(Final) Exam 3

Please do not open or sign this packet until you are instructed to do so.

Please write all of your answers for this exam in this exam packet. Although you may use as many blue books for scratch work as you would like, the blue books will not be collected at the end of the exam or graded. Answer each question in the space provided if you can, but feel free to continue your answer on the back of the page if you need more room. (Please write a note by your answer pointing us to the continuation if you do this.) You will be given 2 hours total to finish the exam.

This exam contains two problems, which are split into parts. *Do not get stuck* on one part and then assume that you will be unable to answer the rest of the question—move on. In addition, partial credit will be given for incorrect but plausible or consistent answers, so *guess* on problems you cannot answer perfectly.

At the end of the 2-hour exam period you will be asked to return your exam to the proctor. (You may, of course, also turn the packet in earlier if you choose.) This exam is *open-resource*—you may use any books, notes, calculator, etc. you have brought with you to the exam. However, you are not allowed to communicate with anyone during the exam, or to bring any materials in or out of the room while you are taking the exam. You are also not allowed to use any devices that could be used to communicate with anyone (laptop computers, cellphones, etc.). Please do not take any part of the exam packet with you when you are done; everything will be returned to you after the exams are graded.

This packet should contain 11 pages, including this one. Please check to make sure that your packet contains 11 pages before beginning your exam.
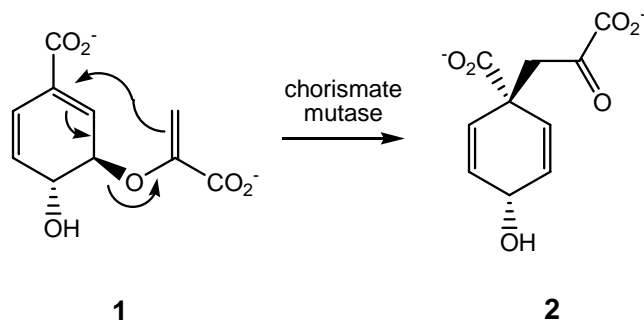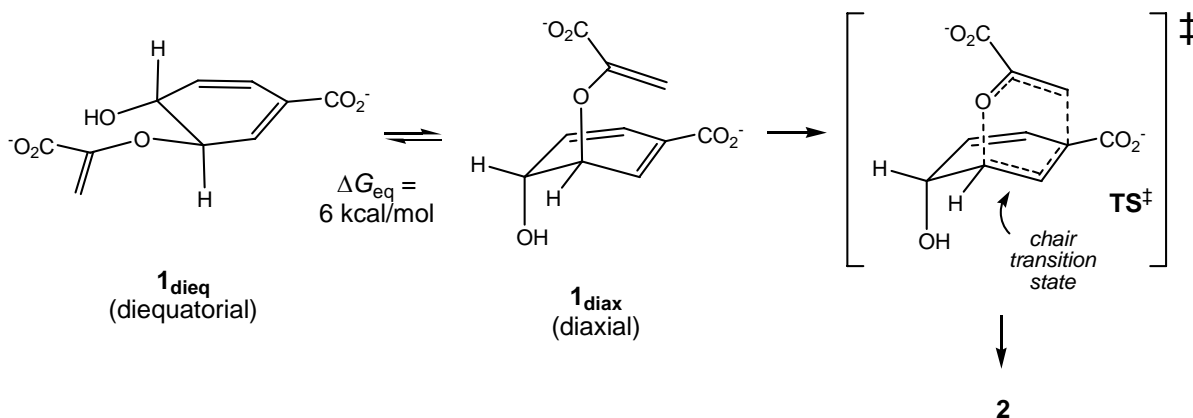
Name: _____

Signature: _____

Helpful constants to know for this exam:

|		
Boltzmann's constant:	$k_B = 2.94 \times 10^{-24}$ cal K^{-1}	$= 1.38 \times 10^{-23}$ J K^{-1}
Gas constant:	$R = 1.99$ cal K^{-1} mol^{-1}	$= 8.314$ J K^{-1} mol^{-1}
Planck's constant:	$h = 1.58 \times 10^{-34}$ cal s	$= 6.626 \times 10^{-34}$ J s
	$e = 2.718$	
	1 J $= 1$ N·m $= 1$ kg m^2 s^{-2}	

1. (60 pts total) Chorismate mutase is an enzyme that catalyzes the transformation of chorismate (**1**) to prephenate (**2**).

