Problem Session

Sep 14, 2019

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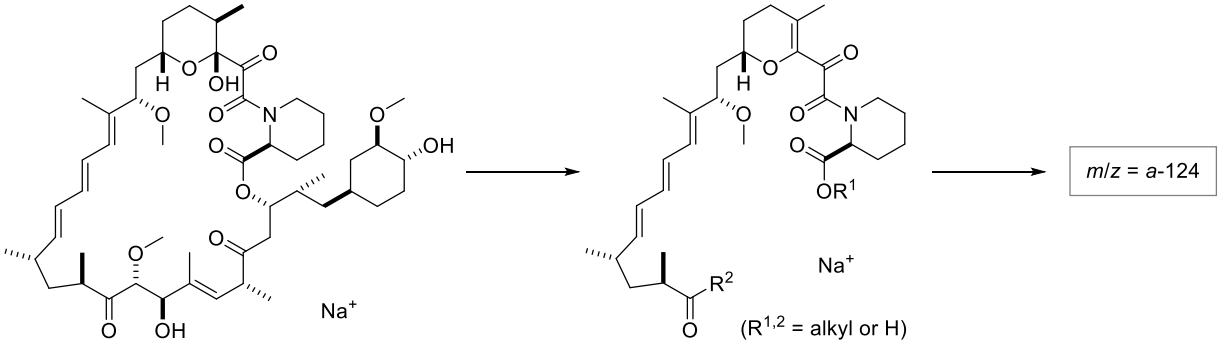
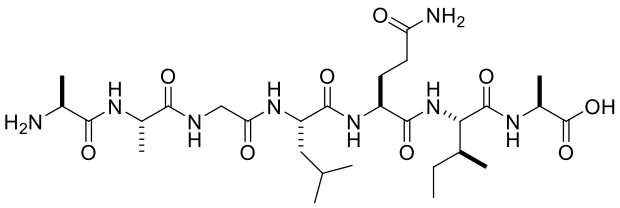
1. Results of ESI-MS^3 (MS/MS/MS) analysis of rapamycin (**1**) are displayed. Please provide a structure of **3** and a possible generation mechanism of **3**.



precursor ion (**1** + Na^+) product ion in MS^2 spectrum (**2**, *m*/*z* = *a*) product ion in MS^3 spectrum (**3**)

2. Please propose an effective <u>chemical modification method</u> for MS fragmentation (high-energy collision-induced dissociation)-based determination of the amino-acid sequence of peptide **4**. Please take notice that leucine and isoleucine must be discriminated. Determination of stereochemistry of each amino acid is unnecessary.



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Mass spectrometry-based structure analysis

1. Introduction

Mass spectrometry-based techniques become more important and helpful for structure determination, which permits the structure analysis by using a *small (or limited) amount of sample* (minimum scale: fmol–amol (10^{-15}–10^{-18} mol))

Current application of tandem mass spectrometry (MS/MS, MS^3 etc) for structure determination

- Omics (e.g. proteomics,[1,2] metabolomics[3])
- Molecular biology (e.g. investigation of biologically active endogenous peptides*), chemical biology (e.g. target identification of natural products)

  *Structure determination using only genome and transcriptome is still difficult due to the lack of information on peptide/protein processing and posttranslational modification
- Structure determination of natural products (combination with other analytical methods)[4]
- Direct "decoding" process of randomly synthesized compound library[5]

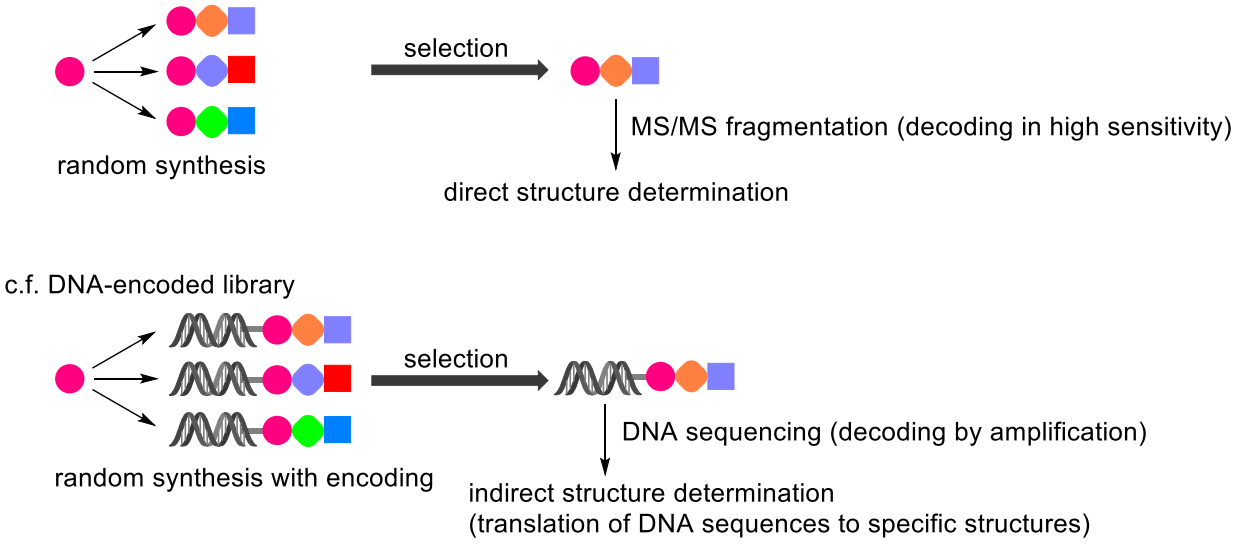


Figure 1. Comparison of coding and decoding methods for randomly synthesized compound library.

