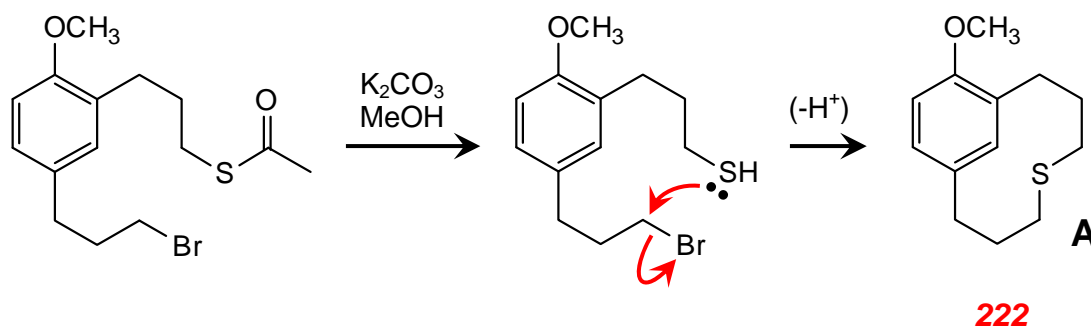


Problem Set 7 Solutions
Mass Spectrometry

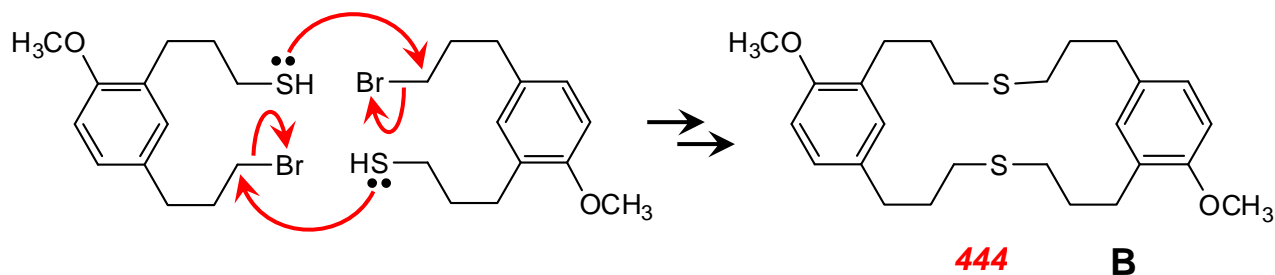
1. a. Jim's starting material has a monoisotopic mass of 344 (using the most prevalent isotopes), and would drop to 302 if removal of the acetyl group was the only thing that happened. Clearly that isn't the case; Jim's two products show parent peaks with $m/z = 222$ and 444, so something else must be happening. In addition, we would expect either the $m = 344$ starting material or the expected $m = 302$ product to have $[A+2]$ isotope peaks with almost the same intensity as the $[A]$ peaks due to the Br atom, which has two prevalent isotopes (^{79}Br , 100%; ^{81}Br , 97%). So the Br must be gone from both of the products. In fact, the mass of product **A** (222) seems to exactly correspond to the loss of the HBr from the expected product ($302 - 79 - 1 = 222$).

This makes sense given that thiols are outstanding nucleophiles, and Br is a great leaving group:



I think the NMR helps with this assignment. The acetyl group is clearly gone (no 3H singlet ~ 2 ppm), and there are the right number of alkyl and aryl protons. Unfortunately, it looks like Jim integrated an impurity water peak at $\delta \sim 1.5$ ppm, and didn't integrate what must be the -OCH₃ singlet at $\delta = 3.8$ ppm.

So where could we end up with something double this mass? Has to be from two thiols combining:



