The Correct Structure of Aquatolide—Experimental Validation of a Theoretically-Predicted Structural Revision

Michael W. Lodewyk,[†] Cristian Soldi,[†] Paul B. Jones,[‡] Marilyn M. Olmstead,[†] Juan Rita,[§] Jared T. Shaw,*[,†] and Dean J. Tantillo*[,†]

[†]Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, California 95616, United States
[‡]Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina 27109, United States
[§]Biology Department, Balearic Islands University, Palma de Mallorca, Spain

S *Supporting Information*

ABSTRACT: Aquatolide has been reisolated from its natural source, and its structure has been revised on the basis of quantum-chemical NMR calculations, extensive experimental NMR analysis, and crystallography.

Aquatolide is a humulane-derived sesquiterpenoid lactone isolated from *Asteriscus aquaticus*.[1] The structure of aquatolide originally proposed on the basis of 1D and 2D NMR analysis (**1a**, Figure 1) contains an exceedingly rare
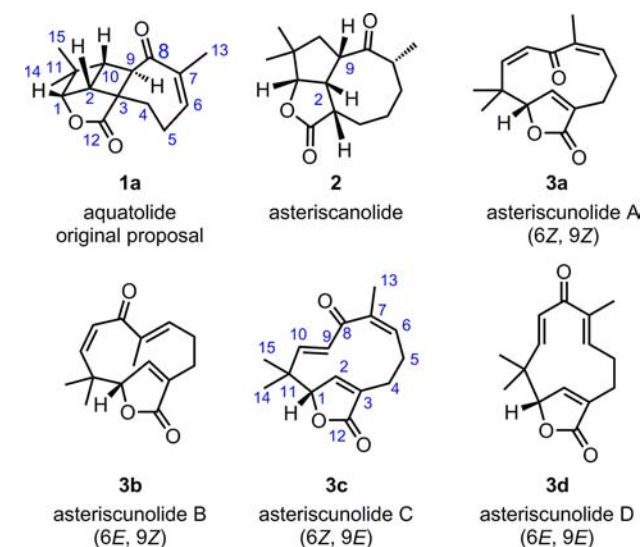
Figure 1. Originally proposed structure of aquatolide and structures of the related natural products asteriscanolide and asteriscunolides A−D.

[2]ladderane substructure.[1−3] Intrigued by this structural unit, we initiated quantum-chemical calculations to verify the reported connectivity in preparation for studies of the biogenesis of aquatolide in nature.[4−6] As described below, however, these seemingly innocuous calculations set us on a journey toward an extensive structural revision of this complex natural product.

Figure 1 also shows the structures of the related natural products asteriscanolide (**2**)[7] and asteriscunolides A−D[8] (**3a−d**, putative precursors to aquatolide via a photochemical 2 + 2 reaction), all isolated from the same plant. **3a−d** have since been

found to display anticancer activity against several cell lines, which in part motivated the recent total synthesis of the D isomer.[9] **2** has received much attention from the synthetic organic community, mainly because of its unusual carbon skeleton, and several successful syntheses have been reported in the literature.[10] Studies of the synthesis or biosynthesis of aquatolide have not been described in the literature.

As shown in Table 1, many of the computed chemical shifts for structure **1a** did not match up well with those reported for the natural product. Significant deviations were found for nuclei throughout the molecule, indicating that the core structure was likely incorrect. It is also noteworthy that experimentally, all three methyl carbon chemical shifts lie within a 1 ppm range, while the computed values for the methyl carbons in the proposed structure span an 8.5 ppm range. While the proposed structure was consistent with all of the reported experimental data, including nuclear Overhauser effect (NOE) and other correlations, our high-accuracy chemical shift calculations indicated that the assigned structure was incorrect.

