

CHEM-643 Biochemistry
Mid-term Examination
8:00 – 10:00, Friday, 5 November 2010

Name _____

Dr. H. White - Instructor

There are 10 pages to this examination including this page. **Write your name on each new page.** Read every question so that you understand what is being asked. If you feel any question is unclear or ambiguous, clearly explain your answer or interpretation. Please call my attention to any errors you encounter.

This examination is closed book until 9:15AM. You may refer to your assignments and your lecture notes, but not textbooks at that time. You may also refer to the hand-drawn metabolic pathway sheets available from the course website.

This examination will assess your learning, problem-solving skills, and ability to communicate clearly. Parts are intended to be challenging even to the best students in the class. Some of the questions will deal with material you have not seen before and is not in your text; however, those questions can be answered by applying basic principles discussed in the course.

Do not expose your answers to the scrutiny of your neighbors. Please fold under each page before you go on to the next.

Breakdown of the examination by sections:

I. Basic Vocabulary	20 Points
II. Problems	88 Points
<u>III. Short Essay</u>	<u>12 Points</u>
Total	120 Points

Exam Statistics

29-106.5/120 Class Range

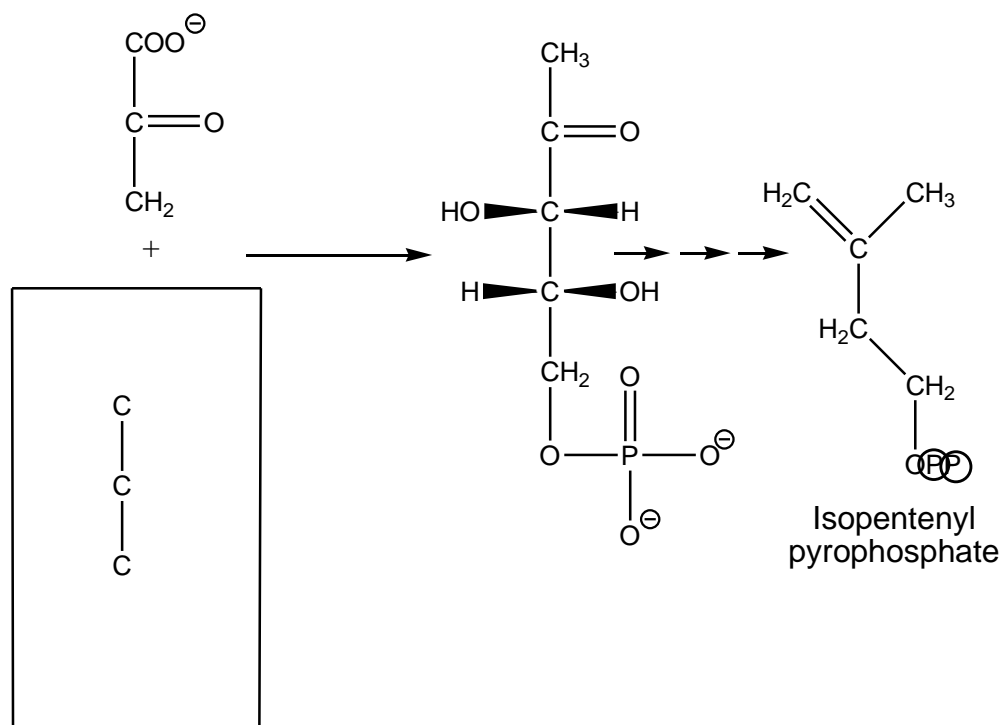
69.4/120 Class Mean

Part I - Basic Vocabulary and Working Knowledge (1 point each)

