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# Complete structure of maitotoxin

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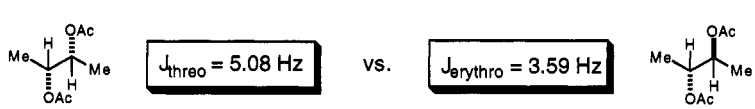
*Abstract*: Using the AAL-toxin/fumonisin class of natural products as an example, the validity and usefulness of a new approach for stereochemical assignment via organic synthesis are discussed. This approach is then extended to the case of the marine natural product maitotoxin.

Synthetic organic chemistry is often conceived of as a relatively matured discipline. However, we would like to point out the fact that the progress made for the past decade or two is truly overwhelming. Our ability to synthesize a molecule with many chiral centers illustrates this point. The limit of our reach in the late 1970's was a molecule with 10 chiral centers. In other words, we were able to synthesize selectively one out of 1,024 stereosiomers possible for lasalocid A [ref. 1]. This number exponentially expanded; it was 66,000 in 1979 [ref. 2], 520,000 in 1982 [ref. 3], and $1.2 \times 10^{21}$ in 1994 [ref. 4]. We realize that this is only one aspect to measure the progress made in the field of synthetic organic chemistry. Nevertheless, it is compelling. Some might consider that synthetic organic chemistry is the subject of labor work, yet we should emphasize that it represents truly the development of new synthetic concepts, new synthetic tactics and strategy, new reactions and reagents, and new techniques. However, we should note that synthetic organic chemistry is not yet perfect, not even close in our view. There are a number of major problems that remain unsolved in this field, but we are convinced that this extraordinary pace of progress will continue, and a perfect solution will be provided to any given synthetic problem someday. At the same time, we recognize that there are many questions which might have been impossible or unrealistic to ask ten years ago but, because of the extraordinary progress we have witnessed, some of them are now feasible.

In this context, we focus on the issues related to the stereochemical assignment via organic synthesis. This program originated from our curiosity on the marine natural product palytoxin (PTX) [ref. 5]. PTX is the toxic principle isolated from marine coelenterates belonging to the genus *Palythoa*. It is one of the most toxic substances known to date. The gross structure of PTX was elucidated in 1981 by two groups independently, the one led by Professor Hirata at Nagoya in Japan and the other by Professor Moore at Honolulu in the United States. Our interests were primarily two-fold: chemical synthesis and conformational analysis. However, we realized that the information given by the gross structure was not sufficient enough to address our chemical questions properly. For this reason, the first priority of our work was to establish the complete structure of PTX.

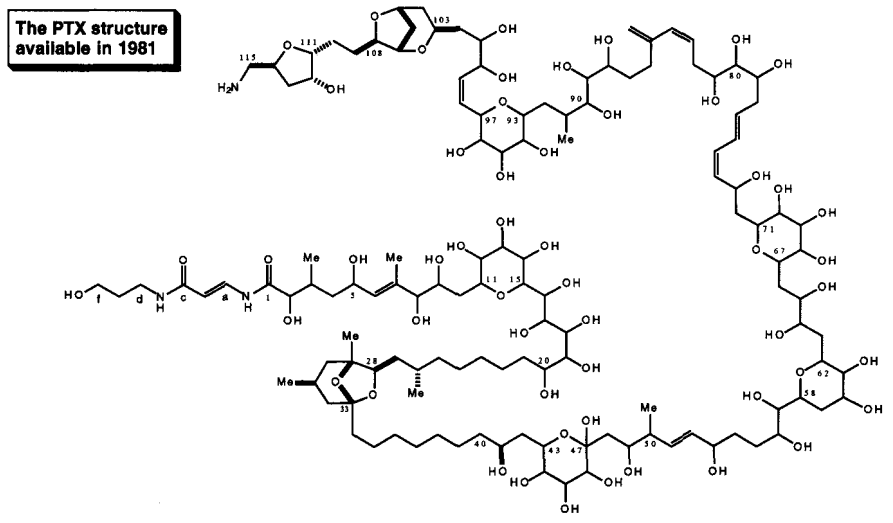
The structure on the next page shows the gross structure of PTX available in 1981. In addition to 4 *trans*- and 3 *cis*-olefinic bonds, PTX contains 64 chiral centers. Twenty-nine of them are in the acyclic portions, and the stereochemistry of 27 of them was unknown then. Our major concern was how to assign their relative and absolute stereochemistry. In this connection, we may note that PTX is amphiphilic, and it or its direct derivatives have not been crystallized even now.

