



Novel azaspiracids produced by Amphidomataceae

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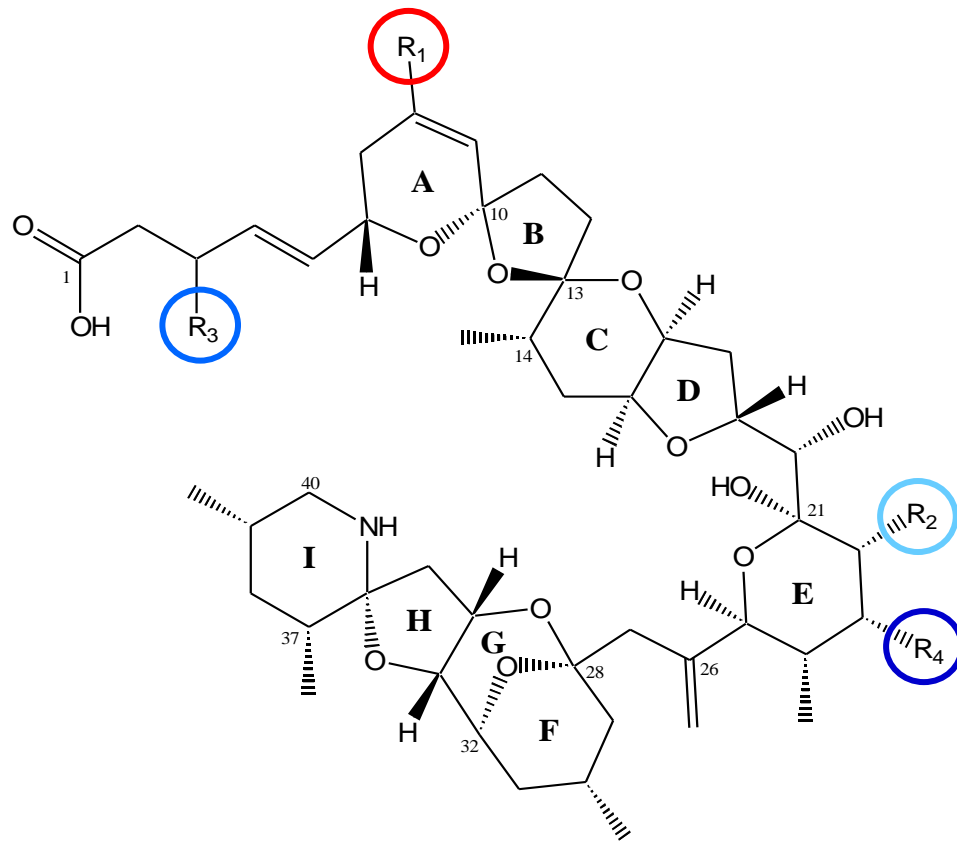
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³ Marine Institute, Oranmore, Galway, Ireland

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introduction: structural variants



Toxin	R ₁	R ₂	R ₃	R ₄	[M+H] ⁺
AZA-1	H	CH ₃	H	H	842
AZA-2	CH ₃	CH ₃	H	H	856
AZA-3	H	H	H	H	828
AZA-4	H	H	OH	H	844
AZA-5	H	H	H	OH	844
AZA-6	CH ₃	H	H	H	842
AZA-7	H	CH ₃	OH	H	858
AZA-8	H	CH ₃	H	OH	858
AZA-9	CH ₃	H	OH	H	858
AZA-10	CH ₃	H	H	OH	858
AZA-11	CH ₃	CH ₃	OH	H	872

To date more than 20 structural azaspiracid variants are known
 Rehmann et al. (2008) Rapid Commun. Mass Spectrom., 22, 549-558

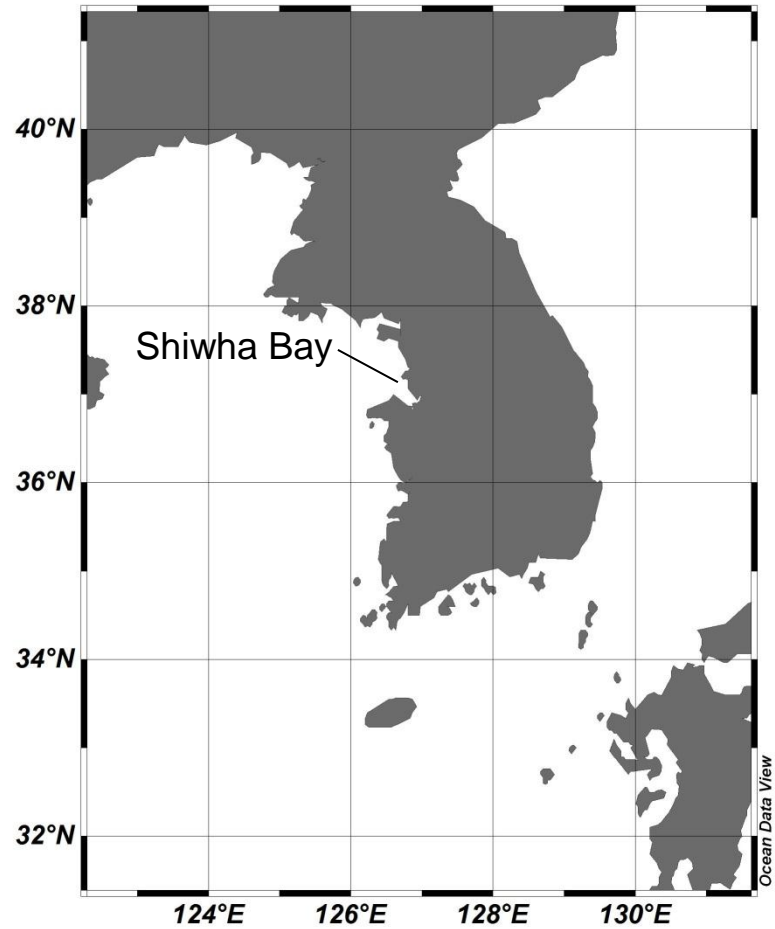


1. *Azadinium* cf. *poporum* Korea



Azadinium cf. *poporum*

Potvin É. et al. 2012. *J. Eukar. Microbiol.* 59, 145-156.

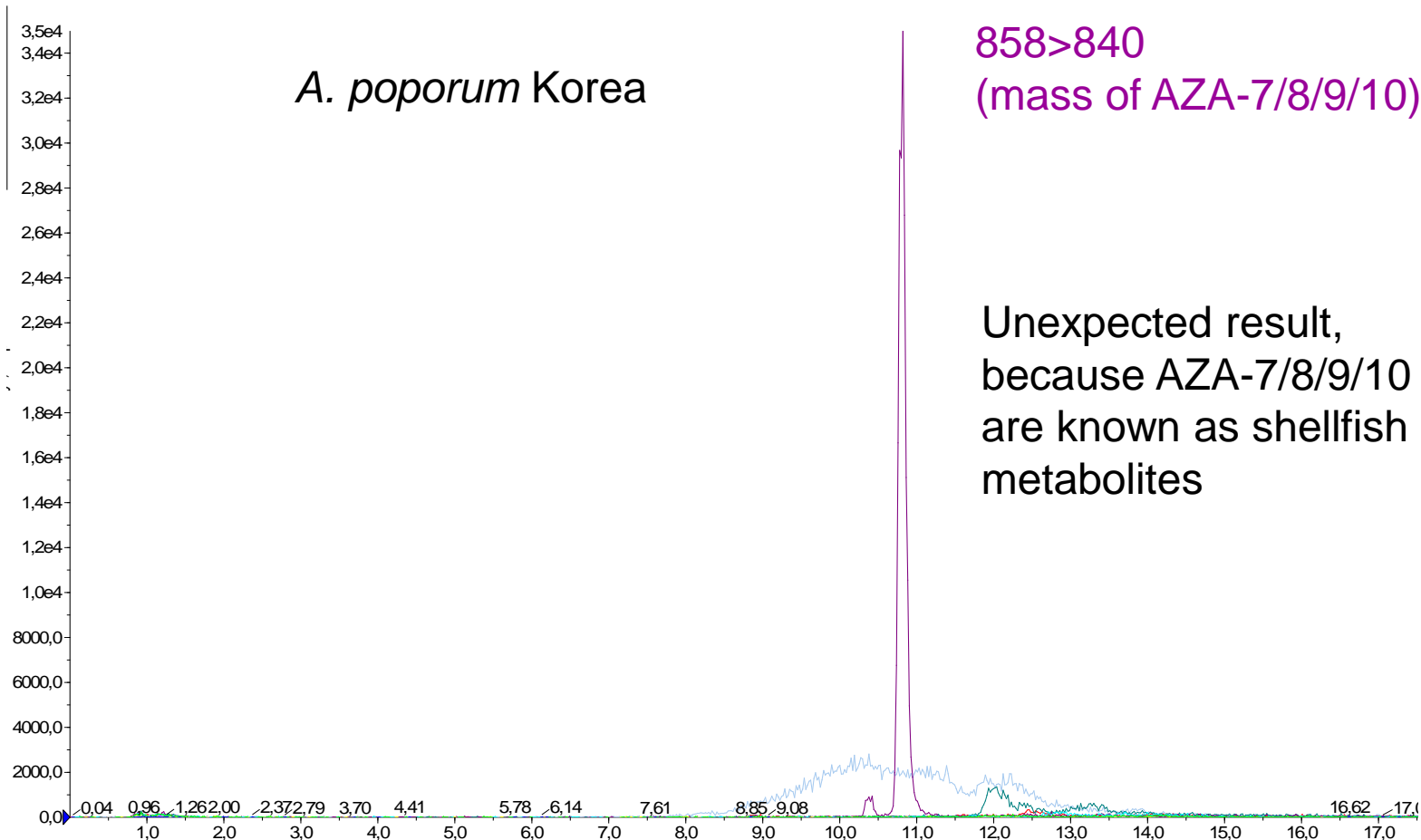


Shiwha Bay (37° 18'N, 126° 36'E)



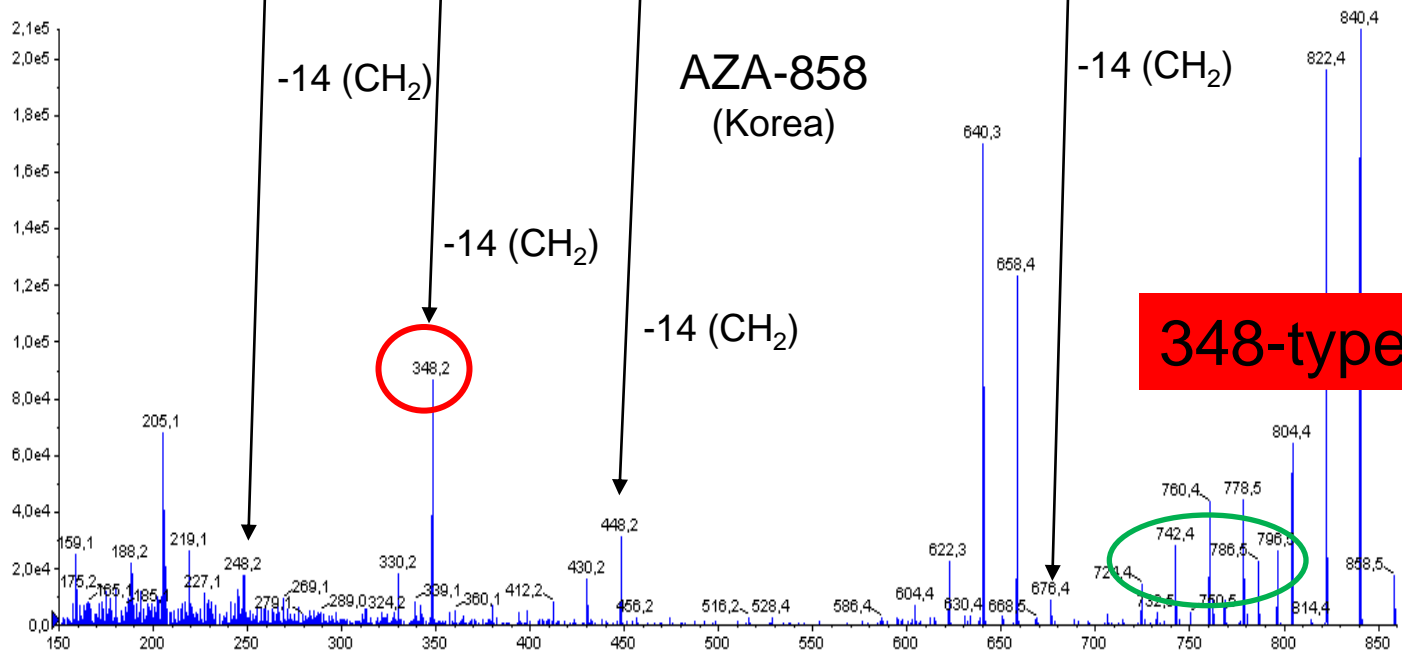
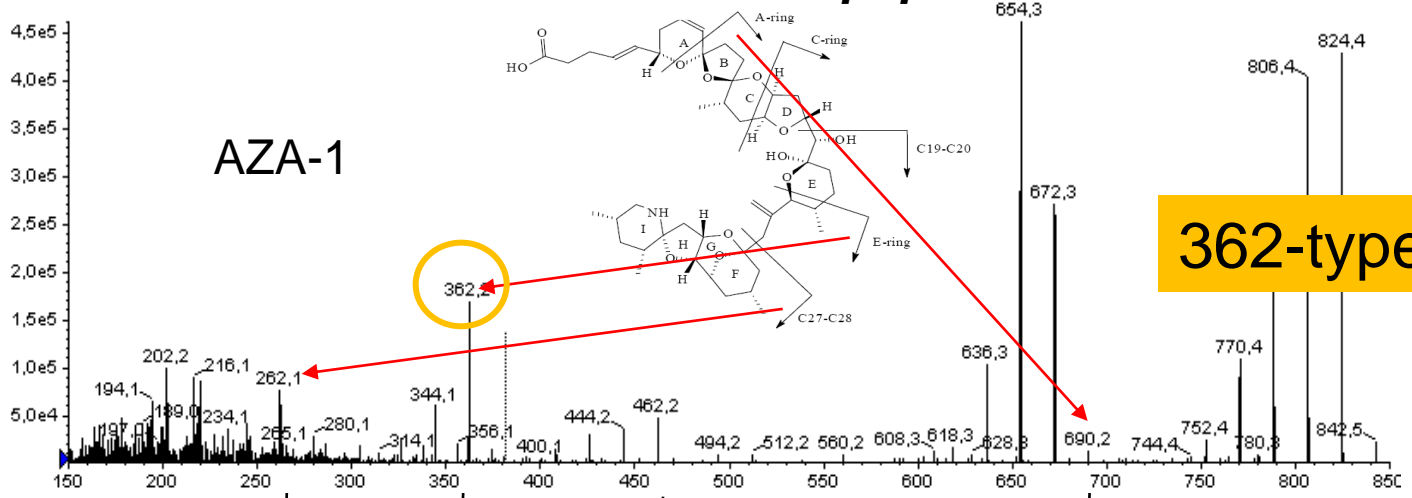
1. *Azadinium cf. poporum* Korea

