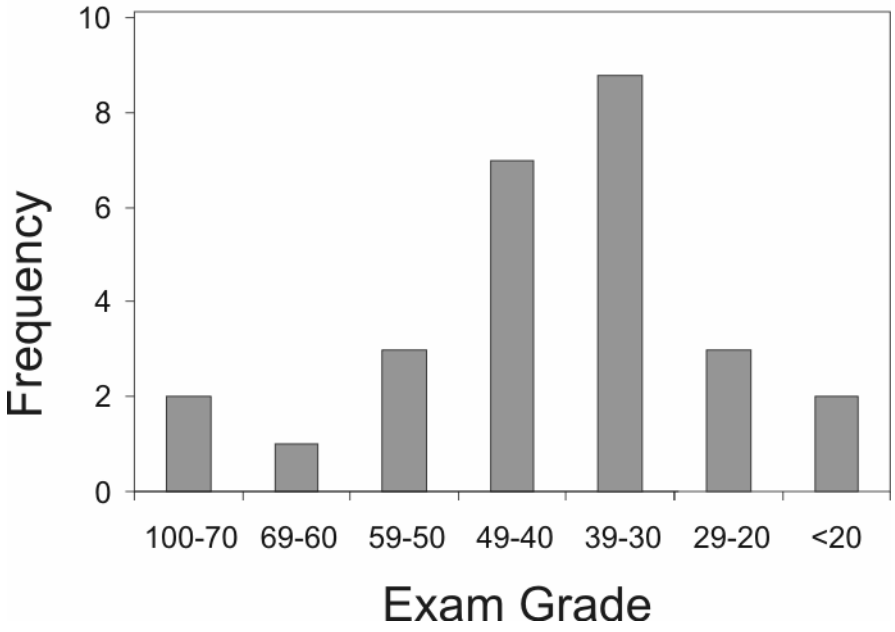
Chemistry 5361/8361 Wednesday, December 3, 2003

Midterm Exam 3
Answer Key

Exam 3 Mean: 41
Exam 3 Median: 39
Exam 3 St. Dev.: 16



EXAM 3 SOLUTIONS

1. a) THE PARENT MOLECULE, 1, HAS MONOISOTOPIC MASS 288 (FOR $C_{12}H_7[^{35}Cl]_3O_2$).

m/z = 218 CORRESPONDS TO LOSS OF 70.

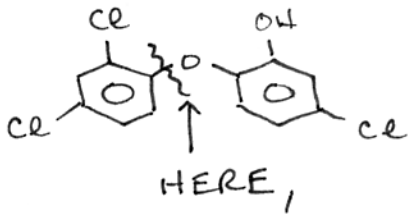
THERE ISN'T ANY EASY WAY TO LOSE 70 AMU WITH CARBONS, OXYGENS & HYDROGENS, BUT IT WOULD BE EASY TO LOSE 2(Cl•) = 70.

SO,

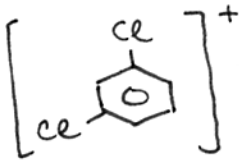
$$m/z = 218 : [C_{12}H_7ClO_2]^+\cdot$$

m/z = 146 CORRESPONDS TO LOSS OF 142.

STRUCTURALLY, CLEAVAGE HAPPENS



HERE,

WITH H• MOVING SOMEHOW FROM RIGHT TO LEFT TO GIVE  (m/z = 146), SO,

$$m/z = 146 : [C_6H_4Cl_2]^+\cdot$$

3 POINTS FOR EACH FORMULA. GIVING STRUCTURE INSTEAD OF FORMULA IS FINE, AND STRUCTURE NEED ONLY CORRESPOND TO CORRECT FORMULA FOR FULL CREDIT.

