Enzymatic Dynamic Reductive Kinetic Resolution (DYRKR) as a Route into Value-Added Building Blocks: D-Glucose as Biorenewable Reductant

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Introduction

My project highlights the emerging use of enzymes in organic synthesis [1]. This project also addresses the growing need for replacing industrial reagents such as H_2 gas and metaloborohydrides with preferable bio-renewable reagents such as cellulosic glucose. The synthetic aspect of my project is to synthesize new compound classes to be applied in biocatalytic reductions with a Clostridial alcohol dehydrogenase (*CaADH*) that was expressed in the Berkowitz lab. This enzyme has shown a high degree of stereoselectivity in the reduction of ketoesters, and ketophosphonates [2]. The resulting chiral alcohols serve as highly valuable synthons to a number of pharmaceutical targets. In this project, the reduced products serve as intermediates in the synthesis of baclofen,pregabalin, and tesetaxel. In the case of tesetaxel, CaADH is envisioned to set two stereocenters in a single catalytic step by dynamic reductive kinetic resolution (DYRKR) [3]. Additionally, the CaADH-mediated reduction employs D-glucose as the terminal, biorenewable reductant. Combined with glucose dehydrogenase (GDH), the costly NADPH can be recycled to produce a 'green' chemical reduction.

CaADH Expression/Purification

Overexpression of *CaADH*. The *adh* gene from *C. acetobutylicum* (GI: 81775727) was obtained from GenScript and ligated into vector pet28c(+) at *NdeI* and *XhoI* restriction sites and cloned into the *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 via Co^{2+}-NTA agarose chromatography.

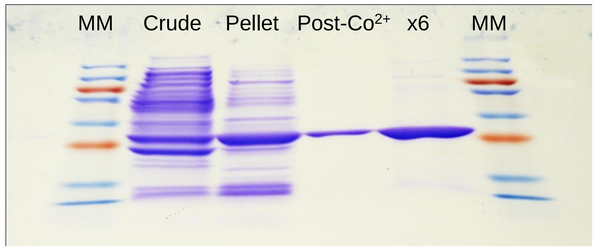
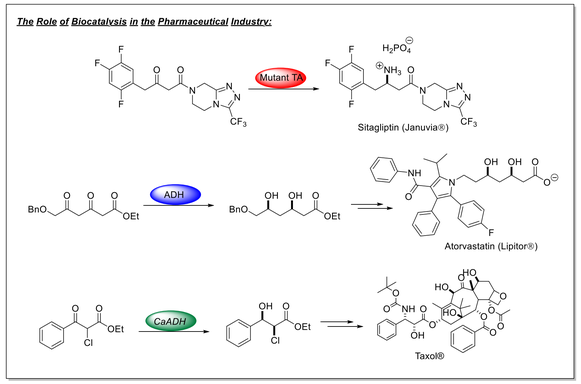


Fig 1. SDS-PAGE (12%) of *CaADH* purification

Fraction	Units*/mL	Total Units	mg/mL	Total mg	Units/mg	Yield	Pur Factor
Crude	38.5	463.1	19.7	236.4	1.95		1
Final	32.1	321.5	0.56	5.6	58.1	70%	30

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

Biocatalysts Emerging as Industrial Catalysts

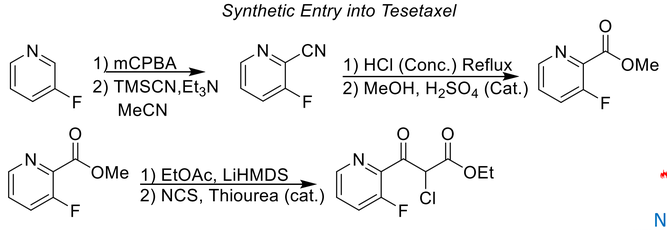


The impressive utility of enzymes in asymmetric synthesis is displayed in the figure shown to the left. While enzymes perform a spectrum of chemical functions, some of the most impressive results are generated by alcohol dehydrogenases, which instill a high degree of chiral purity required by pharmaceutical synthesis. The pharmaceuticals shown amassed $2.3 billion, $3.9 billion, and $649 million respectively in annual revenue in 2012 alone.

