

N-terminal charge-driven *de novo* sequencing by using ASDF-incorporated Curved Field Reflectron

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Overview

- *De novo* sequencing consisted of N-terminal derivatization and informative MALDI-MS/MS bolstered by high resolution MS/MS and HE-CID is reported.
- Differentiations of Ile/Leu are performed with HE-CID successfully.
- A precise assignment of Gln/Lys is conducted.
- Switching PSD to HE-CID rapidly is valuable to realize weak d-ions.

Introduction

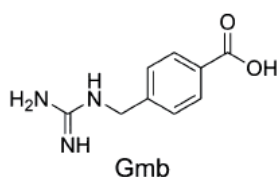
Fixing strong charge at N-terminus of peptide has been reported to be effective derivatization for *de novo* sequencing. While giving high proton affinity at N-terminus of peptide facilitates the generation of a- and b-ions, it is impossible to differentiate isobaric Ile/Leu residues. Side chain fragmentation generated by high energy CID on MALDI-TOF/TOF has a potential to overcome this issue. On

the other hand, a precise assignment of Gln/Lys by the previous MALDI-TOF/TOF is quite difficult due to insufficient MS/MS resolution and accuracy. We will report discrimination of Ile/Leu and a capability of the assignment of Gln/Lys. We will apply newly developed MALDI-TOF/TOF, which has high resolution in MS/MS and a high energy CID at 20keV, to analysis N-terminal derivatized peptides.

Methods

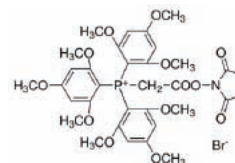
Chemistry of N-terminal derivatization ^{3,4)}

Gmb; 4-Guanidinomethylbenzoic acid



Gmb was synthesized as previously reported. Gmb (1 mmol) dissolved in DMSO at a concentration of 2.5 mM was mixed with peptides (100 nmol, 2.5 mM) and DMT-MM (1 mmol, 2.5 mM). The resultant mixture was stirred at room temperature overnight. The derivatized peptides were purified by RP-HPLC.

TMPP-Ac; N-Succinimidylxylocarbonylmethyl)tris(2,4,6-trimethoxyphenyl)phosphonium bromide



TMPP-Ac-NHS was synthesized as previously reported. TMPP-Ac-NHS (1 mmol) dissolved in 0.5 M phosphate buffer (pH8.5) at a concentration of 2.5 mM was mixed with peptides (100 nmol, 0.25 mM). The resultant mixture was stirred at room temperature overnight. The derivatized peptides were purified by RP-HPLC.

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MALDI-tandem TOFMS

Instrument	: MALDI-7090 (Shimadzu/Kratos)
Measurement	: PSD and high-energy CID-MS/MS in positive ion mode.
Collision gas	: helium
Collision energy	: 20 keV (laboratory frame of reference).



Principle of Axial Spatial Distribution Focusing (ASDF)

MS/MS resolution is improved with correcting the spatial distribution of ions in ASDF cell. This is achieved by applying a pulsed electrostatic field at the point, at which the precursor and fragments are velocity focussed but are spatially resolved.