DON'T NEED POSITIVE CHARGE.

b) FOR BOTH FORMULAE (M+2) AND (M+4) INTENSITIES ARE DETERMINED BY FREQUENCY OF ^{37}Cl, (M+1) IS DETERMINED BY ^{13}C, AND (M+3) IS DETERMINED BY $[^{37}Cl\,^{13}C]$ TOGETHER. CONTRIBUTIONS OF ^{2}H, ^{17}O & ^{18}O ARE NEGLIGIBLE (<1%) BY COMPARISON.

m/z = 218:

(M+1) = 12 × (1.1% ^{13}C) = 13.2%

(M+2) = 1 × (32% ^{37}Cl) = 32%

(M+3) = 32% × 13.2% = 4.2%

(32% ↑ probability of ^{37}C; 13.2% ↑ probability of ^{13}C)

(M+4) = 0%. THERE IS ONLY ONE CHLORINE IN THE MOLECULE.

m/z = 146:

(M+1) = 6 × (1.1% ^{13}C) = 6.6%

(M+2) = 2 × (32% ^{37}Cl) = 64%

(M+3) = 64% × 6.6% = 4.2%

(M+4) = 32% × 32% = 10%

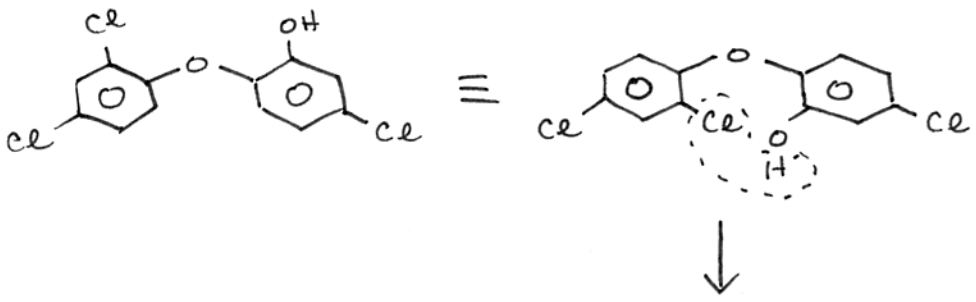
IN BOTH CASES, THE M+2 & M+4 INTENSITIES ARE CONSISTENT WITH DOUG'S SPECTRUM, BUT M+1 & M+3 INTENSITIES ARE TOO HIGH. DON'T KNOW WHY.

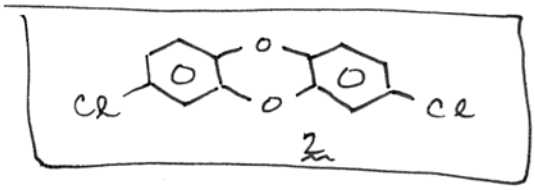
1 POINT FOR EACH ~~[illegible]~~ PERCENT (TO WITHIN 5%);

1 POINT FOR EITHER "YES" OR "NO" IN CONSISTENCY (NO GOOD ANSWER HERE)

c) PARENT MASS IN EI-MS OF 2 IS m/z = 252. ISOTOPE RATIOS ARE CONSISTENT W/ 2 CHLORINES - SO ONE CHLORINE IS GONE FROM 1. ACTUALLY, LOSS OF HCl FROM MASS 288 EXPLAINS m/z = 252 PARENT. SO WHERE DOES HCl COME FROM IN 1?

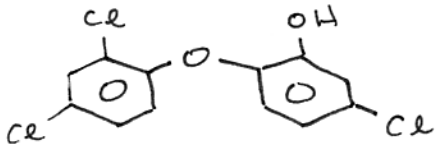
NMR SHOWS THAT PRODUCT 2 IS MUCH LESS COMPLEX THAN PRODUCT 1 (THOUGH IT APPEARS AS THOUGH LOW $\Delta\nu/J$ IS CAUSING SOME COMPLEXITY IN SPLITTING. PRODUCT MOLECULE MIGHT BE SYMMETRIC. ONE WAY OF GETTING THERE WOULD BE





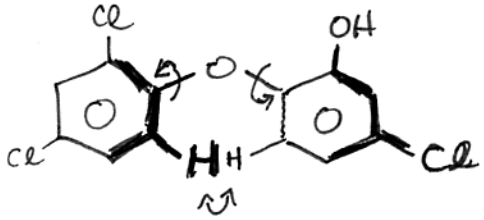
2

4 POINTS.
2 POINTS FOR ANY STRUCTURE IN WHICH HCl HAS BEEN LOST.

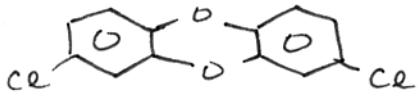
d) 

SHOWS A UV ABSORBANCE AT ~280 nm DUE TO $\pi \rightarrow \pi^*$ TRANSITIONS IN ARYL RINGS.

