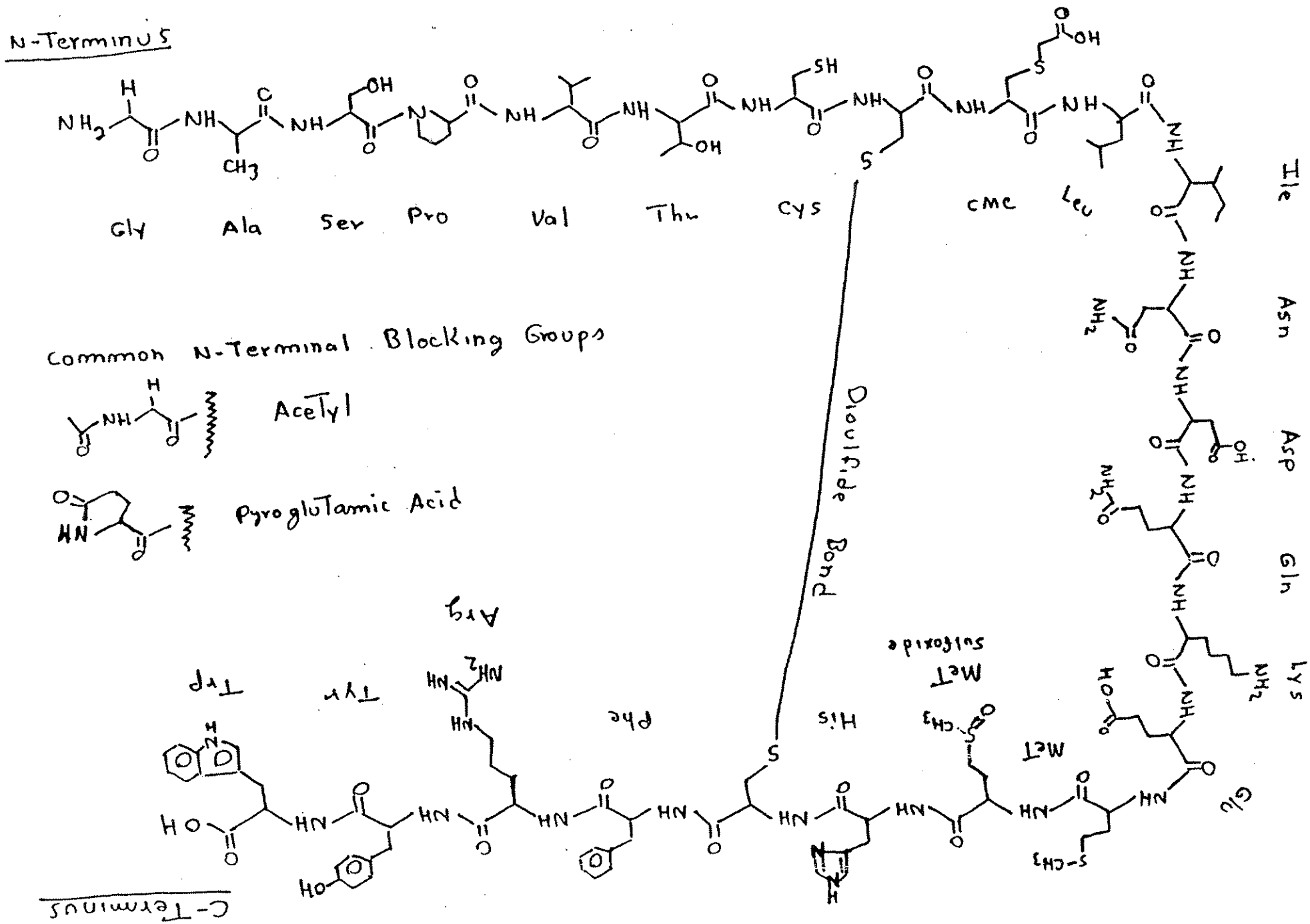
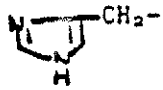
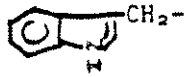
Sample Peptide Structure containing all common Amino Acids



Common N-Terminal Blocking Groups

Table 1. Table of mass values for peptide fragment ions.

	Amino Acid		R-	Residue Mass*	Methyl Ester	Mass Increment
G	Glycine	Gly	H-	57		-
A	Alanine	Ala	CH_3-	71		14
S	Serine	Ser	$HOCH_2$-	87		16
P	Proline	Pro	$-CH_2CH_2CH_2-$	97		10
V	Valine	Val	$(CH_3)_2CH-$	99		2
T	Threonine	Thr	$HOCH(CH_3)-$	101		2
C	Cystine	Cys	$HSCH_2$-	103		
L	Leucine	Leu	$(CH_3)_2CHCH_2-$	113		12
I	Isoleucine	Ile	$CH_3CH_2(CH_3)CH-$	113		-
N	Asparagine	Asn	H_2NCOCH_2-	114		1
D	Aspartic Acid	Asp	$HOOCCH_2-$	115	129	1
Q	Glutamine	Gln	$H_2NCOCH_2CH_2-$	128		13
K	Lysine	Lys	$NH_2CH_2CH_2CH_2CH_2-$	128		-
E	Glutamic Acid	Glu	$HOOCCH_2CH_2-$	129	143	1
M	Methionine	Met	$CH_3SCH_2CH_2-$	131		2
H	Histidine	His		137		6
F	Phenylalanine	Phe	$PhCH_2$-	147		10
R	Arginine	Arg	$H_2N{=}(NH)NH(CH_2)_3-$	156		9
C	Carboxymethyl Cystine	Cmc	$HOOCCH_2SCH_2-$	161	175	5
Y	Tyrosine	Tyr	p-HO-$PhCH_2$-	163		2
W	Tryptophan	Trp		186		23

Peptide structure = $NH_2-CH(R)-CO-NH-CH(R)-CO-NH-CH(R)-COOH$

*Residue mass structure, $-NH-CH(R)-CO-$;

Table 1. Table of mass values for peptide fragment ions.

Amino Acid			R-	Residue Mass*	Residue Mass Methyl Ester	Mass Incre
G	Glycine	Gly	H-	57.02		–
A	Alanine	Ala	CH_3-	71.04		14
S	Serine	Ser	$HOCH_2$-	87.03		16
P	Proline	Pro	$-CH_2CH_2CH_2-$	97.05		10
V	Valine	Val	$(CH_3)_2CH$-	99.07		2
T	Threonine	Thr	$HOCH(CH_3)$-	101.05		2
C	Cystine	Cys	$HSCH_2$-	103.01		–
L	Leucine	Leu	$(CH_3)_2CHCH_2$-	113.08		12
I	Isoleucine	Ile	$CH_3CH_2(CH_3)CH$-	113.08		
N	Asparagine	Asn	H_2NCOCH_2-	114.04		1
D	Aspartic Acid	Asp	$HOOCCH_2$-	115.03	129.04	1
Q	Glutamine	Gln	$H_2NCOCH_2CH_2$-	128.06		13
K	Lysine	Lys	$NH_2CH_2CH_2CH_2CH_2$-	128.09		–
E	Glutamic Acid	Glu	$HOOCCH_2CH_2$-	129.04	143.06	1
M	Methionine	Met	$CH_3SCH_2CH_2$-	131.04		2
H	Histidine	His		137.06		6
F	Phenylalanine	Phe	$PhCH_2$-	147.07		10
R	Arginine	Arg	$H_2N=(NH)NHCH_2CH_2CH_2$-	156.10		9
C	Carboxymethyl Cystine	Cmc	$HOOCCH_2SCH_2$-	161.02	175.04	5
Y	Tyrosine	Tyr	p-HO-$PhCH_2$-	163.06		2
W	Tryptophan	Trp		186.08		23

*Residue masses have the structure, $-NH-\underset{\displaystyle R}{\underset{|}{CH}}-CO-$;

The ion of type b_1, $H-NH-CH(R)-CO^+$, appears at m/z = residue mass of amino acid #1 + H^+ ($AA_1 + H^+$). b_2 apears at m/z = b_1 + the residue mass of AA_2, b_2 = (b_1 + AA_2) etc. The $(M+H)^+$ appears at m/z = $b_{n-1} + AA_n + H_2O$.

The ion of type y_1 has the formula, $H_2-NH-CH(R)-CO-OH^+$, and appears at m/z = $AA_n + H^+ + H_2O$. The y_2 ion occurs at m/z = $y_1 + AA_{n-1}$. The $(M+H)^+$ ion occurs at m/z = $y_{n-1} + AA_1$.

Table of Masses for Neutrals Lost From and Low Mass Fragments Derived from Various Amino Acids.

Amino Acid	Neutral Lost (Da)	Low Mass Fragment Ions (m/z)
Alanine	–	–
Glycine	–	–
Serine	18 (H_2O)	–
Proline	–	70
Valine	–	72
Threonine	18 (H_2O)	–
Cystine	34 (H_2S)	–
Leucine	–	86
Isoleucine	–	86
Asparagine	17 (NH_3)	70, 87
Aspartic Acid	–	–
Glutamine	17 (NH_3)	84, 101, 129
Lysine	17 (NH_3)	84, 101, 129
Glutamic Acid	18 (H_2O)	84, 102
Methionine	48 (CH_3SH)	104
Histidine	–	110
Phenylalanine	–	120
Arginine	17 (NH_3)	70, 98, 129
Carboxymethyl Cystine	92 ($HSCH_2COOH$)	134
Tyrosine	–	136
Tryptophan	–	159

Additional masses of interest:

H	1.01	
H_2O	18.00	
CH_3OH	32.02	
$-CH_2CO$	42.04	(acetylation)

NOTE: The following residue mass combinations = the residue mass of single amino acids.