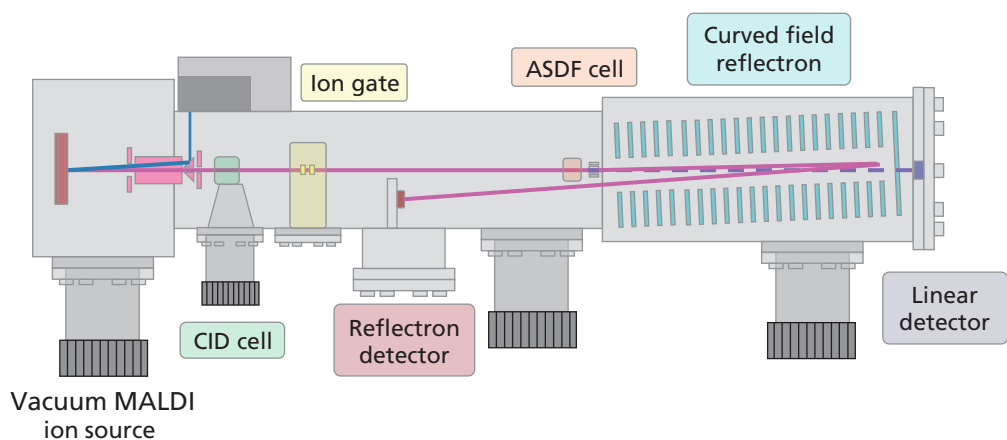


Fig.1 Inside view of MALDI-7090

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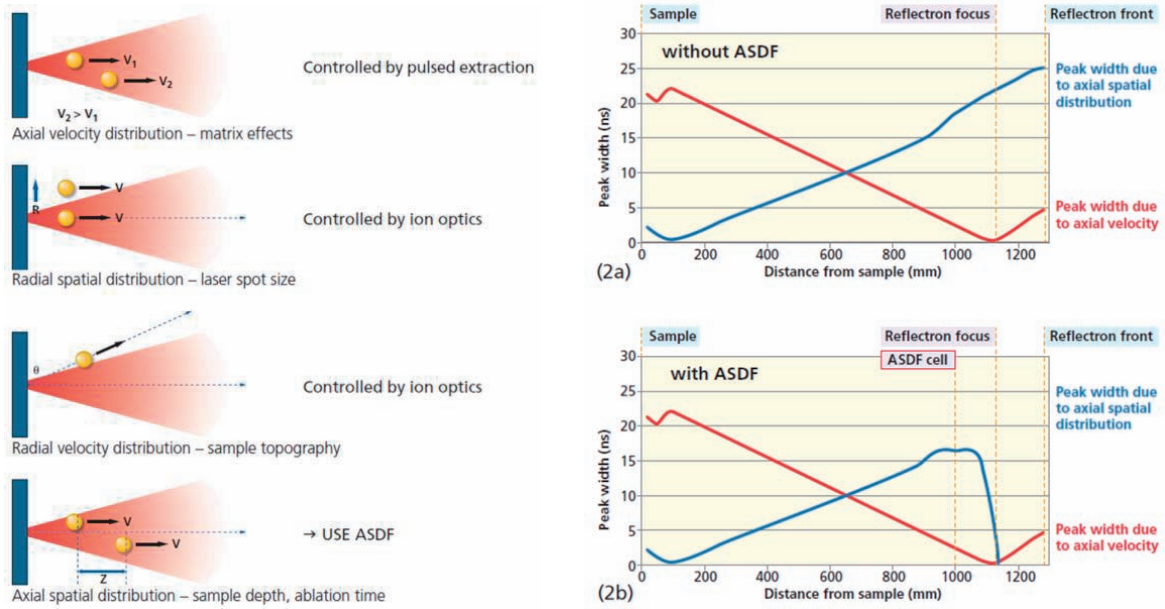


