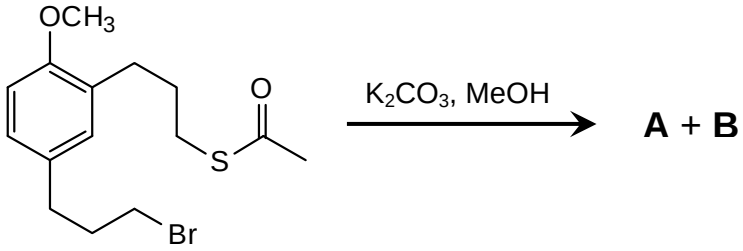
Chemistry 4361/8361 Fall 2012

Problem Set 7
Mass Spectrometry
Due: Wednesday, December 5

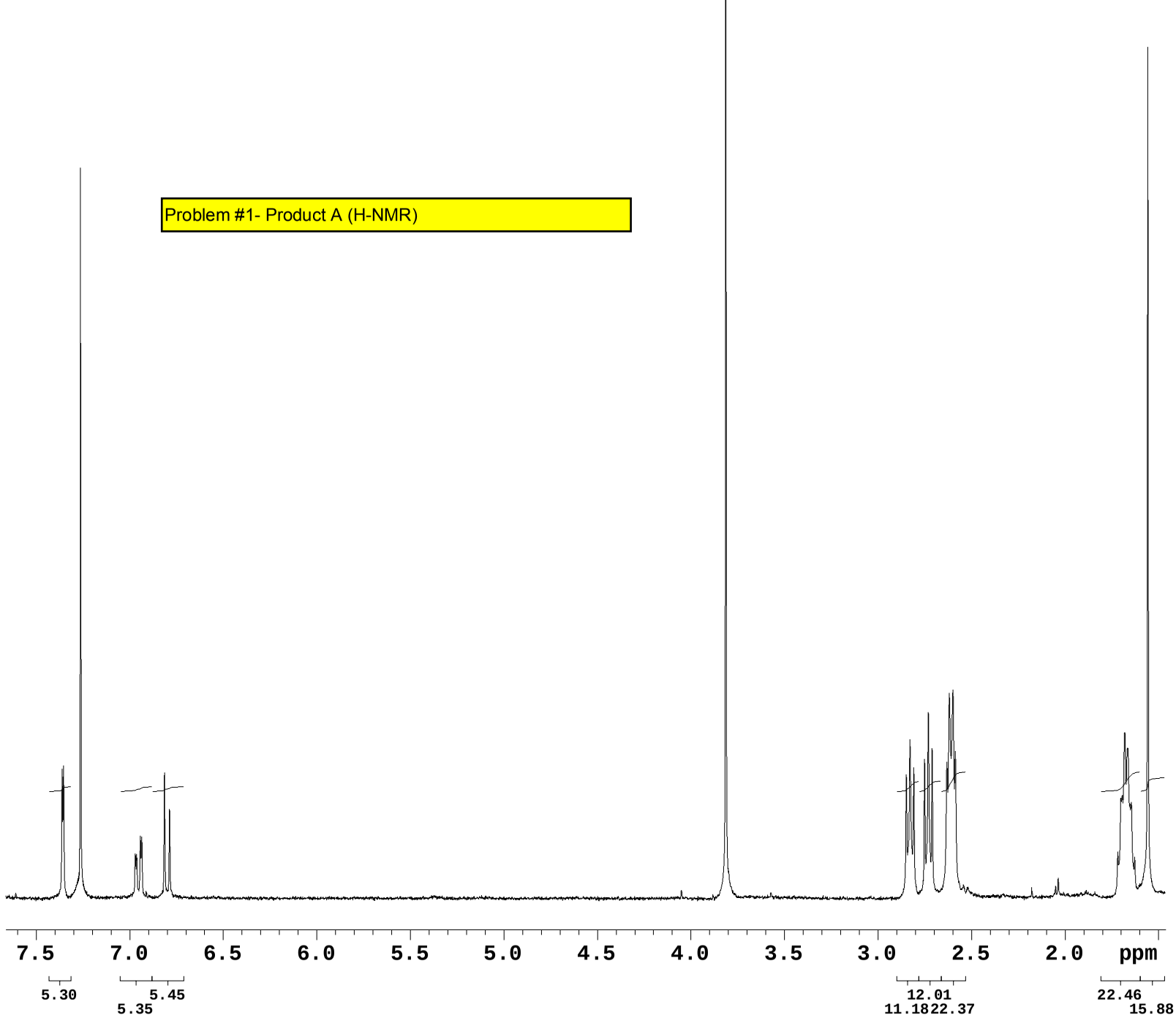
1. Jim Kabrhel (Hoye group) subjected the starting material below to basic conditions in order to convert the thioester into a thiol (to deprotect the thiol group). Unexpectedly, Jim obtained two different products (**A** and **B**) from this reaction. ^{1}H NMR (300 MHz, in $CDCl_3$) and EI-MS spectra of each molecule follow this problem.

