Chemistry 4361/8361 Monday, December 10, 2012

Exam 4
Answer Key

Exam 4 Mean:	58
Exam 4 Median:	59
Exam 4 St. Dev.:	17

1. Before answering the first question, there are a few things we can say about our molecule from the first couple of mass spectra. The CI-MS spectrum shows just one cluster of high-mass peaks, while the EI-MS shows many lower-mass peaks. This is consistent with fragmentation occurring in electron ionization or our molecule, and with that being solved by using a milder ionization technique like chemical ionization. In this problem, our chemical ionization reagent is isobutane, which—like methane and ammonia—deliver protons to neutral, gas-phase acceptors. That means that the "parent" observed in CI-MS is typically $[\mathbf{M}+H]^+$. If the molecular formula associated with the *m/z* = 199 parent is $C_{10}H_{12}ClO_2^+$, then the neutral molecule has one hydrogen less, $C_{10}H_{11}ClO_2$.

   So then what is going on with the peaks higher than *m/z* = 199? Each of the atoms in our molecule has heavier isotopes that can contribute to the mass of an ion. ^{12}C, ^{1}H and ^{16}O all have "A+1" isotopes (^{13}C, ^{2}H and ^{17}O), but of the three, only ^{13}C is present to a significant extent in nature. So our *m/z* = 200 peak probably corresponds to $C_{10}H_{12}ClO_2^+$ with one ^{13}C instead of a ^{12}C.

   Chlorine, on the other hand, is an "A+2" atom, meaning its two common isotopes—^{35}Cl and ^{37}Cl—result in a distinctive pattern of peaks in mass spec separated by two mass units. The *m/z* = 199 and 201 peaks follow this pattern, with the 201 peaks corresponding to one ^{37}Cl.

m/z = 200: $C_9(^{13}C)_1H_{12}(^{35}Cl)O_2^+$ (5)

m/z = 201: $C_{10}H_{12}(^{37}Cl)O_2^+$ (5)

Rubric: 5 points each box. (10 points total.)

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The format of your answer wasn't important, but isotope elements and formula was.

m/z = 200:

-1 point for not labeling "^{35}Cl", and just writing "Cl". It's less than 90% abundance, so instructions say it needs to be labeled.

-1 point for omitting charge.

2 points partial for picking heavy isotope of H or O instead of C.

No partial if formula doesn't add to 200 amu.

No partial for adding a proton to m/z = 199 formula.

m/z = 201:

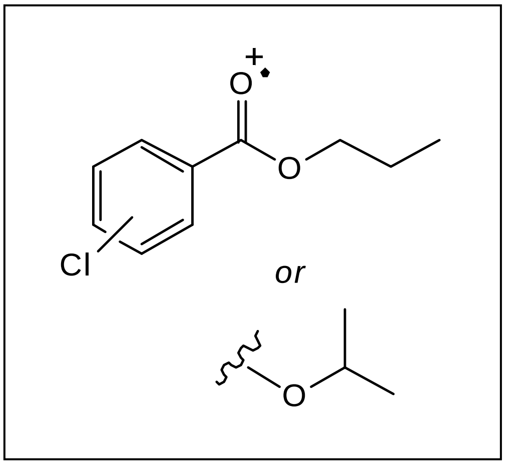
-1 point for omitting charge.

No partial for double ^{13}C or any other double A+1 isotope.

No partial if formula doesn't add to 201 amu.

No partial for adding a proton to m/z = 200 formula.

2. We were actually asking two questions in one here: (a) Can you propose a structure that matches the spectra you have? And (b) can you identify the most ionizable site on whatever molecule you drew as an answer? We graded these questions separately.



Rubric:

4 points for removing one of two lone-pair electrons (i.e., drawing a radical cation on oxygen).

1 point partial for any radical cation.

4 points for drawing any propyl chlorobenzoate.

Chlorine can be anywhere on benzene ring. Any propyl group is fine.