It is widely recognized that vicinal $^1H$-$^1H$ coupling constants observed for acyclic threo isomers are smaller than those of the corresponding erythro isomers. This observation is explained in the terms of the preferred conformation of a given substrate. However, in order to apply this generally recognized trend for the stereochemical assignment of an unknown system, one has to be sure that there is no exception for this trend. In this context, the work on 2,3-diacetoxybutanes by Bothner-By in 1962 is instructive [ref. 6].



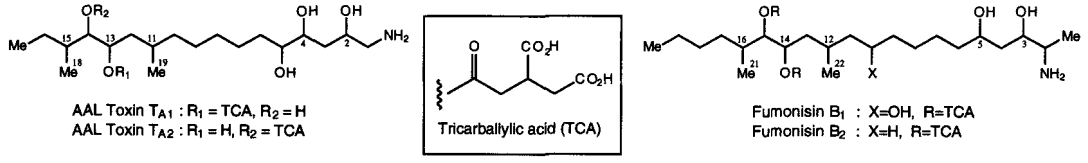

Having given some thoughts on the X-ray- and NMR-based approaches, we opted to rely on organic synthesis. In brief, we decided to synthesize all the diastereomers possible for the eight major degradation products of PTX in order to confirm that all of them can be distinguished by currently available

spectroscopic and/or chromatographic means and then to find which diastereomer matches the given degradation product. On the basis of extensive efforts approximately for two years, we were able unambiguously to establish the complete structure of PTX [ref. 7].




The stereochemistry assignment via organic synthesis did provide the essential foundation for the further chemical investigations on PTX. Under the given circumstances, we can't imagine that any other method could give us an equally unambiguous conclusion. However, we also realize that this work was possible only with enormous efforts by many coworkers. We have been wondering how we might be able to decrease the amount of man-power efforts, yet gain the equally unambiguous conclusion. We shall use the stereochemistry assignment of the AAL-toxin/fumonisin class of natural products as the next example.

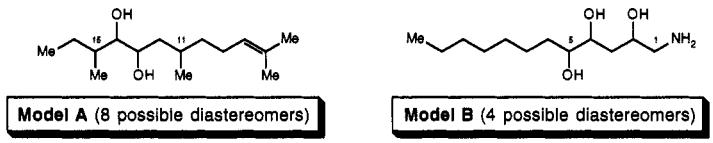
AAL toxins are a family of host-specific phytotoxins found in the tomato pathogen and are known to cause the tomato stem canker disease. The gross structures of AAL toxins were determined in 1981 by Bottini and Gilchrist [ref. 8]. All of the AAL toxins exist as a regioisomeric mixture of the C.13/C.14 tricarballylic acid ester group. No class of natural products was known to have a similar structure until 1988, when Vleggaar reported the gross structures of the fumonisin mycotoxins, found in the corn pathogen [ref. 9]. Fumonisins trigger a variety of biological events [ref. 10]. Most notably, fumonisins are shown to be associated with human esophageal cancer. Coupled with the fact that fumonisins are detected in commercial corn products, this family of mycotoxins has drawn a great deal of attention. Surprisingly, the relative and absolute stereochemistry was not established for any member of these two classes of natural products.