Fig.2 Axial Spatial Distribution Focusing

Results

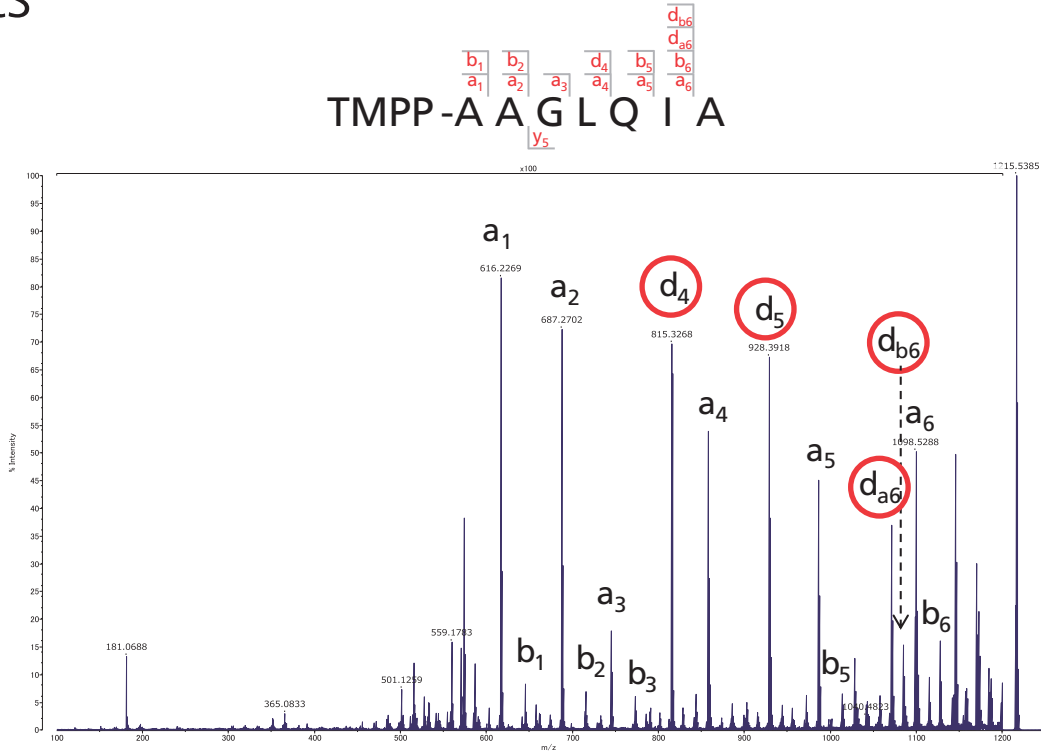


Fig.3 CID-MSMS of TMPP-Ac derivatized peptide

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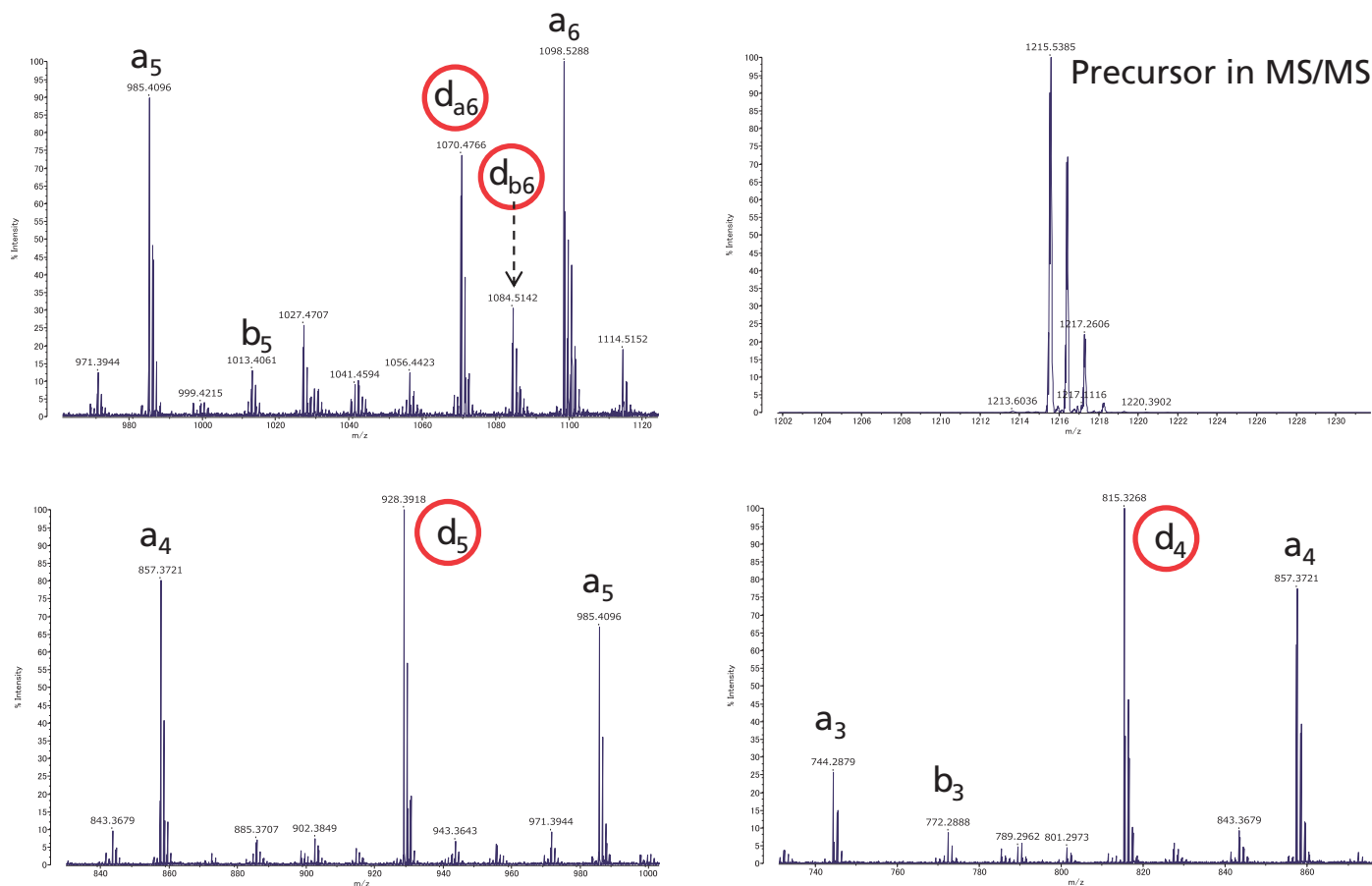