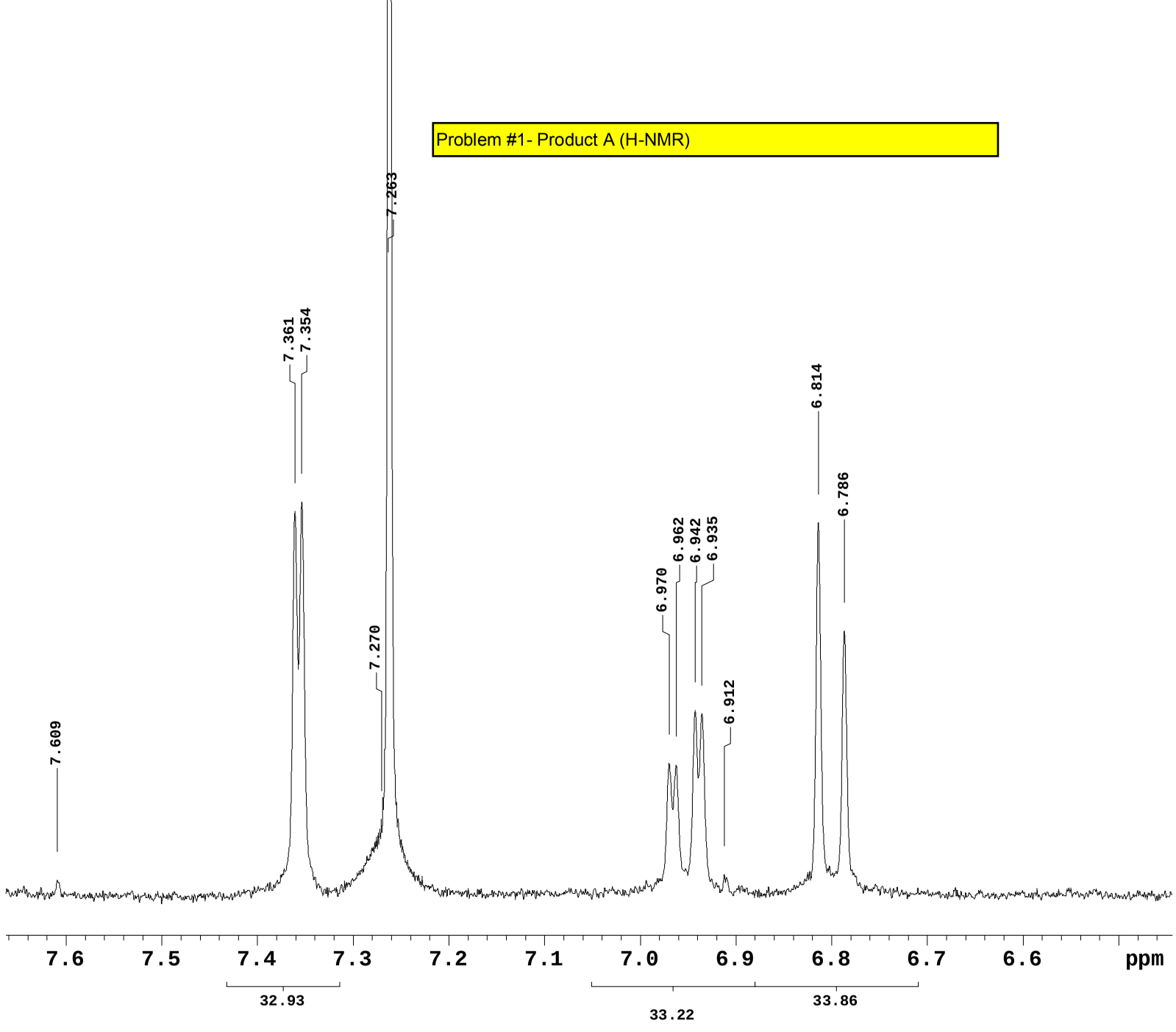


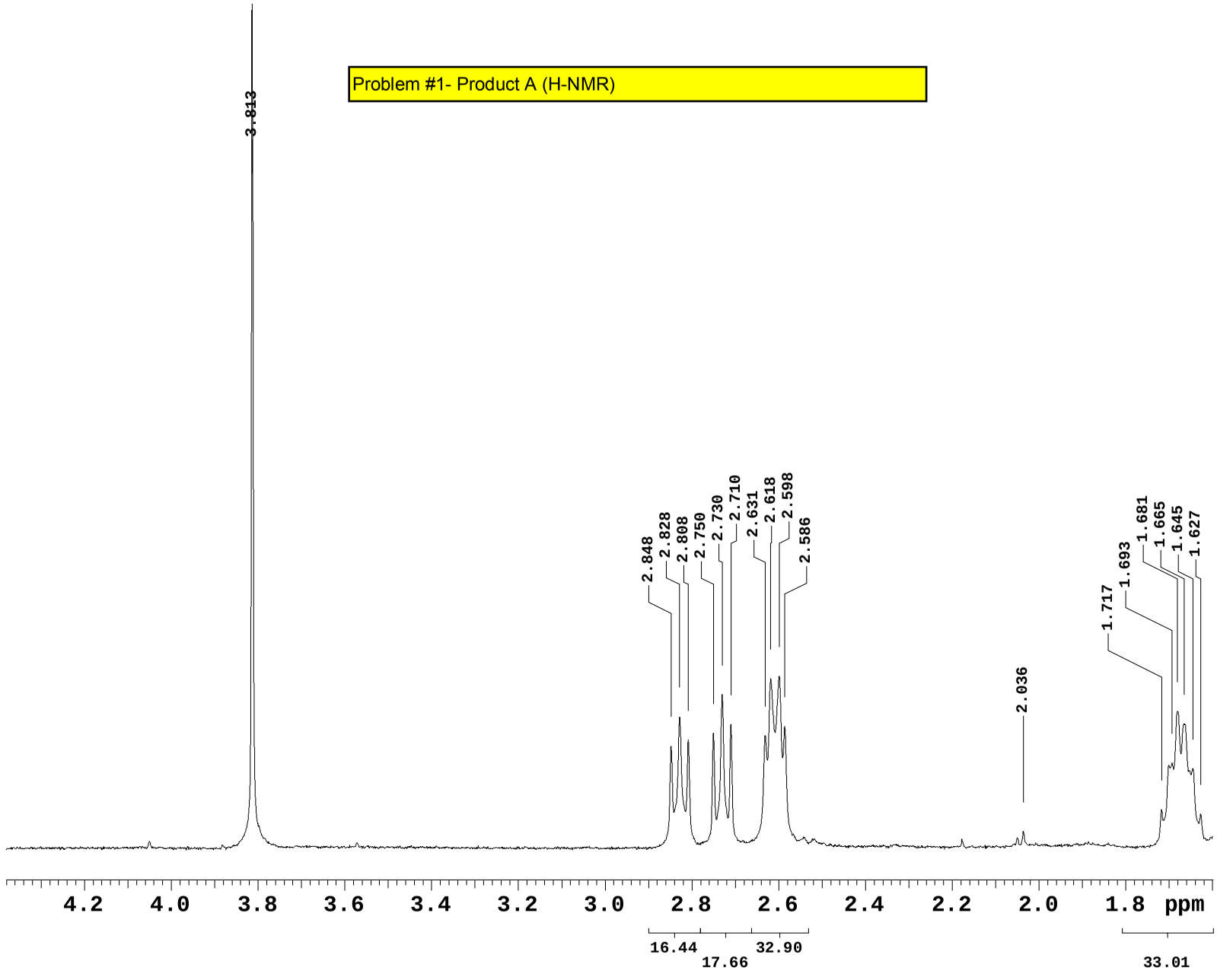
   a. What are the structures of products **A** and **B**?

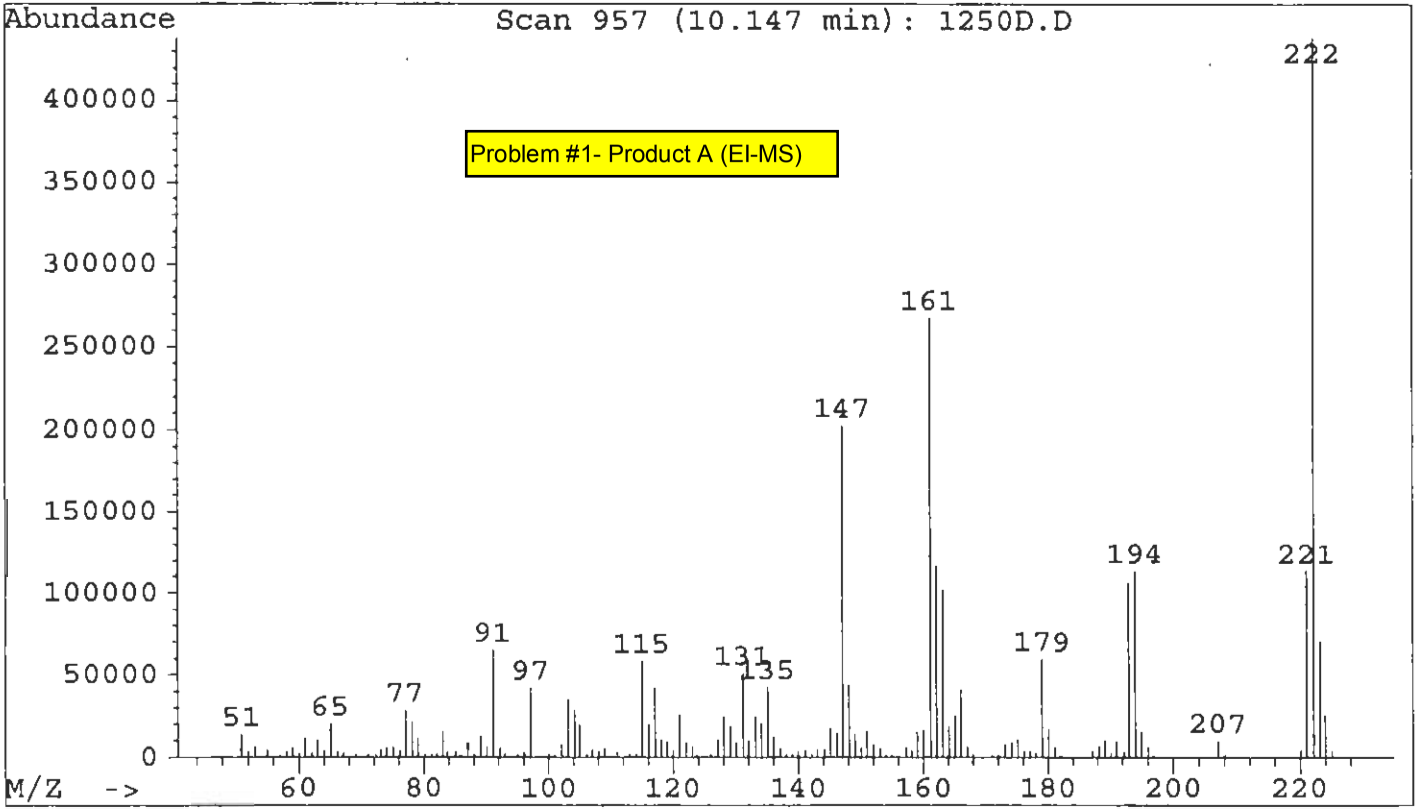
   b. Assuming that the parent ion in the EI-MS spectrum of product **A** has *m*/*z* = 222, there are peaks at *m*/*z* = 223 and *m*/*z* = 224 with significant intensity. What are the relative intensities of these peaks, and do those intensities make sense in terms of the structures you gave in part (a)?