b. Product A :	222	100%
	223	~18%
	224	~7%

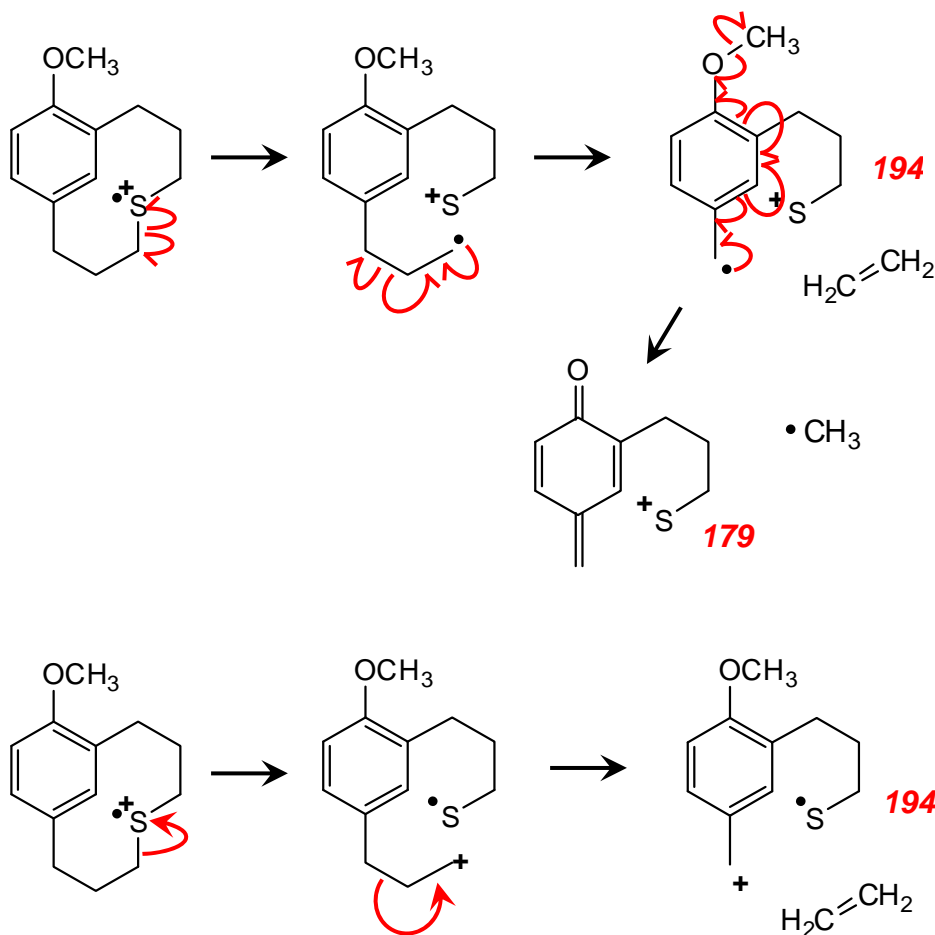
Sulfur has three significant isotopes; ^{32}S (100%), ^{33}S (0.8%), and ^{34}S (4.5%). In addition, we have to consider the contributions of ^{13}C (1.1%), given that product **A** has 13 carbon atoms in it. This will contribute to both the [A+1] and [A+2] intensities (calculating the probability that our molecule has two carbons in it). So, we would calculate intensities for

$$[\text{A}+1]: \{1 \times 0.8\% \} + \{13 \times 1.1\% \} = 15.1\%$$

$$[\text{A}+2]: \{1 \times 4.5\% \} + \{13 \times 1.1\% \} \times \{12 \times 1.1\% \} = 6.5\%$$

Pretty close.

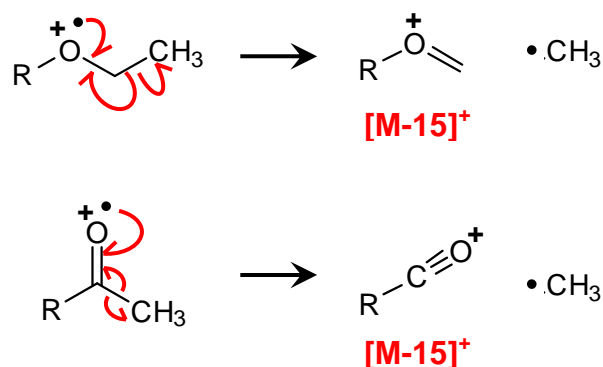
- c. Ionization will occur at the sulfur lone pair, but then Pretsch shows a couple different pathways by which this might fragment:



+ many others. We gave lots of credit for effort here.

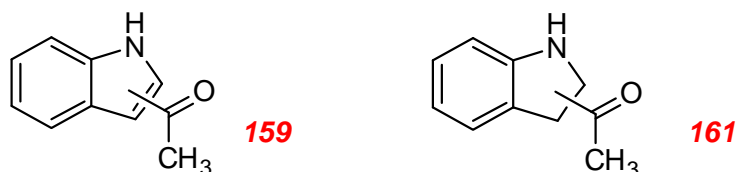
2. a. The starting material in this reaction has mass 117, and the desired hydrogenated product would have mass 119. The parent ions here are $m/z = 167$ (product **C**) and 163 (product **D**). So clearly more has happened here than just hydrogenation; something with a few heavy atoms, maybe three, has added to the molecule. (Each new product is ~ 40 amu higher than we expected; thinking of carbons as model heavy atoms, that's too much mass for two carbons, too little for four.) We don't know where those atoms might have come from, though we might guess that they came either from a second molecule of starting material (which means they might be C or N) or from multiple molecules of MeOH (C or O). Our **C** and **D** parents are odd masses, which means they have an odd number of nitrogen atoms; unless they gained two N's each, the products have just the one indole nitrogen they started with, and our heavy atoms are C's and/or O's.