Fig.4 Enlarged spectrum in "Fig.3"

Theoretical	Observed	Diff.	
a ₁	219.1240	219.1577	0.0337
b ₁	247.1190	247.2139	0.0949
a ₂	290.1612	290.1436	0.0176
b ₂	318.1561	318.1411	0.0150
y ₂	331.1976	331.1648	0.0328
a ₃	347.1826	347.1615	0.0211
b ₃	375.1775	375.1761	0.0014
a ₄	418.2667	418.1995	0.0672
a ₁	460.2667	460.2638	0.0029
b ₄	488.2616	488.2570	0.0046
y ₄	572.3402	572.3271	0.0131
b ₅	588.3253	588.3220	0.0033
b ₅	616.3202	616.3046	0.0156
i _{5a}	673.4093	673.3571	0.0522
i _{5b}	687.4093	687.3678	0.0415
a ₆	701.4093	701.3843	0.0250
b ₆	729.4042	729.3939	0.0103

128.0582

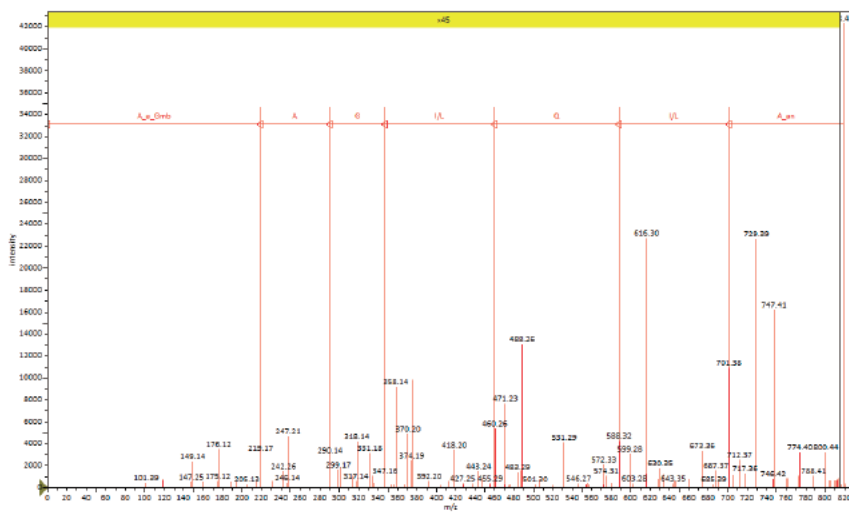


Fig.5 List of fragment ions of TMPP-Ac derivatized peptide

Fig.6 Assignment of TMPP-Ac derivatized peptide by SIMSE in Mass++⁵⁾

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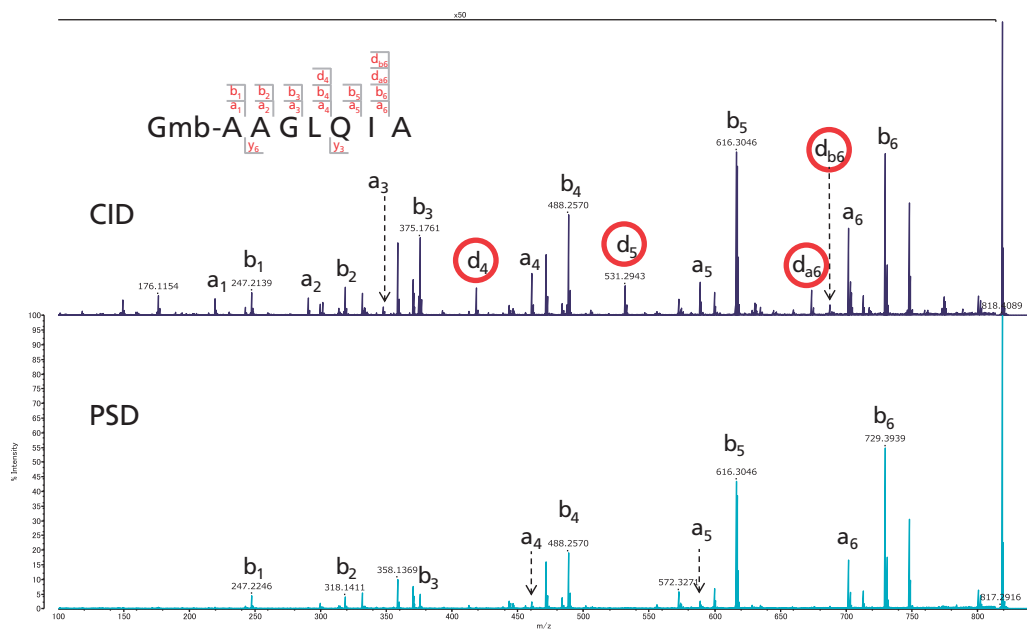


Fig.7 CID (upper) and PSD (lower) of Gmb derivatized peptide

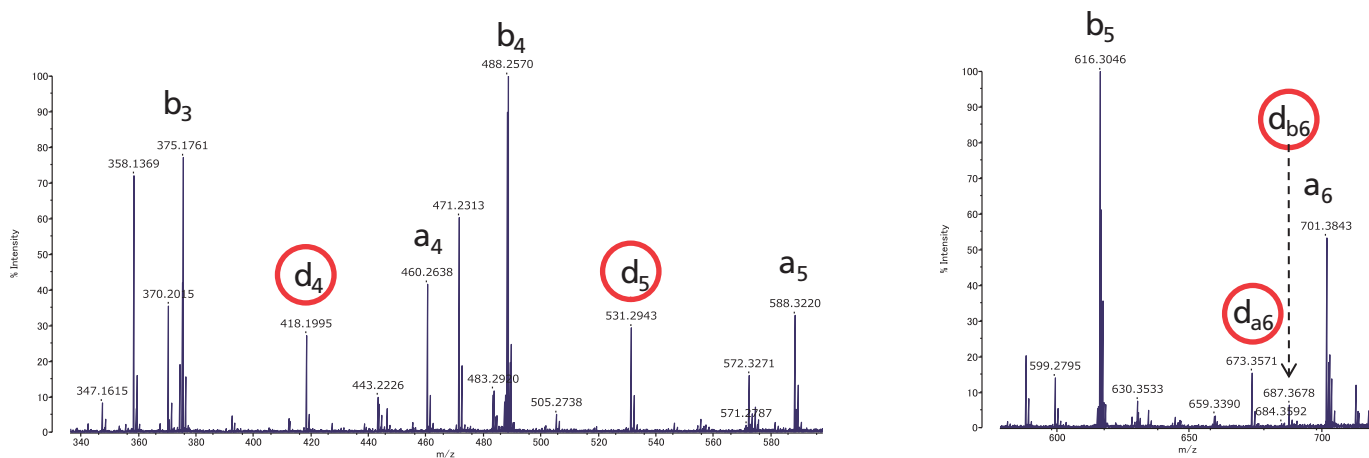


Fig.8 Enlarged CID spectrum in "Fig.7"