- Tandem MS-based analysis as the "decoding" process allows only one-to-one relationship between the structure and the interpretation of fragmentations.

Question: What are the current status and limitation of tandem MS-based structure analysis?
Aim: Understanding of the details of MS/MS fragmentation (reaction) on soft ionization techniques (ESI and MALDI) with collision-induced dissociation (CID)

2. Methods

2-1. Mass spectrum degradation (MSD) method for determining substructures:

- collision-induced dissociation (CID)
- post source decay (PSD, MALDI-TOF (reflectron) specific technique)

2-2. Collison-induced dissociation

Degradation induced by collision of a precursor ion with an inert collision gas (He, Xe, Ar, N_2 etc.)

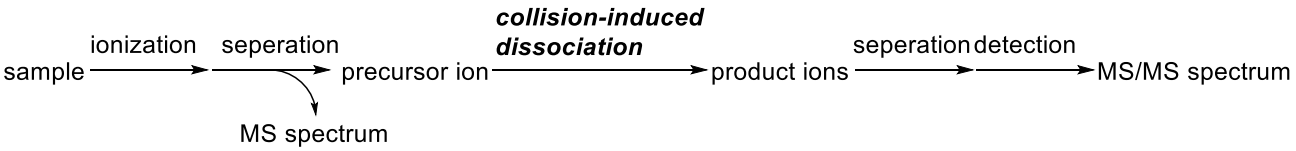


Figure 2. MS/MS analysis using collision-induced dissociation. Multiple tandem mass spectrometry (e.g. MS^3, problem 1) is possible by further degradation/separation/detection of product ions.

The condition of CID affects fragmentation pattern.

- high-energy CID (with magnetic sector, TOF/TOF system): kinetic energy of precursor ions = keV (mainly induces electronic excitation)
- low-energy CID (with quadrupole, ion-trap system): kinetic energy of precursor ions = ~100 eV (vibration excitation)

Pros and cons

High-energy CID: complex spectra derived from charge-remote fragmentation (multiple cleavage)

Low-energy CID: favors charge-driven fragmentation (selective cleavage)

3. Analysis and understanding of fragmentation on tandem MS spectrometry

3-1. Basics of fragmentation by CID

Solvent-free and unimolecular reaction must be considered. Classification based on involvement of a proton or a charge is reasonable for the fragmentation analysis.

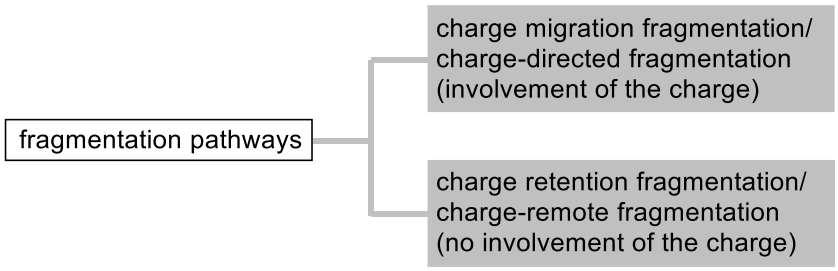


Figure 3. Classification of fragmentation based on a charge.[6,7]

■ The reaction in positive ion mode is depicted here.

1. Charge migration fragmentation/charge-directed fragmentation (e.g. salanin[8])

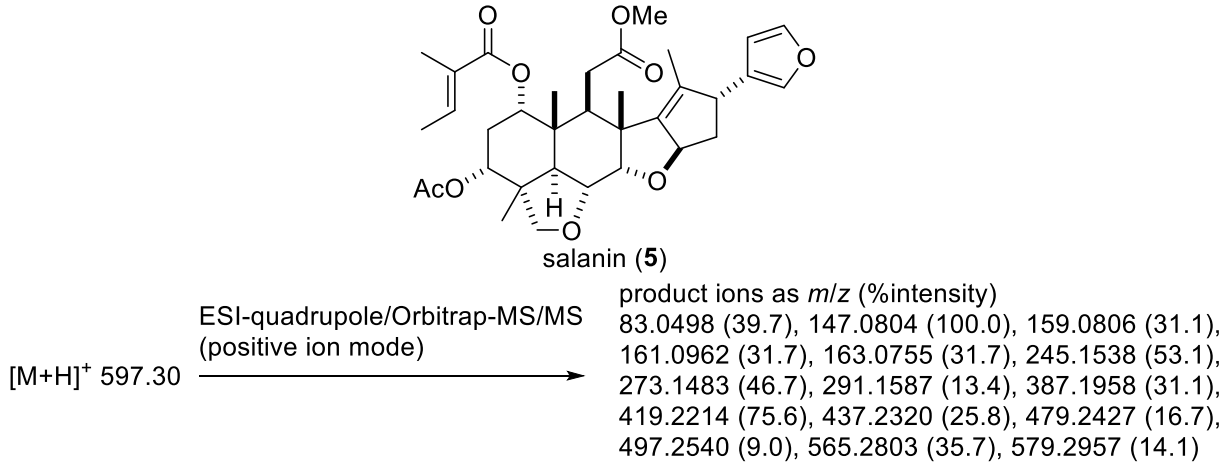


Figure 4. Structure of salanin and observed product ions in ESI-quadrupole/Orbitrap-MS/MS analysis.