Both products fragment by losing 15 amu and generating major daughter peaks at $m/z = 152$ ($[\mathbf{C}-15]^+$) and $m/z = 148$ ($[\mathbf{D}-15]^+$). That would have to be loss of $\bullet\text{CH}_3$, which is not part of the starting indole scaffold. A couple of ways we have described in class for molecules that have ionizable oxygen lone pairs to lose alkyl radical fragments include:

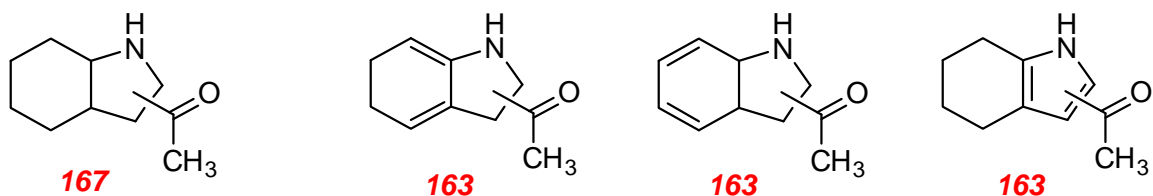


One of the neat things about the second of these two options is that the resulting carboxonium ion can lose C=O to yield another fragment with mass ($m-43$), and both spectra show these daughter peaks at $m/z = 124$ ($[\mathbf{C}-43]^+$) and $m/z = 120$ ($[\mathbf{D}-43]^+$). So my guess is that both of our molecules have the methyl ketone structure shown in the second example above.

Unfortunately, this can't be the only thing that happened; if we just add a methyl ketone at C(2) or C(3) to our starting material or proposed product, we get



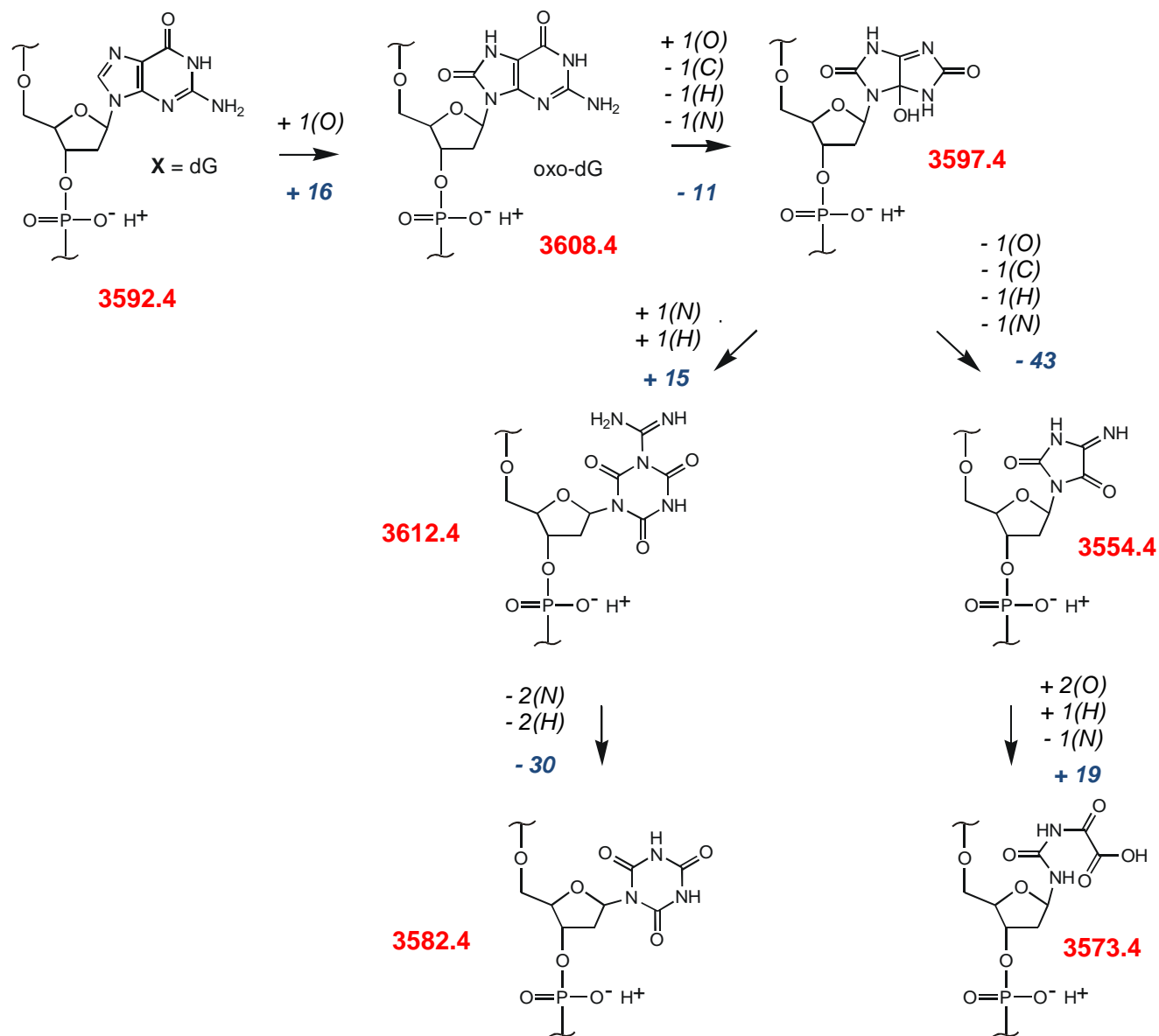
These are too light by a few amu. The reaction was intended to be a hydrogenation, so maybe that hydrogenation actually worked? Proposing some structures that might fit the bill and still have the same skeleton shown above,



