mMass as a Software Tool for the Annotation of Cyclic Peptide Tandem Mass Spectra

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Introduction

Natural or synthetic **cyclic peptides** often possess pronounced bioactivity. Their mass spectrometric characterization is difficult due to the predominant occurrence of **non-proteinogenic monomers** and the **complex fragmentation patterns** observed. Even though several software tools for cyclic peptide tandem mass spectra annotation have been published, these tools are still unable to annotate a majority of the signals observed in experimentally obtained mass spectra. They are thus not suitable for extensive mass spectrometric characterization of these compounds.

The lack of an **advanced and user-friendly software tool** has motivated us to extend the fragmentation module of a freely available open-source software, **mMass** (http://www.mmass.org), to allow for cyclic peptide **tandem mass spectra annotation** and interpretation. The resulting software has been tested on several cyanobacterial and other naturally occurring peptides and has been found to be superior to other tools currently available concerning both usability and annotation extensiveness. Thus it is **highly useful** for accelerating the structure confirmation and elucidation of cyclic as well as linear peptides and depsipeptides.

Main Features and Implemented Fragmentation Pathways

- off-line tool with advanced spectrum manipulation and processing abilities (e.g. support for various data formats, baseline correction, peak picking etc.)
- ability to save spectra, sequences and interpretation results for future use
- monomer database and editor for easy monomer and sequence management
- matching of calculated theoretically possible fragments to and annotation of experimental data

- backbone fragmentation pathways after initial break-up to a b-like ion resulting in a-, b-, c-, x-, y-, and z-like fragment ions (Figure 1)
- alternative initial a/x-like ring opening resulting in +CO fragment ions (Figure 2)
- sequence scrambling
- neutral losses of e.g. H_2O and NH_3 as well as custom side chain losses from individual monomers
- multiple neutral losses from one fragment
- formation of b+H_2O fragment ions (Figure 3)

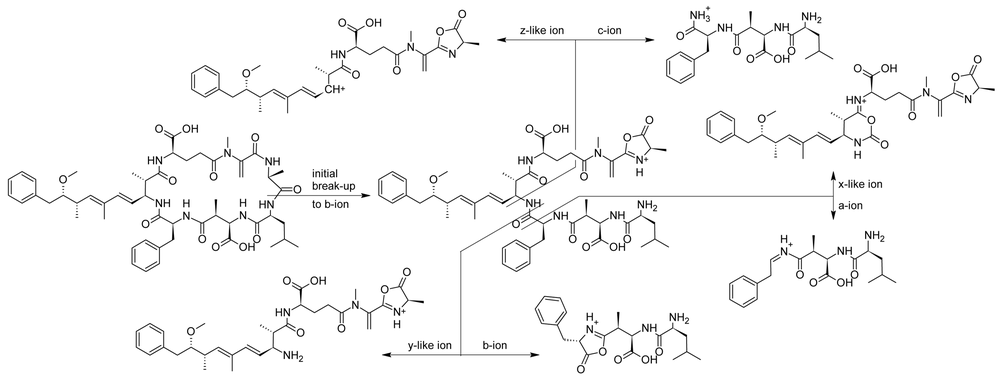

Figure 1 Backbone fragmentation of a cyclic peptide taking Microcystin LF as an example.

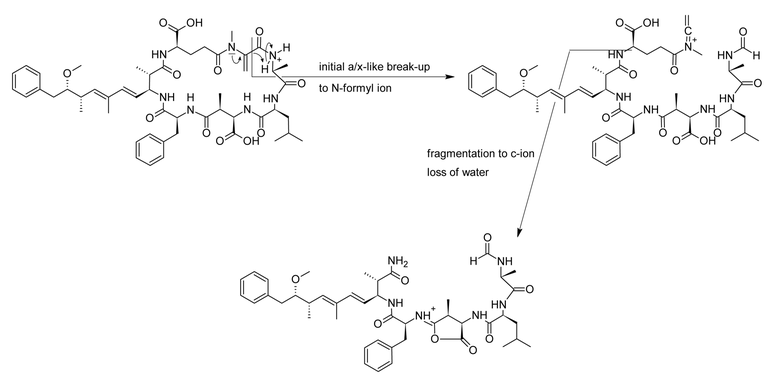

Figure 1 Proposed pathway for the formation of an observed N-formyl fragment ion of microcystin LF after initial protonation of D-alanine.

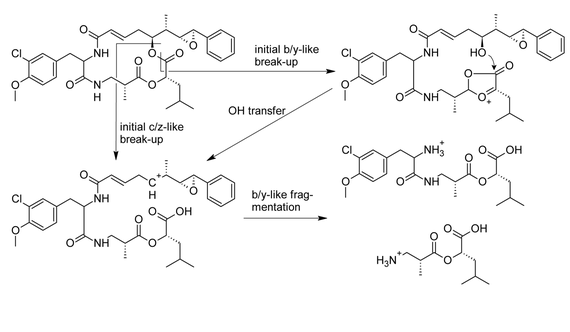

Figure 3 Proposed mechanism for the formation of observed b+H_2O ions of Cryptophycin-1.