The problem of identifying the correct structure of aquatolide thus arose. Our efforts consisted of a combination of both rational and arbitrary changes to the structure followed by recalculation of the ^{1}H and ^{13}C chemical shifts.[11] For example, while structure **1a** could arise from a [2 + 2] photocycloaddition of structure **3c** in which the C2−C10 and C3−C9 bonds are formed, we considered structures that might arise from analogous cyclizations of the other asteriscunolide isomers or [2 + 2] reactions involving the C2=C3 and C6=C7 π bonds. In each case, we considered the bond-formation events that appeared most reasonable on the basis of the reported crystal structures of the asteriscunolide isomers.[8] Such considerations produced structures ranging from reasonable to highly implausible on geometric grounds, but the computed chemical shifts for none of these were an acceptable match to the reported chemical shifts. Although we initially discounted the structure **1b** (Figure 2) that might result from C2−C9/C3−C10 cyclization of **3c** (the lowest-energy asteriscunolide isomer[12,13]) because such a closure seemed unlikely on the basis of the crystallographic conformation of **3c**, this structure could be formed from other conformations. To our delight, the computed chemical shifts for **1b** matched the values reported for aquatolide

Table 1. Comparison of Experimental and Computed NMR Chemical Shifts for Aquatolide

nucleus[a]	^{13}C NMR chemical shifts (ppm)				^{1}H NMR chemical shifts (ppm)			
	exptl[b]	comp **1a**[c]	comp **1b**[c]	exptl[d]	exptl[b]	comp **1a**[c]	comp **1b**[c]	exptl[d]
1	84.12	80.76	83.29	84.20	4.48	4.23	4.21	4.48
2	54.48	41.66	54.75	54.54	3.25	2.99	3.20	3.26
3	62.77	48.84	62.83	62.83				
4	22.07	31.23	22.46	22.15	2.50	1.99	2.48	2.52
					2.02	1.89	1.89	1.96
5	28.53	30.16	30.49	28.63	2.38	3.69	2.29	2.35
					2.07	2.32	2.08	2.03
6	130.94	143.61	135.22	131.10	5.84	6.36	5.94	5.85
7	135.07	139.04	138.11	135.08				
8	211.58	211.93	211.87	211.94				
9	54.37	55.53	54.76	54.45	2.92	3.22	2.78	2.92
10	62.57	38.24	63.89	62.59	2.64	2.97	2.49	2.64
11	41.73	44.89	44.57	41.86				
12	177.27	181.17	177.65	177.50				
13	22.00	21.98	23.19	22.22	1.86	1.89	1.84	1.87
14	22.73	16.72	21.09	22.62	1.18	0.99	0.98	1.05
15	22.46	25.21	20.80	22.84	1.04	1.17	1.09	1.19
	CMAD[e]	7.23	1.37		CMAD[e]	0.35	0.10	
	largest $\Delta\delta$	24.33	4.28		largest $\Delta\delta$	1.31	0.27	

[a]For numbering, see Figures 1 and 2. [b]Data in CDCl$_3$ taken from ref 1. [c]Data computed for the originally proposed structure (**1a**) and the revised structure (**1b**); see the SI for details. [d]Present work. [e]CMAD is the corrected mean absolute deviation, computed as $(1/n)\sum_{i=1}^{n}|\delta_{comp,i} - \delta_{exptl,i}|$, where δ_{comp} refers to the scaled computed chemical shift.
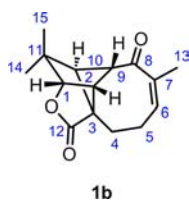

Figure 2. Revised structure of aquatolide.

exceptionally well (Table 1).[14] Structures derived from the same type of cyclization for the other three asteriscunolide isomers did not produce an acceptable match to the experimental data.[15]

While structure **1b** does not possess a ladderane core, the ring system (bicyclo[2.1.1]hexane) is uncommon. Two notable examples that lack the lactone bridge are found in cyclopampeanone isovalerate (**4**)[16] and solanoeclepin A (**5**),[17] the latter of which has been subjected to X-ray crystallographic analysis (Figure 3). Although the biosynthesis of **5** is uncertain, the cyclopampeanone core is thought to arise from an oxidized bisabolene precursor that can undergo [2 + 2] cycloaddition or an intramolecular Alder ene reaction, the latter of which leads to the isomeric pampeanone isovalerate (not shown).[16] All three metabolites are isolated from the same source, supporting the
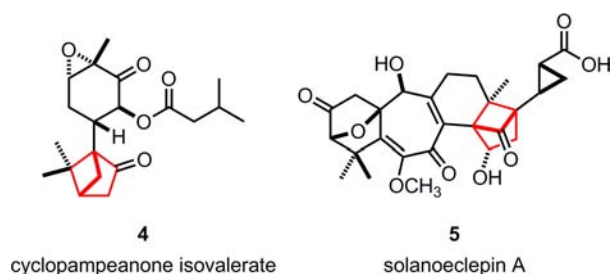

Figure 3. Known natural products with a bicyclo[2.1.1]hexane core.

proposed biosynthesis. The exact number of secondary metabolites that share the bicyclo[2.1.1]hexane core with aquatolide is difficult to discern. In view of the propensity of this core structure to exhibit unusual ^{1}H$-^1$H coupling in the NMR spectrum (see below), the number of structures is likely to grow as more are discovered or reassigned.

