

Today

Section 13.1
Mass Spectrometry

Next Class + 1

Section 13.1 – 13.6
Mass Spectrometry

Infrared Spectroscopy

Next Class

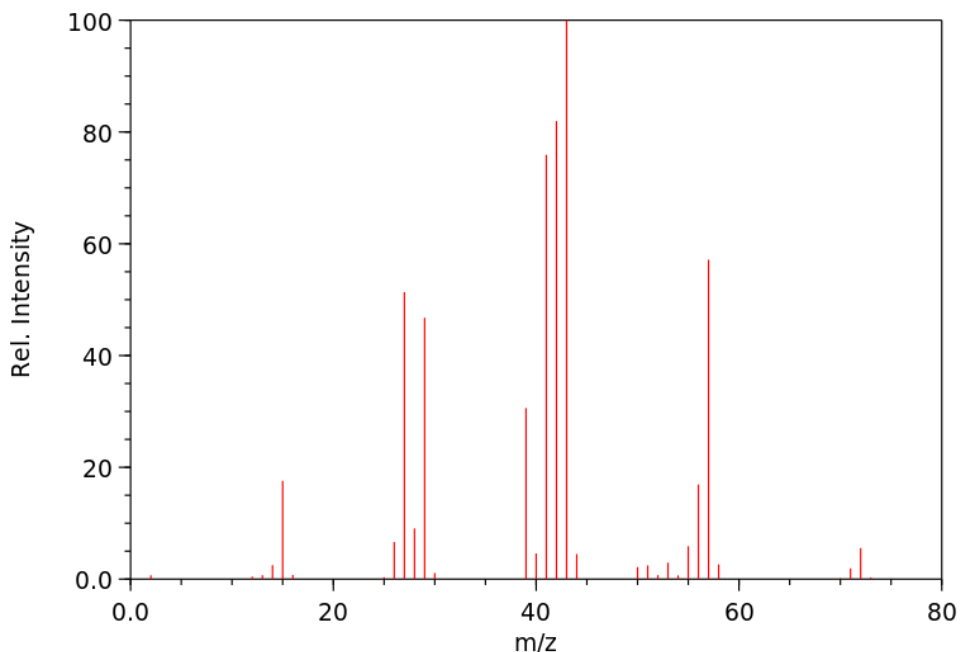
Section 13.1 – 13.6
Mass Spectrometry

Next Class + 2

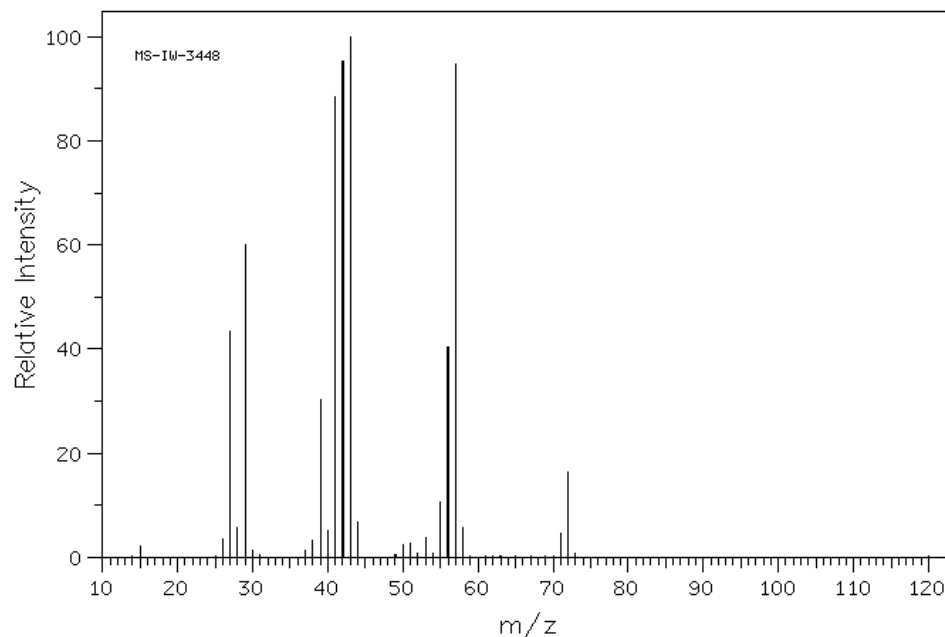
Infrared Spectroscopy

Why Mass Spectrometry?

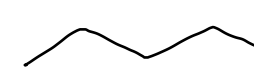
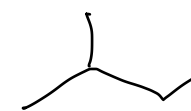
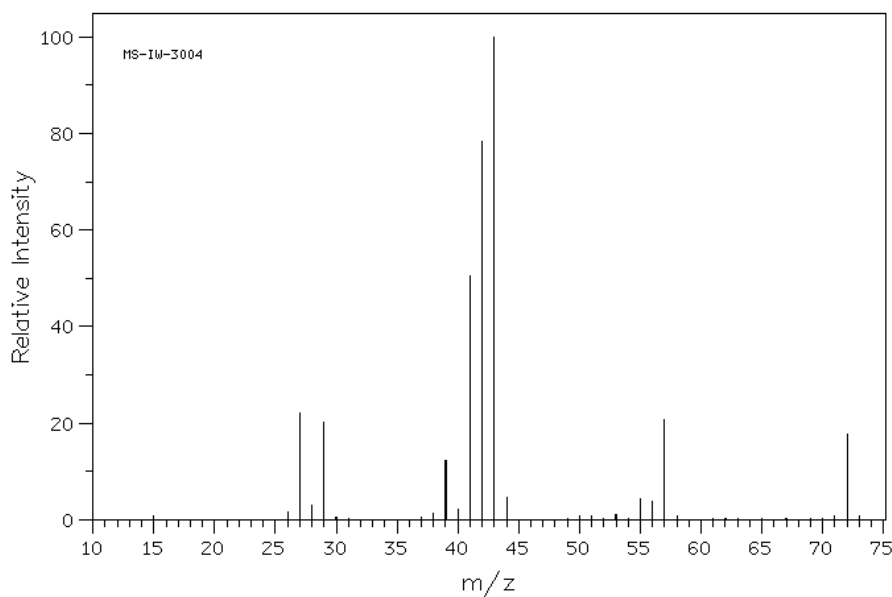
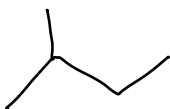
Identify known compounds



NIST Chemistry WebBook (<https://webbook.nist.gov/chemistry>, Jan 2023)

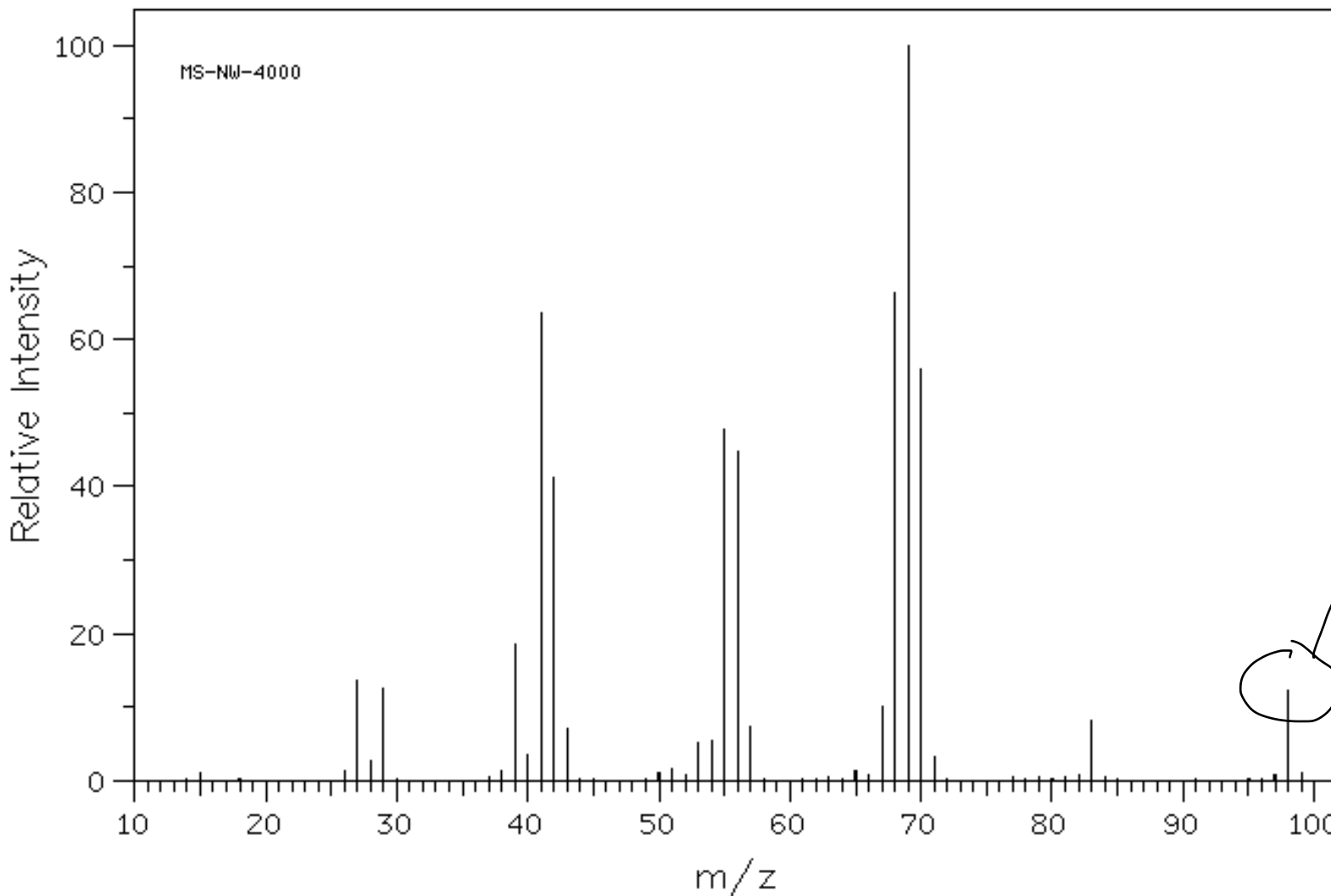


SDBSWeb : <https://sdb.sdb.aist.go.jp> (National Institute of Advanced Industrial Science and Technology, March 2006)



Why Mass Spectrometry?

