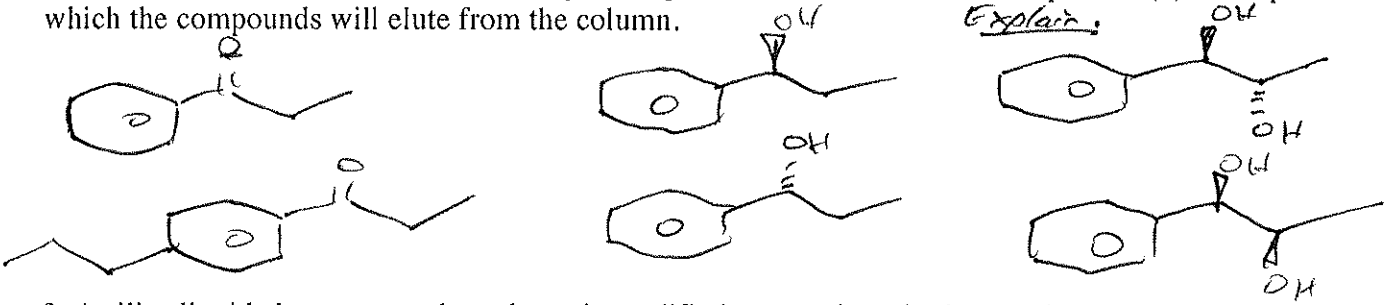
Problem Set 5

Five points. Due Friday, March 22, by the start of class.
Provide answers on a separate sheet(s) of paper, stapling together multiple pages.

1. Propose a structure for an "unknown" compound given the following data:
Molecular weight 146.1, elemental analysis 82.2% C, 6.9% H, 10.9% O (formula $C_{10}H_{10}O$)
How many degrees of unsaturation exist in this compound? Deg. unsaturation = rings + π bonds
^{1}H NMR: 9.7 ppm (doublet, 1 H), 7.2 ppm (doublet, J = 8 Hz, 2 H), 7.0 ppm (doublet, J = 8 Hz, 2 H), 6 ppm (doublet, 1 H), 5.5 ppm (doublet of doublets, 1 H), 2.35 ppm (singlet, 3 H)
^{13}C NMR: 195, 153, 150, 138, 132, 130, 126, 32 ppm
IR: strong band at 1680 cm^{-1}

use handout

2. A mixture of the following molecules is analyzed by each of the following techniques: normal (silica) chromatography and reversed phase chromatography (using hydrocarbon bonded to silica). For each technique, indicate (a) the number of different compounds/peaks observed via the technique and (b) the expected order in which the compounds will elute from the column.



Explain:

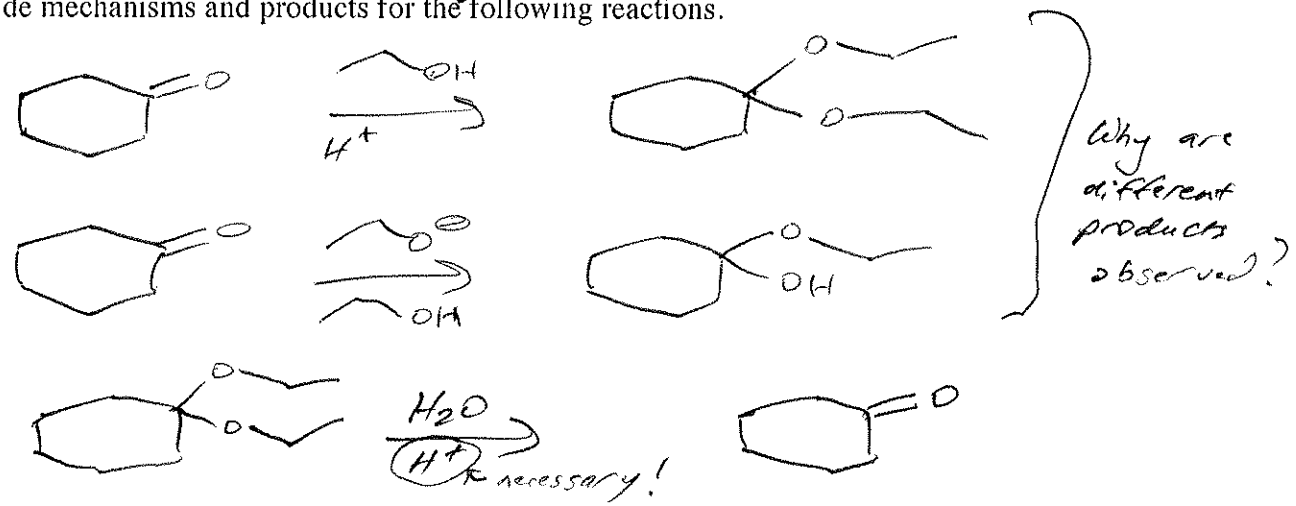
3. A silica liquid chromatography column is modified to contain a single enantiomer of L-phenylalanine (a chiral, single enantiomer amino acid) attached to the stationary phase. When applied to the mixture in 2 above, an additional product is observed compared to what is observed with unmodified silica. Explain.