Test for AZAs:



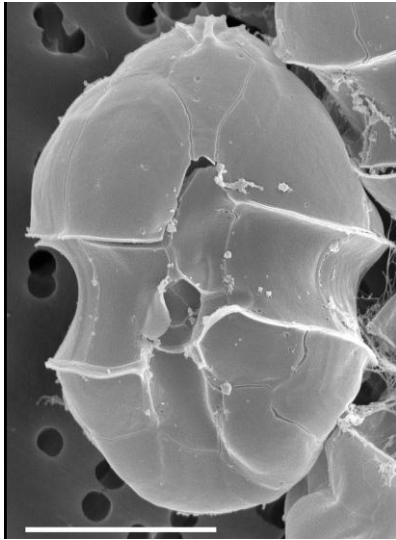


1. *Azadinium cf. poporum* Korea





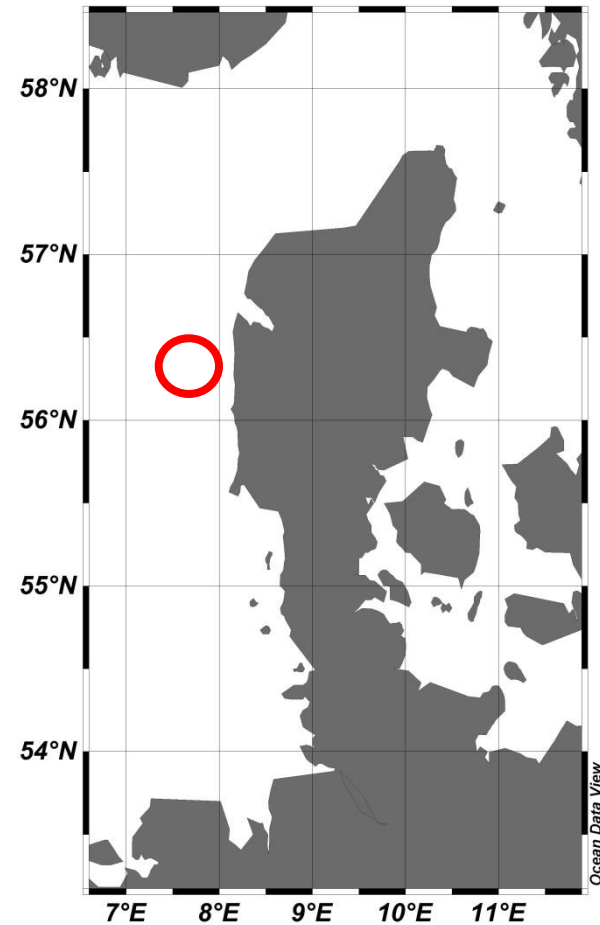
2. *Azadinium poporum* C5 North Sea



A. poporum C5 North Sea

Negative for known AZAs

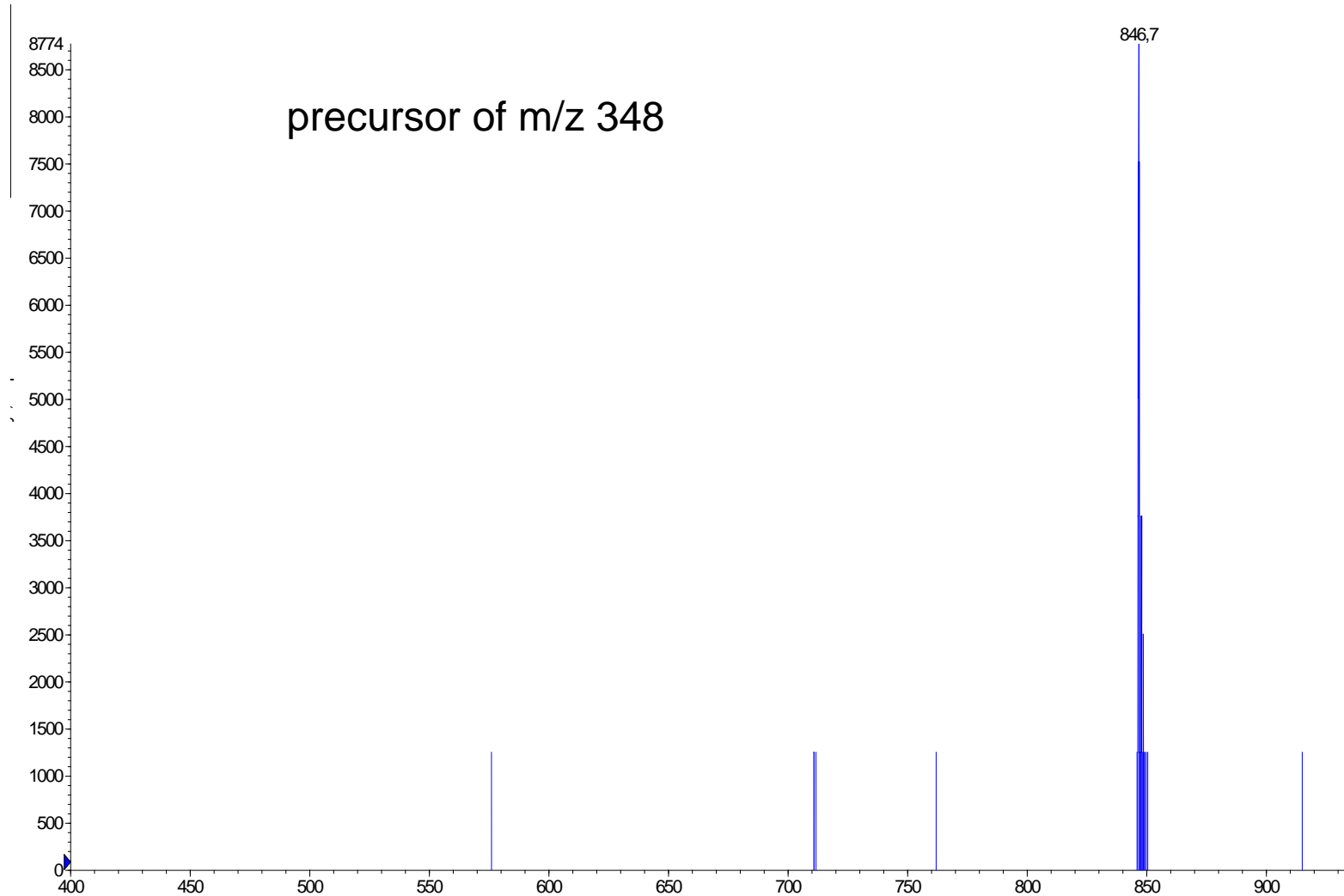
Tillmann et al. 2011. *Eur. J. Phycol.*, **46**, 74 - 87.



