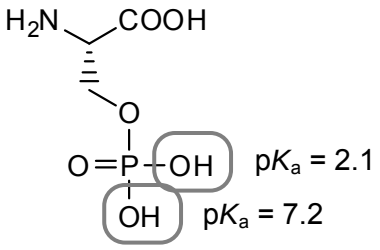
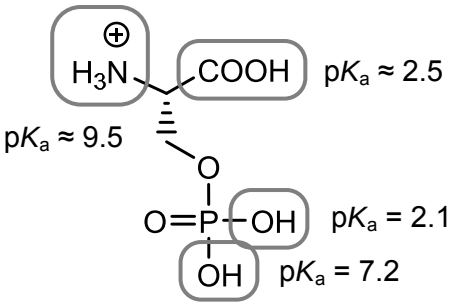
1. (48 pts)

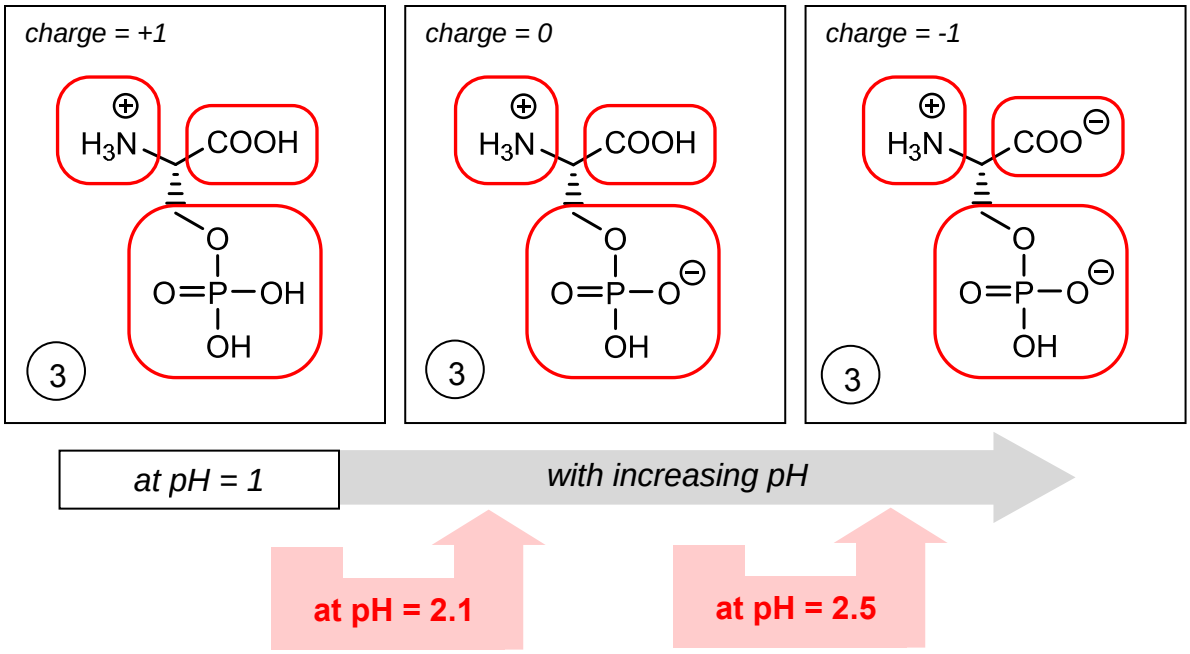
a. L-Phosphoserine is a modified amino acid that is generated in proteins by phosphorylation of serine residues. The amino acid side chain has two acidic protons, which exhibit different pK_a values, as shown at right. What would be the structure and charge state of phosphoserine under extremely acidic conditions, at pH = 1? Then, how would the structure change with increasing pH?



