CHEM-643 Biochemistry Name _____
Final Examination
7:00 – 10:00 PM, Tuesday, 13 December 2011
Dr. H. White – Instructor

- There are 14 pages to this examination. Tear off the last two for use on Question 7.
- **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 suspected errors you encounter.
- This is examination is closed book until 9:00PM. 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. It is 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, the 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. You may use the backs of pages, if you need more space.
- The maximum possible score is 140. Graded Exams can be picked up starting Thursday afternoon and will be held until Spring Semester.

Have a Safe and Happy Holiday!

Exam Statistics:

Class Range ___58-118_____ Total points possible 140

Class Mean ____91.3_____

Your Score _____

Your Rank in Class _____ out of 23

Course Grade _____

1. (24 Points Total) **Group Project**

   a. (2 points) What organism and amino acid pathway did your group study?

   b. (2 points) What enzyme did you study?

   c. (4 points) What is the reaction catalyzed by the enzyme you studied? (Provide structures of substrates and products and indicate any cofactor requirements.)

   d. (4 points) Concisely state the hypothesis your group was testing.

   e. (4 points) What is the reasoning/logic behind the hypothesis?

   f. (6 points) You are presented with a new bacterial genome to analyze. Based on the wisdom and insight you developed on this project, identify ***at least three*** factors/characteristics (not specific pathways or enzymes) that you would consider/use to predict which among the amino acid biosynthetic pathways and enzymes you would expect to find the strongest support for the hypothesis?

      i.

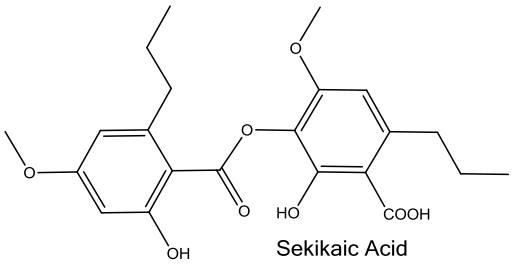
      ii.

      iii.

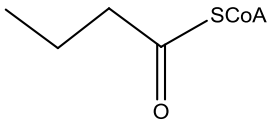
   g. (2 points) What is a lipogram?

2. (8 Points) **Aromatic Amino Acid Metabolism**. Exposed to normal metabolic activity in a rat liver, a ^{13}C label in tyrosine will not appear as a ^{13}C label in phenylalanine. However, a ^{15}N label from tyrosine will appear in phenylalanine (among other amino acids) under the same conditions. Explain and show why this is so using appropriate structures and reactions.

3. (10 Points) **Sekikaic acid**, a compound displayed by Anna Mapp in her seminar on December 2, is a depside natural product isolated from certain lichens. It is a potent inhibitor of prostaglandin synthesis.[1] Examination of its structure below reveals patterns that provide clues to its biosynthesis. Propose a biosynthetic pathway showing the structures of key intermediates building upon butyrylCoA as an initiator and show the source of additional atoms in the final structure.

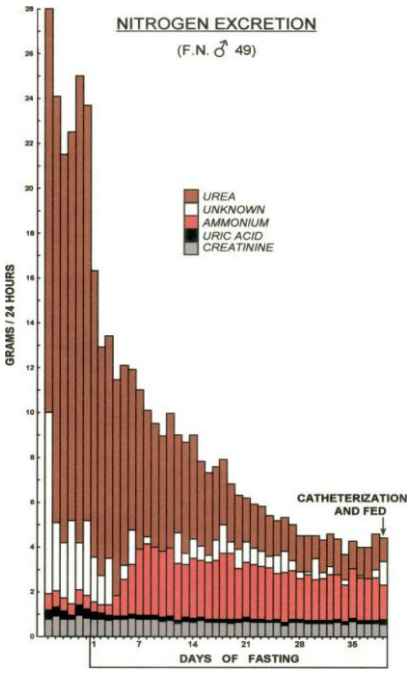


Sekikaic Acid



[1] Saukawa et al. *Prostaglandins* **24**, 21 (1982)

4. (14 Points) **Human Starvation.** The figure below[2] displays the measured daily amounts of various nitrogenous compounds excreted in the urine of an obese patient preceding and during a prolonged fast in which she lost about 50 pounds.