Case Study I – Microcystin LF

The fragmentation behavior of microcystins, cyclic heptapeptides found in several cyanobacterial genera, has been very well studied. Thus microcystin LF (Figure 4) has been used to examine the accuracy and comprehensiveness of our fragmentation algorithms and to compare the results to the software NRP-Annotation.

The tandem mass spectrum of microcystin LF (Figure 5) shows several significant peaks that had not been assigned by NRP-annotation, but were annotated by mMass.

- m/z 478.2644 annotated as $c_{4[7|1][1\text{-}4]}$ (Δ 3.3 ppm)
- m/z 561.3017 annotated as $c_{5[6|7][1\text{-}5]}$ (Δ 2.5 ppm)
- m/z 559.3127 annotated as $z_{5[2|3][3\text{-}7]}$ - $C_9H_{10}O$, at (Δ 0.2 ppm)
- m/z 693.3816 annotated as $z_{5[2|3][3\text{-}7]}$ (Δ 3.1 ppm) *
- m/z 852.4486 annotated as M - $C_9H_{10}O$ (Δ 1.9 ppm)
- m/z 835.4252 annotated as M - $C_9H_{10}O$ - NH_3 (Δ 1.9 ppm)
- m/z 801.4549 annotated as $c_{5[7|1][1\text{-}5]}$ - H_2O + CO (Δ 0.5 ppm)

The annotation of selected fragment ions has been confirmed by MS^3.

The annotations show how important it is to allow for user definable neutral losses, multiple neutral losses as well as all possible known fragmentation mechanisms such as CO adducts and the formation of c-series ions.

* Also annotated by NRP-Annotation

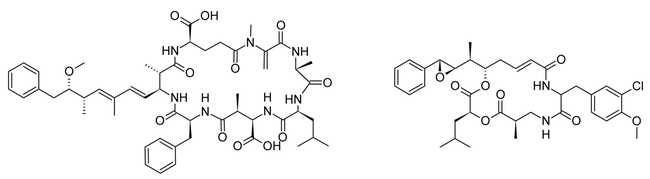

Figure 4 Microcystin LF (left) and Cryptophycin-1 (right).

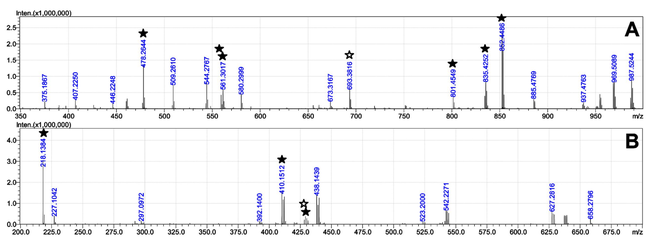

Figure 5 Tandem mass spectra of Microcystin LF (A) and cryptophycin-1 (B). Signals discussed are indicated with a star (filled star: not annotated by NRP-Annotation).

	Microcystin LF	Cryptophycin-1
NRP-Annotation	23	32
mMass*	23	32
mMass**	95	80

Table 1 Percentage of the ion intensity annotated using NRP-Annotation or mMass.
* options chosen reflecting the fragments NRP-Annotation can calculate
** all available options chosen

Case Study II – Cryptophycin-1

Several important peaks of the cryptophycin-1 (Figure 4) tandem mass spectrum (Figure 5) have not been annotated by NRP-Annotation, whereas mMass is able to annotate them.

- m/z 218.1384 annotated as $b_{2[2|3][1\text{-}2]}$ + H_2O (Δ 1.4 ppm)
- m/z 429.1782 annotated as $b_{3[1|2][1\text{-}3]}$ + H_2O (Δ 1.2 ppm)
- m/z 410.1512 annotated as $a_{2[4|1][1\text{-}2]}$ - H_2O (Δ 1.2 ppm)
- m/z 428.1628 annotated as $a_{2[4|1][1\text{-}2]}$ (Δ 1.4 ppm) *

mMass revealed the presence of b+H_2O ions. We expect that b+H_2O ions will be commonly observed ions for all depsipeptides. This shows the advantage of having all known fragmentation pathways implemented in the software used. Also, it can be seen that it is important to allow neutral losses from all types of initial fragment ions.

Conclusions

mMass now is the most concise tool for NRP tandem mass spectra annotation available today. However, fragmentation pathways being possible does not mean they will occur – the annotations still need the attentive assessment of the analyst.

The software can conveniently be used in early steps of structure elucidation / dereplication (matching of *in-silico* fragmentation with experimental tandem MS spectra) and as one of the last steps of structure confirmation (extent of assignable peaks).