

No Analytical crib available

9-23-06

Written by Professor Hovis

CUMULATIVE EXAMINATION IN BIOCHEMISTRY

Sep 23, 2006

It is expected that the answer should contain 1-4 succinct straight-to-the-point sentences. If you feel like elaborating, please stay within an 8 sentence limit anyway. All questions carry the same weight.

1. The protein Databank coordinate set 1MBO shows the structure of myoglobin with an O₂ molecule bound to the heme group. Close inspection of the structure reveals that there is no path available to O₂ to leave myoglobin. If so, then how can myoglobin unload O₂?

There is dynamics in actual protein that creates a "dynamic channel" (i.e. O₂ can travel through small cavities that are transiently opening in the structure). This has been extensively investigated by laser spectroscopy (e.g. PNAS 101, 18000).

2. In order to study folding kinetics, a stopped-flow fluorescence experiment has been performed on a small protein domain. In doing so, 1 part of a denatured protein solution was rapidly mixed with 9 parts of a refolding buffer; then a fluorescence signal (mainly from a single tryptophan present in the protein) was monitored as a function of time. Predict the shape of the experimental curve (fluorescence intensity vs. time).

Monoexponential. Small proteins typically fold according to two-state, 'all-or-nothing' model. This gives rise to a monoexponential curve, which simply reflects the build-up of the folded species. See Biochemistry 36, 3373 for the real-life example.

3. A protein sample was dissolved in D₂O and left on a bench for 10 mins. Then the sample was rapidly digested and the obtained peptide mixture was used to take a MALDI-TOF mass spectrum. The result: loop regions in the protein showed a higher level of deuteration than α -helices. Why?

In loop regions, backbone amide ¹H are readily exchanged for ²H. Not so in α -helices, where ¹H atoms are protected by hydrogen bonds and the exchange takes much longer than 10 mins.

4. A small protein contains three tryptophan residues. In order to identify the NMR signals of these residues (specifically, three well resolved lines from indole ¹H) we rely on a site-directed mutagenesis strategy. What is the minimum number of mutants that need to be prepared in order to assign all three spectral lines?

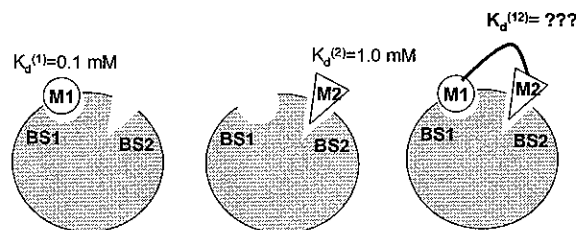
In addition to wild-type, we would need two mutants – in each of the mutants one of the Trp would be 'knocked out'. This strategy will only work if mutations do not cause

a major perturbation in the structure (we are usually safe in this regard, although Trp residues can be structurally important).

5. Briefly describe the principle of 'western blotting' technique.

(1) Run a gel. (2) Transfer proteins from the gel onto a membrane ('blotting'). (3) Detect proteins of interest using antibodies.

6. A protein has two binding sites, BS1 and BS2, located close to each other on the surface. Small molecule M1 binds to BS1 with dissociation constant $K_d^{(1)} = 0.1$ mM. Small molecule M2 binds to BS2 with $K_d^{(2)} = 1.0$ mM.



We now make a construct where M1 and M2 are covalently linked through a tether (assume that the tether is sufficiently long and flexible and does not interact with the protein). This construct, M1-M2, binds to the protein such as shown in the figure (right portion). Estimate the dissociation constant of the construct, $K_d^{(12)}$, and briefly describe your reasoning.

Ideally, we expect $K_d^{(12)} = 0.1$ μ M. Here is the reasoning. To break off the M1 fragment we need $\Delta G^{(1)} = -kT \ln(K_d^{(1)} / K_0)$ (in this expression K_0 describes the standard reference equilibrium where concentrations of all species are 1 M, i.e. $K_0 = 1$ M). To break off the M2 fragment we need $\Delta G^{(2)} = -kT \ln(K_d^{(2)} / K_0)$. To break off both M1 and M2 we need $\Delta G^{(12)} = \Delta G^{(1)} + \Delta G^{(2)} = -kT \ln(K_d^{(1)} K_d^{(2)} / K_0^2)$. Hence, $K_d^{(12)} = K_d^{(1)} K_d^{(2)} / K_0$, which is 0.1 μ M. Of course, in real life there could be binding cooperativity, interference from the linker, etc.