Determine molar mass and structure of unknown compounds.



SDBSWeb : <https://sdb.sdb.aist.go.jp> (National Institute of Advanced Industrial Science and Technology, Jan 2019)

Section
peak
Furthest to the right can tell us the molar mass of the molecule

$$\frac{m}{z} = \frac{98 \text{ u}}{+1}$$

$$m = 98$$

Unfortunately, this isn't always true for all functional groups, so we often need more information for greater certainty

Why Mass Spectrometry?

Determine 1° structures of polypeptides (protein ladder sequencing).

1. 5% phenylisocyanate 95% phenylisothiocyanate (PC)
2. Trifluoroacetic acid
3. repeat

[Glu1]fibrinopeptid

PC-Glu-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

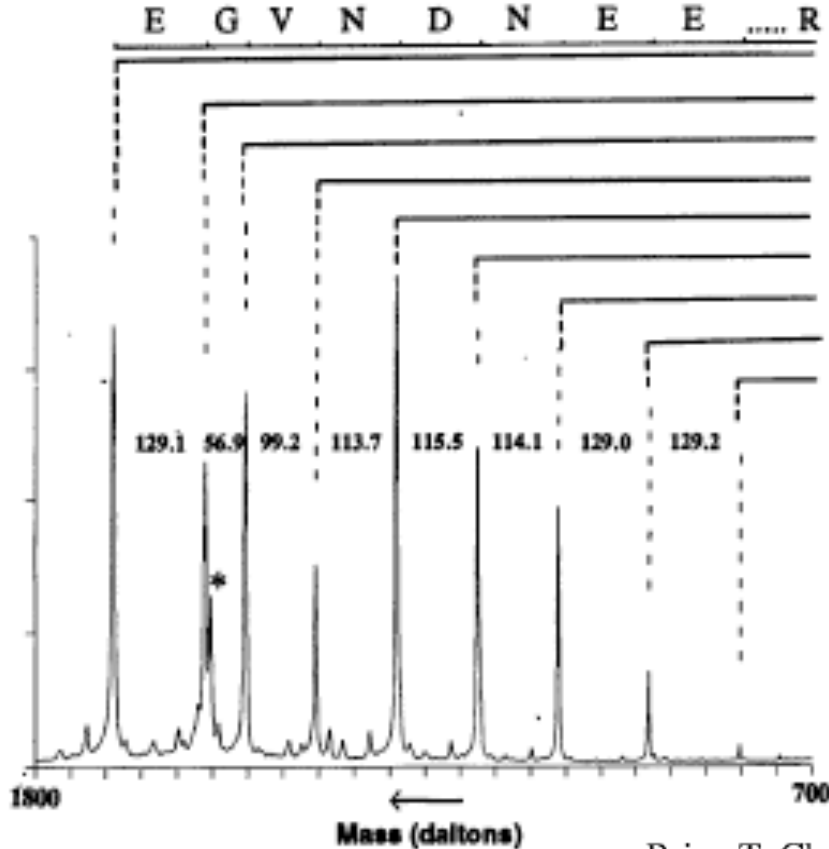
PC-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

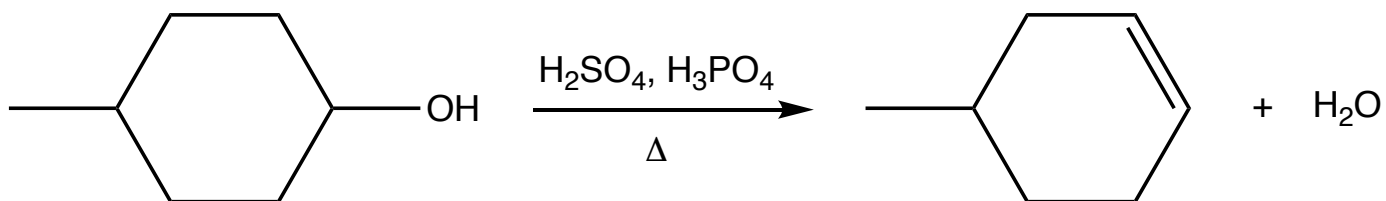


Brian T. Chait; Rong Wang; Ronald C. Beavis; Stephen B. H. Kent

Science, New Series, Vol. 262, No. 5130, Genome Issue. (Oct. 1, 1993), pp. 89-92. 4

Why Mass Spectrometry?

Confirm synthesis of target compound.



IR Data: OH vs no OH, no C=C vs C=C

MS Data: molar mass and high-resolution mass spectrometry to confirm formula

NMR Data

HRAM GC-MS/MS



For comprehensive characterization of samples in a single analysis with high-confidence compound discovery, identification and quantitation, a GC system can be combined with a high resolution accurate mass (HRAM) mass spectrometer.

QSign® Triple Quadrupole LC/MS/MS Platform for Clinical Research



An exceptional solution for a wide range of academic and research applications, including metabolomics, proteogenomics, pharmacology, and biomarker discovery,

Overview

Describe the basics of how mass spectrometry works.

Examine the affects that isotopes and their natural abundance has on the mass spectrum

Consider three methods for determining the formulas of compounds

Predict common fragmentation patterns for different functional groups

Unless indicated otherwise, all mass spectra that follow have been downloaded from the SDBSWeb : <https://sdb.db.aist.go.jp> (National Institute of Advanced Industrial Science and Technology)

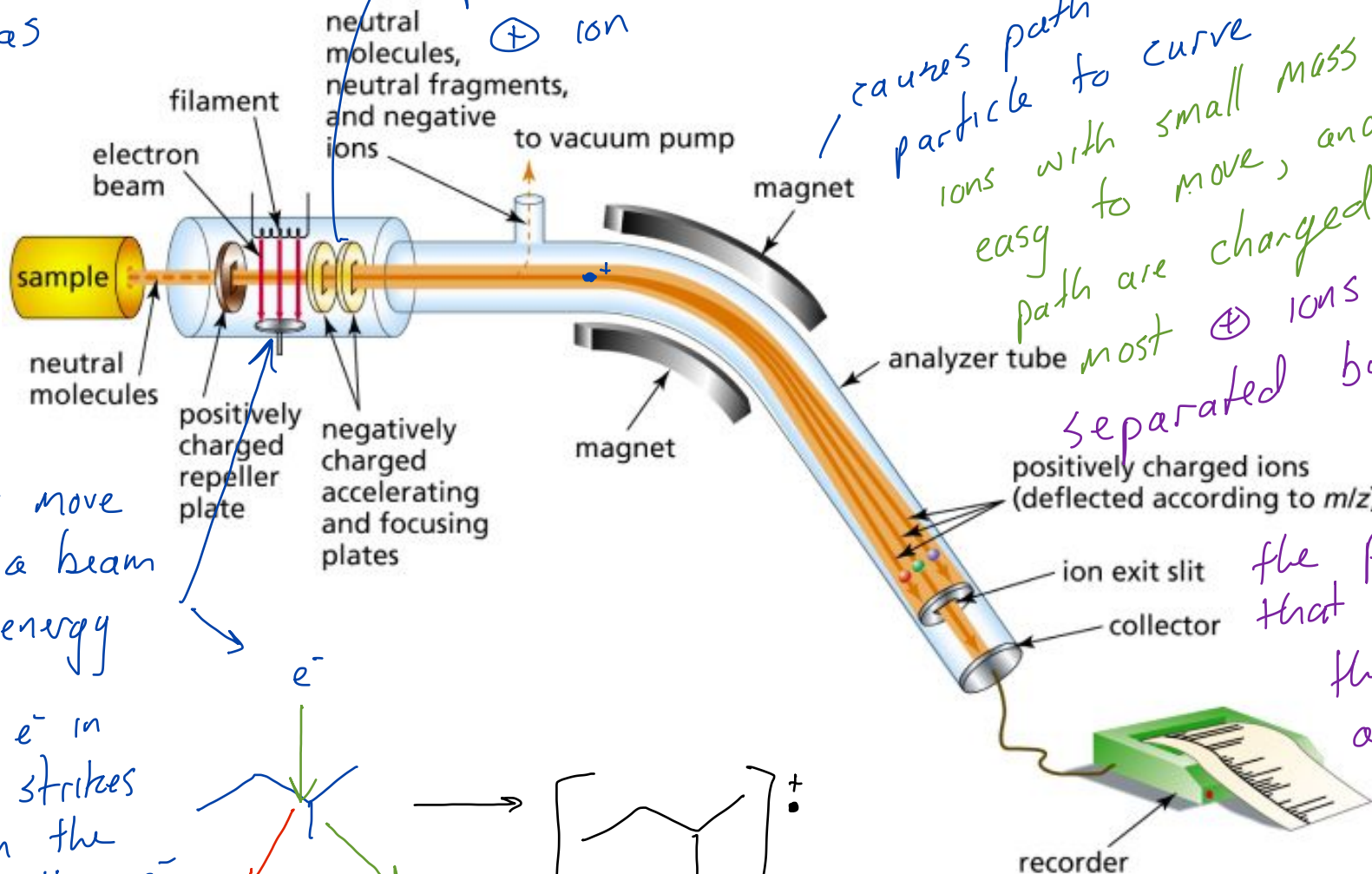
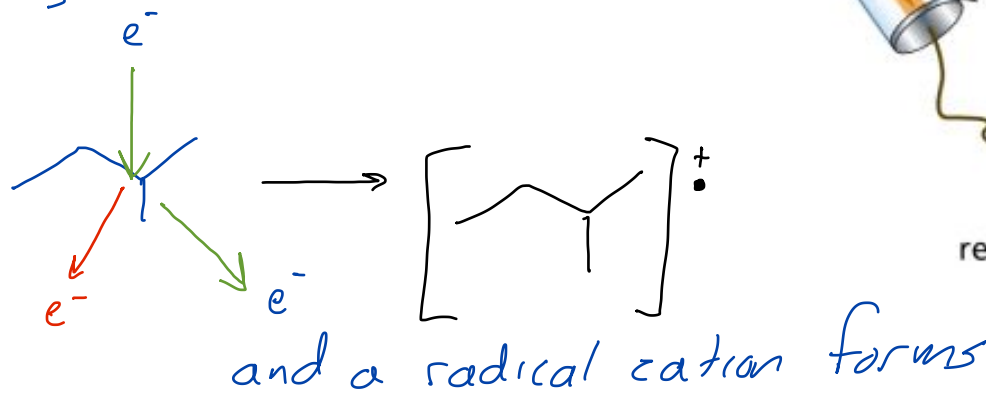
Schematic Representation of a Mass Spectrometer

