Revisiting the toxin profile of *Alexandrium pseudogonyaulax*; formation of a desmethyl congener of goniodomin A

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Highlights:

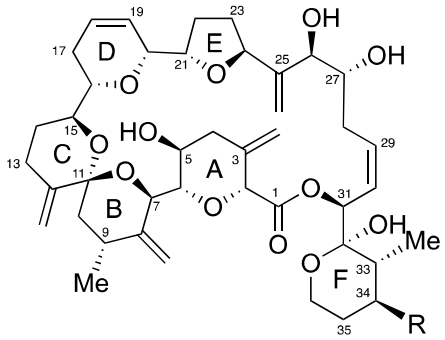
- A previously published truncated congener of goniodomin A had been incorrectly assigned as goniodomin B.
- Mass spectroscopic evidence indicates that the deletion had occurred on ring F.
- Based on biosynthetic considerations, it is hypothesized that it involved deletion of the methyl group from C-34.

ABSTRACT

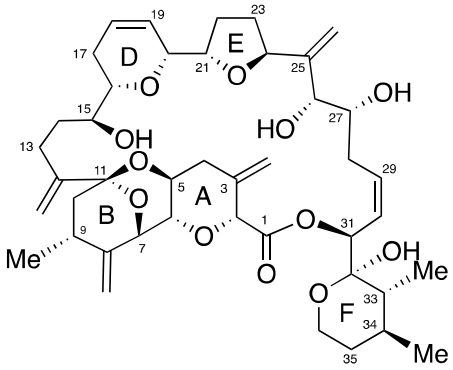
During a survey of the production of goniodomin A (GDA) by *Alexandrium pseudogonyaulax* in Danish coastal waters, Krock et al. (2018) obtained mass spectral evidence for the presence of a truncated congener, herein termed GD754, having a molecular weight 14 Da lower than GDA and assigned it as goniodomin B (GDB). An erroneous structure of GDB involving deletion of a methylene group between rings B and D had previously been reported by Espiña et al. (2016) but without experimental details. HPLC properties reported by Krock for GD754 point to it being a homolog of GDA. Comparison of mass spectral fragmentation data reported for GD754 with fragmentation data for GDA, show it to be a truncated form of GDA with the deletion involving a CH_2 group from ring F or one of the two methyl substituents on ring F, not elsewhere on the molecule. On biosynthetic grounds, the GD754 congener is proposed to be 34-desmethyl-GDA. Further experimental work will be required to confirm this hypothesis.

1. Introduction

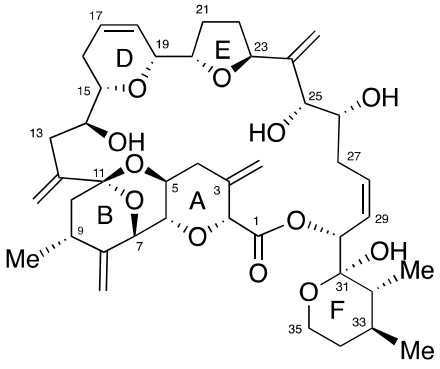
The marine phycotoxin goniodomin was reported by Sharma et al. in 1968 as a metabolite of an unidentified *Alexandrium* species (Sharma et al., 1968). They carried out extensive characterization but were unable to establish the structure. Goniodomin A (GDA) is a metabolite of *A. hiranoi*, *A. monilatum* and A. *pseudogonyaulax* (Murakami et al., 1988; Hsia et al., 2006; Zmerli Triki et al., 2016). The structure of GDA was established by Murakami et al. (1988) via NMR and the absolute configuration by Takeda et al. (2008) on the basis of NMR and synthetic studies. We have recently shown Sharma's goniodomin to be identical to GDA (Harris et al., 2020) and confirmed both the absolute configuration and conformation of GDA by X-ray crystallography (Tainter et al., 2020). In the course of Takeda's studies of GDA (Takeda et al., 2008), an isomeric species, goniodomin B (**2**, GDB), was isolated. Takeda was unsure whether GDB was truly a congener or only an isolation artifact. Takeda's structural studies have not been published but a detailed description is contained in Takeda's dissertation (Takeda, 2008). In 2016, Espiña et al., while collaborating with Takeda on toxicological studies of GDA and GDB, published an incorrect structure (**3**) for GDB in which a methylene group had been deleted from the macrolide ring between rings B and D, leading to a molecular weight of 754 Da versus 768 Da for GDA (Espiña et al., 2016). Subsequently, Krock et al., assuming Espiña's 754 molecular weight to be correct, found small amounts of a 754 Da compound (herein designated GD754) mixed with GDA in planktonic field samples collected during a survey of *A. pseudogonyaulax* in Danish coastal waters (Krock et al., 2018). Not unreasonably, they assumed GD754 to be GDB. The error was further propagated in Kremp et al. (2019). In the present communication, we show that GD754 is neither GDB nor a congener of GDB but is a truncated form of GDA. The deletion is from ring F not the macrolide ring and, on the basis of biosynthetic information for GDA, we propose that GD754 is 34-desmethyl GDA.