7. How many PCR cycles are needed to obtain $\sim 10^6$ -fold DNA amplification?

About 20 cycles. Since the number of strands of DNA doubles every cycle, $2^{20} \approx 10^6$.

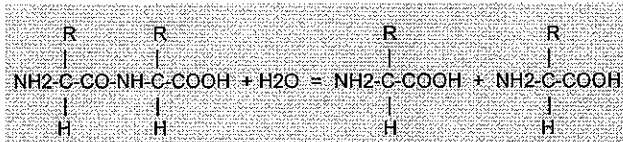
8. Vectors used for bacterial expression of proteins carry genes for resistance to antibiotics (e.g. ampicillin). What is the purpose of inserting these genes there?

To kill off bacteria which do not carry the vector and thus eliminate the competition.

9. What is the difference between “native” gel and SDS gel?

“Native” gel does not use SDS to denature proteins. As a result, the electrophoretic mobility of the protein in the “native” gel depends not only on mass (as in SDS-PAGE) but also on the protein’s charge and shape.

10. To confirm the result of protein expression, amino acid analysis is performed. Briefly, the protein is hydrolyzed into individual amino acids, the products are run through HPLC, and the resulting chromatogram is used to determine the content of different amino acids. Write the reaction of hydrolysis of peptide bond (you do not need to specify intermediates, catalytic species, etc.).



11. What is the main difference between a conventional fluorescence microscope and a *confocal* fluorescence microscope?

In the case of a confocal microscope, there is a screen with a pinhole in front of the detector. The pinhole position is conjugate to the focal point of the lens. As a result, only the light from a small segment of the sample (specifically, the segment around the focal point) is detected. “Out-of-focus” light is blocked. The sample can be scanned then point-by-point, and 3D image can be reconstructed on the computer. A very sharp 3D image can be obtained even from a relatively thick tissue sample – something that a conventional fluorescence microscope cannot do.

12. Consider a certain protein for which both X-ray and NMR structure is available. The resolution of the X-ray structure is 1 Å. The precision of the NMR structure (backbone heavy atom rmsd) also happens to be 1 Å. Which structure is more accurate?

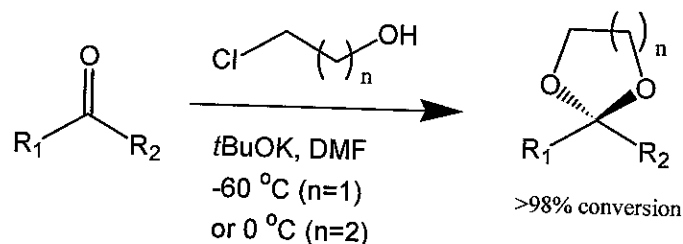
X-ray. The accuracy of the atomic coordinates in the case of X-ray structure with resolution 1 Å is, roughly, about 0.1 Å. On the other hand, in the case of the NMR structure with 1 Å precision the accuracy is about 2 Å.

No Inorganic crib available

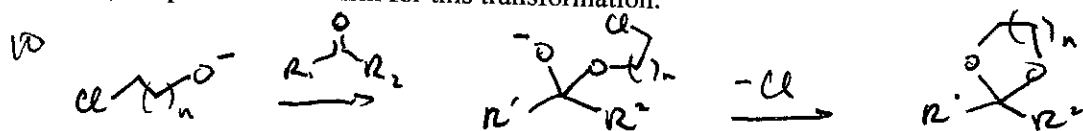
9-23-06

Written by Professor Robinson

1. (25 pts.) Aldehydes and ketones are usually converted into acetals under acidic conditions. Barbasiewicz and Małkośza have developed a useful alternative for preparing cyclic acetals using basic conditions (*Org. Lett.* 2006, 8, 3745-48).



a) Propose a mechanism for this transformation.

