sup>4</sup> asymmetric hydrogenation of enamines,<sup>5</sup> and kinetic resolution via enantioselective *N*-oxide formation.<sup>6</sup> Although lipase-catalyzed kinetic resolution is a favored method for obtaining enantiomerically pure primary amines,<sup>7</sup> this method can only be applied to specific classes of secondary amine<sup>8</sup> and is unavailable for tertiary amines.

In light of the lack of a general method for the preparation of optically active tertiary amines, we sought to adapt our recently developed chemo-enzymatic deracemization procedure to accommodate chiral tertiary amine substrates. We recently reported the deracemization of both primary<sup>9</sup> and secondary<sup>10</sup> chiral amines via a two-step, one-pot process involving an enantioselective amine oxidase in combination with a nonselective chemical reducing agent (Figure 1). In the process, as shown, the enzyme oxidizes only the *S*-enantiomer to the corresponding imine, which is then reduced in situ back to the racemic amine. Repeated cycles result in eventual accumulation of the *R*-enantiomer in high yield and enantiomeric excess.

By subjecting the monoamine oxidase from *Aspergillus niger* (MAO-N) to several rounds of directed evolution,<sup>11</sup> we have identified variant enzymes that possess enhanced catalytic activity and considerably broader substrate specificity toward primary and secondary chiral amines.<sup>12</sup> One of these variants, Ile246Met/Asn336Ser/Met348Lys/Thr384Asn/Asp385Ser (MAO-N-5), showed high activity and enantioselectivity toward the *S*-enantiomer of the cyclic secondary amine, 2-phenylpyrrolidine **2**. This discovery prompted us to examine whether the MAO-N-5 variant would display similar activity toward tertiary amines, such as *N*-methyl-2-phenylpyrrolidine **3**. Gratifyingly, we found **3** to be an excellent substrate for the variant amine oxidase, with an activity even higher than that of our reference substrate,  $\alpha$ -methylbenzylamine **1** (Figure 2). We therefore selected a range of tertiary amines with which to characterize the MAO-N-5 variant.<sup>13</sup>

Among the tertiary amine substrates screened, those containing a pyrrolidine ring flanked by bulky aryl groups (**3** and **4**) displayed the highest activity for the MAO-N-5 variant. This finding was consistent with the activities shown by structurally similar secondary amines that were used in the screening process to select this particular variant. Good activities were also obtained for substrates **7** and **10**, suggesting that a potentially wider range of tertiary amines could be accessed via further rounds of directed evolution. Despite some of the other substrates tested having somewhat lower activities, the initial objective of identifying a variant enzyme with high enantioselectivity toward chiral tertiary amines had been realized.

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Figure 1. Deracemization of  $\alpha$ -methylbenzylamine 1 with an enantioselective amine oxidase in combination with ammonia borane as the reducing agent.



**Figure 2.** Relative activities of various amines toward the MAO-N-5 variant. Assay conditions: amine (10 mM), purified MAO-N-5 enzyme, phosphate buffer pH 7.6 (0.1 M).



*Figure 3.* Deracemization of racemic *N*-methyl-2-phenylpyrrolidine **3** to (*R*)-*N*-methyl-2-phenylpyrrolidine as monitored by capillary electrophoresis.

Substrates **3** and **4** were then used as test candidates to assess the potential of employing the MAO-N-5 variant to carry out preparative deracemization reactions or, as in the case of **4**, stereoinversion of the *S*- to the *R*-enantiomer. Initial small-scale reactions were carried out at 10 mM substrate concentration with 10 equiv of ammonia borane and washed whole cells (*E. coli*) expressing the MAO-N-5 variant amine oxidase. The complete deracemization (ee = 99%) of **3** was achieved within 24 h as monitored by chiral capillary electrophoresis (Figure 3). Similarly, complete stereoinversion of (*S*)-nicotine **4** to the corresponding



Figure 4. Possible forms of pseudooxynicotine 11 present in aqueous solution

R-enantiomer occurred over the same time period. Access to analogues of amine 4 is likely to be of interest to those groups studying the action of drugs at nicotinic acetylcholine receptors.

To demonstrate the application of our method toward preparative scale reactions, the deracemization of 3 was carried out at 25 mM substrate concentration. The reaction was complete after 24 h and yielded (R)-N-methyl-2-phenylpyrrolidine in 75% isolated yield (ee = 99%). A simple workup procedure was developed to ensure yields were as high as possible. This procedure involved initial acidification to denature the whole cells, which were then removed by centrifugation. The supernatant was then basified and continuously extracted overnight with tert-butylmethyl ether (TBME) to afford the optically pure tertiary amine after concentration in vacuo.

We next sought to probe the identity of the achiral intermediate generated by the amine oxidase catalyzed oxidation, using substrate 4 as an example. It has previously been shown that in aqueous solution at neutral pH the nicotine metabolite, pseudooxynicotine 11, exists predominantly as the iminium ion 13 rather than the enamine 14 or aminal 12 (Figure 4).<sup>14</sup>

We therefore were interested to see if our chemo-enzymatic procedure could be used to convert 11 to (R)-nicotine 4 since any intermediate iminium ion 13 formed would be expected to be reduced much faster than 11 (or 14). Pseudooxynicotine 11 was synthesized according to a published procedure<sup>15</sup> and reacted under the conditions used for the stereoinversion of 4 as described above. Satisfyingly, (*R*)-nicotine (ee = 99%) was formed after 24 h with no other species detectable by chiral HPLC. Not only does this finding support the presence of an iminium species that is amenable to reduction, but it also raised the possibility of using our approach to carry out enantioselective intramolecular reductive amination reactions.

Wills and co-workers recently reported a one-pot procedure for the enantioselective synthesis of cyclic amines via intramolecular reductive amination under transfer hydrogenation conditions.<sup>16</sup> Although their procedure worked well with certain substrates, in a number of cases (e.g., 2-phenylpyrrolidine 2), the enantioselectivities obtained were low. We employed their method for the synthesis of N-Boc-protected ketone 16 (Scheme 1). Deprotection yielded the imine 17 which was reduced in situ with ammonia borane to the corresponding amine, which then underwent deracemization, furnishing (R)-2 (ee = 99%) after 24 h. This process offers an efficient and flexible route to a wide range of enantiomerically pure chiral secondary amines from the corresponding prochiral aminoketone precursors.

Scheme 1. Enantioselective Synthesis of (R)-2 via Intramolecular Reductive Amination<sup>a</sup>



<sup>a</sup> Reagents and conditions: (i) PhMgBr, THF, -78 °C; (ii) TFA, 1.5 h; (iii) 10% w/v whole cells (E. coli) expressing MAO-N-5 variant, NH<sub>3</sub>BH<sub>3</sub> (10 equiv), K<sub>i</sub> buffer (pH 7).

In summary, we have developed a practical procedure for the deracemization and stereoinversion of cyclic tertiary amines, which are difficult to obtain in enantiomerically pure form by alternative methods. In addition, we have shown that the procedure can be used for the preparation of enantiomerically pure cyclic secondary amines via intramolecular reductive amination. Ongoing work is focusing on further rounds of directed evolution to identify amine oxidase variants with activities toward other tertiary amines of interest.

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Supporting Information Available: Experimental procedures for deracemization reactions and preparation of substrates. This material is available free of charge via the Internet at http://pubs.acs.org.

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