Synthesis of Terpenoid Estrogen Precursors

Thesis, Purpose, Objective and Approach

Tamoxifen (common name is Nolvadex®) is one of the most popular preventative treatments for breast cancer. The drug stops or slows the growth of breast cancer cells present in the body by binding to the estrogen receptors. Tamoxifen is, therefore, considered an anti-estrogen in breast tissue. The effects of estrogen are mediated by the estrogen receptor functioning as a transcription factor. Estrogen receptors are over expressed in malignant breast lesions as opposed to normal tissue[1].

Breast cancer is both the most common malignancy in women worldwide as well as the leading cause of cancer-related deaths in non-smoking women in the United States. Breast cancer can occur as a result of high estrogen levels, which can induce the proliferation of breast cancer cells. An excess amount of estrogen can also enhance the metastatic capability of breast cancer cells; the resultant disease spreads rapidly to other areas in the body. Tamoxifen is used to inhibit interactions between estrogen receptors and estrogen. However, tamoxifen is only partly selective in binding to estrogen receptors and, therefore, it also binds estrogen receptors associated with negative side effects. For example, tamoxifen is known to cause two different types of cancer that can develop in the uterus. The contradicting actions of Tamoxifen were reproduced in cell culture models[2].

Tamoxifen exemplifies the difficulty in developing an efficient preventative treatment for breast cancer. Our goal is to identify a molecule with binding activity to specific estrogen receptors associated with cancer prevention. We would like to preserve the positive effects estrogen has on the body. In the brain, for example, estrogen improves cognitive function. Estrogen also prevents bone loss, and lowers cholesterol in the liver. Also, this hormone helps keep the pelvic floor organs in place, and plays a role in retaining voluntary control of urinary excretions. Therefore, a treatment must be found that will maintain the positive functions of estrogen, while eliminating those effects that can lead to cancer[3].

Through the use of enzymatic engineering and synthetic chemistry new estrogen analogs that are expected to show novel activity towards estrogen receptors will be produced. For this approach, a linear terpenoid substrate will be synthesized that will then be cyclized to estrogen analogs by terpene cyclase enzymes. In order to find a suitable enzyme mutant that is capable of accepting the unnatural substrate, the substrate will be fed to a library of ~10^9 enzyme mutants. Once an enzyme is isolated that shows the desired activity, the unnatural estrogen analogs can be extracted and tested for efficiency. This class of enzymes has been studied extensively, and our laboratory focuses on tobacco epi-aristolochene synthase (TEAS), whose natural substrate is farnesyl-pyrophosphate[4].

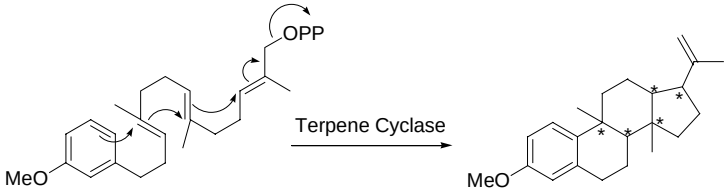

Figure 1. Potential enzymatic cyclization of an estrogen precursor

The cyclization reaction, as shown in figure 1, would most likely yield several different products, depending on how specific the terpene cyclase works and the type of residues that are attached to the substrate. Also, due to the many stereocenters that are observed in the final product, there would also be diversity in the stereochemistry of the molecule. The specific goal of my project is to synthesize the uncyclized terpenoid substrate that will be fed to the enzymes. The following will illustrate my responsibilities by describing methods I have used thus far.

Responsibility

A post-doctoral fellow in the Weiss laboratory planned the basic experimental designs presented in this proposal. For every experiment, first I review procedures found in publications. I then scale the reaction to the necessary amount, and run the experiment based on the different procedures outlined in the literature. Once the reaction is complete and, depending on whether a work-up procedure is required, I isolate the desired product and purify it through column chromatography. An example of my responsibilities is demonstrated through the synthesis of a terpenoid estrogen precursor I worked on earlier this year.

The synthesis of *1-methoxy-3-(4,8,12-trimethyl-trideca-3,7,11-trienyl)-benzene* was attempted through the use of a sulfone group which would direct the addition of a benzylic group to farnesol. The final product was obtained after a series of four reactions, ending with the removal of the sulfone group. The first reaction involved a simple bromination of the hydroxyl group of the farnesol.

