Structure Elucidation

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On the Structure of Maitotoxin**

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In memory of Luigi Gomez-Paloma

The renowned toxicity of maitotoxin has elicited the attention of isolation and structural chemists over the last few decades.[1–4] As a result of their investigations, maitotoxin (**1**, Figure 1) was found to not only be the most potent, but also the largest nonprotein substance known. The overall structure of maitotoxin, which contains 32 rings and 99 elements of stereochemistry (98 stereogenic centers and one trisubstituted double bond), and its absolute stereochemistry was proposed in a series of studies by the research groups of Yasumoto,[2] Tachibana,[3] and Kishi[4] between 1992 and 1996.

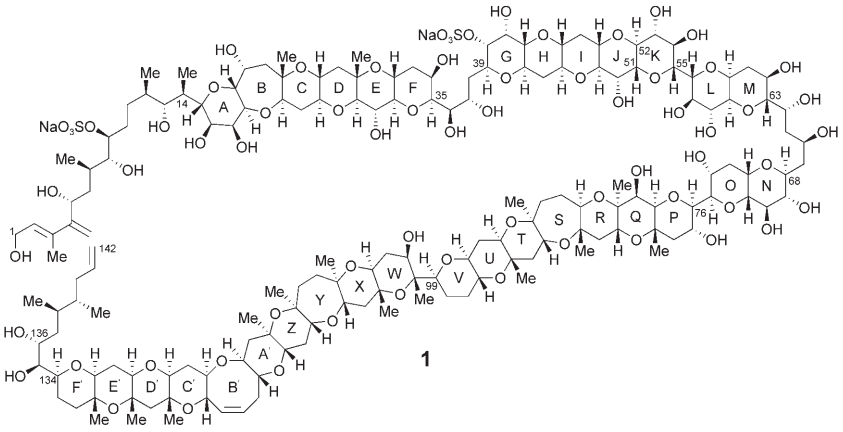

Figure 1. Originally proposed structure of maitotoxin (**1**).[2–4]

In 2006, however, this structure came under the scrutiny of Gallimore and Spencer.[5] These investigators questioned the stereochemistry at the J/K ring junction (C51/C52) which was originally proposed as the opposite of that expected on the basis of biosynthetic considerations, and they noted the exceptional nature of the C51/C52 structural motif of the assigned ladderlike structure of **1** in comparison to other similar marine biotoxins. Indeed, a comparison of polyether marine ladders revealed regularity in the stereochemistry of all the members of this class of naturally occurring substances isolated to date, with the exception of this one site of maitotoxin. Specifically, Gallimore and Spencer implied that the C51 and C52 positions should have the opposite relative configuration.

This revision would bring maitotoxin in line with the other polyether marine biotoxins and thus maitotoxin would then conform to the biosynthesis-based stereochemical hypothesis of Gallimore and Spencer that states that all internal ether rings within fused polyether ladders of natural origin should possess *syn* stereochemical relationships across the oxygen bridge. The authors argue that this stereochemical regularity essentially arises from the assumed common biosynthetic origin of these structures which involves the enzymatic epoxidation (from the same face) of polyunsaturated precursors followed by enzymatic openings of the epoxide with predictable stereochemical outcomes.[5] It should also be noted that the stereochemical assignment of the J/K region of **1** is especially complex. Indeed, as a result of the overlap of signals in the ^{1}H NOESY NMR spectra, advanced three-dimensional NMR techniques were required to assign the stereochemistry of that domain of the molecule.[2] In view of this, and given the importance of the marine neurotoxins, the reexamination of the structure of maitotoxin is warranted. Herein, we discuss some alternative structures and conclude that the originally proposed structure[2–4] is most likely correct.