L-phosphoserine



Under strongly acidic conditions—at pH = 1—every functional group in phosphoserine that can pick up a proton, does. Then, as pH is increased, these groups lose protons in order, from most acidic to least acidic. The most acidic proton (the one with the lowest pK_a, at 2.1) is on the phosphate group, followed by the carboxylic acid proton. (Carboxylic acid groups in amino acids all have pK_a values around 2.5, and ammonium cation groups in amino acids all have pK_a's near 9.5.) That means our charge states look like this:



Rubric for this part:
3 points for each box.
+1 point for each correctly illustrated protonation/charge site (circled in red above, for a total of 3 points each box).
No partial credit if the total molecular charge doesn't match the box.

b. What would you predict for the isoelectric point (pI) of phosphoserine?

pI = 2.3 (3)

for any answer between 2.1 and 2.4

We can calculate pI by averaging the pK_a's of the acid-base transitions that border the charge = 0 state. In this case, those pK_a's are 2.1 and 2.5, so pI = 2.3.

c. If you analyzed a mixture of phosphoserine and serine by ion exchange chromatography, using a solvent gradient of increasing pH, would you expect phosphoserine to elute

earlier (2) **later** *or* **at the same time**

relative to serine? *(Circle one answer.)*

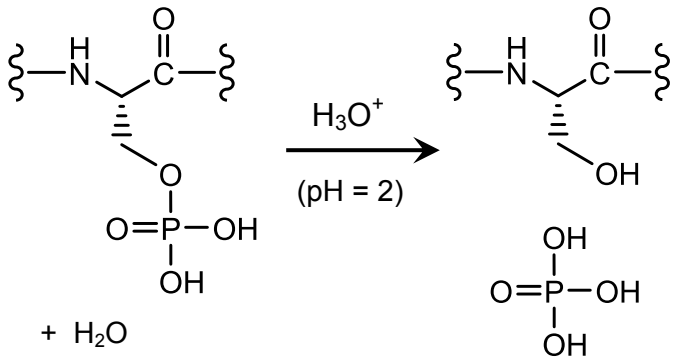
In ion exchange chromatography, a mixture of molecules is loaded onto a charged column at low pH, where the molecules are positively charged, and bind to the negatively charged material in the column. Then, the column is exposed to a solvent gradient of gradually increasing pH; as molecules turn from positively charged to neutral, when each molecule reaches pH = pI, it loses its grip on the column and elutes. Phosphoserine has a much lower pI than serine (pI = 6.0, which you can calculate from the acid and ammonium pK_a's in serine), so phosphoserine elutes earlier.

d. At pH = 7, if a kinase enzyme were to convert serine residues on the surface of a target protein to phosphoserines, would the protein

become **more positively charged?** | become **more negatively charged?** (2) | *or* retain **the same charge state?**