So how do we know what kind of molecule we have? One place to start could be to calculate the degree of unsaturation in the molecule:

$$\text{degree of unsaturation} = \#C - (\#H/2) - (\#Hal/2) + (\#N/2) + 1$$
$$= 10 - (11/2) - (1/2) + 0 + 1$$
$$= 5.$$

Our molecule has a combination of 5 rings and multiple bonds. Most of these must be multiple bonds, and it seems likely that at least some of them would be part of something aromatic, maybe a benzene ring. This is consistent with the set of peaks around *m/z* = 75, which could be a benzene ring fragment. Other than this *m/z* = 75 set, all of the other sets of peaks have the characteristic A+2 chlorine substitution

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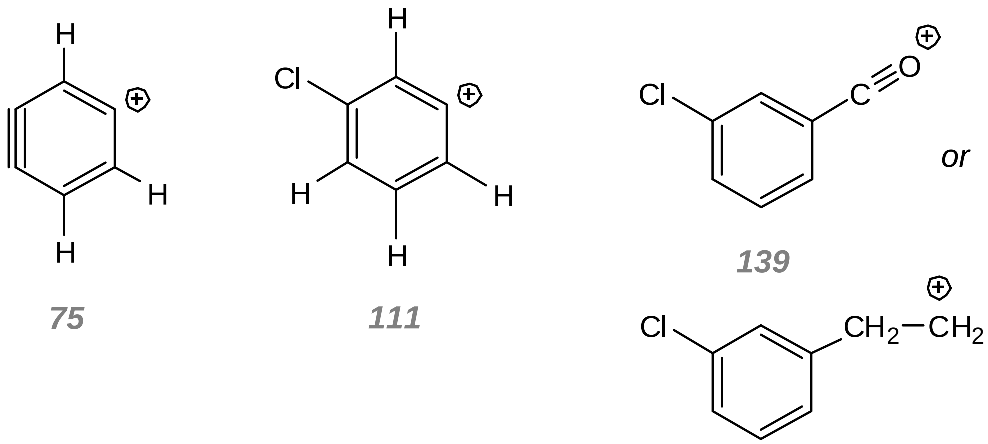
pattern, so they all have a chlorine atom. The *m*/*z* = 111 mass is just phenyl + chlorine. And m/z = 139 is 28 amu higher than that, which could be a CO piece or a CH_2CH_2 piece. That means our fragments look like:



Our parent has an even mass (198). That means that, to fragment to *m*/*z* = 156, another even mass, two bonds will have to be broken. (*Note:* This might not be true if our molecule had nitrogens in it. But it doesn't.) I'm not sure how we could add atoms to the bottom *m*/*z* = 139 fragment to make the *m*/*z* = 156 fragment work, but, as the subsequent problem shows, I think the parent structure I've shown works.

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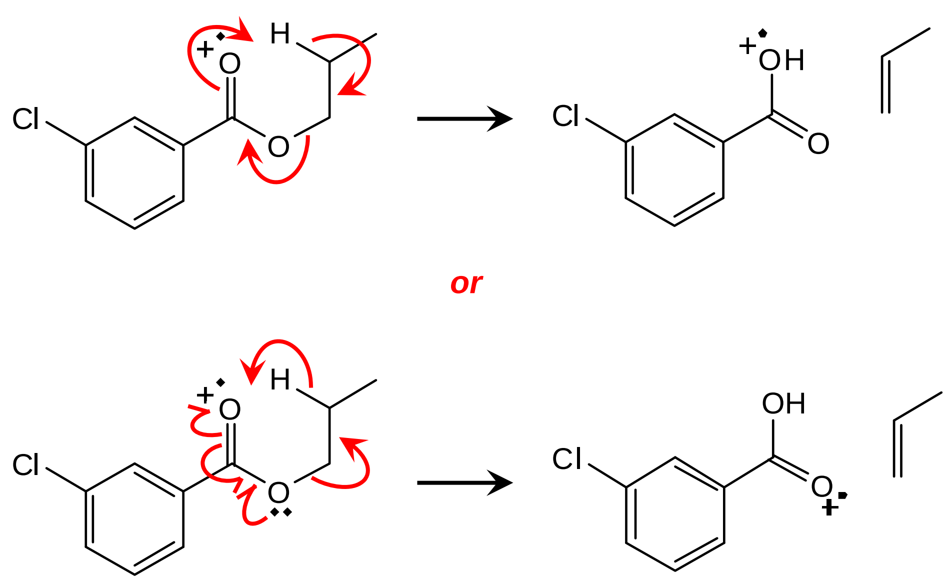
_m/z_ = 156