1   **2**

The concerted mechanism shown above occurs only when the substituents of **1** are in a "diaxial" conformation.[1] Computational studies predict that this is not the ground-state conformation of **1**, and that flipping the ring substituents from **1**$_{\text{dieq}}$ to **1**$_{\text{diax}}$ costs 6 kcal/mol.

1$_{\text{dieq}}$
(diequatorial)

$\Delta G_{eq} =$
6 kcal/mol

1$_{\text{diax}}$
(diaxial)

TS‡

chair
transition
state

2

Although this is definitely the mechanism of the uncatalyzed reaction, it is not clear whether the enzyme-catalyzed reaction occurs by this same mechanism, or if it does, whether the enzyme specifically stabilizes the transition state (**TS**‡) more than it stabilizes **1**$_{\text{diax}}$. An X-ray crystal structure of the chorismate mutase enzyme bound to inhibitor **3** (structurally similar to **1**$_{\text{diax}}$ and **TS**‡)[2] shows a number of specific H-bonding and electrostatic interactions, illustrated in the diagram on the right. The crystal structure also makes it clear that the diequatorial conformation **1**$_{\text{dieq}}$ would not be able to fit in the enzyme active site. Given this information, this problem explores the catalytic mechanism of chorismate mutase.


*from crystal structure,
chorismate mutase*

---
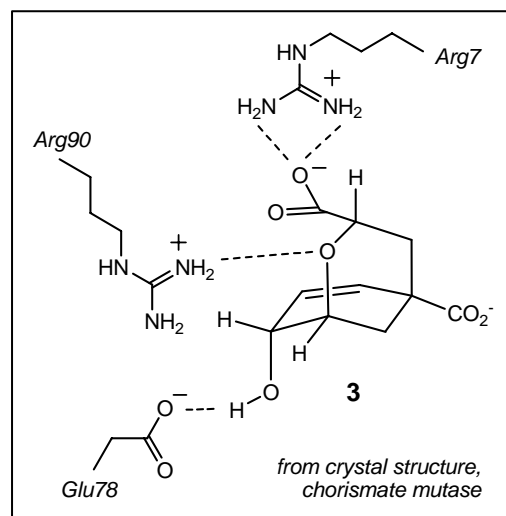
[1] Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 5306.
[2] From *B. subtilis*. Chook, Y. M.; Ke, H.; Lipscomb, W. N. *Proc. Natl. Acad. Sci USA* **1993**, *90*, 8600.

a. (15 pts) For parts (a-c) of this problem, assume that chorismate mutase catalyzes the same concerted reaction mechanism observed in the uncatalyzed reaction, and that the enzyme binds $\mathbf{1_{diax}}$ and $\mathbf{TS}^{\ddagger}$ with the same affinity (ΔG). **Draw a potential energy diagram** that illustrates

- The energy profile of the uncatalyzed reaction;
- The overall activation energy of the uncatalyzed reaction ($\Delta G^{\ddagger}_{overall,uncat}$);
- The energy profile of the enzyme-catalyzed reaction;
- The overall activation energy of the enzyme-catalyzed reaction ($\Delta G^{\ddagger}_{overall,cat}$).

.

Label each ground state in your diagram with an abbreviation that describes its chemical state. (E.g., "$\mathbf{E \bullet 1_{diax}}$" would be the complex of the enzyme with $\mathbf{1_{diax}}$.) Also label the rate-determining transition states for the uncatalyzed ($\mathbf{TS}^{\ddagger}$) and catalyzed ($\mathbf{E \bullet TS}^{\ddagger}$) reactions.