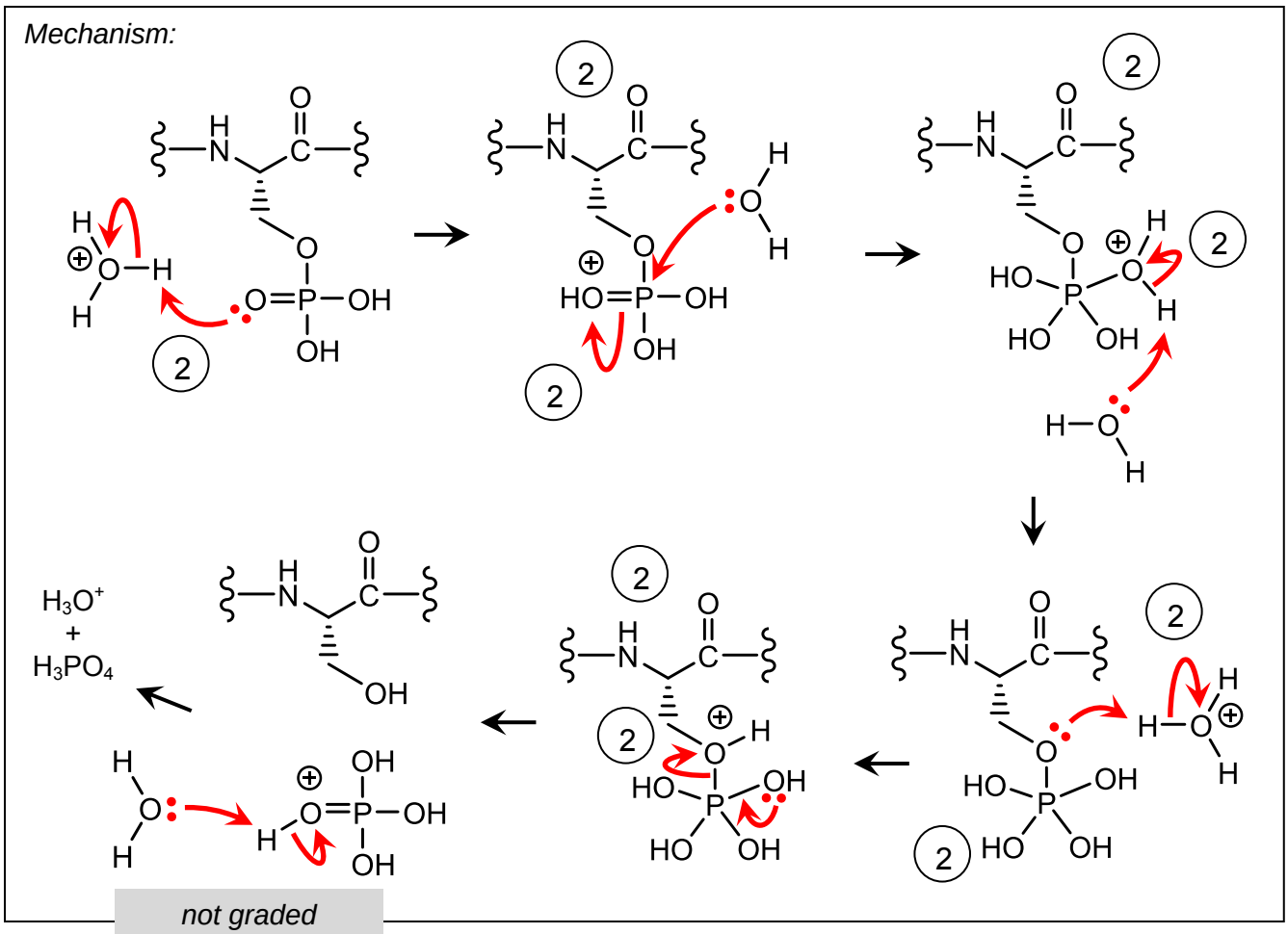
(Circle one.)

e. Ingested, phosphoserine-containing proteins may not survive our stomachs. Under acidic conditions, phospho-serine residues on the surfaces of proteins can undergo acid-catalyzed, nucleophilic phosphoacyl substitution, and can be hydrolyzed back to serines. Draw a mechanism (using "electron pushing") for this process. Draw each molecule and mechanistic step explicitly; don't cheat by combining multiple processes in a single step.



In class, I argued that the chemistry of P=O bonds is very similar to the chemistry of C=O bonds—that the same patterns we observe for carbonyl esters, we also observe for phosphoesters. That means that the phosphoester above reacts a lot like a carbonyl ester, via nucleophilic phosphoacyl substitution (analogous to nucleophilic acyl substitution). This reaction occurs under acidic conditions, so we'll avoid drawing negatively charged intermediates by using protonation under acid:

Mechanism:



not graded

Rubric: *(18 points total.)*

2 points for each electron-pushing step.

2 points for each intermediate.

Overall notes:

Overall, the minimum score for each step is zero; errors in a step cannot earn you negative points that count against another, correct step.

Spectators may be omitted.

Resonance is NOT a mechanistic step; it is just multiple ways of drawing the same intermediate. As a result, we did not evaluate electron-pushing that showed resonance—you can push electrons for resonance, or not, your choice. In addition, resonance does not have to be shown as an explicit "step"—it can be combined with adjacent steps, for full credit.

