Introduction
An alcohol dehydrogenase from Clostridium acetobutylicum (CaADH) recently reported by our lab (Chem Comm 2011, 47, 2420), and deployed in the stereoselective reduction of ketoesters to medicinally relevant chiral synths, has now been applied to the reduction of α,α-difluoro-β-ketophosphonates bearing γ,Δ-unsaturation. To our knowledge, these represent the first examples of a dehydrogenase-based entry into valuable enantio-enriched α,α-difluoromethyl phosphonate building blocks (J. Amer. Chem. Soc., 2015, 137, 3600).

The enzymatic reductions proceed in both high yield and ee, and D-glucose/glucose DH serve to regenerate the NADPH cofactor. Importantly, the resulting phosphonate-bearing, chiral aliphatic alcohols are found to undergo an exceptionally facile thia-Claisen rearrangement (RR) when derivatized with the appropriate thio-ester functionality. In fact, a remarkable SRR is observed for the dependence of rate upon thio-ester structure in this rearrangement, with more than three orders of magnitude separating the observed rate constants for the slowest and fastest thio-esters. Indeed, the most favorable rearrangement proceeds with a half-life of 3 min at rt, and proceeds with complete conservation of ee, within experimental error. This hybrid bio/chemocatalytic approach serves as entry into a new family of potent zinc-aminopeptidase A inhibitors.

CaADH Expression and Initial Reductions

Over expression of CaADH αdh gene from C. acetobutylicum (GenScript) was purchased from GenScript and ligated into vector pET28(c) at NdeI and Xhol restriction sites and cloned into E. coli host strain. E. coli cells were grown at 37°C, and then CaADH protein was induced by adding IPTG. The CaADH protein was purified with Co²⁺-NTA agarose.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Units</th>
<th>Total mg</th>
<th>Yield</th>
<th>Por Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>463.1</td>
<td>236.4</td>
<td>1.95</td>
<td>1</td>
</tr>
<tr>
<td>Final</td>
<td>321.5</td>
<td>5.6</td>
<td>58.1</td>
<td>70%</td>
</tr>
</tbody>
</table>

Notes: Units defined as units of dehydrogenase produced by 1 mg of substrate (mmol of glucose/ml).

Dynamic Reductive Kinetic Resolution Entry into the Taxane Sidechain

Taxane therapeutics belong to a class of tubulin-targeting drugs used to treat ovarian, breast, and pancreatic cancer. The 10-deacetylbaccatin core can be isolated from the needles of few trees, requiring only the sidechain to be chemically synthesized. We have demonstrated that CaADH serves as an excellent biocatalyst in the production of a precursor to the sidechain of taxane therapeutics, using cellulose-derived glucose as the chiral, biorenewable reductant.

Value-Added Products from Lignin-Derived Aromatics

While the hemicellulosic fractions can be converted to glucose and cellulosic ethanol, the oxygen-rich lignin fraction is typically burned to generate power in biofineries. This presents a unique opportunity to convert these aromatic monomers into value-added products.

Purine Nucleoside Phosphorylase Inhibitors (Gout Treatment):

Using Cellulose-Derived Fumarate for Zn-Aminopeptidase A Inhibitors

Exploring a New, Facile [3,3]-Sigmatropic Rearrangement

Conclusions

We describe three different approaches to use hybrid bio/chemo-synthesis to access value-added building blocks from lignocellulose. Each lignocellulosic component is examined in these studies:

1. The celluloflavone fraction provides D-glucose, herein utilized as the terminal reductant to deliver hydroxyl equivalents via a glucose dehydrogenase/NADPH couple to CaADH, a synthetically useful and very versatile enzyme discovered in these studies. These CaADH studies led to value-added enantiomeric α,β; and γ,δ-hydroxy esters and α,α-difluoro-(α,β)-hydroxyphosphonates, both of high optical purity.

2. The hemicellulosic fraction provides fumarate, the starting point for a bio/chemocatalytic process route into new potential Zn-APA (aminopeptidase A)-emerging hypertension target inhibitors, a route that features both the aforementioned CaADH-asymmetric reduction and a new and remarkably facile (3,3)-sigmatropic thio-Claisen rearrangement that consumes the enzyme-derived stereocentral information.

3. In ongoing work, the lignin fraction is being examined as a possible source of building blocks for the hybrid bio/chemo-synthetic and new inhibitor candidates for PNP (purine nucleoside phosphorylase), a target for gout and related metabolic diseases.

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