Problem Set 3 Chemistry 5.08 Mar 1, due Mar 8

1. Stormy Jones was a six year old girl who had FH with plasma cholesterol levels of 1000 mg/dl (6x the levels found in normal children). In an effort to save her life, she had a heart and liver transplant. Subsequent to the transplant she was then given mevinolin, a statin analog. The plasma levels of cholesterol and radiolabeled [^{125}I]-LDL were monitored and the data is shown in Figure 1.

Given what you have learned about cholesterol homeostasis, provide an explanation for the changes in cholesterol and LDL levels observed after the transplant and after treatement with mevinolin.

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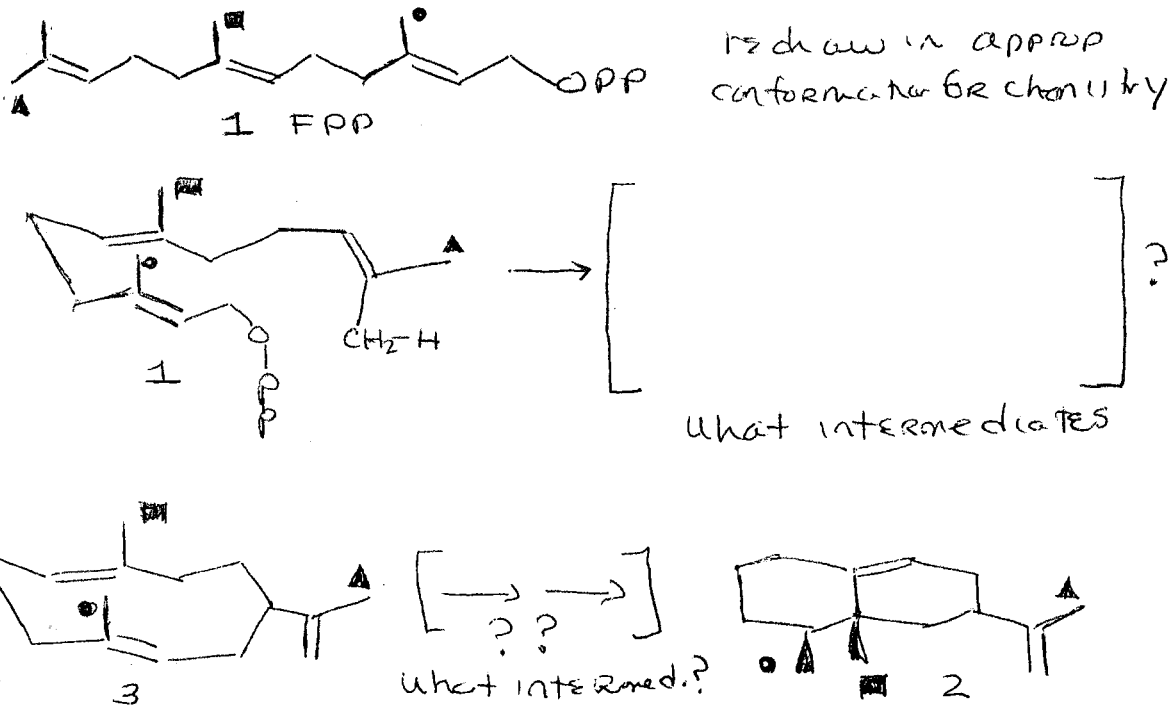
2. Sesquiterpenes synthases (also called terpene cyclases) catalyze the conversion of a universal substrate farnesyl pyrophosphate (FPP) into more than 300 known terpene cyclized products. As seen in class the enzymes that work on the FPP are thus far structurally homologous. Facile mutation and evolution has presented an effective strategy to maximize product diversity. Recently the structure of aristolochene synthase has been solved. This enzyme catalyzes the reaction shown in Eq. 1.

i. Show mechanistically how isopentenyl pyrophosphate (IPP) and dimethylallypyrophosphate (DMAPP) are converted to FPP, that is, show the biosynthetic pathway and the chemistry.

ii. Propose a mechanism by which FPP (**1**) is converted to aristolene (**2**) through the intermediacy of the 10 membered ring (**3**) shown in Figure 2 . Show each step and the intermediates involved. [Hint, think about lanosterol biosynthesis and the types of reactions that you have learned about

given the intermediates in part i and the propensity of carbocations to undergo rearrangements through hydride shifts, CH_3 anion shifts or loss of a proton.]

Figure 2



●, ■, ▲ denote CH_3 groups specifically labeled.

It might help to make a model for rearrangement within ring 3 to get to 2.

