Novel azaspiracids produced by Amphidomataceae

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[2] School of Earth and Envir. Sci., Seoul National Univ., Seoul 151-747, Republic of Korea
[3] Marine Institute, Oranmore, Galway, Ireland
[4] Third Institute of Oceanography, SOA, Xiamen 361005, PR China

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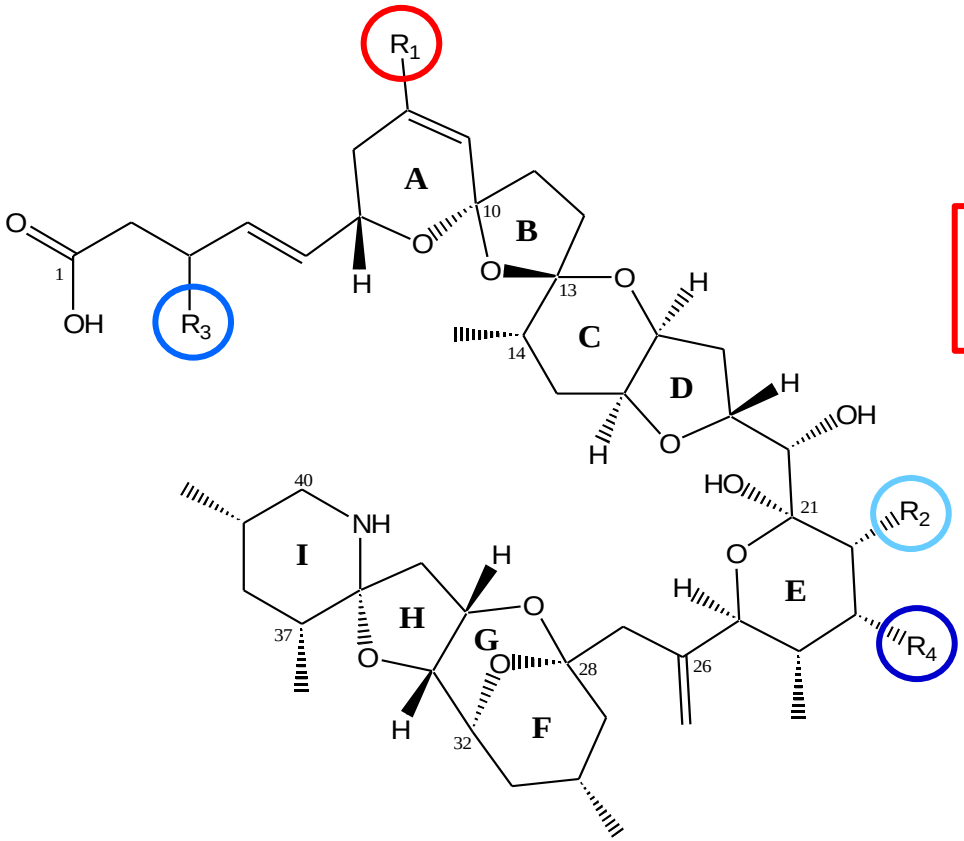
introduction: structural variants



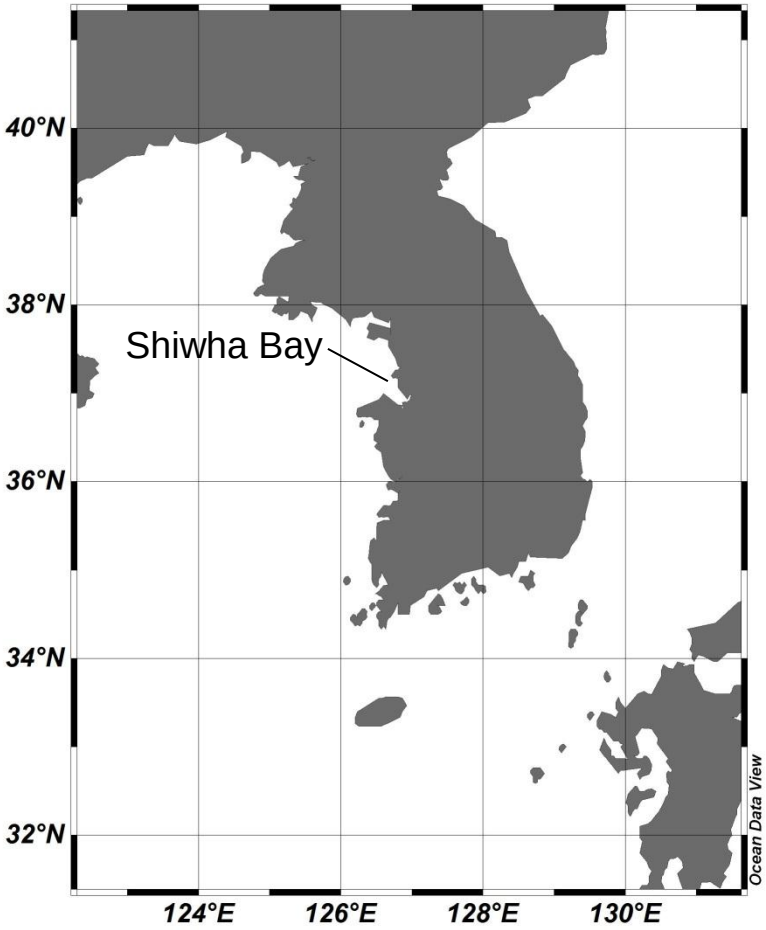
Toxin	R_1	R_2	R_3	R_4	$[M+H]^+$
AZA-1	H	CH_3	H	H	842
AZA-2	CH_3	CH_3	H	H	856
AZA-3	H	H	H	H	828
AZA-4	H	H	OH	H	844
AZA-5	H	H	H	OH	844
AZA-6	CH_3	H	H	H	842
AZA-7	H	CH_3	OH	H	858
AZA-8	H	CH_3	H	OH	858
AZA-9	CH_3	H	OH	H	858
AZA-10	CH_3	H	H	OH	858
AZA-11	CH_3	CH_3	OH	H	872

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*



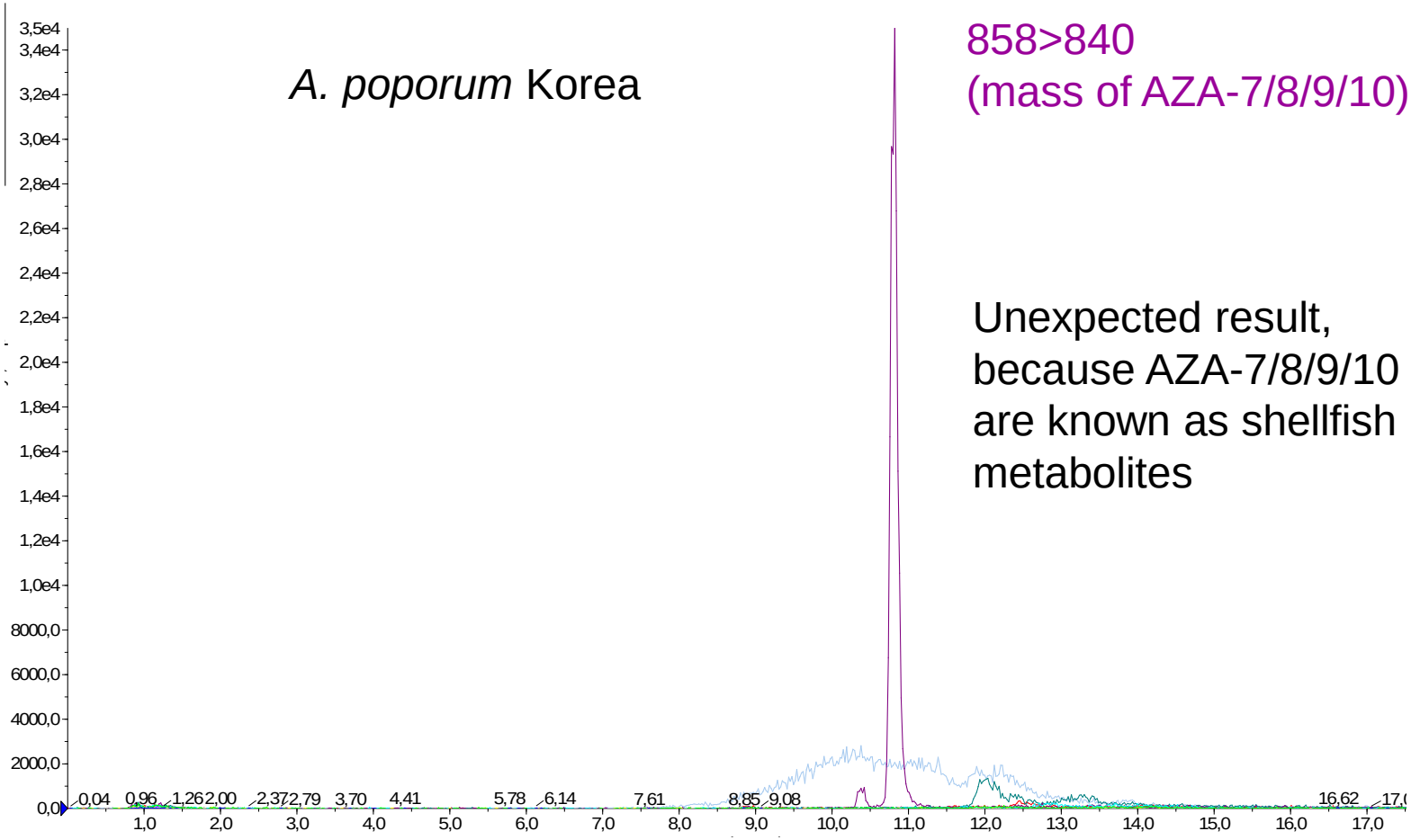
Shiwha Bay (37° 18'N, 126° 36'E)

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

1. *Azadinium* cf. *poporum* Korea

Test for AZAs:

A. poporum Korea



858>840
(mass of AZA-7/8/9/10)

Unexpected result, because AZA-7/8/9/10 are known as shellfish metabolites

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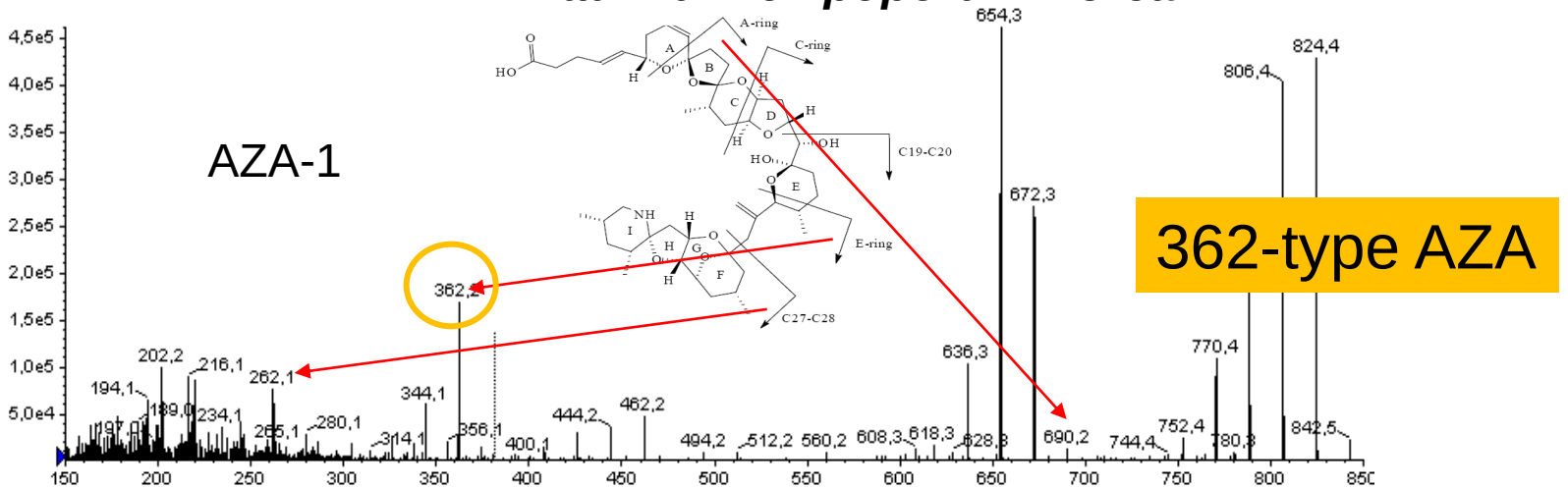
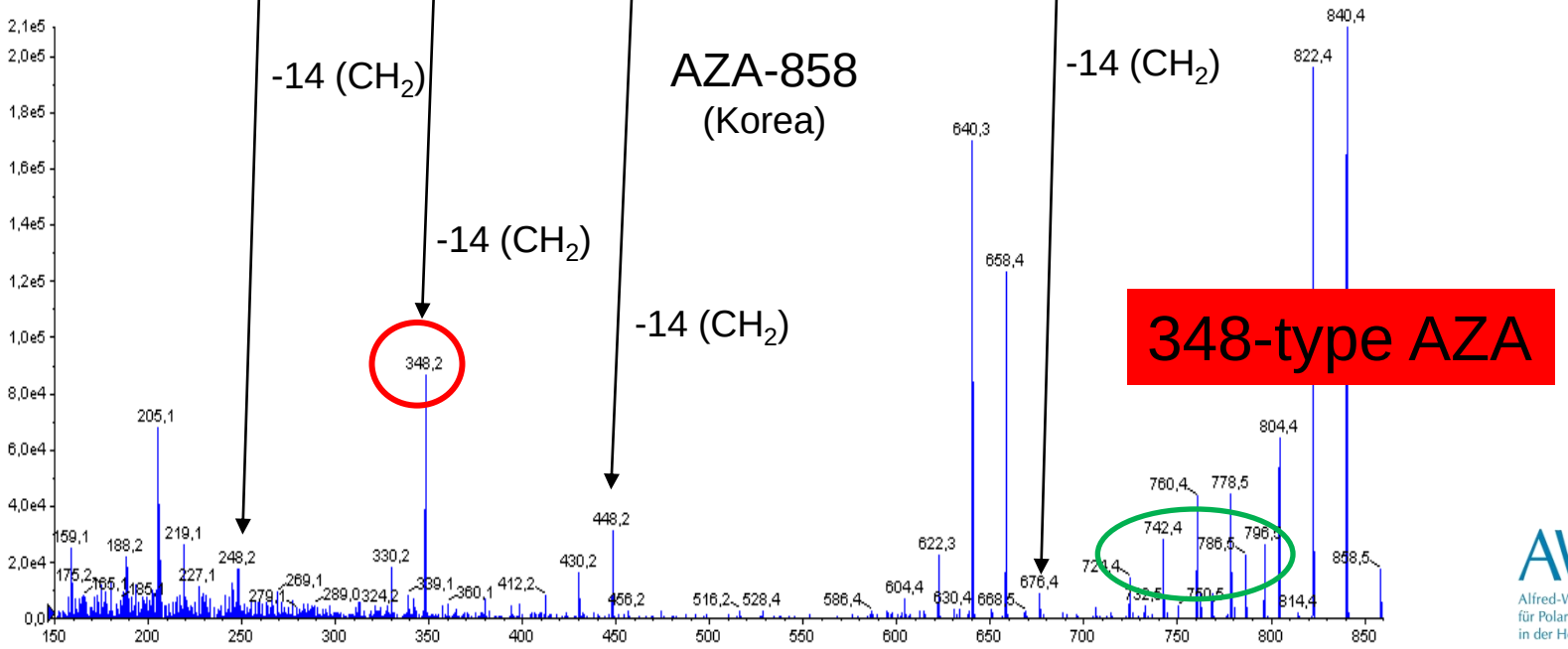
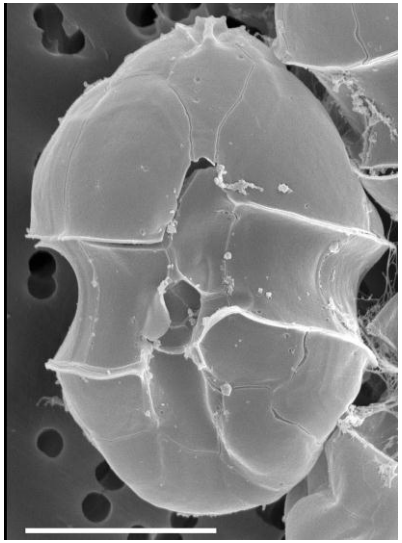
1. *Azadinium* cf. *poporum* Korea

