Analytical Cumulative Examination
March 5, 2005

ANSWERS

QUESTION 1

EACH part is worth 2 points. Give approximate answers to the following questions.
IT IS ESSENTIAL TO GIVE UNITS

No particular units req'd — others equally correct.

What is the value of

1) the Boltzmann constant 1.38×10^{-23} J/K [$(8.6\times10^{-5}$ eV/K)]
2) kT at room temperature 25 meV
3) the number of molecules (of typical size, not macromolecules) that occupy 1 cm^2 of a surface under ordinary conditions 10^{15} to 10^{13}
4) the number of molecules that occupy a liter of gas under ordinary conditions 2×10^{22}
5) pKa of acetic acid 5 (4–6)
6) the proton affinity of ammonia 854 kJ/mol (800–900 kJ/mol) [204 kcal/mol (195–210)]
7) the electron affinity of dioxygen +0.4 eV (0–1 eV)
8) the oxidation potential of water −1.23 V (−1 to −1.5 V)
9) the fundamental wavelength of the Nd YAG laser ~~260~~ 1064 nm (250–280 nm)
10) the rate constant of an ion/molecule reaction that occurs on every collision 10^9 molecules/cc.s
11) the refractive index of glass 1.5 ~~(1.3–2.0)~~ (1–2)
12) the bond energy of a C-C bond 85 kcal/mol (70 to 100 kcal/mol) 340 kJ/mol
13) the bond energy of a typical hydrogen bond 5 kcal/mol (3–12 kcal/mol)
14) the mass of a typical small protein 5–30 kDa
15) the velocity of a nitrogen molecule at STP 500 m/s (300–800 m/s)
16) the dipole moment of chloroform 1.04 D (0.5–1.5)
17) the pressure at which air exhibits maximum conductivity (0.1–1 Torr)
18) the number of theoretical plates in a typical lc capillary column 10^5 (10^4–10^6)
19) the field strength of a typical "high field" magnet (NOT in MHz) 3–15 Tesla
20) the position of the OH stretch in anhydrous ethanol 3300–3500 cm^{-1} (3100–3700 cm^{-1})
21) the absorption wavelength for the electronic transition in a saturated ketone 270–300 nm (250–320 nm)
22) the mass of the characteristic neutral fragment of an aromatic nitrocompound 16, 30 or 46 Da
23) the penetration depth of a photon undergoing total internal reflection 1 μ
24) the energy of visible light photons 10^3 s^{-1}, 2 eV, 20000 cm^{-1}
24) the resolution in confocal microscopy 1 nm
25) the dependence of Raman intensity on excitation wavelength high power (4th ω)
26) the sampling depth in XPS (ESCA) 2 nm (1–4 nm)
25) the mass measurement accuracy of time of flight mass spectrometry 1–20 ppm (0.01 u)
26) the mass of the electron $\frac{1}{2000}$ u (Da) ~~1.9×10^{-27} kg~~ 9.1×10^{-31} kg
27) the detection limit for fluorescence spectroscopy in a favorable case 10^{-8}–10^{-10} M (1 ppb ok)
28) the difference in chemical shifts between methyl and methylene groups 1–3 ppm
28) the diffusion constant of a small hydrophilic compound in water 10^{-5} cm^2/s
29) the quantity varied in the Michelson interferometer to measure wavelength velocity (cm/s)
30) the quantity varied in a grating to measure wavelength angle (degrees)

QUESTION 2

EACH part is worth 4 points; define all units and be as EXPLICIT as possible

a) Write the names of the four common DNA bases
b) Name and write the structures of any three of the natural amino acids
c) Draw the mechanism of the McLafferty rearrangement
d) Give the equation for any type of mass spectrometer
e) Give the fundamental equation for nmr
f) Give Beer's law

NERNST

$$E = E^{o} - \frac{RT}{nF} \ln K \qquad \text{or} \qquad E = E^{o} + \log \frac{[Red]}{[ox]}$$

electrochemical potential
standard state electrochemical potential
gas constant
temp
no. moles charge per mole reactant
K equil. constant.