	Mass		
Gly-Gly	114	=	Asn
Gly-Ala	128	=	Gln or Lys
Val-Gly	156	=	Arg
Gly-Glu	186	=	Trp
Ala-Asp	186	=	Trp
Ser-Val	186	=	Trp
AcGly	99	=	Val
AcAla	113	=	Leu/Ile
AcSer	129	=	Glu
AcAsn	156	=	Arg

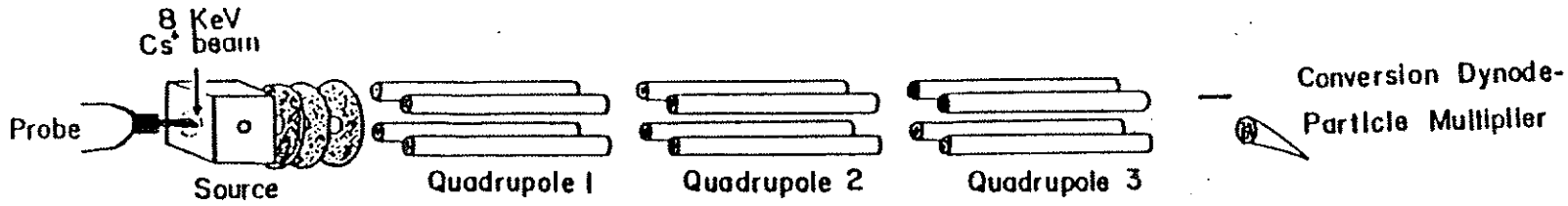


Figure 2 Triple-quadrupole mass spectrometer.

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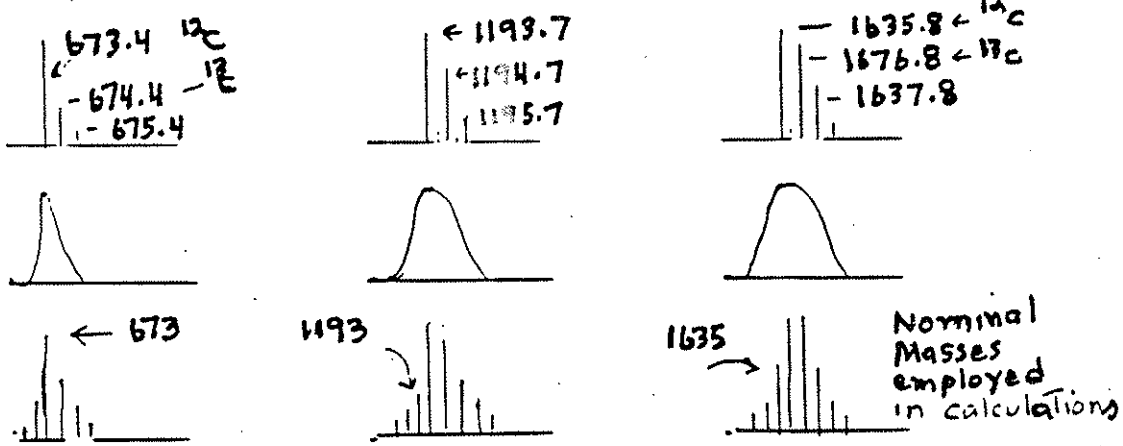
STRATEGY FOR THE INTERPRETATION OF PEPTIDE CAD SPECTRA

The following general strategy will be illustrated with CAD spectra (#2A and 2B) of the model peptide, GXDXQK (X refers to either Leu or Ile). Nominal masses (monoisotopic, ^{12}C, masses rounded to the nearest whole number) for the predicted fragment ions of type b and y from this peptide (free acid and methyl ester) are shown above and below the structure, repectively.

58	171	300	413	541	701	Type b (COOMe)
58	171	286	399	527	673	Type b (COOH)
Gly	Lxx	Asp	Lxx	Gln	Lys	
673	616	503	388	275	147	Type y (COOH)
701	644	531	402	289	161	Type y (COOMe)

Note that all spectra have been generated on a triple quadrupole instrument operating at less than unit resolution. Accordingly the observed signals are often as many as 6-8 mass units wide. Below are shown the predicted isotope patterns for three peptides containing 6, 10, and 14 amino acids, respectively. The actual peak shape recorded on the triple quadrupole instrument is shown on line 2 of the diagram. The third line below shows the experimentally observed pattern after the computer has summed the total signal intensity observed in each 1 mass window within the unresolved multiplet.



Note that at m/z <800 the signal due to the monoisotopic mass of the ion is the most abundant in the cluster and has a value that is essentially identical to that of the rounded nominal mass. At m/z 1100 or so, the monoistopic mass remains the most abundant but is now about one mass unit higher than the rounded nominal mass. Near mass 1600, signal for the ^{13}C isotope becomes approximately equal to that of the ^{12}C isotope and the nominal mass occurs at a value 1 mass unit below the first of the two large peaks. For the purposed of calculating mass values for b and y type ions we will almost always choose the signal on the low mass side of the most abundant signals in a particular cluster. For ions below mass 7-800, we will select the most abundant ion as being that characteristic of the rounded nominal mass value.

1) Measure the mass of the (M+H)+ ions at unit resolution for both the peptide and the corresponding methyl ester. The mass shift observed for (M+H)+ ion on conversion of the peptide to the corresponding methyl ester should be a multiple (n) of 14 Da, where (n-1) = the number of acidic residues (Asp, Glu, and Cmc) in the molecule (assuming that the C-terminus of the peptide is not blocked).

In the above example, the (M+H)+ ion shifts from 673 to 701 (28 mass units) n-1 = 1, therefore, there is a single acidic residue in the molecule.

Caution! Under the conditions employed for converting peptides to the corresponding methyl esters, Gln and Asn residues can also esterify. Here the shift is 15 mass units, $-CONH_2$ ——> $-COOCH_3$.

2) Acetylate the peptide or its methyl ester on the solids probe and remeasure the mass of the (M+H)+ ion. The mass shift observed for the (M+H)+ ion upon acetylation should be a multiple (n) of 42 Da, where (n-1) = the number of Lys residues in the peptide (assuming that the amino terminus of the peptide is not blocked). If no mass shift is observed, the amino terminus of the peptide must be blocked. Lys and Gln, two residues having the same mass, are differentiated by the above procedure. Only Lys suffers acetylation.

In the above example example, the (M+H)+ ion shifts from 673 to 757, (84 mass units. n-1 = 1, therefore, there is a single Lys residue in the molecule.

Caution! In the absence of base catalysis, acetylation usually occurs only only on the N-terminus of the peptide and on the side chain of Lys. Cys will also be acetylated if it has not already been carboxymethylated. If the acetylation reaction is catalyzed by base, phenolic residues (Tyr) and the alcohol side chains (Thr and Ser) can also be acetylated. Partial formation of methyl esters can also result if the peptide is treated with methanolic/acetic anhydride for a prolonged period of time (15-60 min).

3) If the peptides being analyzed are generated in a tryptic digest, look at the low mass end of the spectrum for the ion of type y_1 that is characteristic of the expected C-terminal residues, Lys or Arg. Lys affords a y ion at m/z 147 in the spectrum of the free acid (COOH) and at m/z 161 in the spectrum of the methyl ester (COOMe). The corresponding y_1 ion for arginine occur at m/z 175 (COOH) and 189 (COOMe) respectively. These ions lose ammonia readily from the side chain to produce fragments at m/z 158 (COOH) and 172 (COOMe) respectively. In most cases, the m/e 175 ion will be more abuntant in the COOH spectrum and the 172 ion will be more abundant in the COOMe spectrum.

Spectra 2A and 2B contain ions at m/z 147 and 161, respectively. Accordingly, Lys is assigned as the C-terminal residue and the following partial structure is generated.

	701
	673
	Lys
673	147
701	161

4) Examine the low mass end of the spectrum for fragment ions having the formula, $+NH_2=CHR$. Ions of this type are characteristic of the amino acid composition of the peptide but are not observed for all amino acids. The following fragment ion masses in the spectrum of either the free acid or the methyl ester are particularly diagnostic:

70 = Pro (Arg, Asn)	120 = Phe
72 = ~~Ala~~ Val	134 = Cmc
86 = Lxx	136 = Tyr
104 = Met	159 = Trp
110 = His	

In the present example only m/z 86 characteristic of Lxx is observed.

5) Label signals in the methyl ester spectrum with whole numbers (+0, +1, +2, etc.) to indicate how many methyl groups were incorporated into each fragment as a result of the esterification process. Ions of type y contain the C-terminus of the peptide and thus should all shift to higher mass by at least 14 mass units (more if the fragment contains an acidic residue such as Asp, Glu or CmCys). Ions of type b contain the N-terminus of the peptide and should not shift to higher mass in the methyl ester spectrum unless the fragment also contains an acidic residue such as Asp, Glu, or CmCys

Spectrum 2B is labeled for the present example.

6) Proceed to the high mass end of the spectrum and look for the fragment ion of type b, formed by loss of the C-terminal residue. If the C-terminal residue is Lys, then the highest ion of type b will be observed at $(M+H)^+ - (H_2O)$ - 128 (the residue mass for Lys, see Table 1). In the methyl ester spectrum, the b ion will appear at $(M+H)^+ - 32$ (MeOH) - 128.

If the C-terminal residue is Arg, the corresponding b ions will be observed at $(M+H)^+ - 18 - 156$ and $(M+H)^+ - 32 - 156$.

If the C-terminal residue is not known, the highest mass ion of type b will be found by using the formula $(M+H)^+ - 18$ (H_2O) - X, where X = each of the twenty residue masses in Table 1. In the methyl ester spectrum this ion appears at m/z = $(M+H)^+ - 32 - X$. Note that highest mass ion of type b contains at least one less COOH group than the (M+H) ion (two less COOH groups if the C-terminal residue has an acidic side chain, Asp, Glu, Cmc). According to the labeling scheme discussed in step #5 above, the highest masss ion of type b will be labeled with a whole number that is at least

one less than the label on the (M+H)+ ion.

In the present example the C-terminal residue is Lys, and the high mass b ion is calculated to occur at 673 - 18 - 128 = 527 (COOH) and 701 - 32-128 = 541. The following partial structure results.

	541	701	Type b (COOMe)
	527	673	Type b (COOH)
		Lys	
673		147	Type y (COOH)
701		161	Type y (COOMe)

Note !!! (important). Once you know the mass of any type b ion, the mass of the corresponding type y ion (formed by cleavage of the same amide bond) is obtained by substracting the mass of the b ion from the (M+H)+ ion and adding 1 (y = m/z (M+H)+ - b + 1). Look to see if the spectrum contains a signal for the predicted type y ion each time you locate a type b ion.