get sample into gas phase

negatively charged plates accelerate the \oplus ion

causes path of charged particle to curve
ions with small mass are easy to move, and their path are changed the most \oplus ions are separated by mass

molecules move through a beam of high energy e^- 's. When an e^- in the beam strikes an e^- 's in the molecule, the e^- is ejected...



the particles that hit the detector are recorded

only see \oplus particles

Today

Section 13.1
Mass Spectrometry

Next Class + 1

Sections 13.10 - 13.8
Infrared Spectroscopy

Next Class

Section 13.1 – 13.6
Mass Spectrometry

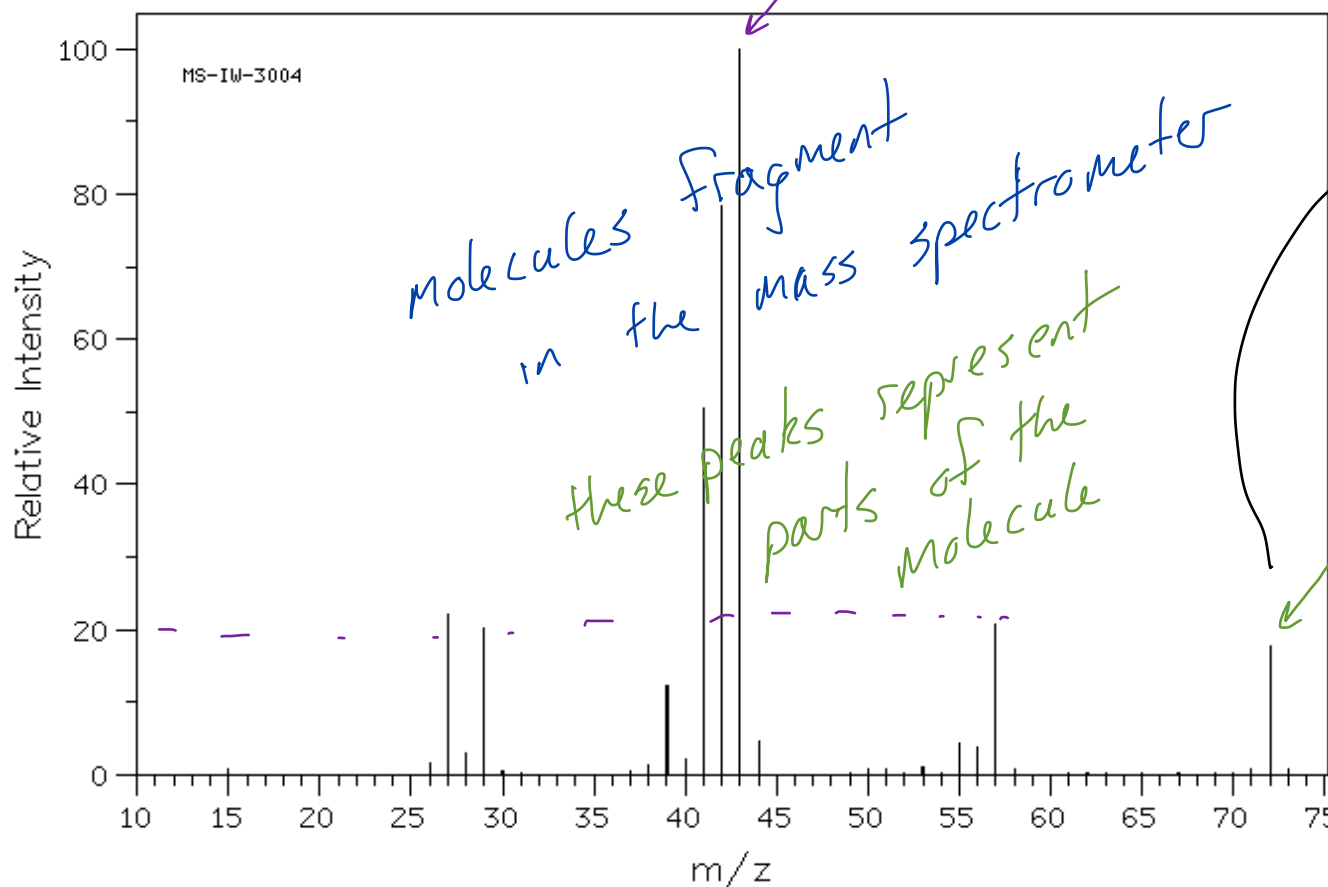
Sections 13.10 - 13.8
Infrared Spectroscopy

Next Class + 2

Sections 13.10 - 13.8
Infrared Spectroscopy

The Basics of the Mass Spectrum

Mass Spectrum of Pentane



100 the tallest peak is called the base peak

molecules fragment in the mass spectrometer these peaks represent parts of the molecule



often, but not always, the peak furthest to the right is

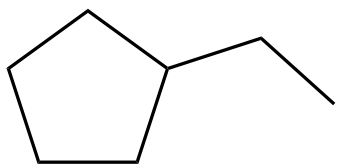
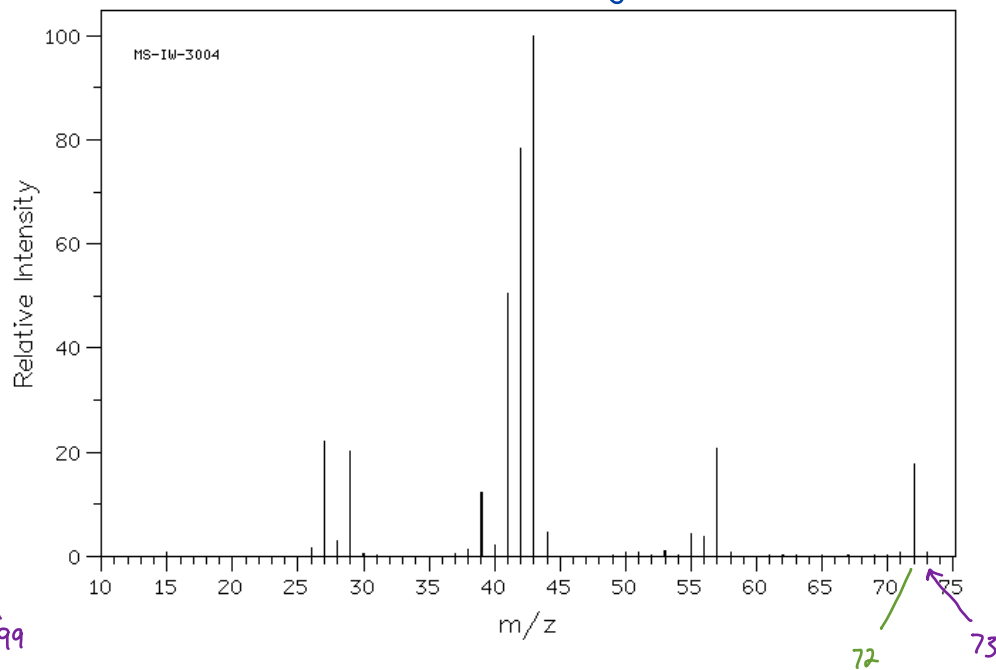
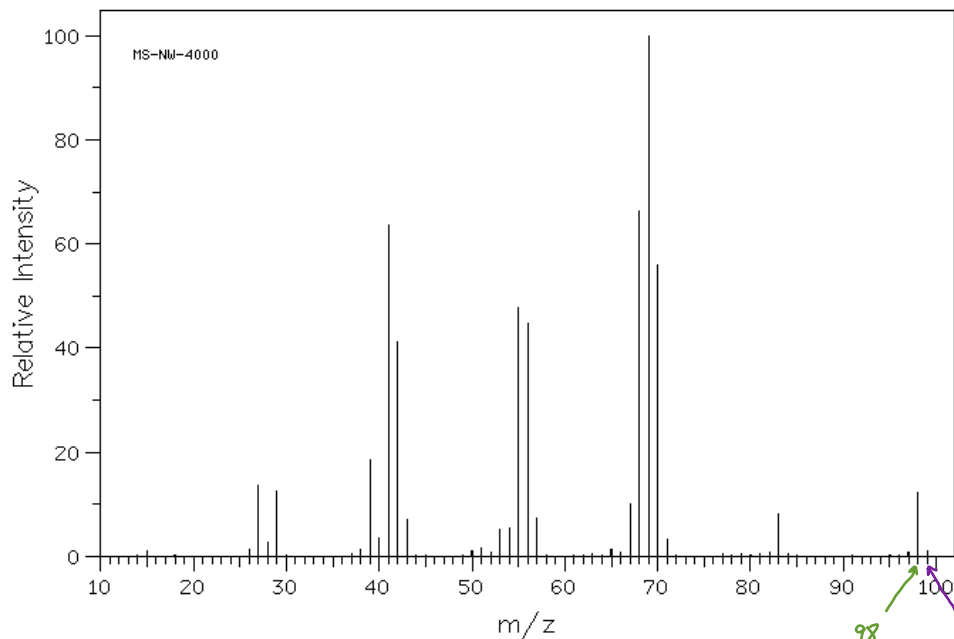
the molecular ion ...