VAN DEEMPTER

$$H = A + \frac{B}{u} + C_u \qquad (C = C_M + C_S)$$

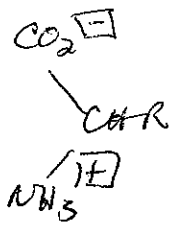
height equiv. theor. plate
A constant, different paths
B mass transfer in mobile phase in direction of flow
C_S mass transfer to stationary phase
u mobile phase velocity
C_M mass transfer in mobile phase orthogonal to flow

ANY ENZYME

ANY SAM

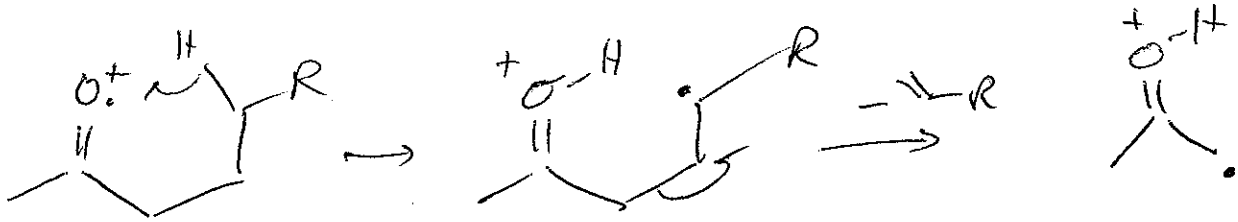
ANY NEUROTRANSMITTER

1) ADENINE, THYMINE, GUANINE, (URACIL), CYTOSINE
[RNA]

2) 

R = CH_3 alanine
R = H glycine
R = CH_2OH serine

3).



4) $m/e = \frac{B^2 r^2}{2V}$

B field
r radius
V acc. voltage

5) $\nu_0 = \gamma \frac{B_0}{2\pi}(1-\sigma)$

$E = B_0 \gamma$

ν_0 res. freq.
γ gyromagnetic ratio
B_0 field
σ shielding

6) $A = \varepsilon b c$

Absorbance
ε molar absorbtivity
path length
conc

Answers to Biochemistry cume, March 5, 2005

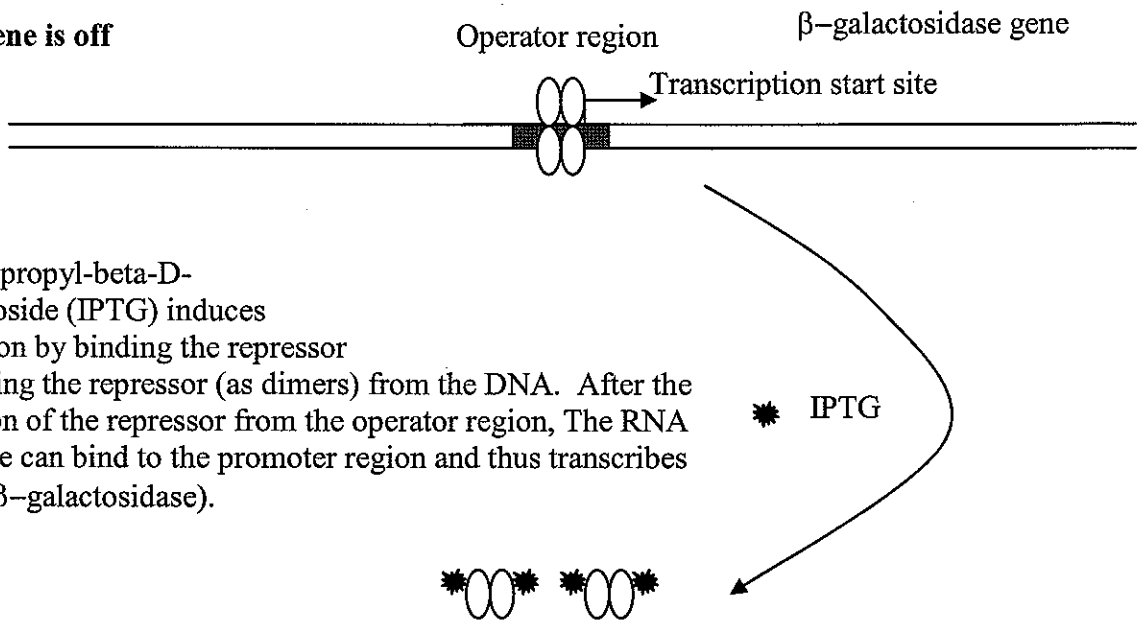
1. (10 points)