Note that loss of CO from ions of type-b is quite common. The resulting species is referred to as a type-a ion. Look for this ion 28 mass units lower than the corresponding b-ion and label it as an ion of type-a. If you find two ions separated in mass by 28 units in a spectrum, they usually belong to an a,b pair.

$$\underset{b}{NH_2CH(R)CO^+} \longrightarrow \underset{a}{^+NH_2{=}CH(R)} + CO$$

Type a and type b ions are often accompanied by other fragments that result from the loss of water and or ammonia. Look to see if these other fragments exist and label them with an ° (water) and * (ammonia). Loss of water occurs in fragments that contain the amino acids Ser, Thr and Glu (only if the latter residue is at the N-terminus of the fragment). Loss of ammonia occurs from fragments that contain the amino acids Arg, Lys, Gln, and Asn.

7) Examine the high mass end of the spectrum and locate the signal corresponding to the fragment of type y formed by elimination of the N-terminal amino acid. The m/z value of this ion will be equal to that of the (M+H)+ ion minus one of 21 possible residue masses in Table 1. Since the smallest residue mass is 57 units (Gly) and the largest is 186 (Trp), the ion in question will be found in a window 57-186 mass units below the (M+H)+ ion. Note that in the methyl ester spectrum this ion and the (M+H)+ ion should be shifted to higher mass by the same increment and thus be labeled with the same whole number, unless the N-terminal amino acid is either Asp, Glu, or Cmc.

Note !!! (important). Once you know the mass of any type y ion, the mass of the corresponding type b ion (formed by cleavage of the same amide bond) is obtained by substracting the mass of the y ion from the $(M+H)^+$ ion and adding 1 ($b = m/z\ (M+H)^+ - y + 1$). Look to see if the spectrum contains a signal for the predicted type b ion each time you locate a type y ion.

Type y ions are often accompanied by other fragments that result from the loss of water or ammonia. Look to see if these other fragments exist and label them with an ° (water) and * ammonia. Loss of water from type y ions occurs in fragments that contain the amino acids Ser, The and Glu (only if the latter is at the N-terminus of the fragment). Loss of ammonia occurs from fragments that contain the amino acids Arg, Lys, Gln and Asn.

Caution! Note that all peptide CAD spectra contain a series of abundant fragment ions within 60 amu of the $(M+H)^+$ ion. These appear to result from the loss of multiple units of water, ammonia, plus 45-46 and 59-60 mass units (HCOOH and CH_3COOH, ?). Signals due to the loss of the smallest amino acid, Gly (57 units) fall within this 60 mass unit window. Accordingly one must always check to see if there is a signal 57 units below the $(M+H)^+$ ion. Note also that several combinations of two residue masses add up to the same mass as a single larger residue (see the table at the front of the notebook). Gly-Gly, for example, has the same residue mass (114) as Asn. Be sure to select the signal closest to the $(M+H)^+$ ion that fits for the loss of a residue mass.

Gly-Gly	114	=	Asn
Gly-Ala	128	=	Gln or Lys
Val-Gly	156	=	Arg
Gly-Glu	186	=	Trp
Ala-Asp	186	=	Trp
Ser-Val	186	=	Trp

AcGly	99	=	Val
AcAla	113	=	Leu/Ile
AcSer	129	=	Glu
AcAsn	156	=	Arg

In the present example the signals at m/z 616 (COOH) and 644 (COOMe) fit for the loss of Gly. The following partial structure is generated.

	58		541	701 Type b (COOMe)
	58		527	673 Type b (COOH)
	Gly		Lys	
673	616		147	Type y (COOH)
701	644		161	Type y (COOMe)

8) If the low mass end of the spectrum contains ions characteristic of Pro (m/z 70) or His (m/z 110), examine the spectrum for fragments that result from internal cleavage at these residues. Often the most abundant signal in the spectrum will correspond to the ion of type y that contains either of these two residues at the N-terminus. Since the structure of this ion is the same as that for the $(M+H)^+$ ion for a shortened peptide containing either Pro or His at the N-terminus, the observed type y ion can undergo further fragmentation to produce a series of type b ions characteristic of this shortened peptide. A search for these ions is often the quickest way to solve the structure of an unkown peptide.

In renin tetradecapeptide, DRVYIHPFHLLVYS, the most abundant ions in the CAD spectrum correspond to ions of type b derived from the y ion of sequence PFHLLVYS. PFVH, PFHLL, PFHLLV, PFHLLVY, and PFHLLVYS are all observed.

None of these types of ions are found in the present example.

9) To continue the sequence analysis, search the spectrum for additional ions of type b or type y. Start at the high mass end of the spectrum and work backwards since the number of signals in that region is generally smaller than that found at low mass. Additional ions of type b or y are found by substracting each of the twenty one residue masses in Table 1 from the m/z value of an existing b or y ion until a new signal is encountered. Note that the signal corresponding to the next lower member of a given series will be labeled with the same whole number from step #5 above, unless the residue lost contains a carboxylic acid (Asp, Glu, Cmc).

The following points should be noted:

a) A unique b_1 ion will not be observed. The only way to determine the order of the first two amino acids in the chain is to find the appropriate ion of type y, acetylate the amino terminus and look for the b_1 ion that has increased in mass by 42 units, or perform manual Edman degradation on the sample and measure the mass of the $(M+H)^+$ that results from the peptide shortened by one residue.

b) The signal intensity for ions of type b drops dramatically when the ~~low~~ next amino acid in the chain is either Pro, Gly or His. Lys and Arg also cause this phenomenon.

c) When a type y or b ion is formed by cleaving an amide bond before or after the residue, Arg, it is not uncommon for the y^* and b^* ions to be much more abundant than the b or y ions themselves.

d) Quite often one will observe the situation where a particular series of b type ions disappears and the corresponding ions of type y become much more abundant. The opposite situation occurs just as frequently. This is usually the case when one encounters either Pro, or a basic residue such as His, Lys or Arg in the middle of the peptide chain.

To determine the identity of the second AA in the chain, we search for the second highest mass ion of type y. This can be found either by substracting all of the residue mass values in Table 1 from the previously identified y ion at m/z 616, or by simply looking for another signal at lower mass that contains the same number of methyl groups as m/z 616. The signal at m/z 503 indicates that a mass of 113 has been lost. Accordingly the Lxx is placed in the structure at position two. The mass of the b_2 ion can now be calculated as 58 + 113 = 171. The existence of this ion in the spectrum provides additional support for the structure.

58	171			541	701	Type b (COOMe)
58	171			527	673	Type b (COOH)
Gly	Lxx			Lys		
673	616	503		147		Type y (COOH)
701	644	531		161		Type y (COOMe)

Since none of the remaining ions in the spectrum have the same number of methyl groups as that of the (M+H)+ ion, we conclude that either the next lower ion of type y is missing or it is formed by loss of a residue containing a carboxylic acid group, Asp, Glu or Cmc. When we substract the residue mass for these three AA's from m/z 503, we find that m/z 388 fits for the loss of Asp. This becomes the third residue in the chain and the corresponding ion of type b is calculated, 171 + 115 = 286. This ion appears in the spectrum.

58	171	300		541	701	Type b (COOMe)	
58	171	286		527	673	Type b (COOH)	
Gly	Lxx	Asp		Lys			
673	616	503	388		147	Type y (COOH)	
701	644	531	402		161	Type y (COOMe)	

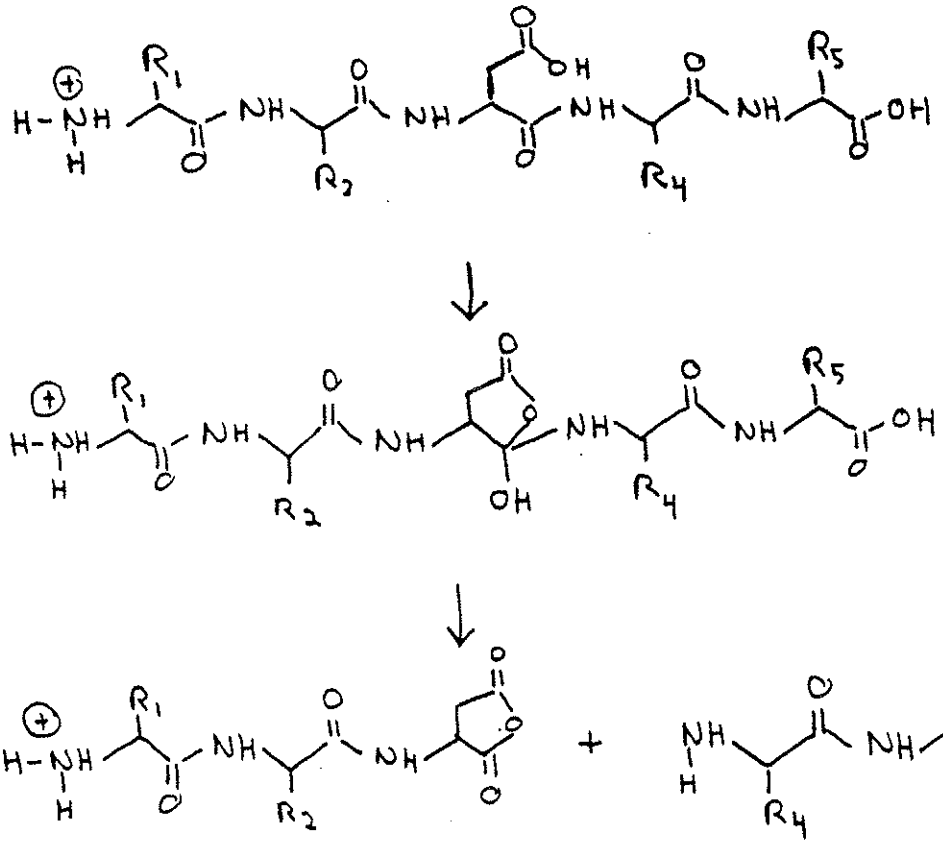
The fourth residue in the chain is deduced from the mass separation between the signals at m/z 388 and 275, 113 = Lxx.