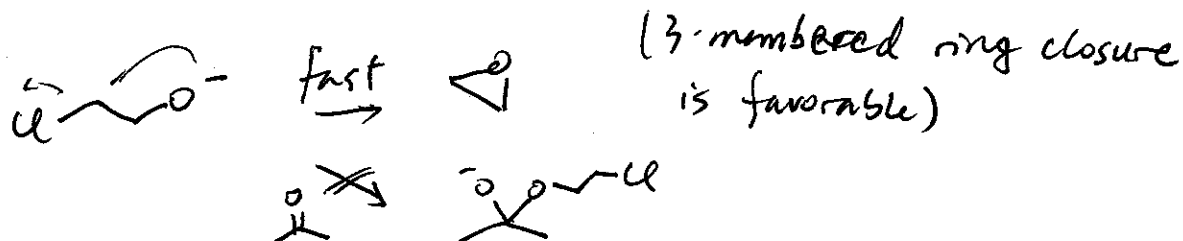


b) In the experimental procedure, *t*BuOK is added last (and slowly) to the reaction mixture. Why?

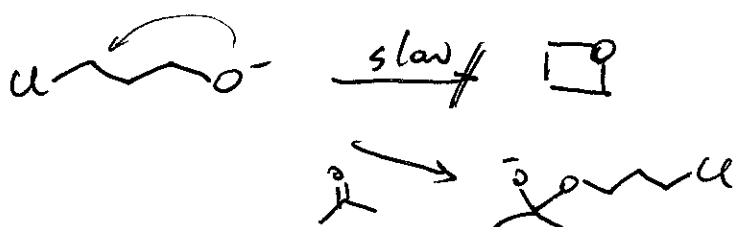
5 * To prevent ring ether (epoxide + oxetane) formation; also, to ~~reduce~~ reduce enolization (leads to aldol side product)

c) The formation of 5-membered cyclic acetals (1,3-dioxolanes) requires 1.5 equiv of chloroethanol at -60 °C for efficient conversion, whereas the formation of 6-membered cyclic acetals (1,3-dioxanes) only requires 1.05 equiv. of 1,3-chloropropanol and can be performed at 0 °C, making the latter more tractable for scaleup. Provide reason(s) for the difference in reaction conditions.

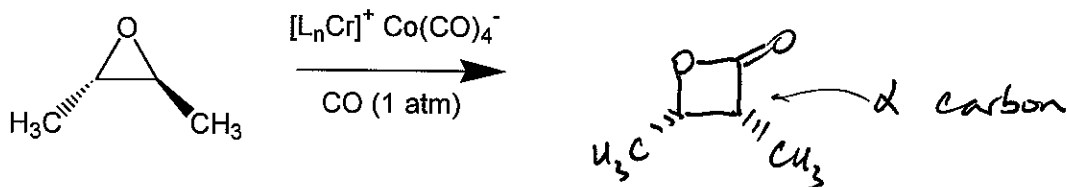
The epoxide formation is a significant side reaction:



Oxetane formation is much slower (4-membered ring closure)



2. (30 pts.) Coates and coworkers have developed a bimetallic Cr-Co ionic complex which can catalyze the formation of β -lactones from epoxides and CO in very high yields (*Org. Lett.* 2006, 8, 3709-12). The reaction is remarkable because the CO insertion can be performed at atmospheric pressure (1 atm). The reaction proceeds with inversion of configuration of one stereocenter, which becomes the α -carbon of the β -lactone.

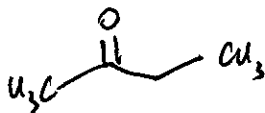


- a) Draw the expected product of this reaction.

