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Synergism of anisotropic and computational NMR methods reveals the likely configuration of phormidolide A

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Characterization of the complex molecular scaffold of the marine polyketide natural product phormidolide A represents a challenge that has persisted for nearly two decades. In light of discordant results arising from recent synthetic and biosynthetic reports, a rigorous study of the configuration of phormidolide A was necessary. This report outlines a synergistic effort employing computational and anisotropic NMR investigation, that provided orthogonal confirmation of the reassigned side chain, as well as supporting a further correction of the C7 stereocenter.

Phormidolide A (Fig. 1C) was isolated from the marine cyanobacterium Leptolyngbya sp. (strain ISB3NOV94-8A) and demonstrated mid-range toxicity in the brine shrimp model. 1 Structurally, phormidolide A contains a 16-membered macrocycle linked to a pendant polyol side chain terminating in bromomethoxydiene; a signature motif shared with the related congeners phormidolide B, C and oscillariolide (see SI). 2, 3 The planar structure of phormidolide A was established from partial structures using 1H, 13C, COSY, multiplicity-edited HSQC, and HMBC NMR experiments. 3 Additional confirmation for this assignment was provided by INADEQUATE and ACCORD-ADEQUATE data acquired on a 13C-enriched sample. 1 The relative configuration of the tetrahydrofuran (THF) ring found in phormidolide A and the related congener oscillariolide was determined to be identical by conventional ROESY experiments. 1 Additional insight pertaining to the relative configuration for the remainder of the molecule was obtained by the then new J-based configuration analysis (JBCA) method. 4–8 Since that time, this method has been shown to be extremely robust except in unusual cases, such as relating the configuration from the 2- or 6-position of a tetrahydropyran moiety to an adjacent oxygenated stereogenic center. 9 By
analogy, the relative conformation from the C17 stereocenter to C16 of the neighboring tetrahydrofuran ring in phormidolide A was also shown to be problematic. In the case of phormidolide A, the configuration at C17 was assigned as anti relative to O16 based on a semi-quantitative utilization of the magnitude of $^{3}J_{	ext{OH}}$ correlations from H17 to C15 and to CH$_{2}$-37, respectively. This anti arrangement between H17 and O16 was corroborated by ROESY correlations from the CH$_{2}$-37 to H18.1 In a recent report, synthetic work by the Paterson and Britton groups has demonstrated that the relative configuration at C17 should be assigned as syn relative to O16 (Fig 1B).10 Their work also showed that while the relative configuration between C17 and the remainder of the polyol chain (C21-C33) was assigned correctly in the original work, the reassignment at C17 inferred the corresponding inversion of configuration at C21, C23, C25, C26, C27 and C29 was necessary.

Furthermore, in the original report, the absolute configuration at C7 was assigned as R through a set of variable temperature NMR experiments performed on a diacetonide-protected phormidolide.1 Since the relative configuration was established through extensive JBCA and NMR analyses, the absolute configuration of C7 was relayed to the THF ring, and to the remainder of the side chain to give the reported structure (Fig 1A). In 2016, the Gerwick group performed a complete biosynthetic gene cluster analysis on phormidolide that cast doubt on the 7R (or L-OH7) absolute configuration and therefore the stereochemistry of the natural product as a whole.11 To address this issue, the phormidolide A macrocycle was hydrolyzed and both the R and S α-methoxy-α-trifluoromethylphenylacetic acid (MTFA) esters were synthesized from the triacetone derivative, and subsequent spectroscopic analysis initially appeared to agree with the original assignment (Fig S5). This apparent conundrum with the biogenetic data was resolved after noting a prior report from the Reynolds group,12 which suggested that the anomalous L-OH configuration (corresponding to the 7R) can indeed be catalytically generated from a type B ketoreductase. Interestingly, a subsequent report from the Piel group on the genomic prediction and isolation of the leptolyngbyalides, a truncated sub-unit of phormidolide A (Fig 2) at GIAO-B3LYP21-24/6-31G(d)//M06-2X25/6-31+G(d,p), uncovered a reversal of the conventional $^{3}J_{\text{OH}}$ values for anti vs gauche configuration in these systems. The 17S configuration provided a single low energy conformation, stabilized by an OH-17 to O16 gauche hydrogen bond. The DFT-derived anti H17-CH$_{3}$-37 $^{3}J_{\text{C,H}}$ coupling constant for this conformation was 3.3 Hz. Conversely, the 17R configuration provided two alternate conformations in a ~1:1 ratio, with OH-17 to O15 and OH-17 to O16 gauche hydrogen bonds, respectively. Whereas the former is in an anti-parallel conformation for H17-CH$_{3}$-37 (with a $^{3}J_{\text{C,H}}$ coupling constant of 3.9 Hz), the latter is in a gauche conformation (exhibiting a $^{3}J_{\text{C,H}}$ of 4.3 Hz). Reanalysis of the original spectroscopic data with modern data processing tools revealed a coupling of 4.1 Hz, which is in seamless agreement with the averaged DFT prediction for the 17R conformers (Fig 2). This anomalous result obfuscates the conclusive assignment of C17 through empirical JBCA, and thus requires more advanced analytical techniques to verify the configuration, as reported here.

