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"IMAGING LIGNIN DEGRADATION: BIO-PROSPECTING FOR NEW ENZYMES FOR USE IN BIOFUEL PRODUCTION"

Objectives and Rationale

Plant biomass is the most abundant renewable carbon source in the biosphere and is comprised mainly of cellulose, hemicellulose and lignin. Lignin encases cellulose and hemicellulose biomass and its chemical recalcitrance is a major obstacle in the efficient utilization of lignocellulosic material [1]. Thus, lignin degradation is of central importance in biomass utilization.

The proposed research focused on improving enzymatic lignin degradation. To date, lignin depolymerization has been demonstrated by only two types of enzymes: peroxidases [2-4] and laccases[5]. The proposed research is aimed at developing methodology to discover new enzymes and organisms that are capable of degrading lignin.

As part of our long term goals of identifying previously uncharacterized enzymes involved in the degradation and further utilization of lignin, this research proposal has three objectives:

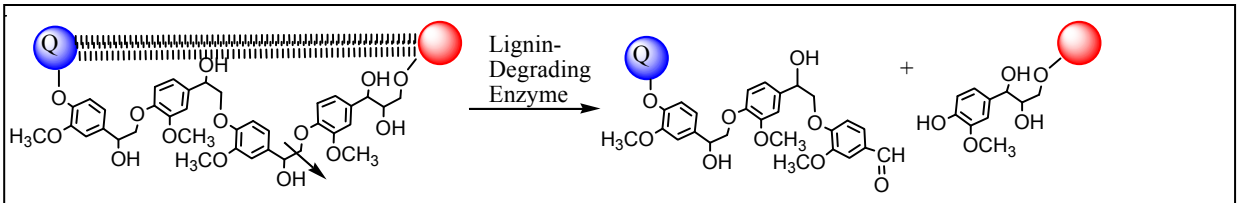
1. Development of new radiological and fluorescent probe molecules for the detection of lignin depolymerization and further degradation of lignin-derived aromatic compounds (LDACs);

2. *In vitro* validation of these probes using known enzymes, including lignin peroxidase and protocatachuate dioxygenase;

3. *In vivo* ecosystem screening for lignin depolymerization and subsequent LDAC degradation in natural environments (fungal mixed cultures and termite gut).

Progress

Fluorescent lignin model substrates.
We strategized to first make non radioactive lignin models to determine whether our approach was feasible. Near infrared fluorescence (NIRF) imaging probes designed specifically for lignin-depolymerizing enzymes Detection of lignin-degrading enzymes will be based on previous methodology described recently by Funovics et al.[6]. The method is dependent upon enzymatic activation of imaging agents. Our stategy involved synthesizing an enzyme-activatible sensor by having a lignin-like substrate with one fluorophore and a spectrally matched “quencher” molecule, which effectively absorbs the energy from the fluorophore via FRET without creation of fluorescence.



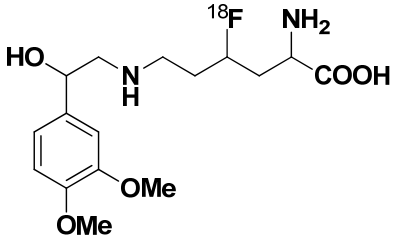
Schematic representation of the lignin depolymerization activatable probes. Arrow shows bond cleaved by enzyme.

The core region of the probe would be composed of a linear lignin substrate which Tien has already used in past studies and is a known lignin peroxidase substrate [7, 8]. Lignin peroxidase cleaves this linear model between C_{α} and C_{β} of the propyl side chain (one arrow is shown in the figure above). We used an azulene dimer (peak absorbance between 700 and 800 nm) and the fluorescein analogue QSY 21 (Invitrogen - peak absorbance between 590 and 720 nm) as quenchers, while the fluorophores will include Alexa Fluor 647 and 680; fluorophores and quenchers will be attached through ether linkages at various positions. Relevant here is the eventual use of these probes for identifying potential sites where lignin is depolymerized. These include the guts of wood-boring beetles, termites and the marine wood-degrading shipworm.

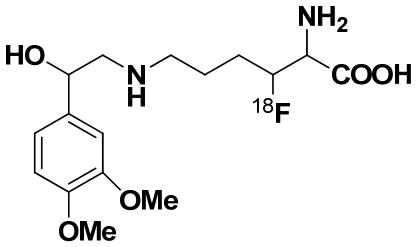
During the time period, the Taylor lab was able to synthesize the lignin substrate however, was not able to successfully synthesize the complete molecule for testing.