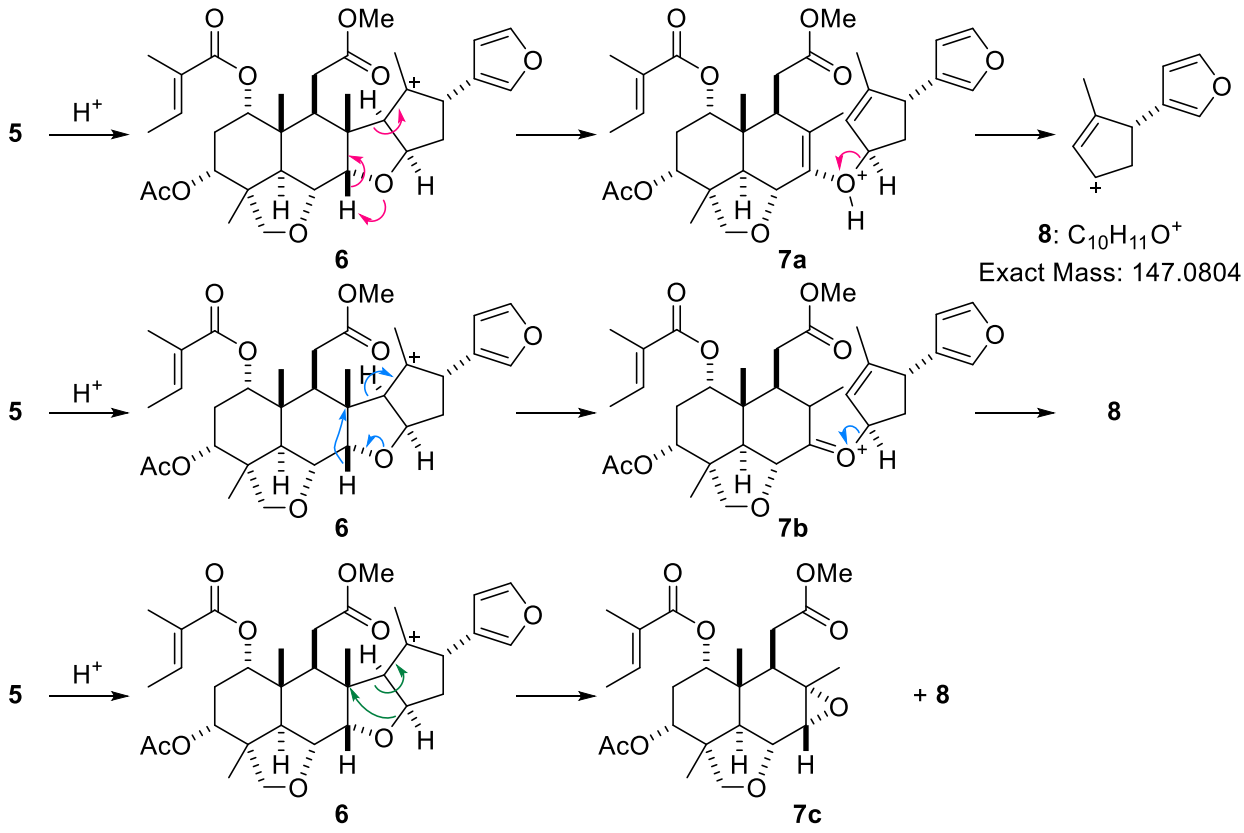


Figure 5. Possible generation mechanisms of the product ion (*m*/*z* = 147.0804)

2. Charge retention fragmentation/charge-remote fragmentation (e.g. retro Diels-Alder reaction, aromatic eliminations (problem 1))

Fragmentation pathways of protonated peptides have been well investigated.

The basic concept of peptide fragmentation on MS/MS is described in the next section for better understanding of MS/MS fragmentation pathways.