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Theoretical	Observed	Diff.	
y ₆	501.3031	501.1259	0.1772
z ₁	616.2306	616.2269	0.0037
b ₁	644.2255	644.2331	0.0076
a ₁	687.2677	687.2702	0.0025
b ₂	715.2626	715.2753	0.0127
z ₂	744.2892	744.2879	0.0013
d ₁	815.3733	815.3268	0.0465
a ₂	857.3733	857.3721	0.0012
H ₂	985.4318	985.4096	0.0222
b ₃	1013.4270	1013.4061	0.0209
d ₂	1070.5160	1070.4766	0.0394
d ₃	1084.5160	1084.5142	0.0018
H ₁	1098.5160	1098.5288	0.0128
b ₄	1126.5110	1126.5200	0.0090

128.0375

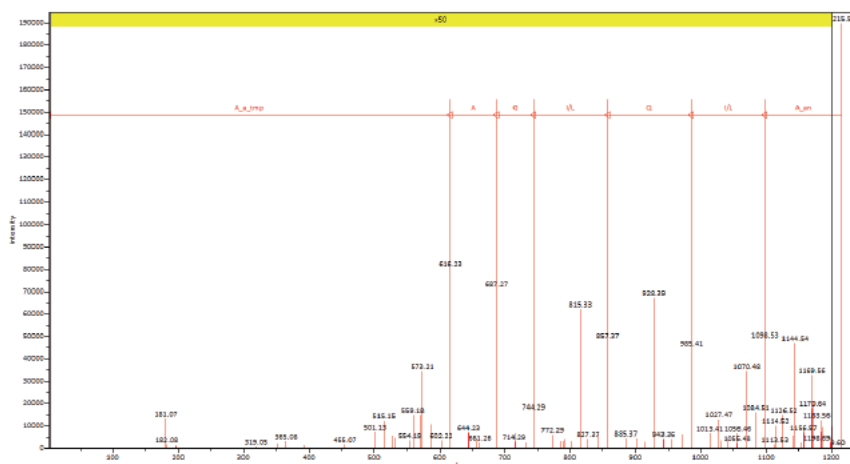
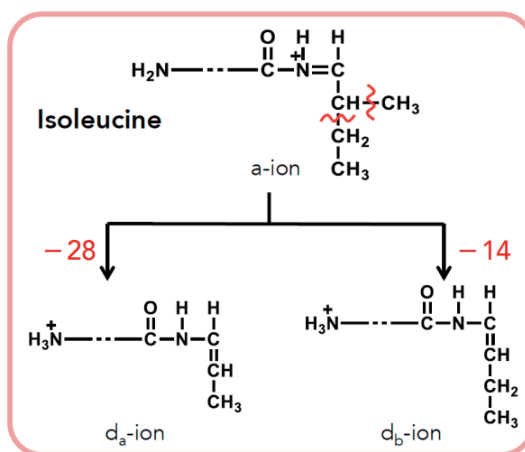
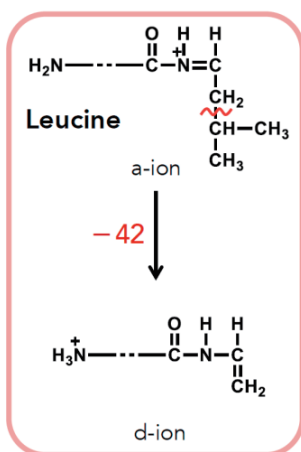


Fig.9 List of fragment ions of Gmb derivatized peptide

Fig.10 Assignment of Gmb derivatized peptide by SIMSE in Mass++⁵⁾



Amino Acid	Residual mass (mono)
Ile	113.0841
Leu	113.0841
Gln	128.0586
Lys	128.0950

Fig.11 Side chain cleavage of Ile/Leu, and exact mass of amino acid residues.

Conclusions

- Ile and Leu in N-terminal derivatized peptides is differentiated successfully by high energy CID-MS/MS, where specific side chain fragmentations of both residues were observed.
- Particular MS/MS accuracy achieved by ASDF enable to apply a precise assignment of Gln/Lys, the mass difference between which is only 0.036 Th.
- High MS/MS accuracy facilitates an interpretation of the data by a dialogical *de novo* software, for instance Mass++.
- Comparing PSD and CID could be useful to recognize fragment ions generated by high energy CID, even if intensities of them will be weak.

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References

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- (3) Miyashita, M., et al; Rapid Commun. Mass Spectrom., 2011, 25, pp1130-1140.
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- (5) download site: <http://www.first-ms3d.jp/english/achievement/software/mass2>