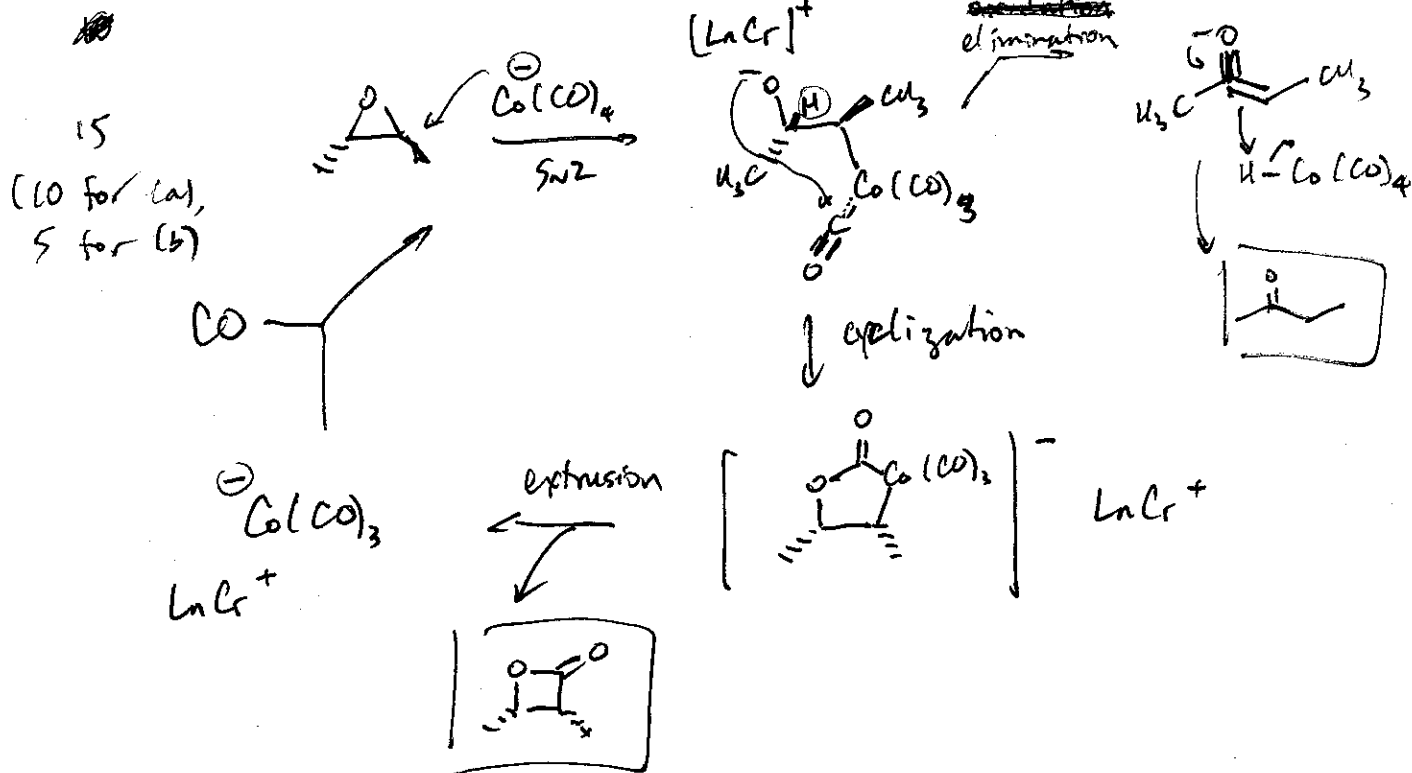
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- b) A ketone is produced as a minor product of the reaction above, with the same MW as the starting material. What is its structure?

