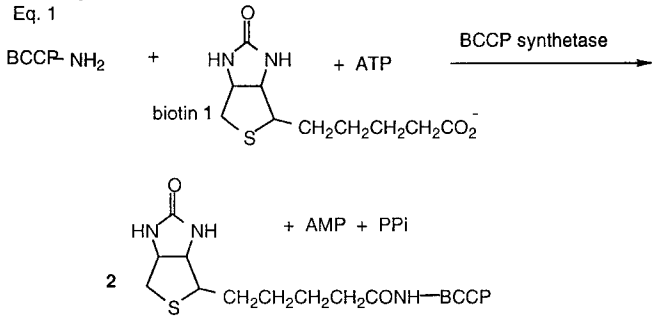
Problem Set 5 Chemistry 5.50 Oct 30 due Nov 6

1. Biotin carboxyl carirer protein (BCCP) is an essential component of acetyl-coenzymeA carboxylase and all other biotin requiring enzymes. Biotin (**1**), a universal CO_2 carrier, is covalently attached to BCCP through a reaction shown in eq. 1, catalyzed by the enzyme BCCP synthetase.

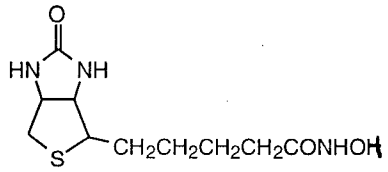
Eq. 1



Recall that ATP is used to convert the hydroxyl group of a carboxylic acid into a good leaving group, in this case so that the biotin is covalently attached to the ε-amino group of a lysine of BCCP. The turnover number of BCCP synthetase is 5 s^{-1}. You are given the following information about this system.

i. In the absence of the substrate BCCP, incubation of biotin, ATP, and ^{32}PPi (inorganic pyrophosphate) with BCCP synthetase, results in the incorporation of radiolabeled inorganic pyrophosphate into ATP giving [^{32}P]-ATP with a rate constant of 20 s^{-1}.

ii. Incubation of biotin, ATP and BCCP synthetase in the presence of NH_2OH and in the absence of BCCP results in the isolation of



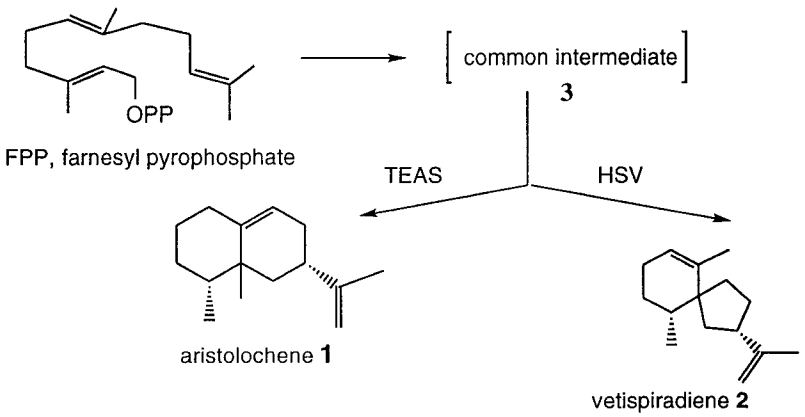
If the same experiment is repeated in the presence of substrate BCCP, no hydroxamate of biotin is trapped.

Questions: a. Propose a mechanism for the role of ATP in this reaction. Explain clearly how this mechanism accounts for the experimental observations given in i and ii.

b Suppose that your mechanism in **1** is correct, but that the exchange reaction described in i was unsuccessful, that is, no incorporation of ^{32}PPi into ATP was observed under the conditions described in part i. What other type of experiment might you attempt to address the same question? Describe the experiment and the controls in detail. Show the consequences of your experiments and state clearly your assumption(s).

1. Sesquiterpene cyclases (or synthases) are a family of enzymes that catalyze the conversion of farnesylpyrophosphate (FPP) to a variety of sesquiterpenes. Epi-artistolochene synthase (TEAS) and vetispiradiene synthase (HSV) have been proposed to catalyze formation of their respective products epi artistolochene (**1**)

and vetispiradiene (**2**) through a common ten membered ring intermediate (**3**). The constellation of residues around the active site of each of these enzymes govern how intermediate **3** is ultimately converted to a specific product. These two synthases are 72 % identical when their sequences are alligned. Interestingly a chimeric protein (a protein composed of both of these cyclases) has recently been made that can catalyze formation of both **1** and **2**.