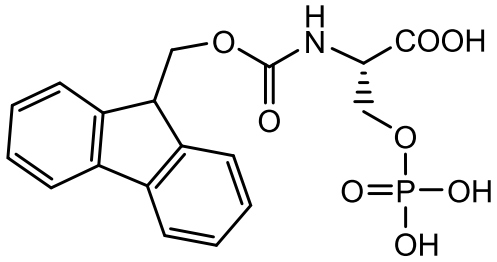
-1 point, for each arrow in each step, for errors in drawing arrows. Arrow must start at an electron pair, and end at nucleus where electrons will newly interact. Can only lose points if you get them.

-1 point for each minor error in charge, valency, structure, etc.; if error propagates, points are taken off only for initial error.

-1 point for each use of a generic or incorrect acid/nucleophile/base. You can pretty much only use H_3O^+ and H_2O as acid and base in this problem.

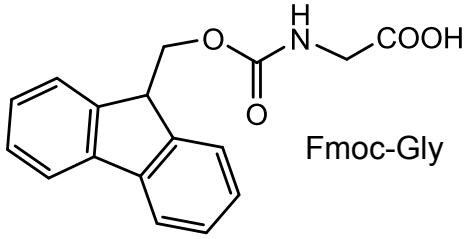
If you combine steps that can't be combined, you can get points for arrows that are in the rubric, but not for the intermediate you skipped.

f. Phosphopeptides—peptides incorporating a phosphoserine residue—can be synthesized via solid-phase peptide synthesis using the Fmoc-protected phosphoserine ("Fmoc-PS") reagent shown at right. In the box below, propose a multistep synthesis of the dipeptide phosphoserinylglycine (H_2N-PS-Gly-COOH), starting from Wang resin (drawn in the box below). You do not need to draw any chemical structures to answer this problem; you can refer to molecules by name or chemical abbreviation. In addition to the starting materials I've drawn, you can use any reagents and reactions we've learned about in class. *(Wang resin is cleaved in acid.)*