   c. Draw mechanisms that explain the appearance of fragments with *m*/*z* = 194 and 179 in the EI-MS spectrum of product **A**. Radical cations that contain sulfur atoms have more fragmentation mechanisms available to them than the ones we've discussed in class; you might want to browse the section in Pretsch's handbook on EI-MS before answering this part.

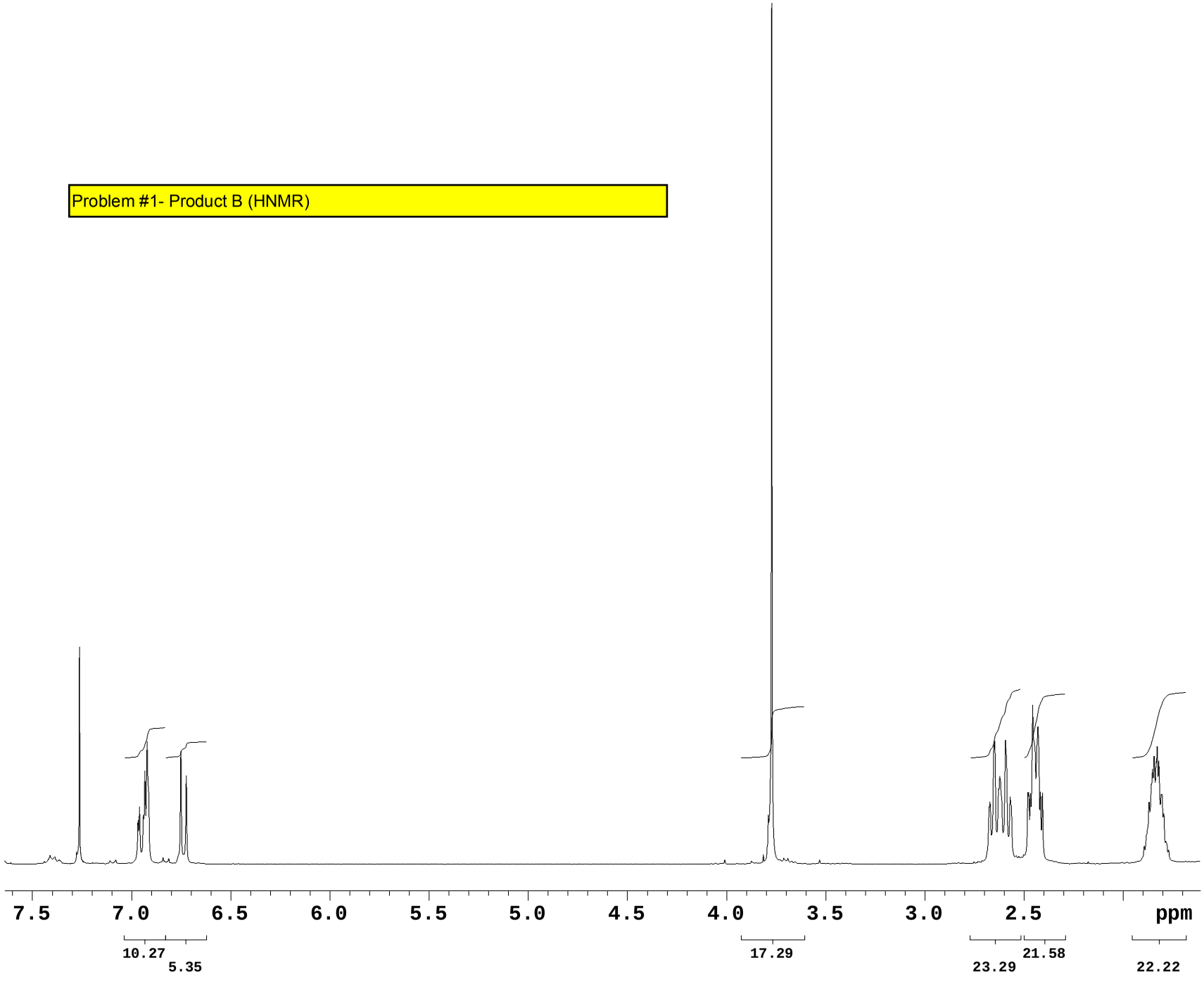

Problem #1- Product A (H-NMR)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
5.30
5.45
5.35
12.01
11.18
22.37
22.46
15.88

