Synthesis of an ABPP probe to target PLP-dependent enzymes

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BACKGROUND

ABPP methods have emerged as versatile and effective tools in proteomic chemistry by utilizing probe molecules specifically designed based on conserved mechanistic or structural features of an enzyme class. Typical ABPP probes are comprised of three parts: a reactive group that binds covalently with the targeted enzymes, a reporter tag that visually labels targeted enzymes or allows for "click" reactions to facilitate characterization and visualization, and a carbon or poly-ethylene glycol (PEG) chain that connects the reactive and tag groups.

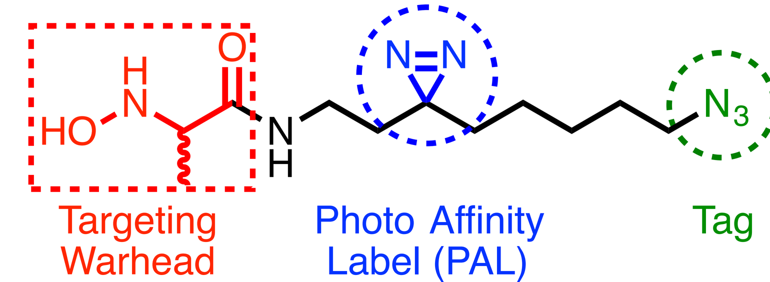

Figure 1. Chemical structure of ABPP probe designed to specifically target PLP-dependent enzymes.

OBJECTIVES

TB is the leading cause of infectious disease mortality worldwide. Existing treatment options for TB often take six to nine months of medication to eradicate the persistent bacterium *Mycobacterium tuberculosis (Mtb)*, which is responsible for TB. Furthermore, treatment of multi-drug resistant TB involves medications that have psychosis-inducing side effects and extends treatment to 18 to 24 months. There is a need for the discovery of novel drug targets for *Mtb*, a major focus of the Aldrich group at the University of Minnesota. The goal of this project was to synthesize an Activity-Based Protein Profiling (ABPP) probe to study the activity of pyridoxal phosphate (PLP)-dependent enzymes in *Mtb*, which have been implicated as potential drug targets. *Mtb* encodes for at least 40 PLP-dependent enzymes that are found in a diverse array of metabolic pathways and include dozens of essential genes, as well as several hypothetical proteins. Our probe was designed to specifically label only PLP-dependent enzymes and will allow us to functionally determine the activity of each labeled PLP-dependent enzyme, as well as globally profile the on-target and off-target activity of several drugs and experimental antibacterial agents that target specific PLP-dependent enzymes.

To target PLP-dependent enzymes in this project, a probe consisting of two reactive groups, a linking carbon chain, and a "clickable" tag was synthesized (see Figure 1). The probe will interact only with the targeted class of proteins, because the targeting warhead reactive group is based on an *N*-hydroxyalanine scaffold, which can mechanistically inhibit PLP by binding irreversibly with the cofactor. Unpublished data from previous work within the Aldrich group shows that the targeting warhead used in this project exhibits non-specific binding activity within this class of enzymes, setting a precedent for the design of this probe. After the warhead binds with PLP, the probe's diazirine PAL, which is activated at 355 nm, reveals an electrophilic carbene group that a nucleophile on targeted enzymes can covalently bond with. Finally, the "clickable" azide handle can be utilized to attach a biotin or fluorescent label to the probe. In *Mtb,* the function of PLP-dependent enzymes includes biosynthesis of biotin, and inhibition of this process has been found to result in cell death. Using this ABPP probe, discovery of target specificity of PLP-dependent enzymes could result in the identification of new drug targets for *Mtb*, and lead to the development of alternative treatment options that are effective, fast-acting, and less dangerous to patients.

METHODS

The initial synthetic route for this project's ABPP probe can be seen in the scheme depicted in Figure 2. The synthesis route includes eleven steps, and starting materials A, B, and C can be synthesized from commercially available compounds. Following each synthetic step, techniques including column chromatography, NMR, and mass spectrometry analyses will be used to confirm the identity of the synthesized molecule.