3-2. Nomenclature of product ions of peptides

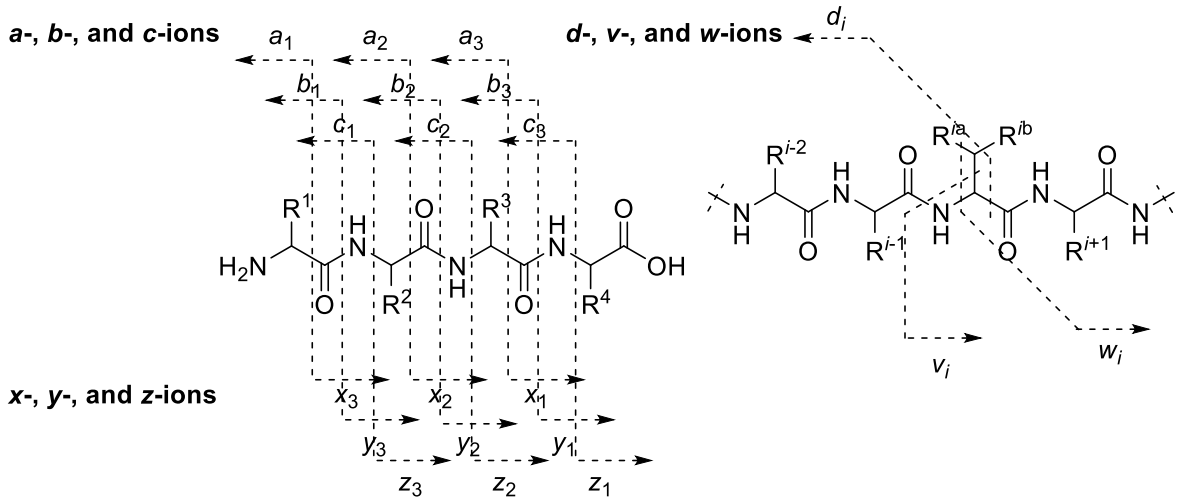


Figure 6. Nomenclature of product ions of peptides on MS/MS analysis. In the case of *d*- and *w*-ions of C_β-disubstituted amino acids, two product ions can be generated (problem 2).

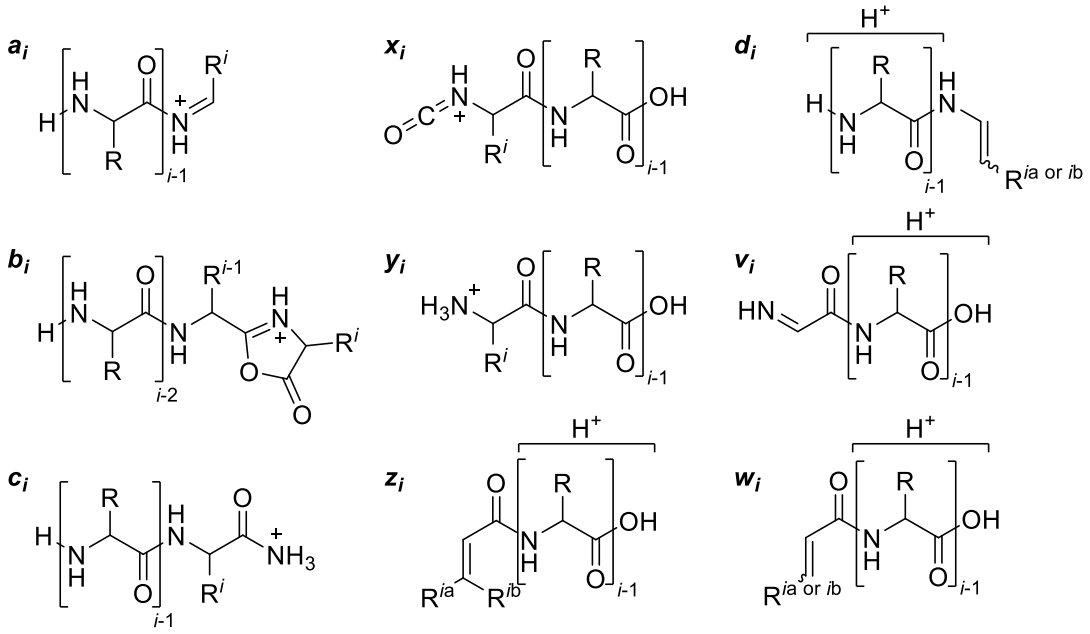


Figure 7. Possible structures of product ions.[9,10]

- Cleavage of C_α-carbonyl C (*a*- and *x*-ions): charge-remote fragmentation by high-energy CID
  a-Ions can also be generated from the degradation of *b*-ions
- **Cleavage of carbonyl C–N (*b*- and *y*-ions): collision-induced dissociation (dominantly occurs by low-energy CID)**
- Cleavage of N–C_α (*c*- and *z*-ions): electron transfer dissociation (ETD)[11] and electron capture dissociation (ECD)[12]
- *d*-, *v*-, and *w*-ions: degradation from other-type ions generated via charge-remote fragmentation

***a*–*c*, *x*–*z* ions: information of sequence**

***d* and *w* ions: information of side chains**

3-3. “Mobile proton model” for charge-directed fragmentation of peptides

(Low-energy CID is considered here) The most comprehensive model to describe how protonated peptides dissociate and form *b*- and *y*-ions: *fragmentation requires the transfer of a proton from a basic site to the amide nitrogen*[13,14,15,16]