- _____ 1. Type of *metabolic* regulation characteristic of biosynthetic pathways.
- _____ 2. Type of *genetic* regulation associated with biosynthetic pathways.
- _____ 3. Name for a grouping of metabolically-related structural genes on a bacterial chromosome.
- _____ 4. General name for a mutant strain that requires a metabolite that it has lost the ability to biosynthesize.
- _____ 5. Prosthetic group/coenzyme that contains two sulfur atoms.
- _____ 6. Vitamin precursor for NAD.
- _____ 7. First compound to be labeled by $^{14}\text{CO}_2$ in a C_4 plant.
- _____ 8. First compound to be labeled by $^{14}\text{CO}_2$ in a C_3 plant.
- _____ 9. Enzyme providing the major metabolic point-of-entry of *reduced* nitrogen into living cells.
- _____ 10. Adenine contains this bicyclic ring system.
- _____ 11. Folic acid contains this bicyclic ring system.
- _____ 12. Enzyme that is responsible for generating oscillations in glycolyzing yeast in an aerobic-anaerobic transition.
- _____ 13. Substrate of an enzyme that contains Mo, Fe, and S.
- _____ 14. Last common precursor for fatty acids and steroids in us.
- _____ 15. $\text{C}_{18:0}$
- _____ 16. Retired UD faculty member who shared the 2010 Nobel Prize for Chemistry.
- _____ 17. Characteristic of enzyme reactions that produce NADPH.
- _____ 18. Compound with a quaternary nitrogen that is required to transport fatty acids into the mitochondria for oxidation.
- _____ 19. Free energy of hydrolysis of ATP is similar to that of what other common metabolite?
- _____ 20. Compound that humans use to dispose of excess nitrogen.

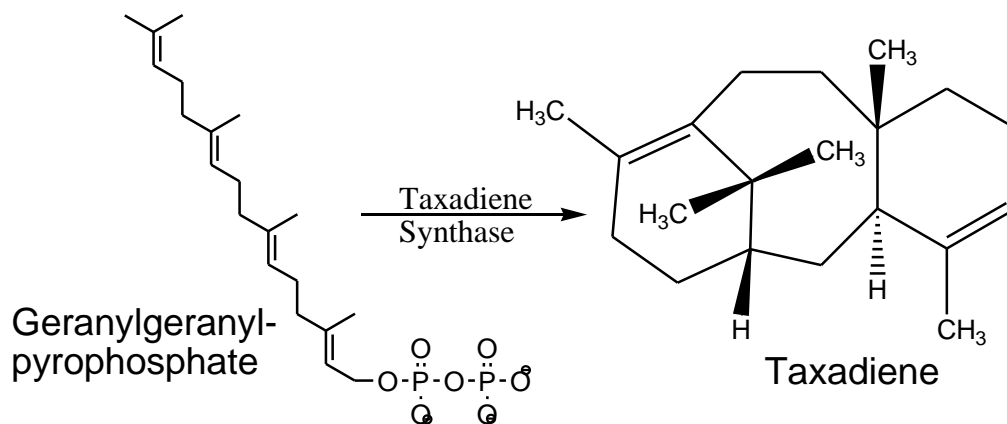
Part II: Problem Solving/ Competency

1. (16 Points total) In contrast to animals, which convert acetyl-CoA to HMG-CoA as a precursor to isopentenyl pyrophosphate (IPP), plants use a condensation between pyruvate and a three-carbon compound to produce the five-carbon precursor shown below. (This is not one of the reactions displayed in the pyruvate reaction work sheet completed early in the semester.)
- (5 points) Complete the structure of the three-carbon compound that reacts with pyruvate.
 - (2 points) Name the three carbon compound that reacts with pyruvate.
 - (5 points) Identify any other reactants, products, or cofactors for this reaction.
 - (4 points) Put circles around the carbons of IPP (Isopentenyl pyrophosphate) that are derived from pyruvate.



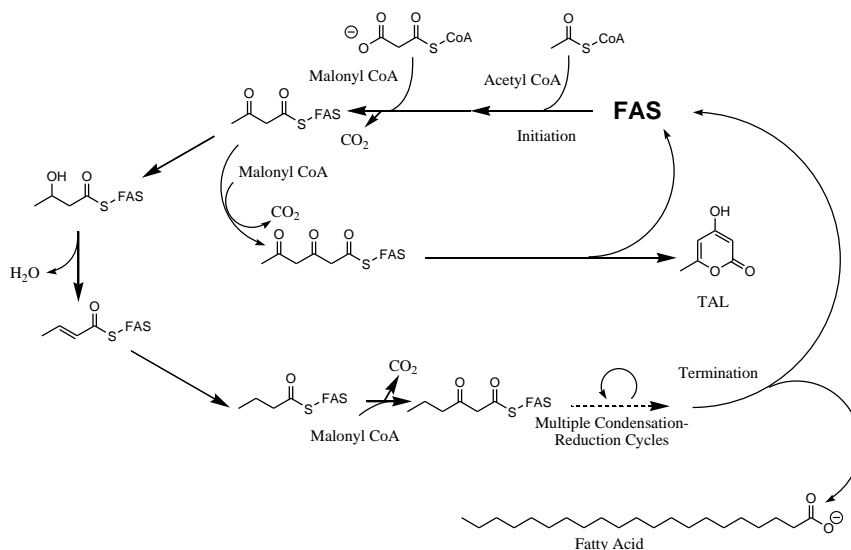
2. (11 Points Total) Taxol and related compounds derived from the bark of the Pacific yew tree are among the most potent anticancer drugs. Because there are not enough yew trees available to supply the demand, finding alternative methods to produce the drug is a major research interest. The October 1, 2010, issue of *Science* magazine [330:70-74 (2010)] reports the biosynthesis of taxadiene, a biosynthetic precursor of taxol. This was achieved by introducing genes from the yew tree into *E. coli*. The diagram below shows the enzymatic step that converts geranylgeranyl pyrophosphate into taxadiene. No carbon-carbon bonds are broken in this reaction, but several new carbon-carbon bonds are made. On the diagram:

- (6 points) Draw a line across every new carbon-carbon bond that is made in this reaction.
- (3 points) Draw a circle around the carbon atom that was attached to the pyrophosphate.
- (2 points) Metabolic precursor that would be the source of the methyl groups in taxidiene.



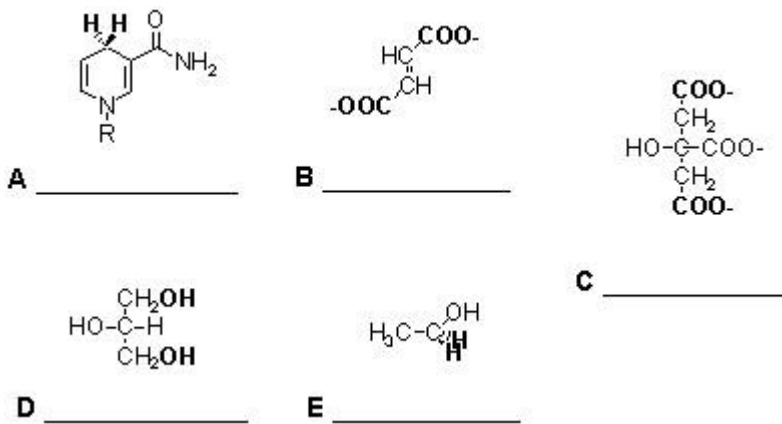
3. (10 Points) Unlike many bacteria, *Mycobacteria* have a multienzyme Fatty Acid Synthase that produces fatty acids with chain lengths of ≥ 24 carbons. It is unusual in other ways as well in that it requires FMN and NADH in addition to NADPH. Researchers suspected that the two reductive steps required different pyridine nucleotide coenzymes and wished to determine which reaction required which coenzyme. They designed an experiment in which NADH and NADPH were used together or separately in the reaction mixture and they extracted polar products (e.g. TAL) and non-polar products (fatty acids) and got the results below. Based on these data, assign NADH and NADPH to different reactions in the Mycobacterial Fatty Acid Synthase reaction shown below. [Taken from *J. Biol. Chem.* **246**, 4751-4754 (1971)]

Explain your reasoning,

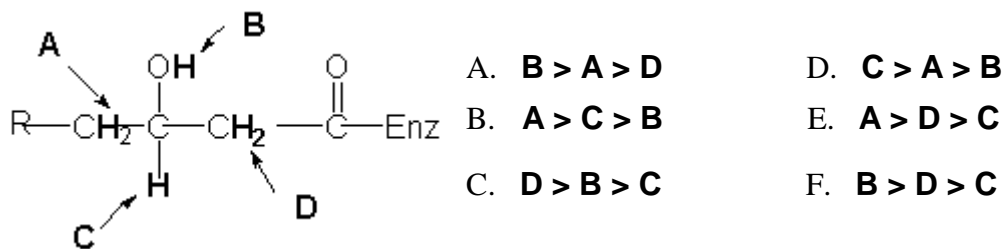


Additions		¹⁴ C from Malonyl CoA incorporated into	
[NADH] μM	[NADPH] μM	Non-polar (fatty acids) pmoles	Polar (TAL) pmoles
0	0	4.3	371
5.1	0	9.1	571
0	5.1	75.6	80.6
5.1	5.1	1976	Not measured

4. (8 points) The following metabolic intermediates have elements of symmetry in their structure that are highlighted in bold letters. Put a circle around the one or more compounds in this group for which an enzyme could not distinguish between the two highlighted functional groups. For one additional point each, name each intermediate in the space provided.