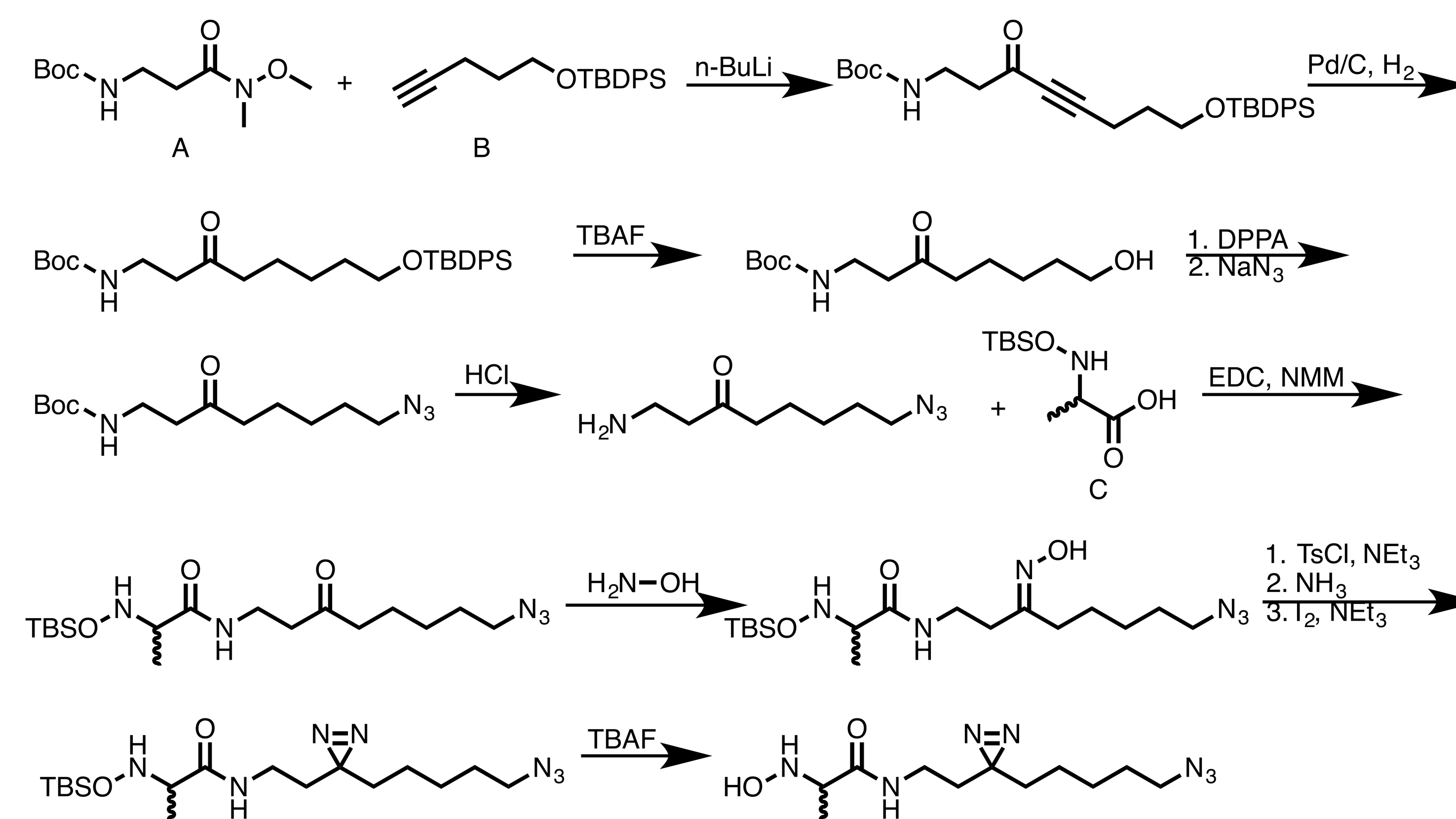

Figure 2. Synthesis of ABPP probe.