The radical cation of the $m = 167$ structure on the left would definitely fragment to generate fragments with $m = 152$ and 124 , and any of the three radical cations from the right would generate fragments with $m = 148$ and 120 . Out of these three, however, only the one on the right would easily lose mass 28 to give a fragment $m/z = 135$:

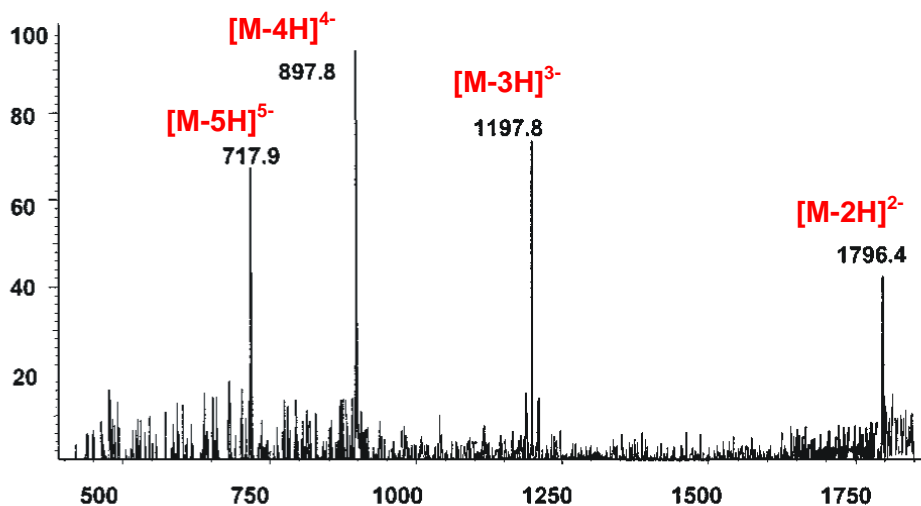


3. I thought it was easiest to start this problem by re-tracing Prof. Tretyakova's mechanistic proposal, seeing what atoms were added and subtracted in each step, and calculating what effect that had on the original parent mass. So, starting with a parent mass of 3592.4,



So these are the masses we might be looking for in this problem.