Note: solvent-free and unimolecular reaction

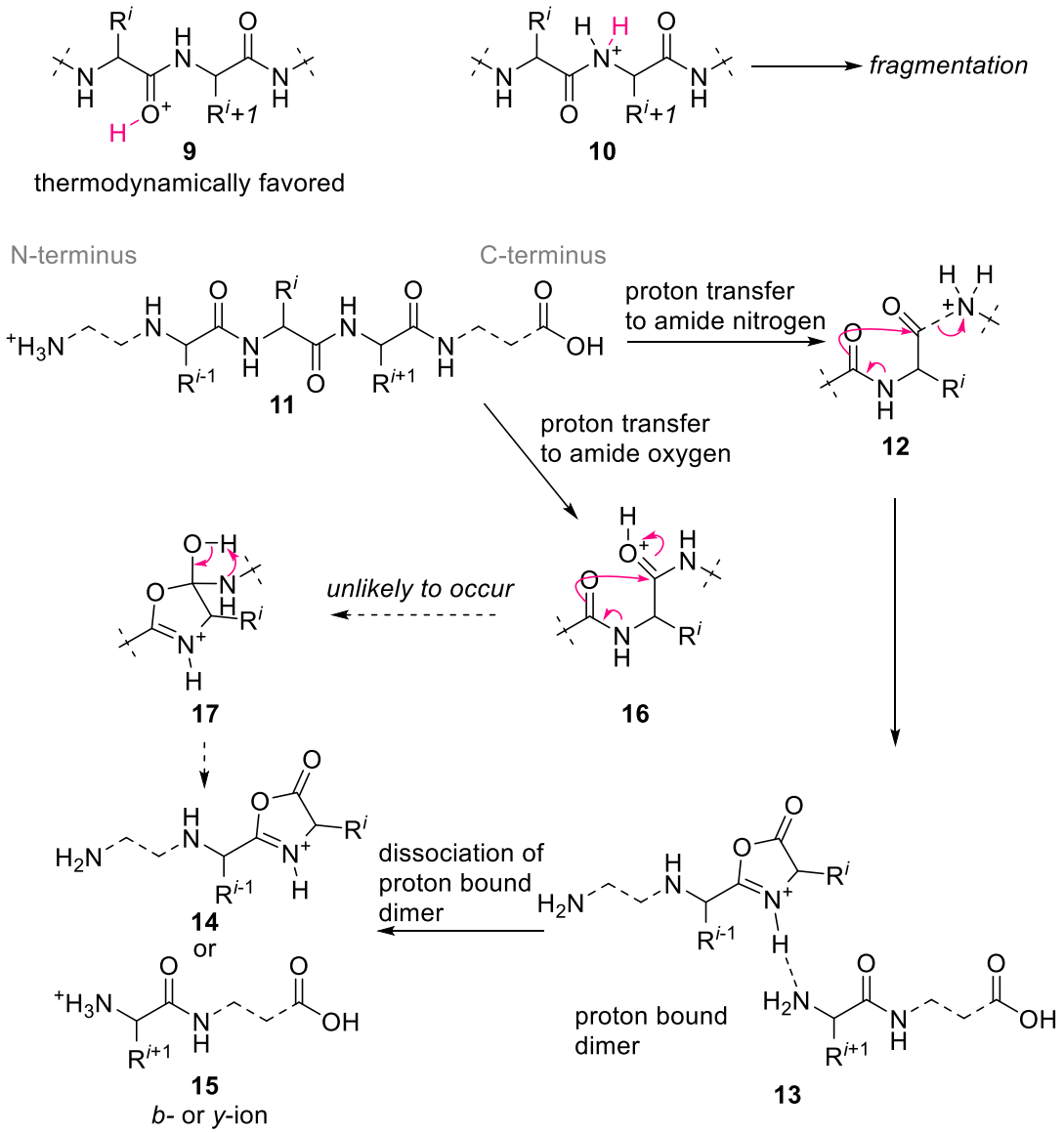


Figure 8. Proposed mechanisms of generation of *b*- and *y*-ion based on mobile proton model.