You are given the following information about this system:

1. The structure was solved in 1997 of TEAS and is now deposited in a folder labeled problem set 5 (pdb.5EAT_cyclase)
2. The pre-steady state kinetics of the three cyclases (synthases) have been studied by rapid chemical quench and they all appear to behave in a similar fashion. The experiments involved the use of [^{3}H]-FPP and rapid chemical quench with KOH and EDTA (ethylene diamine tetraacetic acid). After quenching, the precipitated protein was removed and the product(s) (P) were extracted into hexane and then analyzed directly for radioactivity by scintillation counting. The results are shown in Figure 1.

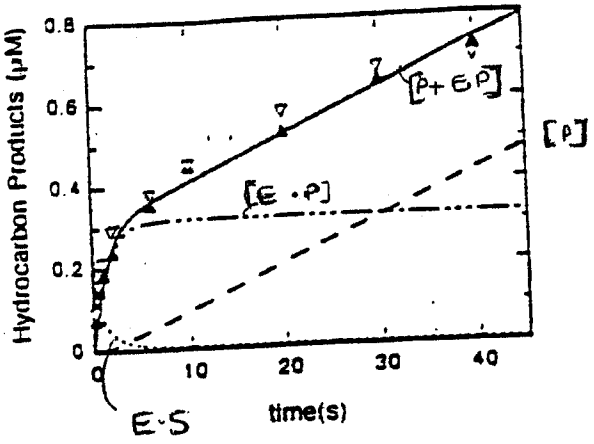
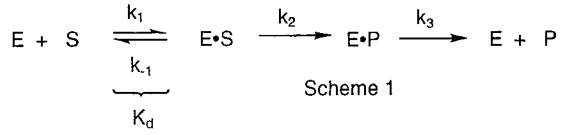


Figure 1 legend: Time course for the formation of sesquiterpene hydrocarbons by TEAS. Concentrations were 12.5 μM [^{3}H]-FPP and 560 nM enzyme. The size

of the data points represent the scatter in duplicate determinations. The open and closed triangles represent two different data sets. The displayed curves are simulated concentrations of intermediates in the reactions ([E•S], ; [E•P], ; [P], ; and [P] + [E•P],) based on the mechanism in Scheme 1. The rate constants employed are in Table 1.

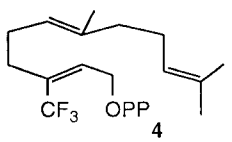


Scheme 1

Table 1 Kinetic Constants for TEAS Synthase

K_d (μM)	69 ± 25
k_2 (s^{-1})	0.7 ± 0.2
k_3 (s^{-1})	0.01 ± 0.003

3 Several FPP analogs have been designed to be inhibitors of the cyclases based on an understanding of the mechanism:



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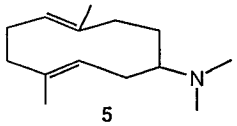

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Questions:

1. Pull up the structure of the cyclase complexed with farnesyl hydroxyphosphonate (FHP). Show the ligands to the metals and look carefully at the conformation of the bound FPP analog. Draw what you see with the appropriate distances to residues that might play an important role in catalysis.

2. Propose a structure for the common intermediate **3** produced by all three cyclases and show the mechanism by which this common intermediate is produced. Propose further, the mechanism by which products **1** and **2** are produced from this common intermediate . Given your proposed intermediates how might they be stabilized chemically to allow one reaction to proceed at the expense of the other?

3. In the pre steady state kinetic analysis, why was EDTA used in the chemical quench? What does the data in Figure 1 and Table 1 tell you about the mechanism? How might the authors of this paper have been able, with very little effort, to greatly improve this experiment? (think about the method of analysis)

4. Given your proposed mechanism in part 2, would compounds **4** and **5** be expected to be inhibitors of these cyclases? Provide an explanation for your answer using your mechanistic insight.