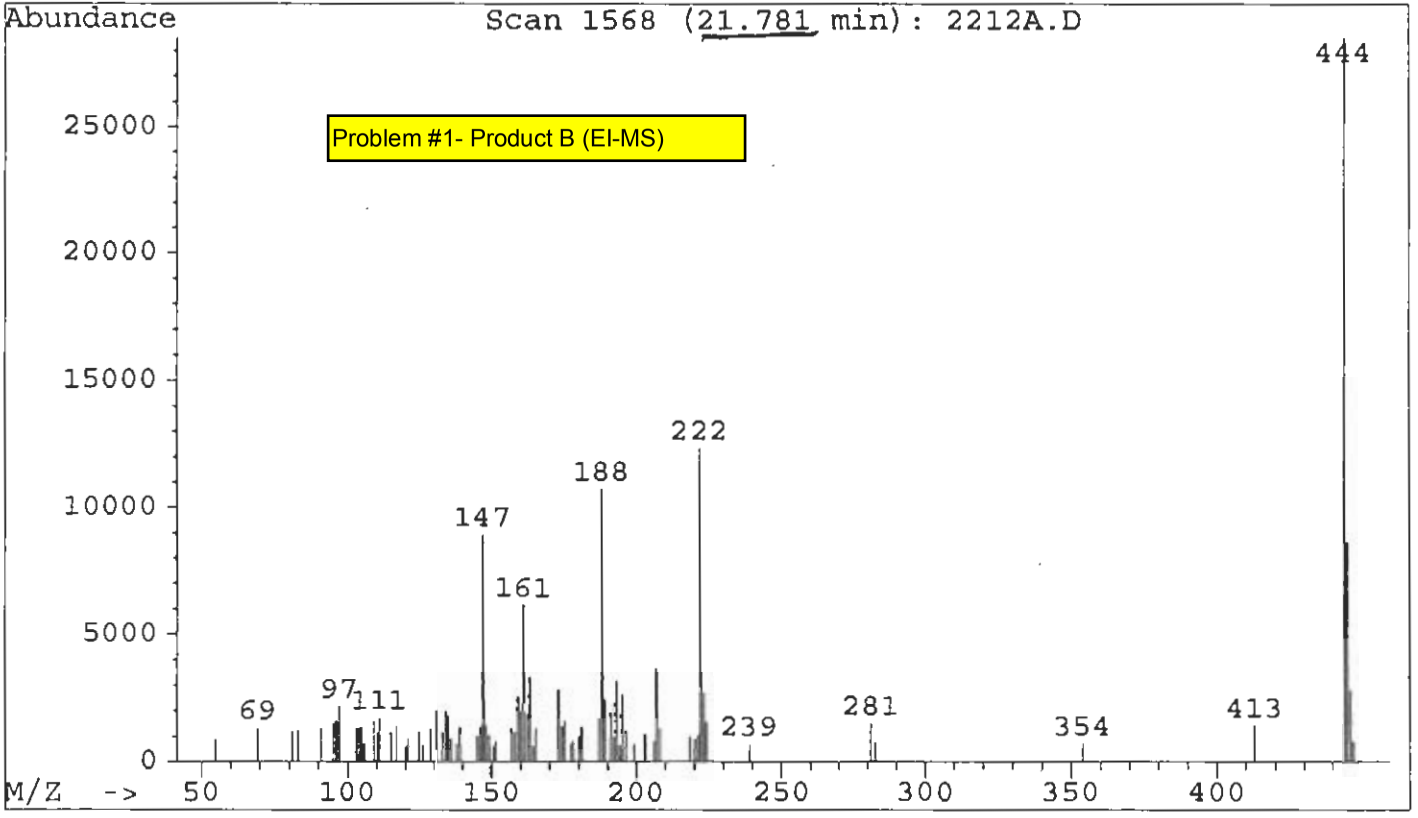

Problem #1- Product A (H-NMR)
7.609
7.361
7.354
7.270
7.263
6.970
6.962
6.942
6.935
6.912
6.814
6.786
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
ppm
32.93
33.22
33.86


Problem #1- Product A (H-NMR)
3.813
2.848
2.828
2.808
2.750
2.730
2.710
2.631
2.618
2.598
2.586
2.036
1.717
1.693
1.681
1.665
1.645
1.627
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
ppm
16.44
17.66
32.90
33.01

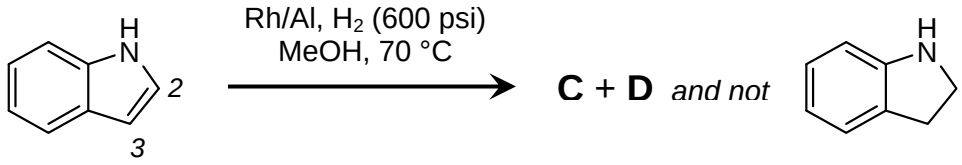

Abundance
Scan 957 (10.147 min): 1250D.D
Problem #1- Product A (EI-MS)
400000
350000
300000
250000
200000
150000
100000
50000
0
M/Z ->
60
80
100
120
140
160
180
200
220
222
161
147
194
221
91
115
131
135
179
97
77
65
51
207

Problem #1- Product B (HNMR)



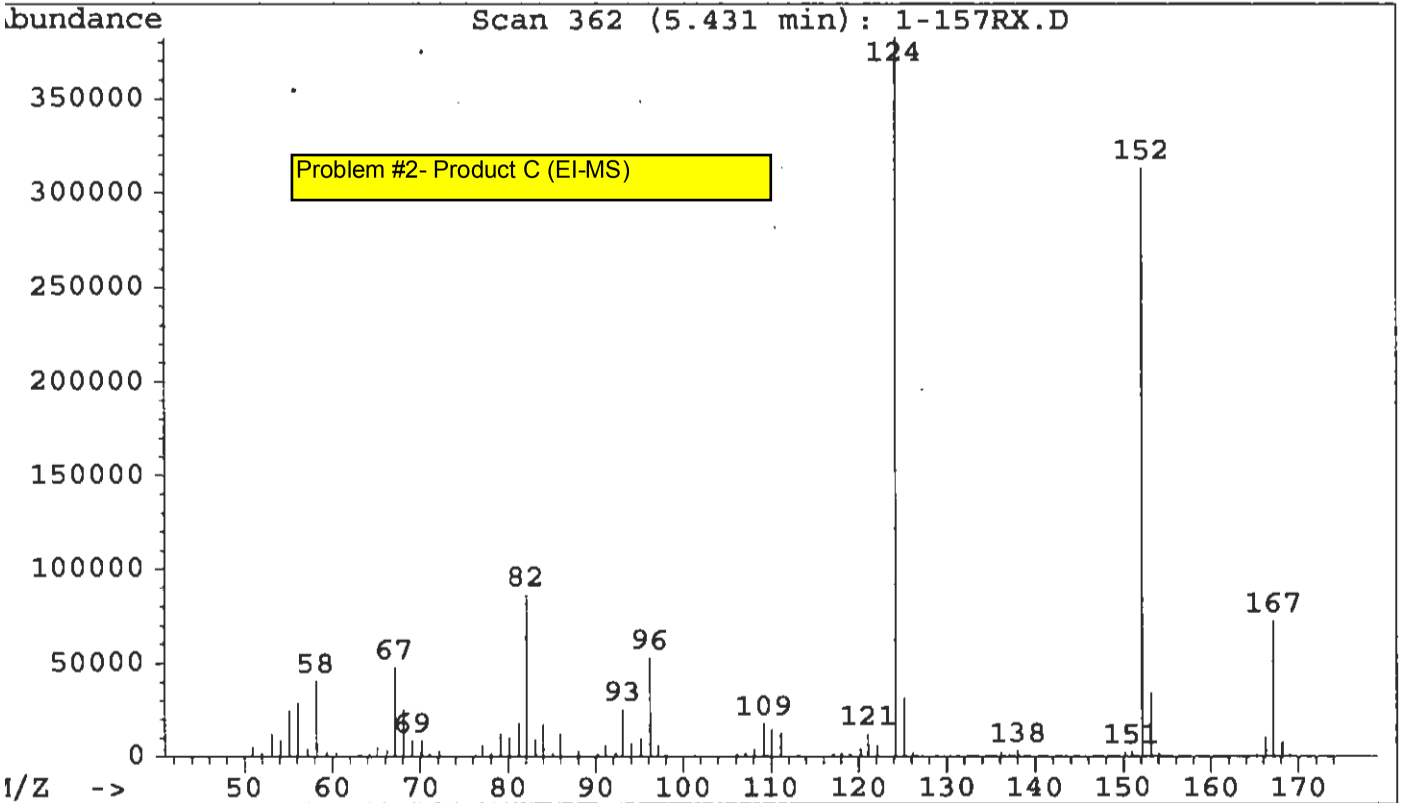

Abundance
Scan 1568 (21.781 min): 2212A.D
Problem #1- Product B (EI-MS)
444
222
188
147
161
97
111
69
239
281
354
413
25000
20000
15000
10000
5000
0
M/Z ->
50
100
150
200
250
300
350
400