- a. All of the mass spectra have peaks that are fractions of the expected parent mass, so each one is multiply charged. In the negative-ion mode spectra, each ion is charged by virtue of deprotonation; this means that the $z = -2$ ion is 2 amu less than the parent, the $z = -3$ ion is 3 amu less than the parent, etc. Using the first spectrum as an example,



In principle, we can calculate the parent mass M from any one of these peaks. In practice, it is a good idea to calculate for multiple peaks, and then average/compare the results.

$$m/z = (M - 2)/2 = 1796.4;$$
$$\mathbf{M = 3594.8}$$

$$m/z = (M - 3)/3 = 1197.8;$$
$$\mathbf{M = 3596.4}$$

$$m/z = (M - 4)/4 = 897.8;$$
$$\mathbf{M = 3595.2}$$

$$m/z = (M - 5)/5 = 717.9;$$
$$\mathbf{M = 3594.5}$$

$$\mathbf{M_{average} \text{ (top spectrum)} = 3595.}$$

If we do the same thing for the middle and bottom spectra, we get $\mathbf{M_{average}}$ (middle spectrum) = **3571** and $\mathbf{M_{average}}$ (bottom spectrum) = **3612**. It's pretty clear that there is some error in this however; the individual values we calculated

above vary over 2 amu, and we should probably keep that in mind when we are making assignments.

It's tempting to directly match the masses we found to specific structures in the degradation map, but I think it's also likely that one of the masses should be the starting oligo; one of the masses should probably have oxo-dG, a stable base variant; and the third mass could be anything. If we apply those biases, then the top spectrum could be the unmodified oligo, the bottom spectrum could have oxo-dG instead of dG, and the middle spectrum could be a degradant, maybe the final product in the right-hand pathway.

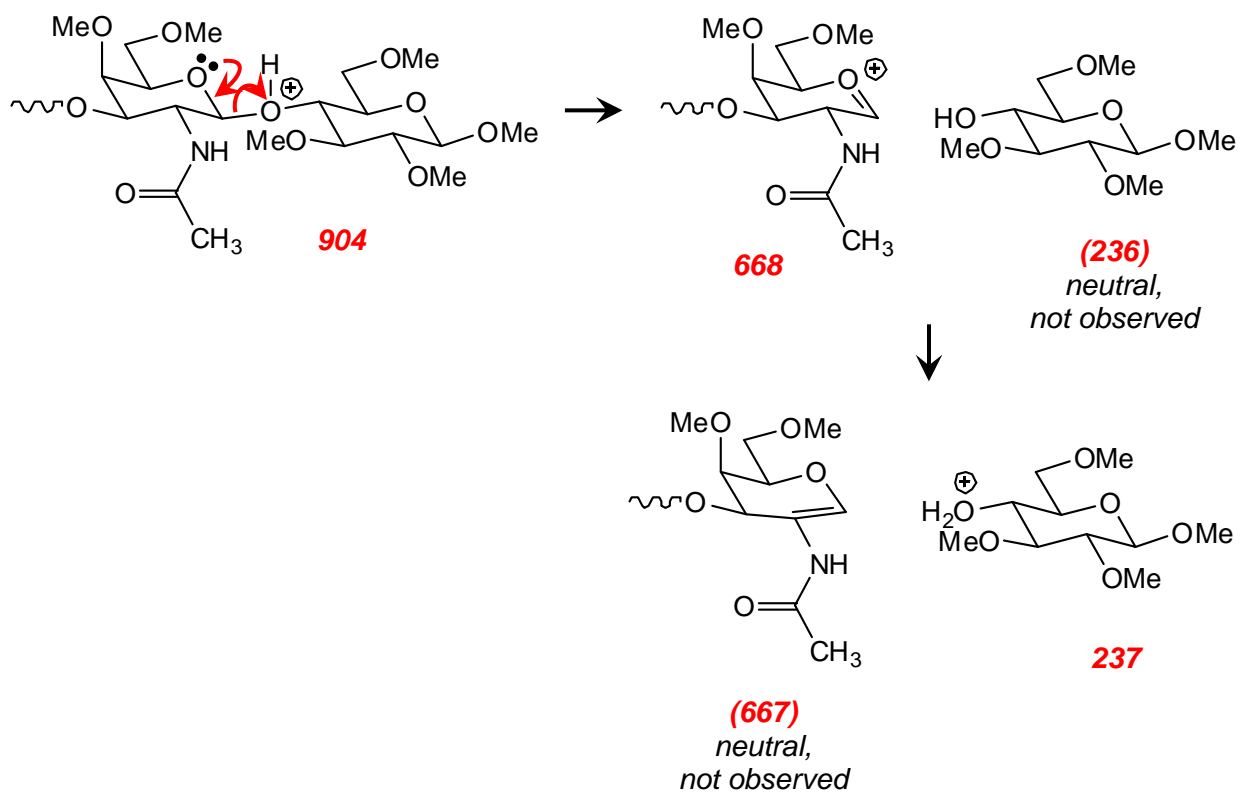
- b. Before I started this problem, I tested the online calculator at <http://library.med.utah.edu/masspec/mongo.htm> on the unmodified oligonucleotide CCACAACGCAAA, just to make sure it worked. It gave an *average* molecular mass of 3592.4, just as given in the problem. That makes sense—the resolution of the spectra in the problem isn't good enough for us to resolve monoisotopic peaks anyway, and the average mass will be the highest point in the sum of individual isotopic peaks. The calculator also points out that the ends of the oligonucleotide are both -OH groups (even though the structures given in the problem don't make this clear, and might have made you wonder whether one of the ends had a phosphate group on it).