During this project, the initial synthesis route was modified after spectroscopic methods revealed low yields or low success of synthetic steps. For example, failure to successfully deprotect the TBDPS group using TBAF prohibited further synthetic progress. Additionally, the purification of compound C proved more difficult than anticipated; although the reductive amination reaction was successful, purification of the extremely polar *N*-hydroxyalanine product using ion-exchange resin chromatography did not provide a high enough yield of the substance to move forward with the synthesis either. To account for these unexpected obstacles, the initially proposed synthesis route was modified using the scheme shown in Figure 3.

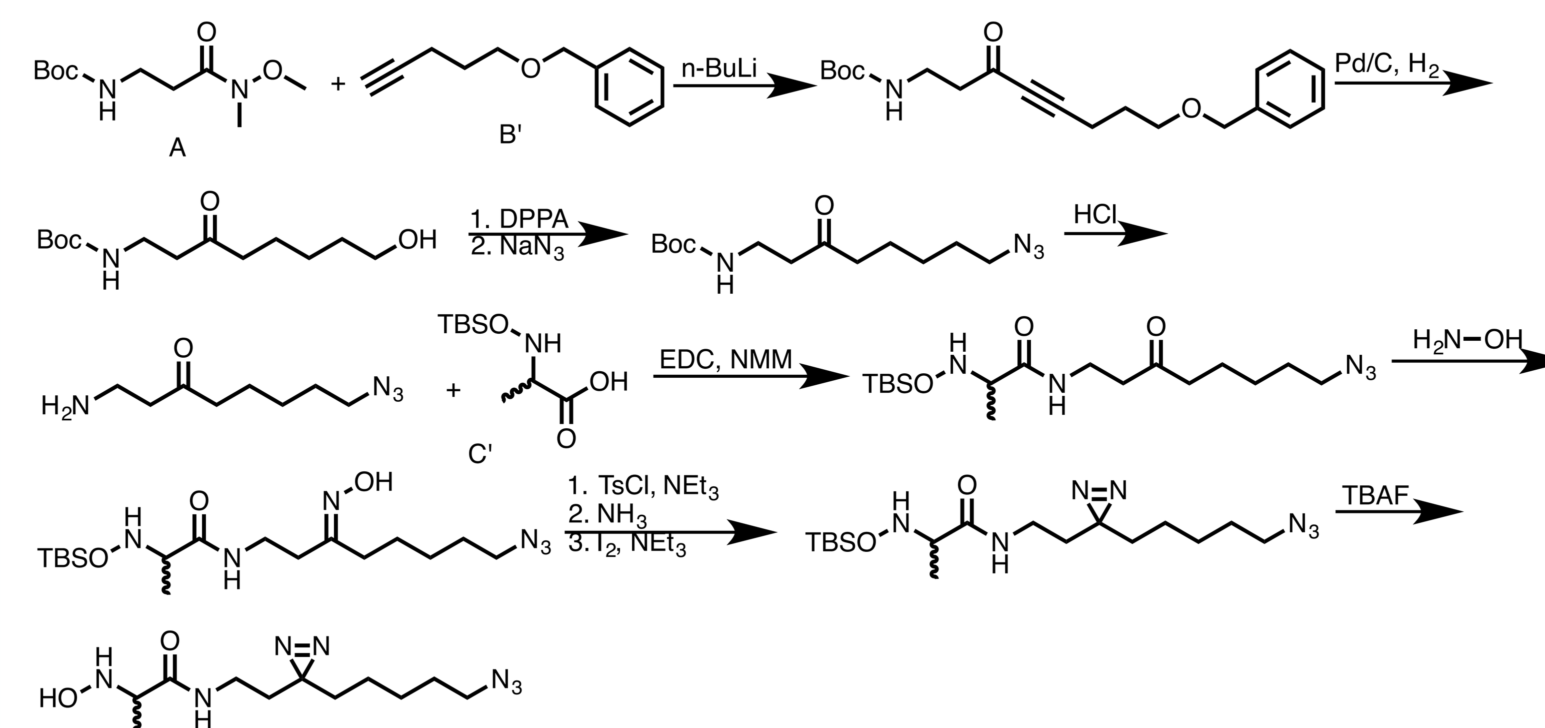

Figure 3. Modified synthesis of ABPP probe.