Danish Coast at
56° 14.52' N, 07° 27.54' E

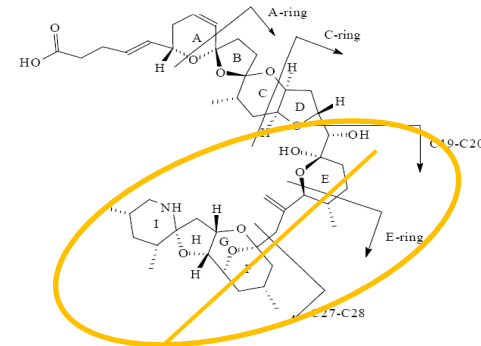


2. *Azadinium poporum* C5 North Sea





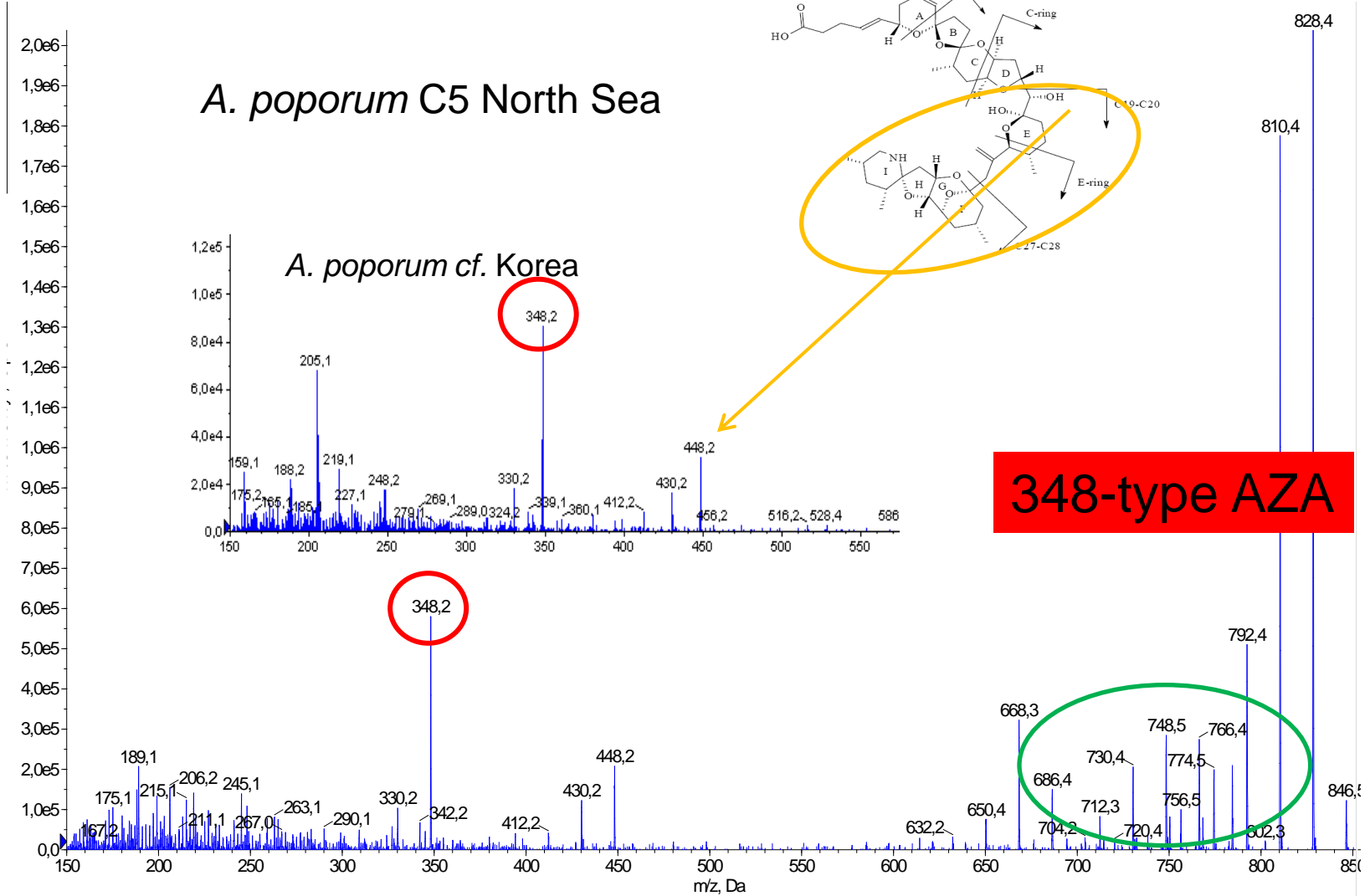
2. *Azadinium poporum* C5 North Sea



A. poporum C5 North Sea

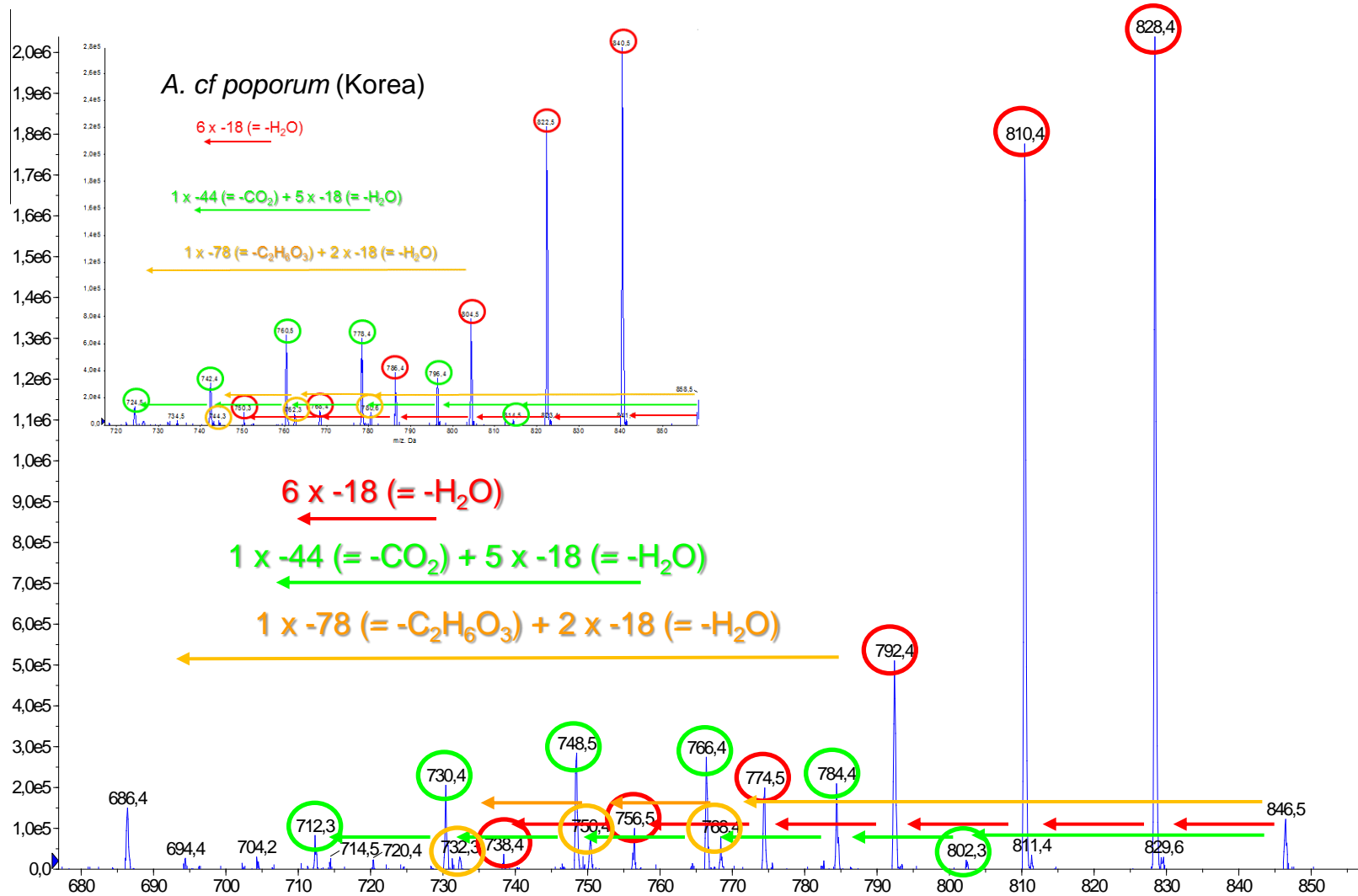
A. poporum cf. Korea

348-type AZA





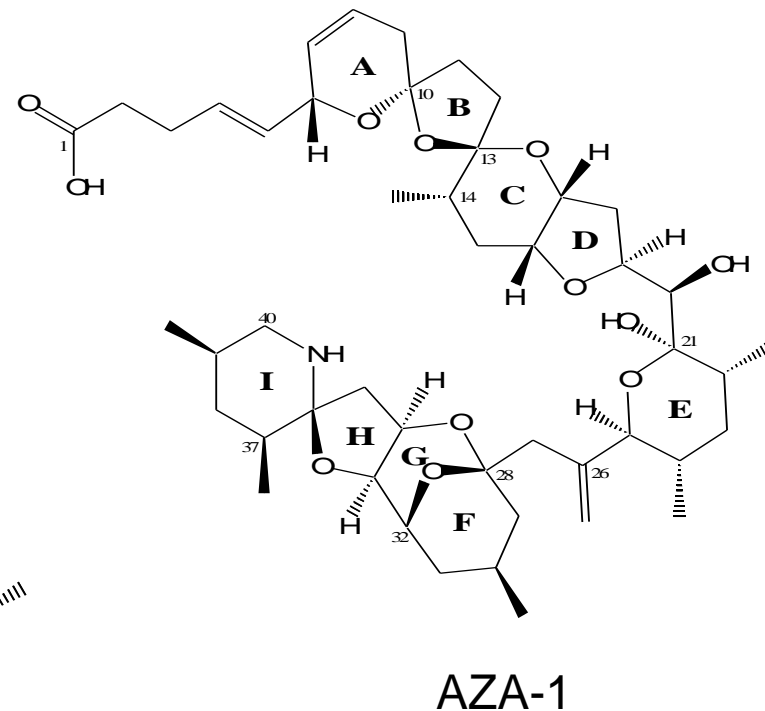
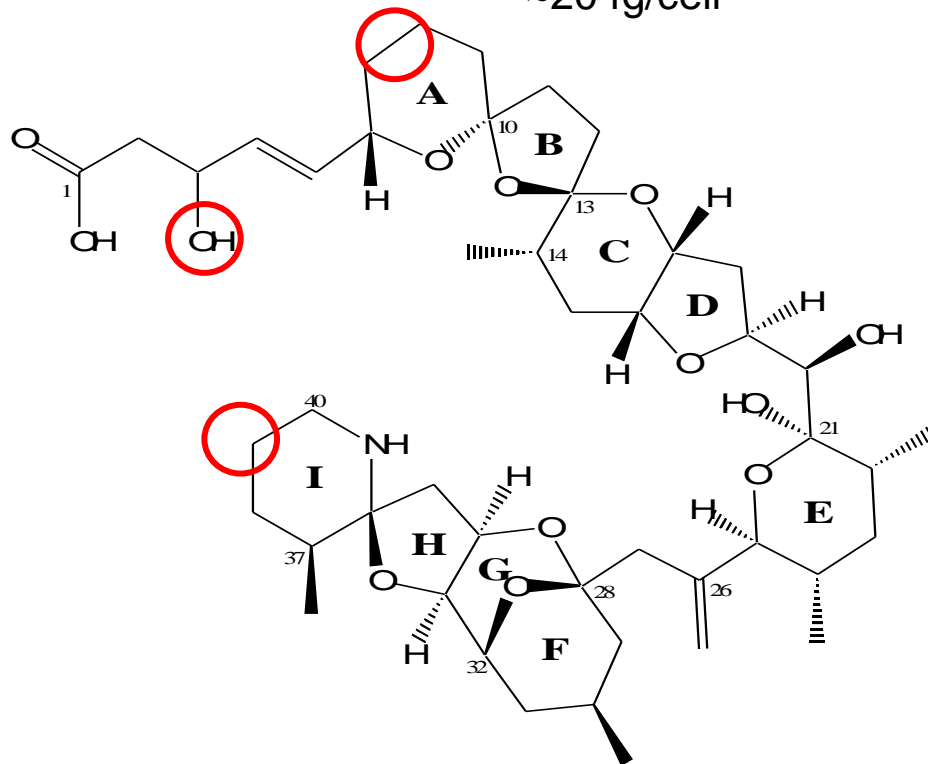
2. *Azadinium poporum* C5 North Sea





2. *Azadinium poporum* C5 North Sea

Toxin cell quota:
~20 fg/cell



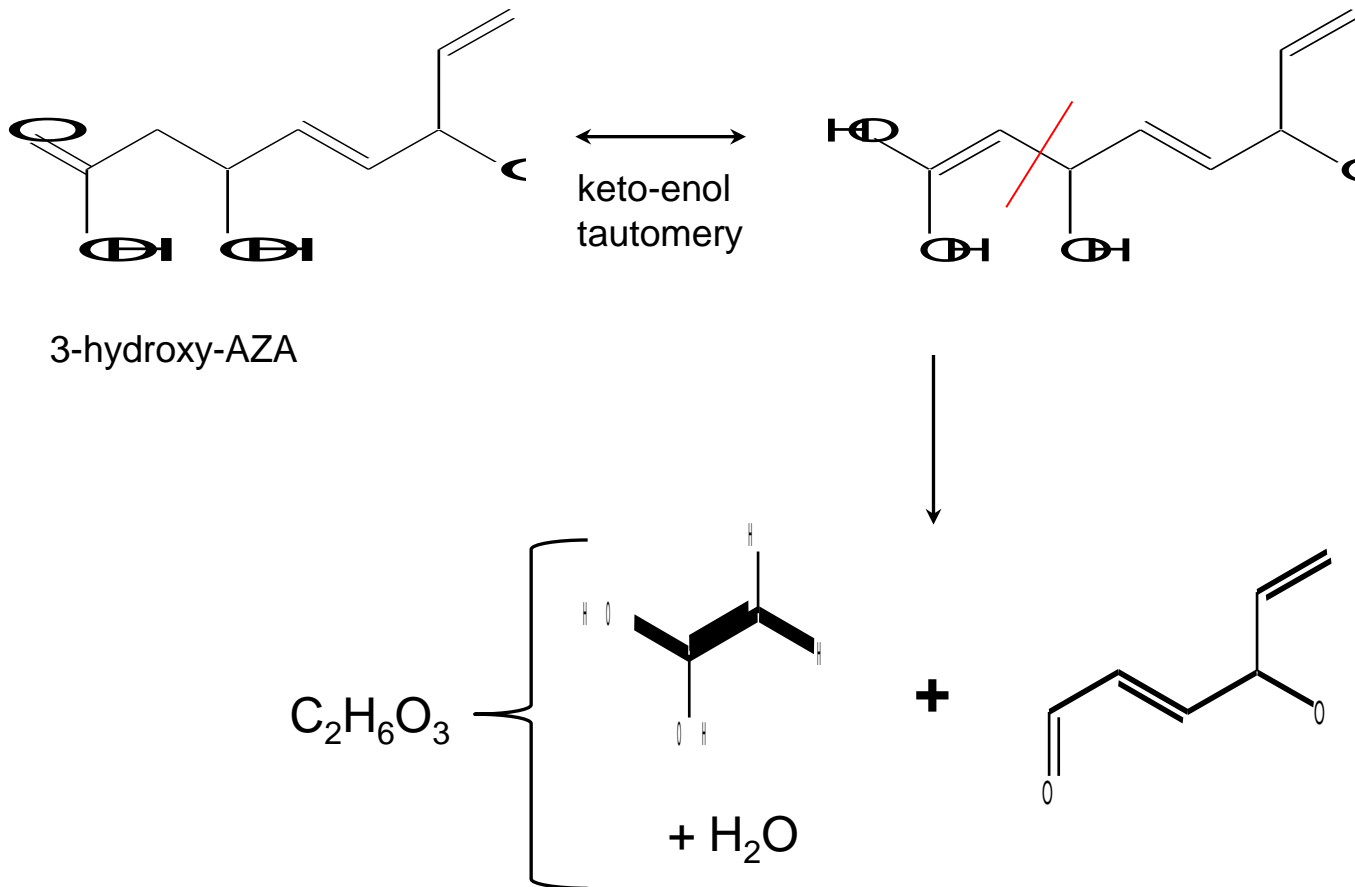
AZA-846; structure (without stereochemistry) elucidated by NMR
(Krock et al. in preparation)

AZA-846: 39-desmethyl-7,8-dihydro-3-hydroxy-AZA-1



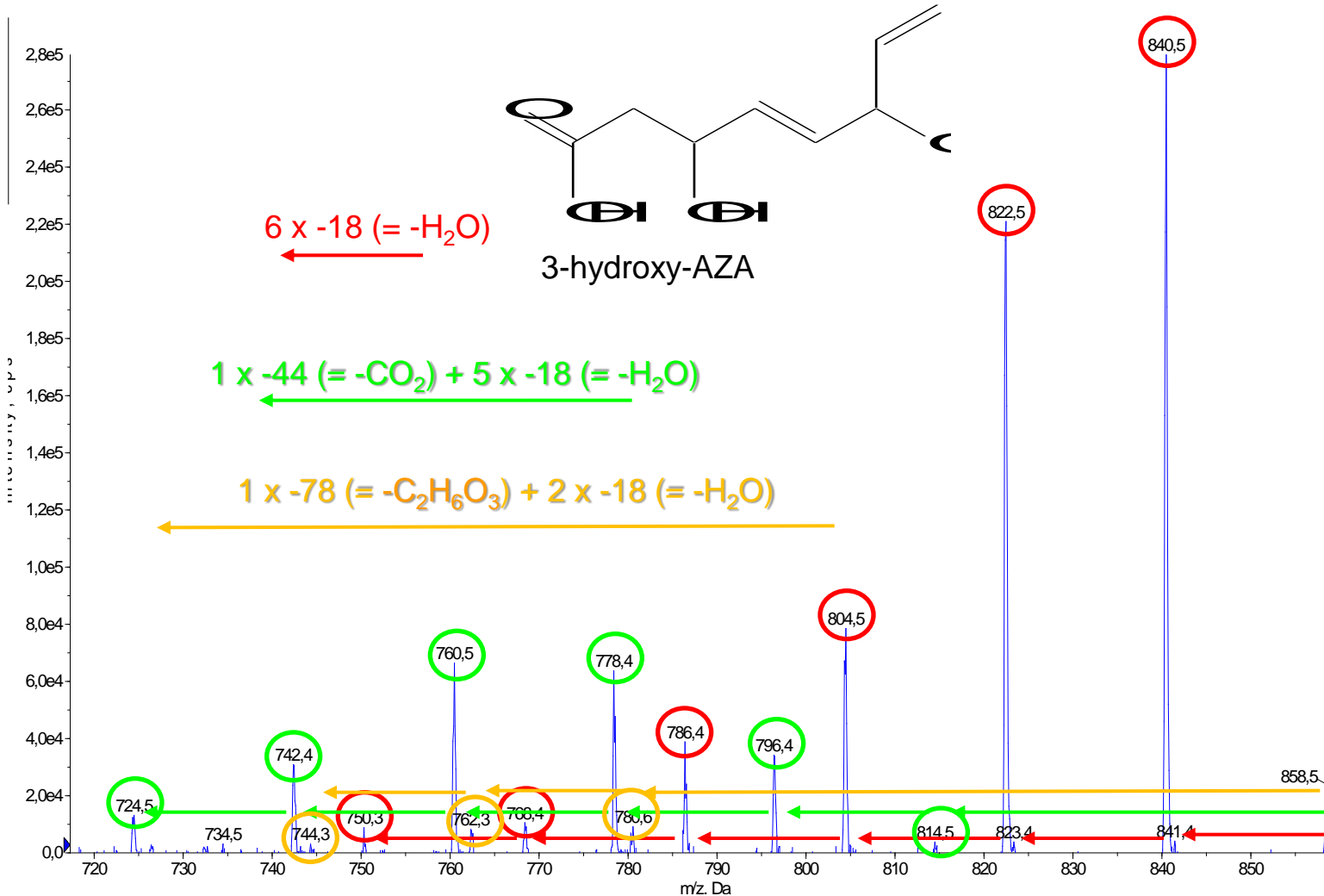
2. Azadinium poporum C5 North Sea

Fragmentation pattern for the cleavage of m/z 78 ($= C_2H_6O_3$)