The starting oligonucleotide in this experiment isn't CCACAACGCAAA, it's CCACAACXCAAA, where dX is 22 amu less than dG. In the calculator, we can define our own base "N"; the guanidine (G) base by itself has $m = 150$, so that means base N has $m = 128$. Plugging that into the calculator, the masses we'd expect to see would be

CCACAACNCAAA	3570.1
CACAACNCAAA	3281.1
ACAACNCAAA	2991.9
CAACNCAAA	2678.7
AACNCAAA	2389.5
ACNCAAA	2076.3
CNCAAA	1763.1
NCAAA	1473.9

We do see all these masses, but (a) we don't see any smaller masses than these; and (b) the peak intensity drops a lot for the last two. I think that means that the modified base N stops the endonuclease, but it also maybe slows it down a couple of bases earlier.

4. As we discussed in class, a common fragmentation pathway in soft-ionization/CID is for a leaving group to leave, sometimes followed by β -elimination.



Of these, the $m = 668$ ion is observed in the CID-MS, but neither of the bottom masses is. Cleavage could also occur on the other side of the molecule:

3. a. Plugging the sequence ILGCWCYLR into an online peptide mass calculator (like <http://ca.expasy.org/tools/peptide-mass.html>) gives an $[M+H]^+$ mass of 1126.55. (With a mass M of 1125.55.)
- b. Beth's spectrum showed four peaks, at (from highest m/z to lowest) $m/z = 1147.60, 1125.60, 574.33,$ and 563.27 . The third and fourth peaks are almost exactly $\frac{1}{2}$ the values of the first and second peaks, suggesting that maybe they are multiply charged ($z = 2$) species. If that were true, and we call the two masses we are looking for M_1 and M_2 ,

$$m/z = (M_1 + 2)/2 = 563.27;$$

$$\mathbf{M_1 = 1124.5}$$

$$m/z = (M_2 + 2)/2 = 574.33;$$

$$\mathbf{M = 1146.7}$$

This matches up nicely with the first two peaks, for which $\mathbf{M_1 = 1124.6}$ and $\mathbf{M_2 = 1146.6}$. I think it's pretty clear that $\mathbf{M_1}$ corresponds to the expected parent. (Even though it is ~ 1 amu off, this only represents an error of $<0.1\%$ in the mass assignment; this might be an instrument calibration error.) M_2 is 22 amu higher than M_1 . What weighs mass 22? Nothing organic, but it might represent our peptide being sodiated instead of protonated; $m/z([M_1+Na]^+) = 1147.6$ (peak 1), and $m/z([M_1+Na+H]^{2+}) = 574.3$ (peak 3). The problem doesn't mention the presence of sodium in the experiment, but it could be a contaminant from the peptide.

- c. To answer this problem, you needed the chemical formula of the peptide. Not too hard to calculate by hand, but I think it was easier to find a calculator online. I got $C_{52}H_{79}N_{13}O_{11}S_2$ from <http://pept.n2.cz/>. I was also able to find a number of downloadable freeware packages that would predict the isotope series intensities (<http://ncrr.pnl.gov/software/> has a few), but I'll calculate it explicitly here.