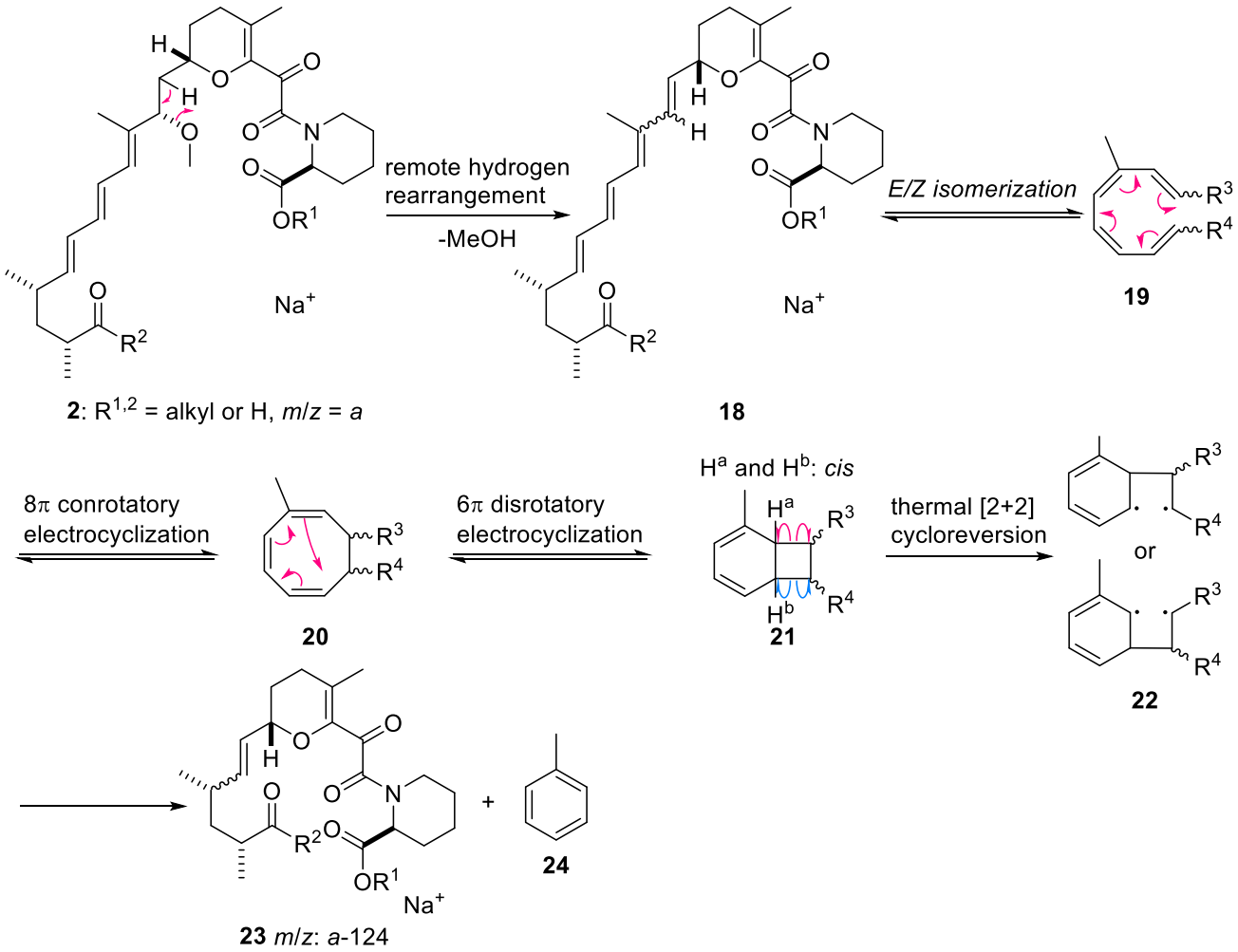
Rationale of mobile proton model

- **H/D exchange experiment**[17] indicated that complete randomization of all hydrogen atoms attached to N and O atoms occurs upon collisional activation prior to the dissociation.

- **IR-MPD spectroscopy (infrared multiple-photon dissociation)**[18] of CID fragments indicated that gradual decrease in the relative population of oxazolone-protonated *b* ion and corresponding increase in N-terminal-protonated *b* ion.,
- **Computational analysis (B3LYP/6-31G(d), RRKM method) of a model protonated peptide (protonated H-Gly-Gly-Gly-OH)**[19] supported the mechanisms (proton transfer from OH to N via four-centered transition state should have high barrier/although oxazolone ring formation from possible conformation **16'** was tried, geometry optimization afforded only bond-cleaved **16**).[20]

4. Answer for problem 1

(low-energy CID is considered)[21,22,23]



■ The loss of aromatic molecule from other polyene compounds was also reported.

Table 1. Specific examples of aromatic loss of polyene compounds[24]

compound	precursor ion	product ion	difference
amphotericin B (**25**)	946.4746 $[M+Na]^+$	868.4298	C_6H_6
rapamycin (**1**)	564.3280[25]	440.2413	C_7H_8

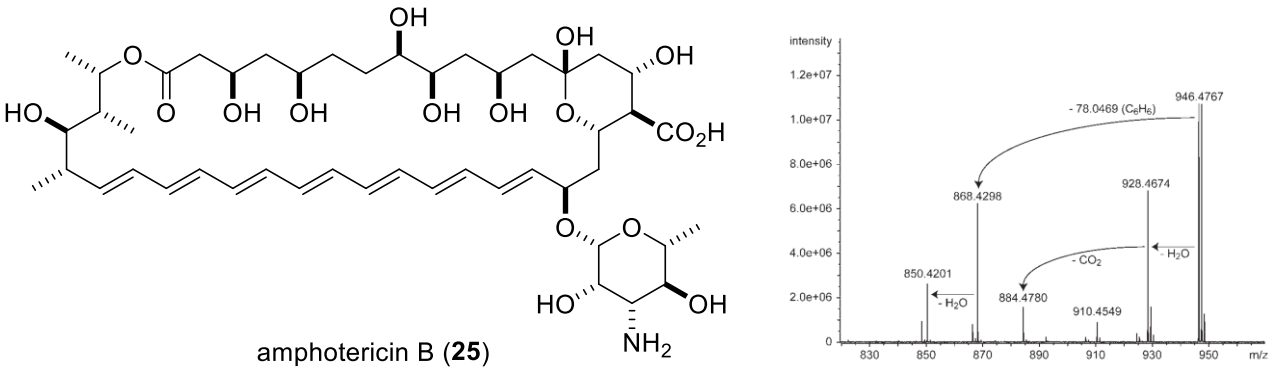


Figure 9. ESI-FTICR-MS/MS of amphotericin B (**25**). MS/MS chart of **25** was taken from ref 24.

5. Answer for problem 2: introduction of a cation (e.g. alkyl ammonium salt,[26] phosphonium salt)[27] or strong basic group (guanidine moiety)[28] to **4** for efficient generation of *d*-ions to discriminate the leucine and isoleucine residues

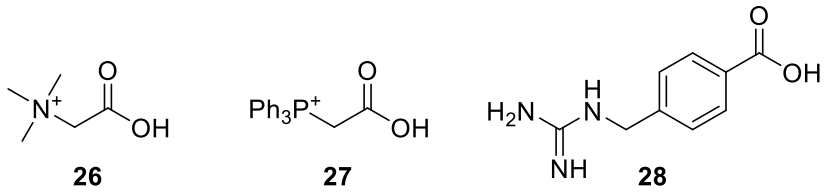


Figure 10. Specific examples reported in the literatures for N-terminal modification.