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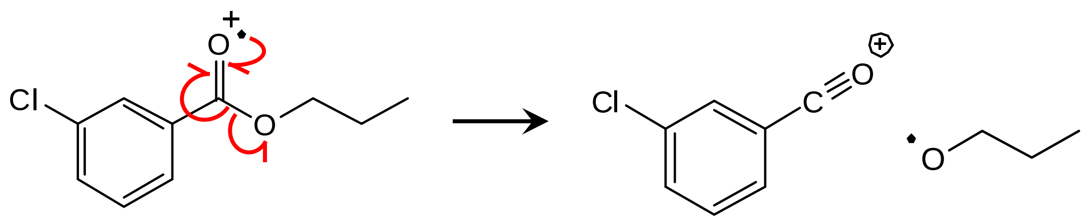
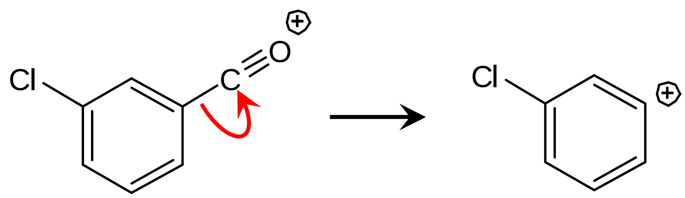
Or I suppose there are a number of ways to push the electrons around to get to one of those two places.

m/z = 139



m/z = 111



<u>Rubric:</u> *(8 points each box; 24 points total this problem.)*

You did not have to have the correct answer to problem 2 in order to get full credit on this problem; all you needed to do is to show how your parent molecule fragmented to the masses indicated.

In each box,

4 points for showing a fragmentation that yields the desired mass.

Fragmentation needs to be a reasonable type.

4 points for pushing electrons to show your fragmentation.

4. To lose H_3PO_4, our molecule needs to lose the -OP(O)(OH)$_2$ leaving group, and a proton. The most common fragmentation mechanism for even-electron ions like our peptide is β-elimination, and that's probably what's happening here. As we mentioned in class, there are a number of ways you can draw β-elimination, but a concerted, one-step mechanism is best if the leaving group will allow it (i.e., if you don't have to draw a four-membered transition state to make it happen). That works here:

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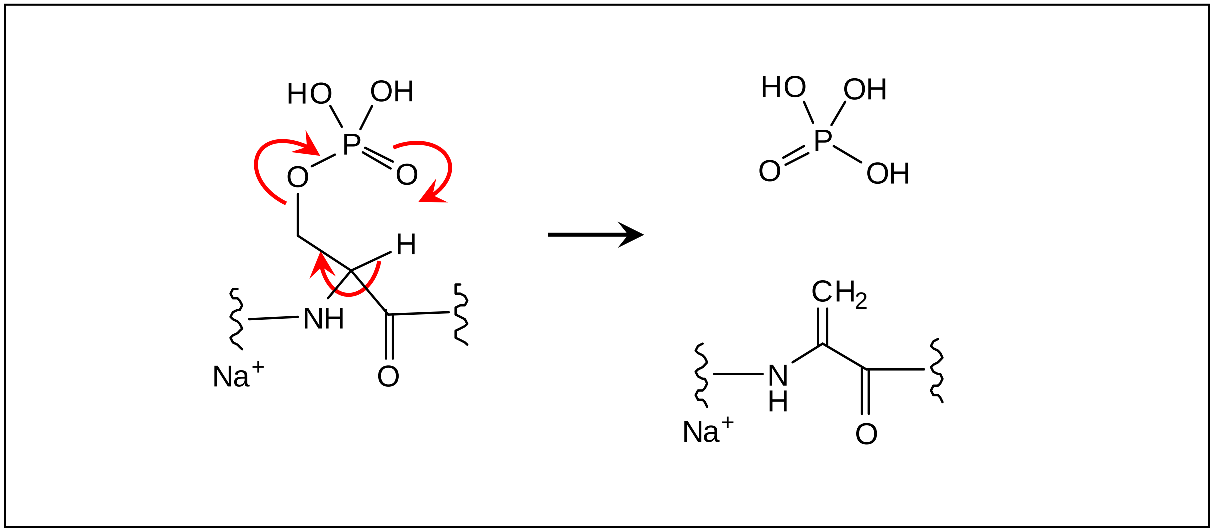
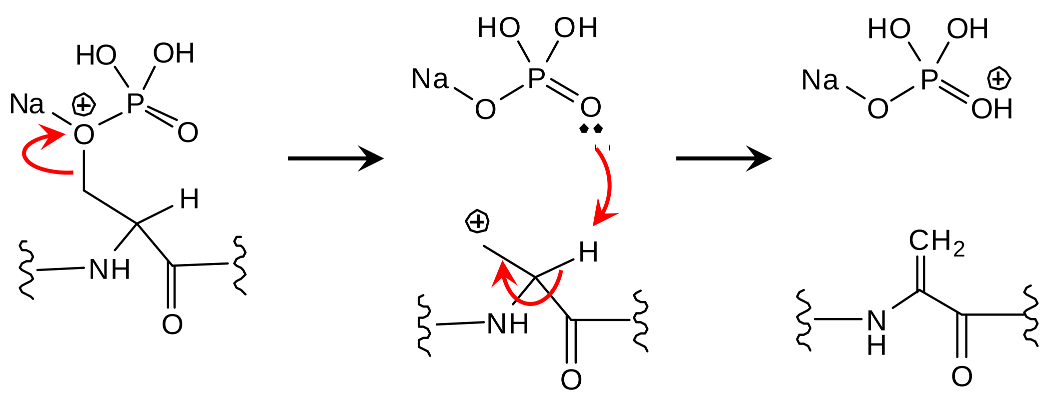