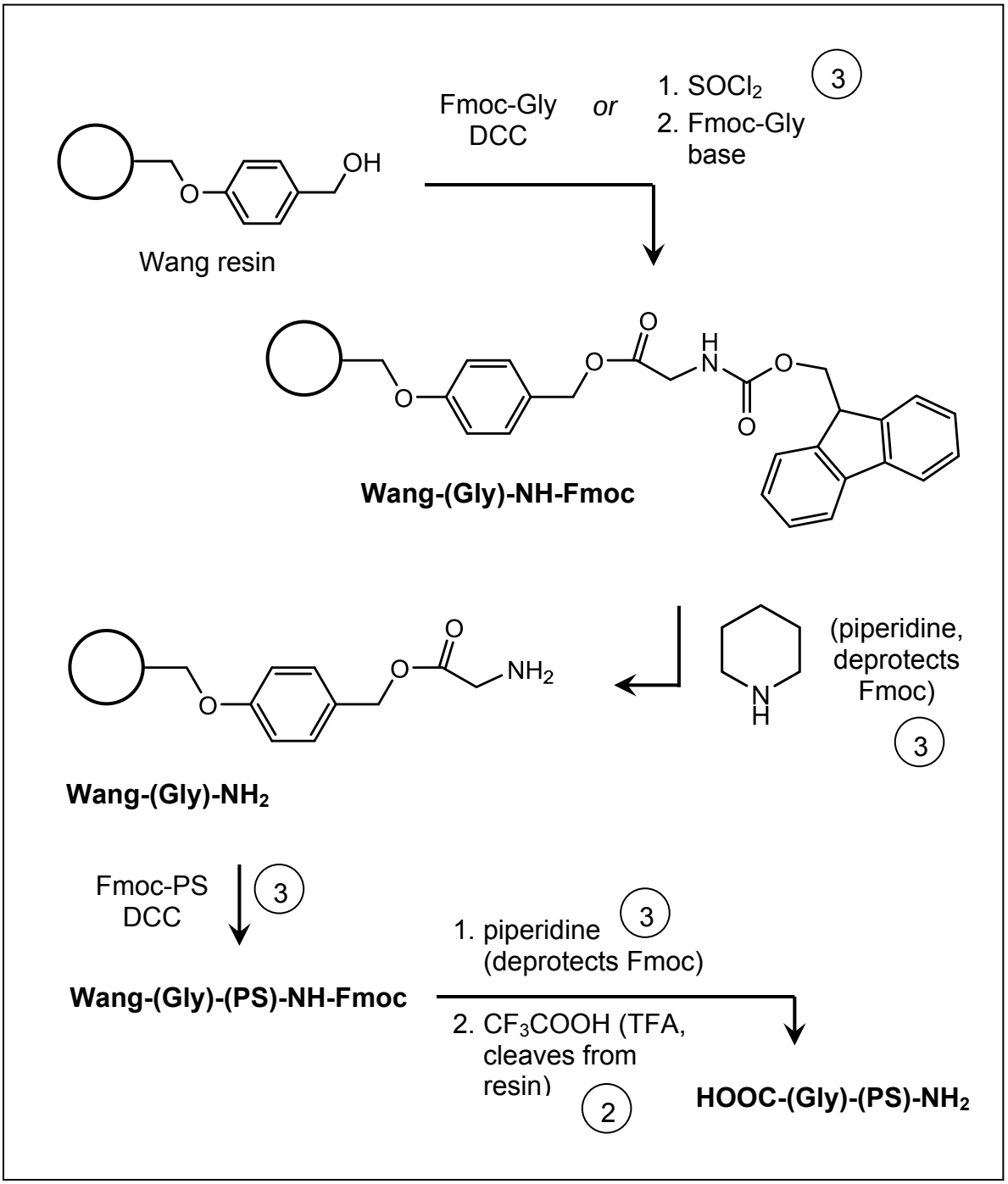


Fmoc-PS

Solid-phase peptide synthesis goes from the C-terminus of a peptide to its N-terminus, so we need to start by attaching Fmoc-Gly to the resin, and then Fmoc-PS. We **must** use Fmoc-Gly, and not *t*Boc-Gly, because *t*Boc deprotection would prematurely cleave the peptide from the resin. I've illustrated the synthesis with some chemical structures on the next page, but you could have answered with chemical names or abbreviations instead.



Fmoc-Gly



Rubric for this part (14 points total for above box):

This synthesis requires five tasks, listed below. Each task is judged separately, and does not require that the synthesis makes sense, or that other tasks are correct. However, you could lose points for putting steps out of order, if having them out of order caused the synthesis to fail.

We did not judge structures in this problem; you could abbreviate reagents, intermediates and products how you liked, as long as they were identifiable. We judged each element of your synthesis only on conceptual content.

-2 points if step reagents are incorrect, but reaction could otherwise be accomplished with correct reagents; or if reagents were correct, but gave the wrong outcome.

1. **Combine Wang resin with Fmoc-Gly (3 points).**
   For full credit, you needed to add Fmoc-Gly first (and not Fmoc-PS).
   -2 points for adding Fmoc-PS first instead, or for not identifying the amino acid used.

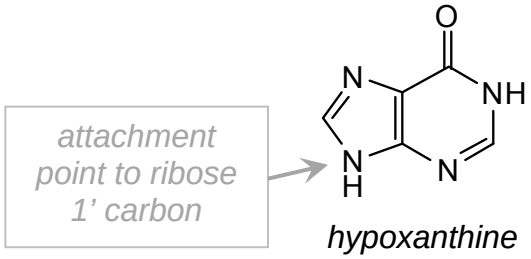
2. **Deprotect first Fmoc with piperidine (3 points).**
   -2 points for deprotecting before adding amino acid to support (before step 1).

3. **Add Fmoc-PS with DCC (3 points).**
   -2 points for adding Fmoc-Gly *instead, or for not identifying amino acid.*
   -2 points for omitting DCC.

4. **Deprotect Fmoc protecting group with base (3 points).**

5. **Cleave dipeptide from solid support with acid (2 points).**
   TFA would normally be used here, but any acid was accepted.