The molecule that one e⁻

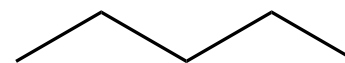
The peak at $m/z = 43$ is the base peak all other peaks are compared to the height of that peak. The relative intensity of the peak at $m/z = 57$ is 20%

Carbon and Those Tiny Peaks Next to the Molecular Ion

$^{12}\text{C} = 12.00000 \text{ g/mol}$ Section 13.4
 $\text{C} = 12.011 \text{ g/mol}$



Molecular Weight: 98.1890



Molecular Weight: 72.1510

$1.1 + 1.1 = 2.2\%$ of the time

$\text{H}_3\text{C}-\text{CH}_3$ 1 will be all ^{12}C

$1.1\% \times 1.1\% = 0.011\%$

^{13}C
M

1 of the C's is ^{13}C

M+1

NO M+2 'cauz ^{14}C is not abundant enough and

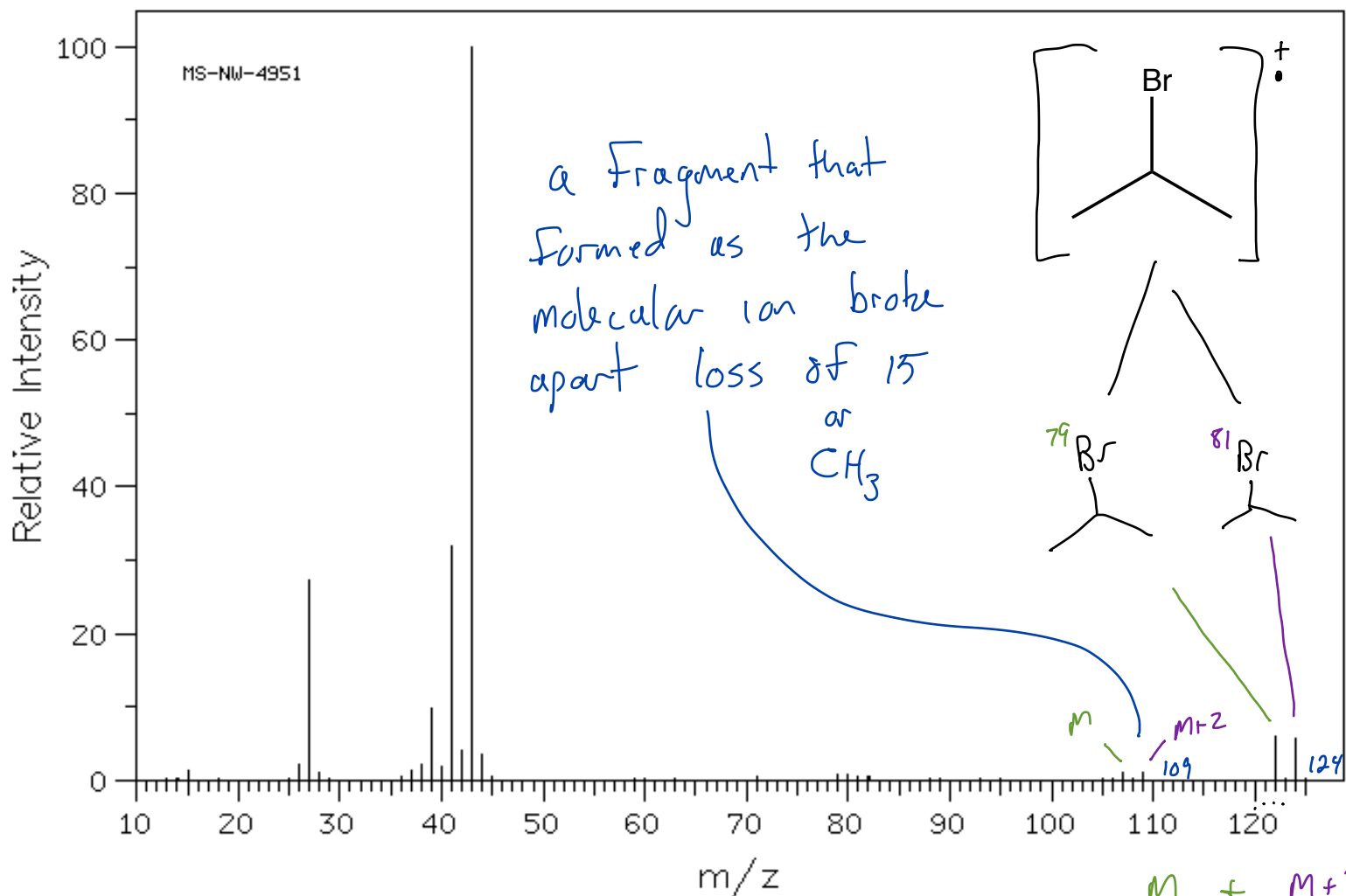
why not M+2?

1.1% ^{13}C in carbon on Earth

abundance of ^{13}C is too low for us to see two

Bromine Atoms and the Missing Peak?

Section 13.4

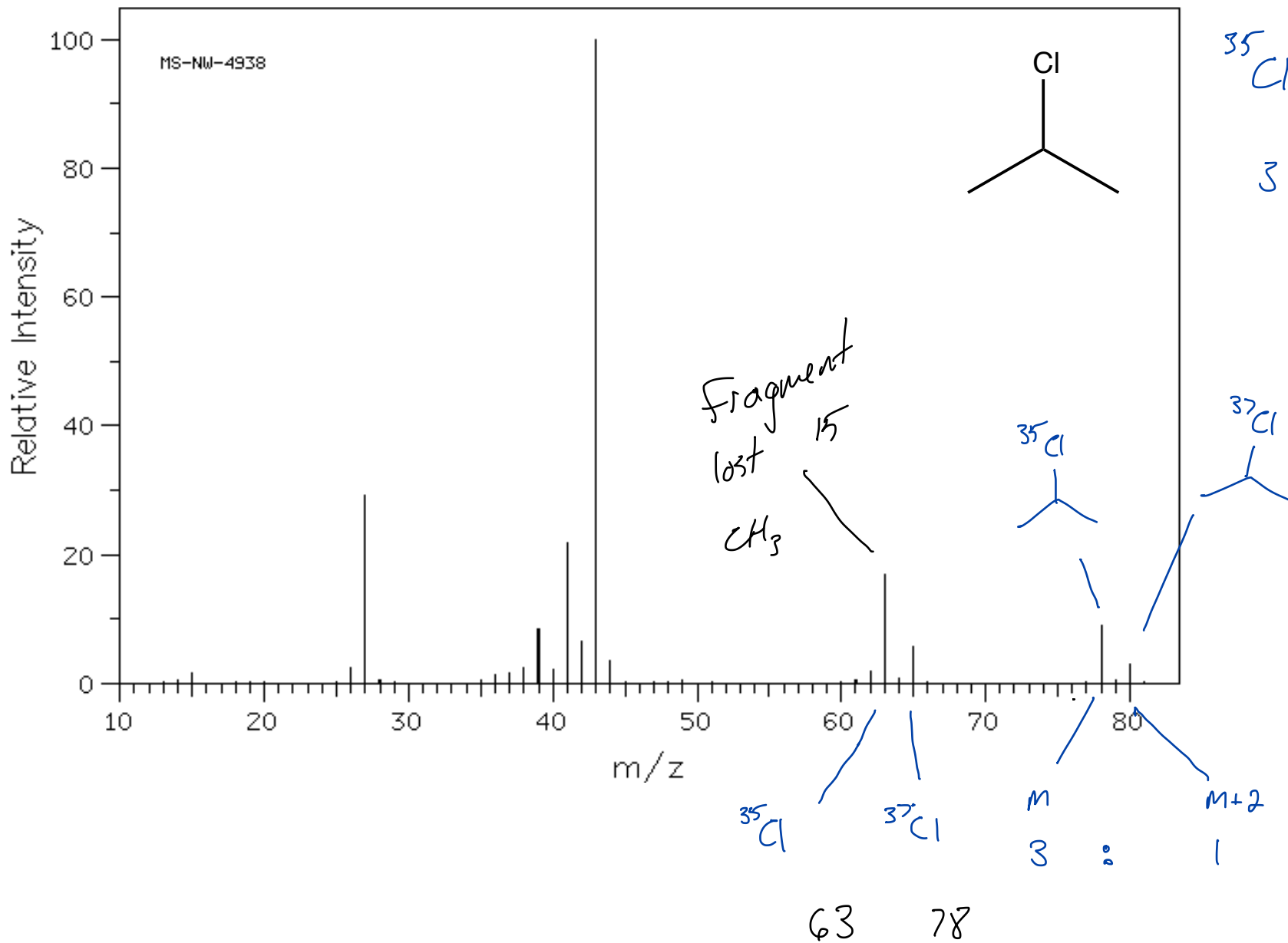


$3(12) = 36$
 $7(1) = 7$
 $1(80) = 80$
 \uparrow
 $\underline{123}$
 79.9...
 $^{79}\text{Br} + ^{81}\text{Br}$