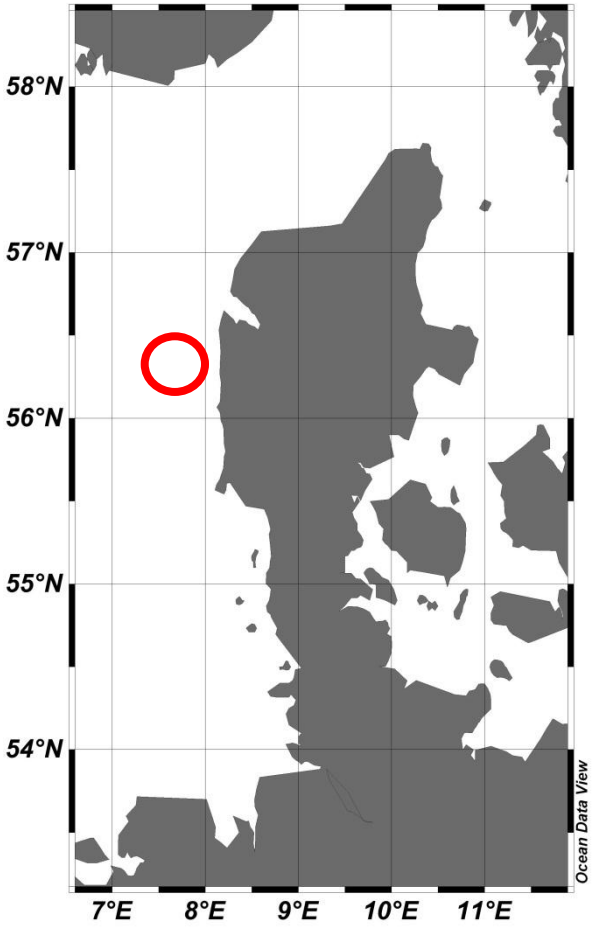




2. *Azadinium poporum* C5 North Sea



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

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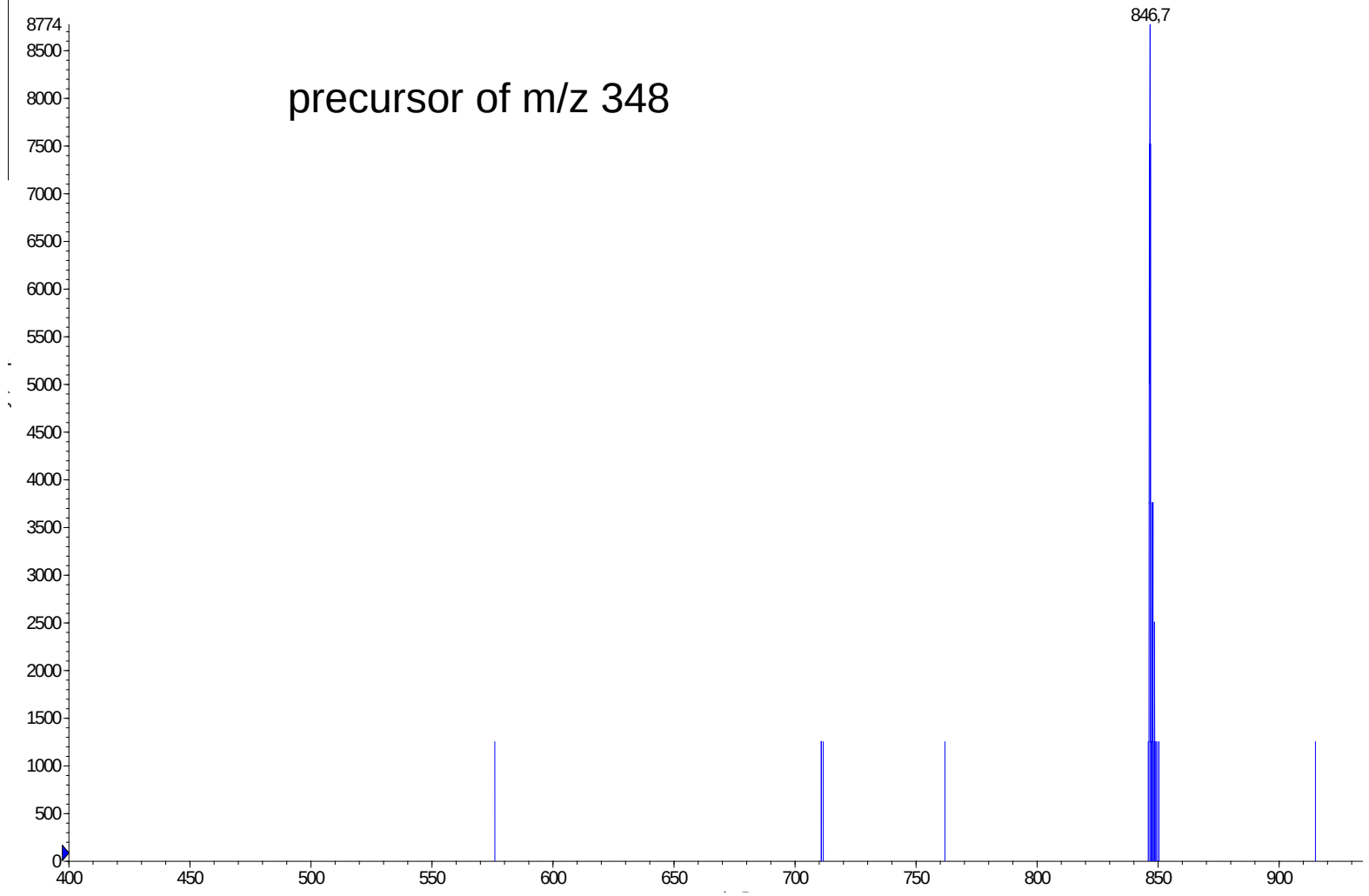
2. *Azadinium poporum* C5 North Sea

