Enantioselective, Ketoreductase-Based Entry into Pharmaceutical Building Blocks: Ethanol as Tunable Nicotinamide Reductant

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Abstract

The use of NADH- and NADPH-dependent ketoreductases to access enantiomerically enriched pharmaceutical building blocks is reported. Seven structurally diverse synthons are obtained, including those for atomoxetine (KRED 132), talampanel (RS1-ADH and CPADH), Dolastatin (KRED 132), and fluoxetine (KRED 108/132). Ethanol may be used as stoichiometric reductant, regenerating both nicotinamide cofactors, particularly under four-electron redox conditions. Its favorable thermodynamic and economic profile, coupled with its advantageous dual cosolvent role, suggests a new application for biomass-derived ethanol.

As has been pointed out in a recent overview from the Merck Process Group,(1) advances in ketoreductase (KRED or alcohol dehydrogenase = ADH) technology have increased their potential for process chemistry. Asymmetric enzymatic reductions, ex vivo, are now more easily investigated in the research laboratory and may be optimized there, under controlled conditions, offering a viable and complementary alternative to in vivo approaches, for example, in genetically engineered yeast(2) or E. coli.(3) The ex vivo system circumvents issues of substrate, product, and cosolvent toxicity, provided that enzyme activity and enantioselectivity are preserved.

We have a standing interest in the use of enzymes in asymmetric synthesis, for example, to access enantiomerically enriched podophyllum lignans(4) or quaternary, α-vinyl amino acids.(5) More recently, that focus has turned to ADHs, as catalytic reporting enzymes to facilitate the evaluation of organometallic catalysts via ISES (in situ enzymatic screening).(5, 6) Parallel to these studies, we have undertaken to exploit ketoreductases in target-directed asymmetric synthesis. Indeed, the repertoire of enzymes in modern asymmetric synthesis continues to expand, including lipases,(7) amidases,(8) amine oxidases,(9) alcohol(10) and amine DHs,(11) epoxide hydrolases(12) and aldolases,(13) among others.(14)

In this work, we have focused upon an array of ketones, the asymmetric reduction of which provides valuable pharmaceutical building blocks. In Table 1, each chiral secondary alcohol product is mapped (red shading) onto the pharmaceutical for which it is a synthon. The Aprepitant-leading ketone 1 served as a model for our ex vivo conditions, giving high (S)-selectivity with CPADH and HLADH, consistent with reports from Merck(15) and Rhodia.(16)
respectively. The second ketone screened serves as the substrate for a classic biocatalytic process (Zygosaccharomyces rouxii whole-cell route, Zmijewski group at Lilly(17)) for the production of Talampanel. Our screen identified two new DHs here, CPADH and RS-1 ADH, each of which also gives the correct antipode (S)-4, with high selectivity.

### Table 1. Asymmetric Ketoreductase-Mediated Access to Pharmaceutical Building Blocks

<table>
<thead>
<tr>
<th>KETONE</th>
<th>ENZYME(s)</th>
<th>CORRECTIVITY (%)</th>
<th>Ee (%)</th>
<th>Prodi (conf.)</th>
<th>Pharmaceutical Target</th>
<th>Chiral HPLC</th>
<th>Application</th>
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<td>5</td>
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<tr>
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<td>99%</td>
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<td>Emend</td>
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<tr>
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<td>99%</td>
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<td>56%</td>
<td>Talampanel</td>
<td>NMR</td>
<td>Merck</td>
</tr>
</tbody>
</table>

a Color code: NADH and NADPH enzymes in black and green, respectively.

Abbreviations: ADH = alcohol dehydrogenase from HL (horse liver), LK (Lactobacillus kefir), both from Sigma-Aldrich; CP (Candida parapsilosis), RS1 (Rhodococcus species-1), both from Jülich; KREDs = ketoreductases, all from Codexis.
b All substrate screening reactions were run with stoichiometric cofactor and conversion was judged by NMR (see Supporting Information for details).
c Percent ee established by chiral HPLC [Chiralcel OD or (S,S)-WHHELK O1].
d Absolute stereochemistry established by comparison of the sign of optical rotation or relative retention time (chiral HPLC) with literature values (see Supporting Information).
e The (S)-selectivity of HLADH with this ketone has been observed by others (ref 16).
f The (S)-selectivity of CPADH with this ketone has been observed by others (ref 15).
g While Emend itself has the (R)-stereochemistry at the secondary alcohol center in question, Merck is investigating NK-1 receptor antagonists with the (S)-stereochemistry at this center (ref 15).
h Closure of the 7-ring here is via N-attack at a secondary mesylate, inverting the stereochemistry at the key center.
i The (S)-stereocenter of MA-205665 is set via double inversion: first, conversion of the alcohol to the (R)-benzylc chloride and then backside displacement with a hydroxylamine nucleophile (ref 20b).
j Even though fluoxetine is FDA-approved as the racemate, the (R)-antipode of the major metabolite, norfluoxetine, more effectively inhibits serotonin reuptake (ref 24).
k The (R)-center in both fluoxetine and atomoxetine is set via inversion of the (S)-alcohol, via Mitsunobu conditions with the appropriate phenolate nucleophile (ref 24).