For the originally proposed structure of maitotoxin (**1**), Yasumoto et al. reported NOE signals between the protons situated on the GHIJK ring system (H39–H55) as shown in the partial structure **1p** (Figure 2a).[2c,d] Reversal of the stereochemistry at the J/K ring junction (H51/H52) leads to partial structure **2p** (Figure 2b), which leaves much to be desired in terms of the observed NOE signals. In other words,

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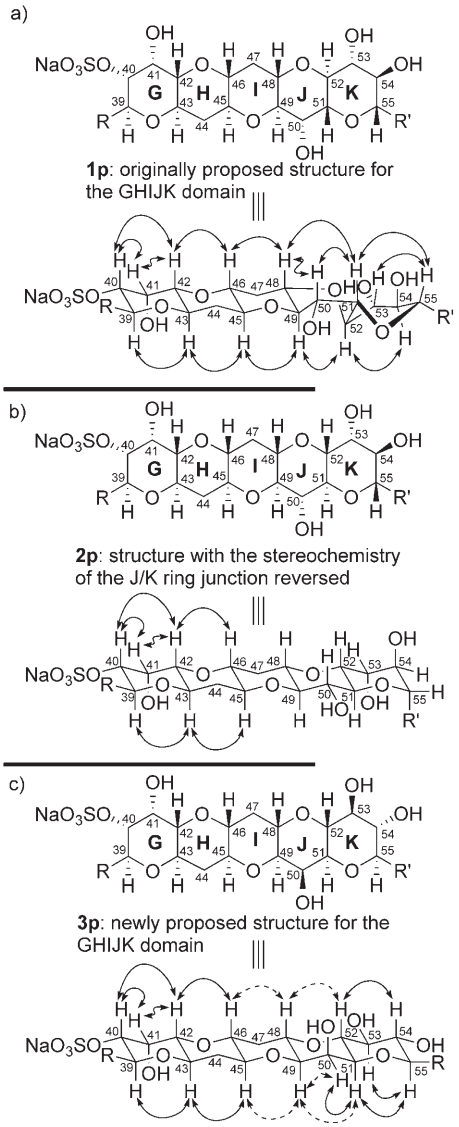


Figure 2. a) Originally proposed partial (GHIJK domain) structure **1p** of maitotoxin; b) revised partial (GHIJK domain) structure **2p** with stereocenters at C51 and C52 reversed; c) revised partial (GHIJK domain) structure **3p** with stereocenters at C50–C55 reversed. Solid arrows indicate the reported NOEs; broken arrows in **3p** indicate reported NOEs that can be explained if the assignments at H48 and H49 are reversed.

although the revised structure **2p** conforms to the biosynthetic principle proposed by Gallimore and Spencer,[5] the structure is not sufficiently supported by the reported NMR spectroscopic data for maitotoxin. However, if the configurations of the stereocenters at the positions C50, C53, C54, and C55, in addition to those at C51 and C52, are inverted, the resulting structure **3p** (Figure 2c) restores the potential for the NOE signals to be in accord with the observed data.[2] This scenario becomes viable with the assumption that the positions H48 and H49 were misassigned with each other, a possibility that could arise from the overlap of the signals for H45 and H46 (both at $\delta =$ 2.98 ppm),[2] of which the NOE signals must have been considered in the assignment of H48 and H49. This hypothetical occurrence would elevate structure **3p** above **2p** as a probable alternative to **1p** in view of some of the spectroscopic data for maitotoxin. It should be noted, however, that even structure **3p** does not satisfy all the reported NMR data for this region of the molecule. Be that as it may, this could not be the end of the story, since these changes to the initially proposed structure[2–4] of maitotoxin would necessitate further revisions along its backbone as discussed below.

The absolute stereochemistry of the overall structure of maitotoxin was determined from the absolute stereochemistry of the C136–C142 domain, which was established by comparison of fragments derived from degradation and synthetic studies. The absolute stereochemistry of the C136–C142 domain was used to assign the absolute stereochemistry of the F′E′D′C′B′A′ZYXW domain, which was then used to assign the absolute stereochemistry of the VUTSRQP domain, which was used to assign the absolute stereochemistry of the ONML domain, which, in turn, was relied upon to assign the absolute stereochemistry of the GHIJK domain. If the alternative structure **3p** depicted in Figure 2 represents the relative stereochemistry within the GHIJK domain, the relative stereochemistry between the K and L rings should now be the opposite, since the stereocenter at C55 is inverted. As a result, the GHIJK domain should be enantiomeric to the partial structure **3p**, and, since the absolute stereochemistry of the C1–C14 and ABCDEF domains was assigned relative to the GHIJK domain, these segments of the molecule should also be enantiomeric. Thus, assuming that the hypothesis of Gallimore and Spencer is correct, and factoring in all of its consequences, structure **4** (Figure 3) emerges as a logical alternative for the originally proposed structure of maitotoxin (**1**).