We next turned our attention to the experimental data reported by San Feliciano and co-workers.[1] These authors proposed structure **1a** largely on the basis of homonuclear and heteronuclear 2D NMR experiments. While it does appear that the proposed structure is fully consistent with the reported experimental data, the absence of a reported NOE correlation between H2 and H10 intrigued us, and we wondered whether additional NMR experiments might further support our proposed structural revision. We thus decided to attempt to reisolate the original compound and/or **3a−d**, which could potentially serve as synthetic precursors to **1a** or **1b**. *A. aquaticus* was collected in March 2012 on the south coast of Mallorca, Spain, and then dried and shipped to UC Davis. The plant material (231.7 g) was ground and extracted with hexanes, and the crude hexanes-soluble extract (7.6 g) was chromatographed on silica gel. Fractions were analyzed by GC−MS for those containing *m/z* 246. Several of the asteriscunolides were isolated, as was a small quantity (2.5 mg) of a compound with a fragmentation pattern similar to that reported for aquatolide. The isolated compound showed an [M + Na]$^+$ ion at *m/z* 269.1158 (calcd 269.1154) and the same ^{1}H and ^{13}C NMR chemical shifts as reported by San Feliciano and co-workers[1] (Table 1). The observed melting point and optical rotation showed reasonable agreement with reported values as well [see the Supporting Information (SI) for details]. The IR spectrum showed strong bands at 1755 and 1674 cm^{-1} consistent with the γ-lactone and α,β-unsaturated ketone, respectively. These data indicated that the compound isolated was the same compound that San Feliciano and co-workers isolated and named aquatolide.

The ^{1}H−^{1}H coupling constants observed for the reisolated compound (Table 2) also matched those originally reported.

Table 2. Experimental and Computed ^{1}H−^{1}H Coupling Constants (in Hz) for Structure 1b

nucleus[a]	experimental[b]	computed[c]
1	t (2.2)	J_{H2} = 2.3, J_{H10} = 1.8
2	dd (7.3, 2.5)	J_{H1} = 2.3, J_{H10} = 6.8
4a	dd (15.8, 6.7)	J_{4b} = 15.7, J_{5a} = 1.8, J_{5b} = 6.7
4b	m	
5a	m	
5b	m	
6	ddt (4.7, 3.1, 1.5)	J_{5a} = 3.4, J_{5b} = 5.4, J_{H13} = 1.8
9	s	J_{H2} = 0.1, J_{H10} = 0.07
10	dd (7.3, 1.8)	J_{H1} = 1.8, J_{H2} = 6.8, J_{H9} = 0.07
13	q (2.0)	J_{H5a} = 2.5, J_{H5b} = 2.8, J_{H6} = 1.8
14	s	
15	s	

[a]See Figure 2. [b]Present work; these data are fully consistent with those reported in ref 1. [c]Selected J values only, reported as absolute values; see the SI for details and the complete set of computed values.

However, these data initially seemed to be at odds with structure **1b** (and possibly a better fit for structure **1a**). For example, H10 was found to be a doublet of doublets with J = 7.3 and 1.8 Hz. This hydrogen has two ^3J neighbors in structure **1a** but only one in structure **1b**. To understand this apparent discord, we computed the ^{1}H−^{1}H coupling constants for both structures using quantum-chemical techniques.[6] The computed values for structure **1b** (Table 2) displayed an excellent overall match to the experimental data (see the SI for the computed values for structure **1a**, which did not match the experimental data). In fact, the computed results bring to light an atypically large ^4J coupling of ∼7 Hz between H2 and H10. While other ^4J coupling constants in the molecule are not as large (e.g., $J_{H1−H10} \approx 2$ Hz and $J_{H1−H9} \approx 0$ Hz), all are consistent between experiment and theory in this case. Examples of comparably large ^4J values in similar structures are known.[18]