58	171	300	413	541	701	Type b (COOMe)		
58	171	286	399	527	673	Type b (COOH)		
Gly	Lxx	Asp	Lxx		Lys			
673	616	503	388	275	147	Type y (COOH)		
701	644	531	402	289	161	Type y (COOMe)		

The mass separation between 275 and 147 indicates that the missing amino acid has a residue mass of 128. The molecule is acetylated twice, there can only be one Lys in the molecule and it has already been located. Residue 5 is thus assigned as Gln.

58	171	300	413	541	701	Type b (COOMe)
58	171	286	399	527	673	Type b (COOH)
Gly	Lxx	Asp	Lxx	Gln	Lys	
673	616	503	388	275	147	Type y (COOH)
701	644	531	402	289	161	Type y (COOMe)

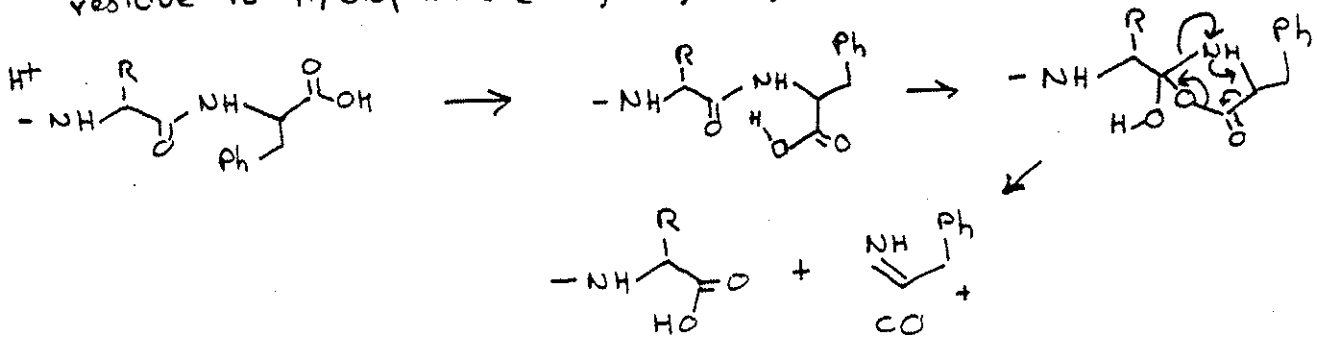
Preferential cleavage C-Terminal To Asp residues:
Neighboring group participation.



ion of Type b_3

{often The most abundant in The spectrum}

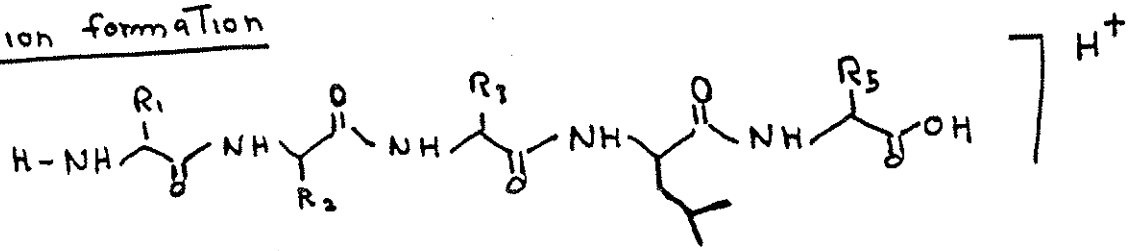
Fragmentation mechanism for loss of c-Terminal residue as a residue mass, $-NH-\underset{\underset{O}{\|}}{\overset{R}{C}}-$ (most often observed when c-Terminal residue is hydrophobic (Leu, Ile, Phe, Tyr, etc)

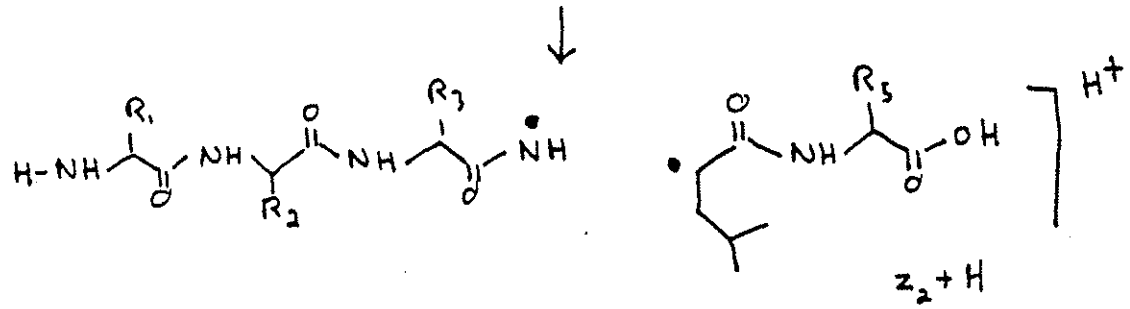


Differentiation of Leu and Ile. Not yet possible on the Triple Quadrupole. Requires collisions in excess of 1 KeV.

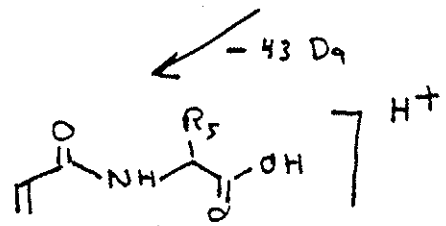
Mechanism for formation of d and w ions.