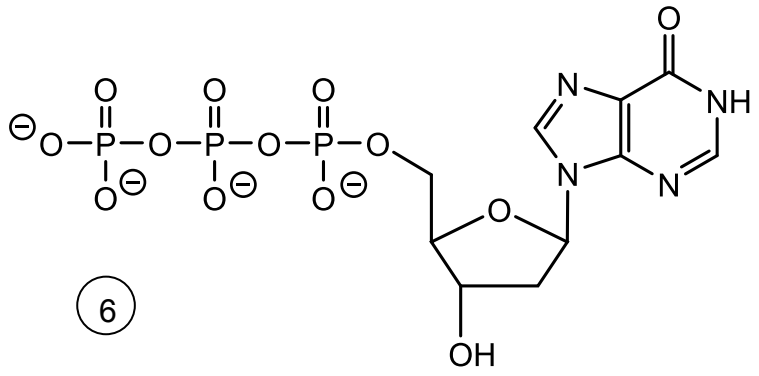
2. (9 pts) Inosine is a non-natural nucleoside that is formed when a hypoxanthine base is attached at the 1' position of a ribose sugar. Inosine can be incorporated into DNA and RNA by both biological and synthetic methods.



hypoxanthine

a. DNA polymerase can incorporate 2'-deoxyinosine into DNA when it accepts 2'-deoxyinosine triphosphate as a substrate. Draw the structure of 2'-deoxyinosine triphosphate.

2'-deoxyinosine triphosphate

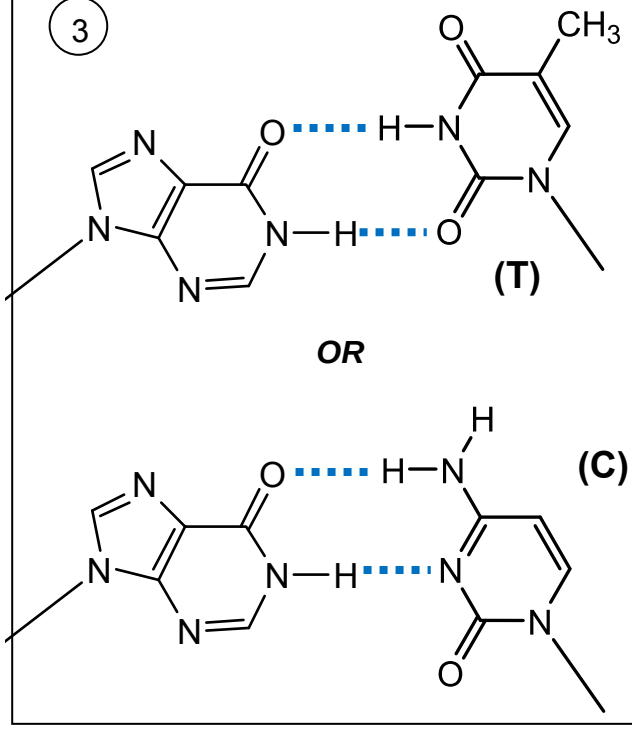


Rubric for this part: *(6 points total.)*
2 points for purine attachment.
2 points for 5'-triphosphate structure/attachment.
2 points for 3'-hydroxy and no 2'-hydroxy group.

b. Inosine can base-pair with any of the four typical DNA bases, though the DNA double helix accommodates a pyrimidine across from inosine better than a purine. Draw a base pair between the hypoxanthine base of inosine (already drawn in the box for you) and either of the two pyrimidine bases typically found in DNA. Illustrate hydrogen bonding in your drawing. You do not need to draw the sugar or phosphate parts of DNA; just draw the base.

hypoxanthine-pyrimidine base pair

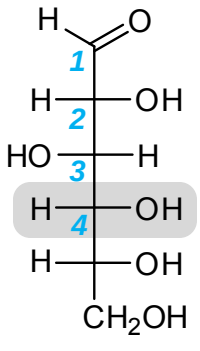
3



3. (30 pts) Lactose is a disaccharide formed from glucose and galactose; it is a glucosyl glycoside of galactose.

a. D-Galactose is the C-4 epimer of D-glucose, shown at right. Draw a Fischer projection of D-galactose in the box provided.