The lactose operon of *E. coli* contains several genes including the gene that encodes β-galactosidase.

The lac-repressor is a tetramer.

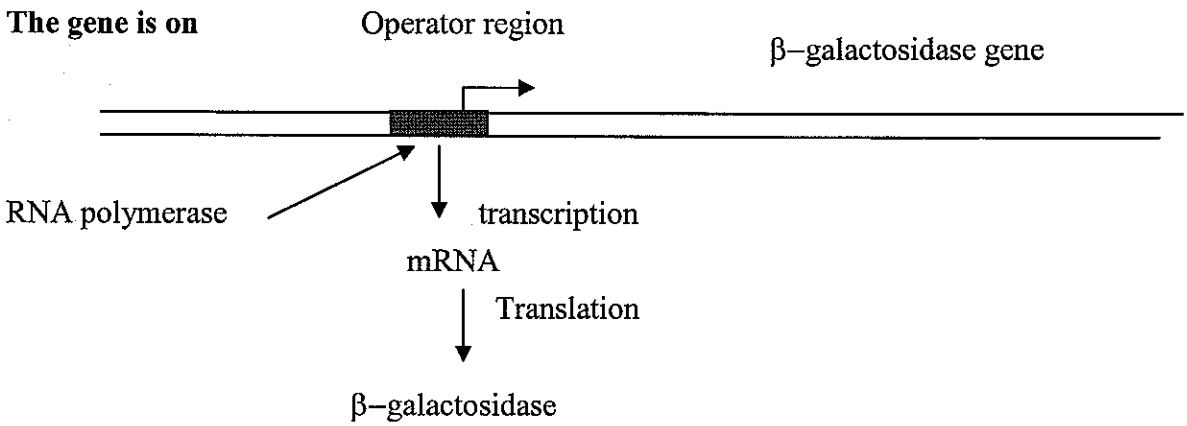
The gene is not transcribed (it is off) when the lac-repressor is bound to its binding site in the operator region.

The gene is off



Isopropyl-beta-D-thiogalactoside (IPTG) induces transcription by binding the repressor and releasing the repressor (as dimers) from the DNA. After the dissociation of the repressor from the operator region, The RNA polymerase can bind to the promoter region and thus transcribes the gene (β–galactosidase).

The gene is on



X-gal (5-bromo-4-chloro-3-indolyl-beta-d-galactosidase) is an artificial substrate of β–galactosidase. X-gal is colorless. Cleavage of X-gal by the enzyme releases a chromophore which is blue and readily detectable.

2. (10 points)

Numerous cloning vectors are available that contain the operator region and the β-galactosidase gene of the Lac-operon. Specific DNA fragments can be cloned into these vectors, at restriction enzyme sites within the β-galactosidase gene. The plasmids are introduced into appropriate E. *coli* host cells. IPTG is used to turn the gene on. X-gal is used for distinguishing cells that contain the original plasmid from those that contain the recombinant plasmid. Details can be found in standard cloning books.

3. (15 points)

TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH are the components of the machinery (basal) that directs the RNA polymerase II to begin transcription of protein coding genes at correct sites.

TBP (TATA binding protein) is the subunit of TFIID that binds the TATA element in the promoter regions of genes.

TBP associated factors (TAFs) correspond to the other subunits in TFIID. They regulate transcription through interactions with transcription factors that bind specific control elements in the regulatory regions of genes and factors that act as mediators of transcription.

SP1 is a well known transcription factor that interacts with specific GC-rich elements in the regulatory regions of genes.