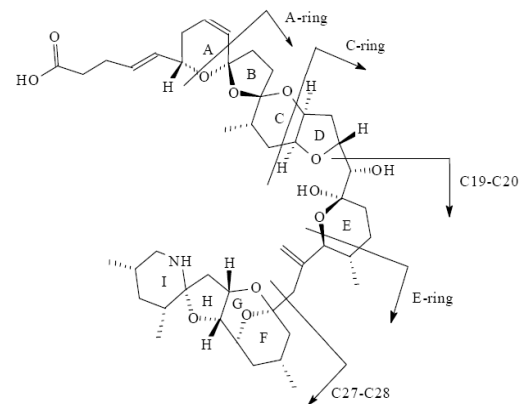
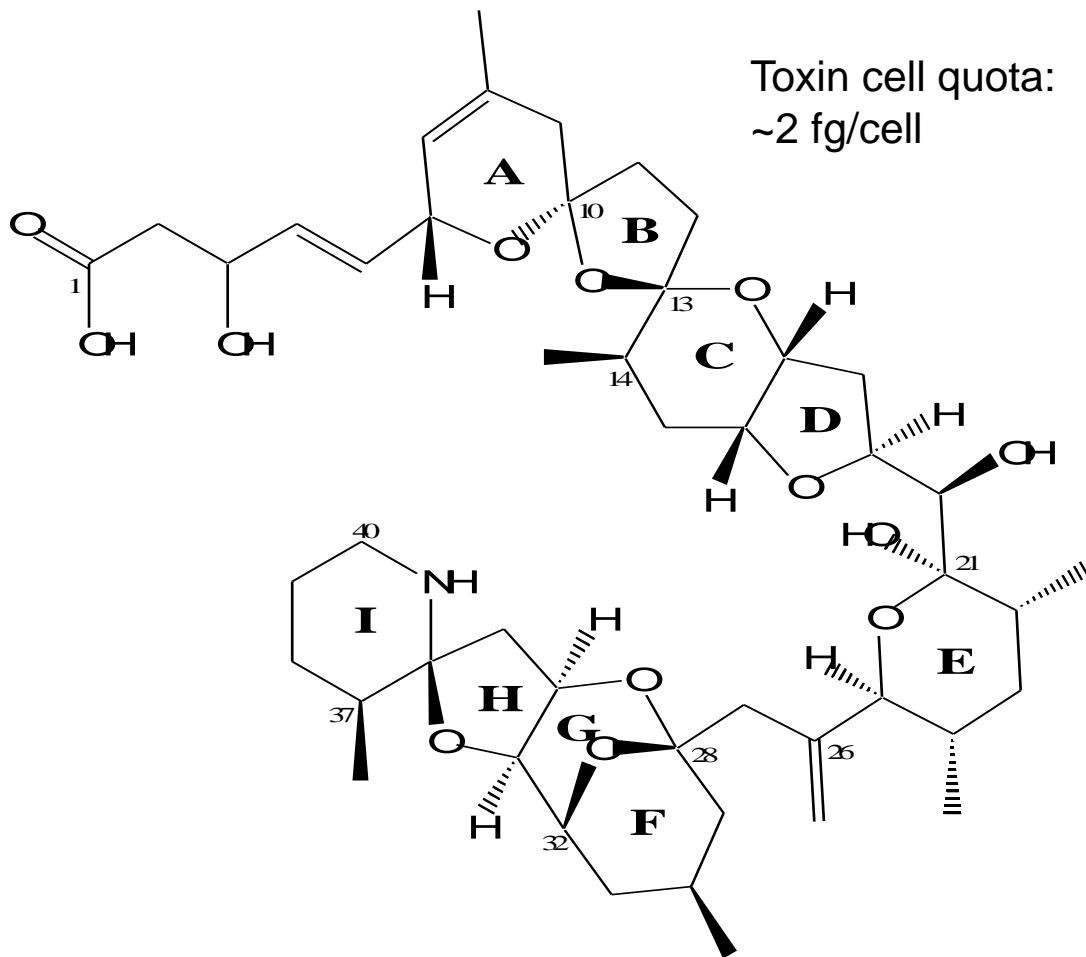
1. *Azadinium cf. poporum* Korea





1. *Azadinium cf. poporum* Korea

Toxin cell quota:
~2 fg/cell



AZA-1

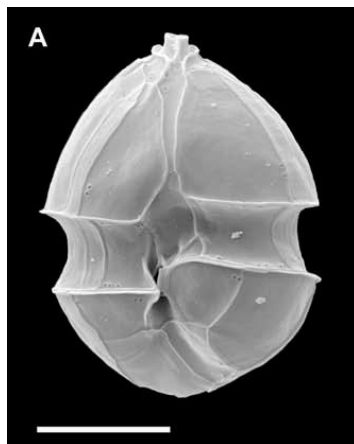
sum formulas
as determined by HRMS:



AZA-858 = 39-desmethyl-3-hydroxy-AZA-2

Structure confirmed by NMR

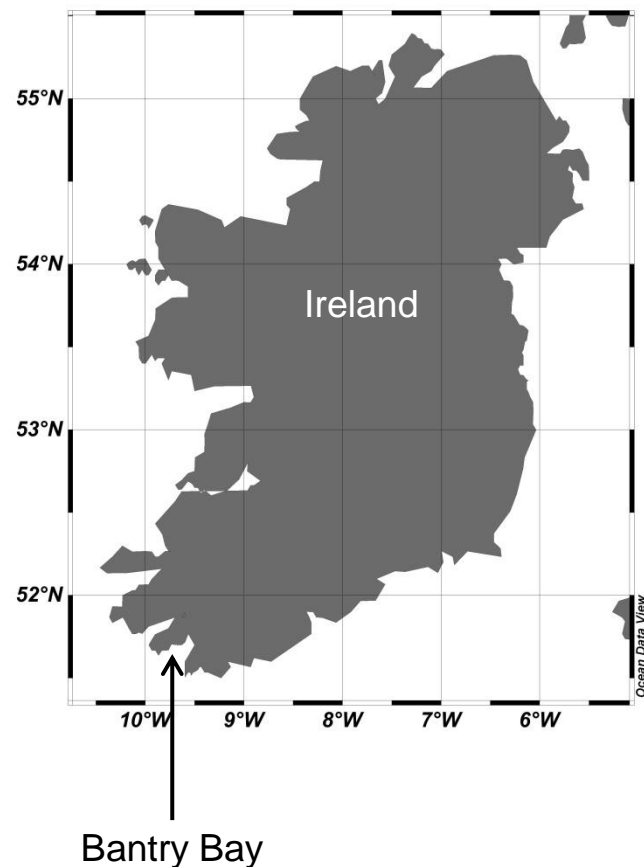
3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland



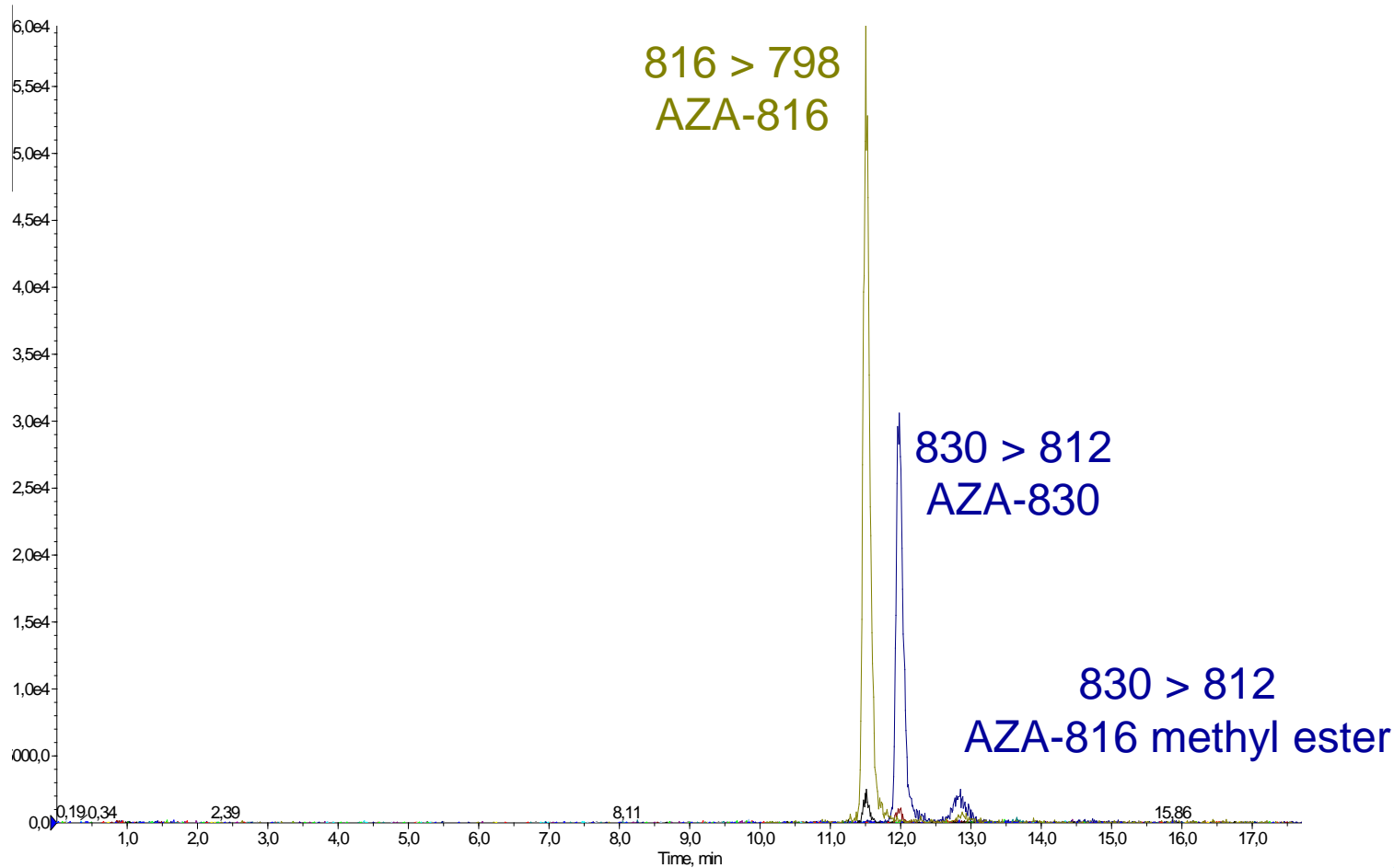
Bantry Bay

51° 39' 4.7" N, 9° 35' 11" E

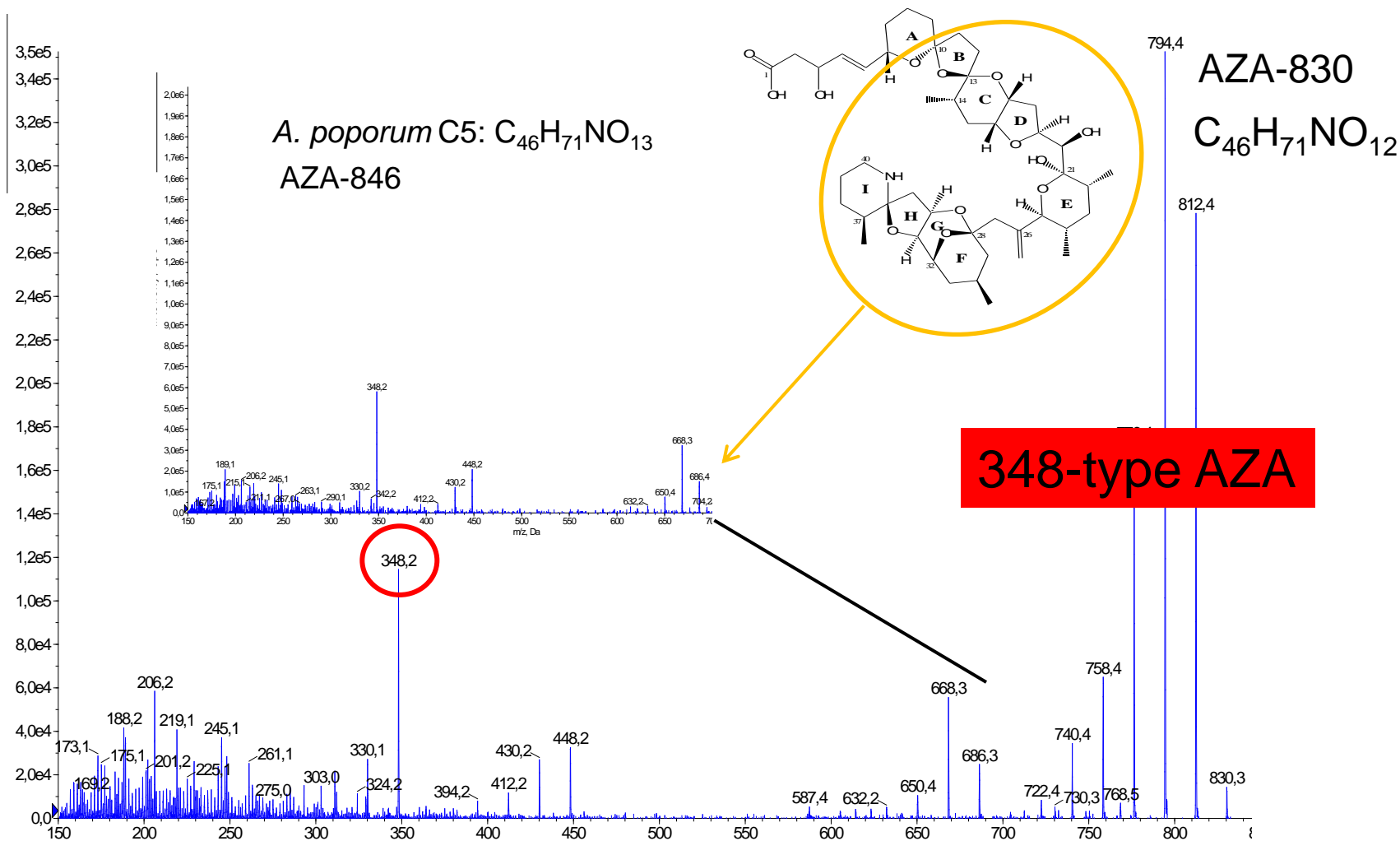
Tillmann U. et al. 2012 *Protist* 163, 701-719.