Following the Piel report, a structural analysis of the ROESY, E.COSY, and HSQMB data utilized for the initial assignment suggested that the configuration at C7 could be inverted without significantly altering the reported correlations for phormidolide A. Interestingly, the molecular mechanics (MM) structure presented in the original report, as well as more recent MM optimizations for both macrocyclic C7 epimers, provided structures that would allow the logical explanation of all scalar and dipolar coupling data (Fig S7).

At this point, three configurations for phormidolide A have been proposed, namely the original assignment (Fig 1A),1 the Paterson/Britton assignment (Fig 1B)10 and the Piel assignment13. In light of our ongoing interest in the total synthesis of phormidolide A and the application of advanced NMR methods to structural/stereochemical assignment, we embarked on a multi-pronged approach focused on conclusively defining the configuration of phormidolide A, which is necessary to pin down an apparently dynamic target. Notably, we sought to leverage the advances in ab initio NMR methods,14 in conjunction with recently reported Residual Chemical Shift Anisotropy (RCSA) techniques,15-20 to independently verify the most likely structure for the natural product, as well as demonstrate the capability of these methods in deconvoluting a flexible and complex structure as challenging as phormidolide A.

Our first step towards verifying the stereochemical identity of phormidolide A was to investigate the apparent stereochemical mis-assignment at C17. DFT scalar coupling constant calculations of C17-epimers (R and S) of the C20-truncated sub-unit of phormidolide A (Fig 2) at GIAO-B3LYP21-24/6-31G(d)//M06-2X25/6-31+G(d,p), uncovered a reversal of the conventional $^{3}J_{\text{OH}}$ values for anti vs gauche configuration in these systems. The 17S configuration provided a single low energy conformation, stabilized by an OH-17 to O16 gauche hydrogen bond. The DFT-derived anti H17-CH$_{3}$-37 $^{3}J_{\text{C,H}}$ coupling constant for this conformation was 3.3 Hz. Conversely, the 17R configuration provided two alternate conformations in a ~1:1 ratio, with OH-17 to O15 and OH-17 to O16 gauche hydrogen bonds, respectively. Whereas the former is in an anti-parallel conformation for H17-CH$_{3}$-37 (with a $^{3}J_{\text{C,H}}$ coupling constant of 3.9 Hz), the latter is in a gauche conformation (exhibiting a $^{3}J_{\text{C,H}}$ of 4.3 Hz). Reanalysis of the original spectroscopic data with modern data processing tools revealed a coupling of 4.1 Hz, which is in seamless agreement with the averaged DFT prediction for the 17R conformers (Fig 2). This anomalous result obfuscates the conclusive assignment of C17 through empirical JBCA, and thus requires more advanced analytical techniques to verify the configuration, as reported here.

Encouraged by the insight provided by ab initio methods, and recalling the uncertainty surrounding the assignment of 7R in the original report, DP4 calculations of the $^{1}$H and $^{13}$C chemical shifts of the two truncated macrocycle C7-epimers of phormidolide A (Fig S11) were undertaken.26 Geometries, shifts and conformer energies were calculated using DFT conditions found to be optimal in recent studies.27,28 The statistical model used was also tailored to the particular computational...
calculated chemical shifts, where initially large prediction errors were significantly reduced in the reassigned structure (configuration. This conclusion was further supported by the 311G*, conformer energies: M06-2X/def2-TZVP.