$m + m+2$ equal intensity
 often means Br present

Isotopic Fingerprint for Chlorine Atoms

Section 13.4



^{35}Cl ^{37}Cl

3 : 1

Comparing m and m+1 peaks

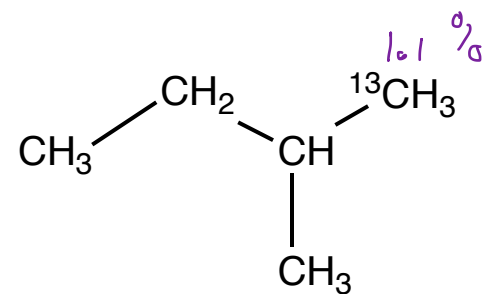
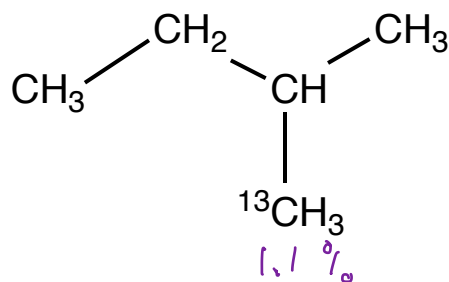
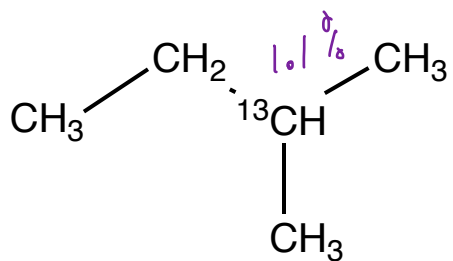
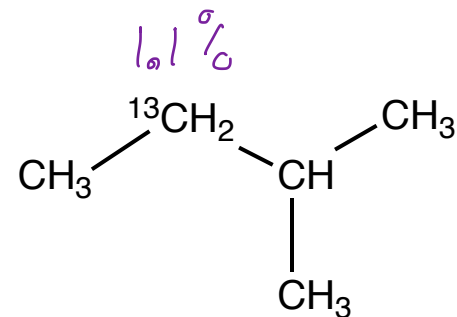
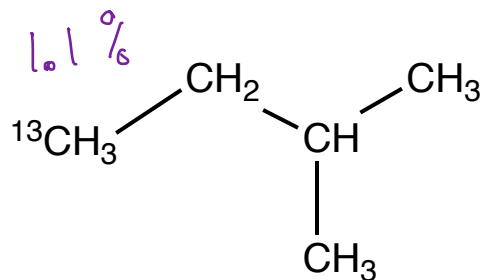
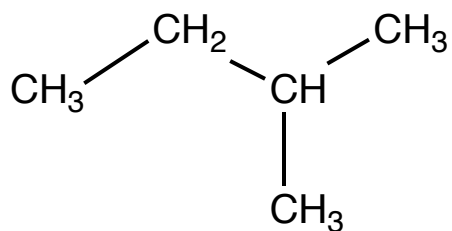
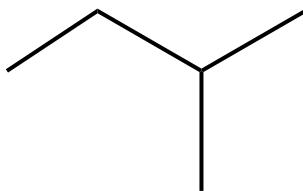
compare ^{12}C + ^{13}C peaks

The "Rule of 13"

see how many CH's can I fit into a peak

High Resolution Mass Spectrometry

spend lots of money



C atoms \times 1.1% = % of ^{13}C
 which relates the height
 of m to m+1 present in
 molecule

5.5% of the
 time this molecule
 has a ^{13}C in it

Today

Section 13.1 – 13.6
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Sections 13.10 - 13.8
Infrared Spectroscopy

Second Class from Today

Sections 13.10 - 13.8
Infrared Spectroscopy

Next Class

Sections 13.10 - 13.8
Infrared Spectroscopy

Third Class from Today

Section 14.1
Introduction to Nuclear Magnetic Resonance

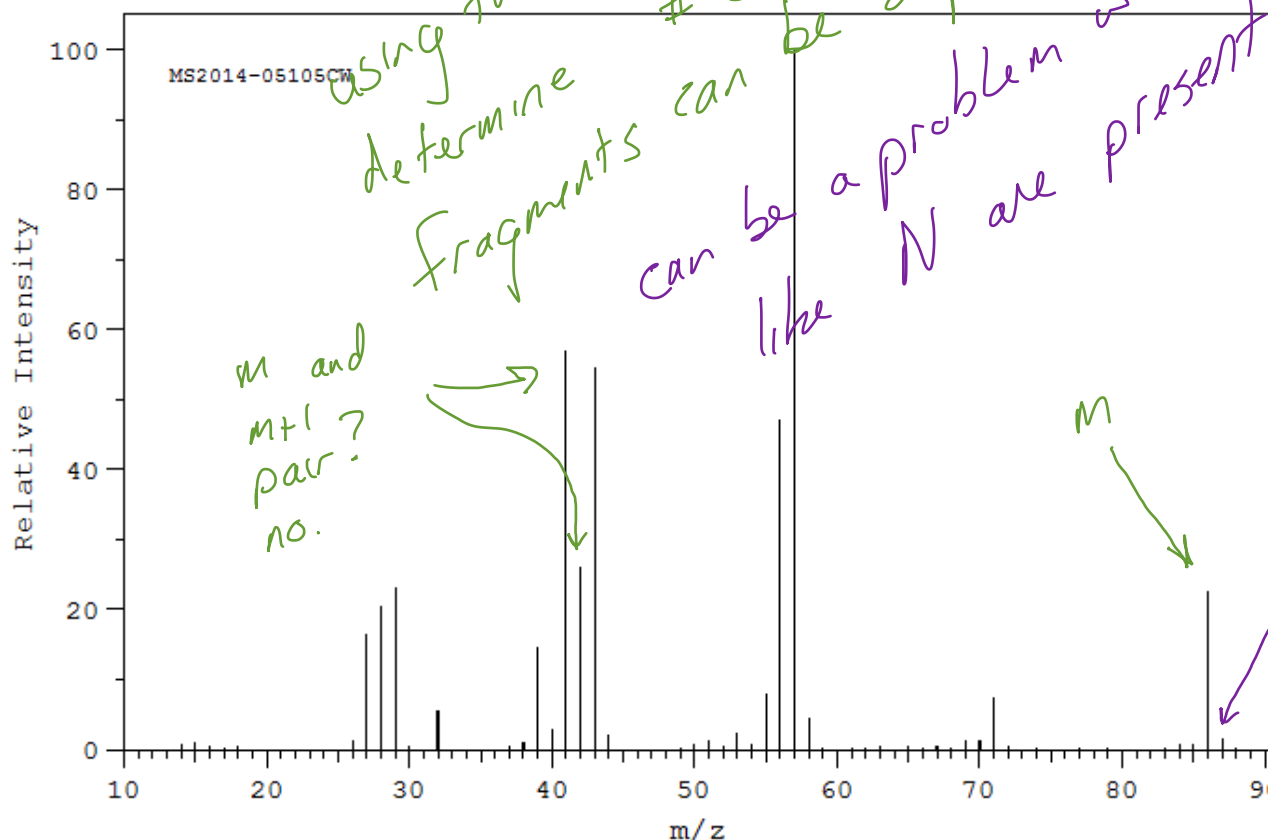
Determining Formulas Using m+1 Peaks

Section 13.3

$$\# \text{ C atoms} = \frac{\text{intensity of } m+1 \text{ peak}}{\text{intensity of } (m) + (m+1) \text{ peaks}} \cdot \frac{1}{0.011}$$

$$\# \text{ C atoms} = \frac{1.5}{22.4 + 1.5} \cdot \frac{1}{0.011} = 5.7 \rightarrow 6 \text{ C atoms}$$