<u>w ion formation</u>





$z_2 + H$

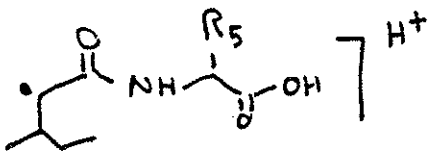


ω_2 ion

Observed at m/z =

<u>$y_1 + 54$</u> or $y_2 - 59$

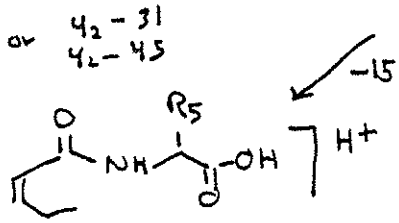
when R_4 = Ile



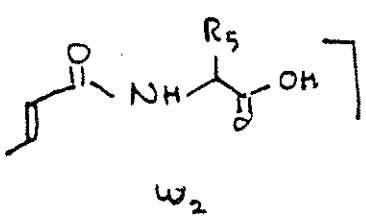
Observed at m/z

y_4 68 and or $y_2 - 31$

$y_1 + 82$ $y_2 - 45$

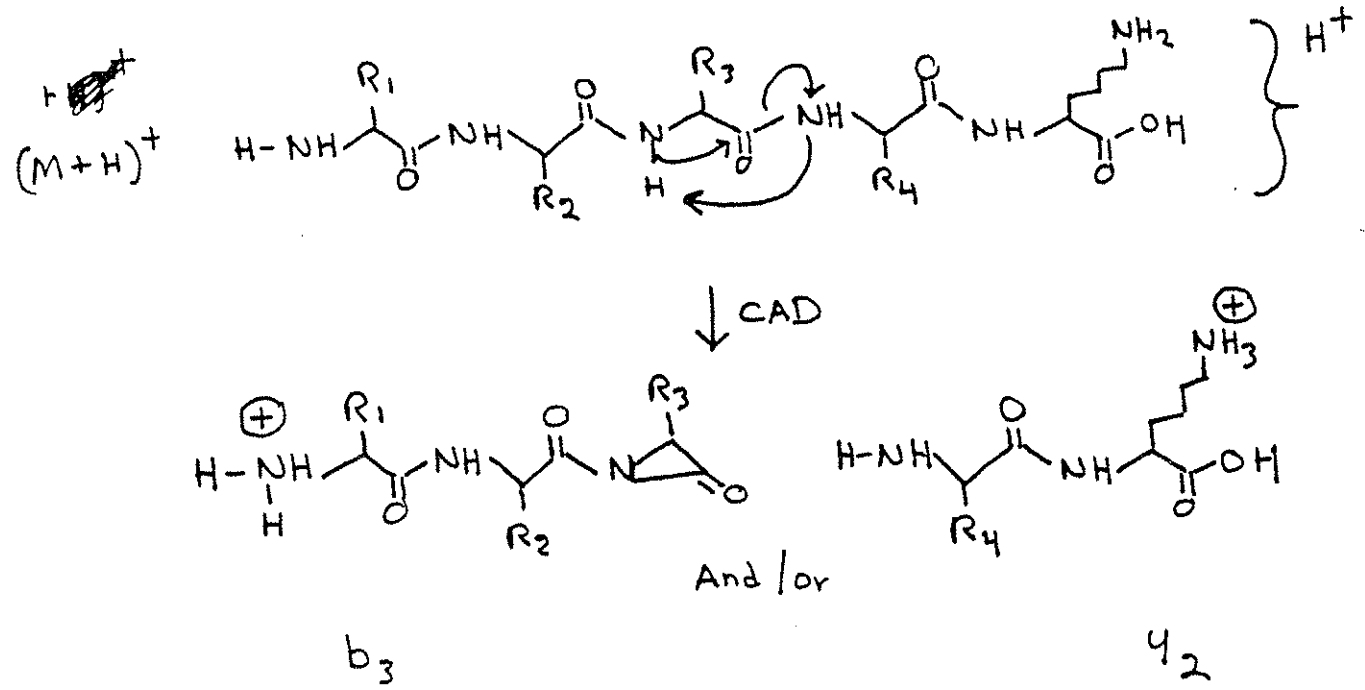


$CH_3^{\bullet}$ + ω_2

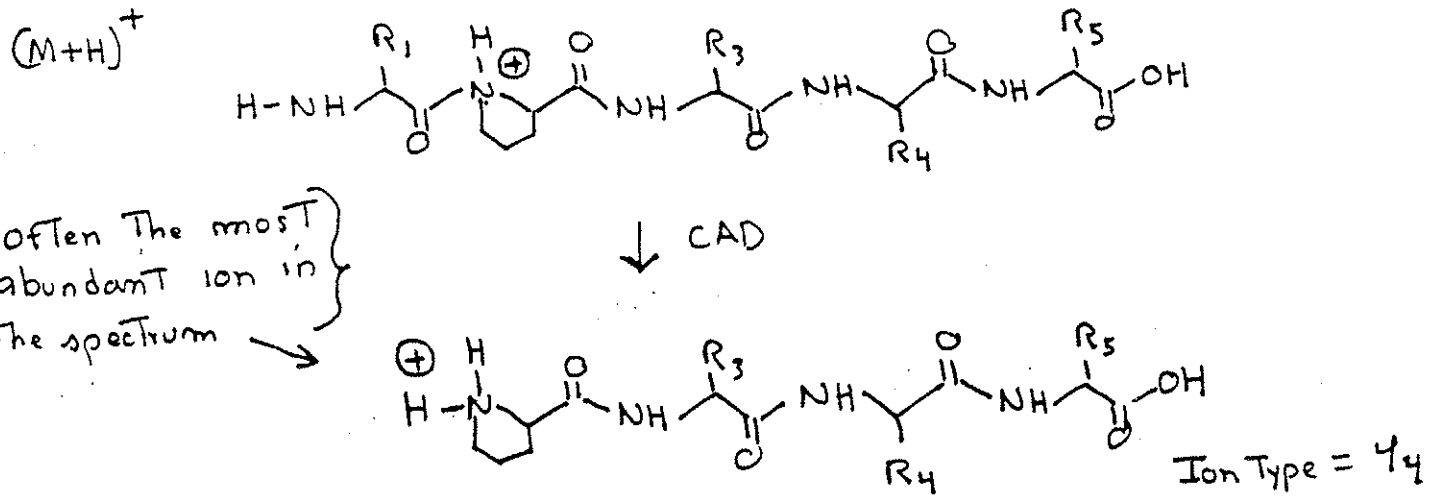


$C_2H_5^{\bullet}$ + ω_2

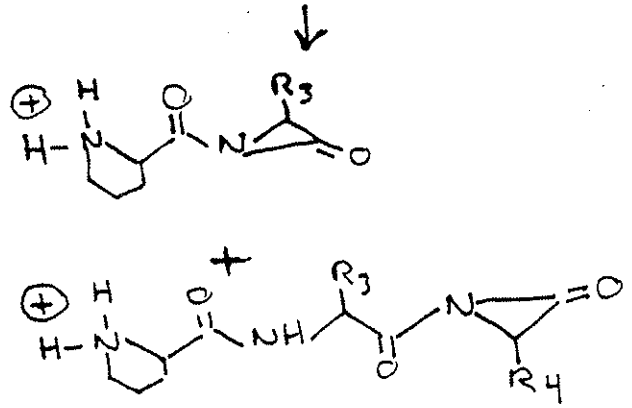
Alternate Fragmentation Scheme: Remote Site Fragmentation



Internal cleavage at either Pro or His (Tryp)



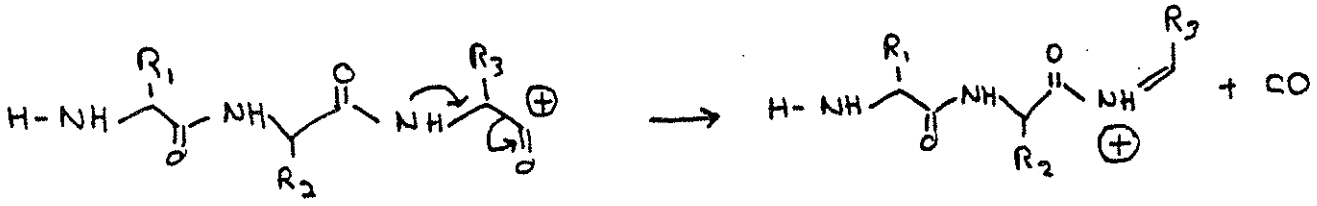
Internal fragments of type b generated from ions of type y_n containing either Pro or His at the N-Terminus



etc.

Production of Fragment ions of Type a_n

Many are formed by loss of carbon monoxide from ions of Type b_n

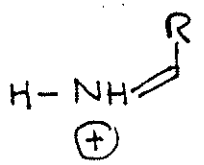


Ion of Type b_3 → ion of Type a_3 + CO

Note that ions of Type b_n and a_n differ by 28 daltons ∴ pairs of signals separated by 28 Da in a CAD spectrum are easily recognized as due ions of Type b and a, respectively.

Ions indicative of amino acid composition:

Ions of Type a_n for many, but not all, amino acids are often observed at the low mass end of a CAD spectrum. These are found at m/z values = to the residue mass − 27 Da (AA + H^+ − CO)



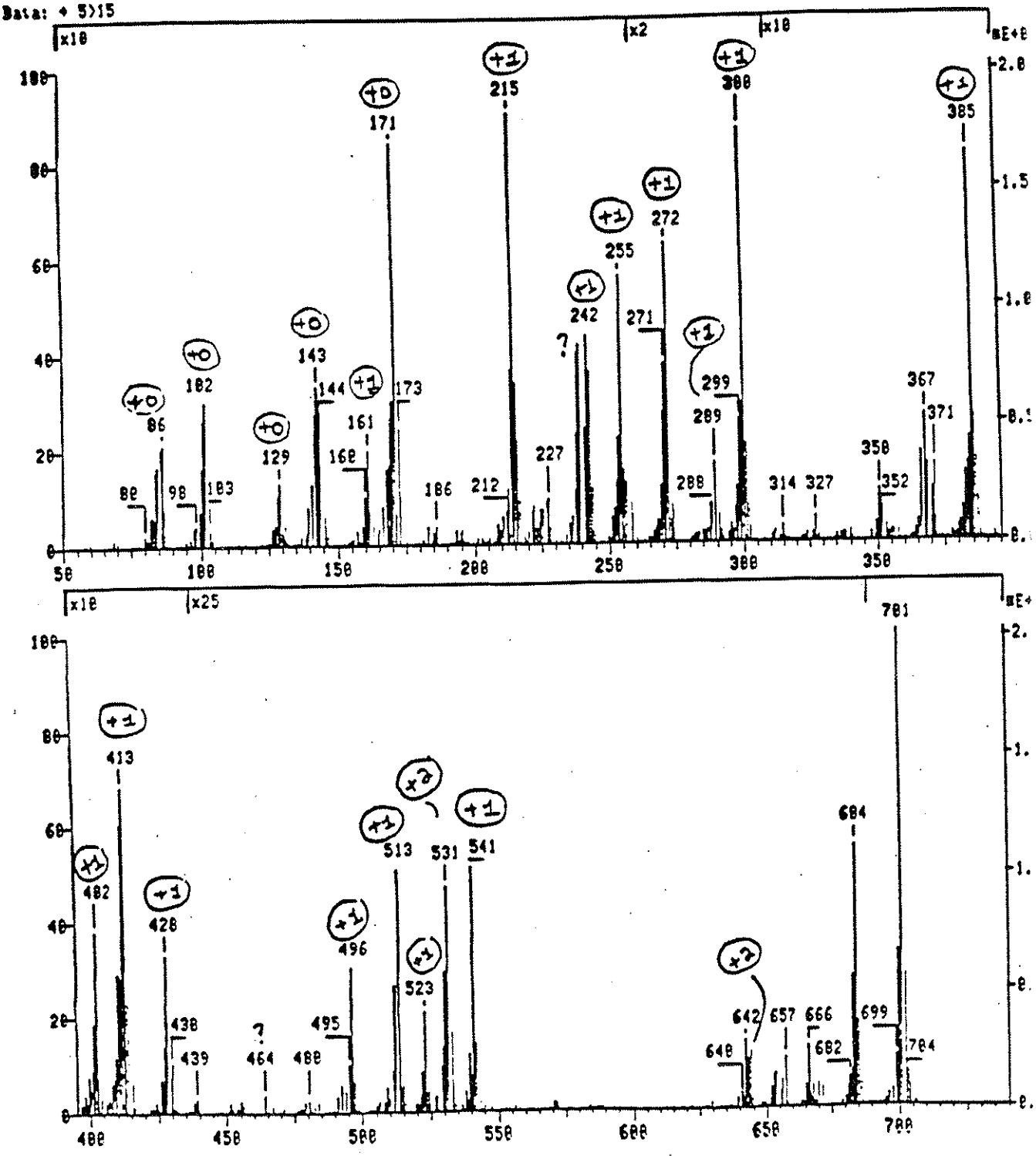
Methyl Ester

MH+ = 701.4

673.4 → 701.4

#2B

```
SPEC: BFH52 ver 1 on UIC 4 1          20-NOV-89 DERIVED SPECTRUM    9
Samp: BLG-TRYPTIC DIGEST-OME                    Start : 01:30:27   15
Cons: 03 CAD 701
Mode: EI +DAUGHTERS OF 701.0 @ -15eV MMR GAS UP LR
Oper: JS                                        Inlet :
Base: 700.9           Inten : 2820832           Masses: 50 > 750
Norm: 700.9           RIC   : 14196592          @ peaks: 672
Peak: 1000.00 mau
Data: + 5>15
```