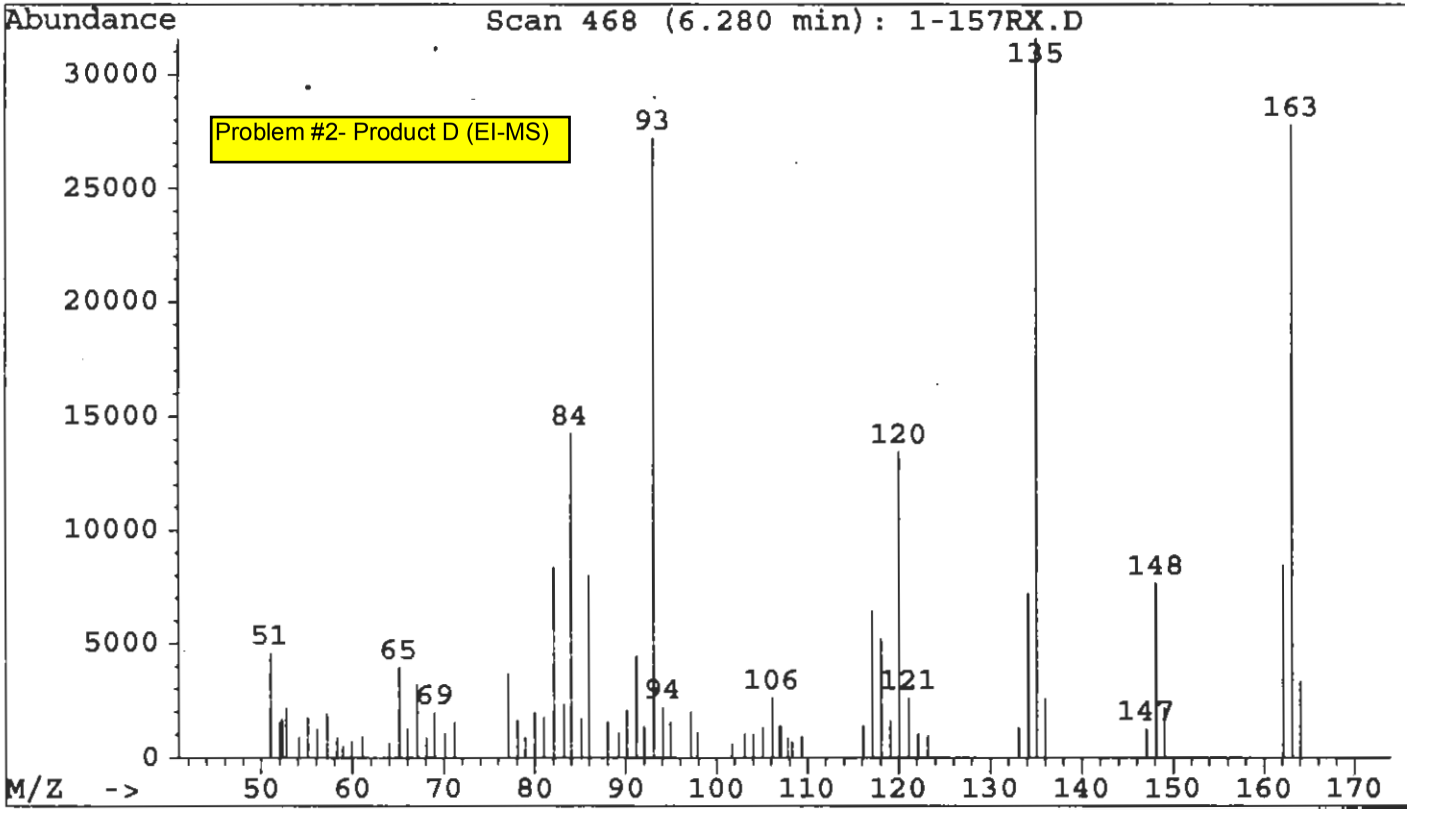
2. Lucas Kopel (Hoye group) subjected the indole starting material to hydrogenolysis, in an attempt to reduce the C(2)-C(3) double bond in the five-membered ring. Lucas obtained two different products (**C** and **D**) from the reaction. EI-MS spectra for both of these products follow this problem.



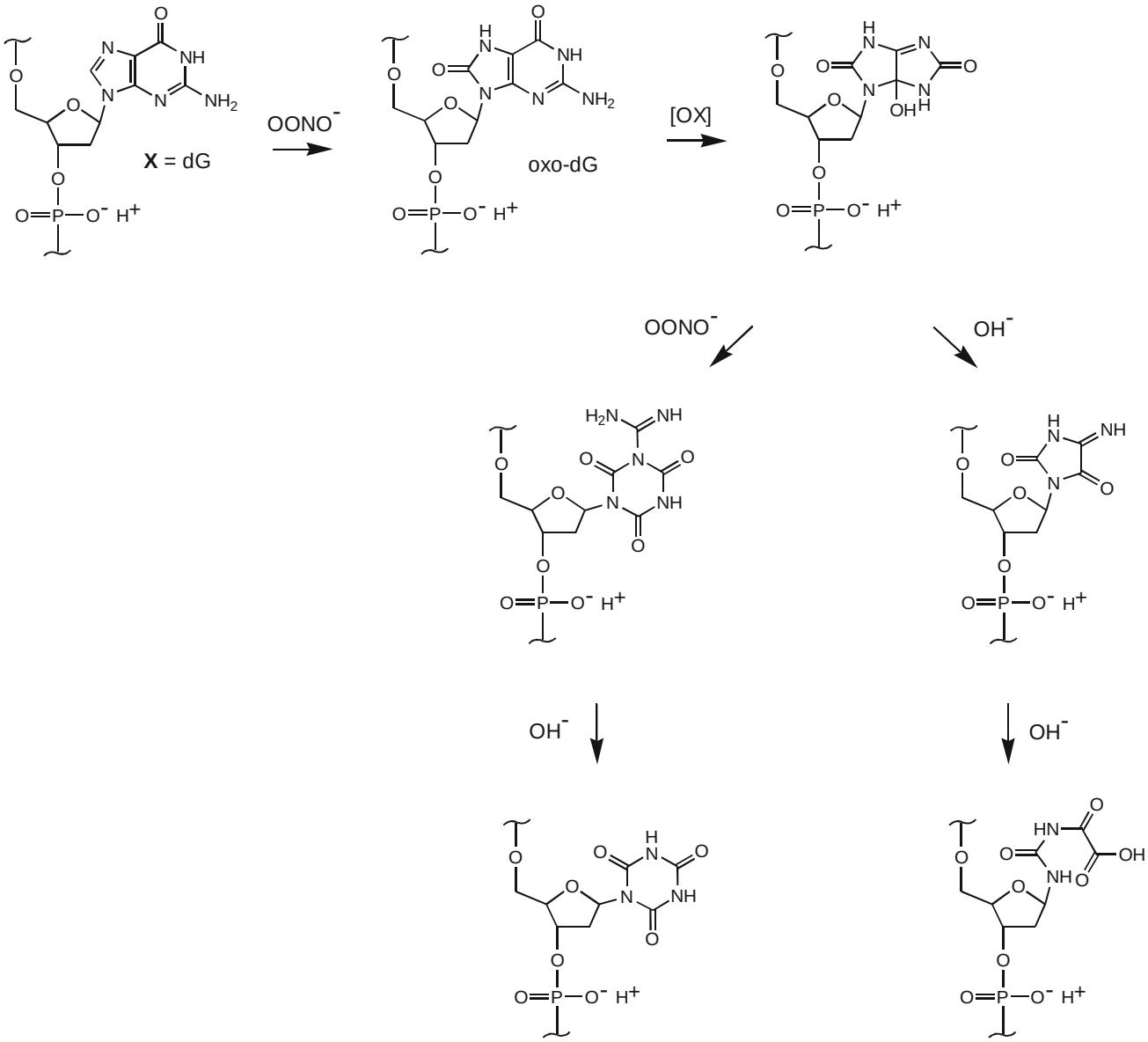
a. The highest-mass (parent) peak in both EI-MS spectra are greater than *m* = 119, and that led Lucas to conclude that he had not made his expected product. Indoles are reactive at C(2) and C(3), and Lucas presumed that some other reaction might have have taken place at one or both of those carbons. Propose structures of products **C** and **D**. (Given the information you have, there are probably a number of possible structures. Propose just one structure for each product.)

b. Based on the structures you proposed, draw mechanisms that explain the peaks at *m*/*z* = 152 and 124 in the spectrum of molecule **C**, and the peaks at *m*/*z* = 148, 135, and 120 in the spectrum of molecule **D**.