GDA (**1**, R = Me)
34-Desmethyl-GDA (**4**, R = H)



Takeda's GDB (**2**)



Espiña's GDB (**3**)

2. Methods

2.1. Material. GDA was isolated by the previously described procedure (Harris et al., 2020) from *A. monilatum* cells that had been collected via plankton nets from blooms in the York River, VA.

2.2. Mass Spectrometry. Mass spectra of GDA were acquired on a Bruker 10 T APEX-Qe FT-ICR mass spectrometer in the Major Instrumentation Center at Old Dominion University, Norfolk, VA using electrospray ionization. The sample of GDA was introduced by direct infusion of a methanol solution. An intense signal at *m/z* 786.4426 was observed for the ammonium adduct of GDA. Calculated for $C_{43}H_{64}NO_{12}^+$: *m/z* 786.4423. CID spectra were acquired using an 8 Da isolation window and -12.2 V CID. An intense $C_{35}H_{43}O_9^+$ fragment ion (8.1 x 10^6 cps) was observed at *m/z* 607.2901. Calculated for $C_{35}H_{43}O_9^+$: *m/z* 607.2902. In addition, a weaker $C_{35}H_{41}O_8^+$ fragment ion (1.2 x 10^6 cps) was observed at *m/z* 589.2796. Calculated for $C_{35}H_{41}O_8^+$: *m/z* 589.2796. Empirical formulas were assigned using ChemCalc (Patiny and Borel, 2013).

3. Results

3.1. GD754 is neither GDB nor a congener of GDB.

Working from a copy of the Takeda dissertation obtained from Tohoku University, we discovered that Takeda had established GDB to be isomeric with GDA with a molecular weight of 768 Da and that GDB was present in an amount comparable to that of GDA. Prior to obtaining access to the dissertation, we had obtained structural data on GDB (unpublished) that confirmed the description in Takeda's dissertation. Therefore, Krock's GD754 is not GDB. Further evidence that GD754 is structurally different from GDB arises from consideration of HPLC retention times. Takeda found GDB to be significantly more polar than GDA, having a retention time 23.4% less than that of GDA on a C18 reverse phase column when eluted isocratically with 80:20 (v:v) MeOH-H_2O (Fig. 1). In contrast, Krock et al. reported the retention time of GD754 to be only 3.3% less than that of GDA using a 9.5-95% acetonitrile-H_2O gradient (Fig. 2, Panel a: GDA standard and Panel b: overlaid chromatograms of GDA and GD754). Even after making allowance for the difference in elution methodology, it is evident that GD754 is significantly less polar than GDB. The solution conformation for GDA proposed by Takeda (2008) and the similar

crystal state conformation which we observed for GDA (Tainter et al., 2020) place the 5-hydroxy group on ring A close enough to the heterocyclic oxygen atom in ring B for hydrogen bonding to occur whereas the 15-hydroxy group of GDB is exposed to solvent. This structural difference accounts for the observed difference in polarities of GDA and GDB. The polarity of GD754 more closely resembles that of GDA than that of GDB which suggests GD754 is a truncated version of GDA not GDB.