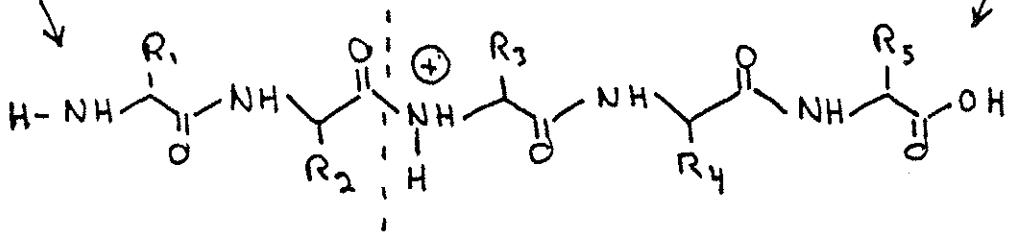


Fragmentation Scheme

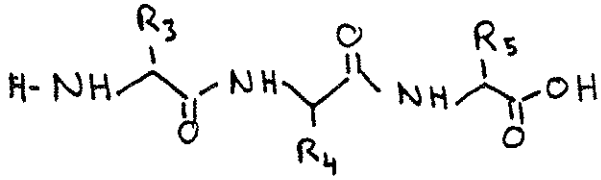
N-Terminus

C-Terminus

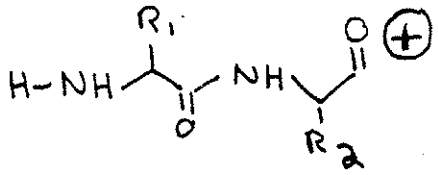
$(n+H)^+$ ion



CAD

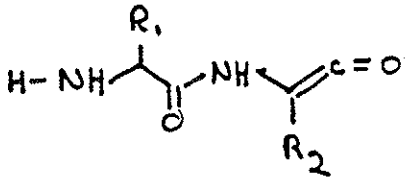


+

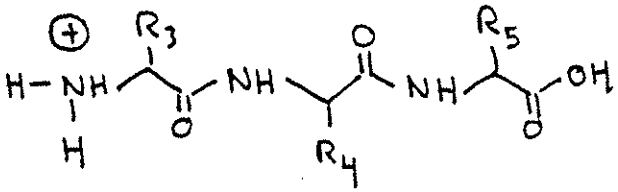


ion Type = b_2

And/or

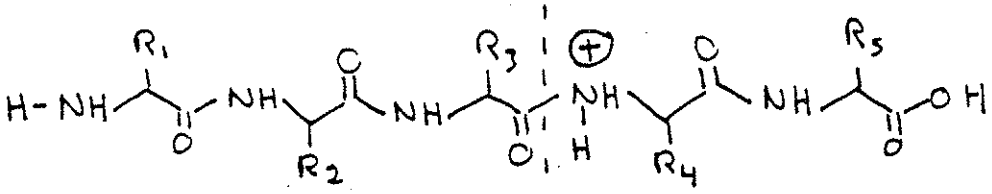


+

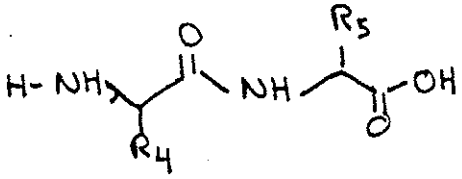


ion Type = y_3

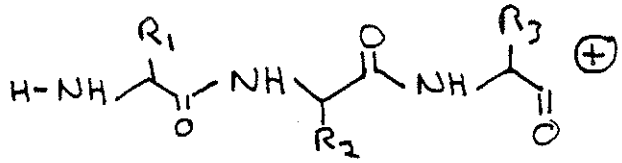
$(M+H)^+$ ion



CAD

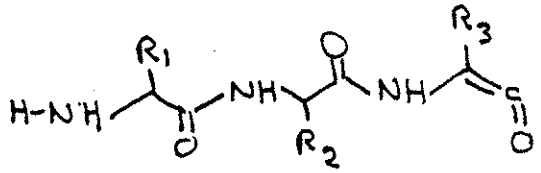


+

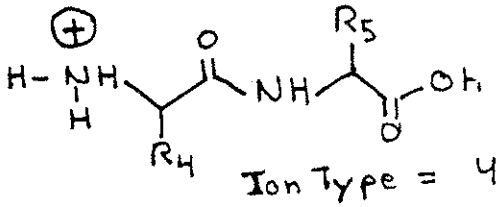


Ion Type = b_3

And/or



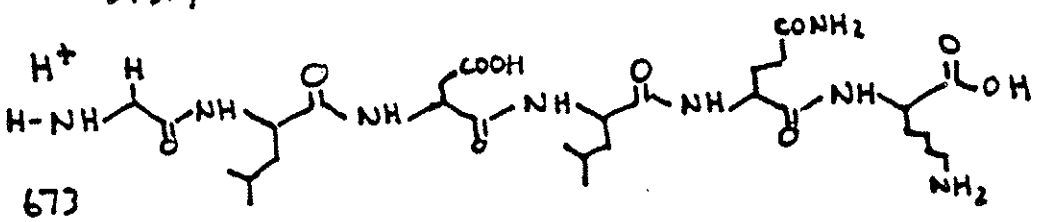
+



Ion Type = y_2

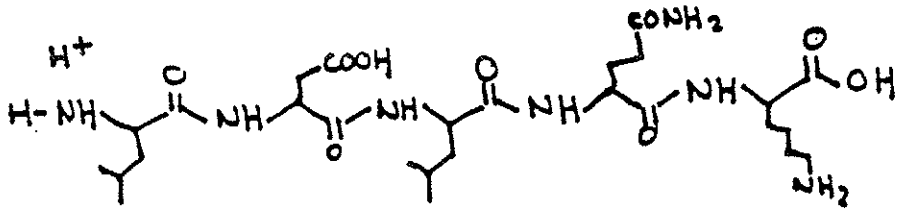
Fragments of Type 4

Gly	Leu	Asp	Leu	Gln	Lys
673	616	503	388	275	147
673.4	616.7	503.2	388.2	275.2	147.1