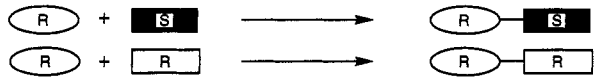

We were intrigued with one specific structural feature commonly found in this class of natural products; they possess two distinct halves bearing chiral centers at remote positions on their backbones. We felt these classes of natural products to be ideal for testing the feasibility and reliability of a new concept for determining relative and absolute stereochemistry via organic synthesis, which is illustrated using the aminopentol backbone of AAL toxin $T_A$, i.e., $R_1$=$R_2$=H in the above structure. The aminopentol backbone of AAL toxin $T_A$ had 7 unknown asymmetric centers, and therefore there were 64 possible enantiomeric pairs of diastereomers to represent its stereochemistry. Its stereochemistry could be established via organic synthesis; namely, as we did in the case of PTX, all of the possible diastereomers could be synthesized and compared with the aminopentol backbone derived from the natural product. However, there were two concerns about this standard approach. First, the synthesis of 64 possible enantiomeric pairs of diastereomers represented a substantial effort. Second, and more critically, even if all of the possible diastereomers were prepared, there was no assurance that all of them could be differentiated by currently available spectroscopic and/or chromatographic techniques.

The new approach consisted of three phases. The aminopentol backbone derived from AAL toxin $T_A$ was composed of two distinct halves with the asymmetric centers separated by five methylene units. This separation was assumed to be great enough for each half to have chemical properties independent from its

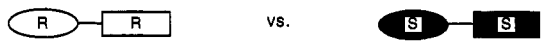
opposite half. Thus, the models **A** and **B** should represent the relative stereochemical characteristics of the left and right halves of the AAL toxin $T_A$ aminopentol backbone, respectively. With this assumption, the first phase of this approach involved: (1) the choice of the models **A** and **B** representing the structural characteristics of the left and right halves of the aminopentol backbone, respectively; (2) the synthesis of all the diastereomers possible for **A** and **B**; (3) the determination of which stereoisomers of **A** and **B** represent the left and right halves of the aminopentol backbone, respectively. There were 8 diastereomers possible for the left half model **A** and 4 diastereomers possible for the right half model **B**. Therefore, this operation requires the synthesis of only 12 diastereomers instead of 64, and would allow us to determine the relative stereochemistry of the left and right halves of the aminopentol backbone of AAL toxin TA.



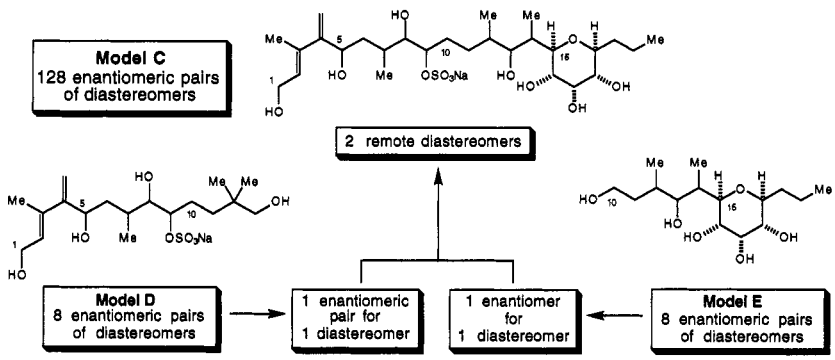

The second phase was to determine the relative stereochemistry between the left and right halves of the AAL toxin $T_A$ aminopentol backbone. The left half is represented by an oval, and since only the relative stereochemistry of the left half would be known at this stage, it could exist in either an *R* or *S* configuration. The same holds true for the right half, which is depicted as a rectangle. Coupling of the *R*-left half with the *S*-right half would provide an *R-S* aminopentol backbone. Likewise, coupling of the *R*-left half with the *R*-right half would yield an *R-R* aminopentol backbone. In the case of diastereomers bearing chiral centers in close proximity, the pair could be distinguished from each other by spectroscopic and/or chromatographic means. However, as the diastereomers in question had chiral centers at remote positions (we refer to such cases as *remote diastereomers*), the degree of chemical communication between the two remote moieties should be negligibly small, if any exists. Indeed, the proposed first phase of this approach relied on this very assumption. We planned to apply the concept of molecular recognition to distinguish these remote diastereomers from each other. Interactions of these diastereomers with an achiral foreign molecule might differ significantly enough to be detected by currently available spectroscopic and/or chromatographic techniques.