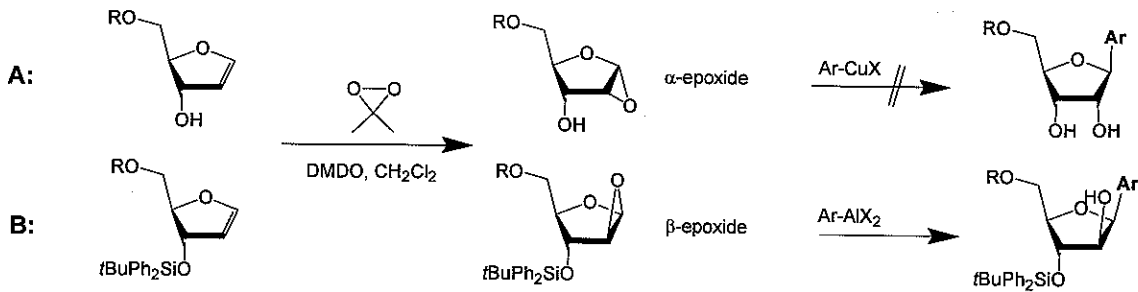
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- c) Propose a reaction mechanism with a catalytic cycle that can generate the products in (a) and (b).
Hint: $\text{Co}(\text{CO})_4^-$ is a good nucleophile.



3. (20 pts.) In their efforts to make unnatural C-nucleosides, Singh and Seitz have developed a methodology from furanoid glycols (*Org. Lett.* 2006, 8, 4319-22). These compounds were stereoselectively epoxidized by dimethyl dioxirane (DMDO), followed by addition of organometallic reagents:



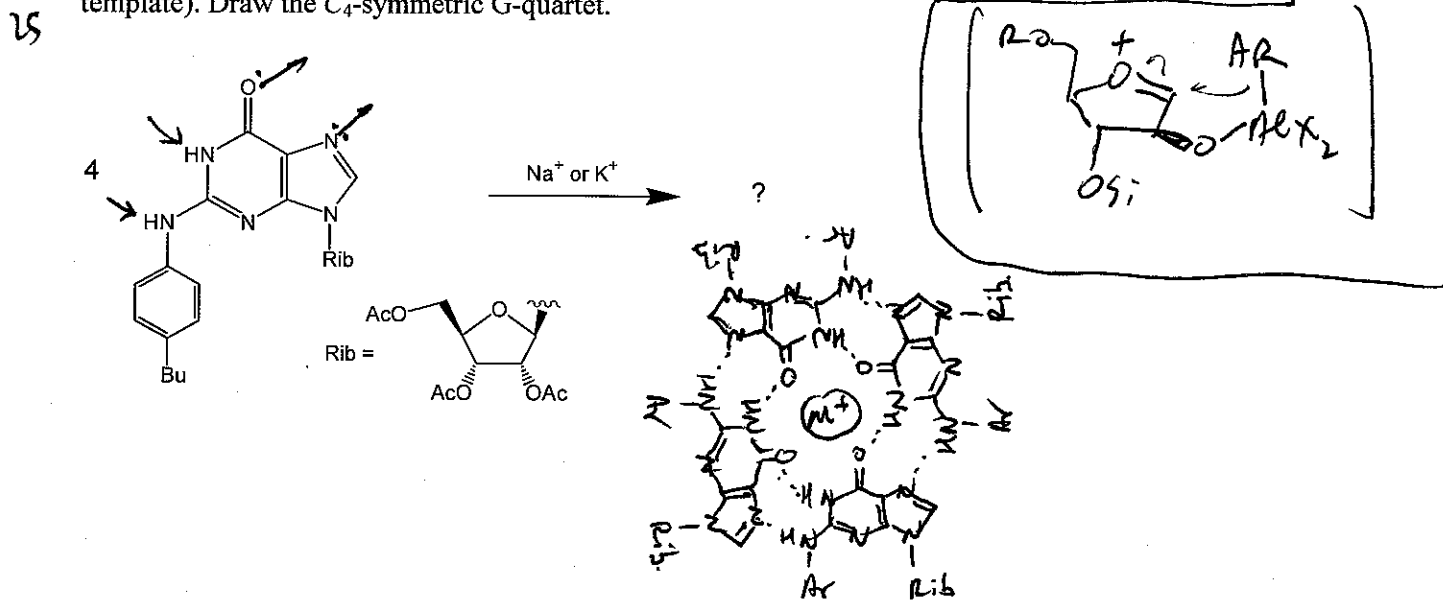
(a) Provide a reasonable explanation for the stereoselective epoxidations in reactions A and B.