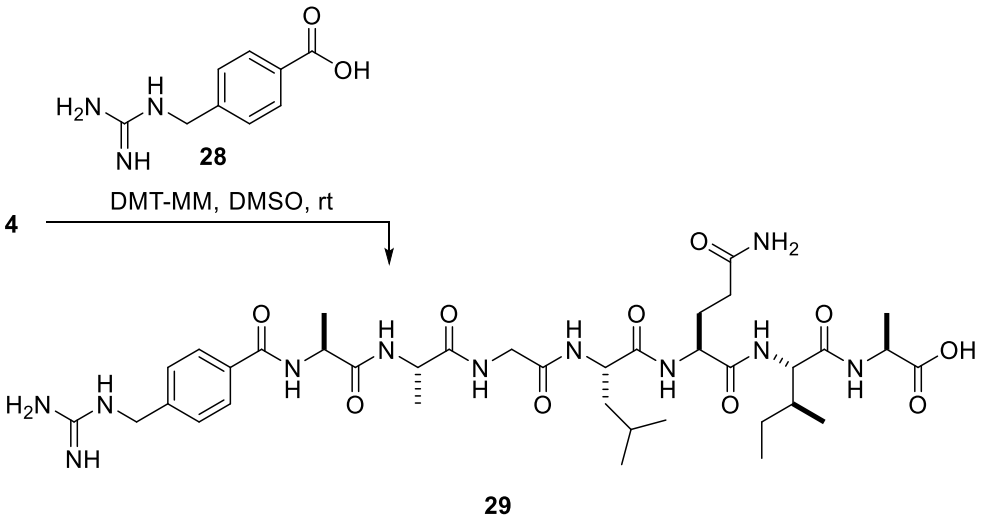


Figure 11. Specific example reported in the literature (**28**).[28]

■ Efficient *d*- (or *w*) ion generation via charge-remote fragmentation is necessary.