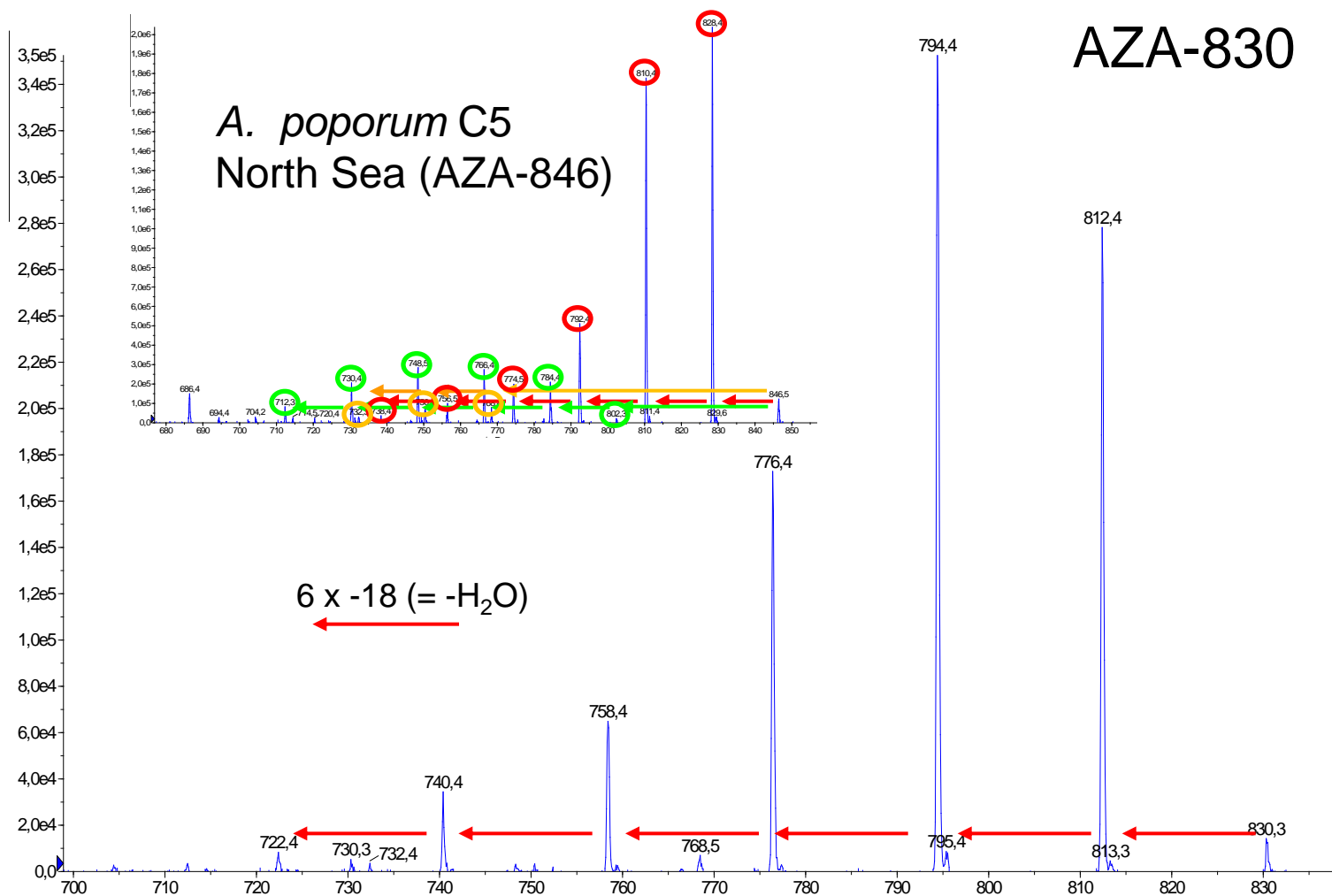
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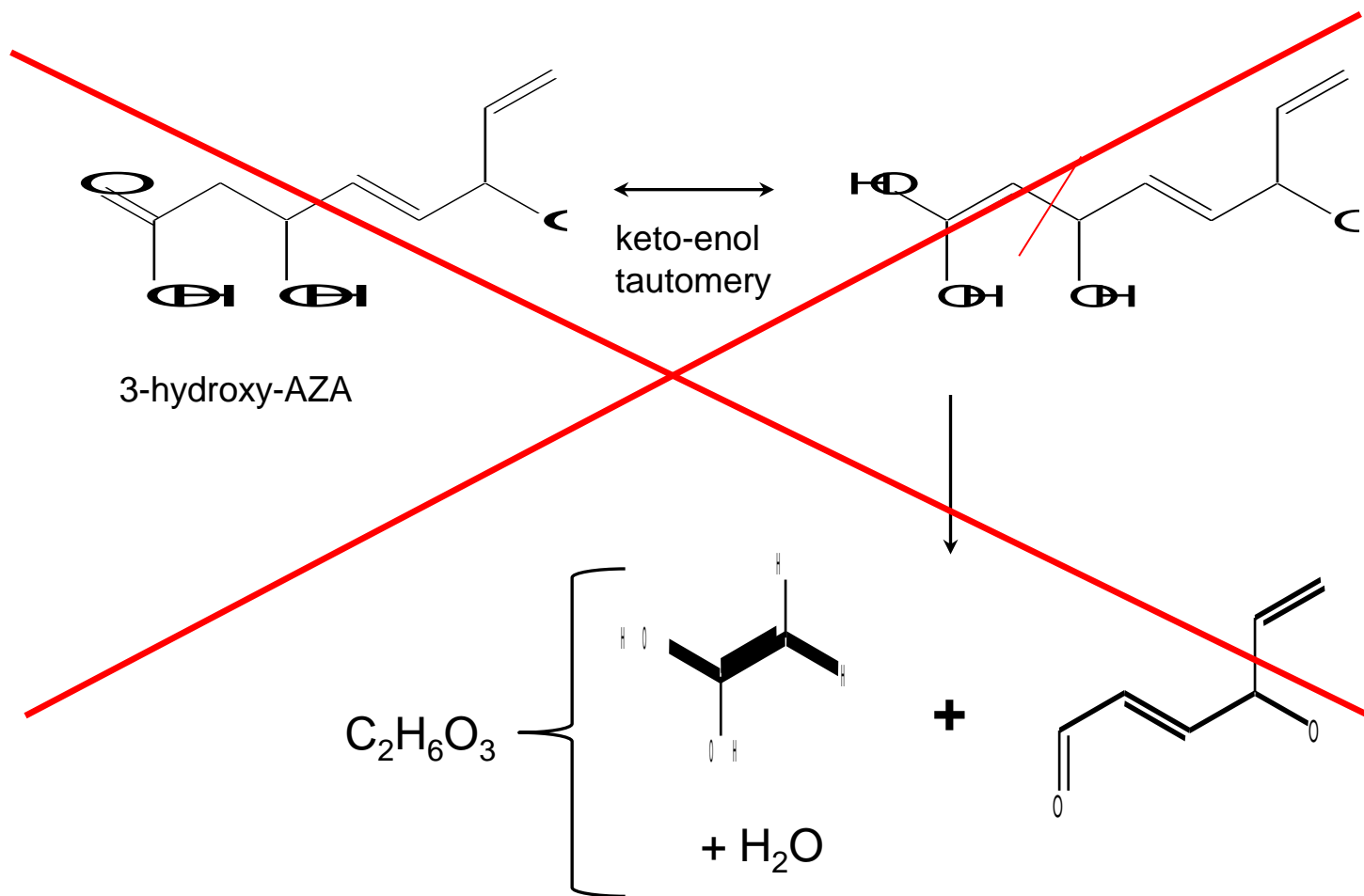


4. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

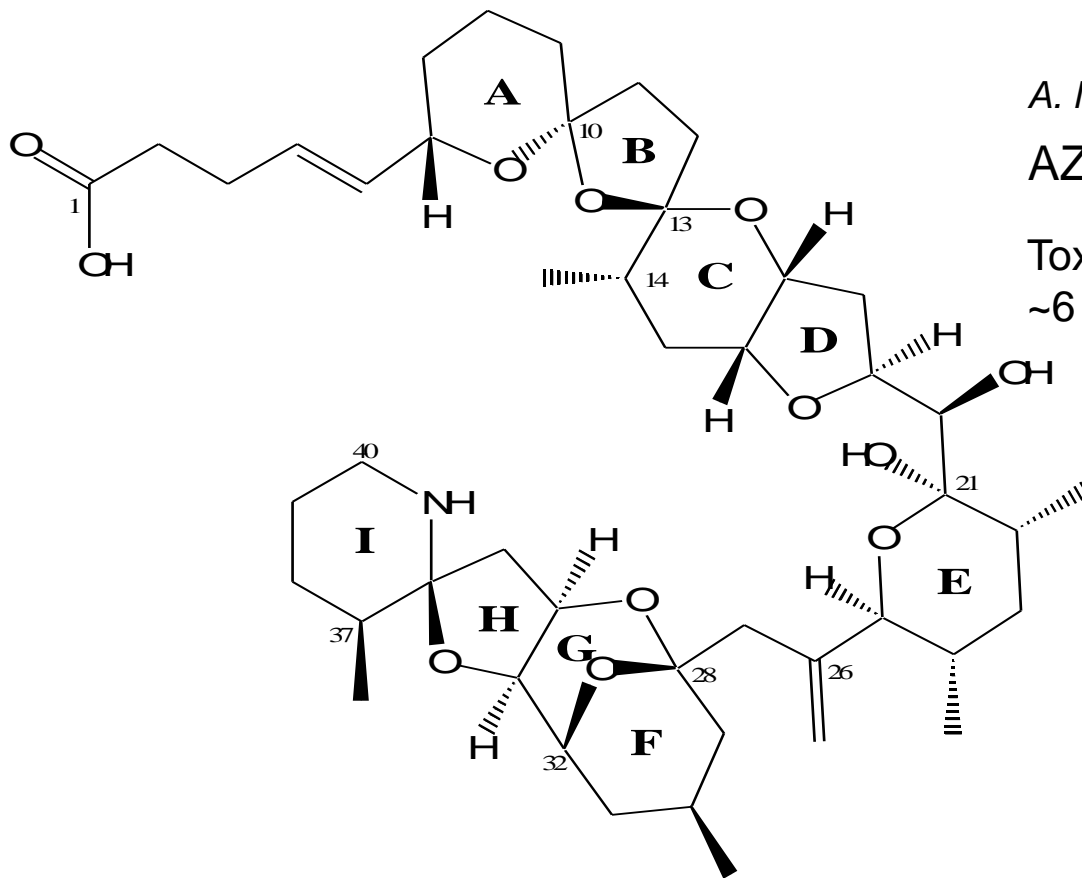


4. *Amphidoma languida* sp. nov., Bantry Bay, Ireland

Fragmentation pattern for the cleavage of m/z 78 ($= C_2H_6O_3$)



4. *Amphidoma languida* sp. nov., Bantry Bay, Ireland



A. languida:

AZA-830

Toxin cell quota:
~6 fg/cell

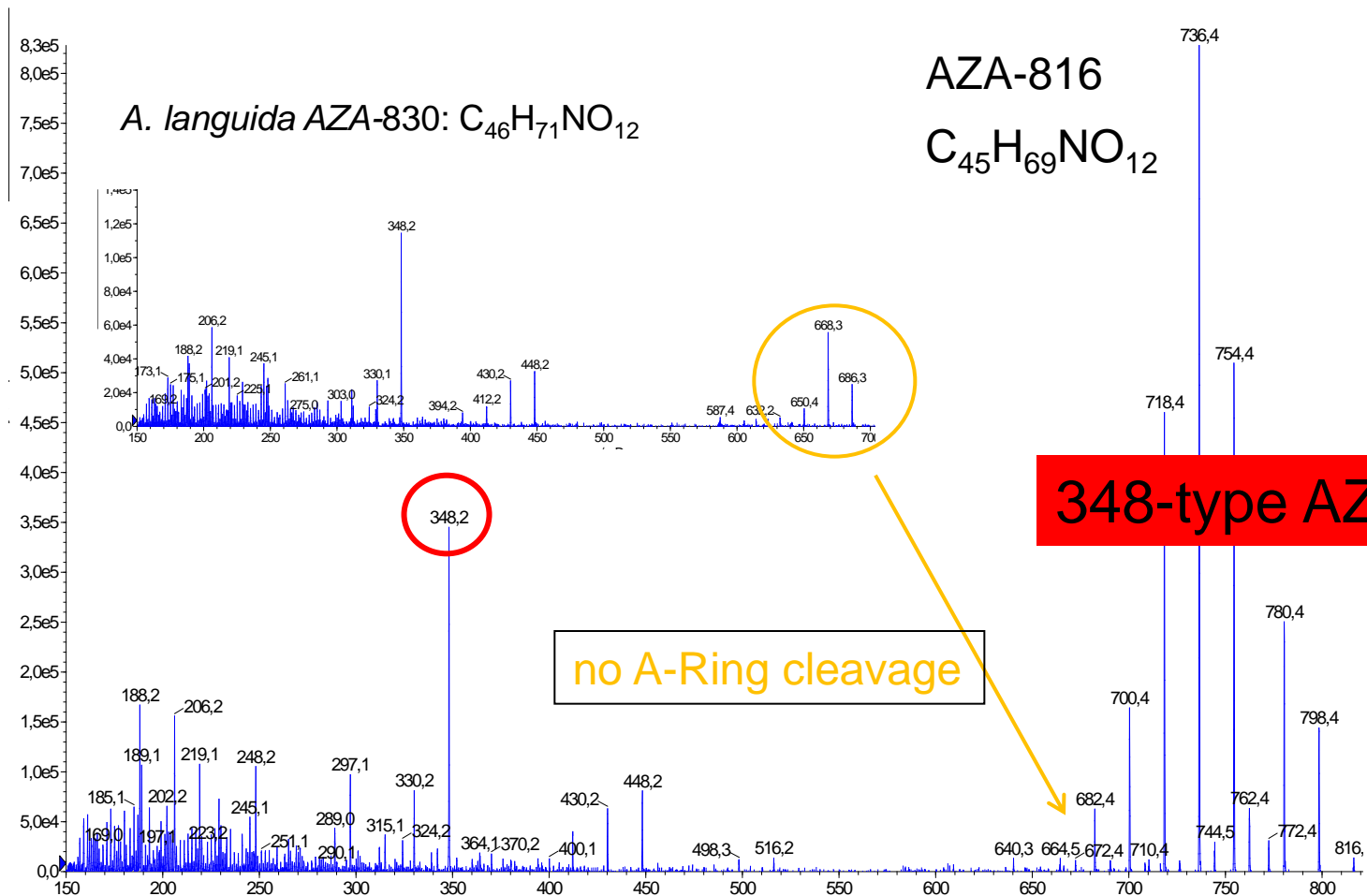
sum formulas
as determined by HRMS:

AZA-1:	$C_{47}H_{71}NO_{12}$
AZA-846:	$C_{46}H_{71}NO_{13}$
AZA-830:	$C_{46}H_{71}NO_{12}$

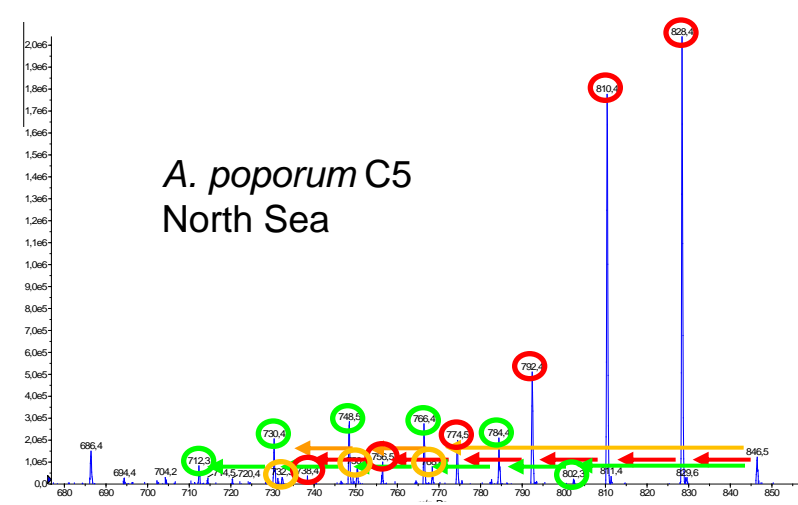
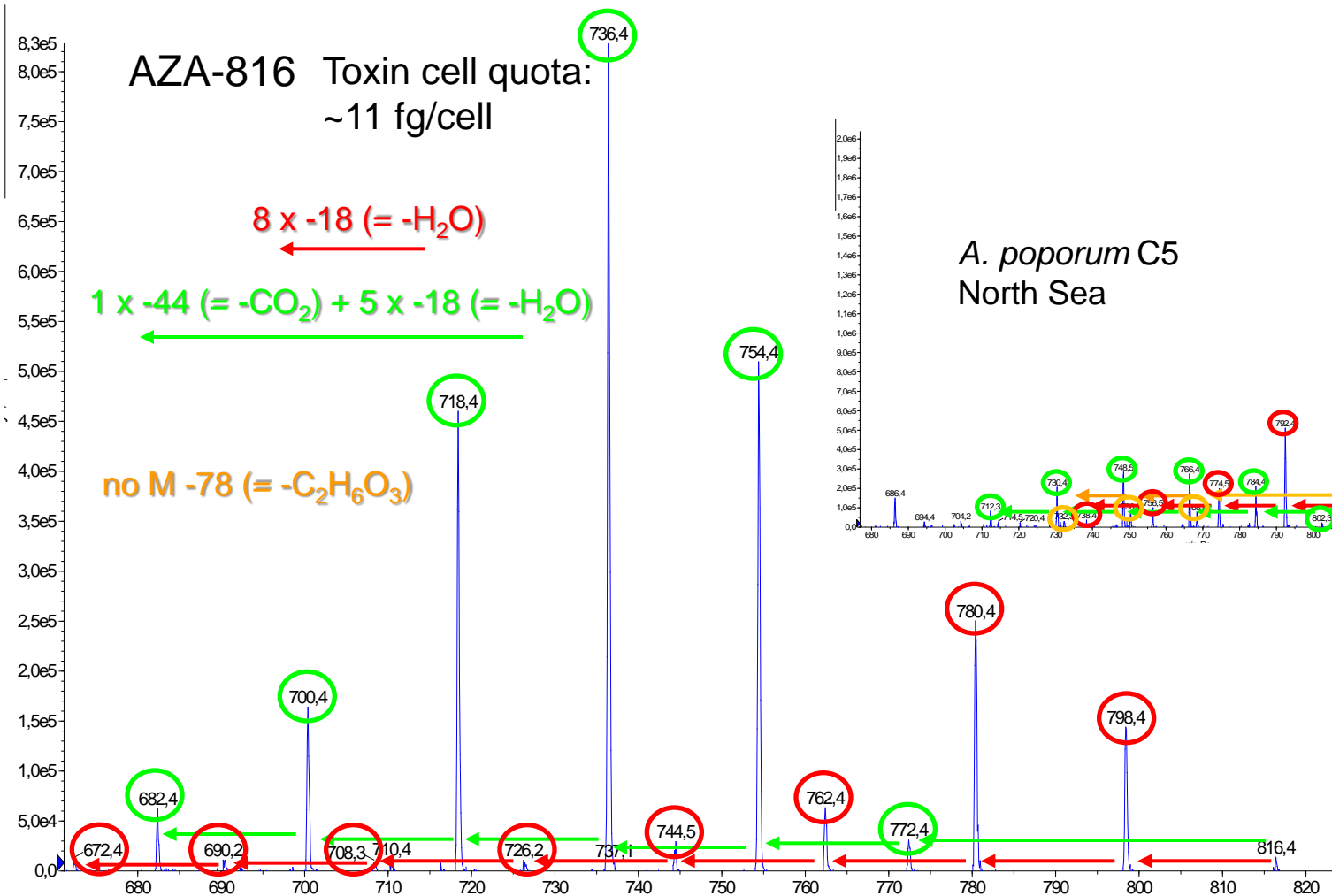
AZA-830 = 39-desmethyl-7,8-dihydro-AZA-1

hypothesized structure!

3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland



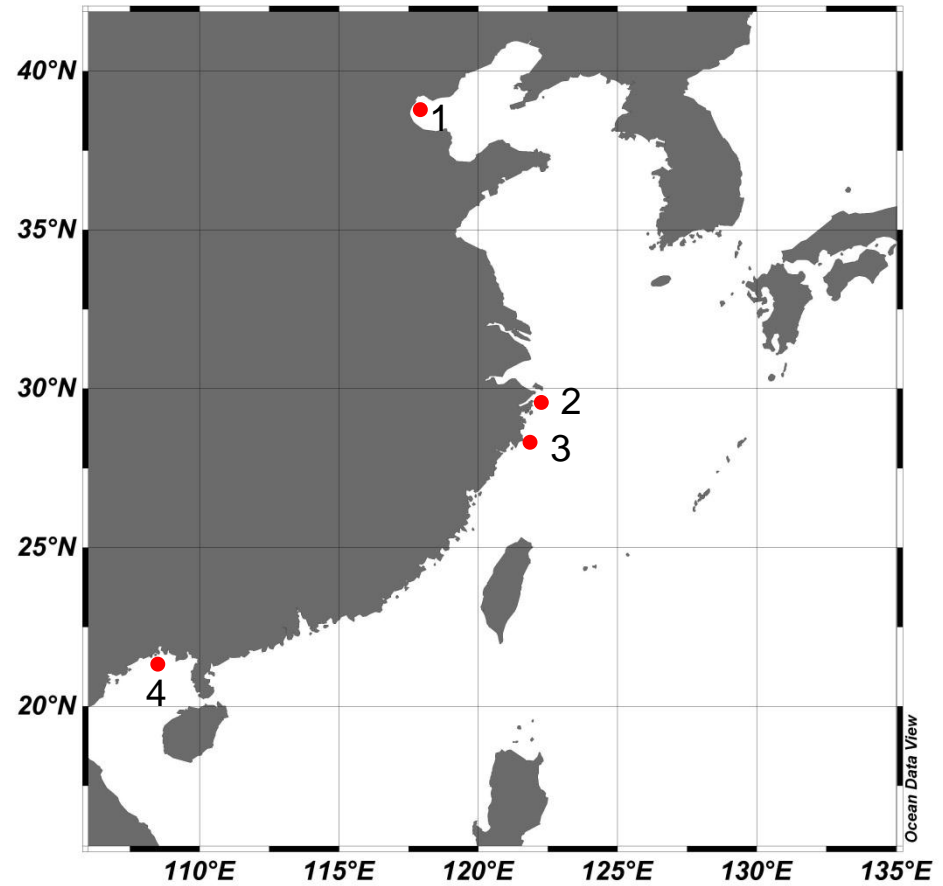
3. *Amphidoma languida* sp. nov., Bantry Bay, Ireland



Structure: ?



4. *Azadinium poporum*, China



Gu et al. 2011. *Harmful Algae.*, accepted manuscript.

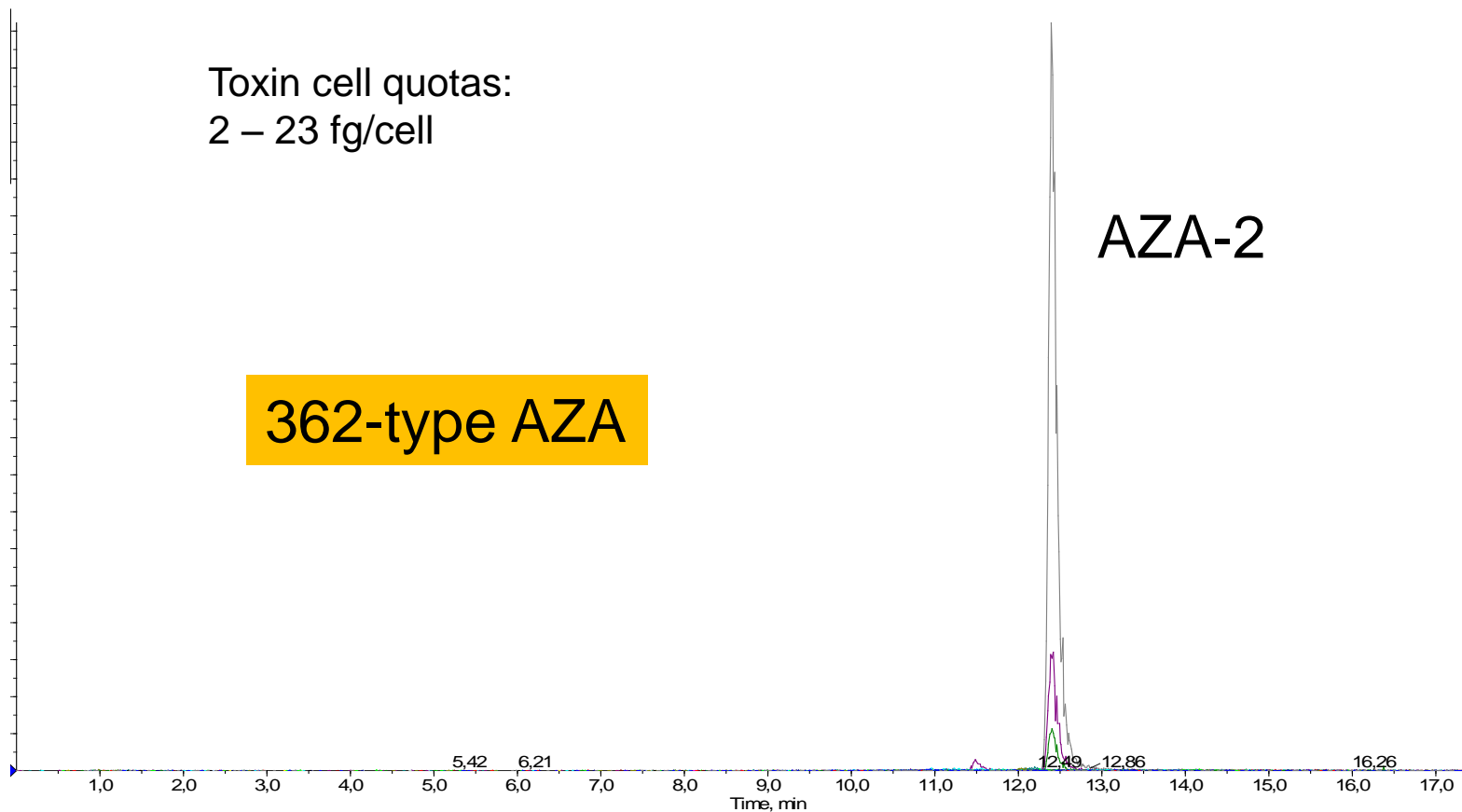


4. *Azadinium poporum* G 42, G 64, G 68, East China Sea, China

Toxin cell quotas:
2 – 23 fg/cell

362-type AZA

AZA-2



4. *Azadinium poporum* G 42, G 64, G 68, East China Sea, China

Toxicon 53 (2009) 680–684



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Isolation of azaspiracid-2 from a marine sponge *Echinoclathria* sp. as a potent cytotoxin

Reiko Ueoka^a, Akihiro Ito^b, Miho Izumikawa^c, Satoko Maeda^b, Motoki Takagi^c,
Kazuo Shin-ya^c, Minoru Yoshida^b, Rob. W.M. van Soest^d, Shigeki Matsunaga^{a,*}