3. In this problem you will design a pair of ssDNA oligos for converting the active site cysteine of the KS module of the yersiniabactin (Ybt) biosynthesis protein HMWP1 to alanine using the site-directed mutagenesis (SDM) method based on digestion of methylated DNA that we talked about in T&D. Show all your work! (i.e., print out your ClustalW results, protein translation results, etc., and include with your problem set answers)

i. Using GenBank, pull up the nucleotide sequence for Yersinia pestis HMWP1 (hint: although the protein is named "HMWP1", the gene is named "irp1").

ii. Translate the nucleotide sequence into protein using this website: http://us.expasy.org/tools/dna.html. Select output format "compact."

iii. Find some other KS protein sequences using GenBank (for example E. coli FabB from the first T&D); then do ClustalW analysis on the HMWP1 protein and these other KS proteins in order to find the KS module of HMWP1. The ClustalW analysis should also tell you which cysteine residue of HMWP1 is likely to be the active site cysteine.

iv. Now use the Expasy website again to translate just the nucleotide sequence of the KS region of the HMWP1 gene into the protein sequence. This time, select output format "includes nucleotide sequence."

v. Using this data which nicely aligns the nucleotide and protein sequences of the KS module of HMWP1, design forward and reverse oligos for changing the cysteine to an alanine. The guidelines for oligo design are:

- Both of the mutagenic primers must contain the desired mutation and anneal to the same sequence on opposite strands of the plasmid.
- Primers should be between 25 and 45 bases in length, and the melting temperature (T_m) of the primers should be greater than or equal to 78°C. The following formula is commonly used for estimating the T_m of primers:

$$T_m = 81.5 + 0.41(\%GC) - 675/N - \%\ \text{mismatch}$$

  For calculating T_m:
  - N is the primer length in bases
  - values for **%GC** and **% mismatch** are whole numbers

  For calculating T_m for primers intended to introduce insertions or deletions, use this modified version of the above formula:

$$T_m = 81.5 + 0.41(\%GC) - 675/N,$$

  where N does not include the bases which are being inserted or deleted.
- The desired mutation (deletion or insertion) should be in the middle of the primer with ~10–15 bases of correct sequence on both sides.
- The primers optimally should have a minimum GC content of 40% and should terminate in one or more C or G bases.

Make use of the genetic code table below in order to covert the cysteine codon to an alanine. Once you design your forward oligo, you can use this website to make the

reverse oligo, which is simply the reverse complement of your forward oligo: http://arep.med.harvard.edu/labgc/adnan/projects/Utilities/revcomp.html

The Genetic Code

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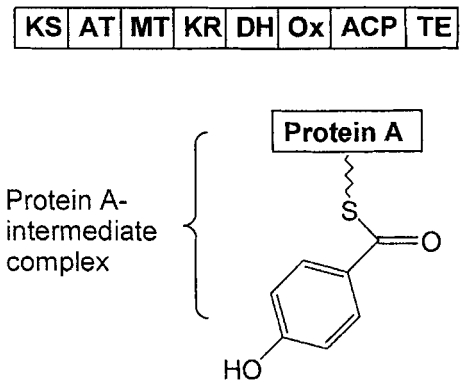
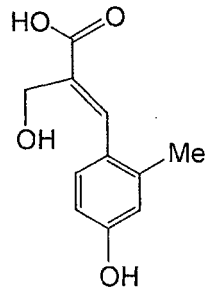
i. Sometimes it is desirable to create a library of mutants using SDM – for example, to change cysteine in the active site not just to alanine, but to any of the 20 natural amino acids. Explain how you would do this with a simple modification of the SDM protocol we discussed in T&D. Hint: it does *not* involve doing SDM 20 times, each time with a different pair of oligos.

4. Protein A and B together biosynthesize compound **1** shown below. The structure of the Protein B gene is shown. In order to investigate the order of the chemical steps catalyzed by B, a mass-spec study was performed. Purified protein B was combined with cofactors, methylmalonyl-CoA, and the proteinA-intermediate complex shown below for 5 minutes at 30 degrees. The reaction was quenched and protein B was digested with trypsin under mild conditions. The resulting fragments were injected onto a reverse-phase HPLC column, and the fragment corresponding to the ACP module was purified and analyzed by ES/FTMS. The following masses were observed: 30,116; 30,194; and 30,208 g/mol (note: the molecular weight of protein B by itself, with pant. arm in place, is 30,000 g/mol). Draw a biosynthetic scheme for protein B to give the compound **1** that is consistent with this mass-spec data.


KS
AT
MT
KR
DH
Ox
ACP
TE
Protein A
Protein A-
intermediate
complex
S
O
HO


HO
O
OH
Me
OH

1