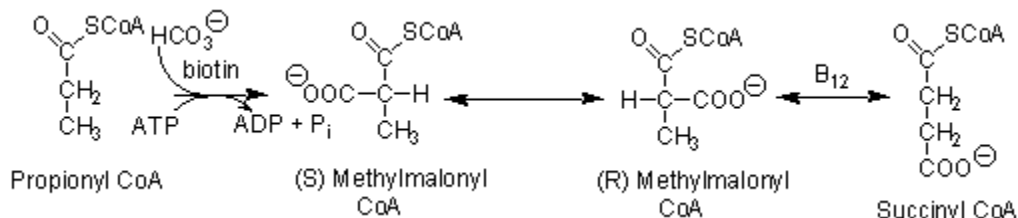
5. (7 Points) When fatty acid synthesis occurs in the presence of tritiated water, tritium is incorporated into the product. The rate at which hydrogens in a compound can exchange with hydrogen from water depends on their relative acidities. Consider the various hydrogen atoms in the β -hydroxyacyl thioester intermediate in fatty acid synthesis below and for 3 points circle the statement that most accurately describes their relative rates of exchange with solvent water protons.



(4 Points) Which of the exchangeable protons above would make the greatest contribution to the tritium label that ends up in fatty acids synthesized in tritiated water? Explain

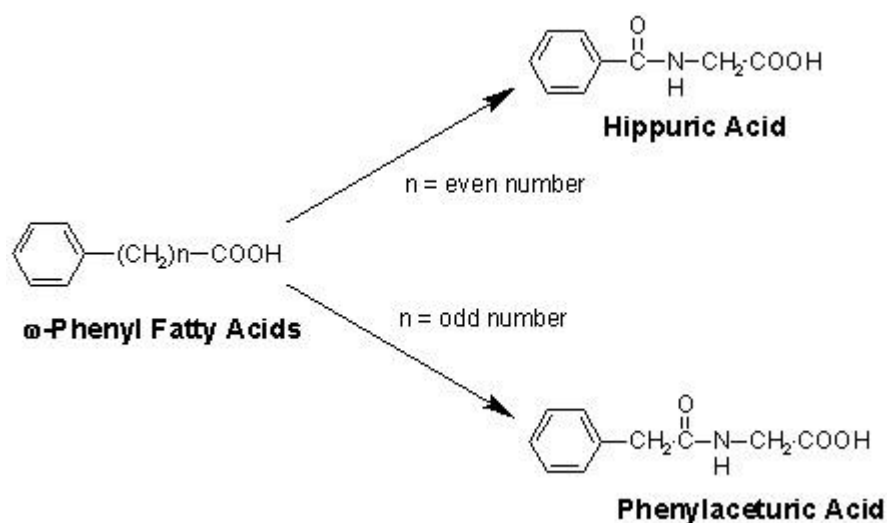
6. (15 Points) Methylmalonyl CoA racemase interconverts S- and R-methylmalonyl CoA on the pathway from propionyl CoA to succinyl CoA. Consider a mutant organism that has a ten-fold overproduction of the normal “wildtype” enzyme without concomitant changes in the other enzymes of the pathway. Consider the five statements that suggest changes that might occur as a result and respond to each with:

- a statement of whether the statement is correct or not, and
- The basis for your answer.



- The intracellular concentration ratio of S to R methylmalonyl CoA would change on the order of 10-fold.
- The rate of succinyl CoA synthesis from propionyl CoA would be stimulated significantly (>2X).
- The concentration of propionyl CoA would be considerably lower than in a normal individual.
- The K_m and V_{max} for the racemase and the K_{eq} for reaction would remain unchanged.
- The concentrations of both S- and R-methylmalonyl CoA would increase at least several fold.

7. (9 Points) More than a century ago, Franz Knoop studied the metabolism of a series of ω -phenyl fatty acids by feeding them to dogs and isolating the products in urine. As shown below, the products were either derivatives of benzoic acid or phenyl acetic acid depending upon whether the number of methylene groups was odd or even, respectively. From these results he deduced the basic chemistry of the β -oxidation pathway. From the structures of products, hippuric or phenylacetic acid make some deductions about the enzyme reaction that generates these end products.

