Hybrid Bio/Chemo-Catalytic Chemistry: Adding Value to the Hemicellulosic and Lignin Fractions of Biomass

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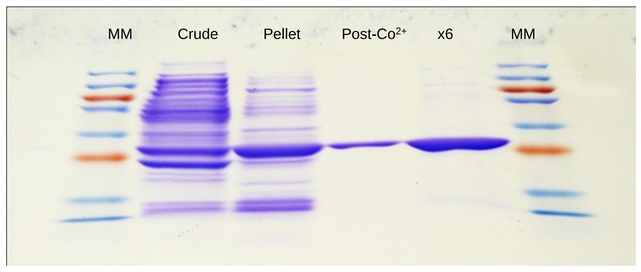
Introduction

An alcohol dehydrogenase from *Clostridium acetobutylicum (CaADH)* recently reported by our lab (*Chem Comm* ***2011*** *, 47*, 2420), and deployed in the stereoselective reduction of β -ketoesters to medicinally relevant chiral synthons, 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 -difluorinated 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 allylic alcohols are found to undergo an exceptionally facile thia-Claisen rearrangement (RR) when derivatized with the appropriate thiono-ester functionality. In fact, a remarkable SRR is observed for the dependence of rate upon thiono-ester structure in this rearrangement, with more than three orders of magnitude separating the observed rate constants for the slowest and fastest thiono-esters. Indeed, the most favorable rearrangement proceeds with a half-time of 3 min at rt, and proceeds with complete conservation of ee, within experimental error. This hybrid bio/chemo-catalytic approach serves as entry into a new family of potential zinc-aminopeptidase A inhibitors.

CaADH Expression and Initial Reductions

Over expression of *CaADH*. *adh* gene from *C. acetobutylicum* (GI: 81775727) was purchased from GenScript and ligated into vector pet28c(+) at *NdeI* and *XhoI* restriction sites and cloned into *E. coli* host strain. *E. coli* cells were grown at 37ºC, and then *CaADH* protein was induced by adding IPTG. The *CaADH* protein was purified with Co^{2+}-NTA agarose.

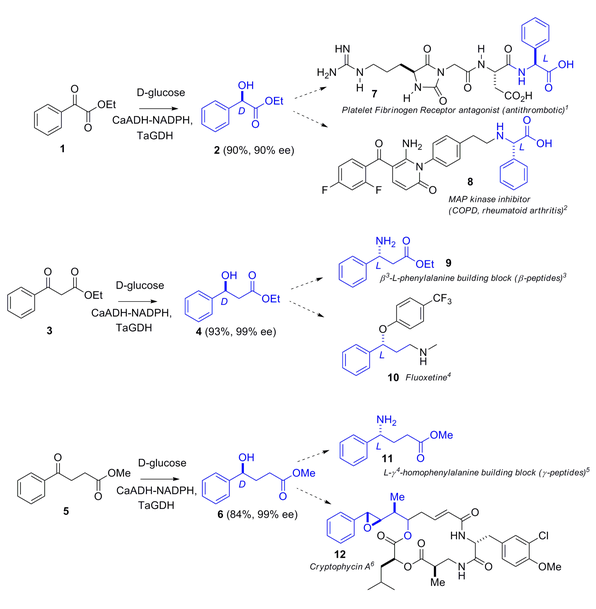


Fraction	Total Units	Total mg	Units/mg	Yield	Pur Factor
Crude	463.1	236.4	1.95		1
Final	321.5	5.6	58.1	70%	30

* - Units defined as µmoles of benzaldehyde reduced to benzyl alcohol per minute

Applegate, G.A., Cheloha, R.C., Nelson, D.L., Berkowitz, D.B. *Chem. Comm.,* **2011**, *47*, 2420-2422.

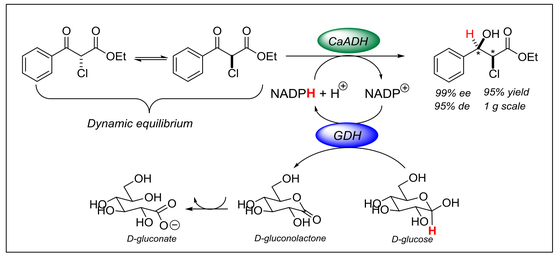
Reduction of Aromatic Ketoesters to Generate Pharmaceutical Synthons