(6 points) Identify three notable changes in nitrogen excretion that occur soon after fasting starts. (1, 3, 6 points for 1, 2, 3 correct)

a.

b.

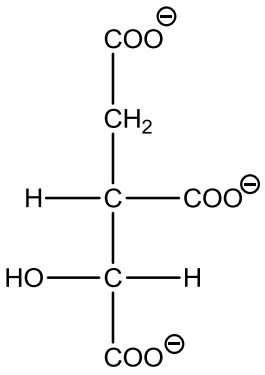
c.

d. (8 points) Describe the metabolic changes during human starvation that explain these changes in nitrogen excretion.

[2] From Owen, *BAMBED* **33**, 246 (2006)

5. (19 Points Total) **Isocitrate Dehydrogenase Mutations.** Understanding cancer has been a major focus of biochemical research for many years. Current research is predicated on the understanding that cancer is a genetic disease often resulting from somatic mutations in genes that regulate the cell cycle, DNA repair, and apoptosis. With new DNA sequencing methods and computational analysis, it is now possible to analyze and compare DNA from a tumor and healthy tissue from the same individual to detect mutations in the cancerous tissue. A genomic analysis of 22 samples from different patients with *glioblastoma multiforme* (an aggressive brain tumor) examined 20,661 protein coding genes.[3] Most of the detected mutations were in genes coding for the usual suspects—P_{53}, CDKN2A, EGFR, and PTEN—that each were found in 30-50% of the tumors and all of which involve signaling pathways, growth control, cell adhesion, or apoptosis. Interestingly a new and unexpected gene was identified in 11% of the tumors—*IDH1* that codes for an NADPH-linked isocitrate dehydrogenase. Furthermore, the mutation was very specific affecting a single amino acid in the enzyme, arginine 132 to histidine, which altered the enzyme's activity.

a. (4 points) Isocitrate is drawn below. Show with reactions and arrow pushing, how it is converted to α-ketoglutarate by isocitrate dehydrogenase (IDH).



b. (3 points) IDH in the TCA cycle is associated with NAD^+. Why does it make good metabolic sense that it should use $NADP^+$?

[3] Parsons et al. *Science* **321**, 1807-1812 (2008)

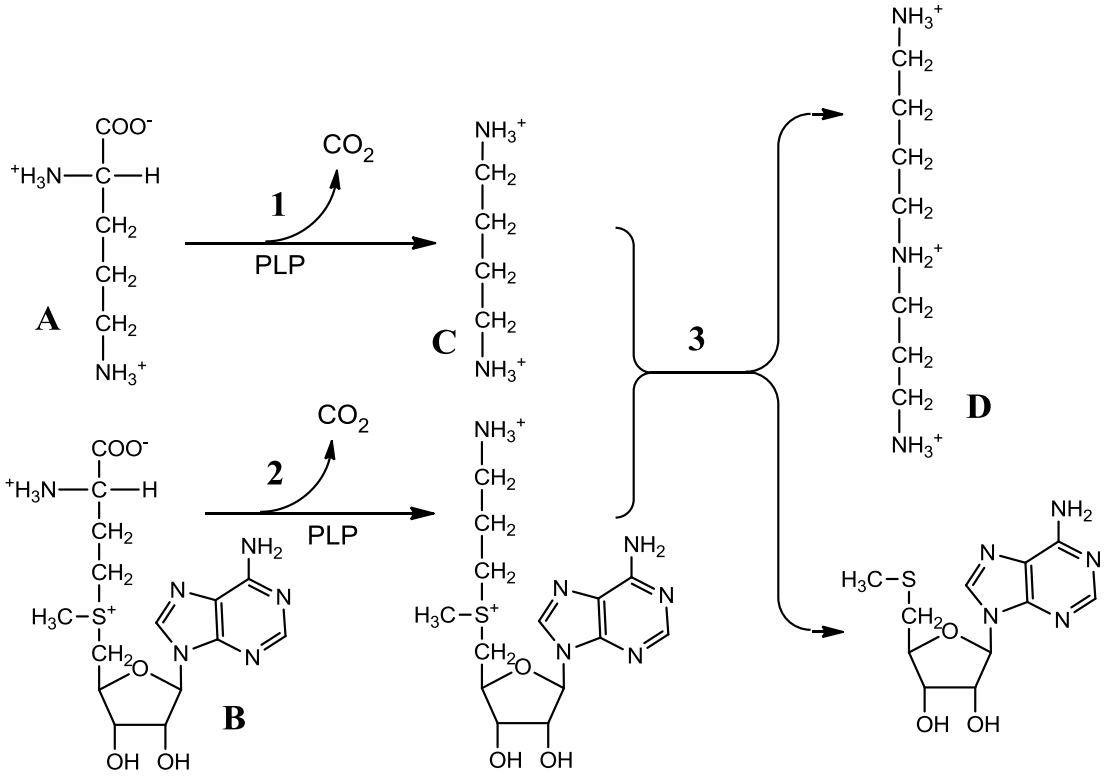
c. (4 points) The R132H mutation converts IDH1 into an $NADP^+$-dependent D-α-hydroxyglutarate dehydrogenase, an enzyme activity not normally found in human tissues. Draw the structures of the reactant and product of this aberrant reaction.