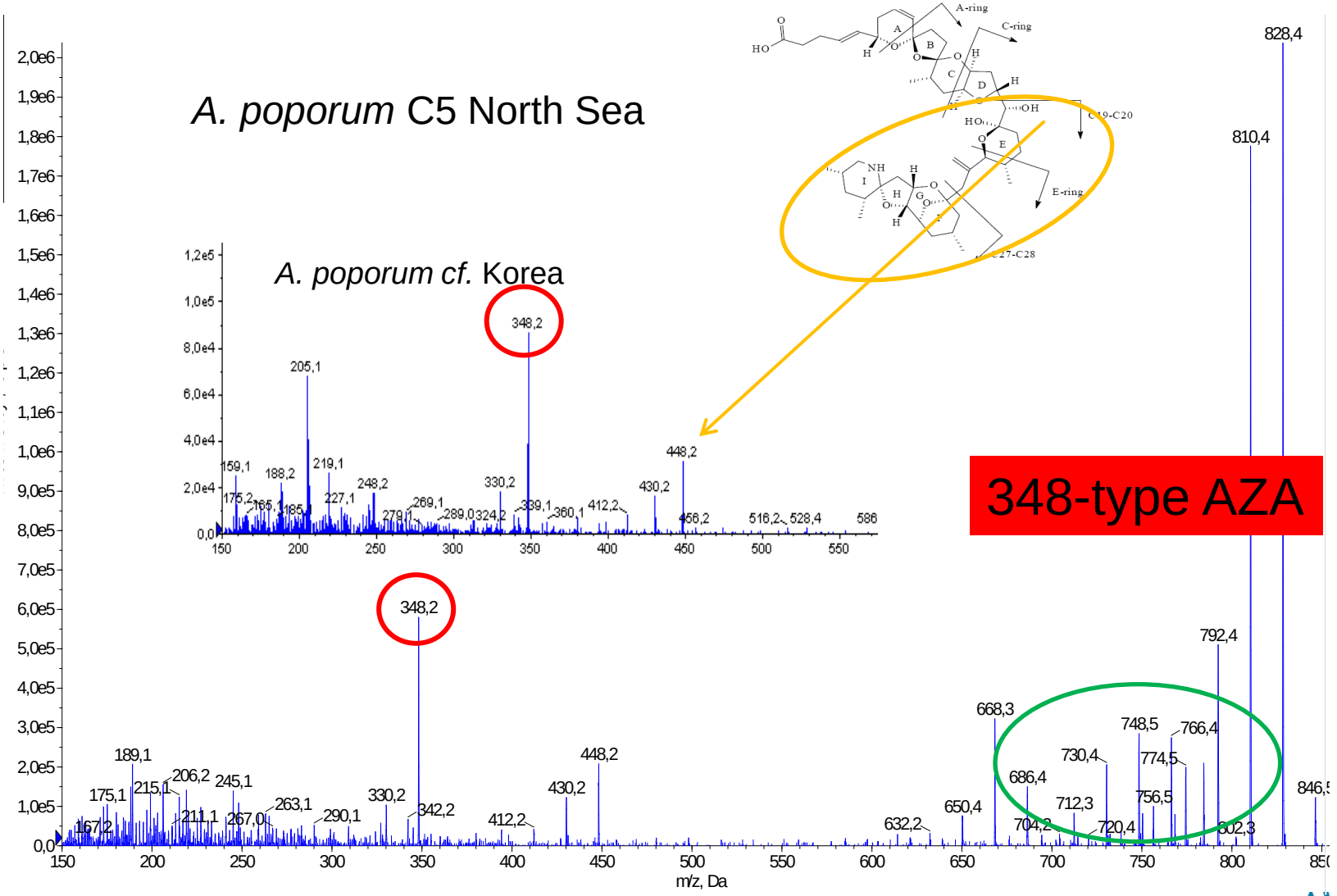


2. *Azadinium poporum* C5 North Sea

A. poporum C5 North Sea

A. poporum cf. Korea

348-type AZA



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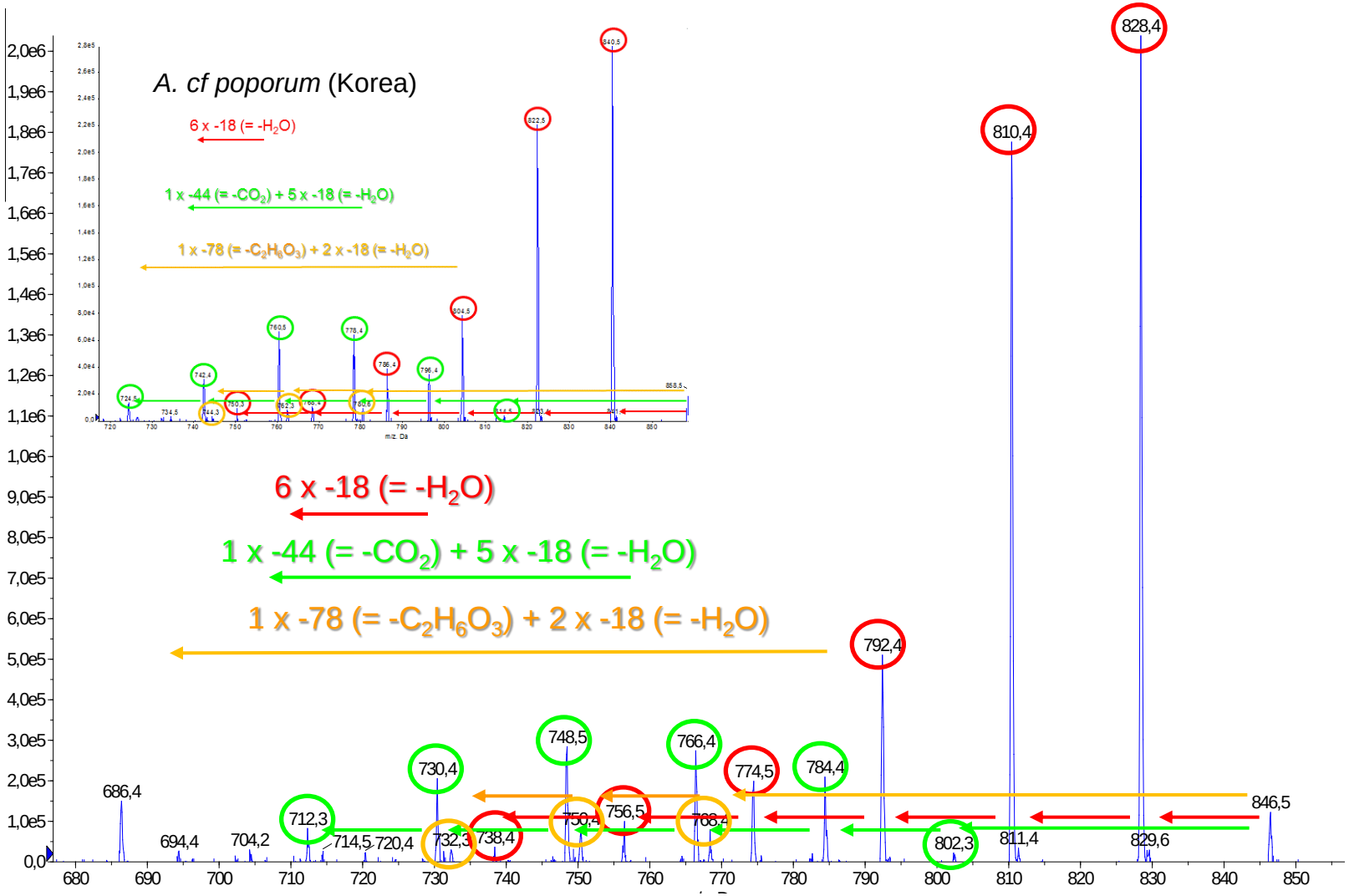
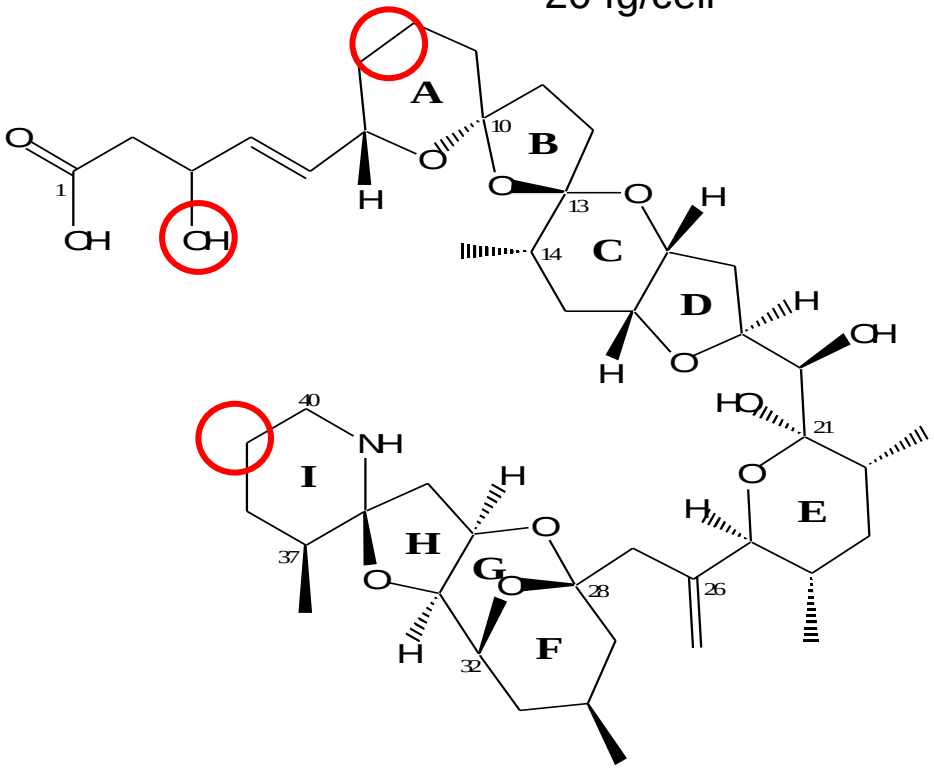
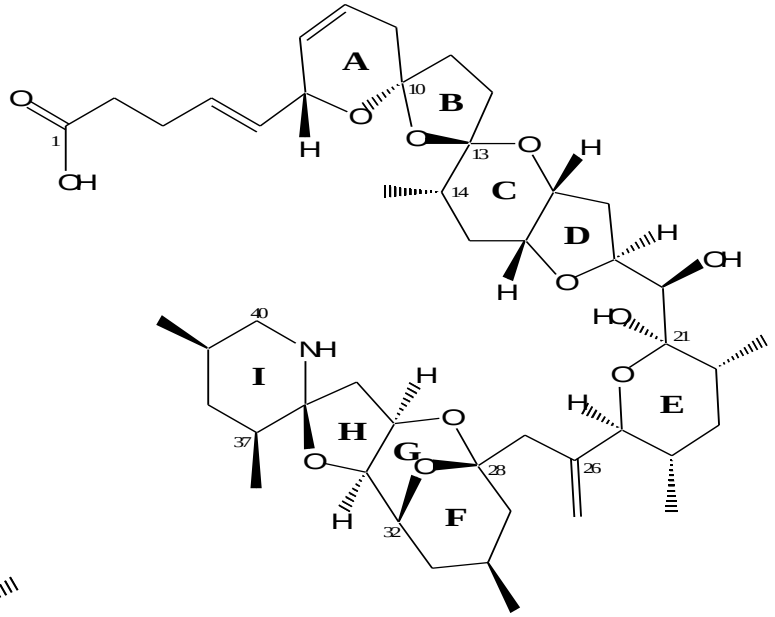
2. *Azadinium poporum* C5 North Sea



2. *Azadinium poporum* C5 North Sea

Toxin cell quota:
~20 fg/cell





AZA-1

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

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

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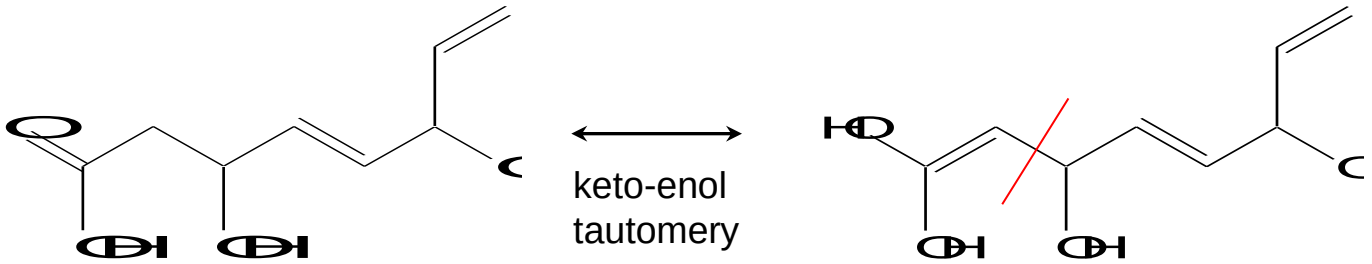
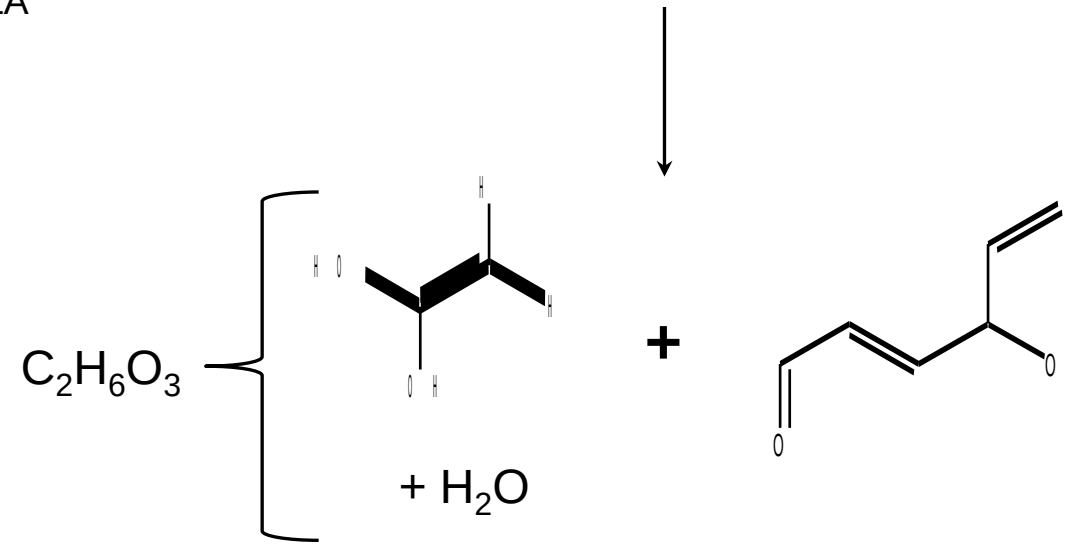
2. *Azadinium poporum* C5 North Sea

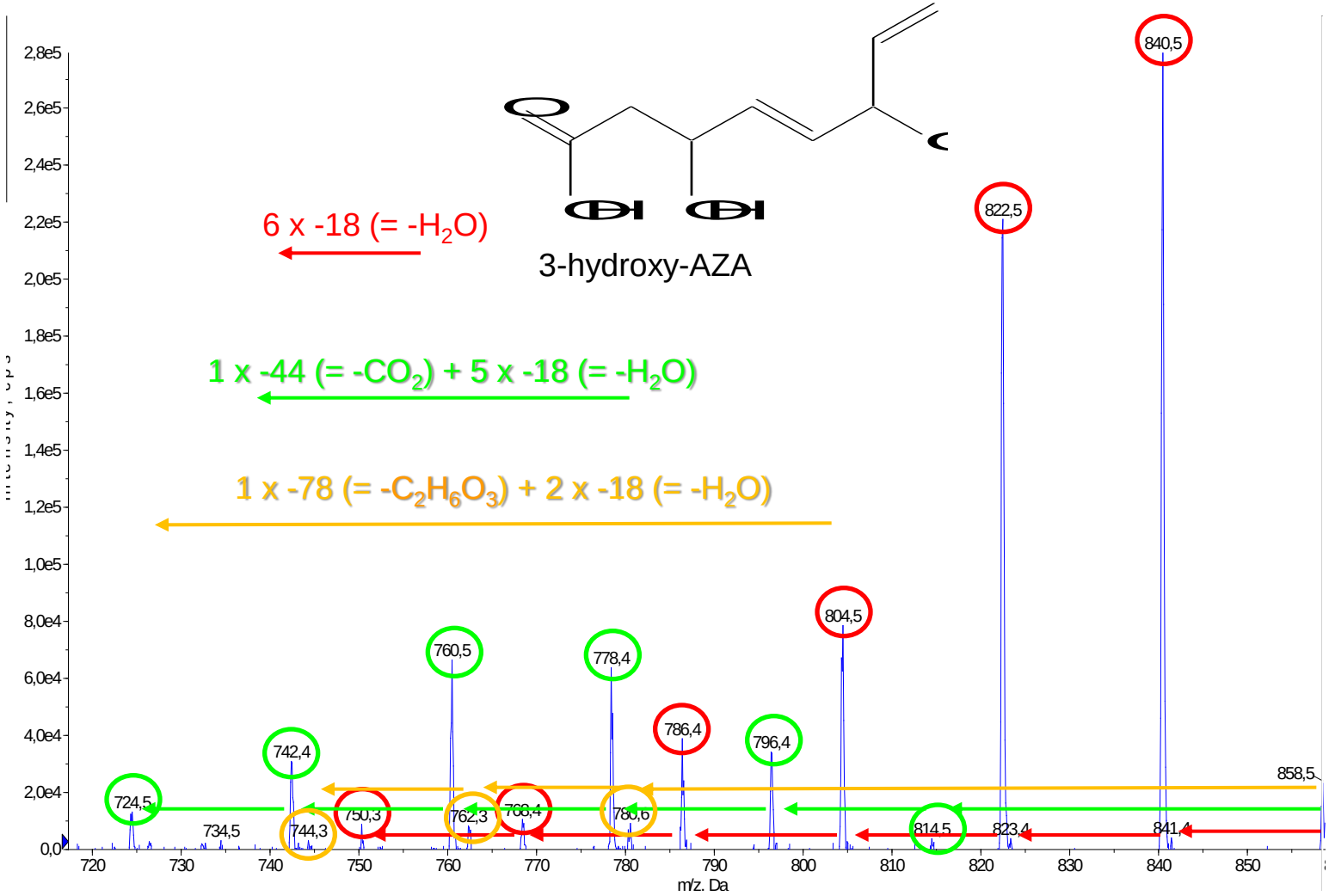
Fragmentation pattern for the cleavage of m/z 78 (= $C_2H_6O_3$)