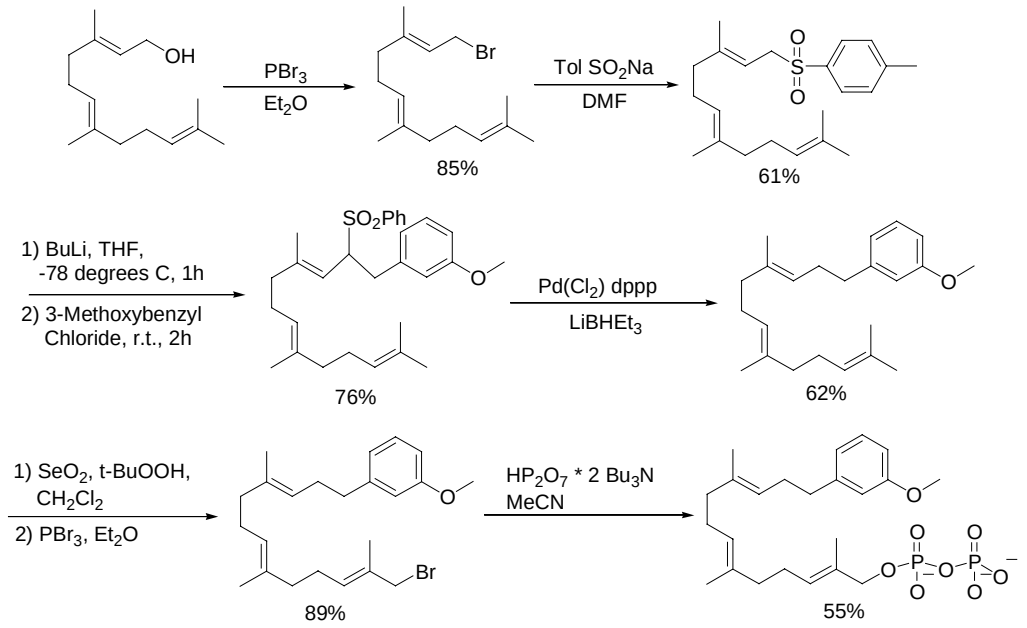

Figure 2. The figure above shows the complete procedural outline of the method attempted

The bromine is installed, because it is needed as a leaving group. Attaching the sulfone group involved a nucleophilic substitution of the bromine with *p*-toluene sulfinic acid. The farnesylsulfone was deprotonated with n-butyl lithium α to the sulfone group. The carbanion was stabilized through the sulfur. Then 3-methoxybenzyl chloride was added and the farnesyl-benzyl-sulfone was formed through a nucleophilic attack (Figure 2). The removal of the sulfone proved to be a lot more challenging than expected. My attempts at removing the sulfone were unsatisfactory and were finished by Dr. Feld who also carried out the last steps to introduce the pyrophosphate leaving group.

During my summer research I will work on the synthesis of a library of unnatural estrogen precursors as shown in figure 3. This design will allow me to use simple propargyl precursors to form a library of various estrogen precursors with different types of substituents on the double bonds.

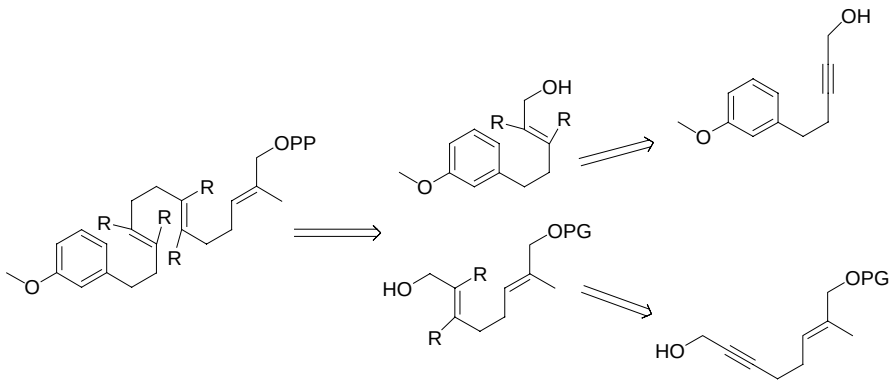

Figure 3. Retrosynthetic analysis of the estrogen precursor library.