d. (4 points) Tumors containing the R132H mutation accumulate D-α-hydroxyglutarate.[4] Would you expect the accumulation to be more or less if the enzyme were coupled to NAD^+ rather than $NADP^+$? Explain.

e. (4 points) There are people who have D-α-hydroxyglutaric aciduria as the result of a similar germ line mutation in the *IDH2* gene.[5] They do not have cancer. In contrast to many inherited metabolic defects, this condition is genetically dominant rather than recessive. Why should that be?

[4] Dang et al. *Nature* **465**, 966 (2010)
[5] Kranendijk et al. *Science* **330**, 336 (2010)

6. (27 Points Total**) Spermine Biosynthesis.** Along with histones, polyamines are important in stabilizing DNA, especially in sperm cells. However, polyamines have other functions that are not so well understood. For example, they are found at high concentrations in the inner ear. Mice that lack spermine synthase [Enzyme 3] in the biosynthetic pathway below are deaf, cannot balance, and die within 5 days.[6] The following questions refer to the pathway below. (Spermine is derived from spermidine [D] by addition of another aminopropyl group by Enzyme 3.)



a. _____ (2 points) Name Compound A

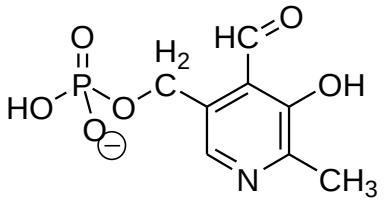
b. _____ (2 points) Name Compound B

c. _____ (2 points) Name Enzyme 1 (not putricine synthase)

d. (3 points) If the alpha amino group of compound A were labeled with ^{15}N, where would the label be in spermidine (compound D)? Circle the labeled atom or atoms.

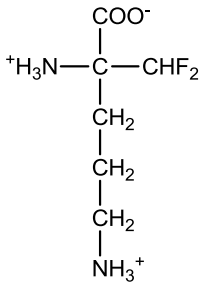
[6] Wang et al. *J. Biol. Chem*. **284**, 930 (2009)

e. (8 Points) Enzyme 1 requires pyridoxal phosphate (PLP) as a coenzyme. Show mechanistically (arrow pushing) how PLP functions as a catalyst in converting compound A to putricine (Compound C).