To test the relative merits of the two structures **1p** and **3p**, as well as **2p** (Figure 2), in which only the C51 and C52 centers are inverted, and inspired by the recent success of Rychnovsky in which the true structure of hexacyclinol was predicted,[6,7] we employed the program Spartan'06[8] to calculate the ^{13}C NMR chemical shifts of the truncated structures **1t**, **2t**, and **3t**, which represent the three alternative maitotoxin structures under consideration. The objective was to compare the calculated values to those reported for maitotoxin and see if any conclusions could be reached regarding the structure. To gain confidence in these computations, we first applied the strategy to brevetoxin B (**2**), whose structure was determined by spectroscopic means and X-ray analysis,[9] and has been confirmed by chemical synthesis.[10] Figure 4 demonstrates the excellent agreement be-

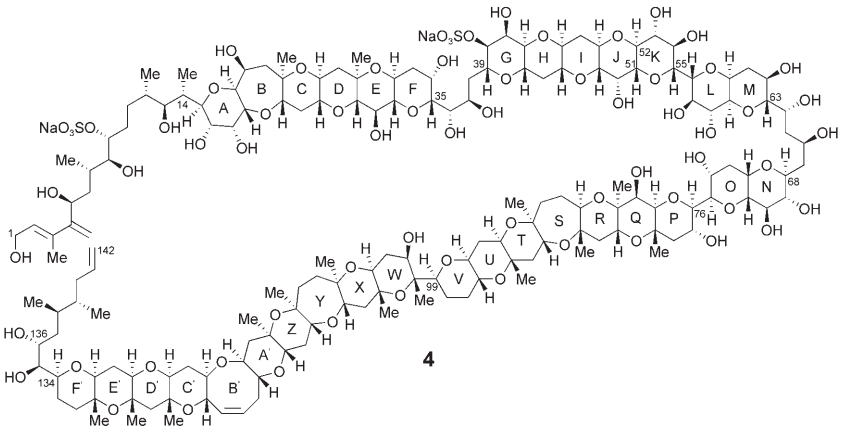


Figure 3. Alternate structure of maitotoxin if the stereochemistry at the J/K ring junction of the originally proposed structure is reversed.

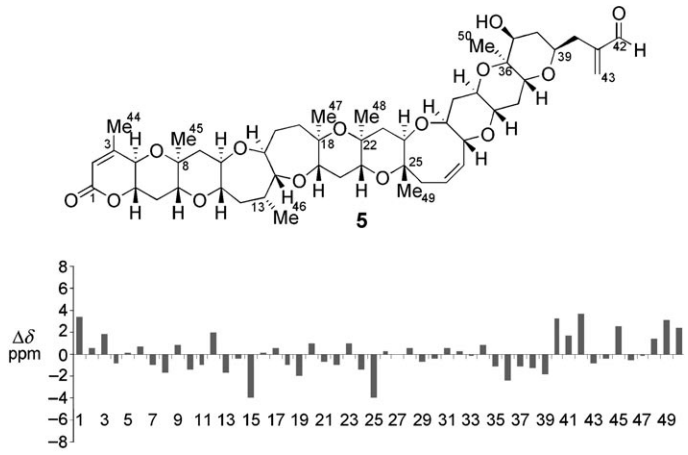


Figure 4. Differences (in ppm) in the calculated and experimental chemical shifts for the ^{13}C NMR spectrum of brevetoxin B (**5**). The average difference was found to be 1.24 ppm with the maximum difference being 3.74 ppm (C15).

tween the calculated and experimental ^{13}C chemical shifts (average difference of 1.24 ppm), a finding that enhanced our confidence in this computational approach to structure elucidation. The method was therefore considered as a valuable tool to probe the issues over the structures in this series of compounds which includes maitotoxin.