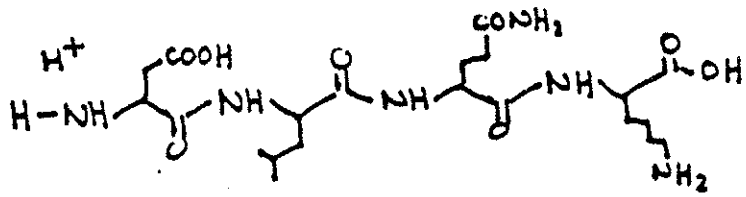


$(M+H)^+ = 673$

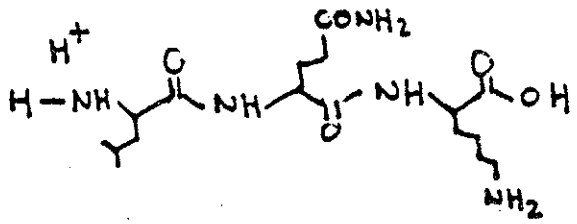
$4_5 = 616$



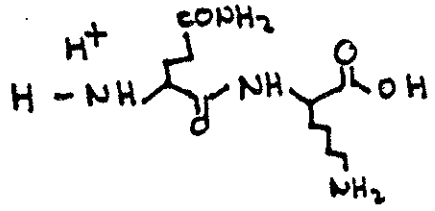
$4_4 = 503$

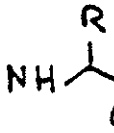


$4_3 = 388$



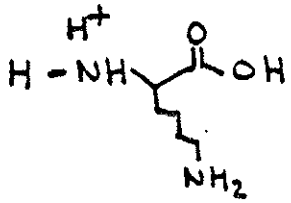
$4_2 = 275$





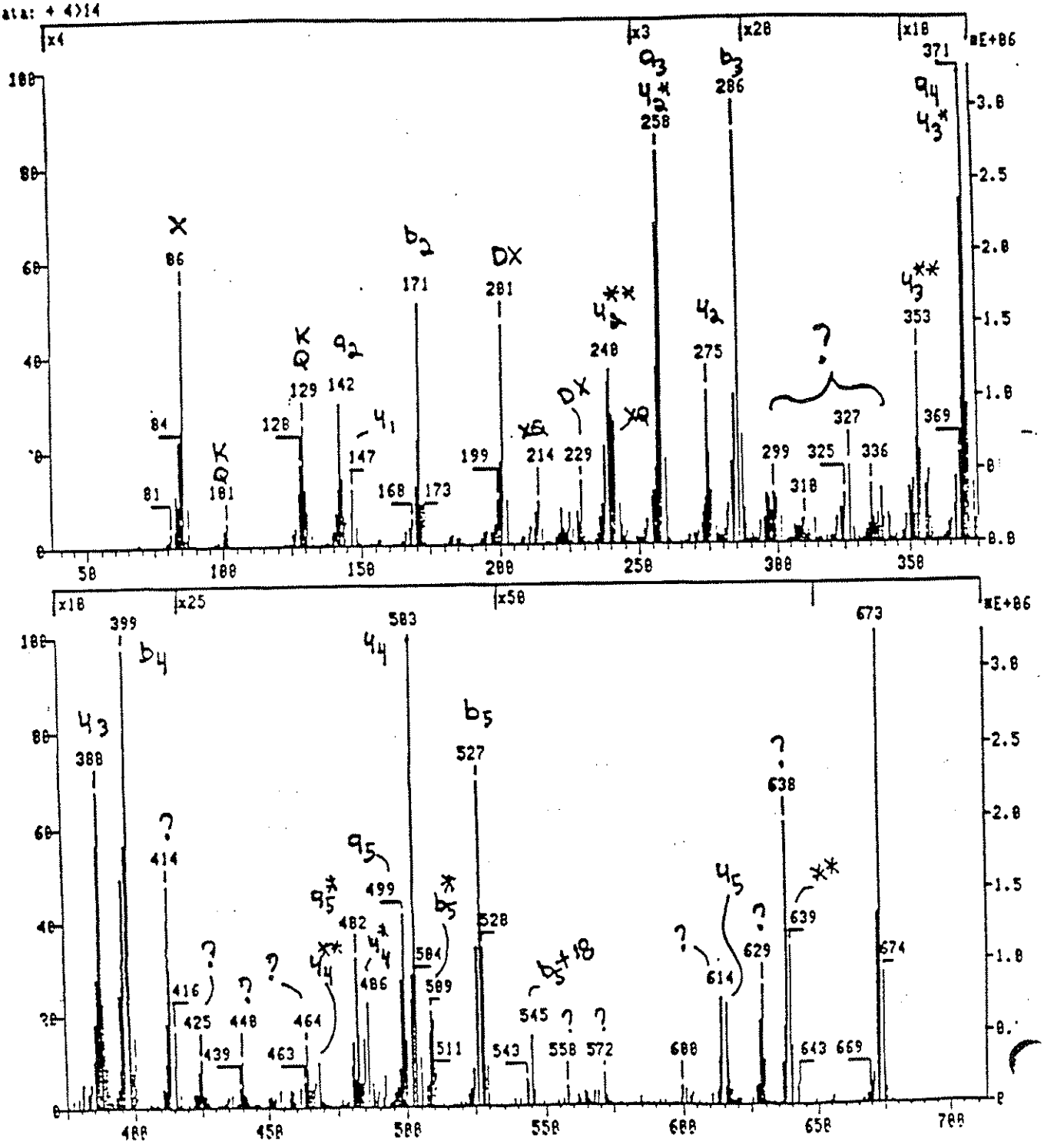
= Residue Mass Table 1

$4_1 = 147$



Tryptic Peptide
CAD 673.4 #2
Acetylation #
673.4 → 757.5

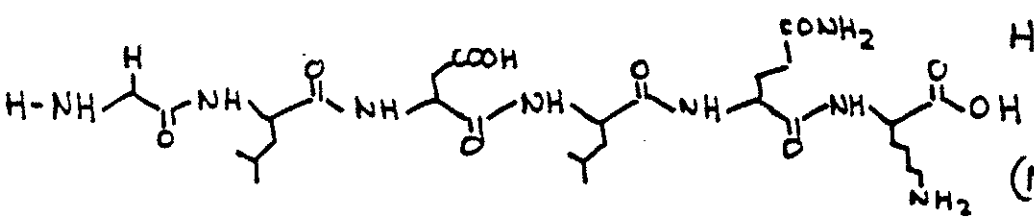
SPEC: DFHS0 ver 12 on UIC 4 1 16-NOV-89 DERIVED SPECTRUM 9
Samp: BLG, TRYPTIC DIGEST Start : 16:43:48 16
Comm: 83 CAD 673
EI +DAUGHTERS OF 673.0 @ -15eV HMR GAS UP LR
Oper: JS Inlet :
Base: 673.1 Inten : 3244798 Masses: 50 > 700
Mora: 673.1 RIC : 26884634 # peaks: 782
Peak: 1000.00 mau
Data: + 4)14



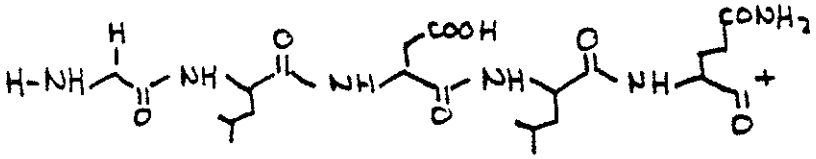
22

Fragments of Type b

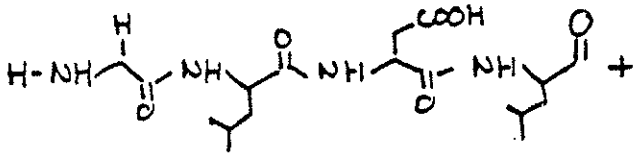
58.0	171.1	286.1	399.2	527.3	673.4
58	171	286	399	527	673
Gly	Leu	Asp	Leu	Gln	Lys



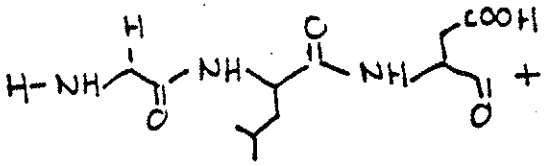
$(M+H)^+ = 673$



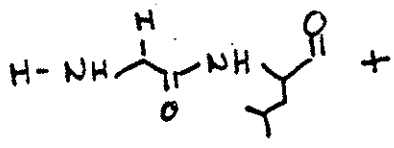
$b_5 = 527$



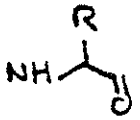
$b_4 = 399$



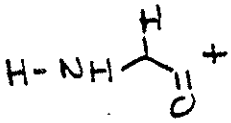
$b_3 = 286$



$b_2 = 171$

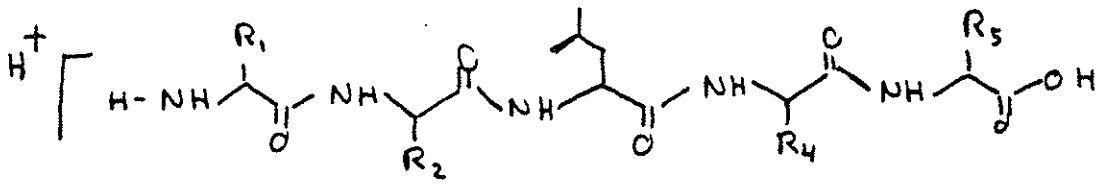


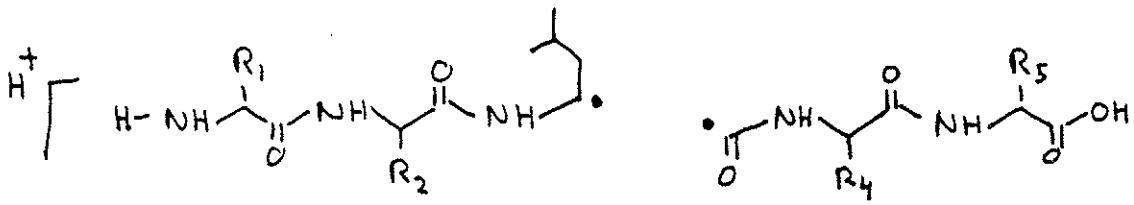
≡ Residue Mass Table 1



$b_1 = 58$

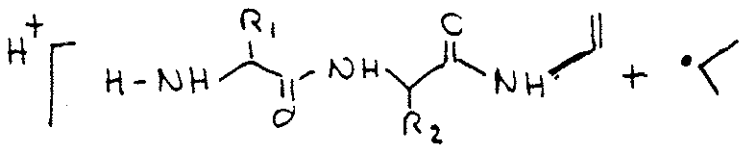
d ion formation





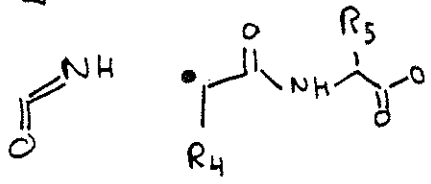
$a_3 + 1$ ion

$\downarrow$ − 43 Da



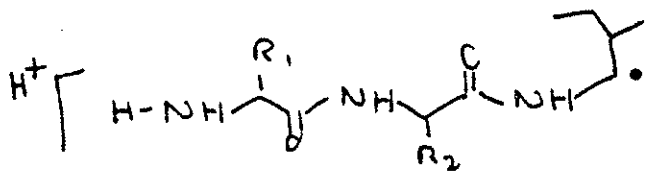
d_3 ion

observed at m/z =
$b_2 + 43$ or $b_3 - 70$

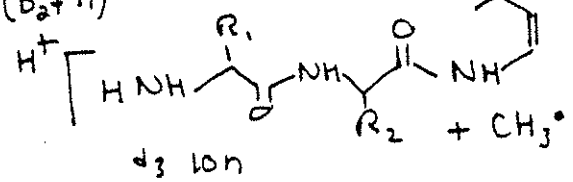


when R_3 = Ile

observed at m/z =
$b_3 - 42$ or $b_3 - 56$



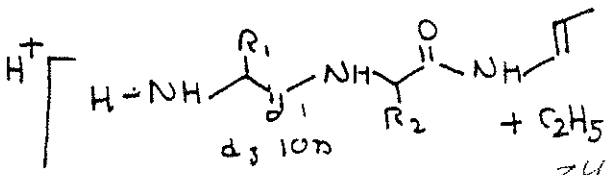
Observed at m/z =
~~(b_2+71)~~ + $(b_2 + 57)$