PLP

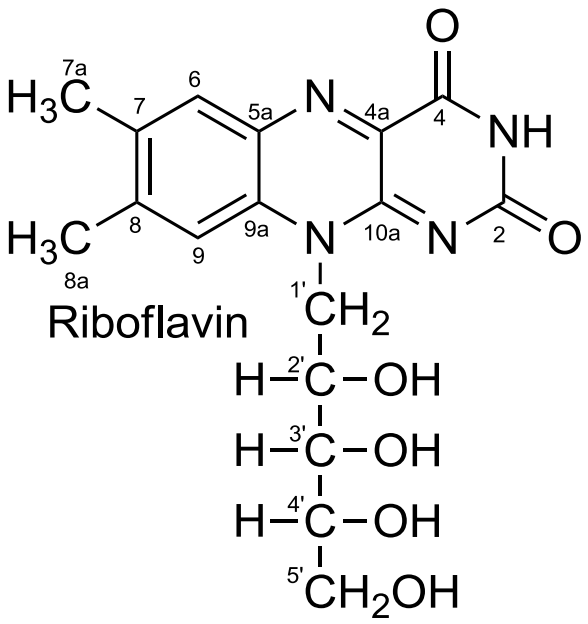
f. (5 Points) DMFO (shown below) is an irreversible inhibitor of Enzyme 1. It is used to kill trypanosomes that cause African sleeping sickness.[7] The inhibitor becomes covalently bound both to PLP and the enzyme. The sulfur from an active site cystiene bonds to the carbon which loses one fluorine after the loss a carboxyl group in forming the complex. Based on this information, draw the structure of the inhibited complex.



g. (5 points) Show mechanistically how this complex might form. (Use back of this page, if needed.)

[7] Barrett et al. *Br. J. Pharmacol*. **152**, 1155 (2007)

7. (28 points) **Riboflavin Biosynthesis**. GTP is a precursor of riboflavin. Draw the structure of GTP to the right of riboflavin below so that the structural relationships can be readily appreciated.
   a. (3 points) Draw circles around those atoms in riboflavin not derived from GTP.
   b. (3 points) Draw circles around those atoms in GTP that are lost in making riboflavin.

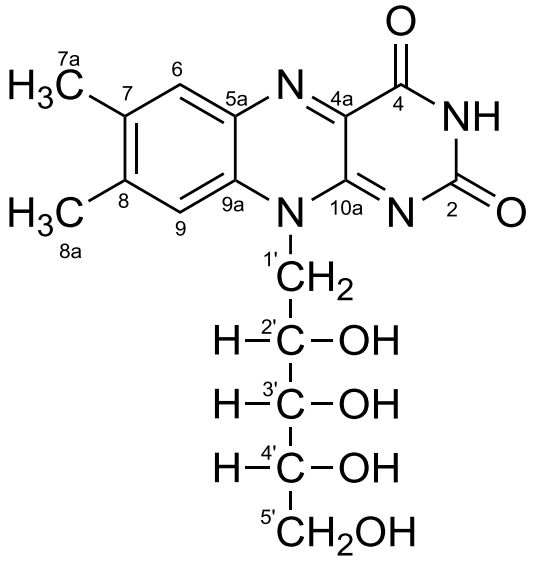


   c. In contrast to ^{12}C, ^{13}C has a nuclear spin that can be detected by nuclear magnetic resonance (NMR). Because the chemical environment of an atomic nucleus influences its chemical shift in NMR, carbon atoms in an organic molecule can be identified nondestructively. The natural abundance of ^{13}C is low (1.1%), thus ^{13}C-enrichment from various precursors can be detected easily in an isolated product. All of the resonances in the ^{13}C-NMR spectrum of riboflavin have been assigned and are indicated in the table on the next page. Also shown are the relative ^{13}C-enrichment for each carbon atom of riboflavin after feeding the indicated ^{13}C-precursors. As with any experiment of this type, there will be dilution of the isotope by endogenous precursors and product. These experiments were done with the mold *Ashbya gossypii.*[8]

[8] Bacher et al. *J. Biol. Chem.* **258**, 13431 (1983).