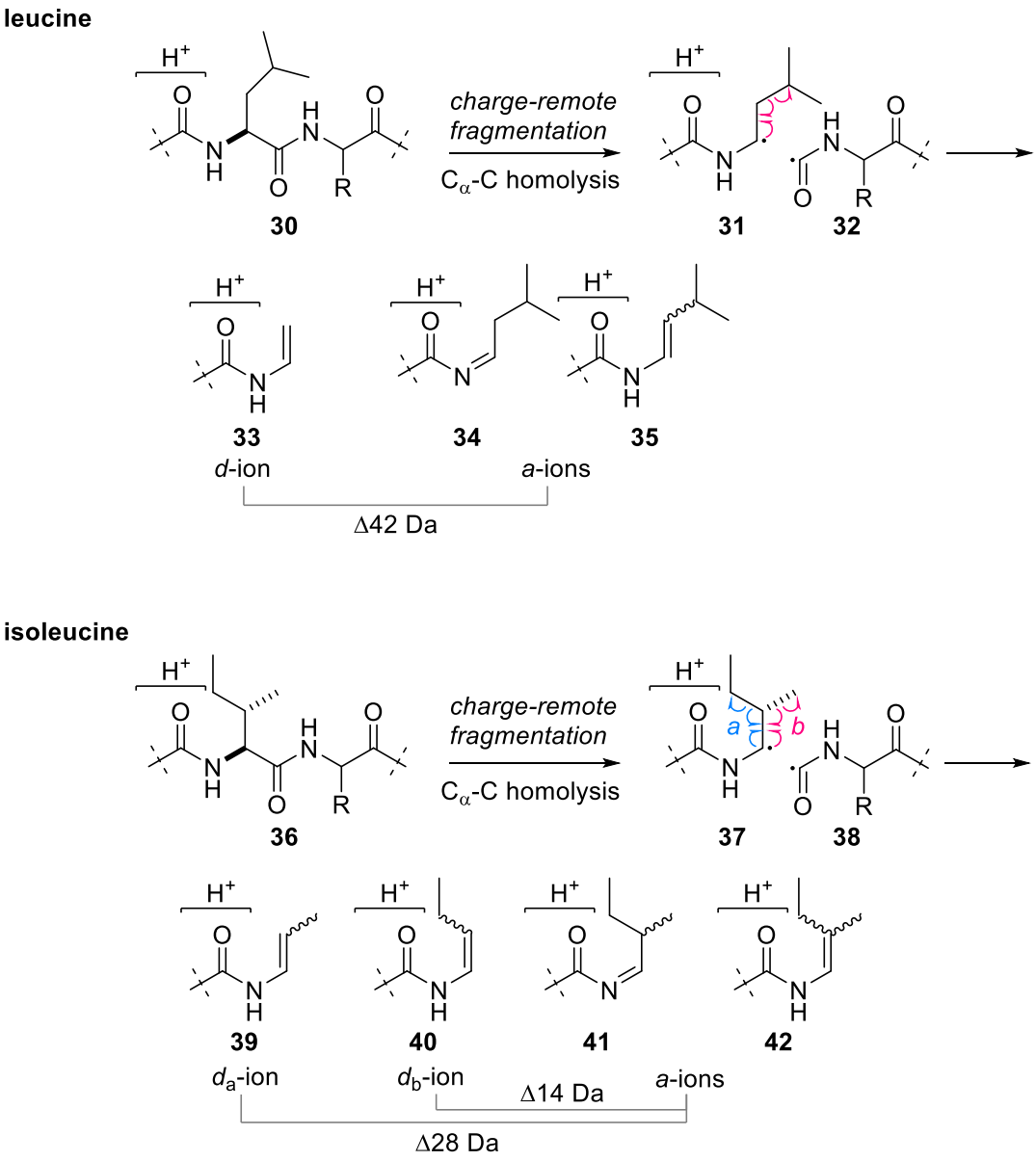


Figure 12. Generation of *d*-ions of leucine and isoleucine residues by high-energy CID[29]

■ To efficiently induce charge-remote fragmentation, competitive charge-directed fragmentations should be suppressed (see also Figure 3). As possible methods, followings could be considered:

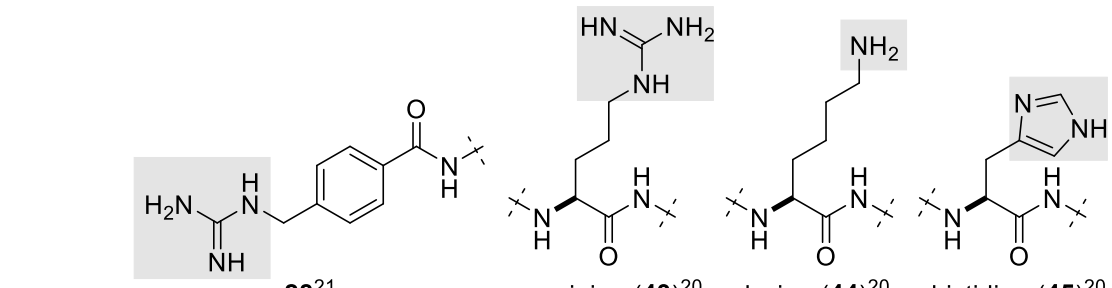
1. exclusion of mobile proton from the ion of interest (deletion of cationic groups and addition of the cation)
2. capture of a mobile proton by introducing strong base

- An arginine residue effectively induces charge-remote fragmentation.[30]

  magnitude of the effect on the induction of charge-remote fragmentation:

  arginine > lysine, histidine

Proton affinity (basicity of gas phase)[30,31]



	28[21]	arginine (**43**)[20]	lysine (**44**)[20]	histidine (**45**)[20]
proton affinity (kcal/mol)	244.5	245.0	230.0	231.7

Proton affinity for the reaction: $B + H^+ = BH^+$

is defined as $-\Delta H$ (negative of the reaction enthalpy at 25 °C)

a **b**

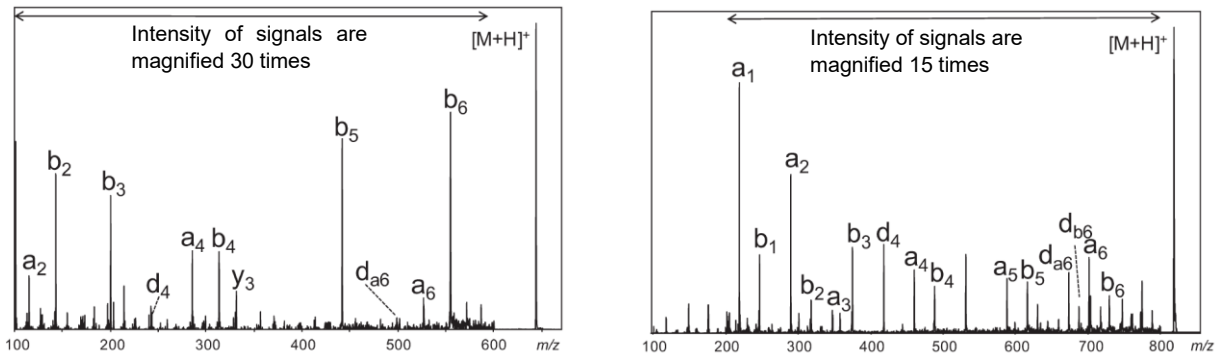


Figure 13. (a) MS/MS spectrum of **4**. (b) MS/MS spectrum of **5**. Charts were taken from ref 28.

6. Misc

- Resolution = high-energy CID < low-energy CID

  To accurately discriminate lysine and glutamine residues, fragmentation analysis using low-energy CID is preferred.
- Even in the presence of arginine, *b*- and *y*-ions can be generated. In that case, alternative pathways are proposed (involvement of a C-terminal carboxylic acid to form salt bridge/acid anhydride or involvement of an amide proton of -COH=N-).[32]
- By using cations such as **26** and **27** with low-energy CID, fragmentation patterns are limited and intensities of *b*- and *y*-ions decrease due to the unavailability of the mobile proton.
- In several cases, diastereomers provided different fragmentation patterns (product ion species and their intensities).[33]

References

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