IN THIS MOLECULE, CONJUGATION IS NOT CONTINUOUS BECAUSE STERICALLY, TWO ARYL RINGS CAN'T LIE IN THE SAME PLANE:



SO, IN 1, CONJUGATION IS NOT MAXIMIZED.
IN 2, HOWEVER, MOLECULE IS COMPLETELY PLANAR & CONJUGATED:



THIS SHIFTS UV-vis ABSORPTION TO LONGER WAVELENGTH.

---

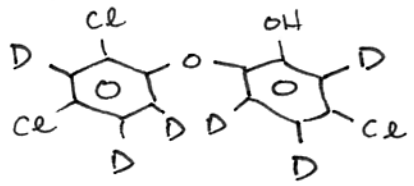
10 POINTS FOR "INCREASE IN CONJUGATION DUE TO PLANARITY." NO NEED TO EXPLAIN $\pi \rightarrow \pi^*$ TRANSITION.

6 POINTS FOR ANY INCREASE IN CONJUGATION ARGUMENT.

10 POINTS ALSO AVAILABLE FOR FUNCTIONAL GROUP-RELATED ARGUMENTS, BUT ONLY IF CONSISTENT W/ YOUR STRUCTURE IN PART (c) AND IF THEY ARE TRUE (BACKED BY CHARTS). THIS IS RESTRICTIVE.

e) MOST IMPORTANT QUESTION HERE: HOW DO WE DISTINGUISH ENDOGENOUS TRICLOSAN FROM OUR TRICLOSAN? IN CLASS, WE TALKED ABOUT RUNNING PARALLEL EXPERIMENTS W/ BACTERIA IN WHICH ONE SAMPLE WAS ISOTOPICALLY MODIFIED; HERE, SIMILAR STRATEGY WOULD BE EFFECTIVE, USING ISOTOPICALLY LABELED TRICLOSAN AS <u>OUR</u> TRICLOSAN:

e.g.,



DEUTERATED TRICLOSAN,
MW = 294
(monoisotopic)

THIS WOULD SHOW SAME SERIES OF PEAKS & PRODUCTS AS NORMAL TRICLOSAN, ONLY 6 MASS UNITS HIGHER.

SO, EXPERIMENT GOES SOMETHING LIKE

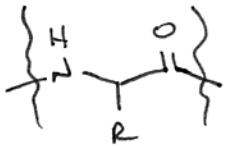
- ADD TRICLOSAN-d_6 UPSTREAM ON A SUNNY DAY;
- COLLECT WATER DOWNSTREAM;
- INJECT THIS INTO GC-MS, MAYBE AFTER EXTRACTING W/ CHLOROFORM;
- LOOK AT EI-MS OF PEAKS FROM TOTAL ION CHROMATOGRAM FOR SAME COMPOUNDS 1 & 2, ONLY 6 MASS UNITS HEAVIER.

5 POINTS FOR SUBSTRATE THAT IS DISTINGUISHABLE FROM ENDOGENOUS TRICLOSAN.

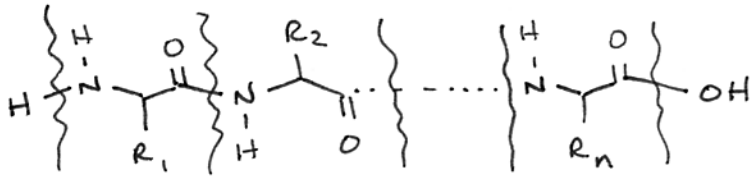
- FULL CREDIT FOR ANY ISOTOPIC SUBSTITUTION THAT MAKES PARENT MORE THAN 4 MASS UNITS HEAVIER. IF NOT, (M+4) PEAK INTERFERES
- 3 POINTS FOR ANY OTHER ISOTOPE SUBSTITUTION (e.g. ALL ^{37}Cl; ^{18}O; MONODEUTERATED)
- 2 POINTS FOR OTHER CHEMICAL VARIANT OF TRICLOSAN (e.g. DICHLORINATED STARTING MATERIAL) – CHEMISTRY WON'T BE THE SAME, BUT WILL GIVE INFO.