Carbon Atom	Chemical Shift *ppm*	Relative Enrichments					
		[1-^{13}C] Acetate	[2-^{13}C] Acetate	[1-^{13}C] Ribose	[1-^{13}C] Glucose	[6-^{13}C] Glucose	[2-^{13}C] Glycerol
2	154.9	1.8	2.7	0.9	1.1	1.6	5.4
4	159.5	4.9	1.9	0.8	1.2	1.2	1.4
4a	136.0	0.9	0.6	0.9	1.3	1.1	1.9
5a	136.6	6.3	5.2	3.3	2.4	2.2	15.9
6	132.8	3.3	5.8	10.3	5.5	3.2	12.9
7	137.1	8.3	3.0	0.8	1.4	1.6	3.0
7a	19.5	1.2	7.0	0.9	3.3	9.0	2.4
8	148.1	5.5	3.6	3.7	1.8	1.9	12.1
8a	21.5	3.3	6.0	9.1	5.0	2.7	12.3
9	115.6	1.2	6.4	1.1	3.4	9.0	2.1
9a	131.2	8.5	2.1	0.6	1.2	1.6	3.0
10a	150.6	1.3	0.8	0.6	0.7	0.9	1.1
1'	45.0	3.2	4.6	19.4	4.5	2.3	8.5
2'	69.4	4.4	4.0	2.9	1.8	1.6	7.4
3'	70.4	6.9	2.7	0.6	1.5	1.7	2.1
4'	69.0	1.0	4.9	1.3	1.9	1.9	12.9
5'	61.9	1.1	4.7	1.1	3.2	6.8	1.6
CH3CO	20.4-21.1	1.0	1.0	1.0	1.0	1.0	1.0
CH3**C**O	169.8-170.7	1.0	0.8	0.9	0.9	0.8	0.9
The final two entries are from the four acetyl groups on the tetraacetylated riboflavin. The values are the average of the four signals and serve as an internal, natural-abundance standard.							

d. (8 points) Using circles, squares, triangles, etc. or different colors, identify carbon atoms in riboflavin that have a common pattern (origin) based on the ^{13}C labeling pattern from different precursors. Arbitrarily, consider ^{13}C enrichment values above 3.0 as significant.



e. (4 points) Examine the ^{13}C-labeling pattern for riboflavin from the various labeled precursors. Is the labeling pattern of those carbons derived from GTP consistent with the known biosynthetic pathway for GTP? (Tear off pathway sheet at back of this exam.) Justify your answer.

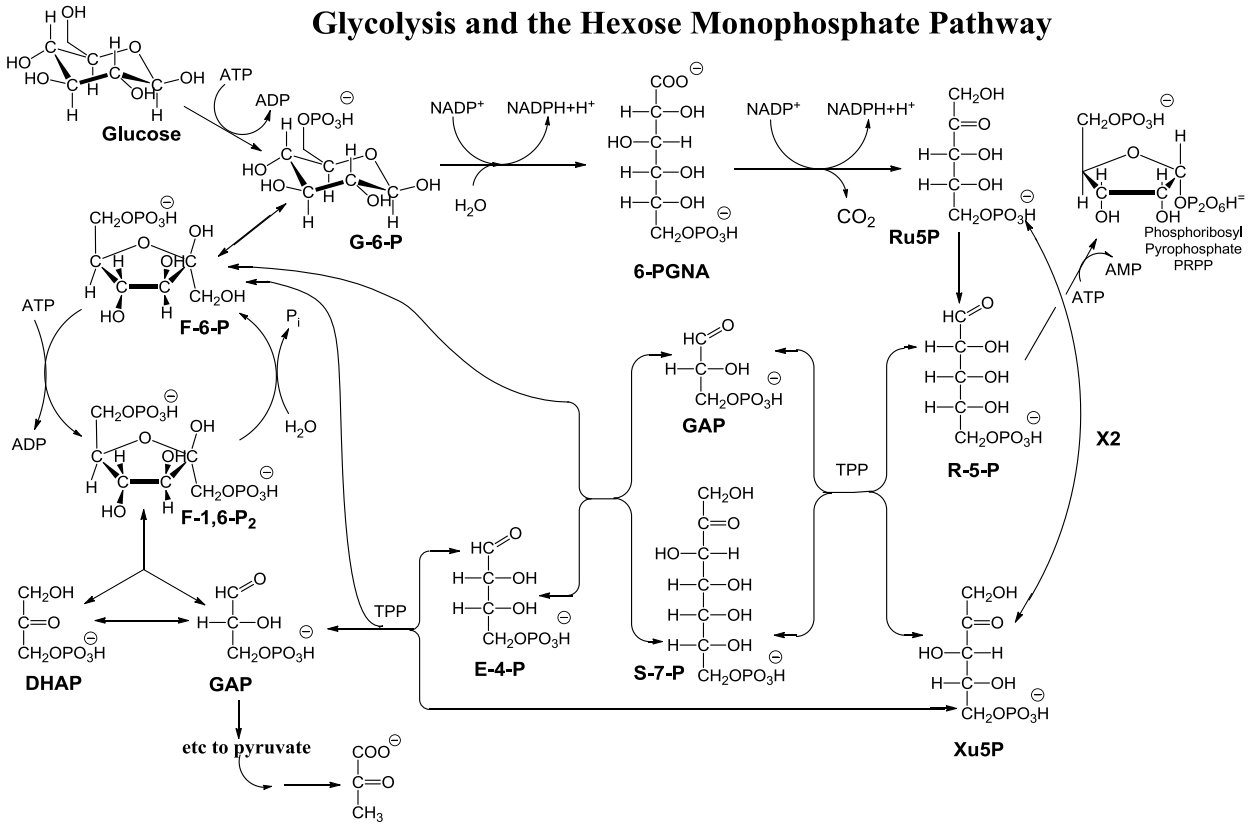
f. (6 points) What distinctive labeling pattern is apparent in the xylene ring moiety (Carbons 5a, 6, 7, 7a, 8a, 8, 9, 9a) of riboflavin? Based on this pattern, what can you deduce about the biosynthesis of the xylene ring portion of riboflavin?