^a Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

^b Chemical Genomics Research Group, RIKEN, Wako, Saitama 351-0198, Japan

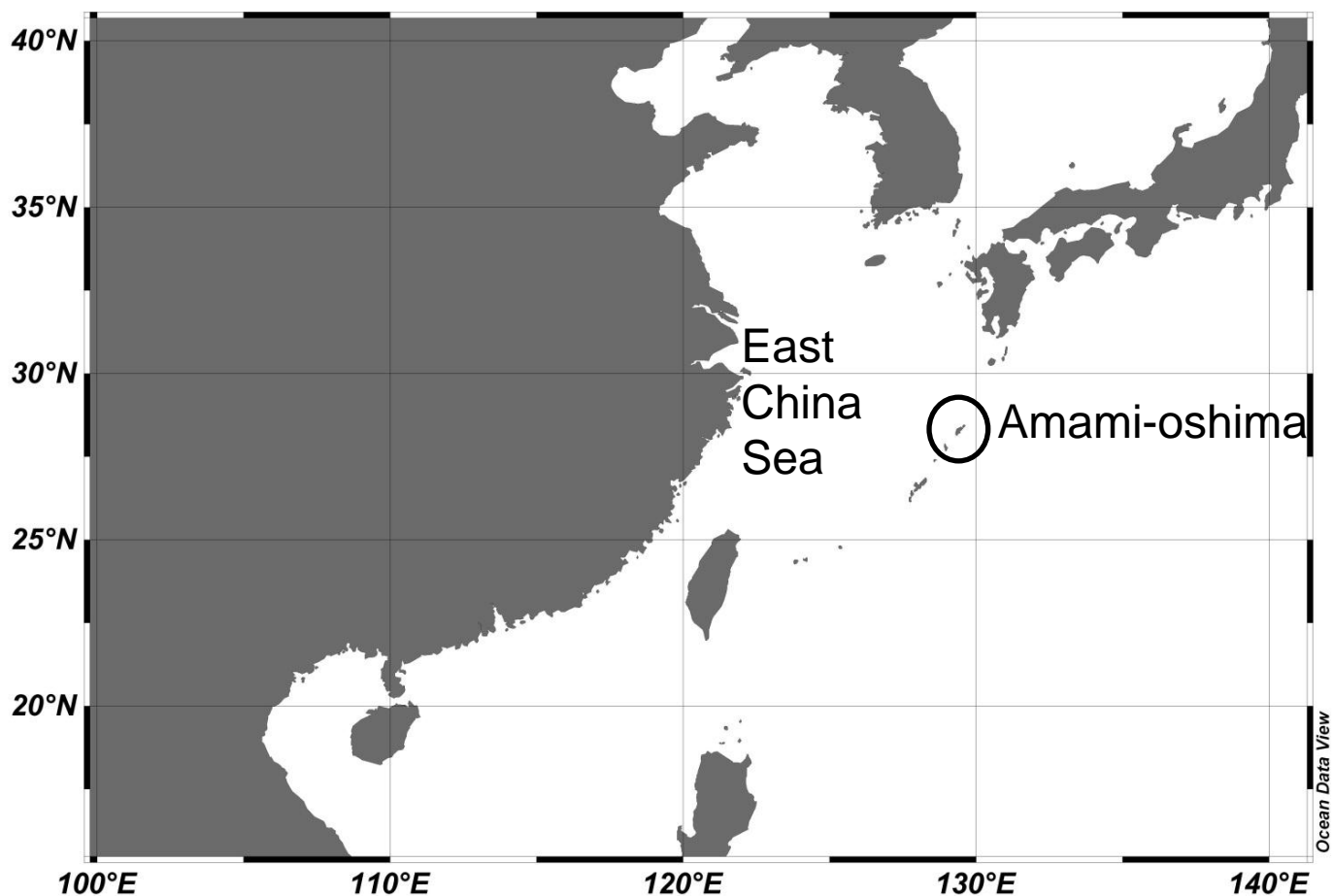
^c Biomedical Information Research Center, National Institute of Advanced Industrial Science and Technology, Koto-ku, Tokyo 135-0064, Japan

^d Zoological Museum, University of Amsterdam, 1090GT Amsterdam, The Netherlands

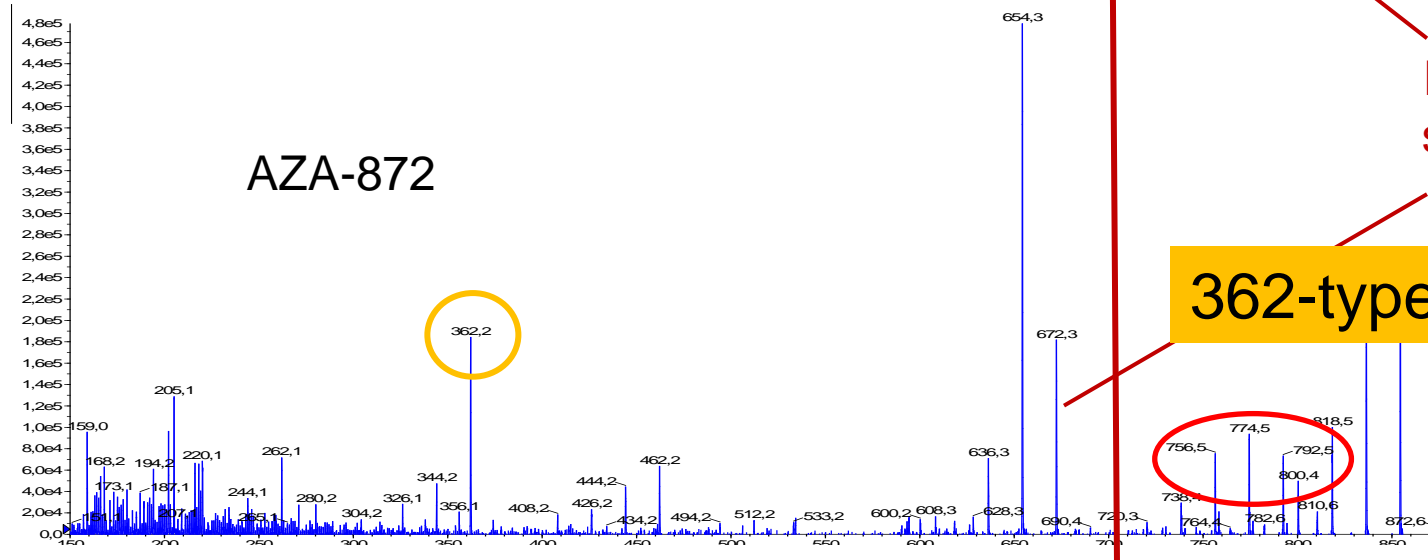
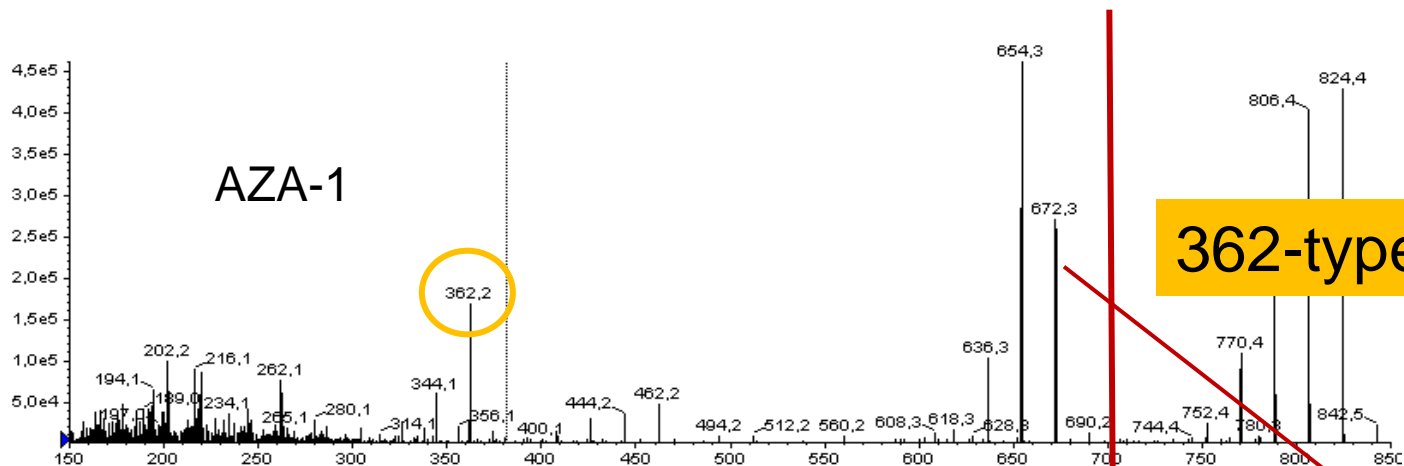


4. *Azadinium poporum* G 42, G 64, G 68, East China Sea, China

A. pororum strains G 42, G 64 and G 68 are probably the source of the sponge contamination with AZA-2



5. *Azadinium poporum* G 66, East China Sea, China



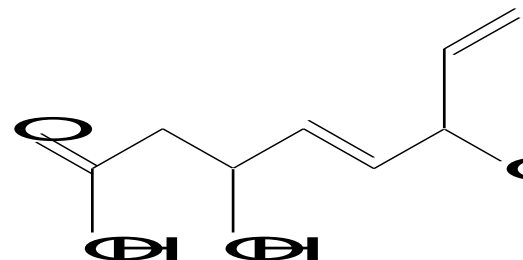
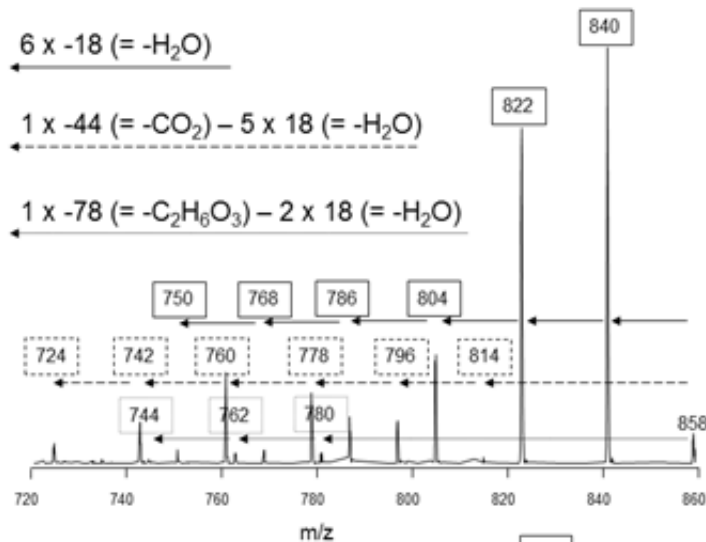
362-type AZA

362-type AZA

Identical CID spectra up to $m/z = 672$

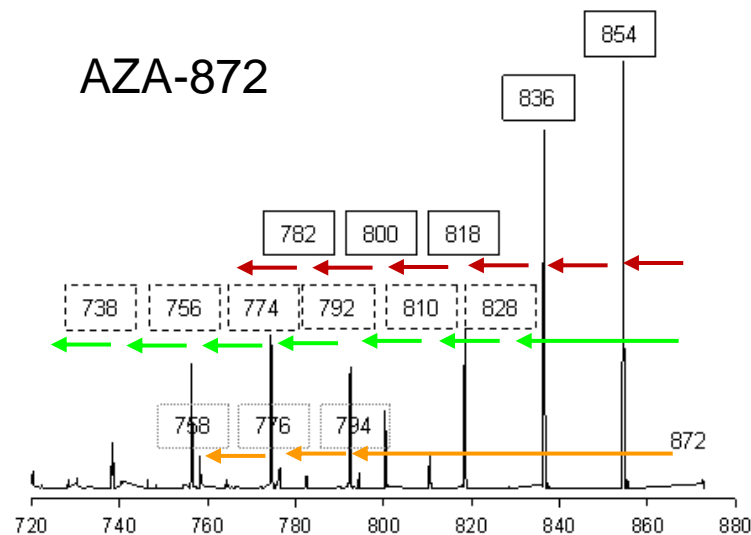
5. *Azadinium poporum* G 66, East China Sea, China