We next examined data from several homonuclear and heteronuclear 2D NMR experiments. The ^{1}H−^{1}H COSY and ^{1}H−^{13}C HSQC spectra confirmed the connectivity among H1, H2, and H10 predicted by the computed ^{1}H−^{1}H coupling constants and previously reported for the original structure.[1] These data also confirmed the absence of coupling for H9, which is nearly orthogonal to both H2 and H10 (the computed dihedral angles are 80° and 85°, respectively). The HMBC spectrum of the central core of the molecule (H1, H2, H9, H10) was inconclusive. Although this experiment was optimized to show ^3J heteronuclear coupling, the frameworks of both structures places these H atoms 2−4 bonds away from the relevant carbon atoms, making it difficult to draw any firm conclusions on the basis of these data. A NOESY experiment, on the other hand, was more conclusive. We observed the same NOE correlations as originally reported, and these appeared to be consistent with both possible structures.[19] However, correlations between H9 and H10 and between H2 and H9, which are more consistent with structure **1b**, were also observed. In particular, the observed H2/H9 correlation seems unlikely for structure **1a**. In addition, an NOE correlation between H2 and H10 was not observed, a result that further supports the revised structure since the distance between these two atoms is computed to be >4 Å in structure **1b** but <3 Å in structure **1a**. Shown in Figure 4 are the observed NOE
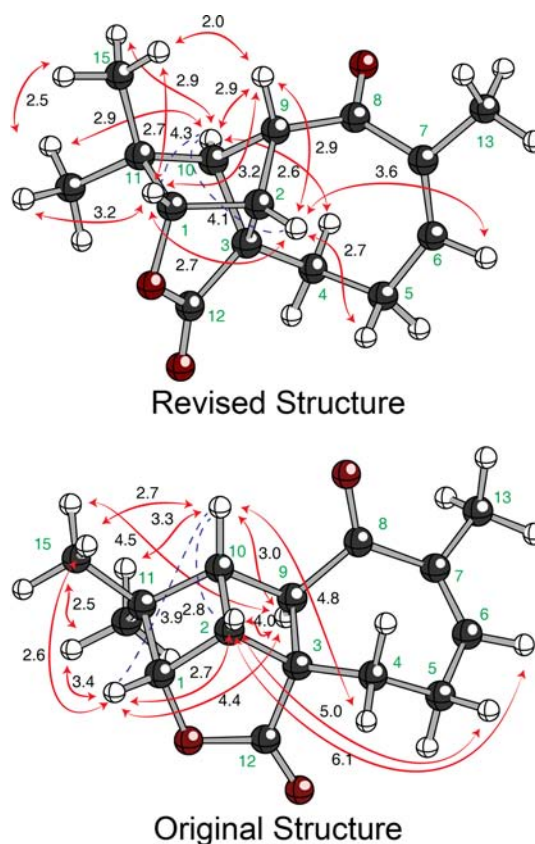
Figure 4. Observed NOE correlations overlaid on the computed geometries (lowest-energy conformers) for the revised and original aquatolide structures. The value near each arrow is the distance between the two atoms in Å. Blue dashed lines are selected distances for which no NOE correlation was observed.

correlations from the present work overlaid on the original and revised structures of aquatolide. It is clear that the revised structure is more consistent with the observed correlations, with the longest NOE distance being 3.6 Å in the revised structure compared with 6.1 Å in the original structure.

The revised structure **1b** assigned on the basis of quantum-chemical calculations and NMR experiments was subsequently confirmed by X-ray crystallography.[20] In addition to confirming the core connectivity, the crystallographic data confirmed the absolute configuration on the basis of the observed Flack parameter of −0.07(15).

