Synthesis of protected 3-aminopiperidine and 3-aminoazepane derivatives using enzyme cascades†


Multi-enzyme cascades utilising variants of galactose oxidase and imine reductase led to the successful conversion of N-Cbz-protected L-ornithinol and L-lysinol to L-3-N-Cbz-3-aminopiperidine and L-3-N-Cbz-aminoazepane respectively, in up to 54% isolated yield. Streamlining the reactions into one-pot prevented potential racemisation of key labile intermediates and led to products with high enantiopurity.

Chiral amine moieties are present in many of the most valuable pharmaceutical compounds, with cyclic diamines in particular often used as semi-rigid bifunctional linkers in medicinal chemistry. Hence there is a demand for efficient, stereoselective synthesis strategies of diamines from easily accessible starting materials, preferentially in their semi-protected form to allow direct application in the manufacture of pharmaceutical intermediates. Recent biocatalytic methodologies have proven to be very successful in the design of alternative efficient and sustainable processes in the synthesis of chiral amines, underpinned by an increasing interest in their industrial application. In this regard, we were interested in the chiral enzymatic synthesis of semi-protected 3-aminopiperidines 7 and 3-aminoazepanes 8, being core structures in many valuable pharmaceutical drugs such as alogliptin and besifloxacin.

Current synthetic methods towards 3-aminopiperidines and 3-aminoazepanes encompass various approaches, including Curtius and Hofmann rearrangements, hydrogenation of 3-aminopyridine or the cyclisation of α-amino acids. Although these routes are well established, they often lack chiral control and the use of expensive and toxic reagents can hinder their wider industrial application. The particular challenge of chiral 3-aminoazepanes such as 8 was recently addressed by Feng et al. using a transaminase-based biocatalytic synthesis of 3-aminoazepane from ketone precursors. An attractive strategy is the combination of biocatalysts in telescoped enzymatic cascades resembling biosynthetic pathways to generate unnatural compounds.

When targeting 7 and 8, the challenge was to find enzymes that could tolerate bulky unnatural protection groups such as carbobenzyloxycarbonyl (Cbz) groups, which are needed for subsequent coupling chemistries. Based on our previous expertise in the application of galactose oxidase (GOase) and imine reductase (IRED) biocatalysts, a multi-enzymatic cascade towards amino-piperidine and amino azepanes was designed. This streamlined approach uses readily available amino alcohols such as 1a-c and 2a-c as substrates, (Fig. 1), which were directly accessible by chemical synthesis from natural amino acids L-ornithine and L-lysine (ESI†).

The enzymatic cascade would then proceed by initial oxidation of amino alcohols 1 and 2 to the respective amino aldehydes...
3 or 4 by GOase, followed by spontaneous formation of cyclic imine intermediates 5 or 6 that would finally be reduced by an IRED to generate the desired enantiopure products 7 and 8.\textsuperscript{17} Both individual enzymatic reactions have already shown promise in industrial applications.\textsuperscript{23,24}

The proposed strategy presented multiple challenges: the α-amino aldehyde intermediates from substrates 1a–2c would be predicted to be very unstable in a biotransformation. Considering the need for selective protection of the target compounds for subsequent chemical coupling reactions in API synthesis anyway, Boc- and Cbz-protected starting materials 1b–c and 2b–c were investigated as leading to reagents that are more valuable. However, the use of monoprotected substrates would require the tolerance of the chosen biocatalysts towards bulky hydrophobic protecting groups. To minimize side-reactions, purified enzymes rather than whole cells or enzyme lysates were used.

For the first step of the enzyme cascade, suitable recombinant GOase variants F2 and M3–5 were tested for the oxidation of amino alcohols 1a–c and 2a–c (ESI†).

An initial activity screen showed that the Cbz-protected amino alcohol derivatives 1c and 2c gave highest initial activity, whilst unprotected and Boc-protected derivatives 1a–b and 2a–b were not well accepted as substrates (ESI†). The poor results for the unprotected substrates 1a and 2a might be due to instability of the amino aldehyde intermediates and problems with amino alcohol functionality, which can result in potential chelation of the active copper center in GOase.\textsuperscript{25}

Given that Cbz-protected substrates 1c and 2c demonstrated good activity, a colorimetric HRP-ABTS assay was used to determine kinetic constants comparing the two GOase mutants F2 and M3–5 (Table 1).\textsuperscript{26} Both mutants showed overall comparable activity, l-lysinol derivative 2c appeared to be preferred over l-ornithinol 1c, with significant higher catalytic efficiency, particularly for the M3–5 variant.