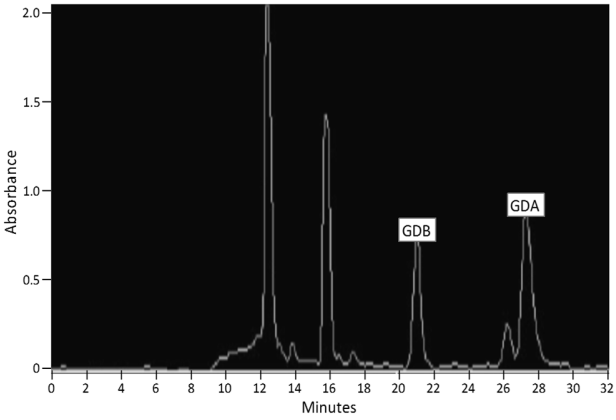
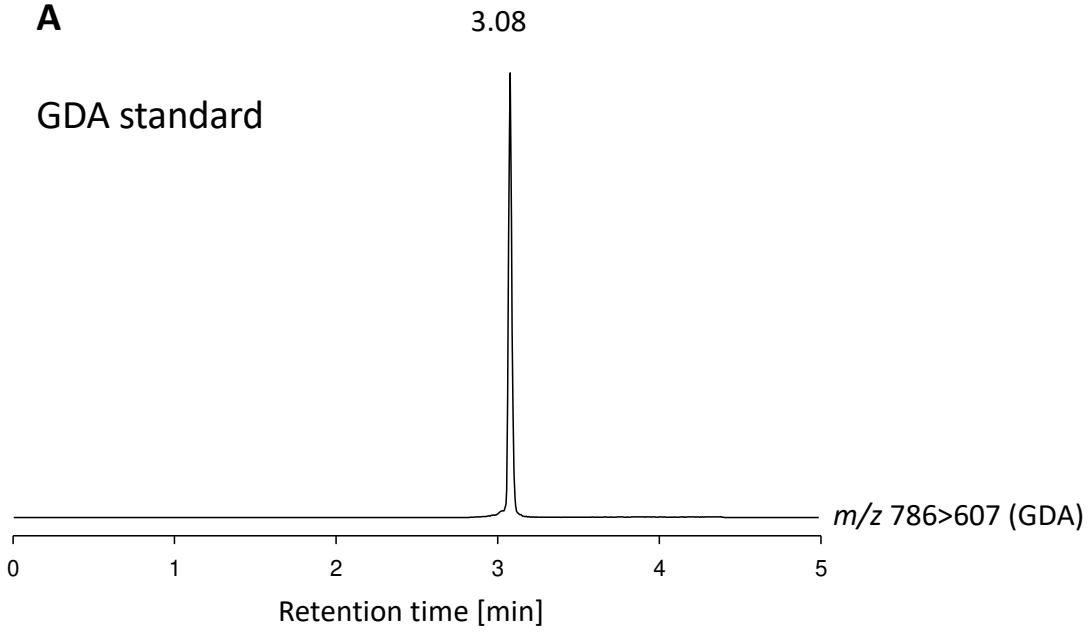


Fig. 1. Takeda's HPLC chromatogram of GDA and GDB: C18 column, isocratic elution with 80:20 MeOH-H_2O, UV detection at 203 nm, retention times of 27.0-28.7 min and 20.8-21.9 min for GDA and GDB, respectively. (Adapted from Takeda's dissertation)