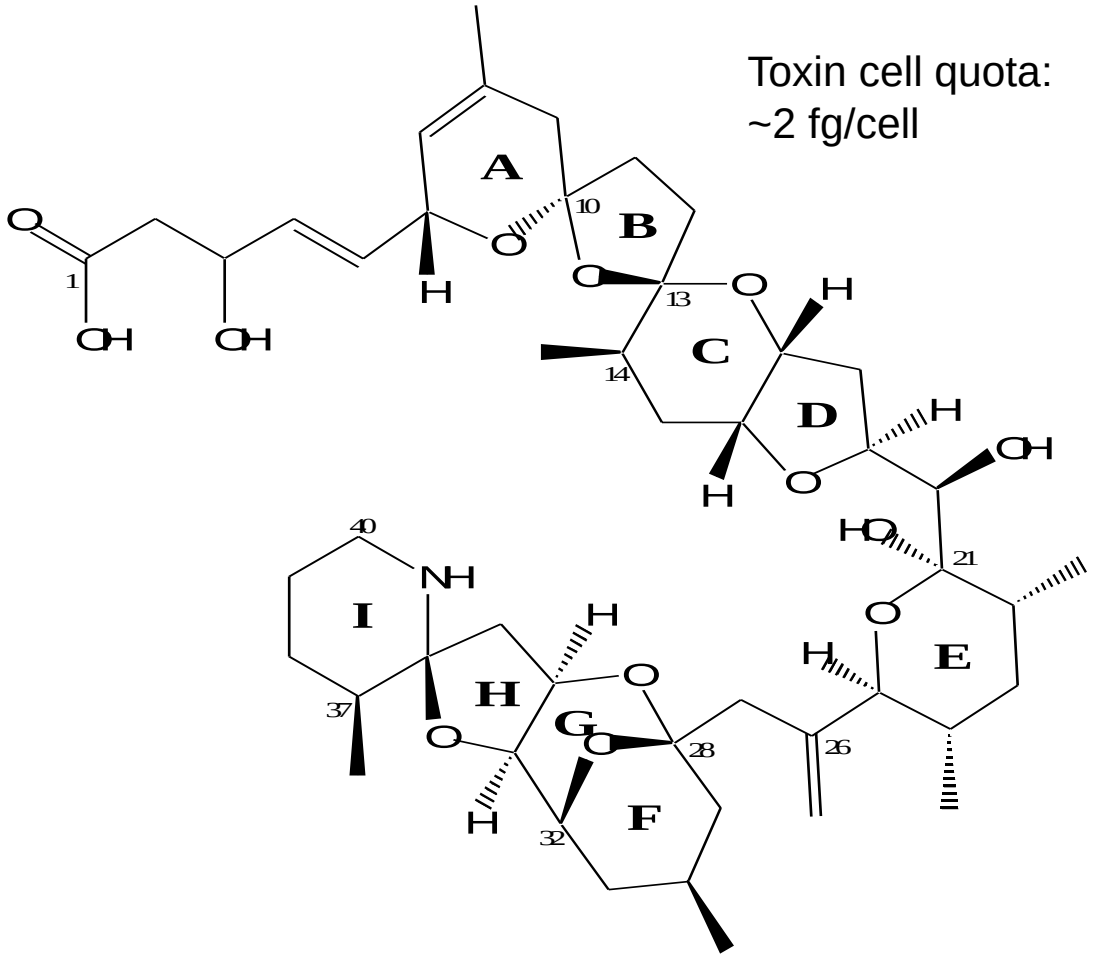
3-hydroxy-AZA



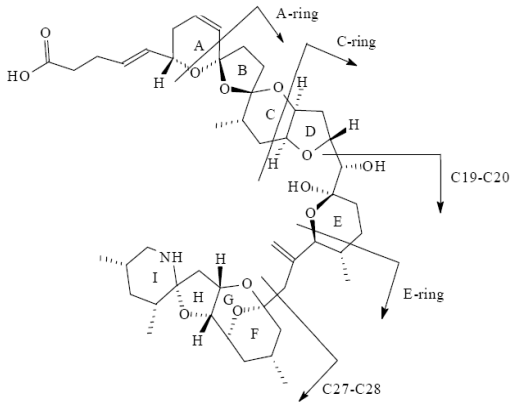
1. *Azadinium* cf. *poporum* Korea



1. *Azadinium* cf. *poporum* Korea



Toxin cell quota:
~2 fg/cell



AZA-1

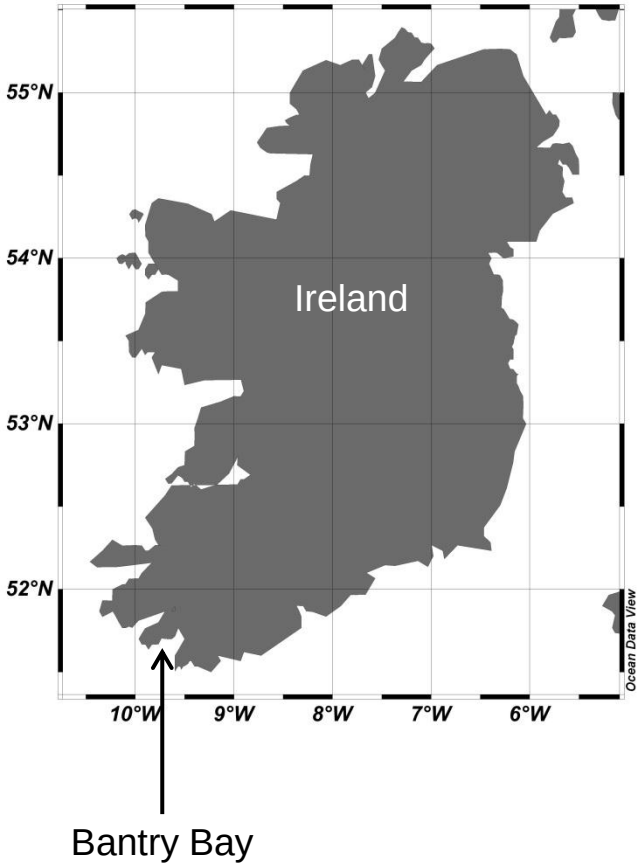
sum formulas
as determined by HRMS:

AZA-1: $C_{47}H_{71}NO_{12}$
AZA-858: $C_{47}H_{71}NO_{13}$

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

Structure confirmed by NMR

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



Bantry Bay

51° 39’ 4.7’’ N, 9° 35’ 11’’ E

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

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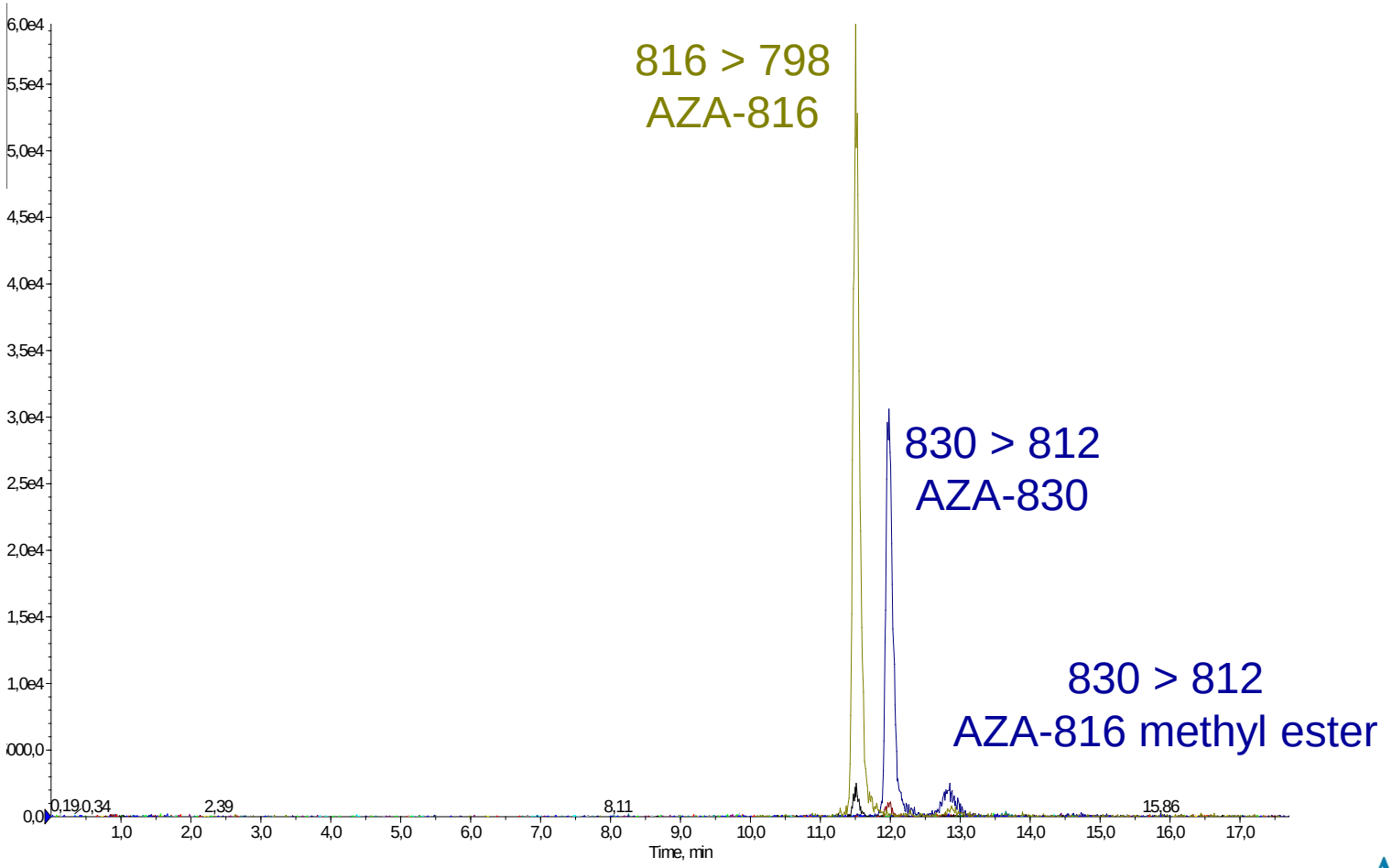
3. *Amphidoma languida* sp. nov., Bantry Bay, Irleand



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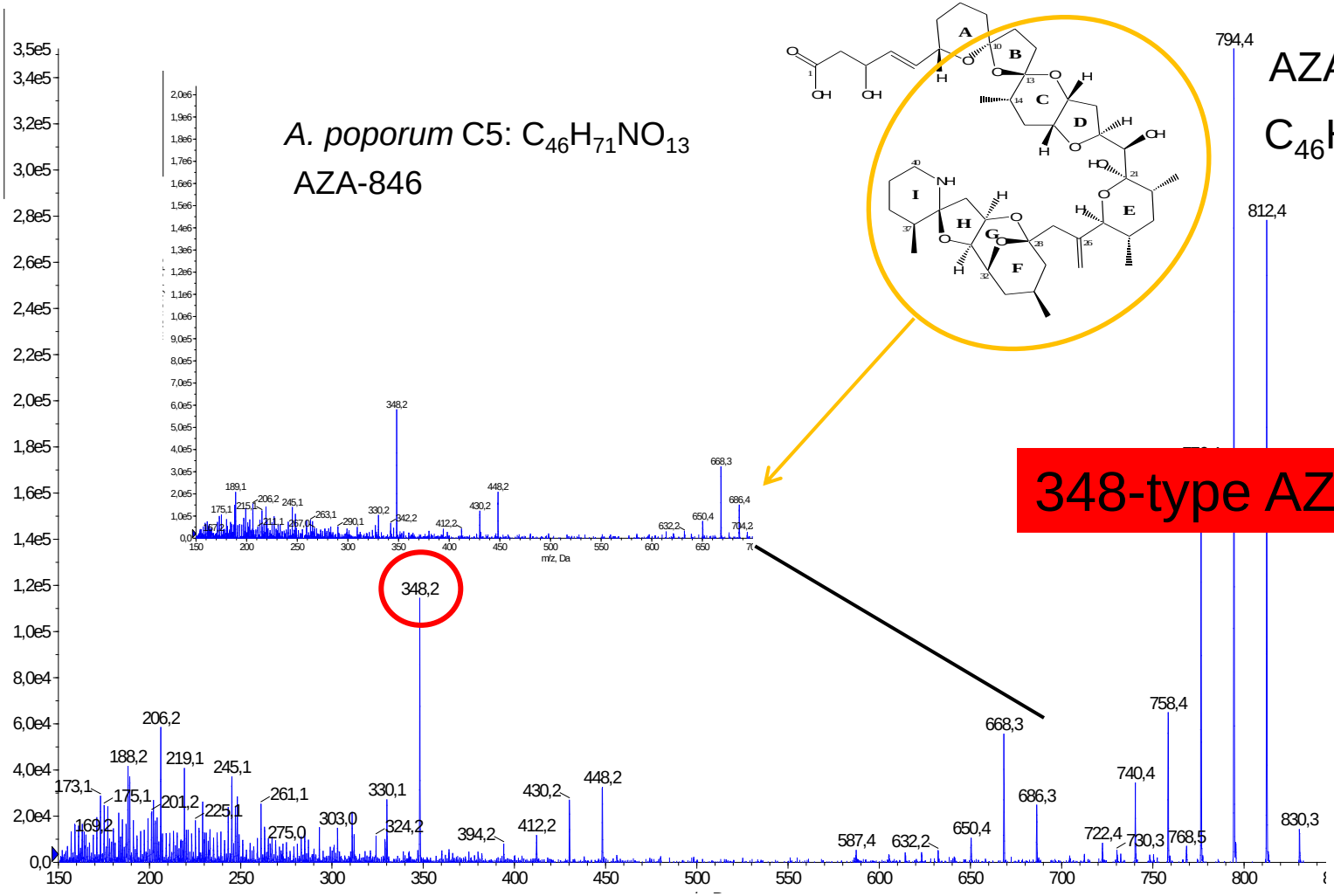
3. *Amphidoma languida* sp. nov., Bantry Bay, Irleand

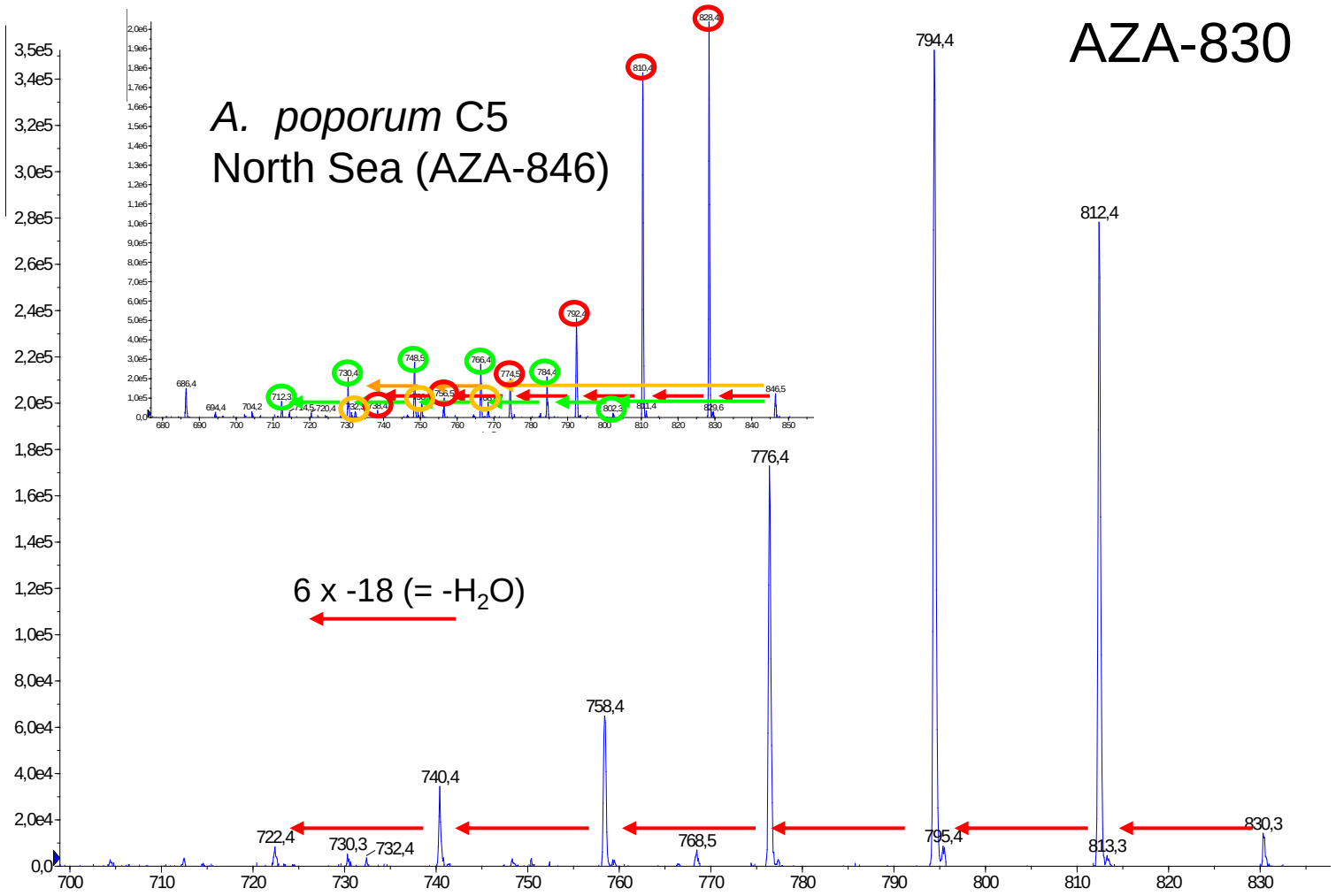
A. poporum C5: $C_{46}H_{71}NO_{13}$
AZA-846