10 **A:** H-bonding directs DMDO to α -face (CH₂OR group too far away for steric effect)
B: Steric hindrance may explain β -facioselectivity

(b) The organocuprate addition in reaction A failed, whereas the organoaluminum addition in reaction B produced the β -C-aryl furanoside in high yield and stereoselectivity. Give an explanation for each of these reaction outcomes.

10 - Free hydroxyl group in **A** quenches organocuprate (protonation)
 - Epoxide oxygen coordinates with Al species, followed by oxonium ion formation and syn-delivery:

4. (25 pts.) Liu *et al.* have synthesized a triacetylated guanosine derivative with an aryl substituent at N2 (*Org. Lett.* 2006, 8, 3685-88). When exposed to Na⁺ or K⁺, four of these molecules can self-assemble into a planar, hydrogen-bonded tetramer known as a G-quartet (the metal ion acts as a template). Draw the C₄-symmetric G-quartet.



Part 1

P. Chem Ans. Key

①

- 1.) $\sim 0.59 \text{ kcal/mol}$
- 2.) $\sim 100 \text{ kcal/mol}$; 418 kJ/mol
- 3.) $\sim 8070 \text{ cm}^{-1}/\text{eV}$
- 4.) $\sim 350 \text{ cm}^{-1}/\text{kcal/mol}$
- 5.) $\sim 3 \times 10^{19} \text{ molecules/l torr-L}$
- 6.) $\sim 30 \text{ GHz/cm}^{-1}$
- 7.) UV
- 8.) 10 nsec ; $100 \mu\text{s}$
- 9.) 5 psec ; 50 psec
10. 1 psec ; 100 fsec

Part 2

- 3.) NMR \rightarrow seconds; EPR, Rotational \rightarrow msec; IR \rightarrow μsec
visible $\rightarrow 100 \text{ nsec}$; UV $\rightarrow 10 \text{ nsec}$
- 4.) NMR \rightarrow Hz ; EPR; Rotational \rightarrow kHz ; IR = MHz
visible $\rightarrow 10 \text{ MHz}$; UV $\rightarrow 100 \text{ MHz}$.
- 5a) IR \rightarrow Intra molecular Vibrational Redistribution.
- b) UV \rightarrow Internal conversion ; Intersystem Crossing.

Part 2 (cont.)

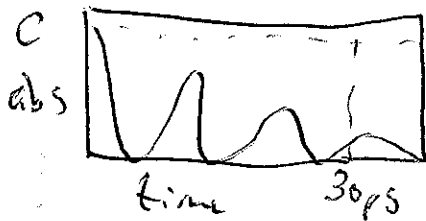
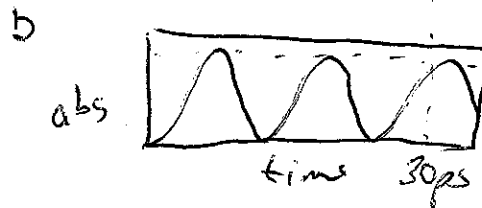
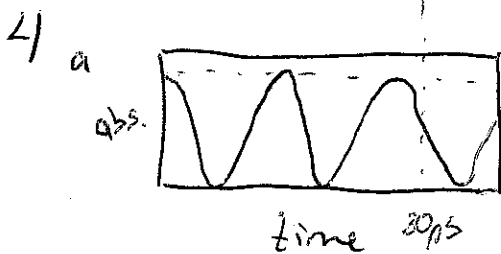
(2)

- 6.) 1.) Power Broadening
- 2.) Pressure Broadening
- 3.) Dopple Broadening

(Any reasonable answer accepted).

Part 3.)

- 1.) $T_1 \rightarrow$ population relaxation ; $T_2 \rightarrow$ Dephasing.
- 2.) ~ 10 ps.
- 3.) - Pulse width in time \ll lifetime of bright state
- Energy levels are in a superposition of states.



5.) No rotations are slow. cf. experiment.

6.) Estimate $\langle v \rangle \sim 100$ m/s @ room temp.

Estimate mean free path @ RT; 1 torr 10^{-5} m

collision frequency 100 ns