Probability of [A+1] relative to [A]:

$$^{13}\text{C}: 1.08\% \times 52 = 56.2\%$$

$$^2\text{D}: 0.012\% \times 79 = 0.9\%$$

$$^{15}\text{N}: 0.37\% \times 13 = 4.8\%$$

$$^{17}\text{O}: 0.038\% \times 11 = 0.4\%$$

$$^{33}\text{S}: 0.79\% \times 2 = 1.6\%$$

$$\mathbf{Total: 63.9\%}$$

Probability of [A+2] relative to [A]:

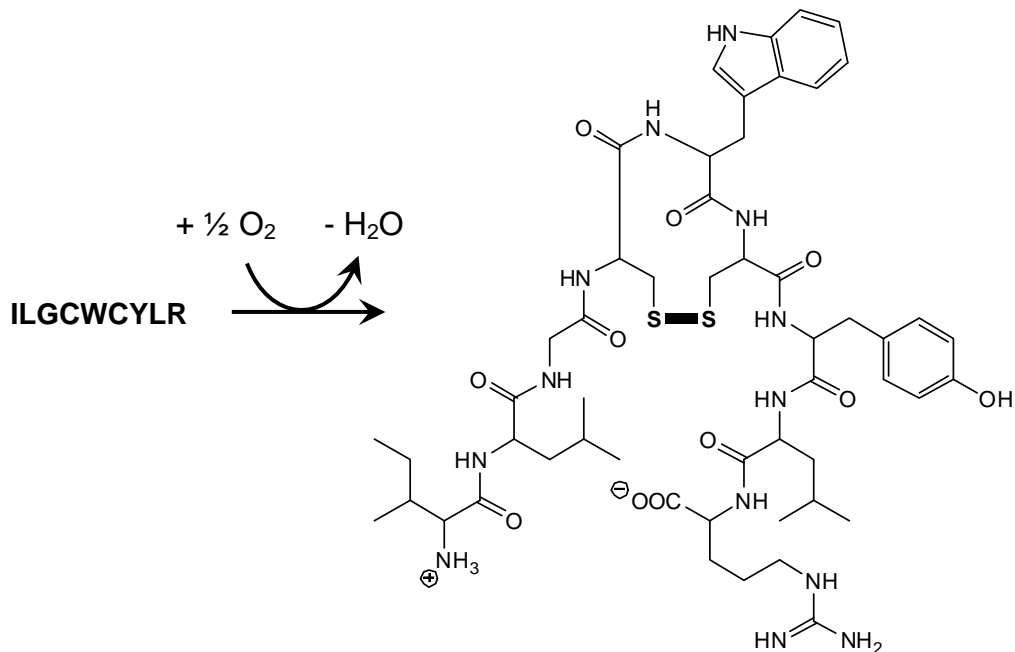
To do this, we need to include the probability of finding an A+2 element (e.g., ^{34}S) as well as the probability of finding two A+1 elements (e.g., two ^{13}C 's). The probabilities of all of the A+1 elements were low except ^{13}C and ^{15}N , so those are the only two I'll consider.

$$\begin{aligned}^{18}\text{O}: & 0.21\% \times 11 = & 2.3\% \\ ^{34}\text{S}: & 4.5\% \times 2 = & 9.0\% \\ 2 \times ^{13}\text{C}: & (1.08\% \times 52)(1.08\% \times 51) = & 30.9\% \\ ^{13}\text{C} + ^{15}\text{N}: & (1.08\% \times 52)(0.37\% \times 13) = & 2.7\% \\ 2 \times ^{15}\text{N}: & (0.37\% \times 13)(0.37\% \times 12) = & \text{negligible}\end{aligned}$$

Total: 44.9%

Looking at the MS closeup, the [A+1] number looks perfect, but the [A+2] number looks a little higher than the peak intensity Beth actually observed in the mass spectrum (~33%).