The determination of the unusual core structure of aquatolide posed a significant challenge. Extensive NMR analysis, including a synergistic interplay between experiment and calculations, was required to elucidate the structure. Although the originally proposed structure was consistent with the set of data reported, it was not the only possible structure that could fit the data. In fact, certain features such as large four-bond ^{1}H−^{1}H coupling constants, small three-bond ^{1}H−^{1}H coupling constants, and other unusual correlations found with this molecule initially seemed to support the original structure better. Such features could therefore easily lead researchers toward incorrect determinations in some cases.[21] These pitfalls can be avoided by using quantum-chemical calculations of chemical shifts and coupling constants, especially in combination with the careful implementation of state-of-the-art NMR experiments. Such computational data were useful here not only for ruling out candidate structures and supporting the correct one but also for

18552

dx.doi.org/10.1021/ja3089394 | *J. Am. Chem. Soc.* 2012, 134, 18550−18553

reconciling the unusual coupling constants and NOE data observed for aquatolide, further demonstrating the utility of quantum-chemical calculations in making structural assignments for small quantities of natural materials for which crystallography may be impossible or impractical.[22]

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Computational and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
jtshaw@ucdavis.edu; djtantillo@ucdavis.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) San Feliciano, A.; Medarde, M.; Miguel del Corral, J. M.; Aramburu, A.; Gordaliza, M.; Barrero, A. F. *Tetrahedron Lett.* **1989**, *30*, 2851.

(2) (a) Nouri, D. H.; Tantillo, D. J. *Org. Biomol. Chem.* **2012**, *10*, 5514. (b) Nouri, D. H.; Tantillo, D. J. *Curr. Org. Chem.* **2006**, *10*, 2055.

(3) For the only example, to our knowledge, of a sesquiterpenoid containing a [2]ladderane substructure (confirmed by crystallography), see: Fedorov, S. N.; Radchenko, O. S.; Shubina, L. K.; Kalinovsky, A. I.; Gerasimenko, A. V.; Popov, D. Y.; Stonik, V. A. *J. Am. Chem. Soc.* **2001**, *123*, 504.

(4) Calculations were performed with Gaussian 03, Gaussian 09 and Spartan'10 (see the SI).

(5) For leading references on NMR chemical shift calculations, see: (a) Quasdorf, K. W.; Huters, A. D.; Lodewyk, M. W.; Tantillo, D. J.; Garg, N. K. *J. Am. Chem. Soc.* **2012**, *134*, 1396. (b) Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J. *Chem. Rev.* **2012**, *112*, 1839. (c) Lodewyk, M. W.; Tantillo, D. J. *J. Nat. Prod.* **2011**, *74*, 1339. (d) http://cheshirenmr.info (accessed Sept 14, 2012).

(6) For ^{1}H−^{1}H coupling constant calculations, see: Bally, T.; Rablen, P. R. *J. Org. Chem.* **2011**, *76*, 4818.

(7) San Feliciano, A.; Barrero, A. F.; Medarde, M.; Miguel del Corral, J. M.; Aramburu, A. *Tetrahedron Lett.* **1985**, *26*, 2369.

(8) San Feliciano, A.; Barrero, A. F.; Medarde, M.; Miguel del Corral, J. M.; Aramburu, A. *Tetrahedron* **1985**, *41*, 5711.

(9) Trost, B. M.; Burns, A. C.; Bartlett, M. J.; Tautz, T.; Weiss, A. *J. Am. Chem. Soc.* **2012**, *134*, 1474.

(10) For syntheses of asteriscanolide, see: (a) Wender, P. A.; Ihle, N. C.; Correia, C. R. D. *J. Am. Chem. Soc.* **1988**, *110*, 5904. (b) Paquette, L. A.; Tae, J.; Arrington, M. P.; Sadoun, A. H. *J. Am. Chem. Soc.* **2000**, *122*,

2742. (c) Limanto, J.; Snapper, M. L. *J. Am. Chem. Soc.* **2000**, *122*, 8071. (d) Kraft, M. E.; Cheung, Y.-Y.; Juliano-Capucao, C. A. *Synthesis* **2000**, 1020. (e) Kraft, M. E.; Cheung, Y.-Y.; Abboud, K. A. *J. Org. Chem.* **2001**, *66*, 7443. (f) Liang, Y.; Jiang, X.; Yu, Z.-X. *Chem. Commun.* **2011**, *47*, 6659. (g) Liang, Y.; Jiang, X.; Fu, X.-F.; Ye, S.; Wang, T.; Yuan, J.; Wang, Y.; Yu, Z.-X. *Chem.—Asian J.* **2012**, *7*, 593.