AZA-830
$C_{46}H_{71}NO_{12}$

348-type AZA

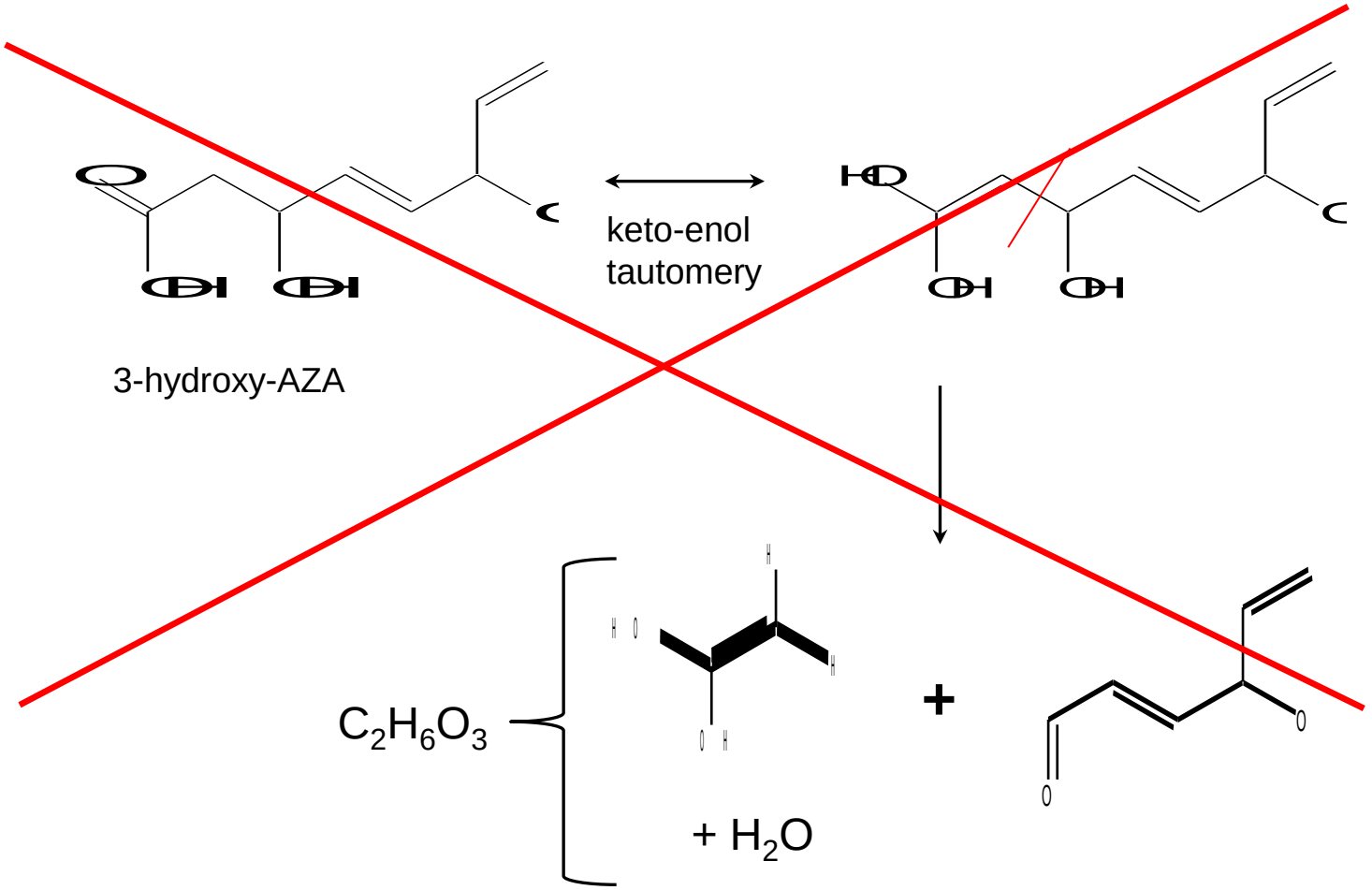


4. *Amphidoma languida* sp. nov., Bantry Bay, Irleand



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

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



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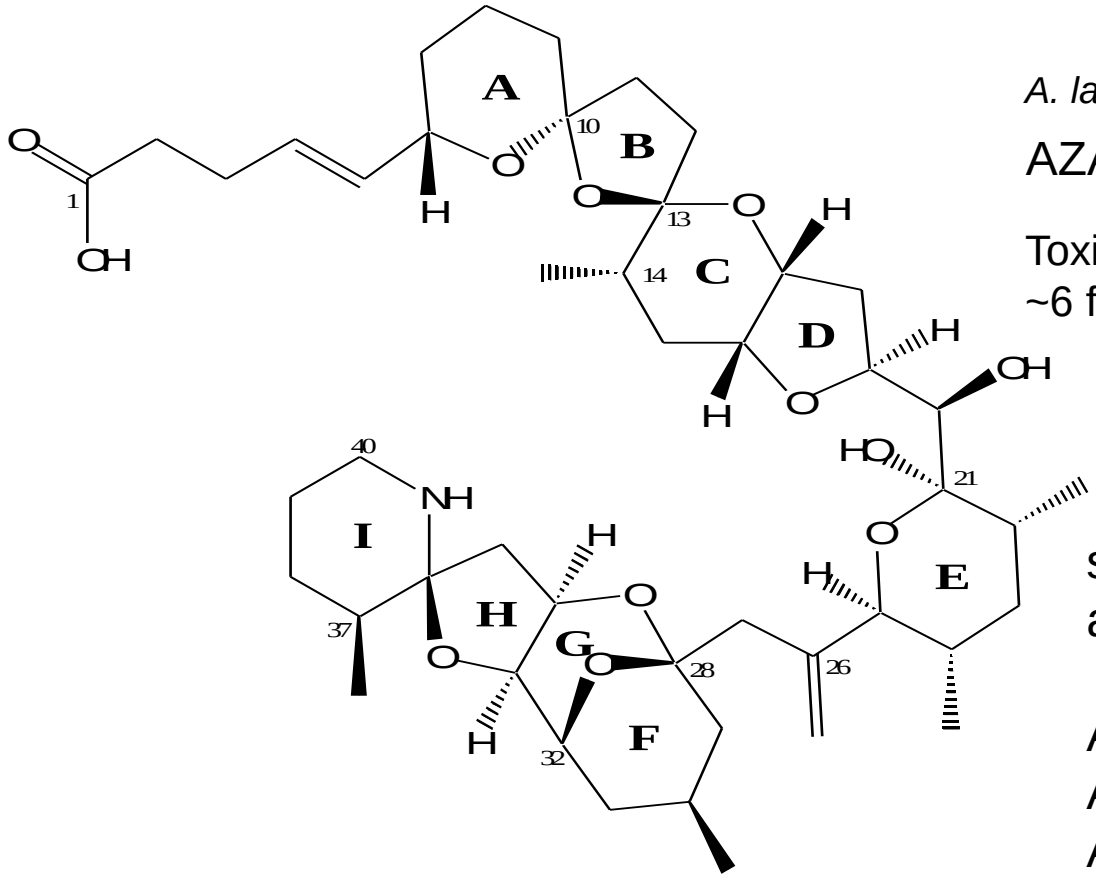
4. *Amphidoma languida* sp. nov., Bantry Bay, Irleand



A. languida:

AZA-830

Toxin cell quota:
~6 fg/cell

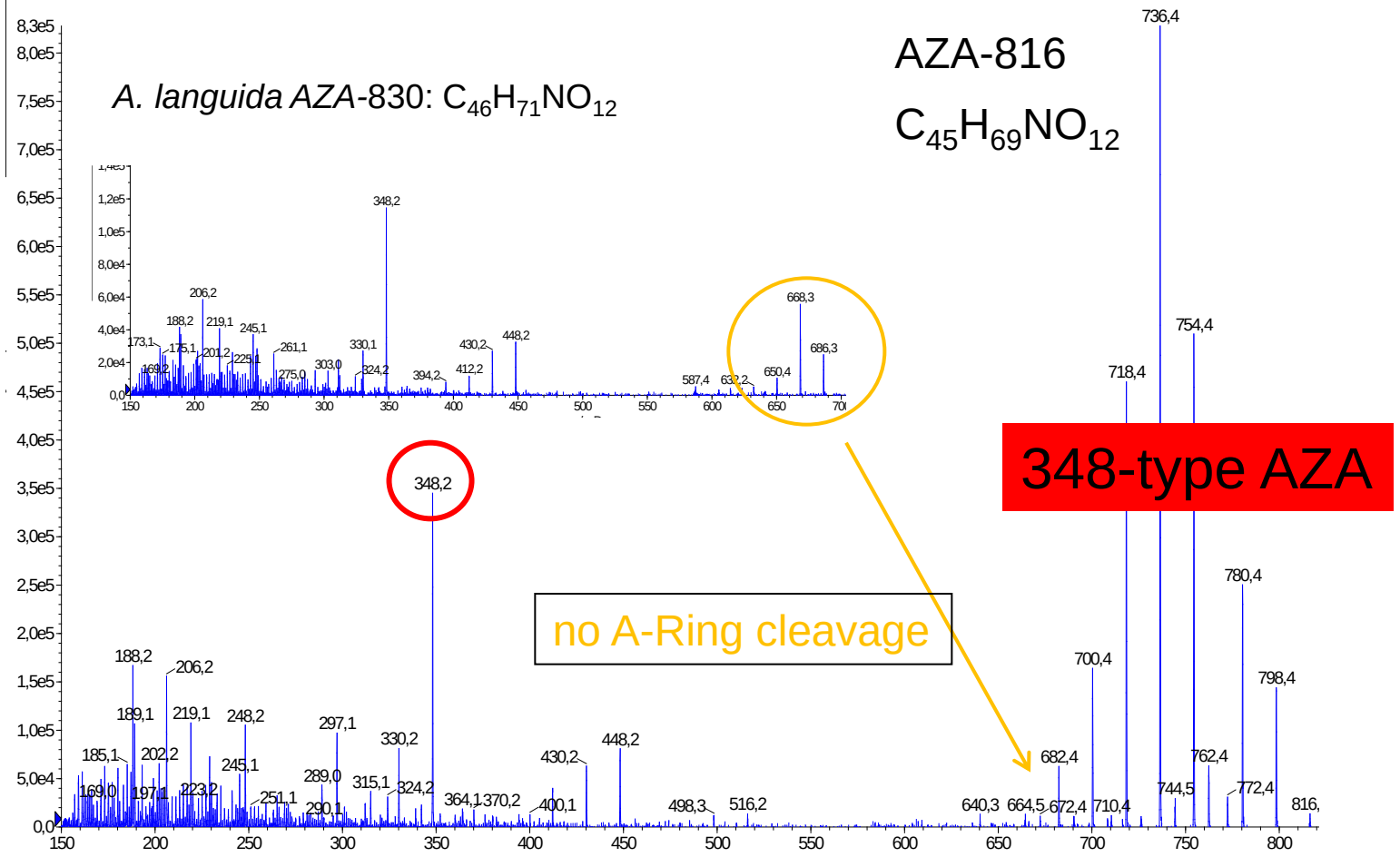
sum formulas
as determined by HRMS:

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

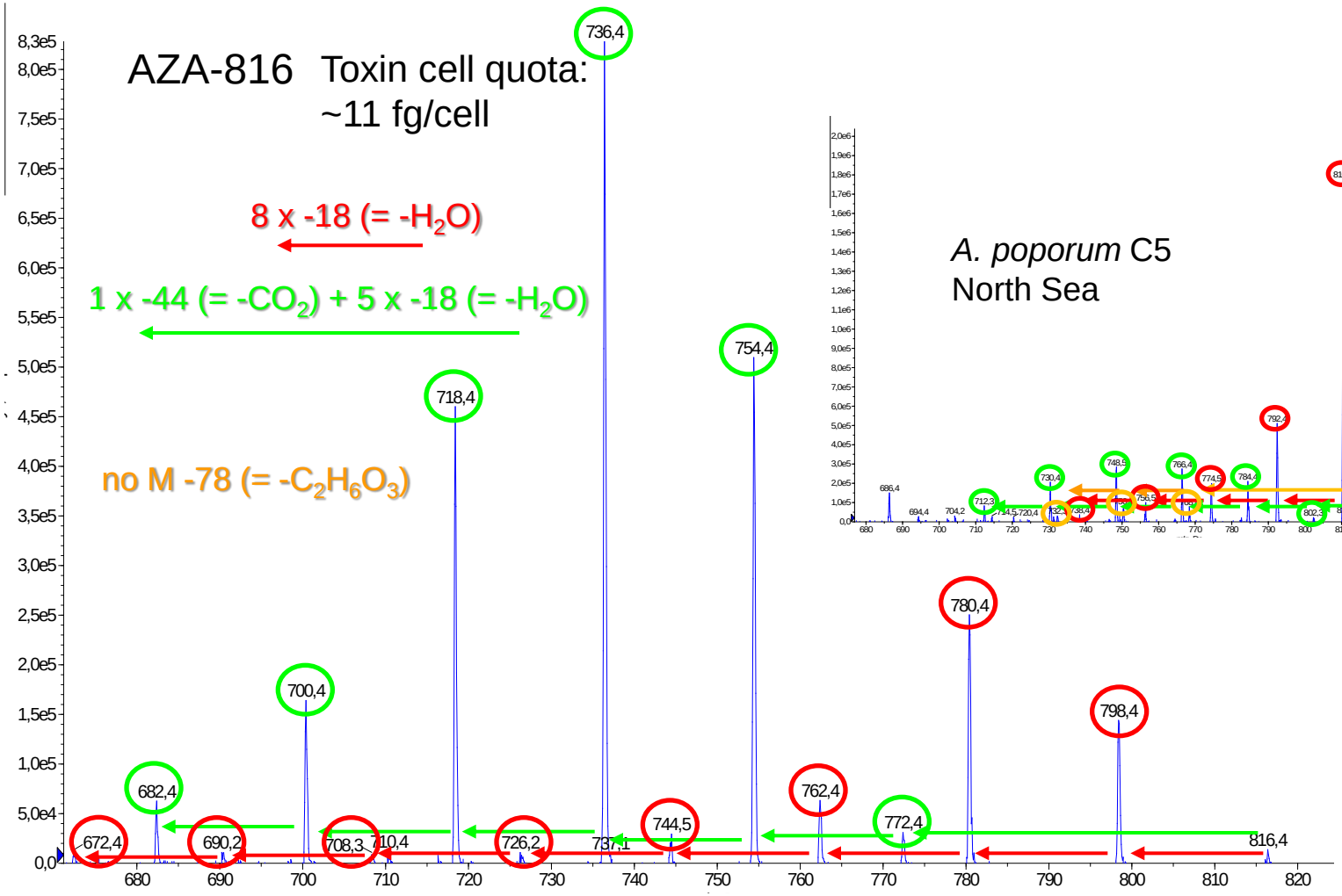
AZA-830 = 39-desmethyl-7,8-dihydro-AZA-1

hypothesized structure!