bundance
Scan 362 (5.431 min): 1-157RX.D
Problem #2- Product C (EI-MS)
350000
300000
250000
200000
150000
100000
50000
0
124
152
167
82
96
58
67
69
93
109
121
138
151
1/Z ->
50
60
70
80
90
100
110
120
130
140
150
160
170

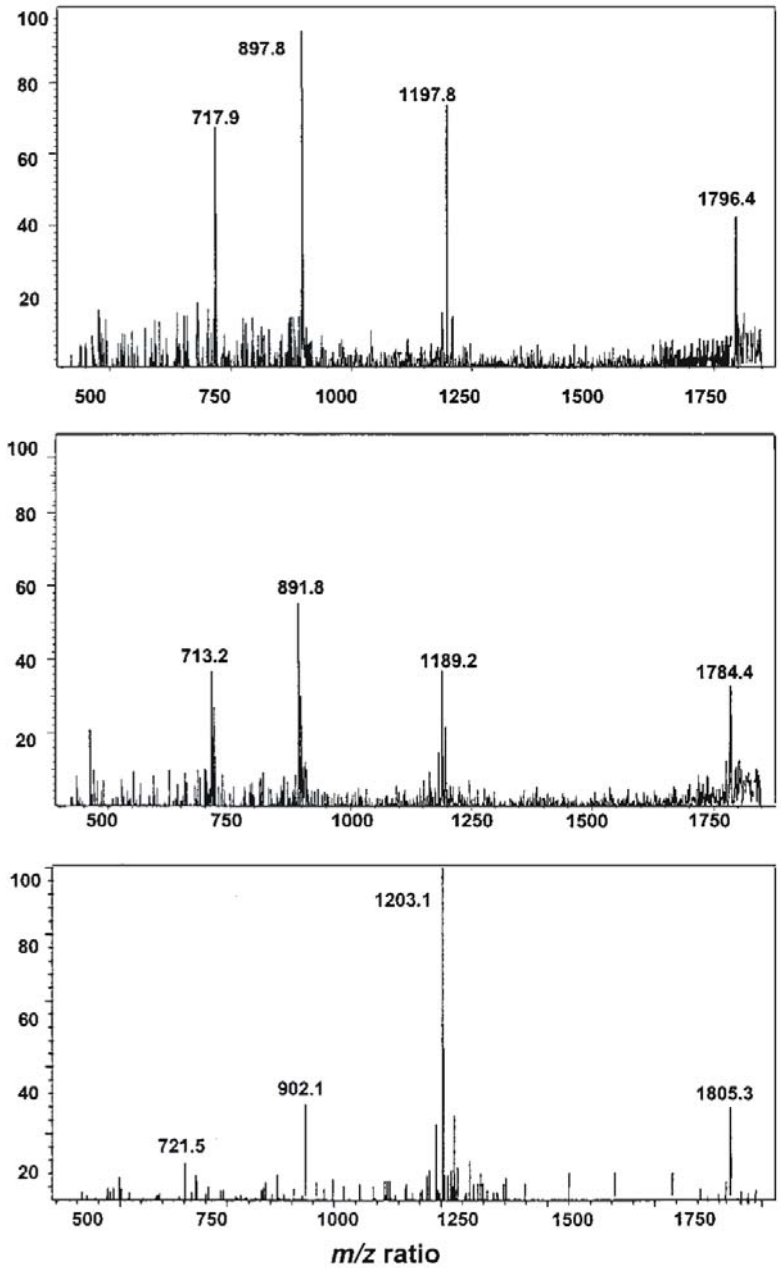

Abundance
Scan 468 (6.280 min): 1-157RX.D
Problem #2- Product D (EI-MS)
30000
25000
20000
15000
10000
5000
0
M/Z ->
50
60
70
80
90
100
110
120
130
140
150
160
170
135
163
93
84
120
148
51
65
69
94
106
121
147

3. Professor Natalia Tretyakova (Medicinal Chemistry) is generally interested in the effect of environmental carcinogens that chemically alter the structure of DNA. (Ref.: N. Tretyakova et al., *Chem. Res. Toxicol.* **2001**, *14*, 1058.) Peroxynitrite anion ($OONO^-$), one species implicated in DNA damage, is generated by the reaction of endogenous NO_2 with chemical oxidants. Peroxynitrite is thought to react selectively with guanosine (dG) bases in DNA by a number of possible mechanisms. Two that might be active under basic conditions are shown below.



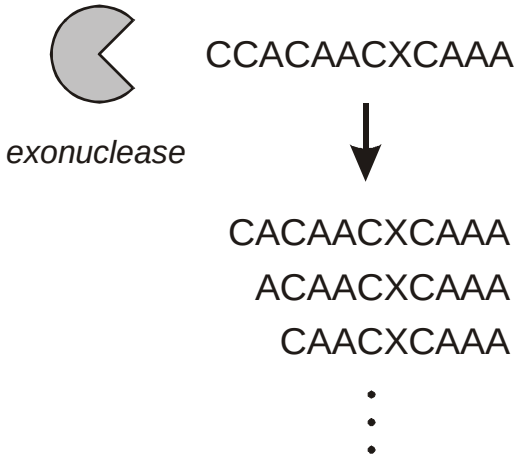
To test these mechanisms, Tretyakova and colleagues have exposed synthetic oligonucleotides (short, single-stranded DNA molecules) to peroxynitrite, and separated the reaction products by HPLC. In all cases, the peroxynitrite is expected to have reacted with dG residues in the oligonucleotide sequence.

a. When the synthetic oligonucleotide CCACAAC**G**CAAA (neutral mass 3592.4) was exposed to peroxynitrite in basic solution, three major reaction products were isolated by HPLC. The negative-ion mode electrospray mass spectra of each of these products is shown below:

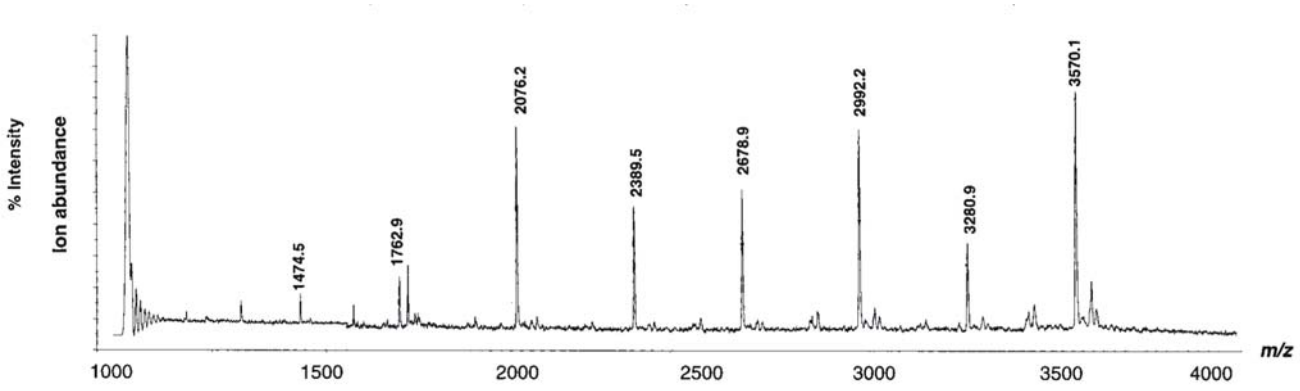


Each of these spectra shows a charge ladder, with each peak corresponding to a multiply charged version of the same basic molecule. What are the masses of each of these three reaction products? What structures from the previous page do these products correspond to?