(11) In all, ∼60 constitutional isomers were considered, and in most cases, various diastereomers of each were considered as well (see the SI).

(12) The bicyclo[2.1.1]hexane framework of **1b** is expected to arise from a "crossed" nonconcerted cycloaddition from the T1 state (typical for enones). Crossed rather than parallel cycloaddition (the latter would lead to **1a**) is expected to predominate for such systems. For example, see: (a) Srinivasan, R.; Carlough, K. H. *J. Am. Chem. Soc.* **1967**, *89*, 4932. (b) Liu, R. S. H.; Hammond, G. S. *J. Am. Chem. Soc.* **1967**, *89*, 4936. (c) Gleiter, R.; Sander, W. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 566. (d) Dilling, W. L. *Chem. Rev.* **1966**, *66*, 373.

(13) The computed gas-phase free energies of **3a**, **3b**, and **3d** relative to **3c** are 5.32, 7.30, and 2.11 kcal/mol, respectively (see the SI for details).

(14) With corrected mean absolute deviations of 1.37 and 0.10 ppm for ^{13}C and ^{1}H, respectively, the closeness of the match is consistent with applications of the same methods to other known complex natural product structures. See the SI for calculations on natural products of comparable complexity, including aplydactone, ingenane, intricarene, echinopine B, and naupliolide, as well as on bicyclo[2.1.1]hexan-2-one. Also see ref 5.

(15) (a) It thus seems that [2 + 2] photocycloaddition of **3c** to form a bicyclo[2.1.1] structural unit is the likely biosynthetic route to aquatolide. The alternative photocycloaddition to form the originally proposed [2]ladderane structure apparently does not occur to any appreciable extent, at least in *A. aquaticus*. Interestingly, [2 + 2] photocycloaddition of myrcene, which could also lead to structures with bicyclo[2.1.1]hexane or [2]ladderane cores, has been shown to produce only the bicyclo[2.1.1]hexane product (see ref 12b). (b) Notably, asteriscanolide may be related to the corrected structure of aquatolide as a product of its fragmentation or as a product of incomplete [2 + 2] reaction, photochemical or not (we thank Dr. Yong Liang of UCLA for sharing the results of his preliminary calculations on these possibilities). Also see: El Dahmy, S.; Jakupovic, J.; Bohlmann, F.; Sarg, T. M. *Tetrahedron* **1985**, *41*, 309. (c) The synthesis of a related bicyclo[2.1.1] hexane system bearing a *gem*-dimethyl group and a fused lactone unit was described previously, but NMR data for this compound were not provided. See: Meinwald, J.; Gassman, P. G.; Hurst, J. J. *J. Am. Chem. Soc.* **1962**, *84*, 3722.

(16) Bohlmann, F.; Jakupovic, J.; Warning, U.; Grenz, M.; Chau-Thi, T. V.; King, R. M.; Robinson, H. *Bull. Soc. Chim. Belg.* **1986**, *95*, 707.

(17) Schenk, H.; Driessen, R. A. J.; de Gelder, R.; Goubitz, K.; Nieboer, H.; Brüggemann-Rotgans, I. E. M.; Diepenhorst, P. *Croat. Chem. Acta* **1999**, *72*, 593.

(18) For example, the bridgehead protons in bicyclo[2.1.1]hexane have a ^4J value of >6 Hz. See: (a) Barfield, M. *Magn. Reson. Chem.* **2003**, *41*, 344. (b) Wiberg, K. B.; Lampman, G. M.; Ciula, R. P.; Connor, D. S.; Schertler, P.; Lavanish, J. *Tetrahedron* **1965**, *21*, 2749. (c) Wiberg, K. B.; Lowry, B. R.; Nist, B. J. *J. Am. Chem. Soc.* **1962**, *84*, 1594.

(19) The only difference between the assignments for the original and revised structures involves the shifts for the methyl hydrogens H14 and H15, which were switched in the revised structure on the basis of the observed H9/H15 correlation. No correlation between H14 and H9 was observed.

(20) See the SI for full crystallographic details. The structural data for **1b** were deposited at the Cambridge Crystallographic Data Centre (CCDC) under deposition number 898298.

(21) Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012.

(22) Molinski, T. F. *Curr. Opin. Biotechnol.* **2010**, *21*, 819.