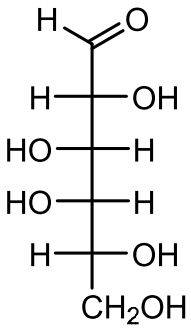
An epimer has its stereochemistry switched at the given position. To draw galactose, we just switch the stereochemistry at C-4.



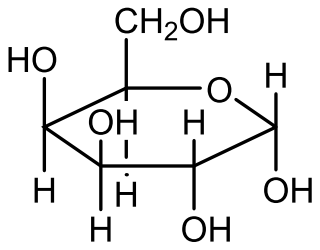
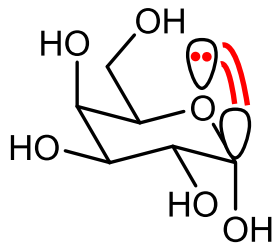
D-glucose

acyclic form of D-galactose (Fischer projection)

3

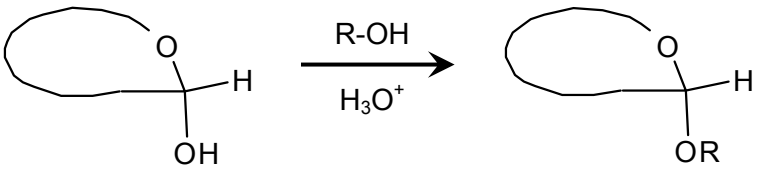


b. Acyclic D-galactose equilibrates with a cyclic, α-galactopyranose (6-membered ring) form. Draw both a Haworth projection and the most stable chair conformer of that cyclic form in the boxes on the next page.

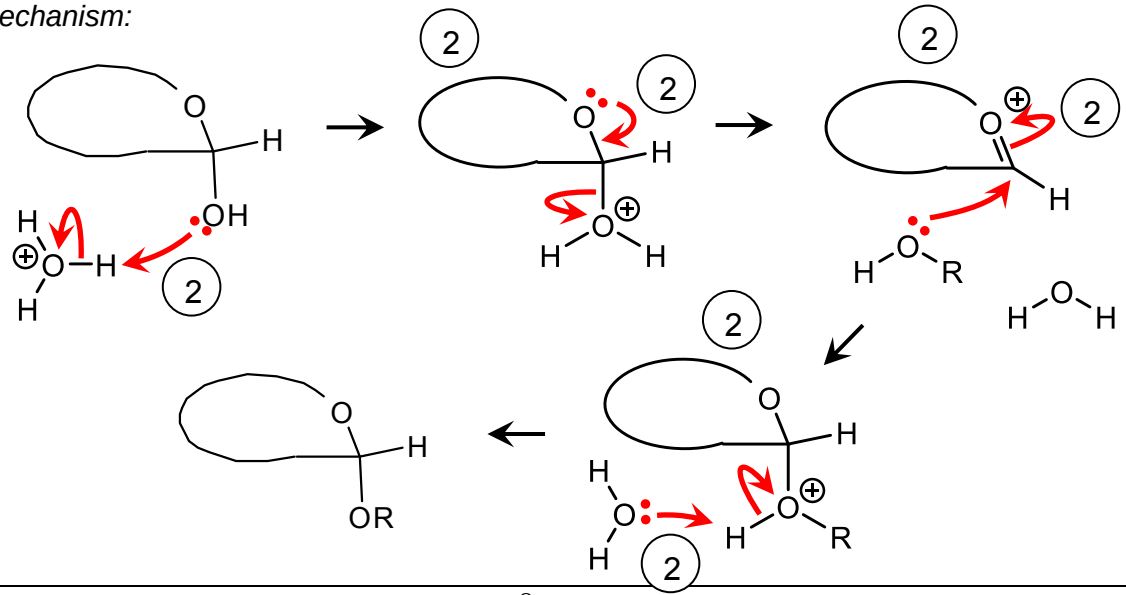
cyclic α-D-galactopyranose (Haworth projection)	*cyclic α-D-galactopyranose (chair conformer)*
	

c. Although the α-anomer of galactose that you drew is less stable than the β-anomer, the α-anomer is still stabilized by the "anomeric effect", a specific molecular orbital interaction. Illustrate that molecular orbital interaction on your chair drawing above, drawing lobes for orbitals.

d. Cyclic α-galactose (a hemiacetal) spontaneously forms glycosides (acetals) with other alcohols, like glucose, in the presence of an acid catalyst. Using my cartoon illustrations below, draw a mechanism on the next page for the formation of glycoside from galactose.