The final phase of this approach was to distinguish the enantiomers of the AAL toxin $T_A$ aminopentol backbone, i.e., *R-S* vs. *S-R* or *R-R* vs. *S-S*. This problem could be solved by several methods, including the possibility of using a chiral, instead of achiral, foreign molecule for molecular recognition.



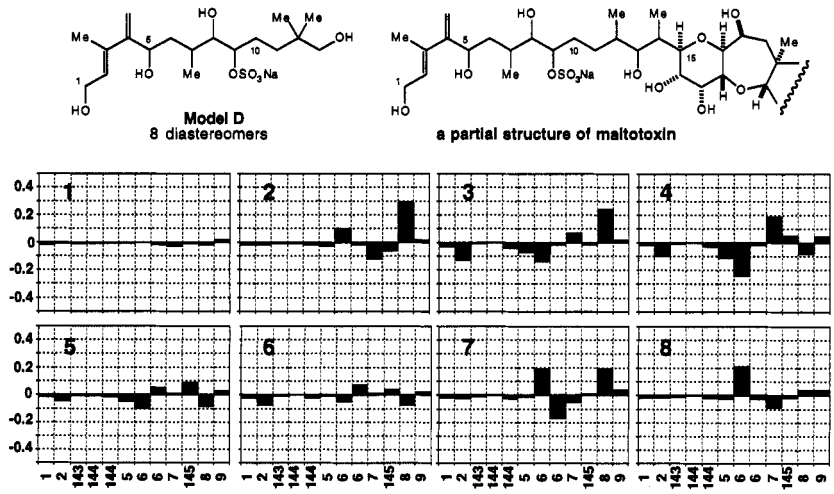

The validity and usefulness of this approach was demonstrated first for determination of the relative and absolute stereochemistry of the aminopentol backbone of AAL toxin $T_A$ and then for the aminotetraol backbone of fumonisin $B_2$ [ref. 11]. Being encouraged by these results, we proceeded to the next level of issues in this approach. We shall use the marine natural product maitotoxin (MTX) as the next example.




Recently, the gross structure of MTX was reported with the stereochemistry assignment of the cyclic portions, but the stereochemistry of the four separate acyclic portions remained unknown [ref. 12]. The C.1-C.15 portion presented the most intriguing opportunity, at least for us. PTX had the structural portions that contained many centers clustered in close proximity, whereas AAL toxins and fumonisins had structural segments that were widely separated. However, the C.1-C.15 portion of MTX has *moderately* separated

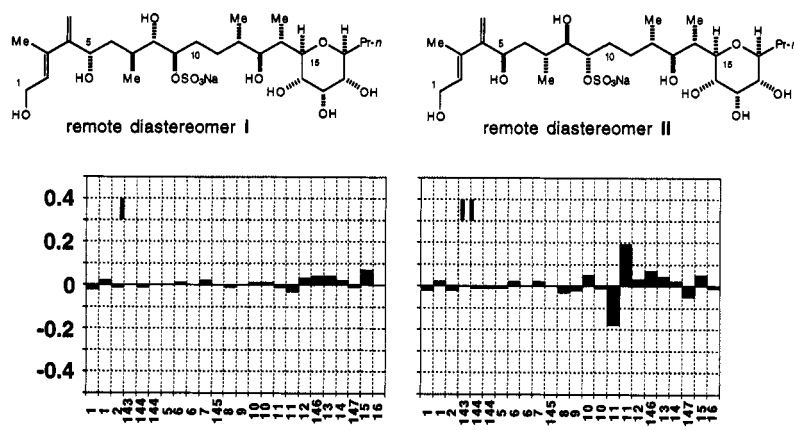
regions of chirality. More specifically, we were curious to test whether two structural moieties spaced by only two methylene groups could be treated independently, in order to determine its relative stereochemistry by synthesizing only 18 compounds, i.e., 8 diastereomers possible for the model **D**, 8 diastereomers possible for the model **E** and 2 remote diastereomers possible for the model **C**, instead of the 128 diastereomers possible for the model **C**—note the C.15 stereocenter to be included for this study. At the same time, however, we still hoped that some residual chemical communication through the two methylene groups could be detected by currently available spectroscopic means. In this context, it should be noted that even a trace amount of the natural product was not available for our work, and we solely depended on the published NMR data to conclude the complete relative stereochemistry assignment.