b. The isolated component shown in the middle mass spectrum from part (a) was then subjected to exonuclease digestion, such that the oligonucleotide was degraded into smaller strands from one end only:

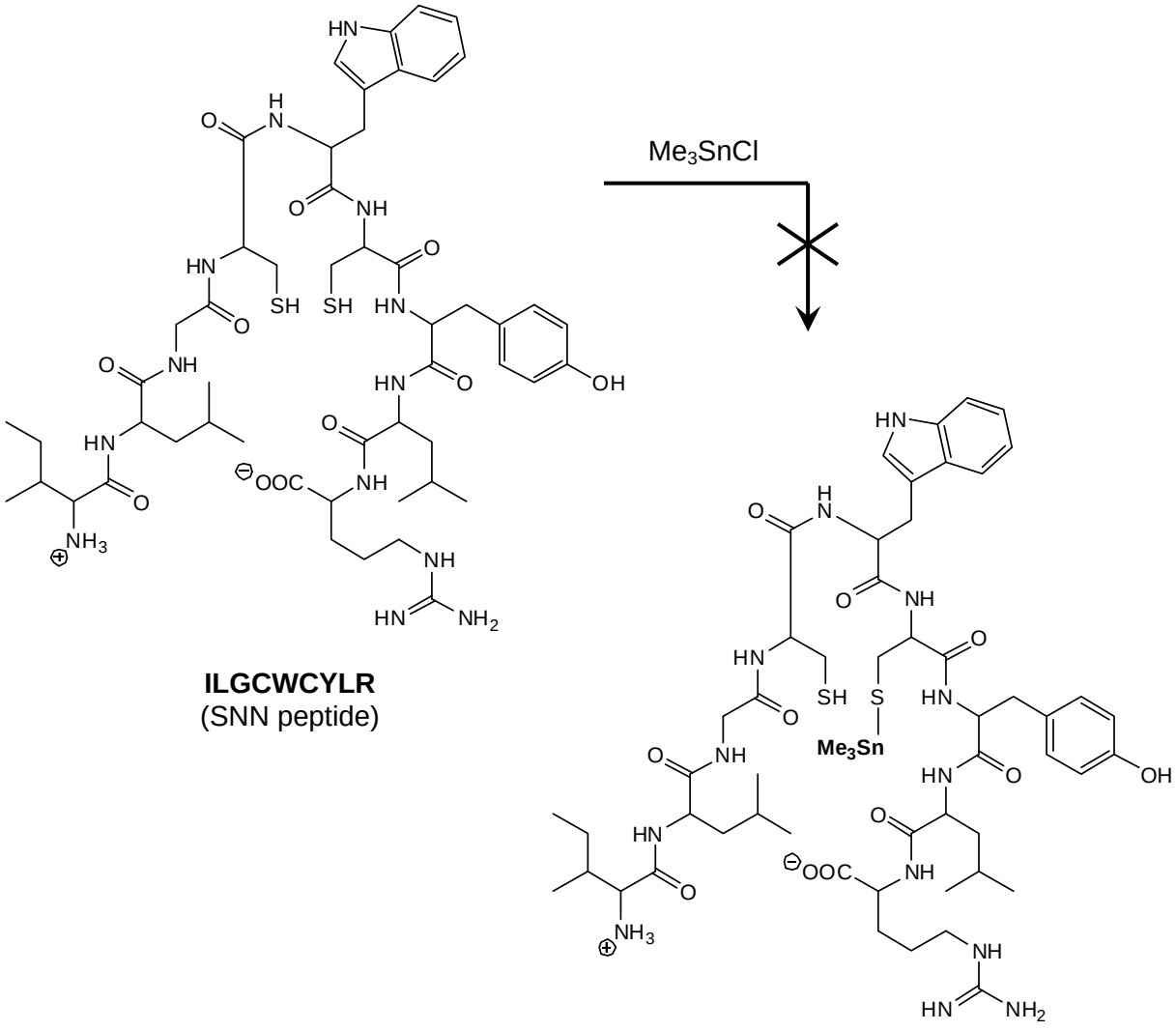


The MALDI-MS of the mixture of exonuclease products is shown below. (The peaks here do not represent a charge ladder, but rather a mixture of molecules.) Where does the exonuclease stop digesting the DNA? From the relative intensities of the peaks, what might you say about the exonuclease's recognition of the DNA strand?



(The oligonucleotide mass calculator at http://medlib.med.utah.edu/masspec/mongo.htm can help you some with this problem.)

4. Trimethyltin chloride (TMT-Cl) is a potent neurotoxin, and is surprisingly specific in its attack on hippocampal nerve cells. Some time ago, researchers isolated stannin (SNN), a hippocampal membrane protein that binds TMT-Cl and is probably responsible for its toxicity. In order to study the interaction between stannin and TMT-Cl, Bethany Buck (Veglia group) synthesized a peptide fragment of stannin, **ILGCWCYLR**, exposed this peptide to TMT-Cl, and investigated the product mixture by ESI-MS. Although Beth anticipated that the peptide would attack TMT-Cl nucleophilically at cysteine thiol (see diagram below), the masses observed were not consistent with a simple addition product. ESI mass spectra of the peptide alone (with closeup) and the peptide + TMT-Cl follow this problem.



ILGCWCYLR
(SNN peptide)

not observed

a. Beth injected her SNN peptide alone into the ESI instrument in 50/50 H_2O:MeOH, with a trace of acetic acid to protonate the molecule to $[M+H]^+$. What parent mass should she have expected to see in this experiment? (Though you might calculate the mass of SNN by hand, it might be easier to use an online peptide mass calculator for this. There are lots—just search for one.)

b. Beth actually observed four main peaks in her ESI-MS. Explain all of these peaks in terms of Beth's peptide. (*Hint:* Two of the peaks demonstrate the presence of a very simple, typical contaminant in biochemical preparations.)

c. Calculate the expected intensities of the [M + 1] and [M + 2] isotope peaks you would expect for the m = 1125.60 peak. Is Beth's closeup spectrum of the *m*/*z* = 1125.7 peakset consistent with your calculations?

d. When Beth exposed her peptide to TMT-Cl, and analyzed the resulting mixture by ESI-MS, she observed a number of new peaks. First, she observed peaks corresponding to a simple loss of 2 amu from peaks observed in the first experiment. Beth found that the relative intensities of these peaks did not depend on the amount of TMT-Cl in the mixture, but that the *m*/*z* = 1123.67 peak was reduced when oxygen was rigorously excluded from the solution. What product was responsible for this new peak?