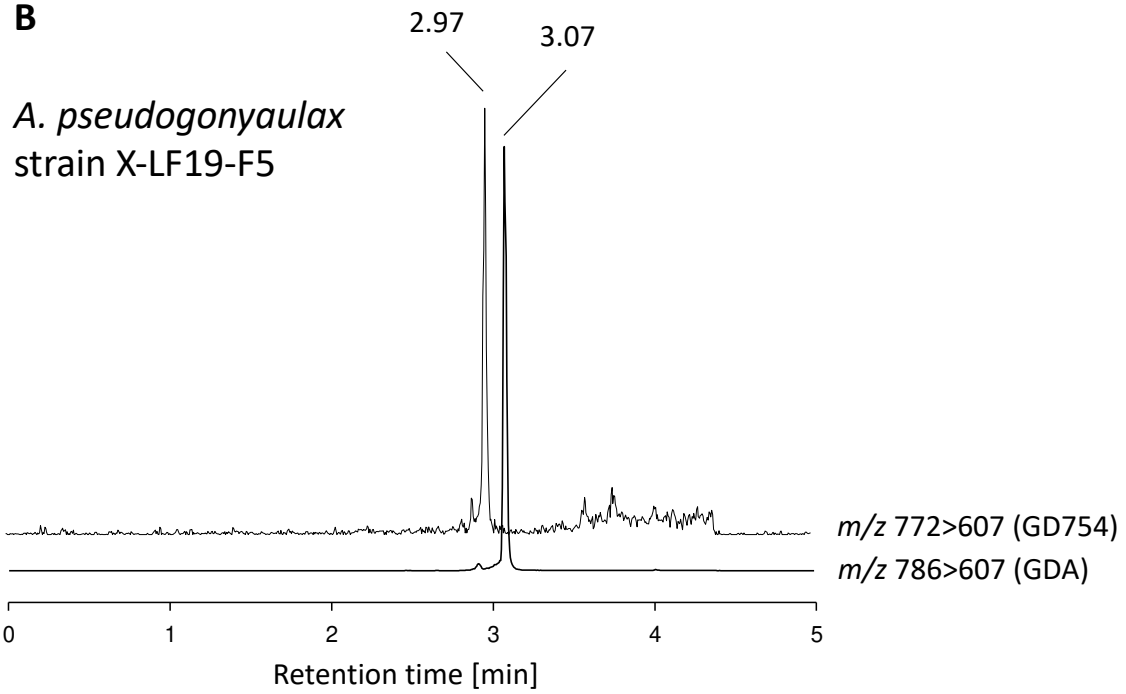


Fig. 2. (A) Ion trace HPLC chromatogram of a GDA standard. (B) Ion trace HPLC chromatogram of a mixture of GDA and GD754 isolated from a *A. pseudogonyaulax* planktonic field sample collected in Limfjord, Denmark: C18 column, gradient elution using a 3.5 min gradient of ACN-H_2O from 9.5% to 95% ACN with addition of 5 mM NH_4HCO_3, ESI-MS detection by MRM transitions: *m/z* 772.5 → 719.5 and *m/z* 772.5 → 607.5 for GD754 (NH_4^+ adduct) and *m/z* 786.5 → 733.5 and *m/z* 786.5 → 607.5 for GDA (NH_4^+ adduct), retention times 3.07 min and 2.97 min for GDA and GD754, respectively. The intensity of GDA is ~ 500-fold greater than that of GD754. (**Figs. 2ab** were provided by Dr. Bernd Krock.)

3.2 GD754 and GDA differ in substitution on ring F.

Information relating to the structure of GD754 is limited to chromatographic observations and mass spectral data (**Fig. 2b**). The latter includes the 754 Da molecular weight and two MRM transitions for the ammonium ion adduct: *m/z* 772.5 → 719.5 and *m/z* 772.5 → 607.5.* Comparable transitions for GDA are *m/z* 786.5 → 733.5 and *m/z* 786.5 → 607.5. Looking first at fragmentation of GDA, the molecular formulas of the fragment ions obtained by collision-induced dissociation (CID) of the ammonium adduct of GDA were established by exact mass measurement (Table 1). A series of ions at *m/z* 751.4051, 733.3946, 715.3836 reflects losses of NH_3 and multiple H_2O molecules. ~~A~~n

intense peak at *m/z* 607.2900 is assigned as $C_{35}H_{43}O_9^+$. Formation of the *m/z* 607 ion is of importance for identification of GD754 because it results from severance of ring F, i.e., the C-31 - C-36 fragment, from the remainder of the molecule. For GDA it involves loss of uncharged species $C_8H_{18}O_3$ (mw 162.1256) (or $C_8H_{16}O_2 + H_2O$). No region in GDA other than ring F contains such a high proton-to-carbon ratio. In formation of the *m/z* 607 cation from GD754, the neutral species would be $C_7H_{16}O_3$ (or $C_7H_{14}O_2 + H_2O$) with a molecular weight 14 Da less than its counterpart derived from GDA. The proposed structure for $C_8H_{18}O_3$ derived from GDA is shown in Fig. 3. For GD754, $C_7H_{16}O_3$ must arise similarly. The fact that the *m/z* 607 ion is being formed from both GDA and GD754 unambiguously places the site of deletion in ring F.

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* Typographical errors in Krock et al. (2018) and Kremp et al. (2019) misrepresent the transitions for GD754 as *m/z* 722.5 → 719.5 and *m/z* 722.5 → 607.5 rather than *m/z* 772.5 → 719.5 and *m/z* 772.5 → 607.5. (Personal communication from B. Krock.)