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

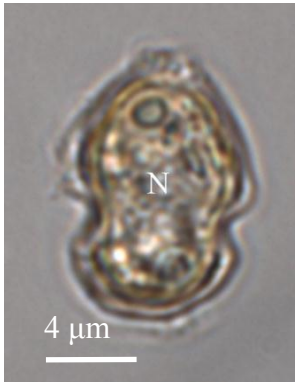


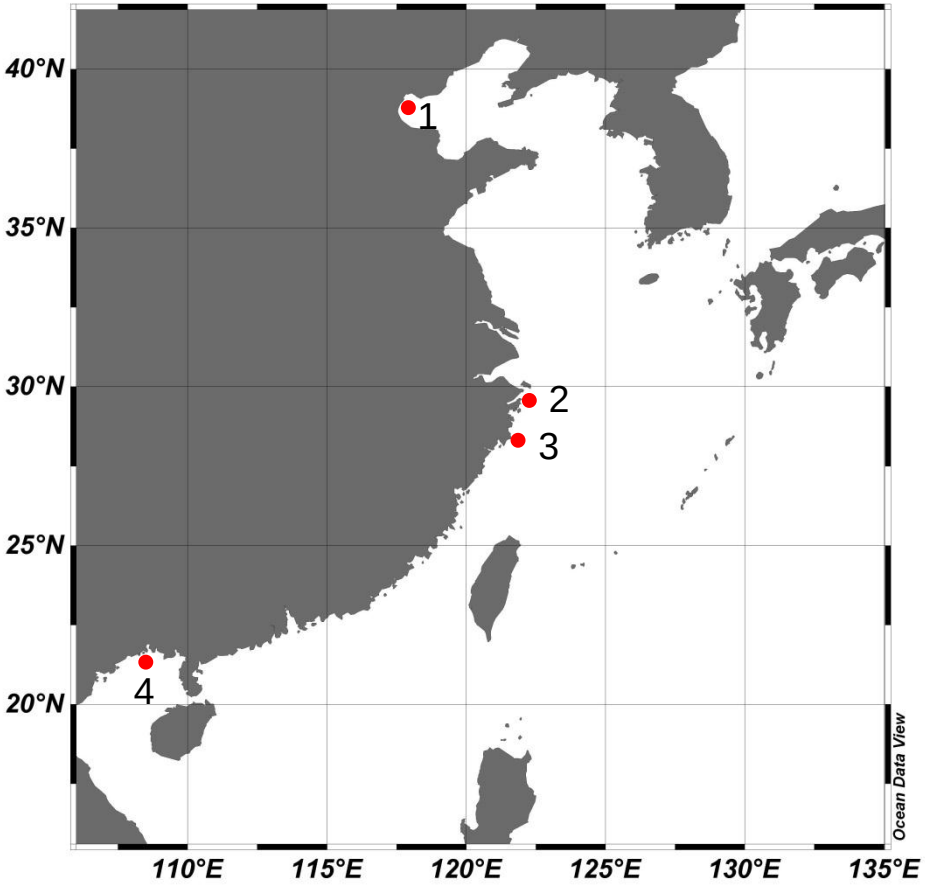
3. *Amphidoma languida* sp. nov., Bantry Bay, Irleand



Structure: **?**

4. *Azadinium poporum*, China





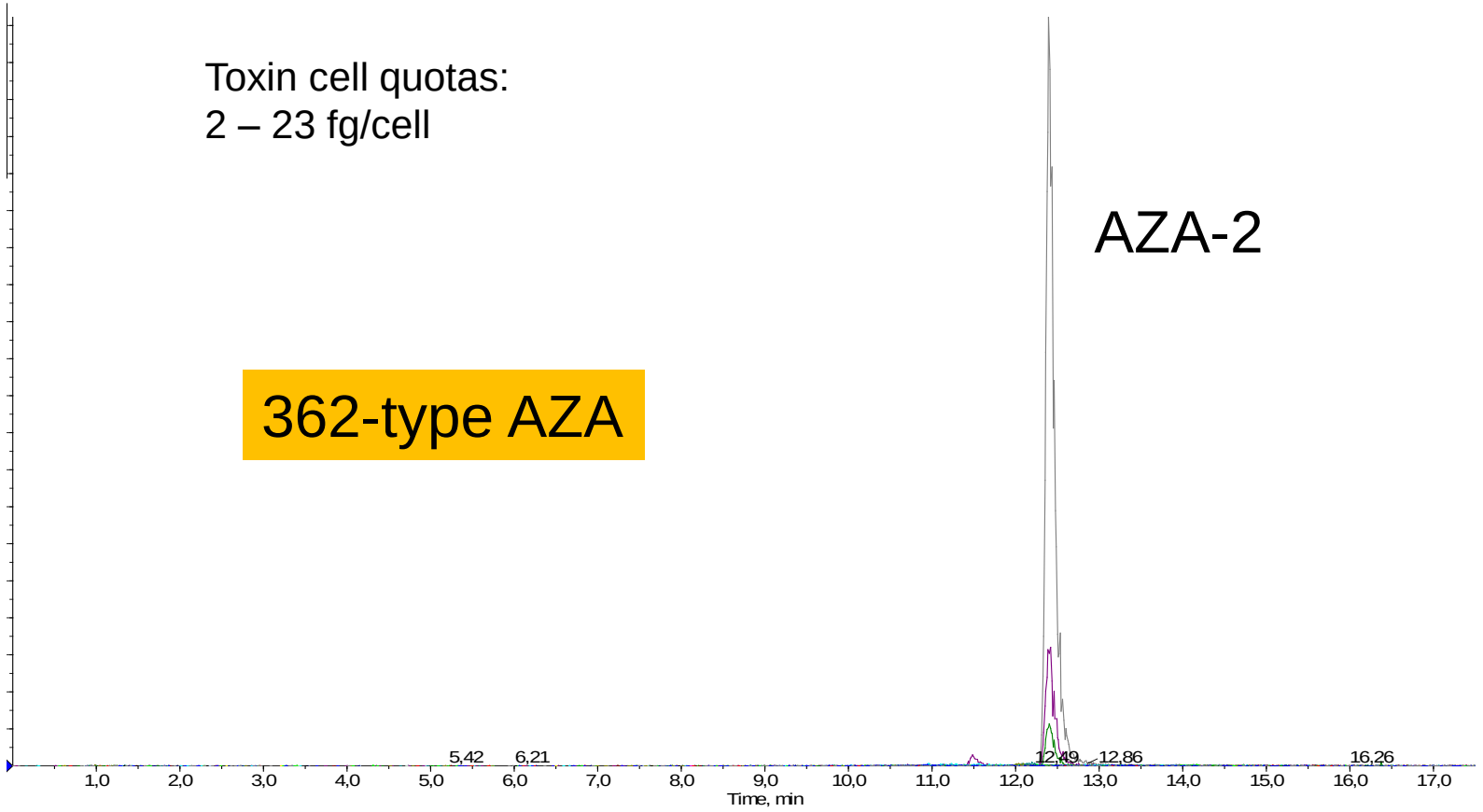
Gu et al. 2011. *Harmful Algae.*, accepted manuscript.

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

Toxin cell quotas:
2 – 23 fg/cell

AZA-2

362-type AZA



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

Toxicon 53 (2009) 680–684

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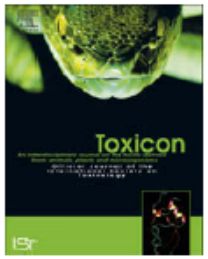


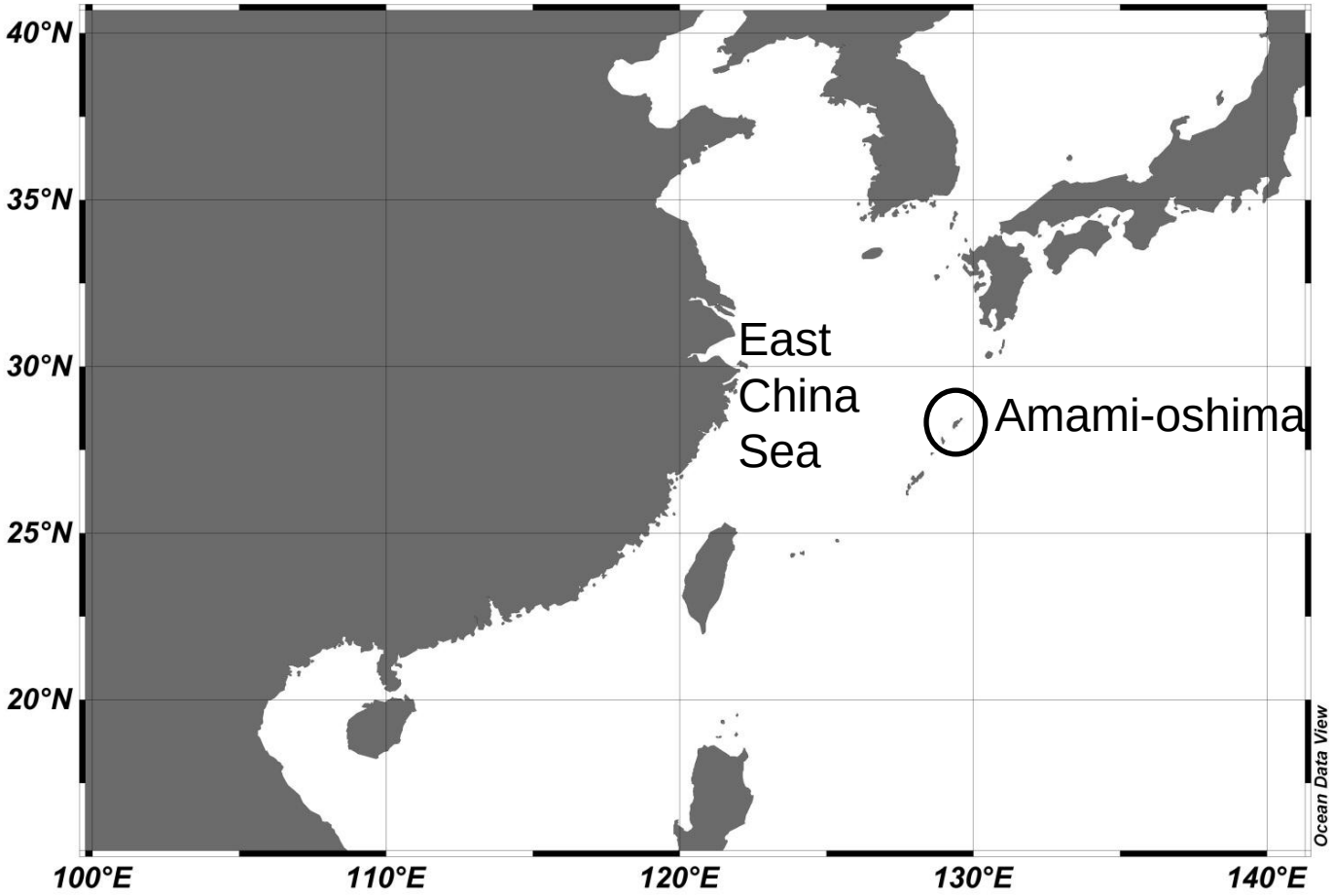
Isolation of azaspiracid-2 from a marine sponge *Echinoclathria* sp. as a potent cytotoxin

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,*]

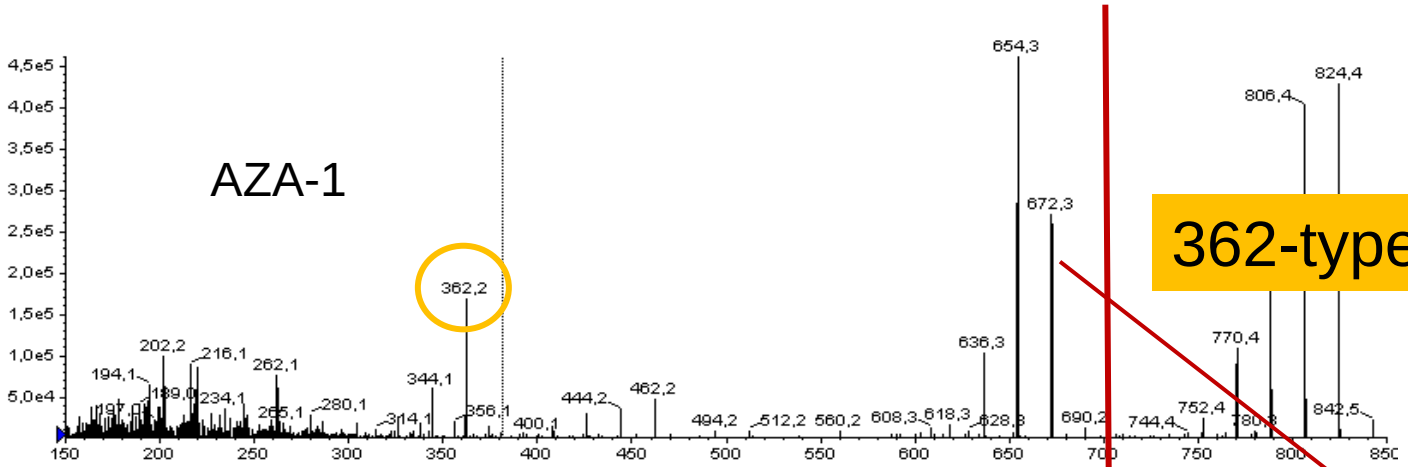
[a] *Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*
[b] *Chemical Genomics Research Group, RIKEN, Wako, Saitama 351-0198, Japan*
[c] *Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology, Koto-ku, Tokyo 135-0064, Japan*
[d] *Zoological Museum, University of Amsterdam, 1090GT Amsterdam, The Netherlands*