Glucocorticoid receptor regulates transcription in response to glucocorticoid (a hormone derived from cholesterol). The receptor includes a domain for binding glucocorticoid and a domain for binding specific sequence elements in the regulatory regions of genes that are controlled by glucocorticoid.

Histone acetylases (HATs) acetylate the histone core of nucleosomes. HATs regulate transcription through chromatin remodeling. Acetylation of histones by specific acetylases results in chromatin remodeling.

4. (12 points)

Examples for known DNA binding domains include helix-turn-helix, zinc finger, helix-loop-helix, leucine zipper.

Helix-turn-helix is found in the homeo domain containing transcription factors (for example Hox1).

Zinc fingers are found in numerous transcription factors including SP1 and nuclear hormone receptors (i.e. glucocorticoid receptor, vitamin D receptor, progesterone receptor, etc.).

5. (10 points)

Differential exon utilization can produce spliced mRNAs that encode distinct but related proteins. Examples of such mRNAs include:

Exon 1 spliced to exon 3

Exon 1 spliced to exon 2, which in turn is spliced to exon 4

Exon 1 spliced to exon 3, which in turn is spliced to exon 4

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6. (45 points) Details of protein synthesis can be found in any standard, graduate level, biochemistry book.

Ribosomes are the molecular machines that translate the sequence of mRNAs to proteins.

IF-1, IF-2, and IF-3 are factors that function in the initiation of protein synthesis in bacterial cells.

EF-Tu and EF-Ts are factors that function in the elongation phase of protein synthesis in bacterial cells. A ternary complex consisting of EF-Tu , GTP and an aminoacyl-tRNA (AA-tRNA) delivers AA-tRNA to ribosomes. During this process, GTP is hydrolyzed to GDP. EF-Ts functions in regeneration of GTP in a complex consisting of GDP and EF-Tu.

Argenyl tRNA synthetase is the enzyme that catalyzes the esterification of argenine to the 3' end of $tRNA_{Arg}$ (the tRNA that carries Argenyl-$tRNA^{Arg}$ to the site of protein synthesis.

EF-G functions in the translocation of ribosomes, along the mRNA, in the elongation phase of protein synthesis.

RF-1, RF2, and RF-3 function in the termination phase of protein synthesis. They release the synthesized polypeptide chain when the ribosome encounters a termination codon in the mRNA.

$tRNA_f^{Met}$ functions in the initiation of protein synthesis in bacterial cells. Met-$tRNA^{Met}_f$ is formylated to produce formyl-Met-$tRNA^{met}_f$. The formylated form functions in the initiation of protein synthesis in bacterial cells

$tRNA^{Ala}$ specifies the tRNA to which alanine is esterified to produce Ala-$tRNA^{Ala}$. Ala-$tRNA^{Ala}$ is delivered (by a complex of EFTu and GTP) to the mRNA codons that specify alanine.

16S rRNA is the RNA component of the 30S ribosomal subunit in E. *coli*.

Inorganic Chemistry Cumulative Exam Crib

Purdue University

March 5, 2005

Topic: Bioinorganic Chemistry

Question 1: (25 points)

According to some recent findings, all of the transition metal ions inside of cells are bound to various biomolecules. The concentration of freely available metal ions is approximately zero. After cellular synthesis of an apoenzyme (i.e., a metalloenzyme that does not yet have a metal), this new enzyme must pick up a metal ion. Explain the process by which metalloproteins obtain the requisite metal ion or ions. Hint: Metalloproteins often contain one, specific ion and not other similar ions.

Question 2: (25 points)

The enzyme superoxide dismutase (SOD) catalyzes the following reaction:

$$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$$

This catalyzed reaction proceeds with a very fast rate constant of $k = 2 \times 10^9\ M^{-1}s^{-1}$. This rate constant shows that the enzyme functions near the diffusion limit, a very fast reaction indeed. Explain the design feature or features of this enzyme system that allow it to have the reaction proceed so rapidly.