4. Proteins contain a diverse array of functional groups. Some (e.g. amines, alcohols) exchanage essentially immediately (< 1 second) with D_2O. Others exchange more slowly, in a manner dependent on structure of the protein and exposure to solvent (structured regions exchange slowly (because the amides do not interact with water, and thus cannot exhcnage), unstructured regions exchange quickly). The rate of H->D exchange thus provides a readout of 3-dimensional structure. H-D exchange is identifiable both by mass spectrometry and by NMR (amide protons are easily observed by NMR (δ = 8-10 ppm) and provide important structural information about proteins (based on their chemical shift and their coupling to other protons).

→ Amides!

For the protein represented schematically on the ~~second~~ final page, indicate the mass of the protein prior to H-D exchange, at an early time in H-D exchange (10 minutes), and after complete exchange has occured (16 hours).

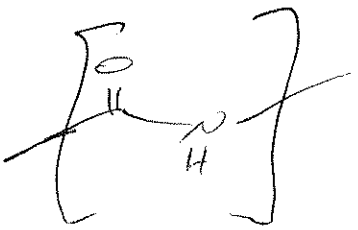
5. Provide mechanisms and products for the following reactions.



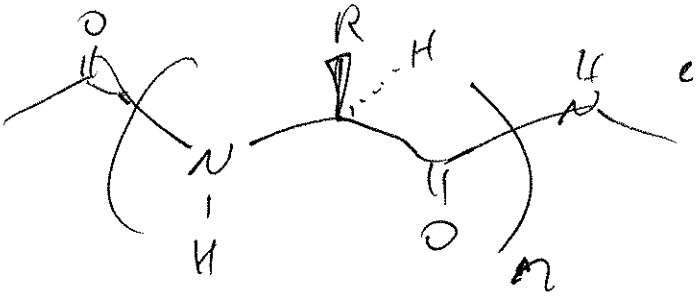
Why are different products observed?

H_2O, H^+ necessary!

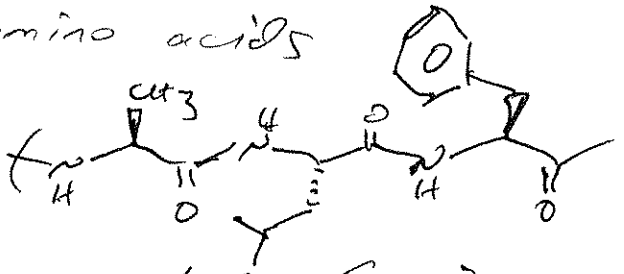
Amide functional group



Protein = polymer of α-amino acids



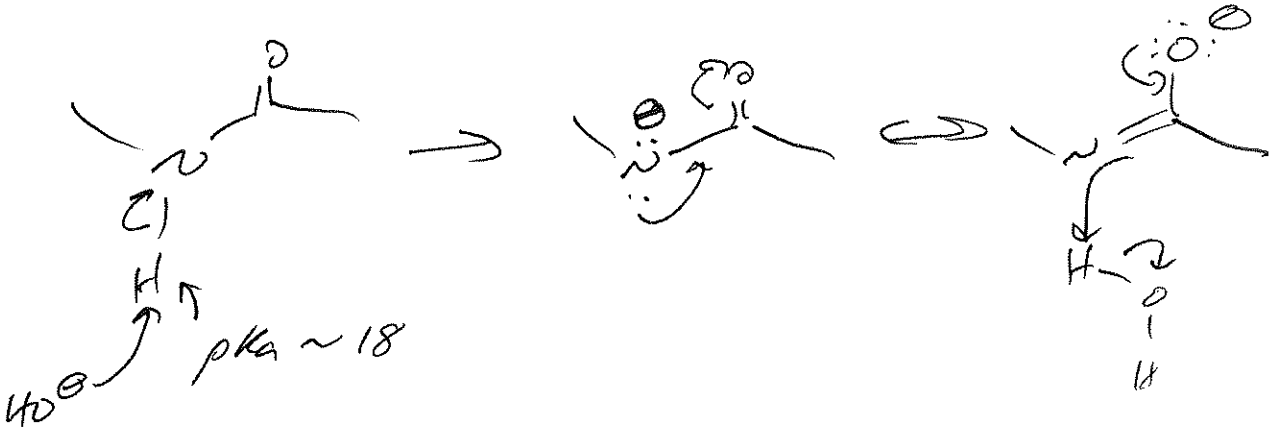
e.g.