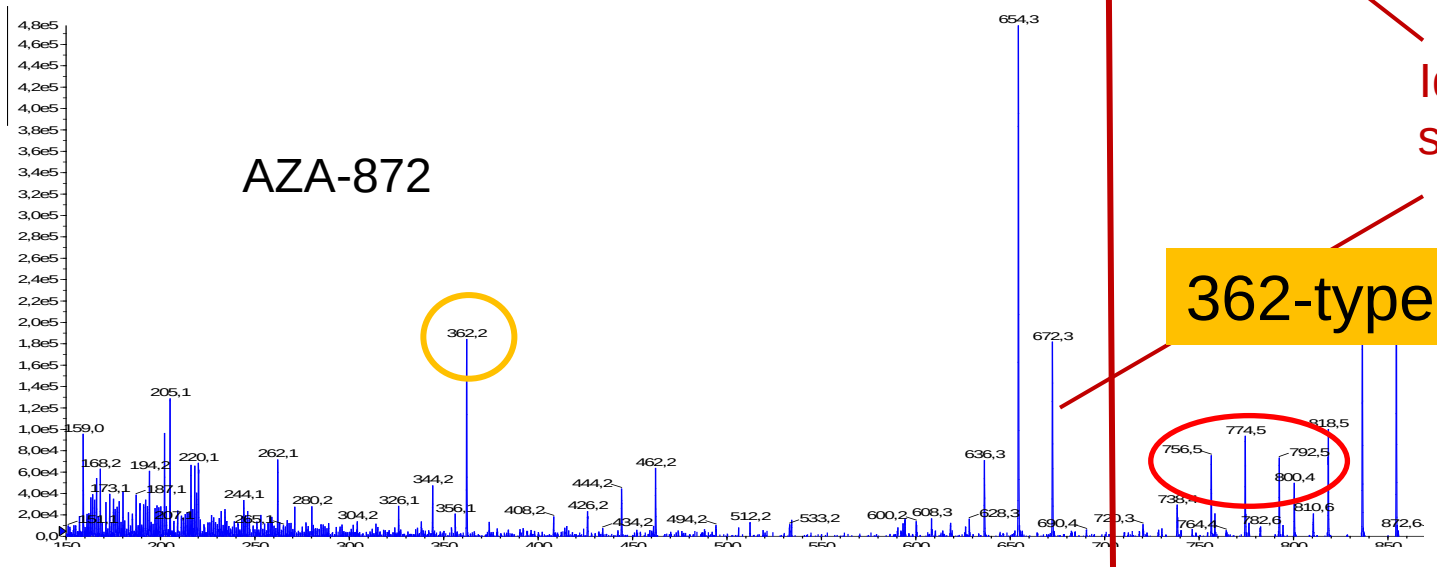
4. *Azadinium poporum* G 42, G 64, G 68, East China Sea, China