Question 3: (25 points)

The ferredoxin class of electron transfer proteins contain a tetranuclear iron-sulfur cluster, $[Fe_4S_4(S\text{-cys})_4]^{2-/3-}$. These clusters cycle between the 3- and 2- states, as noted in the formula. Typical reduction potentials for these ferredoxin clusters are in the range of between -0.65 ⇔ -0.28 volts. The high potential iron proteins (HiPIP's) also have tetranuclear iron-sulfur clusters, $[Fe_4S_4(S\text{-cys})_4]^{1-/2-}$, but these clusters cycle between 2- and 1- states, as noted, and the typical reduction potentials are between +0.28 ⇔ +0.36 volts. Explain why these two protein systems can both have tetranuclear iron-sulfur clusters, but the reduction potentials and redox cycles are so different. Keep in mind the different redox cycles for each system (i.e., 3-/2- versus 2-/1-).

Question 4: (25 points)

We humans transport oxygen through our blood by using the protein hemoglobin. Marine invertebrates such as clams use hemerythrin. Spiders and lobsters use hemocyanin. Each of these three proteins has a metal center or centers for reversible O_2 binding. Provide a drawing of the metal site of each system with and without O_2 bound. Your schemes need not be exactly correct, but should show the general differences between hemoglobin, hemerythrin, and hemocyanin.

-Answer Key-
Inorganic Chemistry Cumulative Exam
Purdue University
March 5, 2005
Topic: Bioinorganic Chemistry

Question 1:

Cells use chaperone systems to transport metal ions into cells and carry them to a desired location. For a given metal ion, there will be a protein contained within the cellular membrane to transport that ion into the cell. There will then be a second chaperone protein that takes the metal ion from the cell membrane. This chaperone protein then carries the metal ion to the apoprotein, thus forming the metalloprotein.

Question 2:

The enzyme has a canyon cut into the structure, leading from the surface to the active site with the Cu and Zn. The surface of this canyon has a positive charge, with the positive charge density increasing as the canyon approaches the copper, thereby drawing the negatively charged superoxide substrate to the active site.

Question 3:

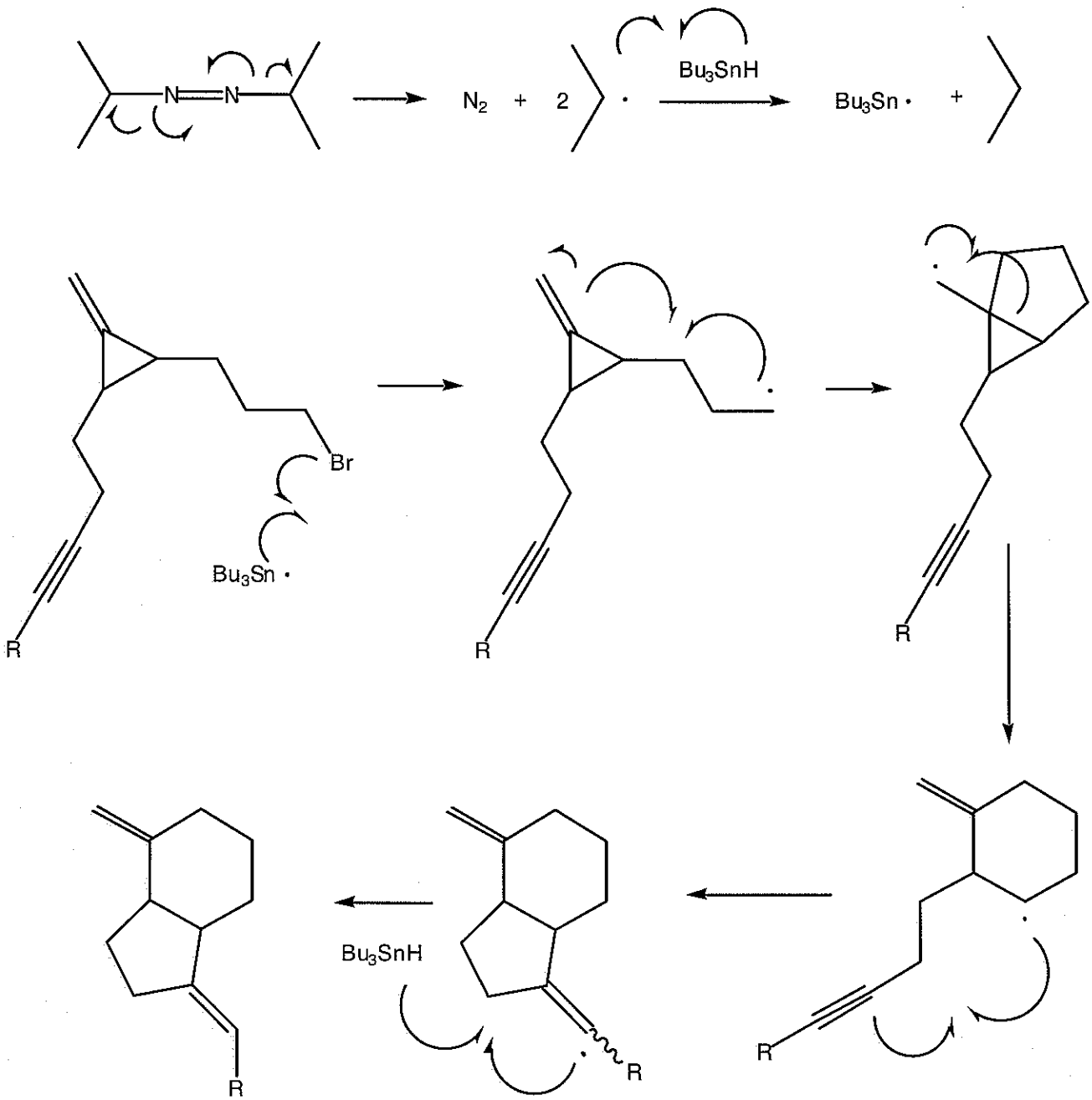
Hydrogen bonds to the cluster sulfurs influence the potentials by withdrawing electron density, thereby making the cluster easier to reduce (i.e., have a higher reduction potential). The ferredoxins have about 9 hydrogen bonds per cluster. The HiPIP's have about 5 hydrogen bonds per cluster.

Question 4:

Hemoglobin uses a heme center. Hemerythrin uses a dinuclear iron site. Hemocyanin uses a dinuclear copper site.

Organic Division Cume Exam Crib
March 2005

1.