Encouraged by these results, the GOase variants were tested in combination with an IRED panel in a one-pot reaction, thus avoiding the need to isolate potentially unstable aldehyde/imine intermediates. Five IREDS were selected from available libraries for the first screen: AdRedAm, IR-23, IR-49, IR-102 and IR-110. AdRedAm was chosen because of its broad substrate range, whereas the other four IREDS have been previously shown to accept the azepane scaffold.\textsuperscript{26,27}

Previous studies directed the reaction design for the one-pot in vitro GOase–IRED cascade, which included HRP for activation of GOase, catalase for H2O2 decomposition, while NADPH in combination with glucose dehydrogenase (GDH) and glucose was added as a cofactor recycling system to ensure electron supply for the IRED reaction.\textsuperscript{26,28}

Considering previously optimized reaction conditions for both GOase and IRED, initial analytical scale reactions were performed in phosphate buffer at pH 7.5 and 30 °C for 16 h. The resulting analytical yields were determined by GC-FID and are given as colour-coded ranges.

![Fig. 2 Result of activity screening for the synthesis of 3-N-Cbz-aminopiperidine 7 and 3-N-Cbz-aminoazepane 8 comparing 2 GOases and 5 IREDS. Reactions were performed with 3 mM substrate in NaPi buffer (pH 7.5) at 30 °C and 200 rpm for 16 h. The resulting analytical yields were determined by GC-FID and are given as colour-coded ranges.](image)

**Table 1** Kinetic constants \(K_m\) and \(k_{cat}\) for oxidations of N-α-Cbz-ornithinol 1c and N-α-Cbz-lysinol 2c using GOase variants M3–5 and F2.

<table>
<thead>
<tr>
<th>Substrate–GOase</th>
<th>(K_m) [mM]</th>
<th>(V_{max}) [U mg(^{-1})]</th>
<th>(k_{cat}) [s(^{-1})]</th>
<th>(K_{cat}/K_m) [mM s(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1c–F2</td>
<td>70.7 ± 7.2</td>
<td>0.83 ± 0.09</td>
<td>0.9 ± 0.1</td>
<td>0.013</td>
</tr>
<tr>
<td>1c–M3–5</td>
<td>47 ± 15</td>
<td>0.77 ± 0.22</td>
<td>0.87 ± 0.25</td>
<td>0.019</td>
</tr>
<tr>
<td>2c–F2</td>
<td>70.5 ± 2.2</td>
<td>1.74 ± 0.05</td>
<td>1.97 ± 0.06</td>
<td>0.028</td>
</tr>
<tr>
<td>2c–M3–5</td>
<td>40.5 ± 7.3</td>
<td>2.29 ± 0.34</td>
<td>2.57 ± 0.42</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Conditions: ABTS-HRP coupled assay in NaPi (100 mM, pH 7.4) with substrates 1c and 2c at various concentrations, \(\lambda = 420\) nm, 25 °C (ESI).
Increasing reaction temperatures above 30 °C resulted in lower conversions, suggesting deactivation of the enzyme at these temperatures. Increasing IRED concentration showed minimal variation in the yield, but increasing the GOase concentration improved product yield, confirming that the IRED does not appear to be rate limiting. It was also noted that conversions for the transformation did not increase further when more than 0.5 mg ml⁻¹ GOase was used for the transformation of 2c to product 8, but did increase up to 1 mg ml⁻¹ for the transformation of 1c to product 7. Combined with the initial kinetic screening results, this further confirms the higher affinity GOase M3–5 has for substrate 2c over 1c.

Given that the GOase is the gatekeeper for the cascade, molecular docking studies were performed to better understand substrate specificity. F2 and M3–5 models were created based on the reported E1 variant structure (PDB 2WQ8). 1c and 2c were used as ligands for the docking simulation into the two GOase mutants. The displayed binding modes in Fig. 3 represent the catalytic binding modes with the highest calculated binding affinity. For catalytic binding, the free copper binding site needs to be occupied by the hydroxy group that is to be transformed to product 8, but did increase up to 1 mg ml⁻¹ for the transformation of 1c to product 7. Combined with the initial kinetic screening results, this further confirms the higher affinity GOase M3–5 has for substrate 2c over 1c.

While the phenyl group binds atop P463 in M3–5, it is bound far away from other copper binding sites. In M3–5, it is bound far away from other copper binding sites. In the E1 crystal structure, a co-crystallized acetate binds the copper in 2.3 Å distance, which is closely mimicked by the calculated binding of 1c and 2c to M3–5. In contrast, more than 1 Å greater distances are calculated for binding to F2, supporting the kinetic data. Interestingly, the ligands adopt two opposing binding modes with respect to the Cbz phenyl group.

![Fig. 3 Molecular docking (AutoDock VINA as implemented in YASARA) of N-α-Cbz-ornithinol 1c and N-α-Cbz-lysinol 2c in GOase variants M3–5 and F2, modelled based on the E1 variant crystal structure (PDB 2WQ8). Substrate (cyan) conformations positioning the target hydroxyl group towards the copper centre (dark orange) are depicted with the respective O–Cu distance in red. Substrate-interacting active site amino acids are highlighted (purple) including residues altered in between the GOase variants (green). Selected substrate–receptor interactions are highlighted (yellow).](image)

Conflicts of interest

There are no conflicts to declare.
Notes and references