E

reaction coordinate
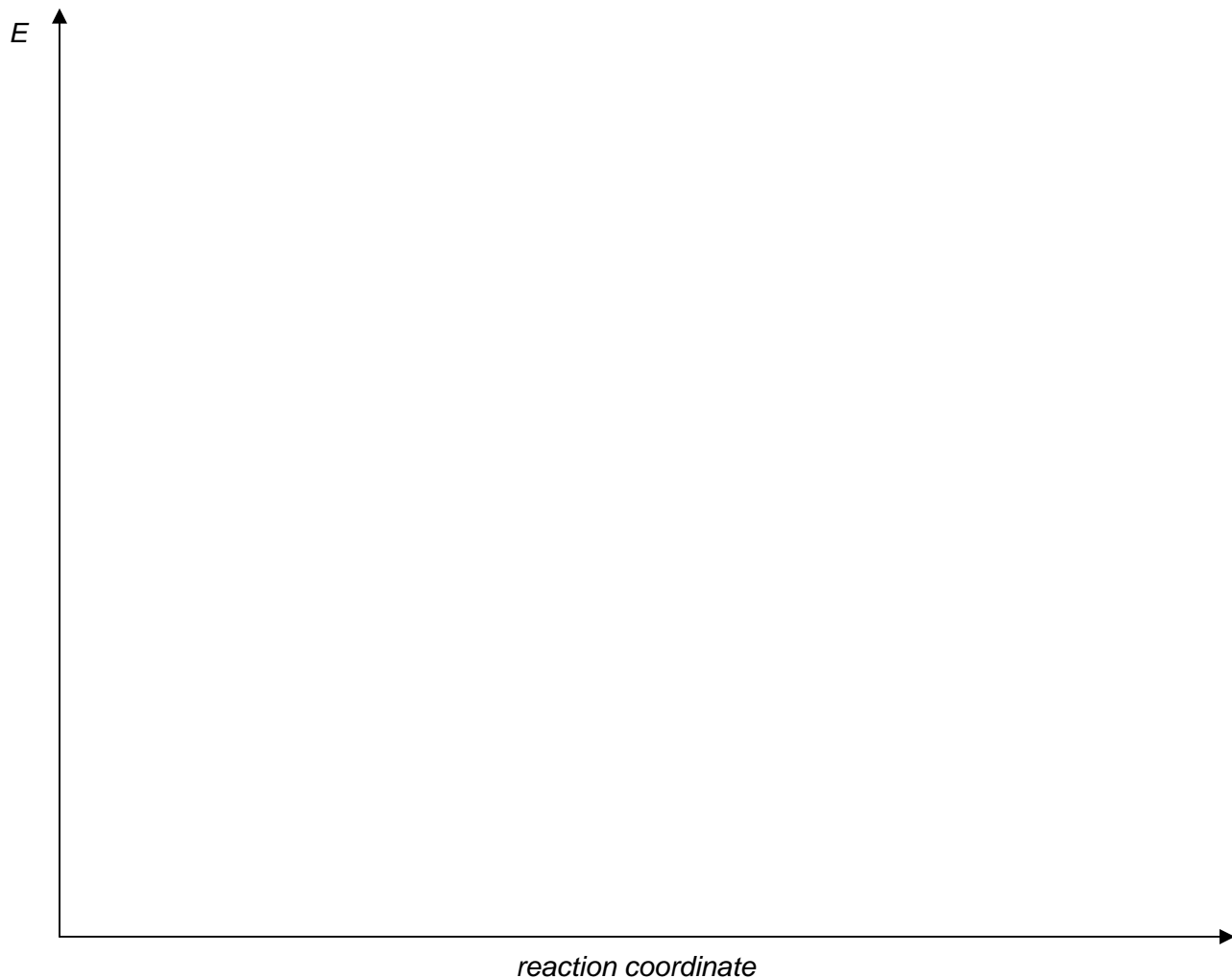
b. (5 pts) In the scenario you drew on the previous page, **what is the maximum value of** $k_{overall,cat}/k_{overall,uncat}$? Assume $T = 298$ K.

$$(k_{overall,cat}/k_{overall,uncat})_{max} = \boxed{}$$

Calculations (for partial credit if answer above is incorrect):

c. (5 pts) Given the reaction profiles you drew in part (a), **what would be the effect of the enzyme on $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the overall reaction?**
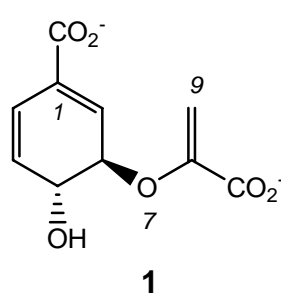
>, < or =

$\Delta H^{\ddagger}_{overall,cat} - \Delta H^{\ddagger}_{overall,uncat}$ $\boxed{}$ 0

>, < or =

$\Delta S^{\ddagger}_{overall,cat} - \Delta S^{\ddagger}_{overall,uncat}$ $\boxed{}$ 0