AZA-858

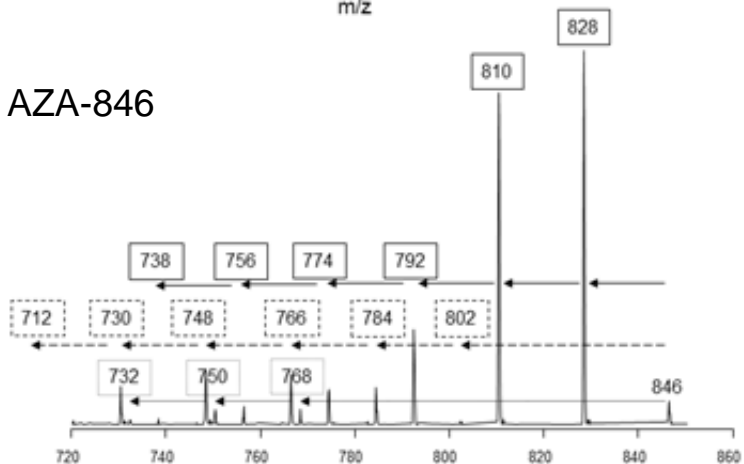


3-hydroxy-AZA

AZA-872

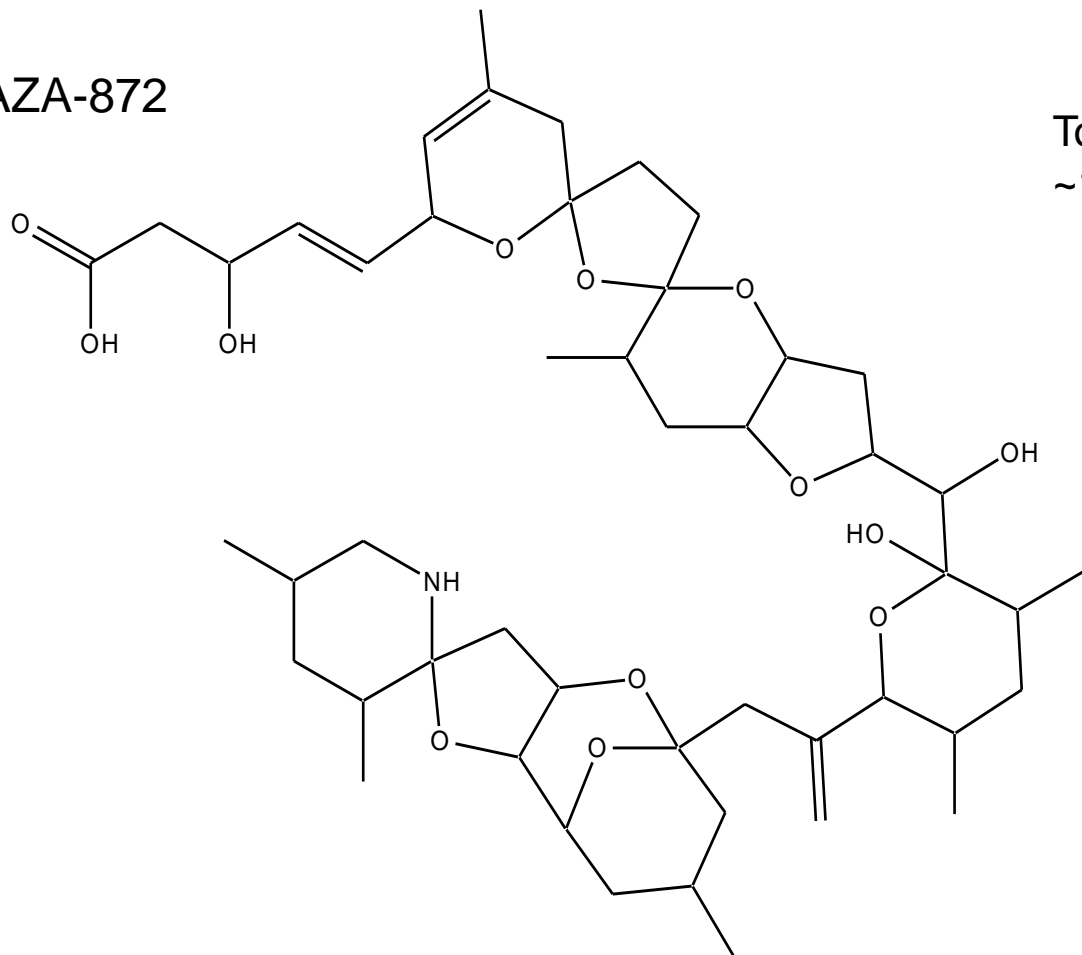


AZA-846



5. *Azadinium poporum* G 66, East China Sea, China

AZA-872



Toxin cell quota:
~1.5 fg/cell

sum formulas
as determined by HRMS:

AZA-1: $C_{47}H_{71}NO_{12}$

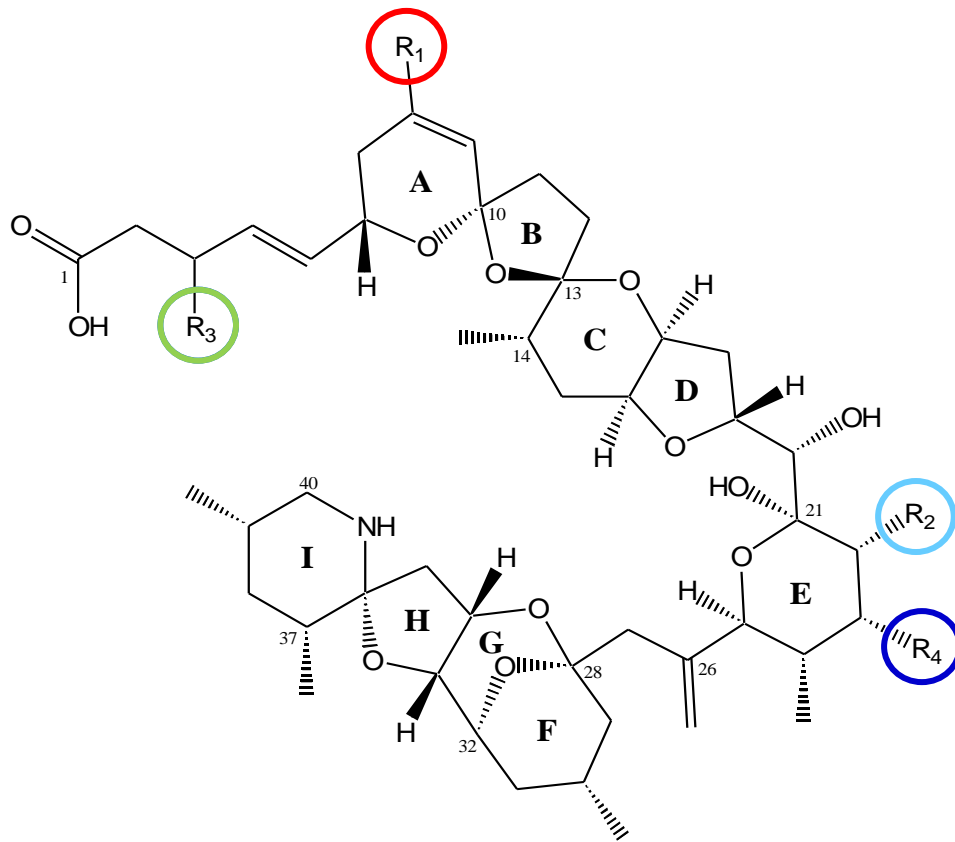
AZA-872: $C_{48}H_{73}NO_{13}$

AZA-872 = 3-hydroxy-8-methyl-AZA-1 = **AZA-11**

confirmed by retention time and CID spectra comparison



introduction: structural variants

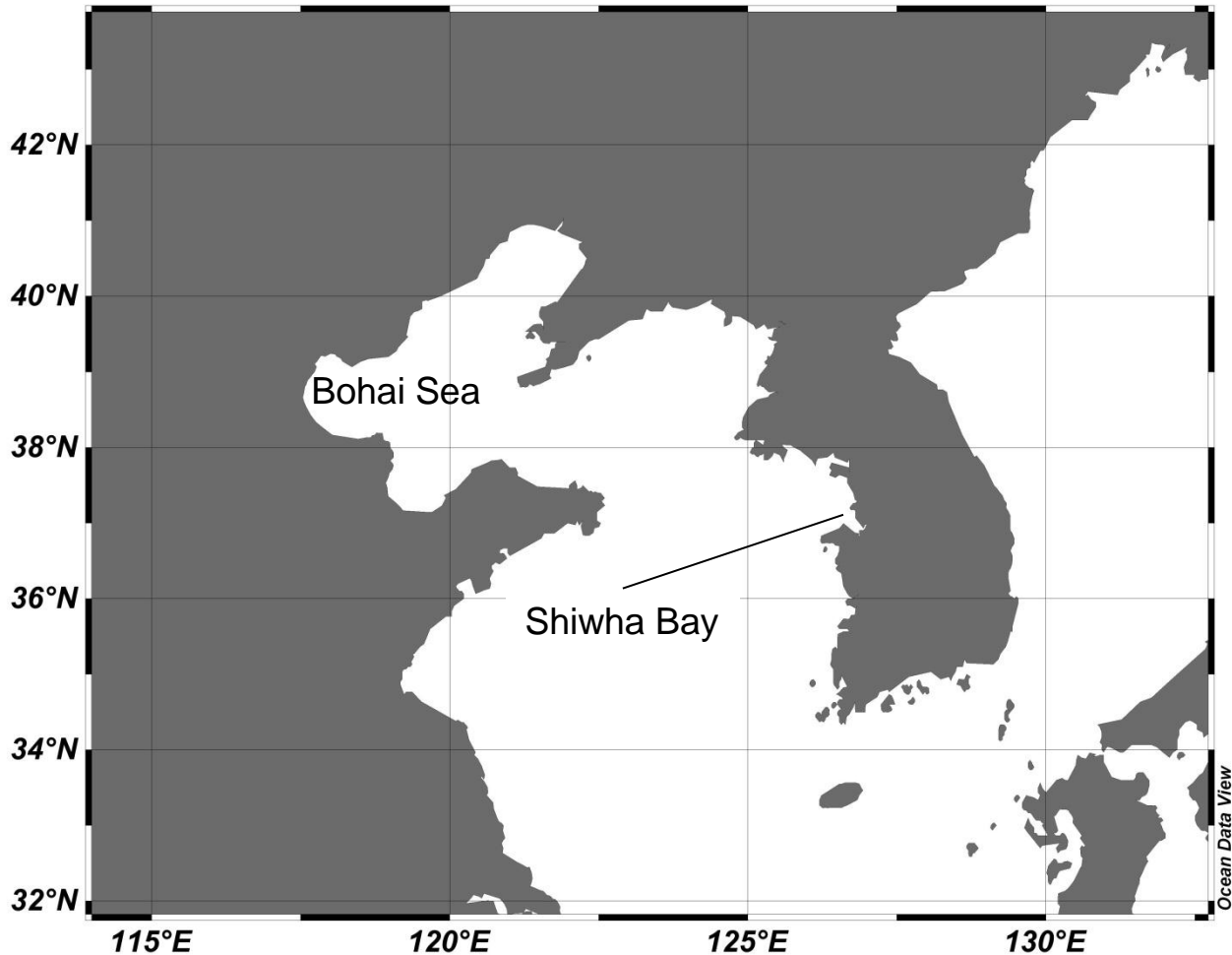


Toxin	R ₁	R ₂	R ₃	R ₄	[M+H] ⁺
AZA-1	H	CH ₃	H	H	842
AZA-2	CH ₃	CH ₃	H	H	856
AZA-3	H	H	H	H	828
AZA-4	H	H	OH	H	844
AZA-5	H	H	H	OH	844
AZA-6	CH ₃	H	H	H	842
AZA-7	H	CH ₃	OH	H	858
AZA-8	H	CH ₃	H	OH	858
AZA-9	CH ₃	H	OH	H	858
AZA-10	CH ₃	H	H	OH	858
AZA-11	CH ₃	CH ₃	OH	H	872



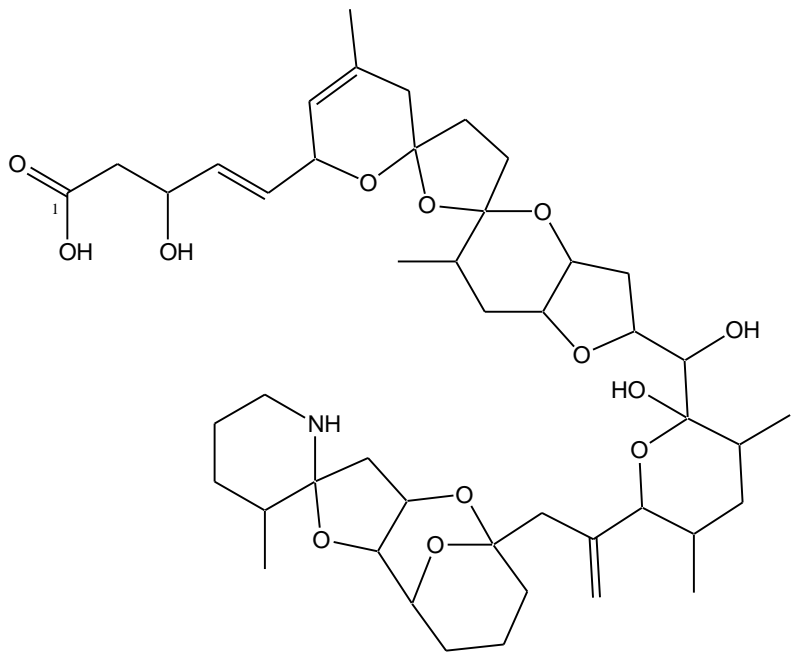
6. *Azadinium poporum* G 25 Bohai Sea, China

AZA-858 producing strains *A. poporum* G25 and *A. cf. poporum* (Korea) are from proximate geographic regions



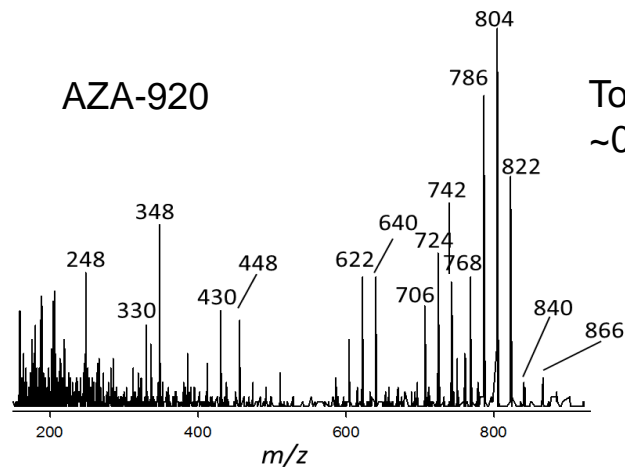


6. *Azadinium poporum* G 25, Bohai Sea, China

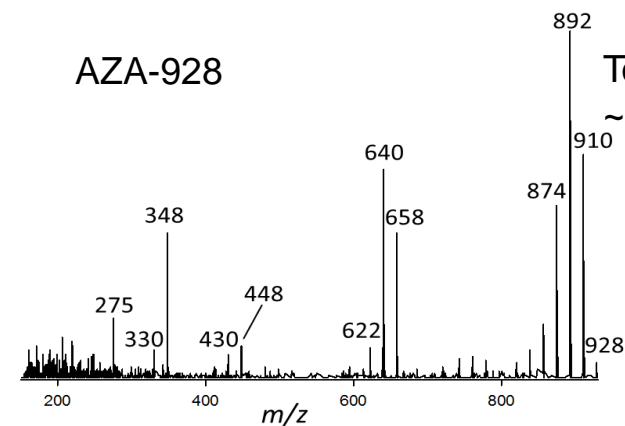


Toxin cell quota:
~1.4 fg/cell

AZA-858



Toxin cell quota:
~0.02 fg/cell



Toxin cell quota:
~0.14 fg/cell



Summary

1. A new 39-desmethyl-AZA-family (348-type AZA) was found
2. 7 new AZAs were detected in *Azadinium poporum* and *Amphidoma languida*
3. The structure of AZA-846 has been fully elucidated by NMR as 39-desmethyl-7,8-dihydro-3-hydroxy-AZA-1
4. *A. poporum* seems to be the species with highest variability in AZA-production

Acknowledgements:

Wolfgang Drebing, AWI

Matthias Witt, Bruker Daltronics

Qun Göthel, Matthias Köck, AWI

Thank you for your attention!