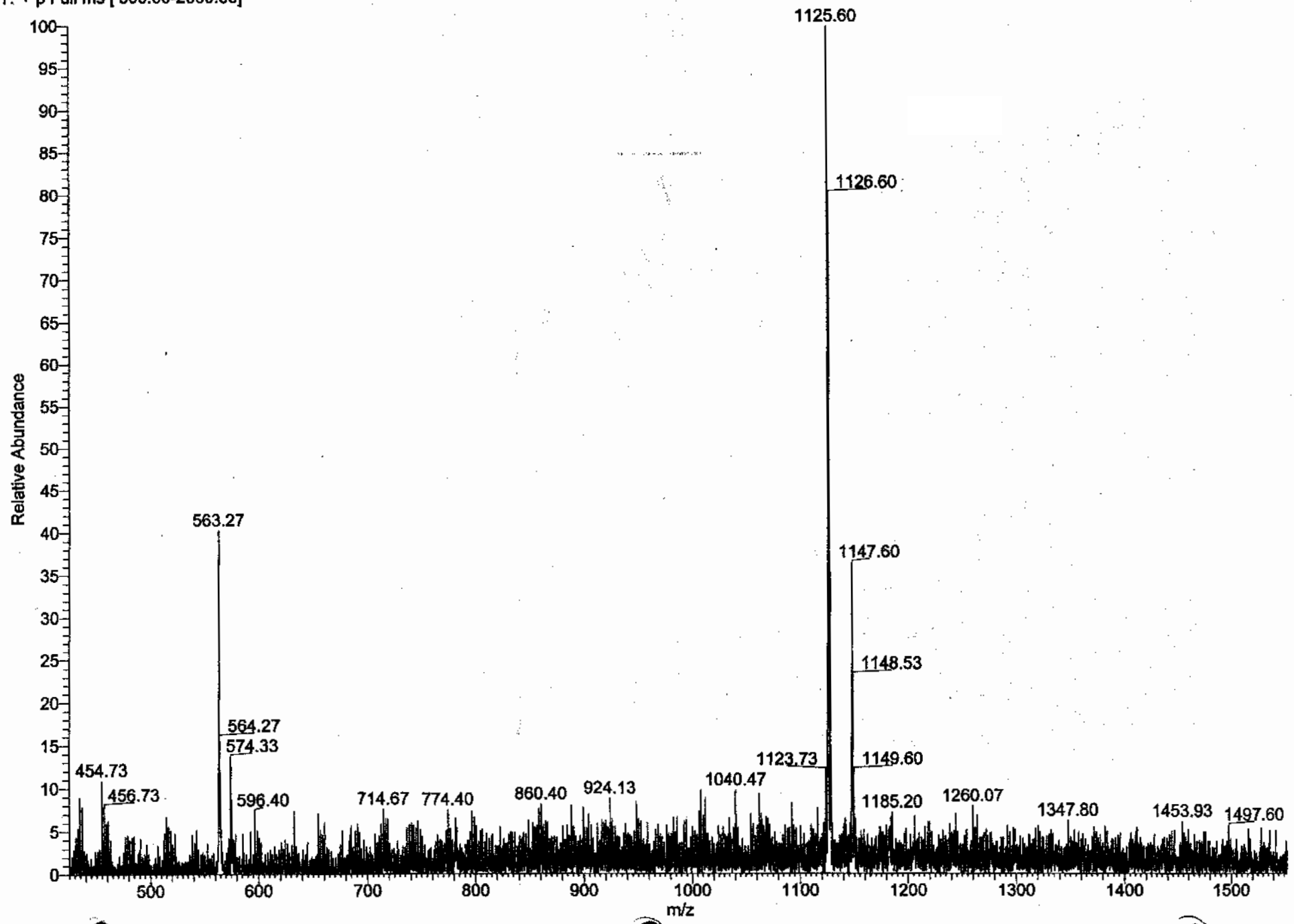
e. What structures do the higher-mass peaks correspond to? There are many more isotopic peaks surrounding the *m*/*z* = 1273.60 and *m*/*z* = 1295.67 peaksets than in the lower mass peaks. Why? Explain the intensities of these peaks as quantitatively as you can.

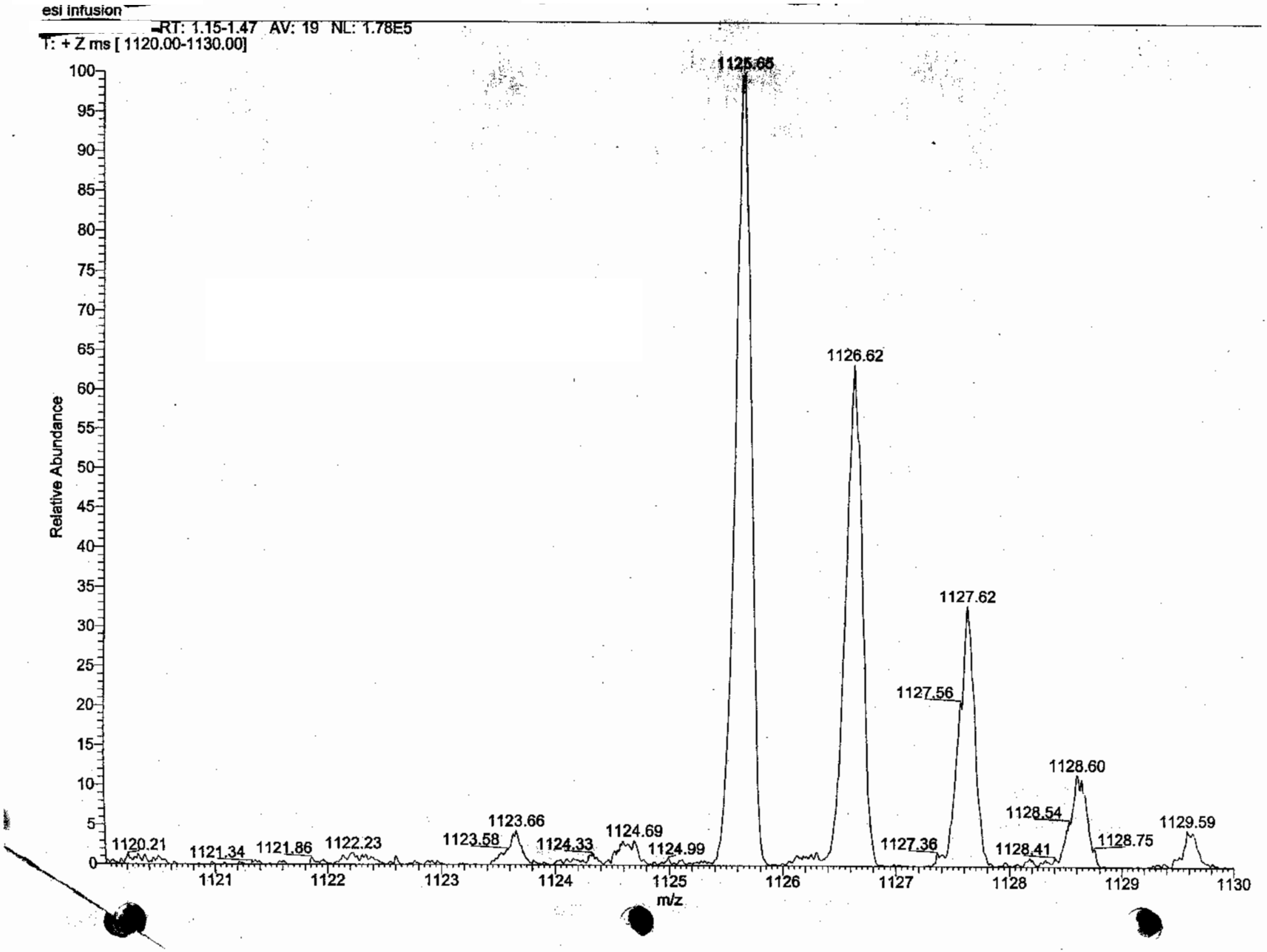
f. In order to verify her conclusions about the structures of the TMT-Cl adducts, Beth also mixed her peptide and TMT-Cl in D_2O:MeOD, such that all exchangeable protons were exchanged with deuterium atoms. The ESI-MS of this mixture yielded sets of peaks at *m*/*z* = 1290.6, 1311.6, and 646.3. Is this result consistent with your proposed product structure?

07/31/02 10:00:54 AM

esi infusion

T: + p Full ms [300.00-2000.00]




esi infusion
RT: 1.15-1.47 AV: 19 NL: 1.78E5
T: + Z ms [1120.00-1130.00]
Relative Abundance
m/z
1125.65
1126.62
1127.62
1127.56
1128.60
1128.54
1129.59
1128.75
1127.36
1128.41
1123.66
1123.58
1124.33
1124.69
1124.99
1120.21
1121.34
1121.86
1122.23

esi

gv619h #44-76 RT: 0.71-1.22 AV: 33 NL: 3.53E5

T: + p Full ms [700.00-1500.00]