Hilvert and coworkers have demonstrated that amino acid 90 in chorismate mutase (shown in the diagram on page 2) must be a cationic residue; enzymes that have lysine instead of arginine at this position are still active, but other amino acids at this position give inactive enzyme, even when they are H-bond donors.[3] This observation suggests an alternative, two-step mechanism for the enzyme-catalyzed reaction in which C-O bond-breaking (to form a cation-stabilized enolate) and C-C bond-making occur in separate steps:
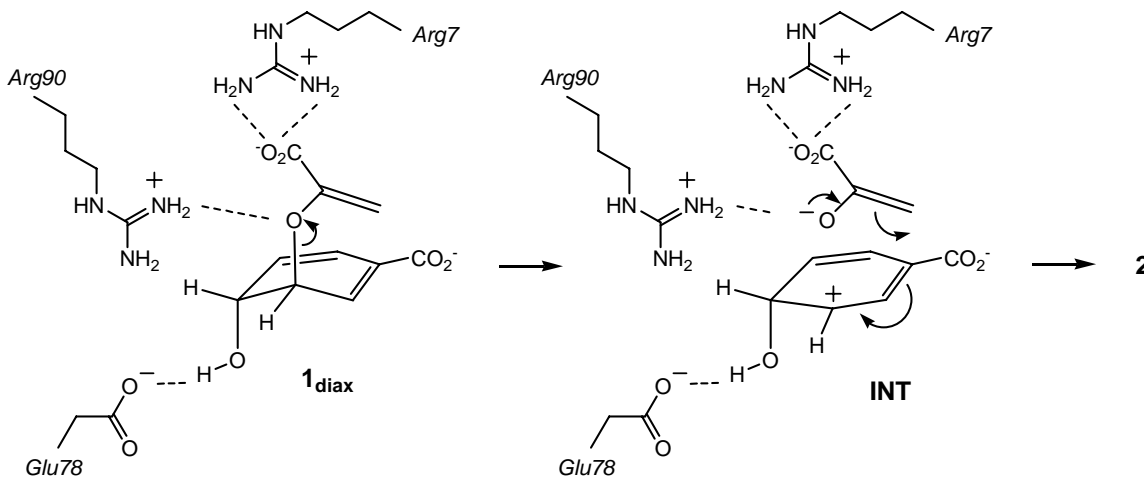
To test this hypothesis (and others), the Hilvert group measured kinetic isotope effects for a number of heavy atoms in **1**.[4]

	$\dfrac{k_{16_{O-7}}}{k_{18_{O-7}}}$	$\dfrac{k_{12_{C-1}}}{k_{13_{C-1}}}$	$\dfrac{k_{12_{C-9}}}{k_{13_{C-9}}}$
enzyme-catalyzed	1.045	1.0043	1.0129
uncatalyzed	1.0482	1.0118	1.0154

d. (15 pts) Clearly, the largest isotope effect the group observed was k_{16_O}/k_{18_O} at oxygen *7*. **What is the largest value of k_{16_O}/k_{18_O} one would expect to see for this substitution?** In your calculation, use $\nu_{\text{stretch}}(^{12}\text{C} - {}^{16}\text{O}) = 1000$ cm^{-1}. Assume $T = 298$ K. Answer on the next page.

[3] Kast, P.; Grisostomi, C.; Chen, I. A.; Li, S.; Krengel, U.; Xue, Y.; Hilvert, D. *J. Biol. Chem.* **2000**, *275*, 36832.
[4] Wright, S. K.; DeClue, M. S.; Mandal, A.; Lee, L.; Wiest, O.; Cleland, W. W.; Hilvert, D. *J. Am. Chem. Soc.* **2005**, *127*, 12957.
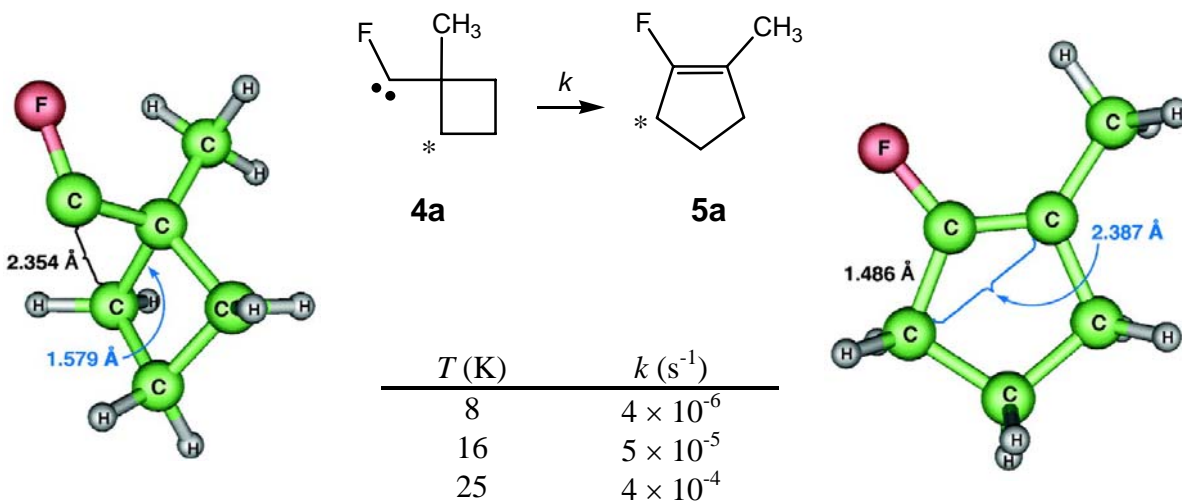
Calculations:
   (be as explicit as you can; we will award partial credit here, but only if we
   can understand what you've written)

