The Stereoselective Reduction of Ethyl Acetoacetate using Enzymes found in Bakers Yeast.

In preparation for this lab you should read up on how the enzymes in yeast reduce ketones and aldehydes to alcohols using alcohol dehydrogenase and NAD reductase. It is acceptable to use Wikipedia on the subject of alcohol dehydrogenase and NAD reductase. You should also read up on oxidation and reduction in your textbook.

By this time you should have found the article on the web concerning the reduction of ethyl acetoacetate with the enzymes found in yeast. You should search using google for an article in Organic Synthesis by Dieter Seebach and coworkers on the Yeast Reduction of ethyl acetoacetate. You must have this with you and scaled for the first week of lab.

Note this lab has been tested and adapted by M. Nerz-Stormes. Before writing this, Dr. Nerz had three successful reactions and isolations. It is possible, however, that things will not go smoothly and you should be prepared for that psychologically.

It is very important before coming to lab, that you write an introduction, a main reaction, that you calculate the moles of ethyl acetoacetate being used and the moles expected (ethyl acetoacetate is the limiting reagent). It is important that you look up the boiling point of the ethyl acetoacetate and the boiling point of the product since, you will be isolating it by distillation. The parts of the lab report will be summarized in more detail below.

Procedure:

You will be carrying out this reaction as written, but with the following variations.

1. The reaction will be done at 1/20th the scale using a 500 mL Erlenmeyer. The one hour incubation period at thirty degrees will be accomplished using an incubator. You are responsible for scaling the reaction.
2. The reaction will be started one week before the actual day we do the workup and isolation.
3. Most of the reaction is carried out on a stir plate at room temperature. You will work in groups of three and one person from the group needs to come in 24 hours after starting the reaction and add the next portion of sugar and warm water to boost up the yeast. One hour after that, you need to add another sample of ethyl acetoacetate.
4. The reaction will stir through the week, so it is important that you label your flask, leave it out stirring and cover it with foil and parafilm. This means that there could be as many as thirty to forty reactions stirring in the lab over the course of the first week.
5. The work up/purification procedure is carried out as written, with the exception that you will not distill.
6. You will want to obtain a yield, percent yield, a boiling point an NMR and an IR. It is possible also that you may do some GC work.

The **write-up** will consist of the following. There will be one write-up per group.

**Introduction with references.** It should be succinct with just a few paragraphs.

Include the background on the reaction you are doing. Why is the reaction significant and what method is specifically being used? The papers read before the lab are probably adequate for this purpose. Points may be merely mentioned and referenced if the references are good.

A **net reaction** should be written

An outline of how the reduction occurs with alcohol dehydrogenase –**a rough mechanism**.

**Data tables** for all data collected in lab and processed after lab, including grams and moles of substrate and product, the percent yield calculation, the spectra and the interpretation of the spectra in tabular form. The color and physical state of the compound should be included.

**Observations** made during the reaction and purification should be recorded. Observations are up to you. You should write down what you observe as you do the reaction.

**Discussion:** In this section you will discuss – yield, purity and identity. Spectra will play greatly into the purity and identity sections and should be discussed extensively. They should be compared to referenced literature spectra. In this discussion, the spectra should be compared to the literature spectra. References to the online spectra should be included.

You should review the following you tubes:

http://www.youtube.com/watch?v=-4Yfr3Vopp0&list=UUgww6yAXD261fbCWjHXXLnw&index=124&feature=plcp

http://www.youtube.com/watch?v=Ekhe3mvF6h0&list=UUgww6yAXD261fbCWjHXXLnw&index=123&feature=plcp

http://www.youtube.com/watch?v=PEO_SIexFho&list=UUgww6yAXD261fbCWjHXLnw&index=116&feature=plcp