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

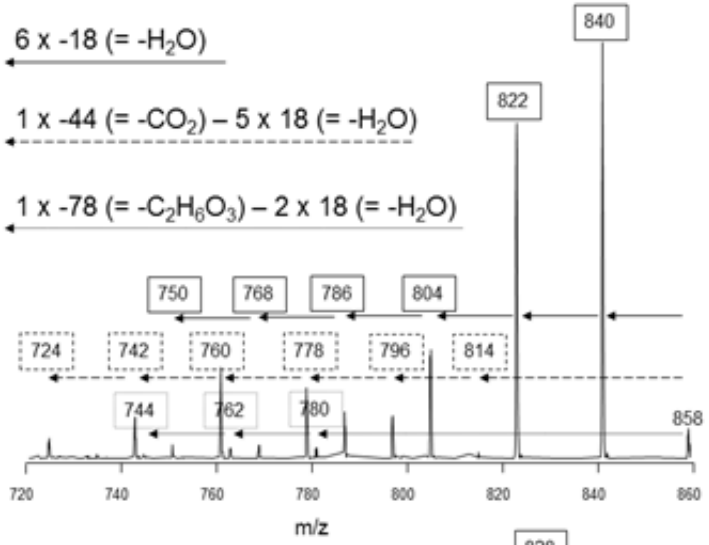


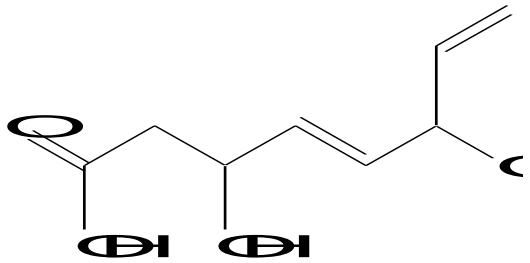


Identical CID spectra up to m/z = 672

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

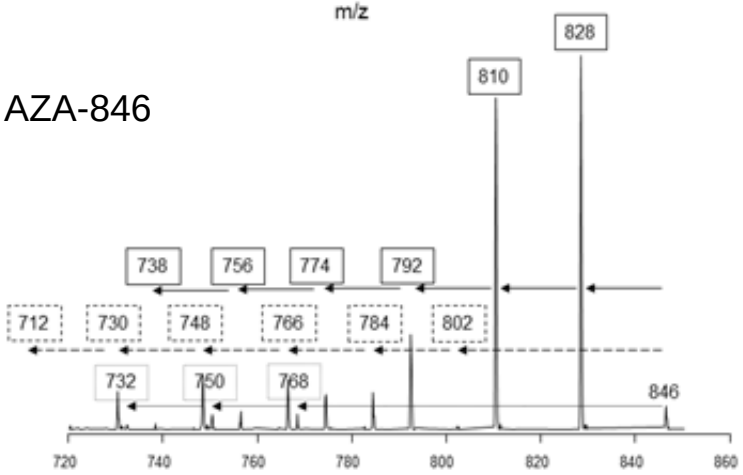
AZA-858



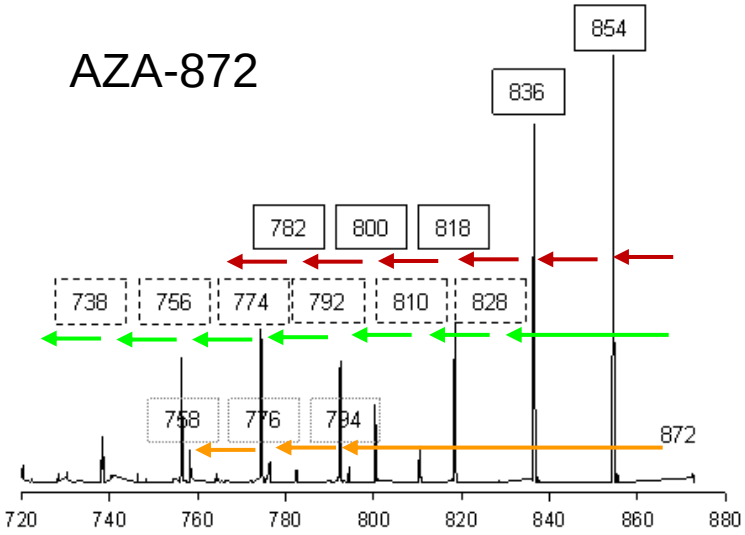


3-hydroxy-AZA

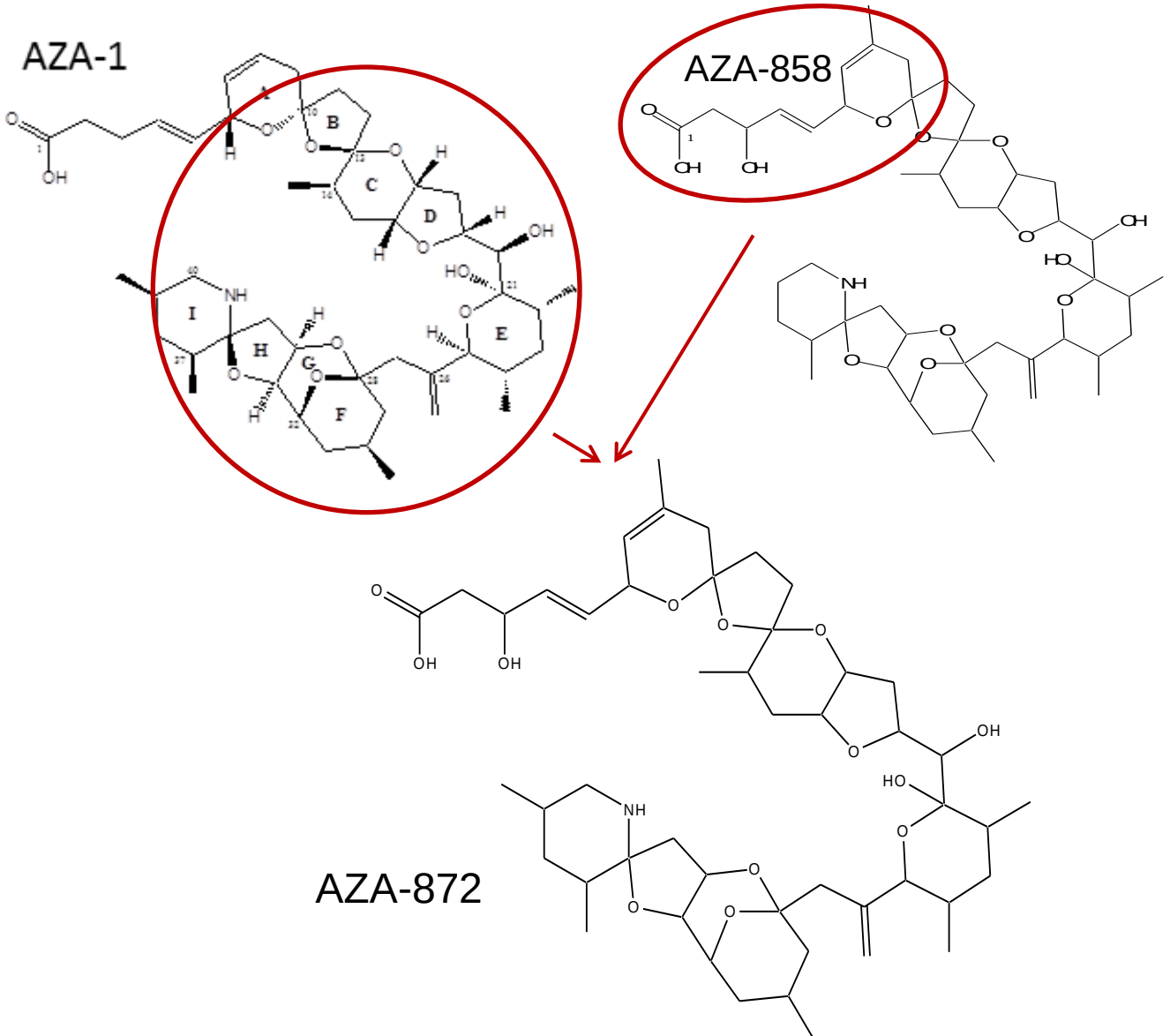
AZA-846



AZA-872

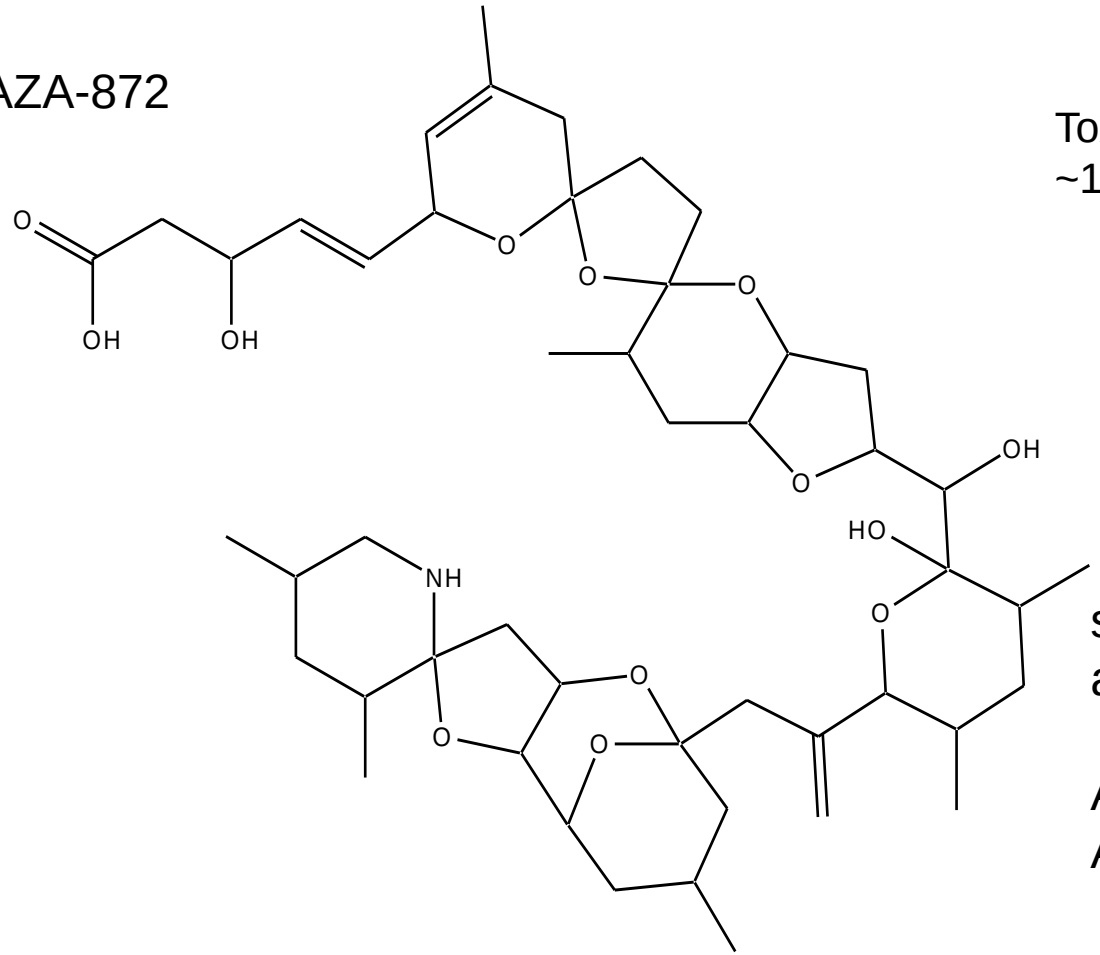


5. *Azadinium poporum* G 66, East China Sea, China



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

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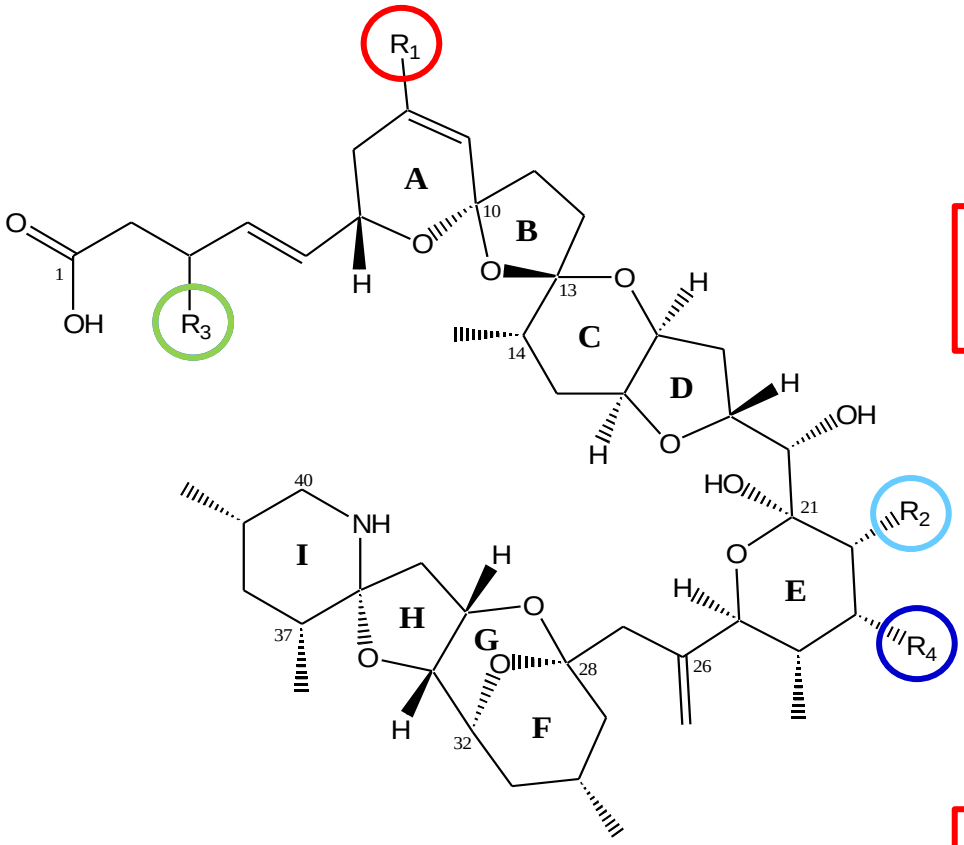
introduction: structural variants



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

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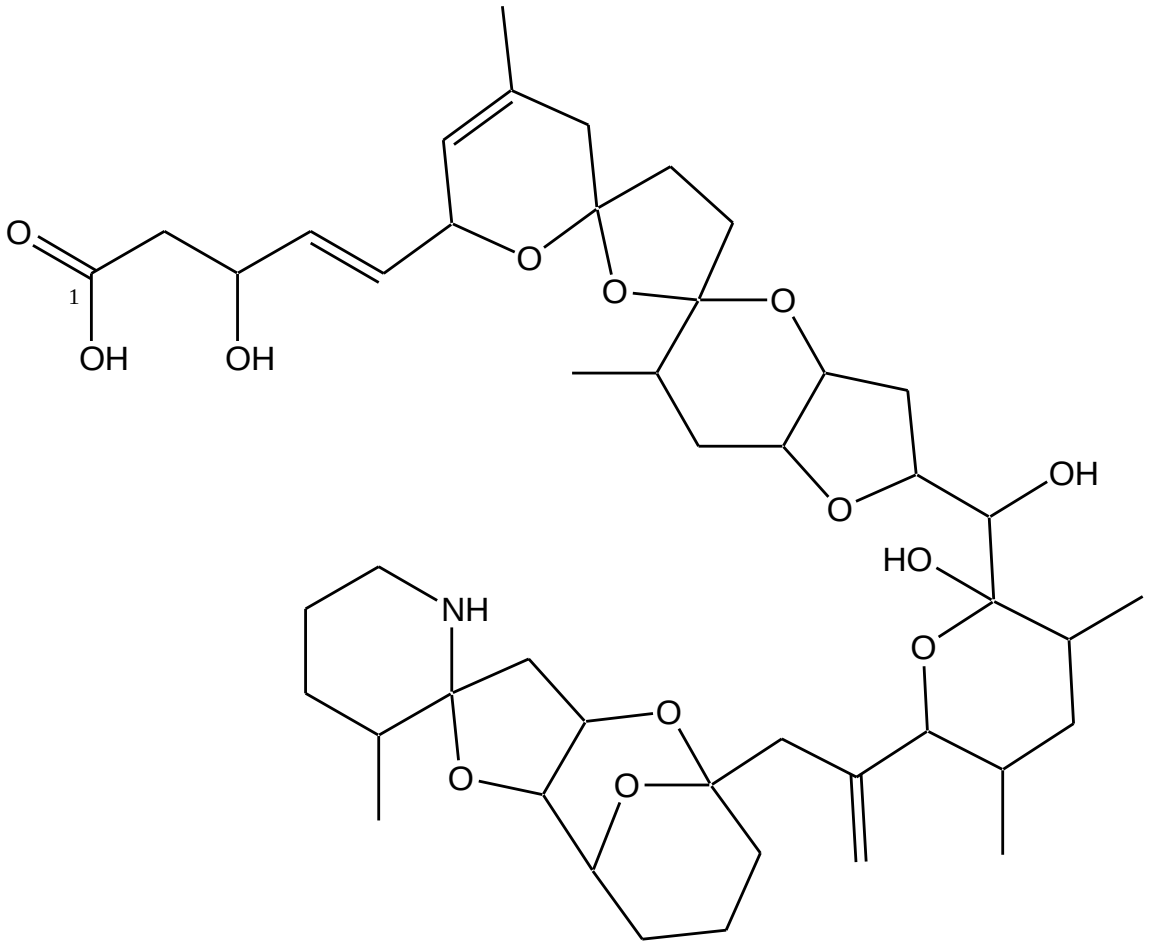
6. *Azadinium poporum* G 25, Bohai Sea, China



AZA-858

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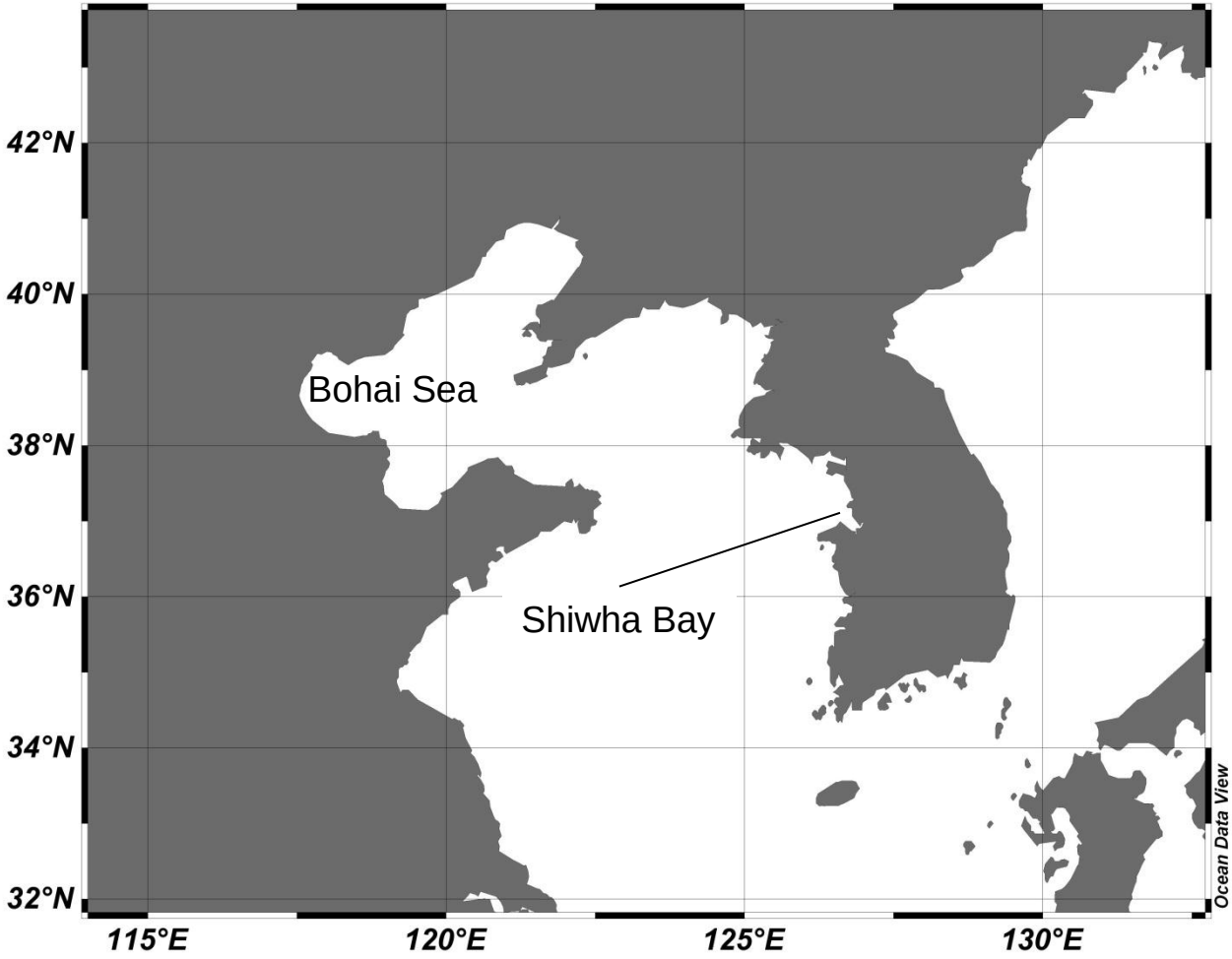
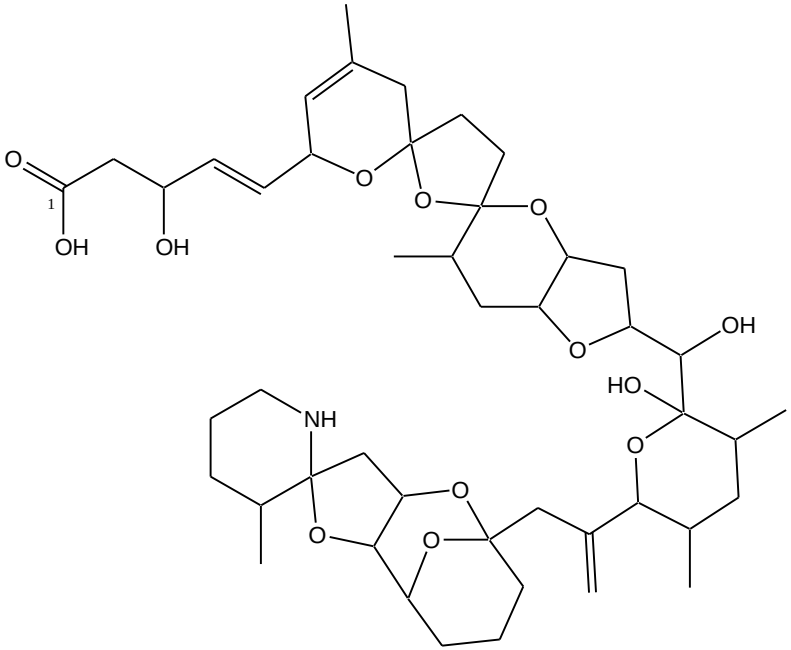
6. *Azadinium poporum* G 25 Bohai Sea, China

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



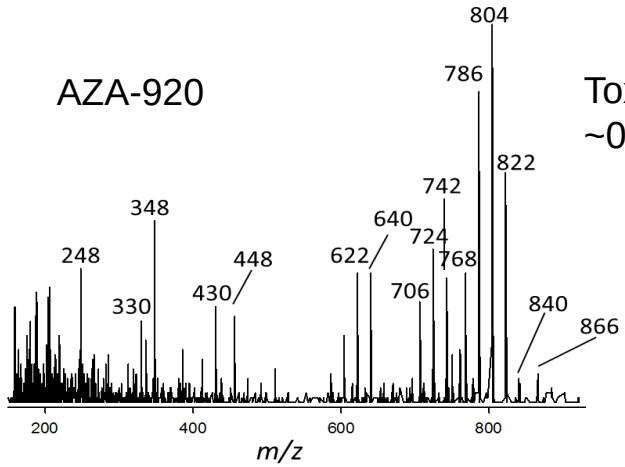
6. *Azadinium poporum* G 25, Bohai Sea, China



Toxin cell quota:
~1.4 fg/cell

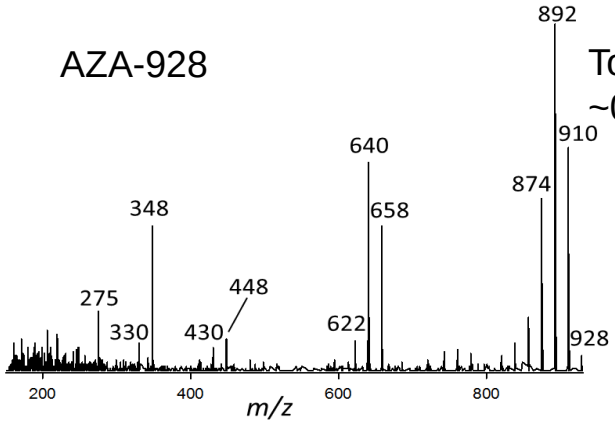
AZA-858

AZA-920



Toxin cell quota:
~0.02 fg/cell

AZA-928



Toxin cell quota:
~0.14 fg/cell

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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

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Thank you for your attention!

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