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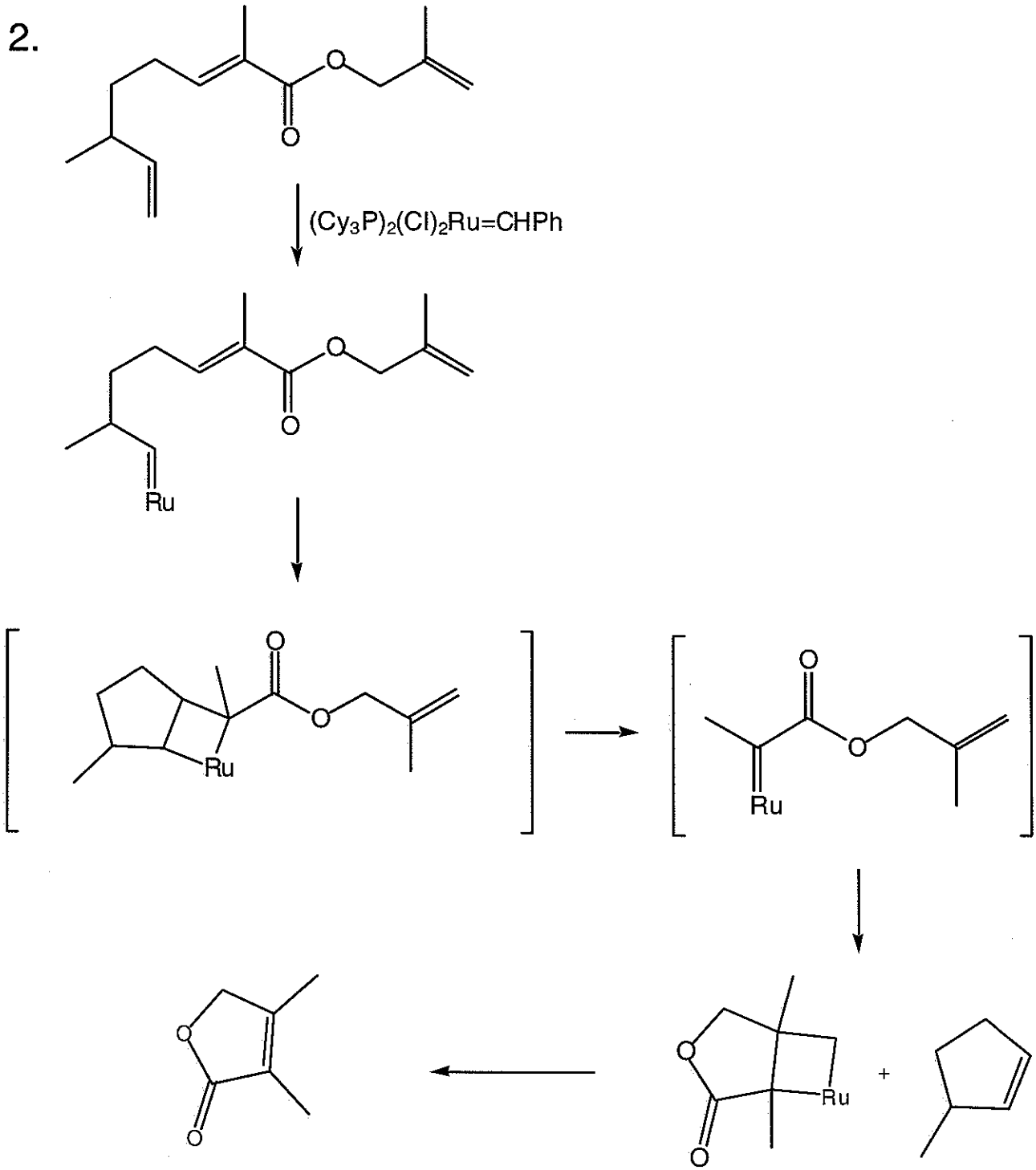
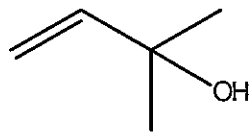


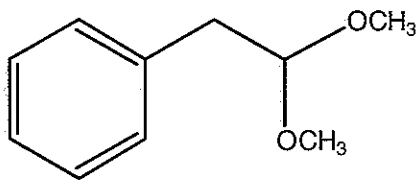
3.a. IR: ~3400 cm^{-1}, -OH; >3000 cm^{-1}, alkenyl CH, <3000 cm^{-1}, alkyl CH
MS: m/z 86 → 71, loss of CH_3; m/z 71 → 43, loss of $CH_2{=}CH_2$
^{1}H NMR: 6.0 ppm, 1H, q
5.1 ppm, 2H, qd
2.9 ppm, 1H exchangeable, s
1.3 ppm, 6H, s
^{13}C NMR: 145 ppm, CH
110 ppm, CH_2
70 ppm, C
30 ppm, CH_3

Data consistent with:



3.b. IR: >3000 cm^{-1}, alkenyl CH, <3000 cm^{-1}, alkyl CH; 1600-2000 cm^{-1}, aromatic ring
MS: m/z 166 → 135, loss of OCH_3; m/z 91, tropylium ion; m/z 166 → 75, loss of tropylium to give a fragment of $C_3H_7O_2$
^{1}H NMR: 7.2 ppm, 5H, m (∴ monosubstituted benzene)
4.5 ppm, 1H, t
3.3 ppm, 6H, s
2.9 ppm, 2H, d
^{13}C NMR: 138 ppm, C
130 ppm, CH
128 ppm, CH
125 ppm, CH
105 ppm, CH
52 ppm, CH_3
40 ppm, CH_2

Data consistent with:



Physical Cumulative Exam Crib
Professor Naumann(IUPUI)