

## A Novel O-Acetylthreonine Directing Peptide Bond Cleavage Reaction Facilitates Sequencing of The Cyclic Hexapeptides Aspergillicins A-E.

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### Introduction:

While sequencing of linear peptides via MS/MS of protonated peptides is widespread and represents the cornerstone for most proteomics applications, sequencing of cyclic peptides via MS/MS techniques can be problematic. This is due to the lack of selectivity in the ring opening reactions to give the formal acylium ions. We have uncovered a novel selective gas phase ring opening reaction directed by O-acetylthreonine residues.

### Methods and Instrumentation:

A search for new antiparasitic agents from a strain of the fungus *Aspergillus carneus* isolated from an estuarine sediment collected in Tasmania, yielded a series of new cyclic hexapeptides, aspergillicins A-E. Complete stereostructures were assigned to aspergillicins A-D on the basis of detailed spectroscopic analysis, together with ESIMS analysis of the free amino acids generated by acid hydrolysis, and HPLC analysis of Marfey derivatives prepared from the acid hydrolysate. Aspergillicin E was identified by spectral comparison to aspergillicin A. The peptide amino acid sequence for all aspergillicins was unambiguously assigned by subjecting their electrospray ionization generated  $[M+H]^+$  and  $[M+Na]^+$  ions to multi-stage mass spectrometry collision induced dissociation experiments in an ion trap (LCQ).

### Results:

In order to assign the sequences of these cyclopeptides, we follow the methodology of Gross, whereby the pseudomolecular ion (i.e.  $[M+Na]^+$ ) is mass selected and allowed to undergo collision induced dissociation (CID) with the helium bath gas [1,2]. MS/MS results in cleavage of a peptide bond to give a  $b_n$  ion, which can undergo loss of an amino acid residue mass to give a  $b_{n-1}$  ion (i.e.  $[M+Na-XNCHRCO]^+$ ). The full amino acid sequence was "read out" from sequential amino acid residue losses by carrying multiple stages of CID on the  $b$  sequence ions in a series of  $MS^n$  experiments. The selectivity of ring opening at the O-acetylthreonine residues is examined by comparing the gas phase fragmentation behavior of the  $[M+H]^+$  and  $[M+Na]^+$  ions and through the use of molecular modeling.

- [1] Ngoka, L. C. M.; Gross, M. L., *J. Am. Soc. Mass Spectrom.*, 1999, **10**, 732-746.
  - [2] Ngoka, L. C. M.; Gross, M. L., *J. Am. Soc. Mass Spectrom.*, 1999, **10**, 360-363.
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