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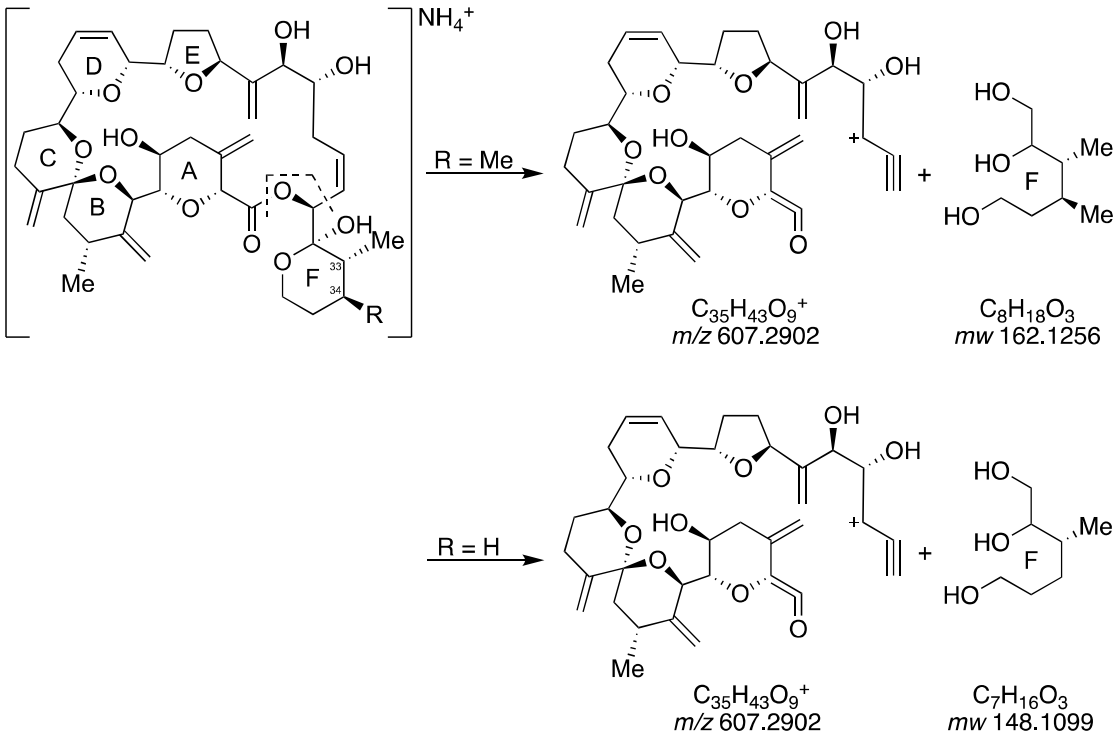

Fig. 3. Proposed fragmentation of GDA (**1**, R = Me) and 34-desmethyl-GDA (**4**, R = H). Neutral fragment from **1** is $C_8H_{18}O_3$; neutral fragment from **4** is $C_7H_{16}O_3$.

Table 1.

Collision-induced fragmentation of the NH_4^+ adduct of GDA.

Observed m/z (Intensity)	Formula	Calculated (m/z)	Proposed assignments Cationic fragment
786.4421 (1.3e7)	$C_{43}H_{64}NO_{12}^+$	786.4423	Parent cation (GDA-NH_4^+)
751.4044 (5.7e5)	$C_{43}H_{59}O_{11}^+$	751.4052	C1-C36
733.3944 (9.0e5)	$C_{43}H_{57}O_{10}^+$	733.3946	C1-C36
715.3836 (5.2e5)	$C_{43}H_{55}O_9^+$	715.3841	C1-C36
607.2900 (5.4e5)	$C_{35}H_{43}O_9^+$	607.2902	C1-C36
501.2483 (2.6e5)	$C_{28}H_{37}O_8^+$	501.2483	C1-C24
491.2791 (3.3e5)	$C_{31}H_{39}O_5^+$	491.2792	C2-C27
483.2376 (2.1e5)	$C_{28}H_{35}O_7^+$	483.2377	C1-C27
457.2222 (3.3e5)	$C_{26}H_{33}O_7^+$	547.2221	C1-C22
439.2115 (2.2e5)	$C_{26}H_{31}O_6^+$	439.2115	C1-C22
431.2064 (3.4e5)	$C_{24}H_{31}O_7^+$	431.2064	C1-C20
421.2373 (7.3e5)	$C_{27}H_{33}O_4^+$	421.2373	C2-C24
421.2008 (2.0e5)	$C_{26}H_{29}O_5^+$	421.2010	C1-C22
415.2266 (2.2e5)	$C_{28}H_{31}O_3^+$	415.2268	Unassigned
415.2116 (2.1e5)	$C_{24}H_{31}O_6^+$	415.2115	C2-C22