The results with truncated structures **1t**, **2t**, and **3t** are shown in Figure 5, and reveal that structure **1t** exhibits the closest agreement between the calculated and experimental values of chemical shift, with an average difference of 2.01 ppm as compared with average distances of 2.85 ppm for **2t** and 2.42 ppm for **3t**. The average differences are even more significant when we focus on the immediate structural domain in question (C48–C55). For this region, structure **1t** exhibits an average difference of 0.78 ppm with a maximum absolute difference of 2.1 ppm for C55 for the calculated and experimental ^{13}C NMR chemical shifts. This difference compares with an average difference of 3.03 ppm and a maximum absolute difference of 7.5 ppm (C48) for **2t**, and an average difference of 2.89 ppm with a maximum absolute difference of 5.0 ppm (C52) for **3t**. It is interesting that these calculations support the originally proposed structure of maitotoxin, although they do not by themselves constitute an absolute proof of its correctness. It is also interesting that the second-best structure in terms of agreement between calculated and experimental values of ^{13}C NMR chemical shift is **3t**, rather than **2t**, which underscores the problems associated with simply switching the configurations at C51 and C52 without further changes. Nevertheless, in light of the highly complex structure of maitotoxin possible errors could occur either in the calculations and/or the interpretation of the NMR spectroscopic data. While the above findings seemingly constitute an intransigent conundrum in view of the sensible hypothesis of Gallimore and Spencer,[5] a solution may be found in further biosynthetic considerations, as outlined below.

An alternative biosynthetic plan for the formation of the JK ring framework (that could be extended to the LM and NO ring systems) is shown in Scheme 1. Here, instead of a diepoxide opening, which involves an anomalous *S*,*S* epoxide and which is initiated by the C55 hydroxy group (**6**, Scheme 1), one can consider the participation of C-glycosidic structural motifs, which have appropriately positioned hydroxy groups, in the epoxide openings (**7**, Scheme 1).

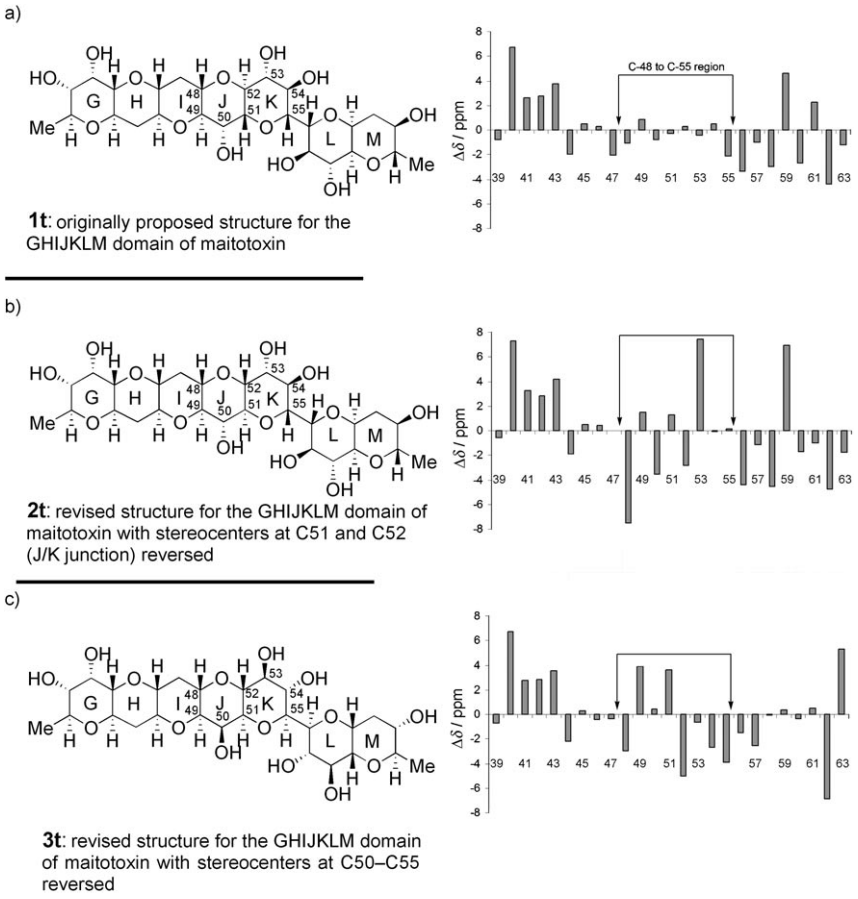
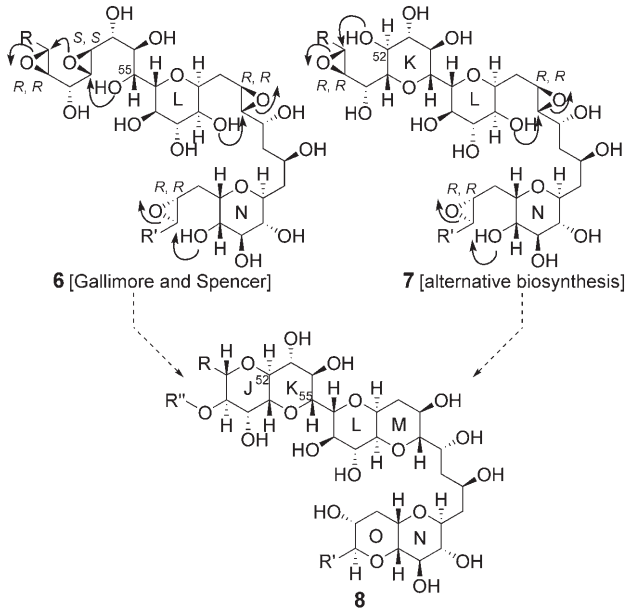