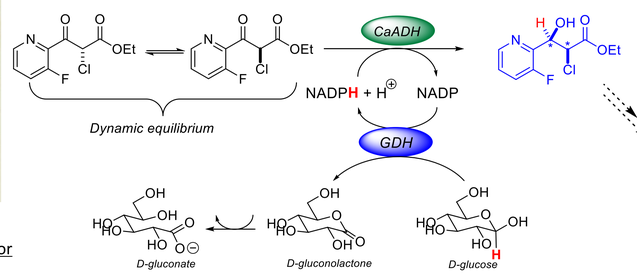
Toward Tesetaxel

My project is centered around the reduction of three pharmaceutical synthons, shown in the top right. Tesetaxel is currently in phase two trials as a novel, orally administered taxane, with high chemotherapeutic activity. The use of a GDH/ D-glucose couple, shown below, couples the oxidation of glucose with the regeneration of the expensive NADPH.

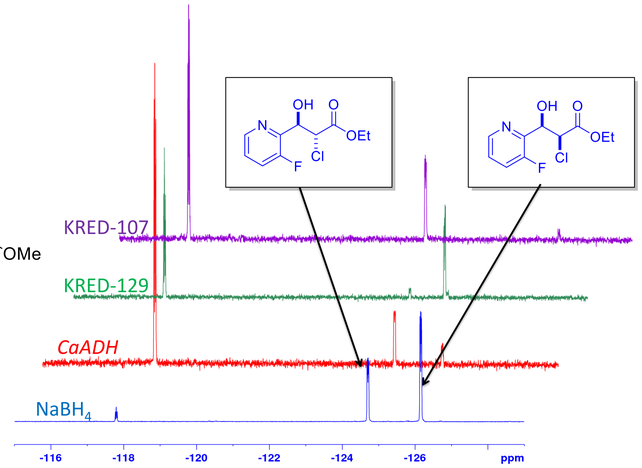
Synthetic Entry into Tesetaxel



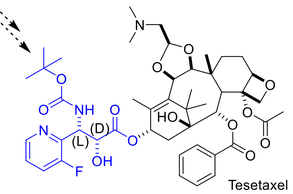
Mechanism of DYRKR and Cofactor Regeneration



Using ^{19}F NMR as a Quantitative Handle

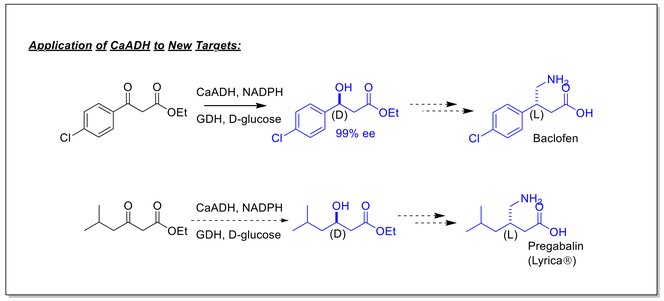


a. % conversion calculated based on crude 19F NMR, enzymatic reactions aged for 3 hours
b. d.r. assigned based on crude 19F NMR, relative stereochemistry assigned based on Taxol substrate
c. % ee calculated from purified HPLC traces (highlights % ee for major diastereomer)



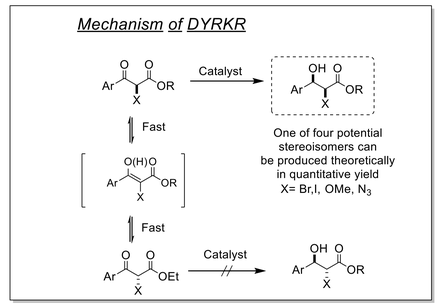
Reductant	% Conv.[a]	d.r. (*syn*:*anti*)[b]	% ee[c]
NaBH4	95	1.5:1	0
CaADH	32	1:4.5	85
KRED – 107	34	<1:20	>99
KRED - 129	52	12.5:1	98

New Synthetic Targets



Conclusions and Future Directions

•Create a focused array of unique functionalized β-keto esters by attaching various substituents at the alpha position and test for enzymatic activity with DYRKR systems.



Acknowledgments

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References

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2. Panigrahi, K., Applegate, G.A., Malik, G., and Berkowitz, D.B. *J. Am. Chem. Soc.* **2015**, *137*, 3600-3609
3. Applegate, G. A. and Berkowitz, D. B. "Exploiting Enzymatic Dynamic Reductive Kinetic Resolution (DYRKR) in Stereocontrolled Synthesis." *Adv. Synth. Catal.* **2015** *357*: 1619–1632.
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