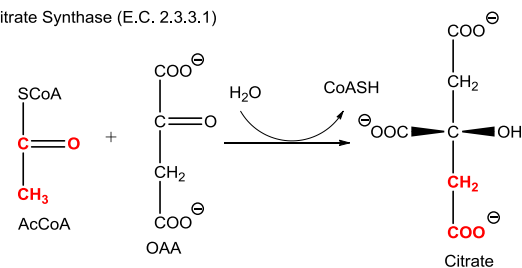


- A. (3 Points) Name and draw the structure of the common nitrogen-containing compound that has been added in the final metabolic step.

- B. (6 Points) Based on what you know about the β -oxidation pathway and the formation of certain bonds in biochemical systems, make a reasonable proposal for the enzymatic reaction (substrates and products) of the final metabolic step.

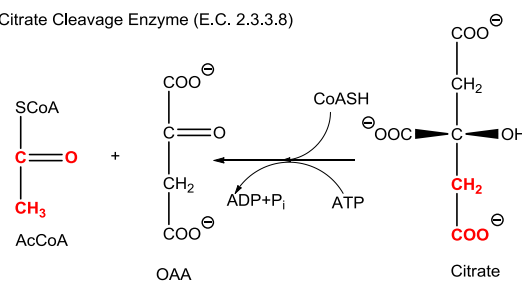
8. (12 Points) The reactions catalyzed by HMG CoA synthase (ketone body formation and steroid biosynthesis), HMG CoA lyase (ketone body formation), citrate synthase (TCA cycle enzyme), and citrate cleavage enzyme (fatty acid synthesis) are chemically similar. All of the enzymes catalyze a common reaction which is why they are classified with similar Enzyme Commission (E.C.) numbers. The four reactions are laid out below in a format that enables easy comparison of the different reactions. The ΔG° for the Citrate Synthase reaction is given. Knowing that the ΔG° values for the hydrolysis of ATP and CoA-thioesters are -30.5 kJ/mol and -31.4 kJ/mol, respectively, estimate the ΔG° values for the remaining three reactions in the direction drawn. (The arrows reflect the direction of flux in the respective metabolic pathway, not necessarily the sign for ΔG°).

Citrate Synthase (E.C. 2.3.3.1)

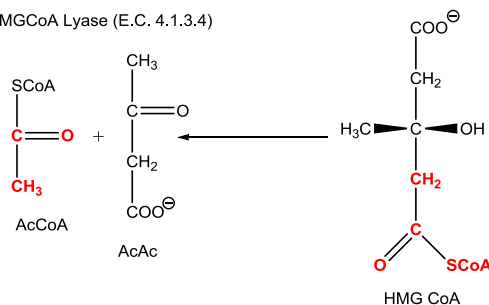


$\Delta G^\circ = -32.2$ kJ/mol

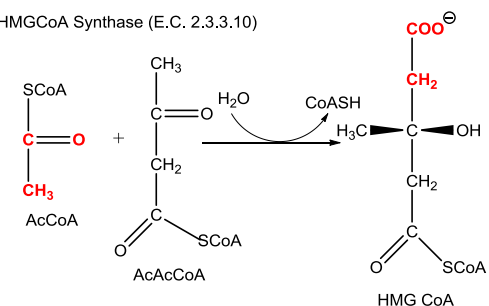
Citrate Cleavage Enzyme (E.C. 2.3.3.8)



HMGCoA Lyase (E.C. 4.1.3.4)



HMGCoA Synthase (E.C. 2.3.3.10)



Part III Essay

Writing reflects how you think. Among the “right answers” I will read for the following question, some will be better than others because they show greater depth of understanding, avoid extraneous or inaccurate information, use knowledge from previous learning, provide a more logical structure, use appropriate examples, and choose words with precision. Better quality answers will receive higher marks. Therefore organize your thoughts before you write. Strive to write not that you may be understood, but rather that you cannot possibly be misunderstood. Stream of consciousness answers are rarely well organized or clearly presented.

1. (12 points) How is it possible for an enzyme to participate in a metabolic pathway if the ΔG° for the reaction it catalyzes has a positive sign in the direction of metabolic flux?