Radioactive substrates.

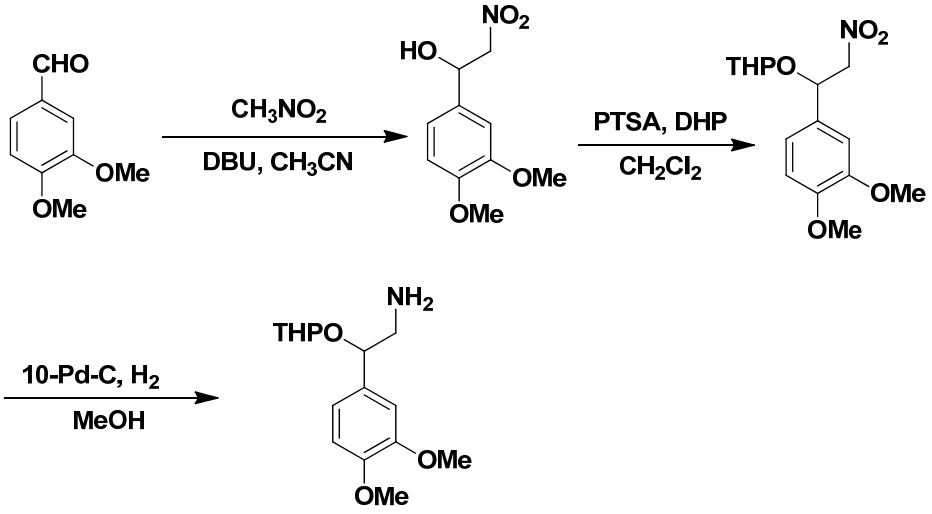
The Kabalka group at U. Tennessee was responsible for synthesis of radioactive substrates to be used for visualization. The original target compound is shown below:



They had difficulty in obtaining the appropriate precursors to assemble the amino acid fragment and thus modified their strategy where they would fluorinate to give the following compound:

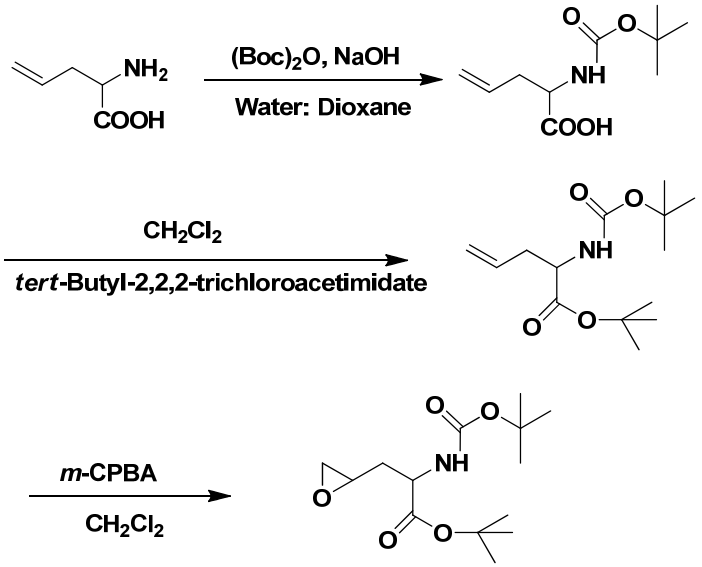


The left side was successfully prepares a number of months ago as shown in Scheme 1:



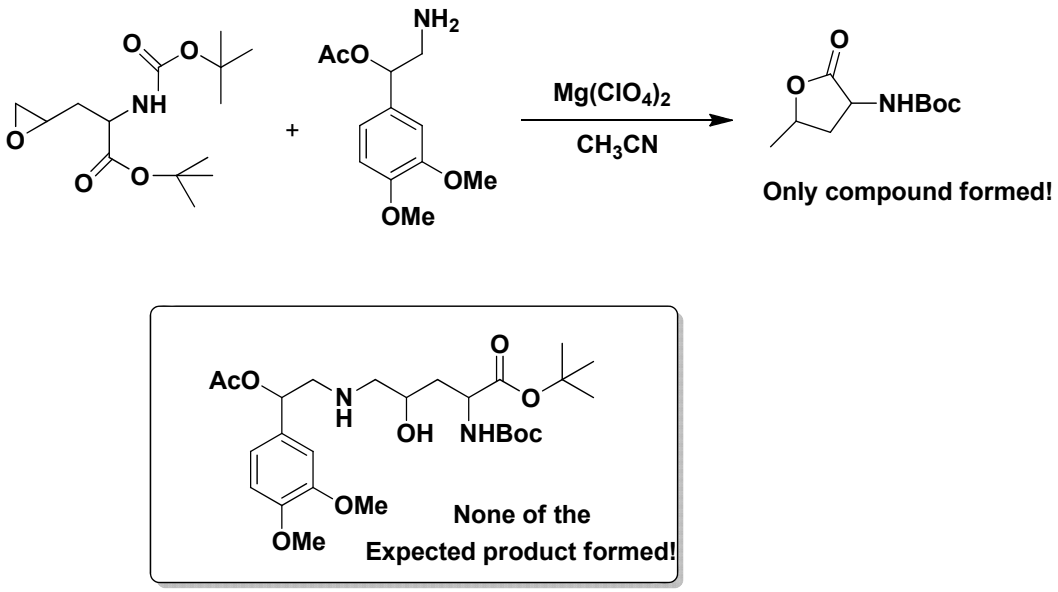
Scheme 1

The precursor for the right hand portion was synthesized via the following synthetic route (Scheme 2):



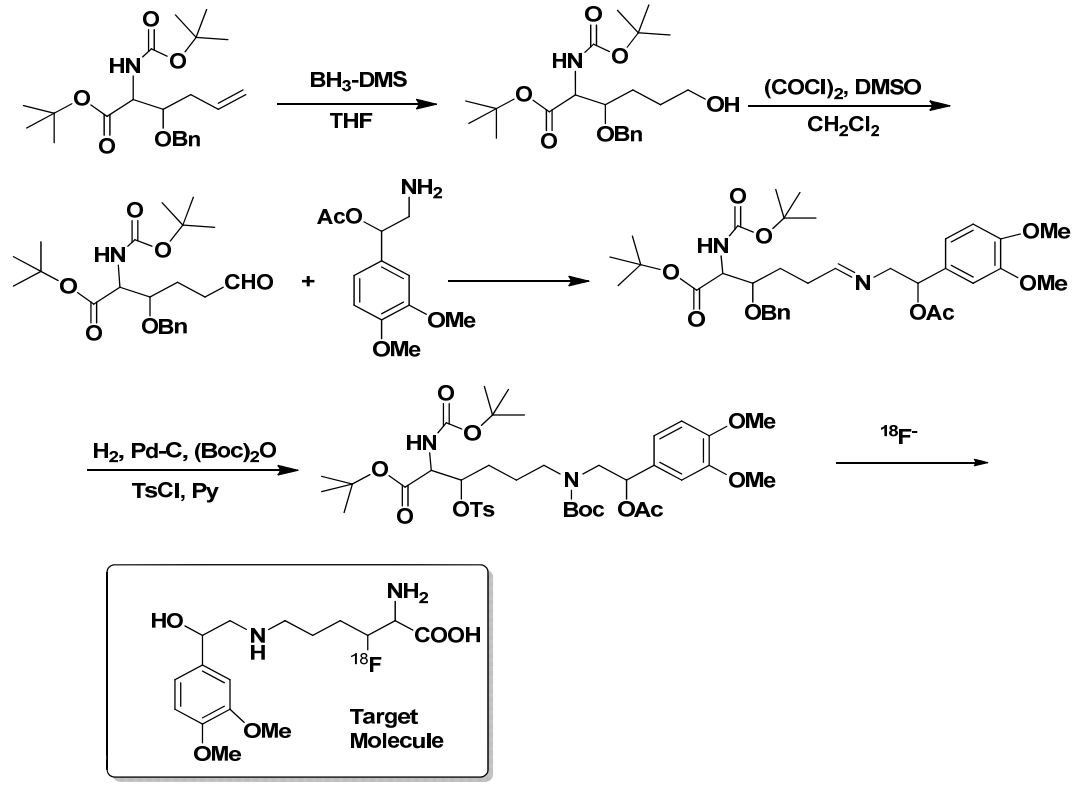
Scheme 2

The coupling reaction was attempted (numerous times under a wide variety of conditions), a stable lactone was formed as shown in Scheme 3:



Scheme 3

A new route as outlined in Scheme 4 was also attempted but unsuccessful:



Scheme 4

Conclusions

The model compounds as proposed in the original aims proved to be problematic to synthesize and thus we were not able to determine the feasibility of their use for detection of lignin degradation.

References

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