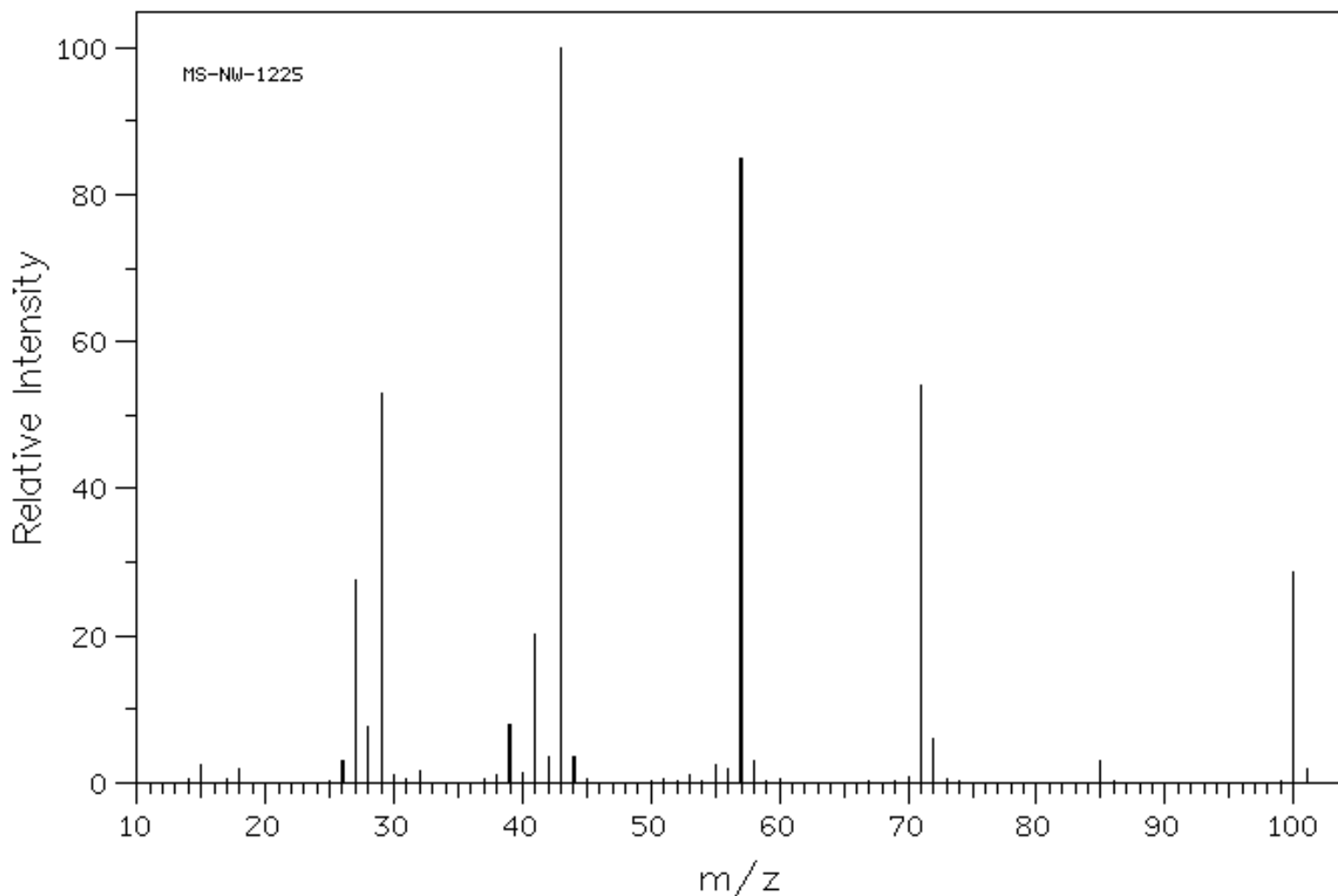
m/z	%
26.0	1.3
27.0	16.5
28.0	20.3
29.0	23.1
32.0	5.6
38.0	1.0
39.0	14.5
40.0	2.9
41.0	56.8
42.0	25.9
43.0	54.4
44.0	2.0
51.0	1.3
53.0	2.3
55.0	7.9
56.0	47.0
57.0	100.0
58.0	4.4
69.0	1.2
70.0	1.1
71.0	7.3
86.0	22.4
87.0	1.5



don't use this # in the formula

Determining Formulas Using m+1 Peaks

Section 13.3



m/z	%
14.0	0.5
15.0	2.4
17.0	0.4
18.0	1.8
26.0	2.9
27.0	27.6
28.0	7.5
29.0	53.0
30.0	1.1
31.0	0.6
32.0	1.6
37.0	0.4
38.0	1.0
39.0	7.9
40.0	1.2
41.0	20.3
42.0	3.6
43.0	100.0
44.0	3.4
45.0	0.4
50.0	0.3
51.0	0.4
53.0	1.0
54.0	0.2
55.0	2.4
56.0	1.8
57.0	84.9
58.0	3.1
59.0	0.3
60.0	0.4
67.0	0.3
69.0	0.3
70.0	0.7
71.0	54.0
72.0	6.1
73.0	0.4
74.0	0.2
85.0	2.9
100.0	28.6
101.0	2.0

Rule of 13 - how many CH units can fit into a peak with a given mass Section 13.3

Determine the number of CH units that "fit into" the peak. $C+H = 12+1 = 13$

If only C and H present, the remainder must be the number of H atoms present.

If other atoms present, "make room" for them by removing CH units.

C_4H_{10} would have a peak at m/z of 58.01 $[C_4H_{10}]^+$

$$\begin{array}{r} 4 \text{ r } 6 \\ 13 \overline{) 58} \\ \underline{52} \\ 6 \end{array}$$



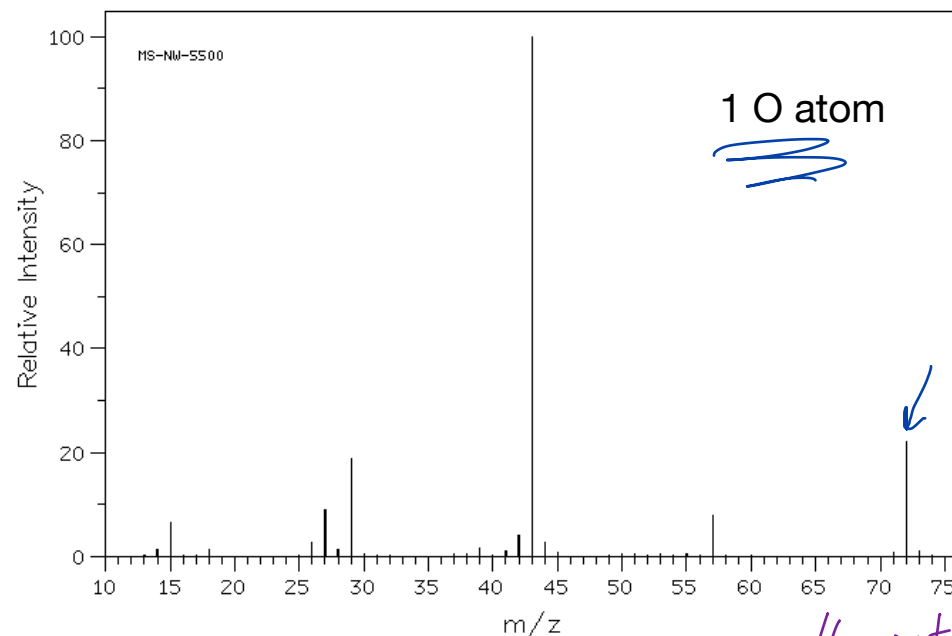
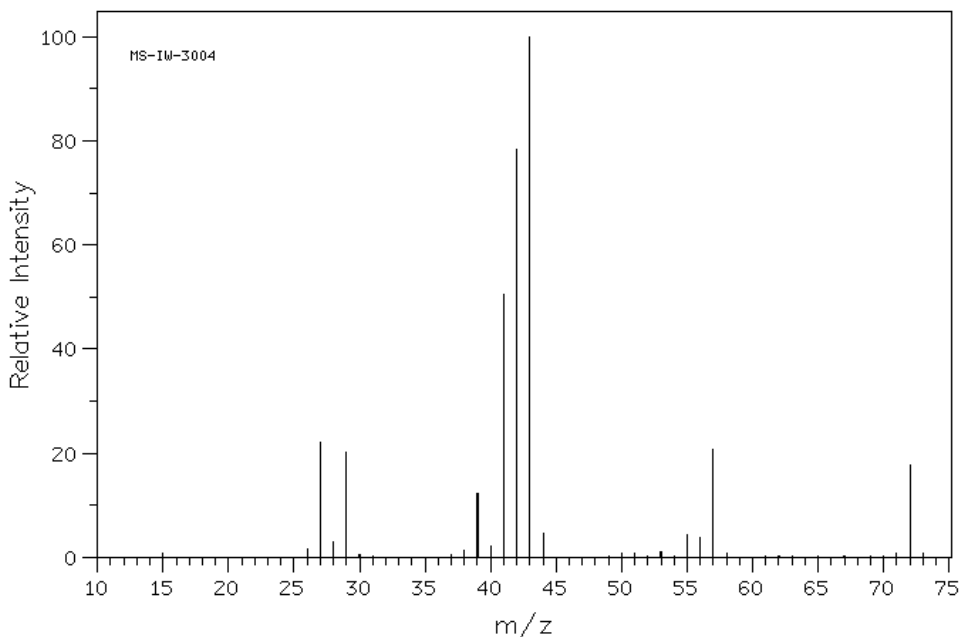
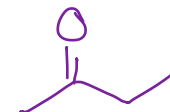
$$13 \overline{) \frac{n}{m/z} \text{ r}}$$



Determine the number of CH units that "fit into" the peak.

If only C and H present, the remainder must be the number of H atoms present.

If other atoms present, "make room" for them but removing CH units.