In this drawing I included the Na^+, but I don't think Na^+ has to be involved in the mechanism, so you didn't have to draw it. In fact, I think it would be pretty difficult to implicate Na^+ in a stepwise mechanism of the H_3PO_4's leaving, because somehow it would have to make it back to the peptide:



…and then the Na can be transferred back to the peptide. This seems a little unlikely—the first step generates a primary cation—but, as I mentioned in lecture, lots of things can happen in even-electron ion fragmentation. We gave partial credit for mechanisms that weren't concerted, as long as they got you to an alkene.

<u>Rubric:</u> *(10 points total.)*

5 points for showing a concerted β-elimination.

3 points partial for a stepwise elimination, or for a four-membered transition state.

5 points for pushing electrons to show your elimination (regardless of what you proposed.)

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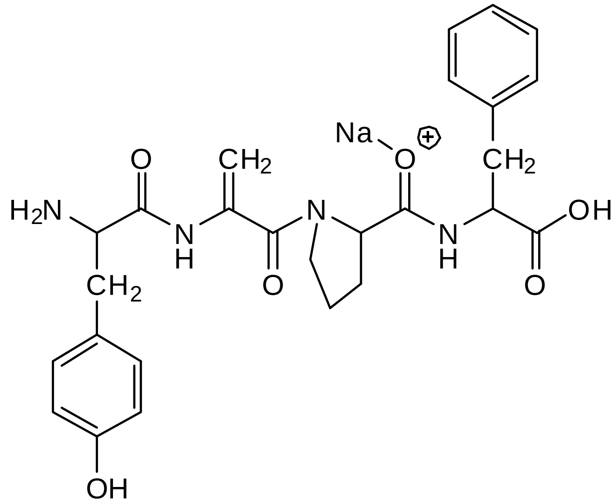
5. Rubric:
   2 points for any ion that has mass 517.
   You did not have to get problem #4 correct to receive these points.
   2 points for your ion including sodium.
   4 points for the right ion.



6. The ions at *m/z* = 517 were selected and, in the ion-trap instrument, subjected to a tandem, MS/MS experiment via collision-induced dissociation (CID).

   a. What do ions encounter in the CID step?

   In CID, ions are accelerated into a collision cell that contains some inert gas, often xenon, heated to a temperature that promotes fragmentation in ions that collide with the xenon atoms.

   I assigned too many points to these simple essay questions, so I gave some automatic points.

   Rubric: *(10 points total.)*
   4 points automatic.
   4 points for mentioning inert gas.
   2 points for mentioning thermalization. (Required for collisions to lead to fragmentation.)

   b. What happens to ions after they undergo CID?

   In a tandem MS experiment, ions that have undergone CID-induced fragmentation are analyzed in a subsequent MS step.