d_3 ion

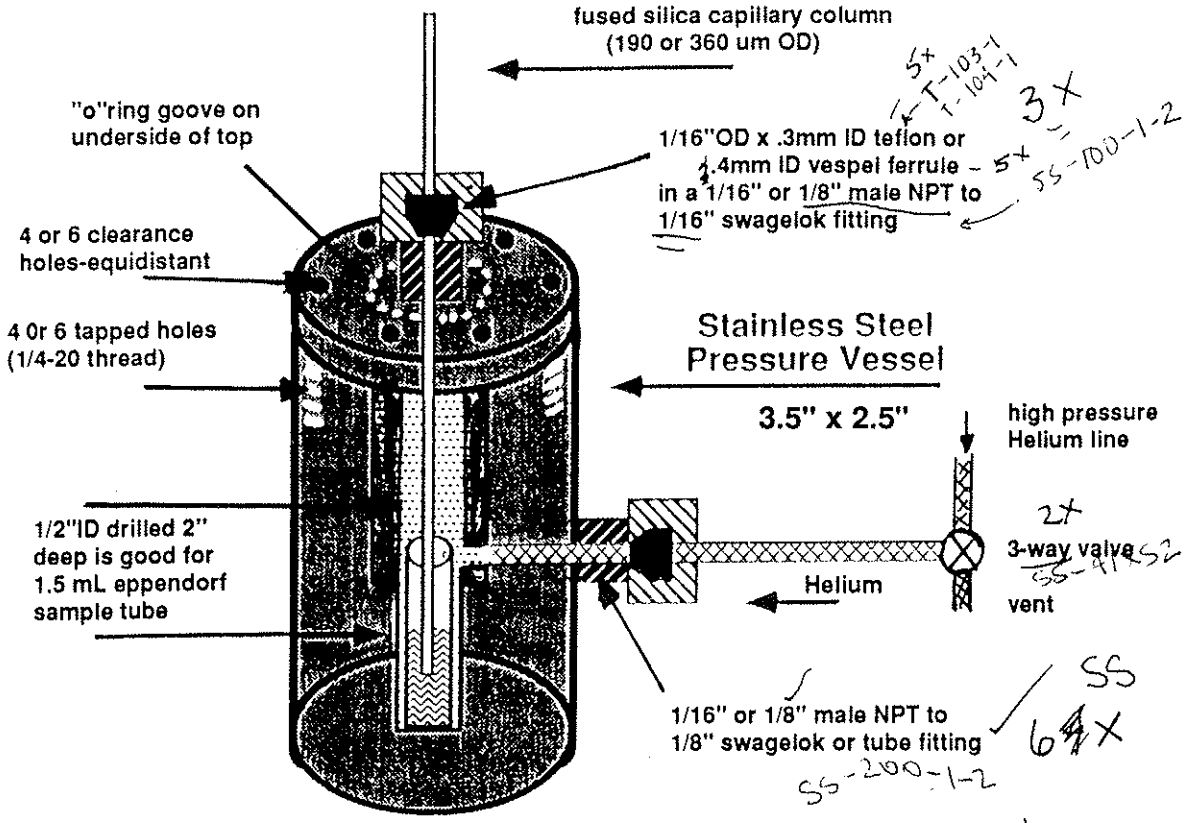
− $CH_3^{\bullet}$
15 Da

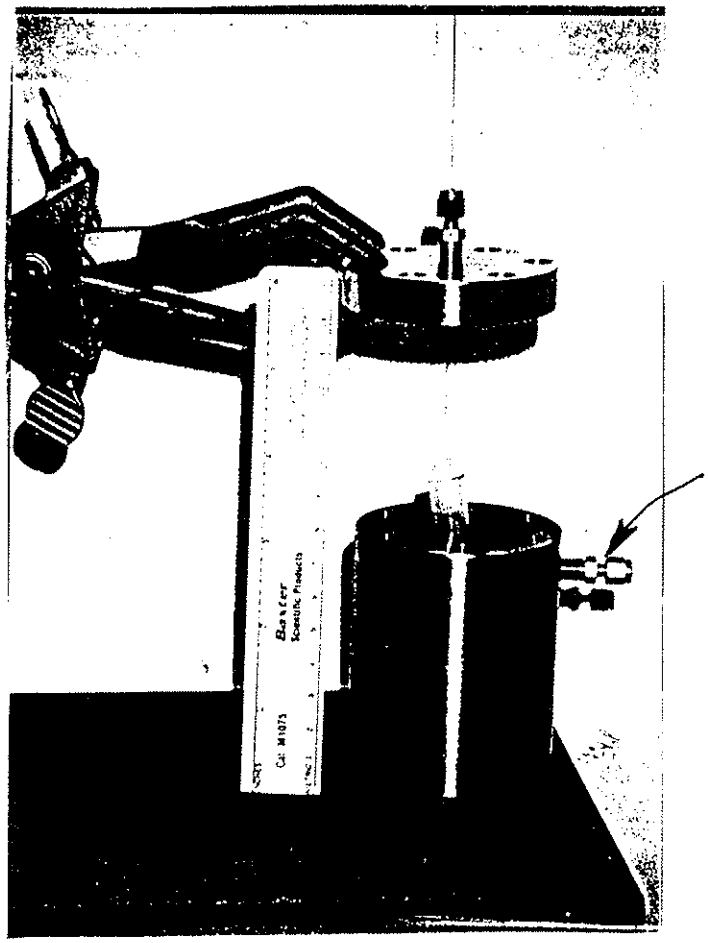
− $CH_3CH_2^{\bullet}$
29 Da



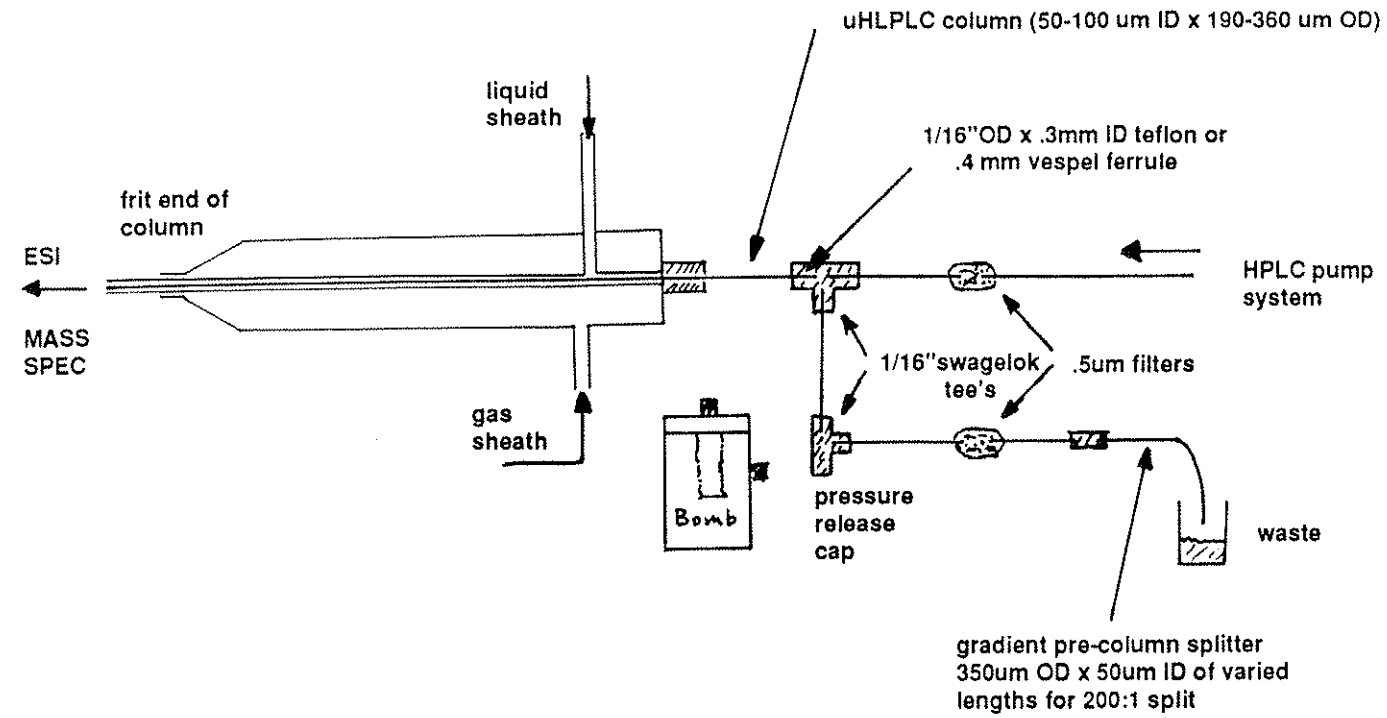
d_3 ion

Micro-Sample Injection System for Capillary LC

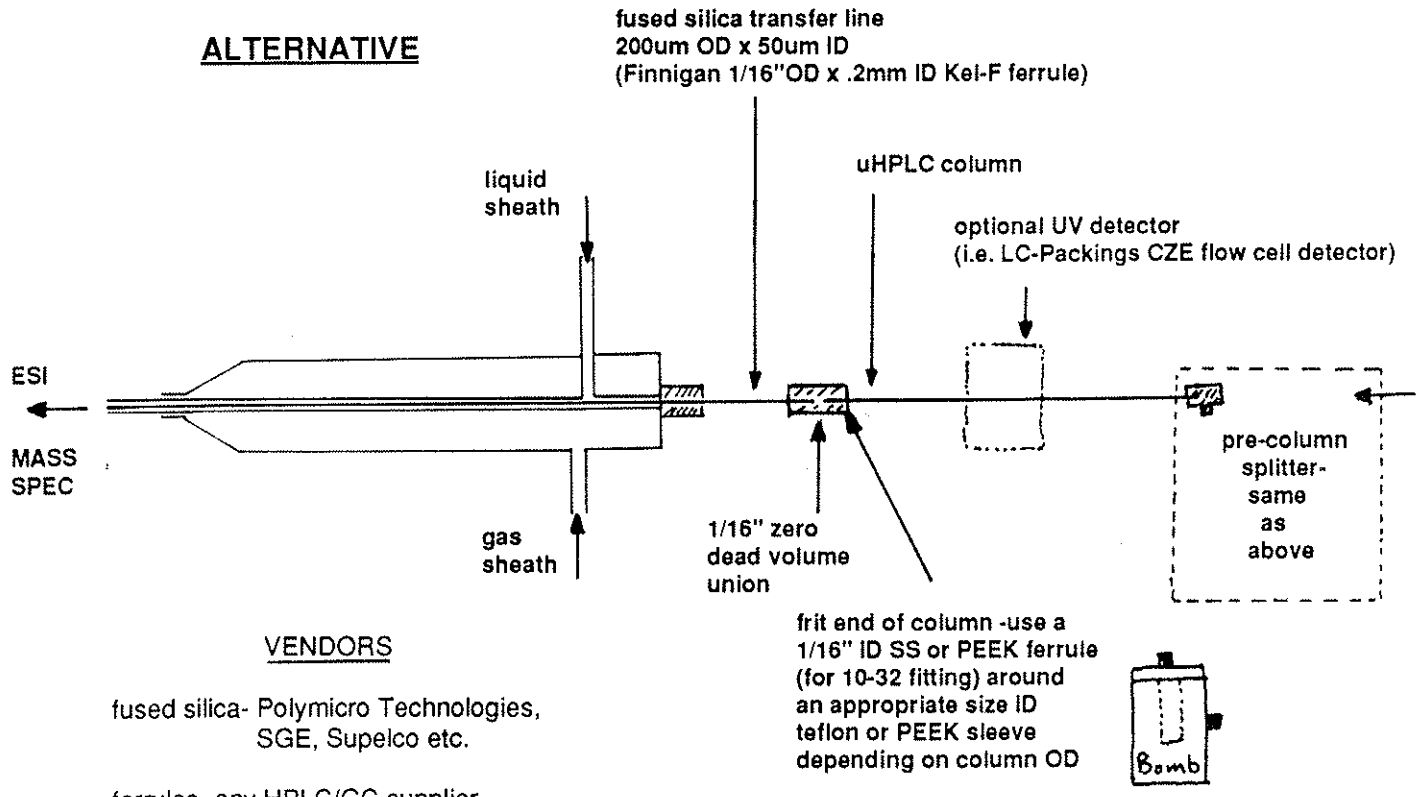




HPLC/micro-column/ESI INTERFACE



ALTERNATIVE



VENDORS

fused silica- Polymicro Technologies,
SGE, Supelco etc.

ferrules- any HPLC/GC supplier-
Supelco, Altech, Anspec etc.

swagelok fittings
& whitey valves- Crawford Fiitings Co.
Solon, OH