Ketones 5 and 7 are precursors to building blocks for the promising chemotherapeutic candidate Dolastatin 10 and Mitsubishi’s broad spectrum fungicide MA-20565, respectively. In the former case, Genet has reported the use of stoichiometric DIP-Cl (92% ee), whereas Masui employs...
The highly enantioselective reductions seen here (KREDs 108 and 132) open up alternative “green” processes. Similarly, while both Ru (II)-diamine-(20) and Rh-diamine-based (21) asymmetric hydrogenations of 7 have been reported, reductions with CPADH, RS-1 ADH, and KRED 132, uncovered in these studies, provide viable biocatalytic alternatives.

The final three entries (9, 11, 13) in Table 1 are precursors to either (R)-Strattera or (R)-Fluoxetine. While there are isolated reports of whole-cell procedures for the asymmetric carbonyl reduction of 11, either with *Saccharomyces* (22) or *Rhodotorula* (23) species, we find no previous literature descriptions of asymmetric biocatalytic reductions of either 9 or 13. In this regard, the success we have had with KRED 132, in both cases, is quite notable. The ee’s are certainly competitive with those seen using Itsuno–Corey oxazaborolidine reduction (Senanayake) (24) in the former case or Pd(II)-sparteine-mediated oxidative kinetic resolution (Stoltz) (25) in the latter.

With a half-dozen promising new DH-based asymmetric reductions in hand, we next set about to examine cofactor regeneration. The most commonly used nicotinamide-regenerating reagents, with favorable thermodynamics, are collected in Figure 1 and compared with EtOH. Note that van der Donk and Zhao (26) have recently opened the door to phosphite-based reductions, with the most favorable redox potential of the group. Although Wong and Whitesides (27) established the potential for using EtOH in biocatalytic reductions with water-soluble substrates, use of this reductant for chemoenzymatic synthesis has lagged behind. However, EtOH is attractive here in (a) having a favorable redox potential, (b) being economically priced and readily available from the biomass fermentation stream, and (c) potentially serving a dual role as organic cosolvent. Regarding the first point, employing EtOH as a four-electron reductant provides for more favorable thermodynamics, which result from the highly exergonic reduction of NAD(P) with acetaldehyde, provided that aldehyde DH (AIDH) activity is present.

This tunability of the EtOH reductant was examined in a model NMR experiment (Figure 2) with KRED 132 and ketone 9. KRED 132 requires NADPH. We have found that LKADH can effectively be used to oxidize EtOH with NADP. In our hands, yeast AIDH also efficiently utilizes NADP. So, this LKADH/YAIDH couple was employed to access the full four-electron reducing capacity of EtOH (panel A) and compared with the reaction under two-electron redox conditions (no YAIDH, panel B, Le Chatelier effect alone). In fact, the reduction run under four-electron reducing conditions proceeds much more rapidly. As expected, one sees the clear AcOH signature in the former case, attesting to the four electron redox cycle in play. Table 2 illustrates the use of these four electron conditions across three different substrates and four different DHs at the millimolar scale.
Table 2. Biochemical Reductions at the Millimolar Scale; Ethanol as Four-Electron Reductant

<table>
<thead>
<tr>
<th>chiral product</th>
<th>ADH system</th>
<th>regen system</th>
<th>cofactor (mol %)</th>
<th>yield</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4)</td>
<td>CP-ADH</td>
<td>YADH/ YADH</td>
<td>NAD⁺ (0.4)</td>
<td>89%</td>
<td>94%</td>
</tr>
<tr>
<td>(10)</td>
<td>KRED 132</td>
<td>LK-ADH/ YADH</td>
<td>NADP⁺ (1)</td>
<td>86%</td>
<td>96%</td>
</tr>
<tr>
<td>(8)</td>
<td>RS-1 ADH</td>
<td>YADH/ YADH</td>
<td>NAD⁺ (1)</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>(8)</td>
<td>LK-ADH</td>
<td>LK-ADH/ YADH</td>
<td>NADP⁺ (2)</td>
<td>64%</td>
<td>86%</td>
</tr>
</tbody>
</table>

All reductions were performed on a 1 mmol scale at 30 °C, 300 rpm, pH 7.5 with the cofactor regeneration systems shown. See Supporting Information for details.

In summary, the first viable ketoreductase-based entries into secondary alcohol building blocks for Dolastatin 10 (5), Prozac (9), and Strattera (13) are presented here, as are new biochemical entries into building blocks for Talaman (3) and MA-20565 (7). The viability of using biomass-derived EtOH for cofactor regeneration is examined, and the advantage of using four-electron redox cycles in such processes is demonstrated. Future studies will further probe the scope, limitations, and optimal conditions for such “green” alternatives to transition metal or boron hydride based chiral carbonyl reductants for asymmetric process chemistry.

Acknowledgment

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Supporting Information

Details of the synthetic and enzymatic chemistry, and spectroscopic and chiral HPLC characterization of products. This material is available free of charge via the Internet at http://pubs.acs.org.

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