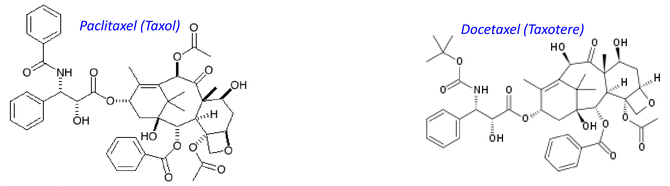


(1) H.U. Stilz; W. Guba; B. Jablonka; M. Just; O. Klingler; W. Konig; W. Wehner; G. Zoller *J. Med. Chem.* 2001, **44** 1158-1176; (2) D.F.C. Moffat; S. Pintat; S. Davies (Chroma Therapeutics Ltd) *PCT Int. Appl. O* 2009060160 (2009), 71 pp; (3a) A.V.R. Rao, M.K. Gurjar; B.R. Nallaganchu; A. Bhandari *Tetrahedron Lett.*, 1993, **34**, 7085-7088; (3b) D. Seebach; A.K. Beck; S. Capone; G. Deniau ; U. Groselj; E. Zass *Synthesis* 2009, 1-32; (4) J.W. Hilborn; Z.H. Lu; A.R. Jurgens; Q.K. Fang; P. Byers; S.A. Wald; C.H. Senanayake *Tetrahedron Lett.* 2001, **42**, 8919-8921; (5) D. Seebach; D.F. Hook, A. Glaettli *Biopolymers (Protein Science)* 2006, 84, 23-27; (6) A.K. Ghosh; L. Swanson. *J. Org. Chem.* 2003, **68**, 9823-9826.

Dynamic Reductive Kinetic Resolution Entry into the Taxane Sidechain

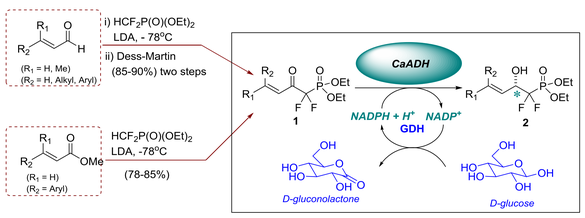
Taxane therapeutics belong to a class of tubulin-targeting drugs used to treat ovarian, breast, and pancreatic cancer. The 10-deacetylbaccatin core can be isolated from the needles of *Yew* trees, requiring only the sidechain to be chemically synthesized. We have demonstrated that *CaADH* serves as an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



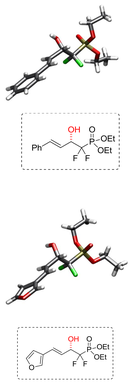


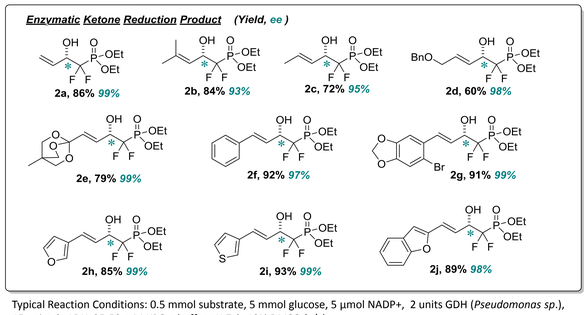
Semi-synthesis of Taxanes: Feske, BD; Stewart, JD. *J. Org. Chem.* **2005**, *70*, 9654.

Reduction of Difluoro Ketophosphonates



X-Ray Crystal Structures solved by Dr Doug Powell (University of Oklahoma)

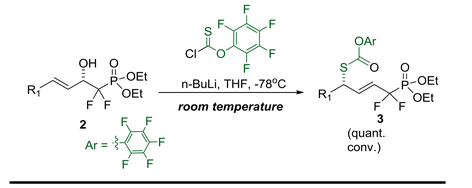


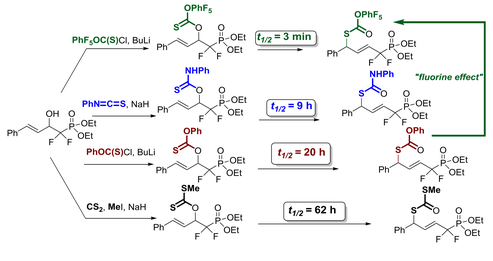


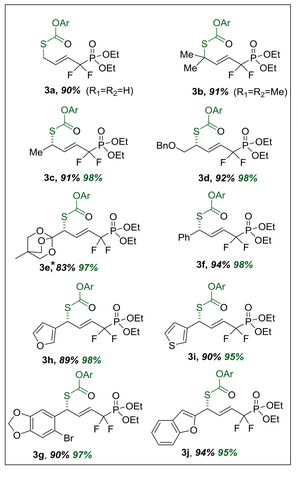
Typical Reaction Conditions: 0.5 mmol substrate, 5 mmol glucose, 5 µmol NADP+, 2 units GDH (*Pseudomonas sp.*), 15 units *CaADH*, 25-50 mM KPO_4^- buffer pH 7.0, 10% DMSO (v/v).

Panigrahi, K., Applegate, G.A., Malik, G., Berkowitz, D.B. *J Amer Chem Soc*, **2015**, *137*, 3600-3609.