5 POINTS FOR ANY DESCRIPTION OF MASS SPEC EXPERIMENT. COULD BE ANYTHING – GC-MS, ESI-MS, JUST ABOUT ANYTHING – MUST BE REASONABLE.

2 a) THE CHART ON PAGE 14 LISTS MASSES FOR EACH AMINO ACID FRAGMENT



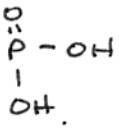
HOWEVER, THE PEPTIDE [redacted] IN FULL LOOKS LIKE



SO THE MASS OF THE PEPTIDE WILL BE THE SUM OF THE FRAGMENT MASSES, PLUS 1 amu FOR THE N-TERMINAL -H, PLUS 17 amu FOR THE C-TERMINAL -OH. IN ADDITION, THE MASSES OBSERVED ARE FOR $[M+H]^+$ IN MALDI-MS, IN ACID MATRIX, SO, ANOTHER 1 amu IS ADDED.

m/z (NON-PHOSPHORYLATED PEPTIDE) =

$$Y + F + R + P + S + G + F + Y^* + D$$
$$+ 17 + 1 + 1 = \boxed{1196.5}$$

IF ONE -OH IS CONVERTED TO  ,

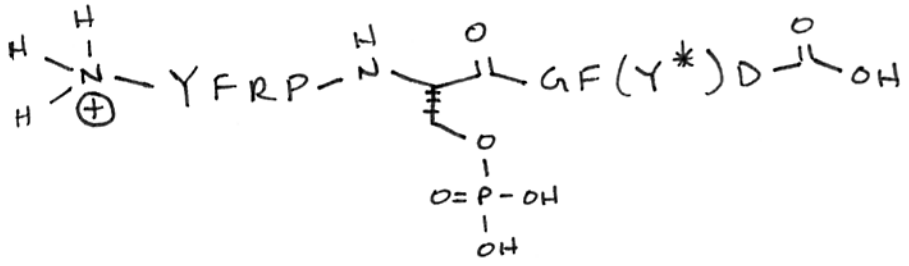
m/z (PHOSPHORYLATED) = m/z (NON-PHOSPHORYLATED)
$$+ 80$$
$$= \boxed{1276.5}$$

OKAY, SO THE PROBLEM PROBABLY WOULDN'T EXIST IF THE PEPTIDE WEREN'T PHOSPHORYLATED, SO THE APPEARANCE OF m/z = 1276.4 IN SPECTRUM (i) IS NO SURPRISE. BUT IMPORTANTLY, m/z = 1178.4 IS <u>NOT</u> NON-PHOSPHORYLATED PEPTIDE - IT MUST BE A FRAGMENT.

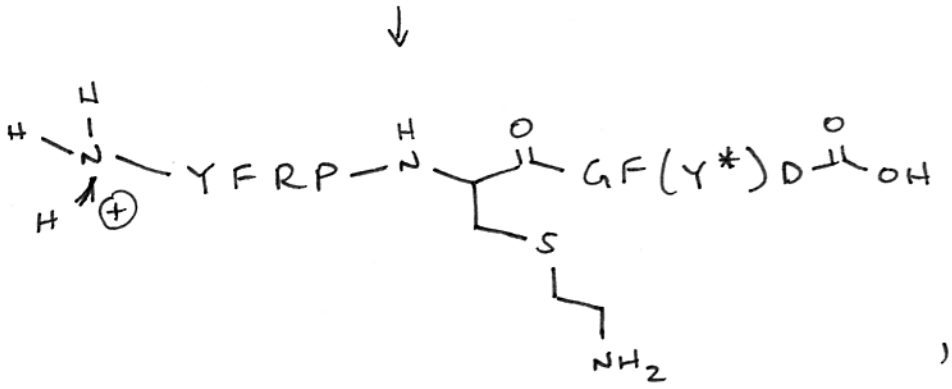
4 POINTS FOR EACH MASS.
2 POINTS FOR "YES".