n = number of amino acids in protein

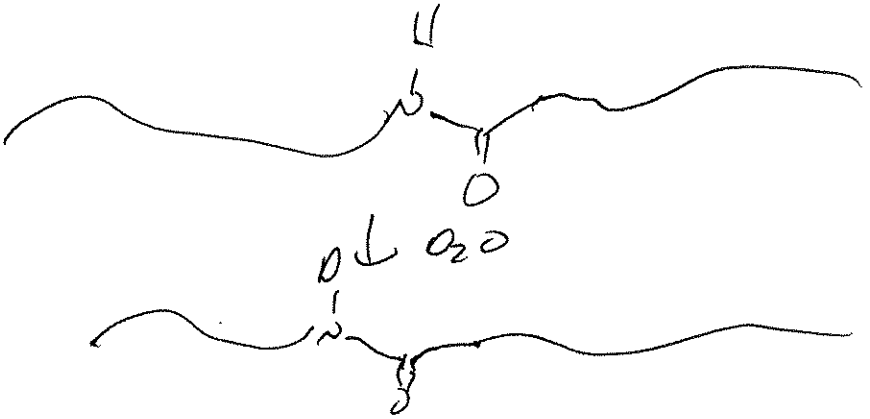
observable by NMR; couples to other protons and spin-½ nuclei. Exchanges on timescale from 1 second to hours (even days), depending on pH and accessibility to solvent.

Exchange faster at higher pH via



So, a protein with 100 amino acids has ~100 amide protons. Exchange rates of amide protons depend on 3-D structure. For example, in an α-helix

amide groups are hydrogen-bonded and exchange more slowly. The same is true for other structured, non-solvent exposed regions. In contrast, regions that are unstructured have amides exposed to solvent, ~~which~~ which causes rapid exchange.



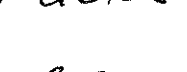
MOM2 is an oncoprotein (cancer-driver).

The key domain (region) of MOM2 is 100 amino acids (molecular weight 11,280)

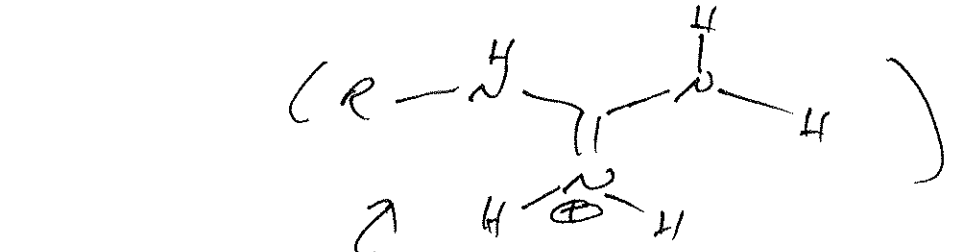
It includes 25 Ser/Thr/Tyr amino acids (serine/threonine/tyrosine)

(R–OH)

8 Lysine amino acids



7 Arginine amino acids



all exchange rapidly

and 14 Asp/Glu amino acids

Under acidic conditions (pH ~ 3), most/all Asp/Glu residues will be protonated (R–COOH).

What protein masses would be observed by electrospray ionization mass spectrometry? (m/z)

At pH 7 (physiological conditions), MOM2 typically adopts a structure where it is 50% structured, 50% unstructured

↑ slower (1 h) amide exchange

↑ rapid amide exchange (< 5 minutes)

Describe the mass of MOM2 at 10 minutes and at 24 hours.

MOM2 in H_2O $\xrightarrow[pH\ 7]{D_2O}$ 10 minutes $\xrightarrow[pH\ 7]{D_2O}$ 24 hours

11,280 MW MW? MW?

The above is MOM2 under normal conditions. In cancer cells, MOM2 will bind to a protein called p53. The MOM2•p53 interaction allows cancer cells to survive and proliferate, and therefore inhibition of this interaction is of intense biomedical interest. The p53•MOM2 interaction results in MOM2 becoming completely structured. Describe how the presence of p53 changes the mass spectral analysis above.

MOM2 + p53 (in H_2O → MW 11,280) $\xrightarrow[D_2O]{pH\ 7}$ 10 minutes MW? $\xrightarrow[pH\ 7]{D_2O}$ 24 h MW?