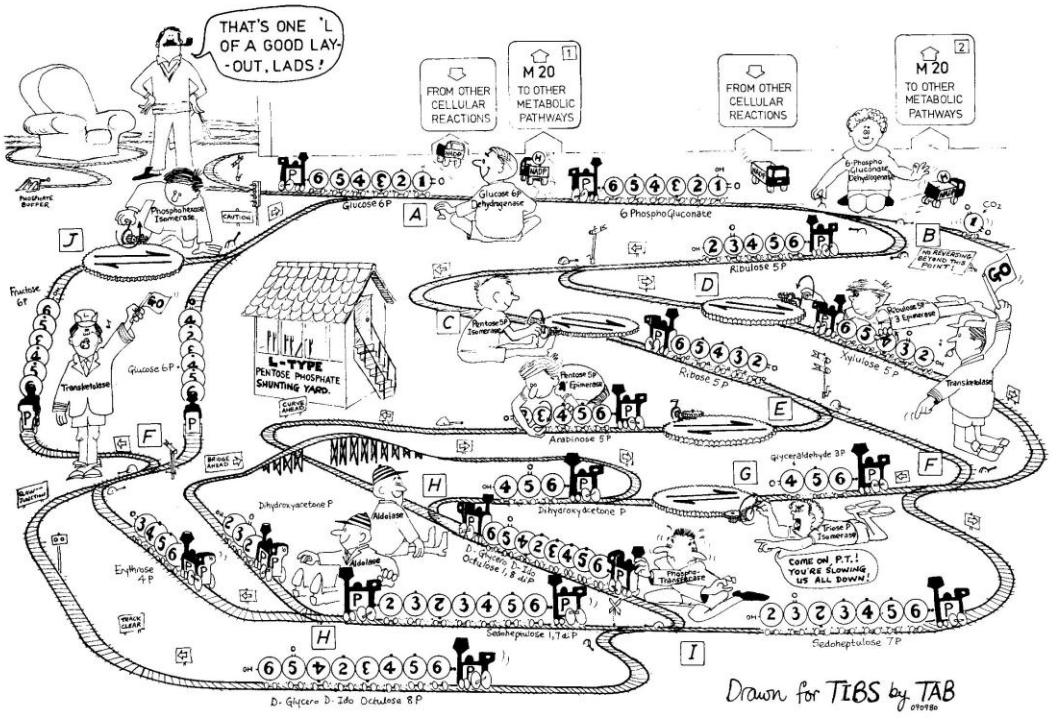
g. (4 points) Considering the coupling of nuclear spins detectable by NMR, What biosynthetically important information could be obtained by comparing the ^{13}C-NMR spectra of riboflavin synthesized from [4-^{13}C] or [5-^{13}C] Ribose versus that synthesized from [3,5-^{13}C]Ribose? Here [3,5-^{13}C]Ribose is doubly labeled, not a mixture of [3-^{13}C] and [5-^{13}C]Ribose.