b) FOLLOWING THE SHOKAT PROTOCOL RESULTED IN NEW CHEMICAL SPECIES - WHICH SUGGESTS THAT IT IS SERINE THAT IS PHOSPHORYLATED. (BECAUSE THAT IS WHAT THE METHOD IS DESIGNED TO DETECT.) SO, THE PHOSPHORYLATED PEPTIDE MUST BE

m/z = 1276.4 : $[YFRP(pS)GF(Y^*)D + H]^+$, OR

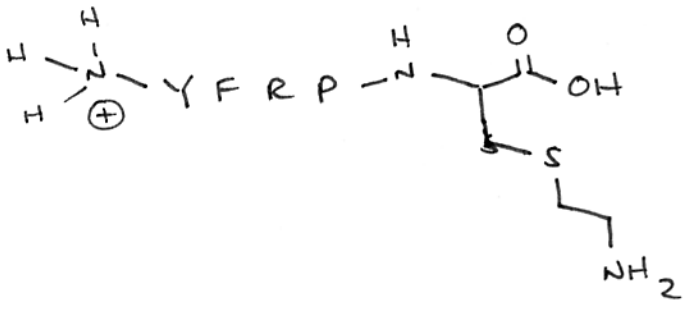


THE SHOKAT PROCEDURE CONVERTS THE (pS) RESIDUE INTO K*:



OR YFRP(K*)GF(Y*)D; m/z = 1255.4.

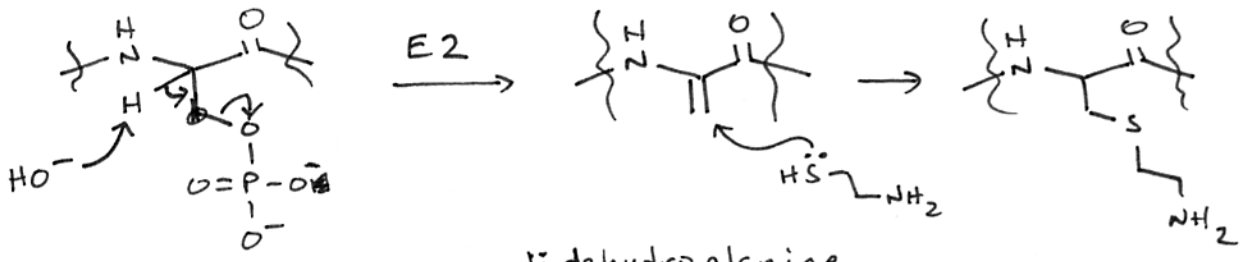
PROTEASE THEN CLEAVES THIS TO THE RIGHT OF K*:



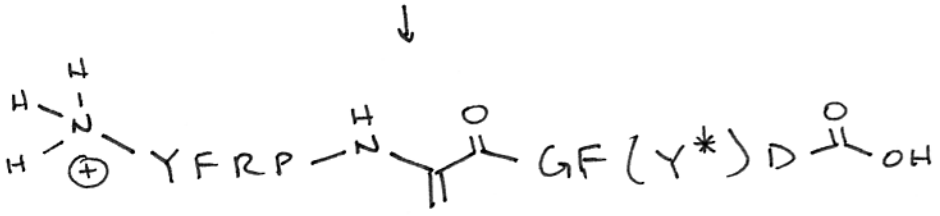
OR YFRP(K*);

m/z = 728.3.

THAT'S 3 OUT OF 4. THE LAST m/z = 1178.4, IS ALREADY PRESENT FROM THE PURE PHOSPHORYLATED PEPTIDE. IT IS ALSO AN INTERMEDIATE GENERATED BY THE KOH.



didehydroalanine
(dhA)



OR YFRP(dhA)GF(Y*)D; m/z = 1178.4.