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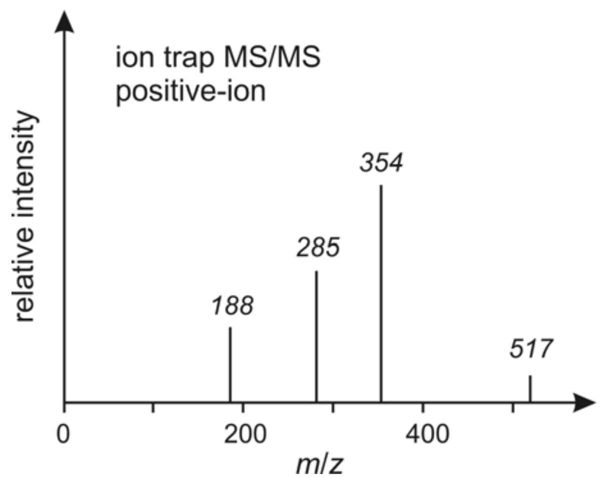
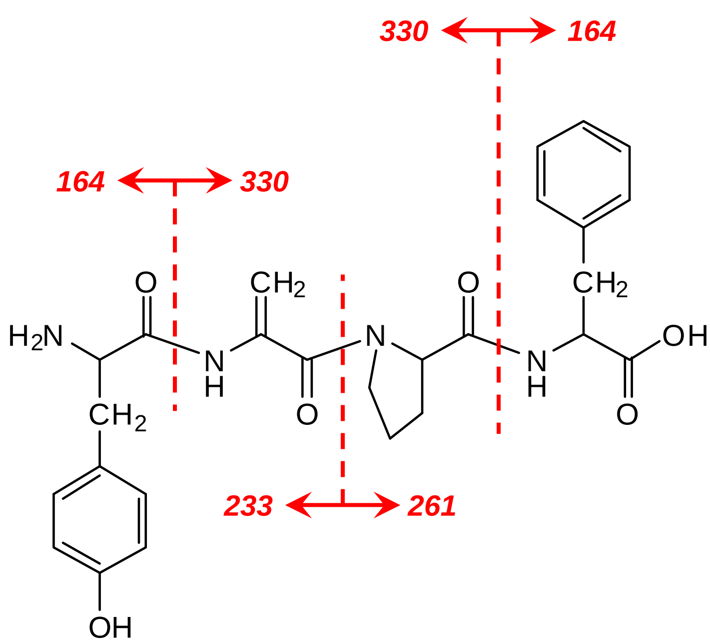
I think nearly everyone in class misread this question. The question asked, “What happens to ions after they undergo collision-induced dissociation?” In this last term, “dissociation” is just another word for “fragmentation”, so the question is asking, what happens to ions after they undergo fragmentation? But many of you read the question as asking, “What happens to ions after they collide with the gas in the CID chamber?” And the answer that most of you wrote was, “They fragment.”

So I decided to throw this question out.

7. As we discussed in class, in peptides, cleavage typically occurs at peptide bonds. In principle, the ion (provided here by the sodium ion) can go with either fragment. So it’s probably most useful for us to figure out what peptide cleavage leads to the fragment masses.

   To get from *m/z* = 517 to 354, we’re losing about 1/4th of the molecular mass, so we’re probably losing just one amino acid off of one end. We can just imagine cutting the peptide, without worrying about mechanism, to come up with the fragments we’re looking for:





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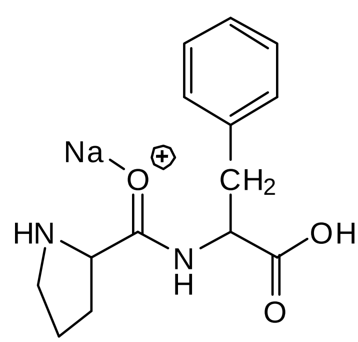
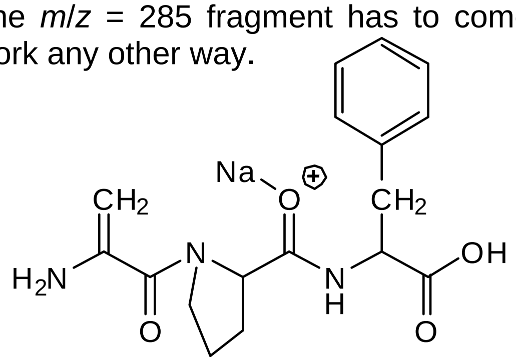
I haven't shown the sodium ion in this drawing; any of these fragments would be 23 amu heavier if they had a sodium ion attached. So, the *m*/*z* = 354 ion could come from either 330 amu fragment, plus the sodium, plus a proton. We accepted either fragmentation, though I think the one on the left—which follows the *b*/*y* fragment pattern—is more likely.

***m*/*z* = 354**

***m*/*z* = 285**



The *m*/*z* = 285 fragment has to come from the right-hand side, the math doesn't work any other way.



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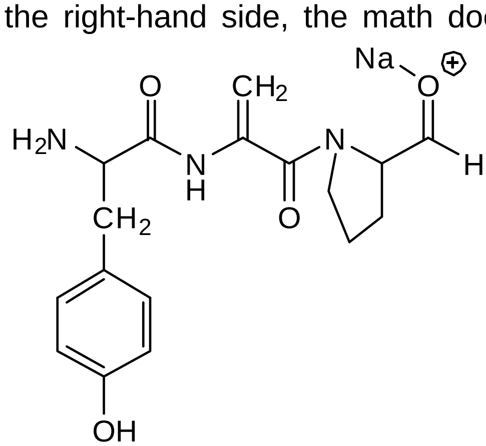

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Rubric: Again, too many points per box. I gave you 4 automatic, and graded each box worth 6.

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