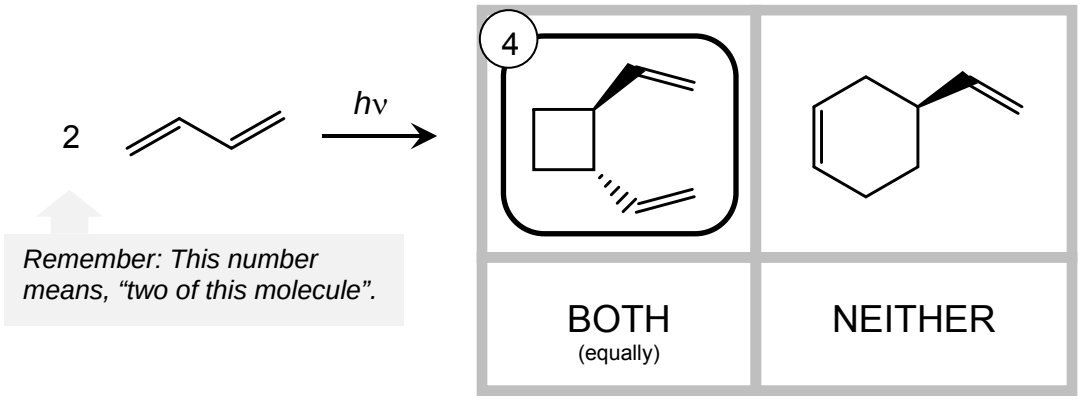
Mechanism:

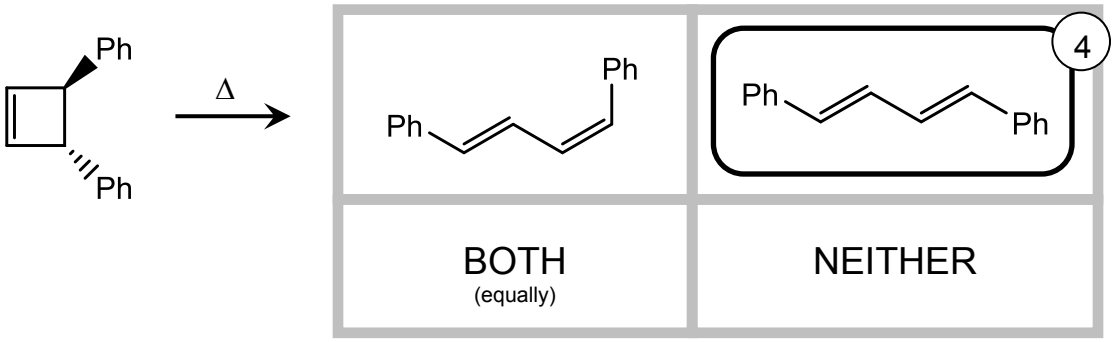


e. What is the timescale of the reaction on the previous page? Does it take place spontaneously over

minutes? **weeks?** *or* **decades?** (2) *(Circle one.)*

4. (8 pts) Each of the reactions below is drawn with two possible products. Circle the preferred product. If the two products are produced equally, circle "BOTH". If neither product would result from the reaction, circle "NEITHER". **Circle one answer only.**





5. (5 pts) For the reaction below, fill in the empty box corresponding to product. Give only one answer. If you expect the reaction to yield multiple products, draw one major product. If the reaction yields multiple enantiomers, draw only one enantiomer in the box, and include the note "+ enantiomer".