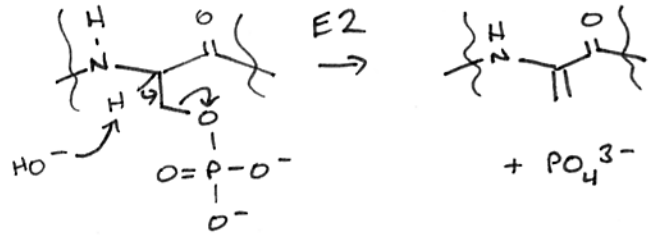
~~EACH BOX~~

IN SUM, m/z = 1276.4 YFRP(pS)GF(Y*)D
1255.4 YFRP(K*)GF(Y*)D
1178.4 YFRP(dhA)GF(Y*)D
728.3 YFRP(K*)

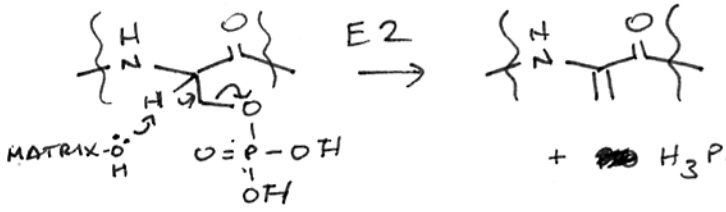
5 POINTS EACH BOX. THOUGH THE QUESTION MAKS SOME RECOMMENDATIONS ON FORMAT, ANY FORMAT IS FINE.

c) THREE EXPLANATIONS:

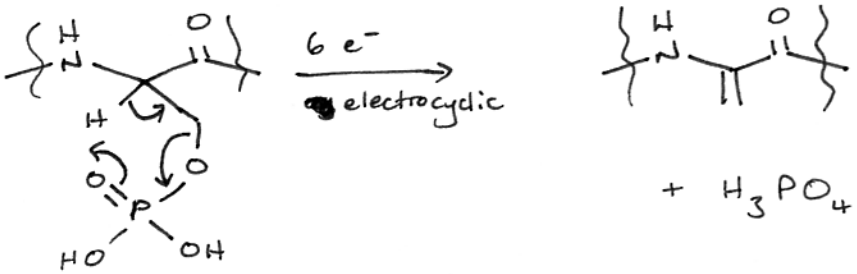
#1: KOH-MEDIATED ELIMINATION (SEE ABOVE). HAPPENS DURING SHOKAT METHOD (SAMPLE PREP).



#2: MATRIX-MEDIATED ELIMINATION. SAME MECHANISM AS ABOVE, BUT HAPPENS DURING IRRADIATION / VAPORIZATION.

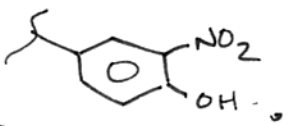


#3: UNIMOLECULAR FRAGMENTATION, HAPPENS DURING VAPORIZATION.



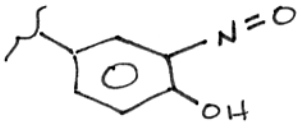
8 POINTS EACH BOX.
6 POINTS FOR MECHANISM
2 POINTS FOR WHERE.

d) ONLY ONE FUNCTIONAL GROUP IN PEPTIDE ABSORBS >300 nm: NITRO GROUP IN Y*,

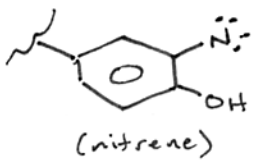


4 POINTS FOR ANY OF THESE ANSWERS
(NITRO, Y*, $-NO_2$, )

e) ALL OF THE OBSERVED PEAKS CORRESPOND TO LOSSES OF 16 AND 32 FROM PEAKS IN (ii). IRRADIATED GROUP IS $-NO_2$, SO MY BET IS THAT OXYGEN ATOMS COME OFF (VIA MULTISTEP PROCESS) FROM THIS.

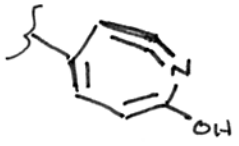


(parent − 16)



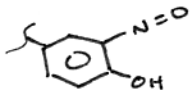
(nitrene)

OR

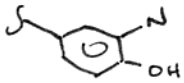


(parent − 32)

3 POINTS EACH STRUCTURE.



MUST BE CORRECT.



CAN BE ANYTHING THAT HAS LOST MASS 32.

2 POINTS (EACH) FOR "16" AND "32".