The syntheses of the propargyl alcohols **A** and **B**[5], as shown in figure 4, have been reported in the literature and have been worked on before by Dr. Feld and myself. Therefore, synthesis of the starting material is expected to proceed quickly. (Figure 4).

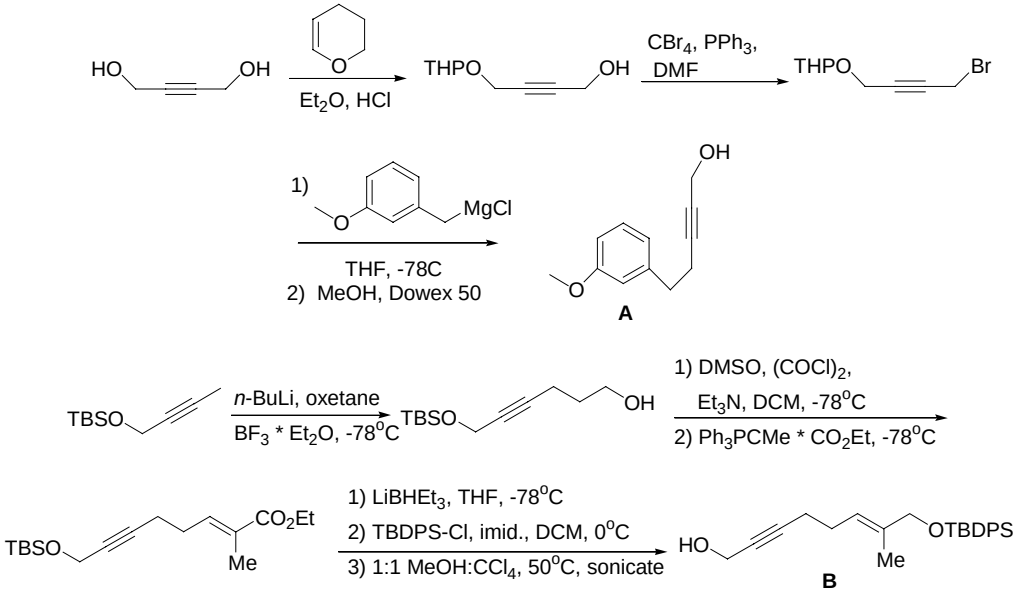

Figure 4. Synthesis of aromatic propargyl alcohol (A) and second propargyl alcohol (B)

The triple bonds can then be reduced selectively to form either a vinyllic iodide[6] or stannane[7] (Figure 5). After this step, the two compounds will be coupled together by the same sulfone chemistry used above to create the desired analog precursors.

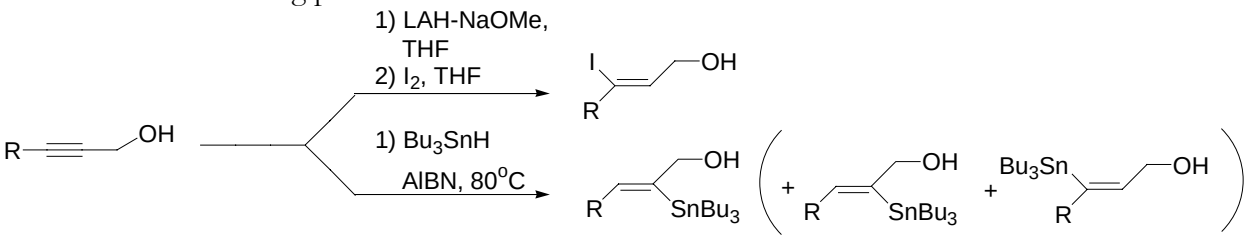

Figure 5. Selective reduction of propargyl alcohols

With the knowledge and experience that I have acquired in the past year, this synthesis is very manageable.

Timeline

The following table outlines the proposed schedule of experiments that will take place during the intensive ten-week period during the summer.

Week 1 – 3	Bring up the starting material as depicted in Figure 4.
Week 4	Selective reduction of triple bonds to form vinyl iodides and vinyl stannanes, shown in Figure 5.
Week 5 – 7	Introduction of different residues in the vinyllic position.
Week 8 – 10	Coupling of fragments to form a library of terpenoid estrogen precursors (Figure 3)

References

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