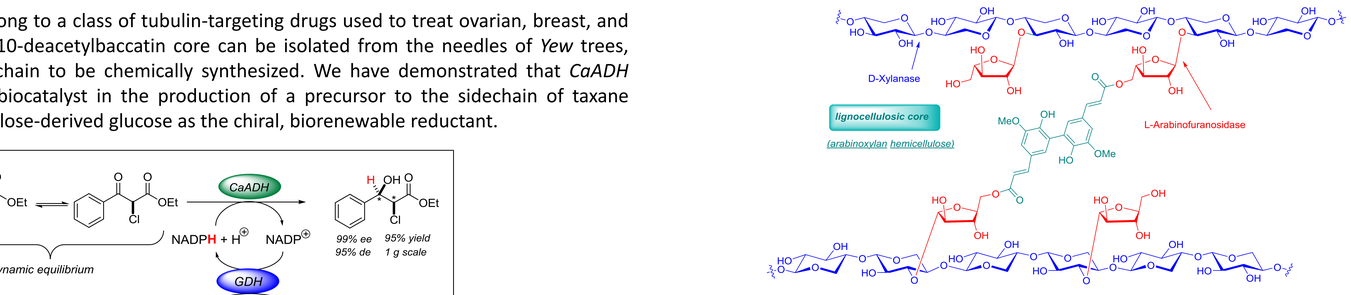
Exploring a New, Facile [3,3]-Sigmatropic Rearrangement





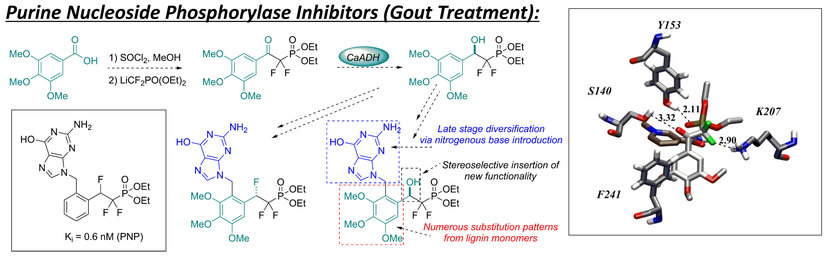


Value-Added Products from Lignin-Derived Aromatics



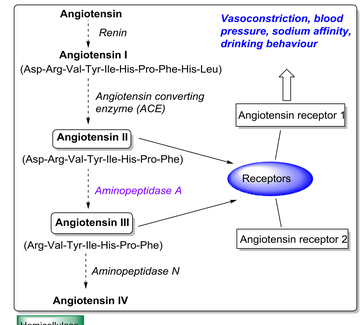
While the hemicellulosic fractions can be converted to glucose and cellulosic ethanol, the oxygen-rich lignin fraction is typically burned to generate power in biorefineries. This presents a unique opportunity to convert these aromatic monomers into value-added products.

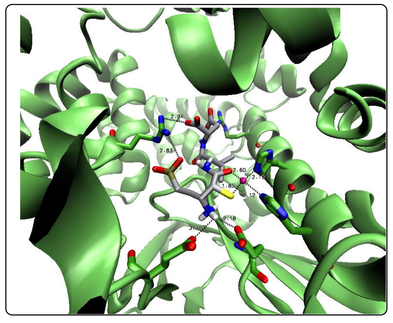
Purine Nucleoside Phosphorylase Inhibitors (Gout Treatment):

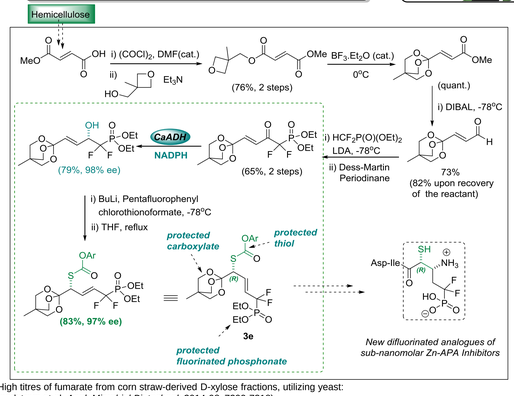


For PNP inhibitors based on benzyl guanine: Halazy, S.; Danzin, C. *Tetrahed.* **1996**, *52*, 1996.

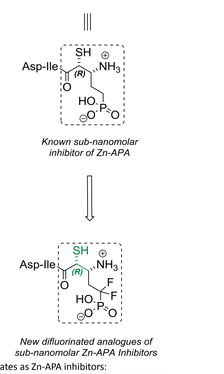
Using Cellulose-Derived Fumarate for Zn-Aminopeptidase A Inhibitors







*High titres of fumarate from corn straw-derived D-xylose fractions, utilizing yeast: Sandstrom et al. *Appl. Microbiol Biotechnol.* 2014 *98*, 7299-7318)



Phosphonates as Zn-APA inhibitors:
Roque, B.P. *et al. J Med Chem*, **1999**, *42*, 5197-5211.

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 **cellulosic fraction** provides **D-glucose**, herein utlilized as the terminal reductant to deliver hydride 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 enantioenriched α-, β- and γ-hydroxy esters and to α,α-difluoro-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 thiono-Claisen rearrangement that conserves the enzyme-derived stereochemical information.

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

Panigrahi, K., Applegate, G.A., Malik, G., Berkowitz, D.B. *J Amer Chem Soc*, **2015**, *137*, 3600-3609. Spotlighted in *JACS*: **2015**, *137*, 3717-3719.

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