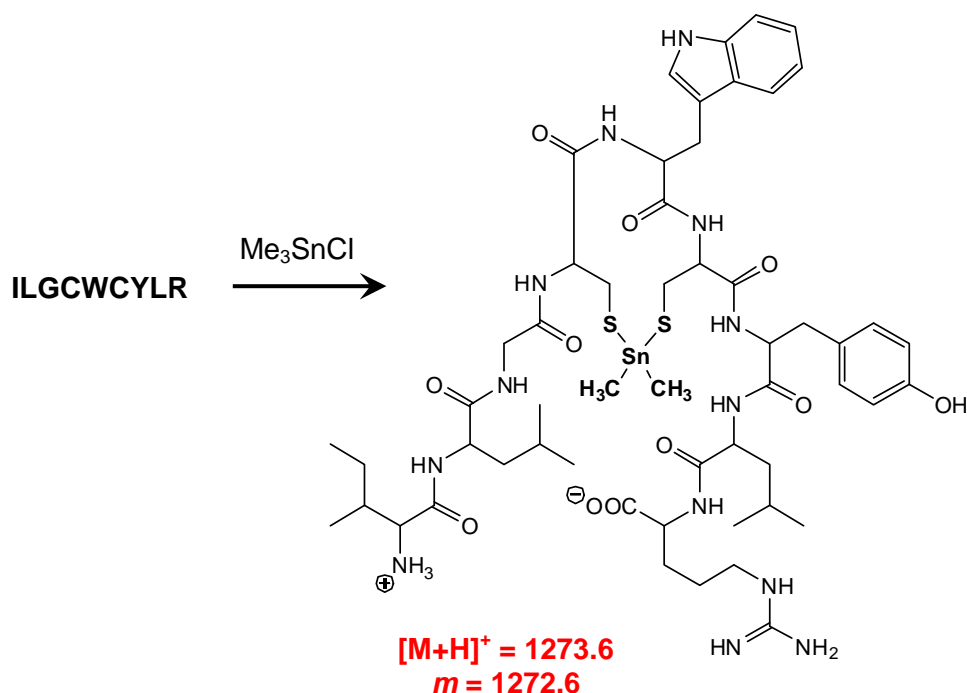
- d. The problem describes some reaction between oxygen and the peptide that would selectively remove two hydrogen atoms (a mass of 2) from the molecule. Dithiols are readily oxidized in air to yield disulfides, and this is probably what Beth observed.



- e. The higher-mass peaks ($m/z = 1273.6$ and 1295.7) must correspond to some reaction with the Me_3SnCl . The two peaks are once again separated by 22 amu, so they might be related to each other by a sodium ion. But how do we explain the increase in mass from starting material ($m/z = 1125.6$) to these?

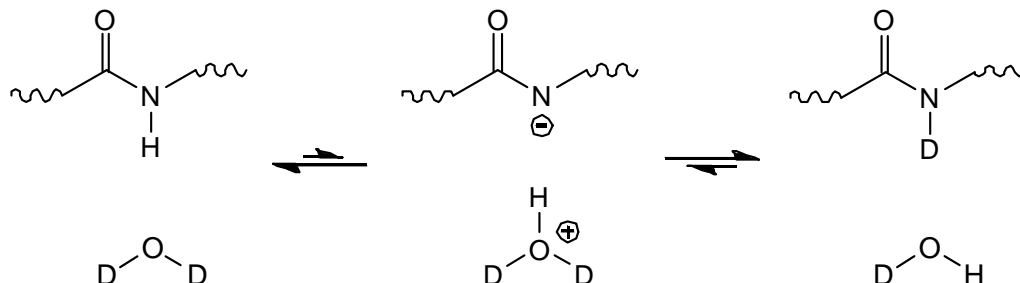
Sn has a mass of 119 all by itself—that could explain much of the mass increase. But just as the initial problem text explains, addition of $\text{Sn}(\text{CH}_3)_3$ to a thiol adds too much mass. ($1125.6 + 164 - 1 = 1288.6$.) In fact, this mass is just one methyl group too much; if somehow we could add $\text{Sn}(\text{CH}_3)_2$ instead of $\text{Sn}(\text{CH}_3)_3$, we would be set.

That is in fact exactly what Beth argued:



Who knows how the third methyl group came off of there. In any case, the presence of the Sn atom explains the broad distribution of isotope peaks; Pretsch's mass spec summary tables show 10 different prevalent isotopes for Sn, with ^{118}Sn (74%) and ^{120}Sn (100%) being the most prevalent. In fact, this pattern might encourage us to label Sn an "A-2" atom (because its second-most prevalent isotope is 2 amu less than its most prevalent isotope), and this would explain the relative heights of the $m/z = 1273.6$ and 1271.5 peaks.

- f. In principle, incubating the peptide in $D_2O/MeOD$ should exchange any acid-base labile protons for deuterons, because of the overwhelming proportion of deuterons present. For example, amides would become deuterated amides:



Looking at the structure of the proposed product, it has 17 protons that would exchange for deuterons. That would give it a neutral mass of $m = 1289.6$. When Beth injected this in $D_2O/MeOD$, she would have seen a monocation peak corresponding to $[M+D]^+$ instead of $[M+H]^+$, at $m/z = 1291.6$. This is 1 amu off from the observed 1290.6, and it isn't clear why. But in general, the MS is consistent with H/D exchange for this product. In addition, she observed $[M+Na]^+$ for this molecule ($m/z = 1311.6$), and $[M+2D]^{2+}$ ($m/z = 646.3$).