$$\left(\frac{k_{16_{O-7}}}{k_{18_{O-7}}}\right)_{max} = $$

e. (20 pts) Hilvert found k_{16O-7}/k_{18O-7} to be much greater than either k_{12C}/k_{13C} value, and these results could, in principle, be used to justify a two-step mechanism in which C-O bond-breaking preceded C-C bond-making. But the results are also fully consistent with the concerted mechanism shown on page 2, with a transition state in which C-O bond-breaking and C-C bond-making are only halfway finished. What do I mean by this second statement? **Explain the large differences between k_{16O}/k_{18O} and k_{12C}/k_{13C} in terms of a *concerted* mechanism.** Illustrate your answer using separate potential energy diagrams and zero-point energy comparisons for both bond making and bond breaking.

(continue on the back of this page if necessary)

7

2. (40 pts total) "Carbenes" are neutral organic molecules that contain a divalent carbon atom, with only six valence electrons and two bonds to other atoms. (This is in contrast to more typical, tetravalent carbon atoms that possess four bonds and have a filled octet of valence electrons.) Carbenes are normally unstable and very reactive. Zuev et al. studied the isomerization of 1-methylcyclobutylfluorocarbene (**4a**) to 1-fluoro-2-methylcyclopentene (**5a**) by first generating the carbene at 8 K in a matrix of solid N_2 or Ar, and then monitoring the conversion to **5a** at different temperatures by infrared spectroscopy.[5] The first-order rate constants measured for the reaction in N_2 are shown below.

T (K)	k (s^{-1})
8	4×10^{-6}
16	5×10^{-5}
25	4×10^{-4}

The authors also calculated structures and energies for the reactant, product and transition state; the calculated structures for **4a** and **5a** are shown above. This computational analysis predicted ΔE^0 = -78.4 kcal/mol and $\Delta E^{\ddagger}$ = 6.4 kcal/mol for this reaction. (For the purpose of this problem, assume that ΔE^0 is like ΔG^0, and that $\Delta E^{\ddagger}$ is like $\Delta G^{\ddagger}$ or E_a.) Based on these calculations, and on the observed rate data, Zuev et al. argued that this reaction proceeds by quantum tunneling *through* the energetic barrier between **4a** and **5a**, rather than over the barrier through the classical transition state.

[5] Zuev, P. S.; Sheridan, R. S.; Albu, T. V.; Truhlar, D. G.; Hrovat, D. A.; Borden, W. T. *Science* **2003**, *299*, 867.

a. (10 pts) The proposed tunneling would primarily involve motion of the carbon labeled with an asterisk in the scheme (and its attached hydrogens). **How far would you expect a carbon atom to tunnel at 8 K?**

(include calculations)

b. (20 pts) **Explain *two* reasons why the kinetic data makes it seem as though tunneling is involved.** Provide quantitative/mathematical justifications for your answers. Assume that the measured rate constants are extremely accurate (even though they probably aren't).

Reason #1:

Reason #2:

c. (10 pts) Zuev et al. argued that the exothermicity of the reaction contributed to rate of tunneling. As evidence for this, the authors also studied the isomerization of *chloro*carbene **4b** to chlorocyclopentene **5b**.

4b   **5b**
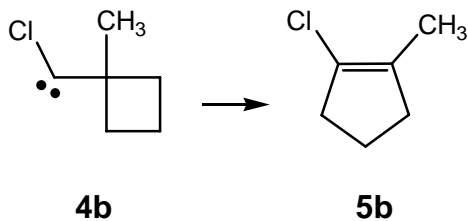
Computational analysis indicated that this reaction was more exothermic (ΔE^0 = -85.6 kcal/mol) and had a lower classical barrier ($\Delta E^{\ddagger}$ = 3.1 kcal/mol) than the conversion of **4a** to **5a**. Experimentally, the authors couldn't even observe **4b** at 8 K, because it isomerized to **5b** so quickly after being produced in the matrix. **Why would the tunneling rate be higher in this system** than in the reaction of **4a**?