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 specific examples with illustrations, 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.

8. (10 points) **Big Picture Question**. Desiring to make chemistry relevant to his CHEM-103 class composed of many biology majors who dreaded chemistry, Dr. Alec Trawn told his class that all life on earth—plants, animals, fungi, microorganisms—was dependent on oxidation-reduction reactions that drive metabolism. Despite his good intentions, Dr. Trawn has never had a biochemistry course and asks you to help him out. What should he include in his *life based on redox* remarks?





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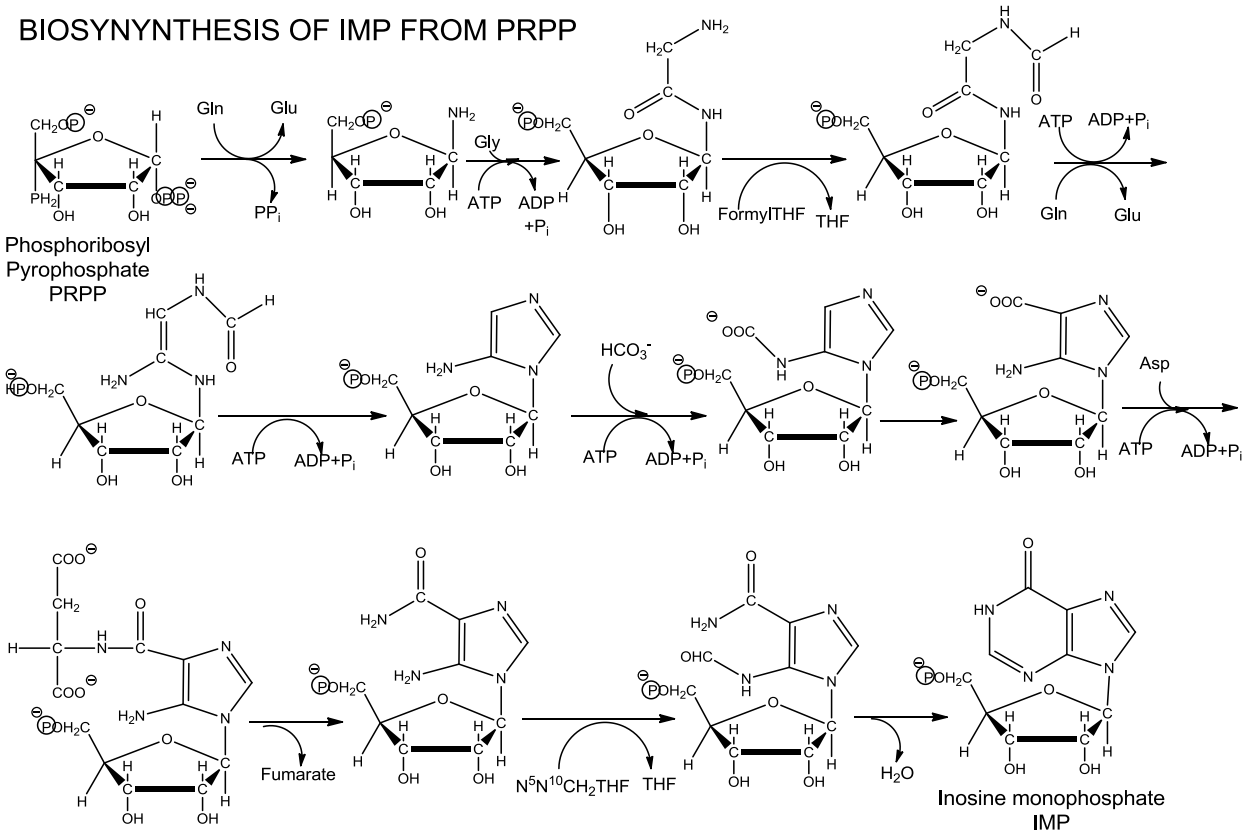
[9] T.A.B., *Trends in Biochemical Sciences* Dec 1980 p. 319.

BIOSYNYNTHESIS OF IMP FROM PRPP



BIOSYNTHESIS OF ATP AND GTP FROM IMP