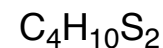
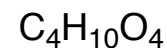
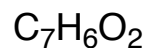
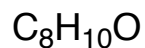
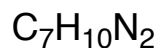
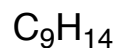
72 # CH units that fit? pull out enough C's + H's to make room for O...
 O is 16 g/mol
 so 1 C + 4 H's have to go

$$\begin{array}{r} 5 \\ 13 \overline{)72} \\ \underline{65} \\ 7 \end{array}$$
 r7

C_5H_{12}
 $-1C - 4H's$
 C_4H_8O

High Resolution Mass Spectrometry: Using exact mass to determine formulae

these are exact masses ... the masses of the molecules made from the most abundant isotopes



122.1096 u

122.0845 u

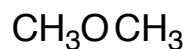
122.0732 u

122.0368 u

122.0579 u

122.0225 u

a computer can crunch numbers to find the distinct formula that will produce the correct exact mass



Exact Mass: 46.04



Exact Mass: 46.04

constitutional (structural) isomers have the same exact mass

so even high resolution mass spectrometers can't determine structure by only examining the molecular ion

Comparing m and m+1 peaks

Advantage: Don't need to know whether other atoms are present as part of the ion

Disadvantage: Can be made inaccurate by overlapping peaks and the presence of atoms with m+1 isotopes like nitrogen

The "Rule of 13"

Advantage: Don't need to worry about other atoms with m+1 isotopes
Don't need to worry about overlapping peaks

Disadvantage: Need to know whether other atoms are part of the ion

High Resolution Mass Spectrometry

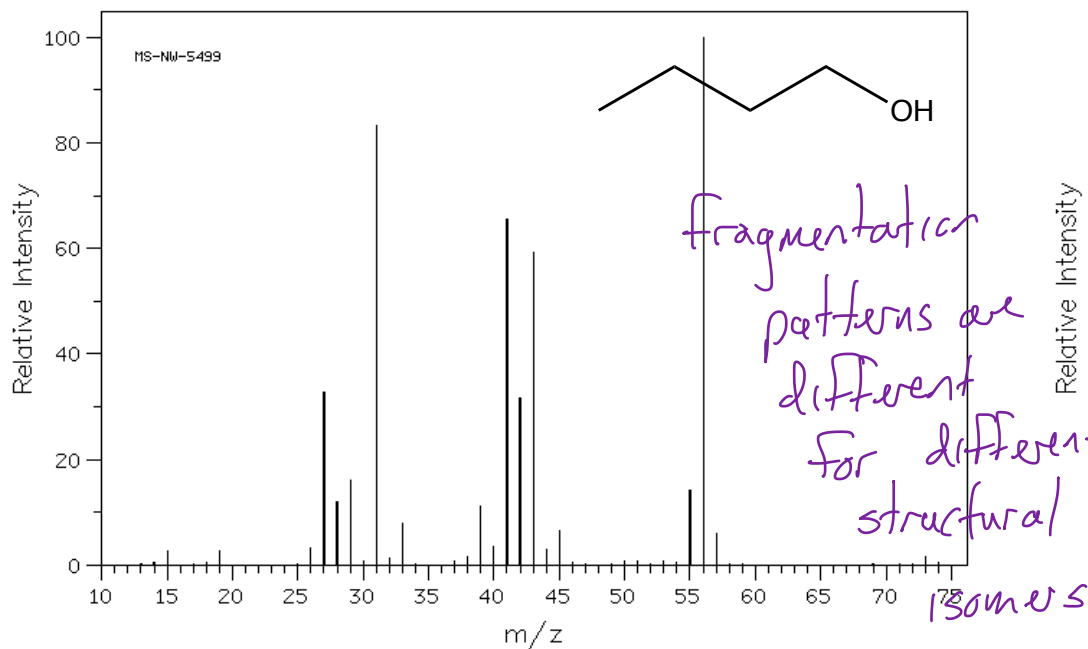
Advantage: Computer and instrument can determine many formulas with minimal operator input

Disadvantage: Very expensive
The larger the molecule the harder it is to determine formula

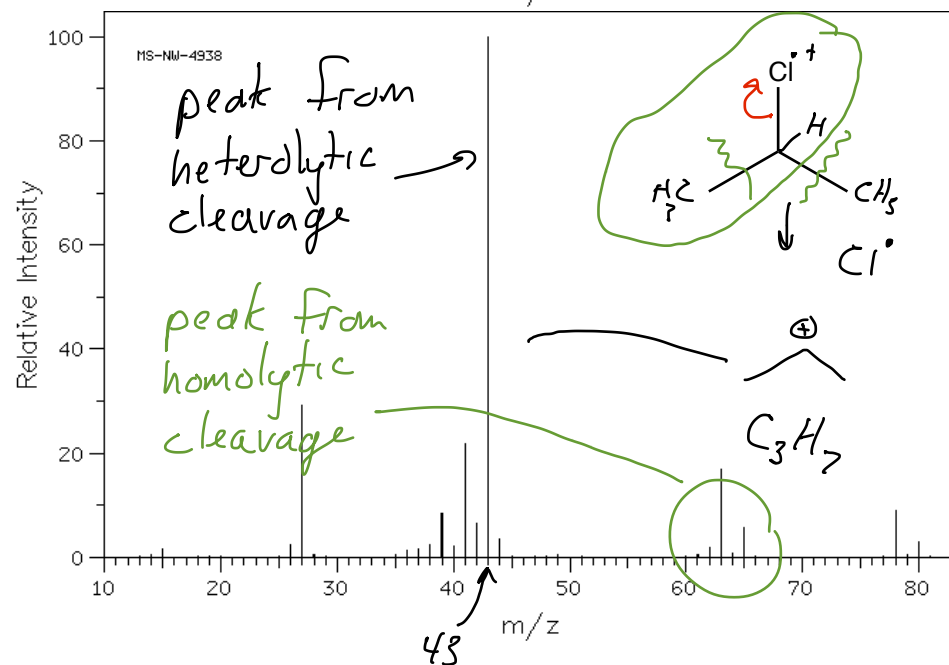
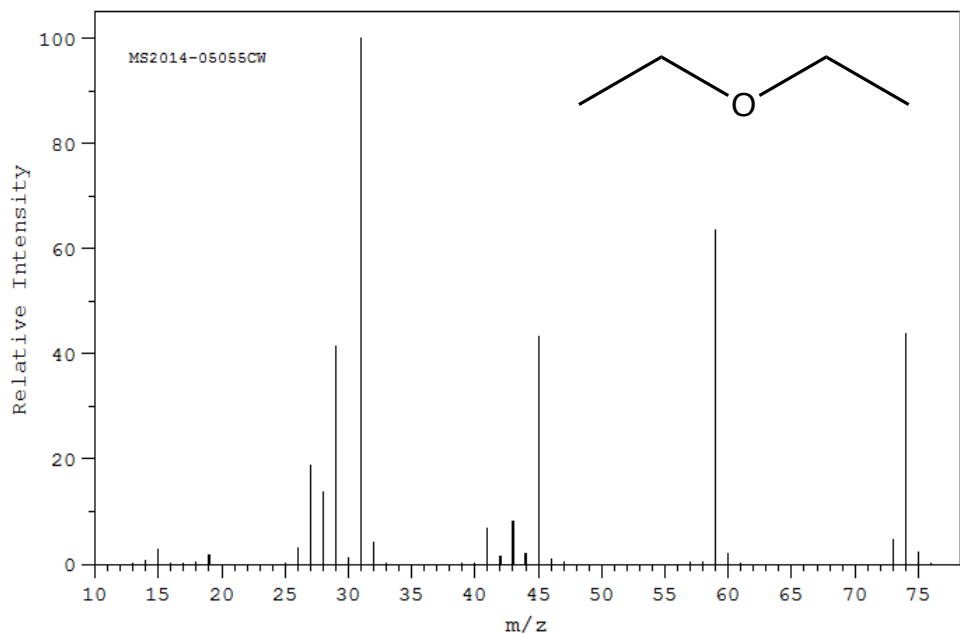
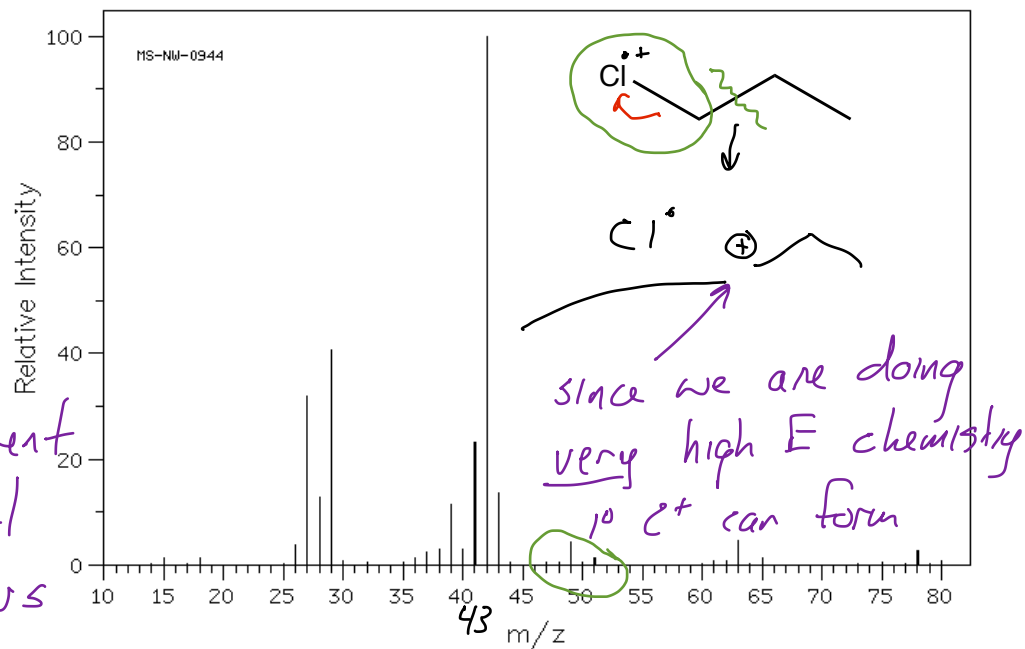
Fragmentation Patterns Can Help Identify Compounds

