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 - alcohol dehydrogenase and NAD reductase - reduce ketones and aldehydes to alcohols
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

Using the internet, you should try to find a specifc journal paper that describes the reduction of ethyl acetoacetate utilizing the enzymes found in yeast. Though there are several papers in the literature that deal with this subject, try to find the paper that wa publishsed 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 to work with our equipment in the lab. Do not be surprised if there are more changes because we treat everything as research in this course. We want things to be in flux, constantly improving. There is not one way to do things. You will see when we carry out the synthetic project that we are constantly adapting and improving. We are interested in any innovations you suggest.

It is very important before coming to lab, that you prepare you laboratory notebook. This means you should write an introduction, a main reaction, the procedure in a format that anyone could follow at $1/20^{th}$ the literature scale. You should draw diagrams of any apparatus that you need and you should review techniques that were learned in the past. You also need to 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. The parts of the lab report will be summarized in more detail below.

Procedure:

Safety

1. **Ethyl acetoacetate and ether are toxic and flammable. Always wear proper lab gear and work in the hood. Any exposure to lab materials (except yeast or sucrose) requires rinsing for fifteen minutes with cold water.**
2. **Avoid ether fumes. Work in hood and take turns in your group carrying out the successive ether extractions.**
3. **Ether is very flammable. Do not put ether on hot plate, near flames or in the centrifuge.**

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 Ehrlenmeyer. 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, clamped over the stir plate 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 following possible variations:
   Centrifuging instead of or before filtering.
   Possibly omitting the celite step.
   Using components of a baby diaper to dry the ether solution.
   Skipping the distillation step.

6. You should obtain a yield, percent yield , an NMR and an IR. It is possible also that you may do some GCMS 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 paper 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 (take notes in lab lecture).**

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 should 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 on line spectra should be included.

You should review the following youtubes:

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_SJexFho&list=UUgww6yAXD261fbCWjHXXLnw&index=116&feature=plcp