**$^1$H NMR comparison of 8 diastereomers of D with MTX**




We have synthesized all the diastereomers possible for **D** and **E** and compared their NMR characteristics with those reported for MTX. For example, the graph shown above indicates the difference in the proton chemical shifts between MTX and all the diasteromers possible for the model **D**. No imagination is required to conclude that the diastereomer **1** represents the stereochemical characteristic of this portion of MTX. We were thus ready to proceed to the next phase to question whether or not we can detect some chemical communication between these two portions through the two methylene groups. In this connection, we noticed the very encouraging phenomena on this model system. We originally opted in placing the geminal dimethyl group at C.12 to simplify the spectroscopic analysis. However, we observed that the geminal dimethyl groups of **1** exhibited the small, but distinct diastereotopic property in its proton NMR spectrum.

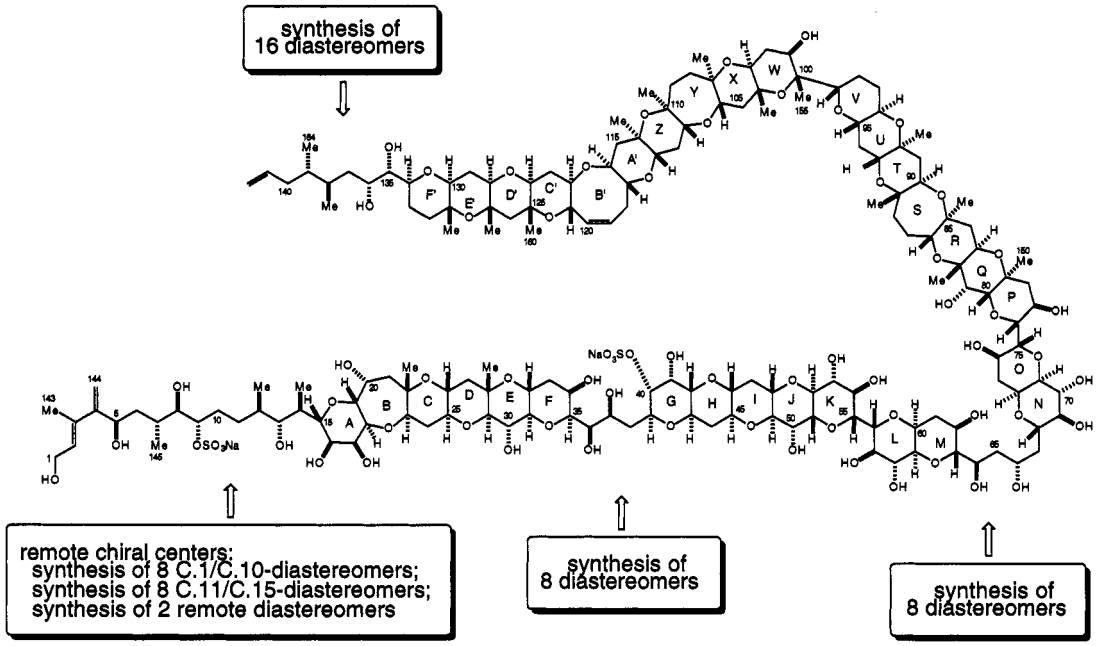
**$^1$H NMR comparison of 2 remote diastereomers I and II of C with MTX**




Being encouraged with this observation, we synthesized the two remote diastereomers **I** and **II** remaining as candidates at this stage, and studied their NMR characteristics, which clearly exhibited a small but distinct difference, particularly at the C.10 and C.11 portions. Thus, the relative stereochemistry of this portion of MTX was established as depicted in the remote diastereomer **I**.