3. Discussion

Murakami, after establishing the structure of GDA, carried out a study of its biosynthesis. The study entailed feeding experiments with [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate and with [*methyl*-^{13}C]-methionine, employing NMR to identify the sites of incorporation (Fig. 4) (Murakami et al., 1998). Of the 43 carbon atoms in GDA, all but three were labeled by [^{13}C]-acetates. The three carbon atoms having other origins are in ring F. C-35 and C-36 are the initiating unit in the polyketide chain. They were assumed to be derived from glycolate, based on analogy with okadaic acid and dinophysistoxins-1 and -4, where initiation of the polyketide chain by glycolate had been demonstrated (Needham et al., 1994 and 1995). The 33-Me was labeled by [2-^{13}C]-acetate but the 34-Me was labeled by methionine. [1,2-$^{13}C_2$]-acetate simultaneously labeled C-33 and C-34, showing that they are derived from an intact acetate group. The 33-Me is attached to the carbonyl position of that acetate unit and 34-Me is attached to the methyl position. Terrestrial polyketides arise uniformly by alternating methyl and carbonyl groups, reflecting the intermediacy of poly-β-keto acids. Polyketide metabolites produced by marine organisms frequently exhibit

anomalous labelling patterns in which some of the adjacent carbons are derived from methyl carbons of acetate. In GDA there are 8 locations where two methyl groups of acetate are directly linked. The phenomenon was originally proposed to result from involvement of TCA cycle metabolism (Chou & Shimazu 1987; Lee et al., 1989) but this hypothesis has been abandoned after further study showed that deletion of carbonyl groups by Favorski reactions appeared more likely (Wright et al., 1996).

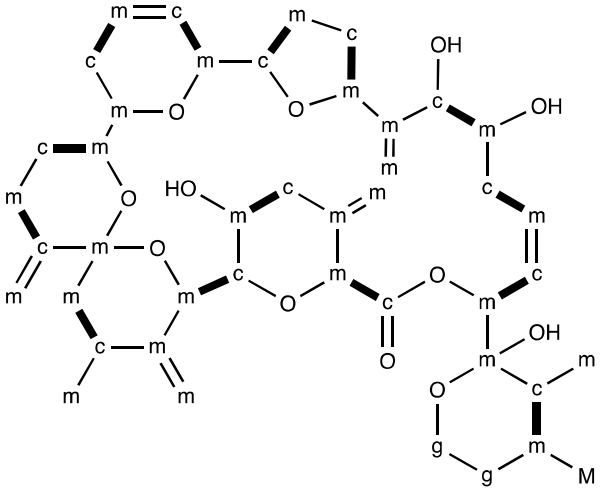

Fig. 4. Labeling pattern of goniodomin A. m: acetate methyl, c: acetate carbonyl, M: methionine methyl, g: glycolate (inferred), bold bond: intact acetate unit. (Adapted from Murakami et al. 1998)

Congeners involving deletion of C-methyl groups are relatively common in marine polyketide metabolites. For example, congeners of spirolide C have been observed that are missing 13-Me and both 13-Me and 19-Me. Biosynthetic experiments carried out on 13-desmethyl spirolide C failed to detect incorporation of ^{13}C-acetate species into the 19-Me group suggesting a methionine origin for 19-Me but methionine incorporation experiments have not been reported (MacKinnon et al., 2006). Gymnodimines have also been reported with varying levels of C-methylation (Zurhelle et al., 2018) but again biosynthetic experiments have not been reported. Four of the seven C-Me groups on brevetoxin B (BTX-B) have been shown to arise from methionine with the other three being derived from the methyl group of acetate (Lee et al., 1989). Desmethyl brevetoxins have not been reported.

4. Conclusions

The structure of GD754 might involve deletion of the acetate-derived 33-Me or the methionine-derived 34-Me. Other potential sites of deletion in ring F are improbable. Of the two methyl groups, 34-Me appears more likely to be the one deleted because a deletion process comparable to that for 33-Me could equally well have occurred with 9-Me. In that case, the deletion would have led to appearance of an m/z 593 fragment in the CID and MRM spectra of the truncated congener. As a consequence, we conclude the most likely structure for GD754 is 34-desmethyl GDA (**4**). Further experimental work will be required to confirm this hypothesis. Manipulation of the biosynthesis of GDA using d_3-methionine or inhibitors of *S*-adenosylmethionine synthetase might be attractive approaches.

Ethical statement

The authors declare to follow the ethics outlined in the Elsevier 'ethics in research and publication procedure'.

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CRediT authorship contribution statement

Constance Harris: Investigation, Resources, Writing - review & editing. **Kimberly Reece:** Funding acquisition, Resources, Writing - review & editing. **Thomas Harris:** Conceptualization, Investigation, Methodology, Visualization, Formal analysis, Writing - original draft, review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Graphical Abstract