Figure 5. Differences (in ppm) in the calculated and experimental chemical shifts for the ^{13}C NMR spectrum of a) **1t**, b) **2t**, and c) **3t**. The average differences for the most relevant carbons (C48–C55) for **1t**, **2t**, and **3t** were found to be 0.78, 3.03, and 2.98 ppm, respectively, with the maximum differences being 2.1, 7.5, and 5.0 ppm, respectively.



Scheme 1. Alternative biosynthetic route to the J/K ring domain **8** of maitotoxin.

This alternative route would forge the J/K ring junction with the originally proposed stereochemistry (**8**, Scheme 1),[2–4] and avoids violating the hypothesis of Gallimore and Spencer.[5] However, this route requires an explanation as to how these C-glycoside-type structures are formed in the first place, a legitimate query that remains to be elucidated.[11] The advantage of this proposal is that it could also explain the formation of the LM and NO domains of the molecule, without the need for infringement on any biosynthetic considerations.

The above discussion provides support for the originally proposed structure **1**[2–4] for maitotoxin despite the doubts cast by the hypothesis of Gallimore and Spencer. However, we wish to point out that other alternatives may still be viable.

Maitotoxin has thrown the gauntlet to chemists yet again. Based on the assumption of Spencer and Gallimore that the J/K ring junction may be wrongly assigned, we have presented logical arguments for a hypothetical alternative structure for maitotoxin (that is, **4**, Figure 3), in which the modifications must go beyond the J/K ring junction. However, on the basis of reported NMR spectroscopic data and computational studies, the originally proposed[2–4] structure of maitotoxin (**1**) still remains the most likely to be correct, a conclusion that does not necessarily have to violate the biosynthetic hypothesis of Gallimore and Spencer, since alternative modes of biogenesis may be envisioned. In the absence of a X-ray crystal structure of maitotoxin, the issue of its impressive structure can only be confirmed by chemical synthesis. As an initial response to this challenge, the construction of partial structures such as the two truncated GHIJK domains (**1t** and **3t**, Figure 5) may be appropriate as a further means to confirm the partial structure of this remarkable molecule.

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[11] A different set of enzymes would most likely be required for the formation of the K, L, and N rings of structure **7** (Scheme 1) in this scenario. Since very little is known about the biosynthesis of maitotoxin and related substances, any suggestions regarding their generation in nature should be considered as mere speculation.