Section 13.6

CH3CH2OCH2CH3 vs CH3CH2CH2CH2OH



ClCH2CHCH3 vs CH3CHClCH3



Homolytic vs Heterolytic Cleavage

Homolytic



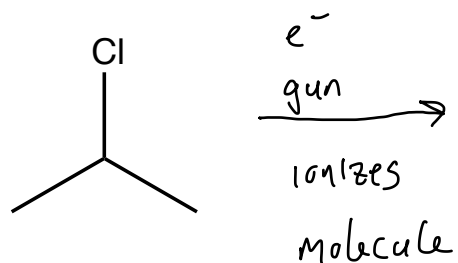
evenly distribute e^- 's in bond when bond breaks

Heterolytic

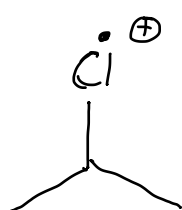


unevenly distribute e^- 's in bond - - - -

Fragmentation of Alkyl Halides: Heterolytic



lone-pair e^- are more easily knocked off



the Cl atom will attract e^- 's to itself

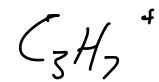
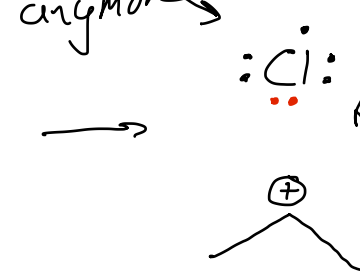


ionized



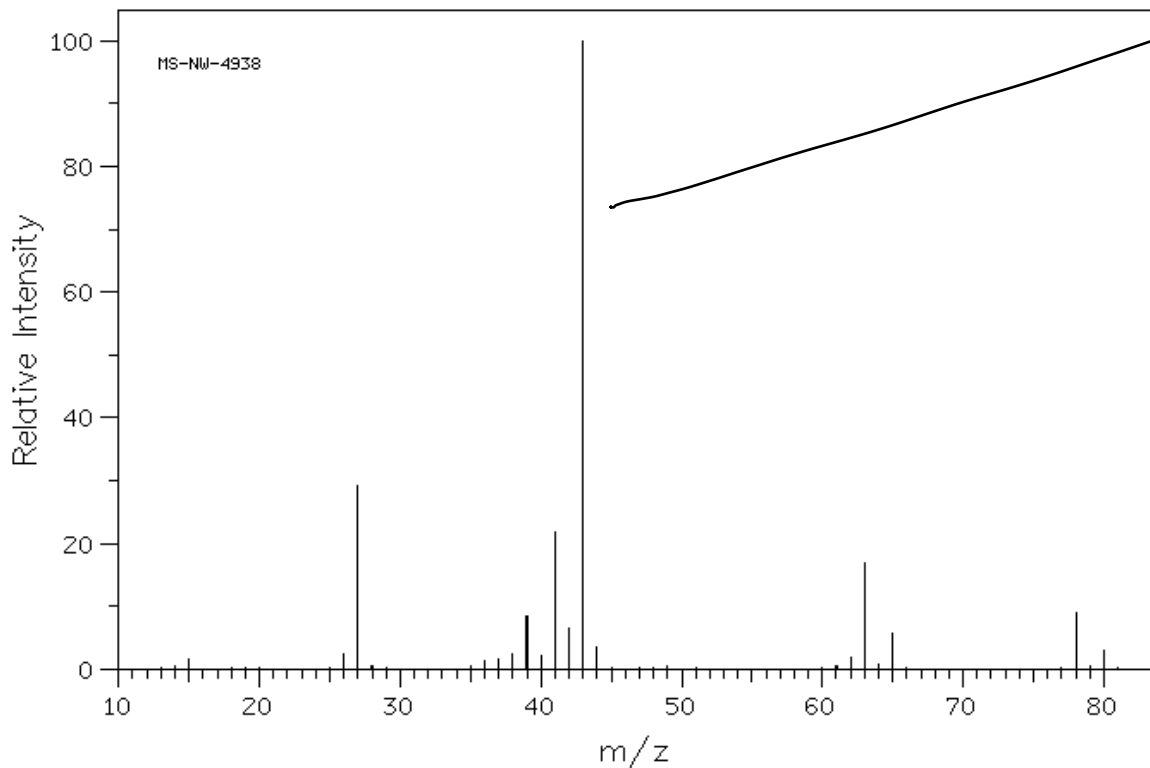
more stable because it's not \oplus anymore

Section 13.6



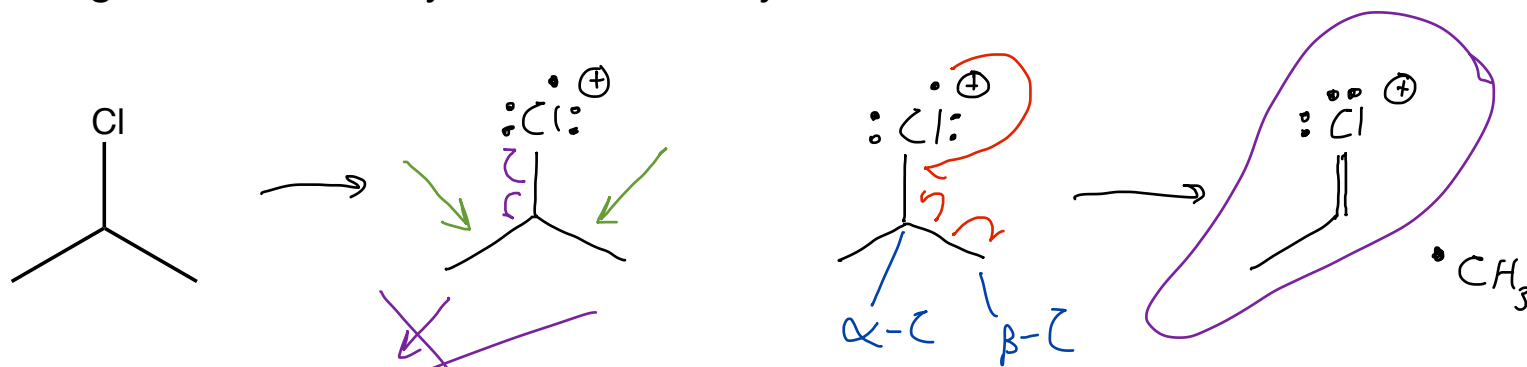
see this

don't see this

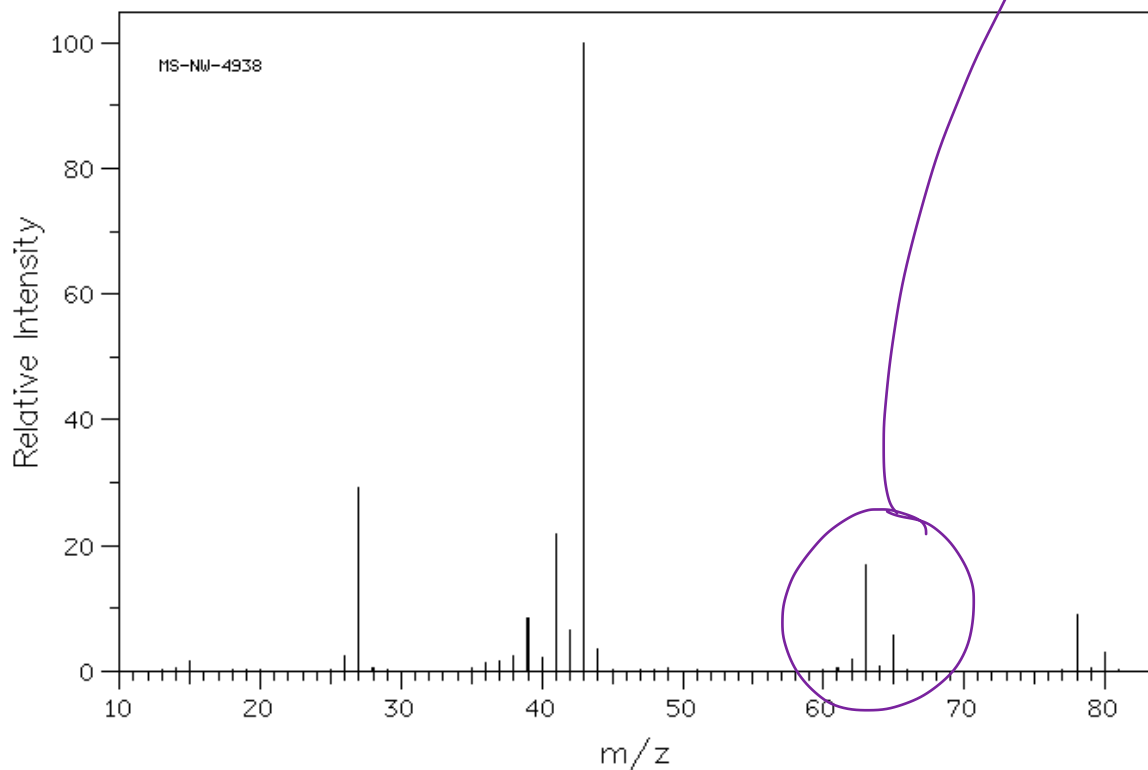


Heterolytic cleavage reactions occur between the $\alpha-C$ and the heteroatom.

Fragmentation of Alkyl Halides: Homolytic



this Cl is worse off



Homolytic cleavage reactions are α -cleavage reactions. The bond between the α -C and the β -C is broken.

The bond between the heteroatom and the α -C is not broken