There are three additional acyclic portions, i.e., the C.35-C.39, C.63-C.68, and C.134-C.142, in the MTX structure. We adopted the same approach as the one used in PTX, i.e., to synthesize all the possible diastereomers, to compare their spectroscopic characteristics with those of the natural product and confirm all of them are distinguishable by NMR spectroscopy, and to identify the matching diastereomer. Indeed, these exercises did allow to conclude the relative stereochemistry for these acyclic portions.

MTX contains 32 rings, all of which exist as fused-ring systems. Using primarily the NMR methods, Yasumoto and coworkers have successfully determined the relative stereochemistry of these fused-ring portions [ref. 12]. Coupled with this information, the present work allowed unambiguously to establish the complete relative stereochemistry of MTX as summarized, with the indication of the type of approaches adopted for each case [ref. 13,14].




Regarding the absolute stereochemistry, Yasumoto reported the periodate cleavage of the C.135-C.136 glycol of MTX [ref. 12], which should obviously provide an ideal handle to address this issue experimentally. Because of the lack of MTX for our disposal, we were unable to conduct any experimental work along this line. However, in comparison with brevetoxins, ciguatoxins, and related marine natural products, we speculated the absolute configuration of MTX [ref. 13]. Meanwhile, with the hope that even a trace amount of the natural compound might become available for us, we were engaged with development of a method to determine the absolute configuration of the C.135-C.136 alcohol on a very small scale. This method was recently applied for a sample (10 μg) of MTX purchased from the commercial sources, concluding the absolute stereochemistry at C.138 and C.139, consequently the absolute stereochemistry of MTX as shown above [ref. 13,14].

As noted, MTX contains 32 rings, all of which exist as fused-ring systems. Except two, they are *trans*-fused, and are expected to be relatively rigid conformationally, which is indeed demonstrated through the extensive NMR studies by Yasumoto and coworkers. At three places, two of these fused-polycyclic portions are connected directly via a single carbon-carbon bond, i.e., the K/L-, O/P-, and V/W-ring junctures. Yasumoto and coworkers have also studied the preferred conformation for these moieties by NMR methods, coupled with force-field computation [ref. 12].

In order to estimate the overall conformational properties of MTX, the conformational properties regarding the remaining acyclic portions, i.e., the C.1-C.15, C.35-C.39, C.63-C.68, and C.134-C.142, must be addressed. The fact that the NMR characteristics observed for the four acyclic models used for this study were virtually superimposable on those of the relevant portions of MTX itself convincingly argue that the preferred conformations found for these models should adequately represent the preferred solution conformation of MTX as well. Thus, the vicinal proton coupling constants observed for the four acyclic models provided indispensable conformational information. Coupled with the preferred conformation recognized for the cyclic portions, the preferred solution conformations found for these models allowed us to suggest the global, preferred solution conformation of MTX to be approximately represented as the shape

of a hook [ref. 13]. Interestingly, MTX is conformationally rather rigid, except the C.7-C.9 and C.12-C.14 portions which are expected to provide with conformational flexibility.

**Closing Comments.** It is intriguing and remarkable that the small models discussed are perfectly representing the structural, including conformational, characteristics of each portion of MTX. Related to this, we should point out the fact that all the eight diastereomers of the left half of the AAL toxin $T_A$ aminopentol backbone as well as all the four diastereomers of the right half exhibited differing and distinct spectroscopic behavior from each other, and this trend was also recognized on all the acyclic portions of MTX. This implies that the structural properties of these substances are inherent in the specific arrangement of the small substitutents and independent from the rest of the molecules. In other words, each of these diastereomers is able to install a unique structural characteristics via arrangement of substituents with a specific configuration on the backbone. Then, it is tempting to suggest a possibility that fatty acids and related classes of compounds may be able to carry a specific information and serve as a functional material in addition to the structural materials.

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