FUTURE DIRECTIONS

After completing the synthesis of this molecule, biological assays will be performed to determine the selectivity of the *N*-hydroxyalanine potential drug scaffold and targeting warhead of this molecule. The ABPP molecule will be administered to *Mtb* cells, and after interacting with PLP-dependent enzymes in the native *Mtb* proteomes, the cells will be lysed or homogenized in order to utilize the "clickable" handle. A fluorophore will be "clicked" allowing for visualization of labeled enzymes in 1D- and 2D-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) assays.

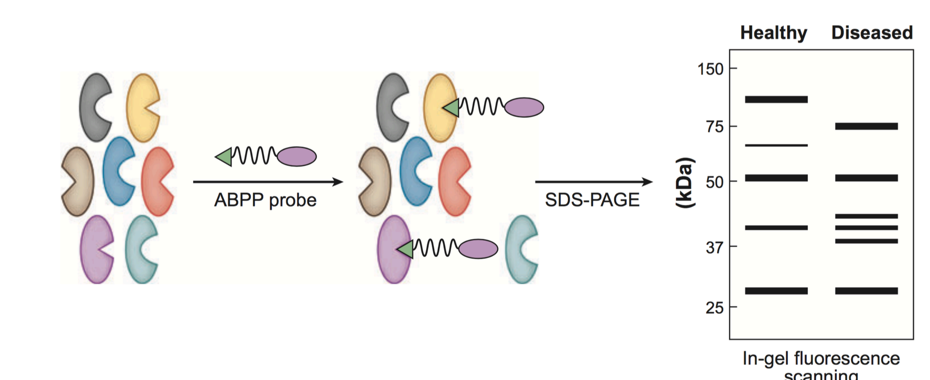

Figure 4. SDS-PAGE assay methodology.

In tangent, biotinylated probes labeling enzymes will be digested with trypsin and enriched using (strept)avidin beads. These peptides will be eluted with organic solvents and analyzed with liquid-chromatography mass spectrometry methods. A protein search algorithm will be used to identify enzyme targets and allow for peptide sequencing to determine the specificity of labeled proteins.

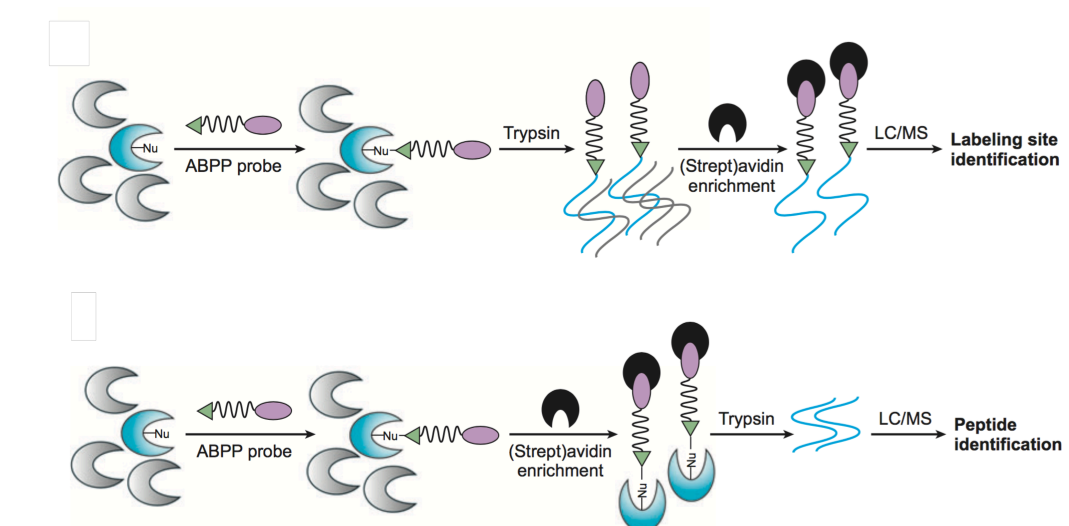

Figure 5. LC-MS/MS assay methodology.

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