In addition, DFT calculations of \( J_{\text{HH}} \), \( J_{\text{CH}} \), and \( J_{\text{CS}} \) coupling constants (GIAO-B3LYP/6-31G(d)//M06-2X/6-311+G(d,p)) and \( ^{13}C \) NMR chemical shifts (GIAO-mPW1PW91/6-311+G(d,p) //M06-2X/6-311+G(d,p)), also indicated the 7S assignment (Fig 3). A recent report has demonstrated the utility of long-range \( J \)-coupling constants for diastereomeric analysis.\(^{29}\) Due to the computational demands of modelling such a highly flexible molecule, the models were truncated at C24 with only eight isomers considered. These isomers corresponded to isomeric permutations at the C7, C17 and C21/C23 stereocenters. In this context, SRS would mean a 7S, 17R and 21S configuration, respectively – with the THF fragment configuration remaining unchanged as shown in Fig 1A (n.b.: this notation also corresponds to 21S/23R, which are considered as a pair in each model and inverted simultaneously). The Mean Absolute Error (MAE) of experimental vs. DFT-derived scalar coupling and chemical shift values (up to C21 but excluding sp\(^2\) carbons due to errors associated with the DFT functional used)\(^{31,32}\) not only favor the 7S configuration but also the 17R configuration (Fig 1C), with values of 0.84 and 1.22, respectively (compared to 0.93 and 1.36 computed for the SSR configuration). Interestingly, including the sp\(^2\) carbon chemical shifts (previously excluded) in the statistical analysis also favor the SRS configuration (Fig S13).

As a final check on the revised configuration of phormidolide A (Fig 1C), we further embarked on an orthogonal structure verification process – leveraging anisotropic NMR parameters to generate an unbiased orthogonal evaluation of structural accuracy. Anisotropic NMR data, including Residual Dipolar Coupling (RDC) and Residual Chemical Shift Anisotropy (RCSA), have developed into powerful parameters for verification of proposed molecular constitution, and thereby increase confidence in assigned structures.\(^{15,17,18,33–39}\) In particular, \(^{13}C\) NMR chemical shift changes due to Residual Chemical Shift Anisotropy (RCSA) in appropriate liquid crystalline alignment media provide information on the relative orientations of nuclear chemical shielding tensors of the molecule that provides detailed information on molecular constitution and configuration that can be applied to molecules of even moderate flexibility. The eight diastereomeric phormidolide A configurations (RRR, RRS, RSR, RSS, SSR, SRS, SSS), truncated at C24, were also used for anisotropic NMR evaluation. Due to the moderate flexibility of these diastereomers (SI), which resulted in the generation of multiple
combinations with >1 % Boltzmann populations, theoretically determined conformer electronic energies were used as additional constraints in the single-tensor singular value decomposition (SVD) analysis of the proposed structures. This approach potentially minimizes SVD overfitting of the comparatively sparse RCSA dataset. The superimposed conformations of the SRS configuration (Fig 5) demonstrates that single-tensor SVD analysis can be applied to these moderately flexible systems. To verify the Paterson/Piel revised side-chain configuration (and thus the revised stereochemistry of phormidolide A, Fig 1C, in general), RCSA data up to C22, including the diastereotopic methyl groups C39/C40, were also used for SVD analysis (summarized in Fig 5). Clearly, the SRS configuration agrees best with the measured RCSA data giving a Q-factor of 0.190, while the nearest configuration SRR gave a Q-factor of 0.418. A revision of phormidolide A to the \( \alpha \) configuration agrees best with the measured RCSA data giving previously unavailable, we highlight that the reassigned relative configuration to the THF ring. Notably, these results confirming the stereochemical reassignment of 7 to the \( \alpha \) configuration (SRS) demonstrates a critical aspect of the total synthesis of phormidolide A, \( \chi \) This journal is © The Royal Society of Chemistry 20xx

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Notes and references


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