Communications to the Editor

Absolute Configuration of Phorboxazoles A and B from the Marine Sponge *Phorbas* sp. 1. Macrolide and Hemiketal Rings

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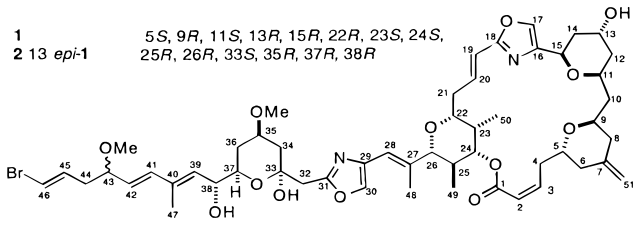
Recently, we reported the structures of two new bis-oxazole macrolides, phorboxazoles A (**1**) and B (**2**), from the marine sponge, *Phorbas* sp.[1] Both compounds exhibit exceedingly potent cytostatic activity against a variety of human solid tumor cell lines. For example, a recently completed round of *in vitro* testing in the National Cancer Institute's panel of 60 human tumor cell lines showed that **1** inhibited growth of colon tumor cells HCT-116 (GI_{50} 4.36×10^{-10} M) and HT29 (3.31×10^{-10} M).[2] The initially reported structures of **1** and **2** were solved by interpretation of MS and 2D NMR experiments. However, the absolute configuration was left undefined. Determination of the relative and absolute configurations of complex natural product macrolides by NMR methods is often made difficult by the need to assign independent molecular segments containing remote stereogenic centers.[3] Our attention was drawn to **1** as an attractive target for total synthesis due to its extraordinary activity and unprecedented carbon skeleton. Consequently, we applied our efforts to determination of the complete stereochemistry, but unfortunately so far all attempts to obtain crystals of either compound suitable for X-ray diffraction studies have failed. We now report the elucidation of the absolute configurations of 14 out of the 15 stereocenters in both **1** and **2** by a combination of techniques including synthesis of a suitable model compound for NMR spectroscopic comparisons.

Our strategy relied upon recognition that **1** contained three independent segments with a total of 15 stereogenic centers—the macrolide ring C1−C26, the hemiketal oxane ring C33−37 and vicinal secondary carbinol C38, and the isolated allylic MeO group at C43. The presence of two secondary OH groups—one within the macrolide ring at C13 and the second at C38 in the side chain—suggested direct determination of these centers by application of Kakisawa's modification of the Mosher's ester



1 5*S*, 9*R*, 11*S*, 13*R*, 15*R*, 22*R*, 23*S*, 24*S*,
2 13 *epi*-**1** 25*R*, 26*R*, 33*S*, 35*R*, 37*R*, 38*R*

(α-methoxytrifluoromethylphenylacetate, MTPA) method[4] and analysis of differential anisotropy between *S*- and *R*-MTPA ester derivatives. Because the relative stereochemistry of the macrolide ring was known,[1] the absolute configuration of the entire macrolide ring then would be revealed. Fortunately, the two secondary hydroxyl groups at C13 and C38 in **1** were sufficiently removed from each other that "crossover" of anisotropy between 13,38-*O*-MTPA groups would be negligible and not complicate independent analysis about each respective *O*-MTPA group. Phorboxazole A (**1**, *ca.* 5 mg) was converted (DCC, 3 equiv of MTPA, DMAP, CH_2Cl_2) to the *S*-MTPA diester **3** and *R*-MTPA diester **4**, respectively.[5] The formulas of the MTPA diesters **3** and **4** were secured by electrospray ionization mass spectrometry.[5] Interpretation of the double quantum filtered (DQF) COSY[6] allowed complete assignment of ^{1}H NMR chemical shifts and calculation of anisotropic chemical shift differences, $\Delta\delta = \delta_S - \delta_R$ for each proton. The signs of $\Delta\delta$s for protons near each of the MTPA groups at C13 and C38 were observed to be dependent upon the absolute configuration of the MTPA unit and conformed to Kakisawa's configurational model of MTPA esters. Chemical shifts consistently fell into regions that lay to the left and right of the secondary MTPA ester groups at C13 and C38, respectively, as drawn in Figure 1.[7] Consequently, the macrolide ring and C38 configurations of **1** were assigned as 5*S*, 9*R*, 11*S*, 13*R*, 15*R*, 22*R*, 23*S*, 24*S*, 25*R*, 26*R*, 38*R*.[8]

The relatively large H37,38 vicinal coupling constant (J = 7.9 Hz) measured for **1** in $CDCl_3$ could be consistent with either *threo* or *erythro* C37−C38 configuration depending upon the degree of intramolecular hydrogen bonding between the C38 hydroxyl and the oxane ring oxygen.[9] To remove any doubt in assignment of configuration of the oxane ring we chose to compare the H37,38 vicinal J of **1** with the corresponding Js in suitable model compounds. Model compounds *erythro*-**5a** and *threo*-**5b** (Figure 2) were designed to embody necessary and sufficient structural elements, including C33, C35, and C37 equatorial substituents, an axial oxygen at the anomeric carbon C33, and an *E*-allylic alcohol, that would match the most important nonbonded and hydrogen-bonded interactions present

(1) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126−8131.

(2) GI_{50} is defined as the concentration at which cell growth is inhibited by 50%. (a) Suffness, M.; Newman, D. J.; Snader, K. *Discovery and Development of Antineoplastic Agents from Natural Sources*; Scheuer, P. J., Ed.; Springer-Verlag: Berlin, 1989; Vol. 3, p 175. (b) Boyd, M. R.; Paull, K. D.; Rubinstein, L. R. In *Antitumor Drug Discovery and Development*; Valeriote, F., Corbett, T., Baker, L., Eds., Kluwer Academic: Amsterdam, 1991; pp 11−34. (c) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* 1995, 34, 91−109. Phorboxazole A also showed selectivity against leukemia CCRF-CBM (GI_{50} 2.45×10^{-10} M), prostate cancer PC-3 (3.54×10^{-10} M) and breast cancer MCF7 cell lines (5.62×10^{-10} M), although high activity was seen across the entire panel of cell lines (mean GI_{50} 1.58×10^{-9} M). Compounds **1** and **2** had essentially the same activity profiles. We are grateful to Drs. Jill Johnson and Anthony B. Mauger, National Cancer Institute, for these data.The phorboxazoles A and B have been selected by the NCI for in vivo antitumor trials.

(3) For the synthesis and properties of complex polyketide macrolides of marine and terrestrial origin see: (a) Norcross, R. D.; Paterson, I. *Chem. Rev.* **1995**, *95*, 2041−2114. (b) Omura, S. *Macrolide Antibiotics: Chemistry, Biology and Practice*; Academic Press: Orlando, FL, 1984; p 635.

(4) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(5) *S*-MTPA diester 3, 68%: high-resolution electrospray ionization mass spectrometry (HRESIMS) MH^+ *m/z* 1455.4925, $C_{72}H_{86}BrF_6N_2O_{17}$, calcd 1455.5014; *R*-MTPA diester 4, 62%: HRESIMS MH^+ *m/z* 1455.5095. See supporting information for 500 MHz 1H NMR, DQFCOSY, and 1H assignments.

(6) Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479−485.

(7) The H47 signal showed an anomalous $\Delta\delta$ (−27 ppb) due to placement of the vinylmethyl syn to the MTPA group and in the shielding region of the phenyl ring.

(8) The correct macrolide ring configuration is, therefore, opposite to that depicted for **1** in ref 1.

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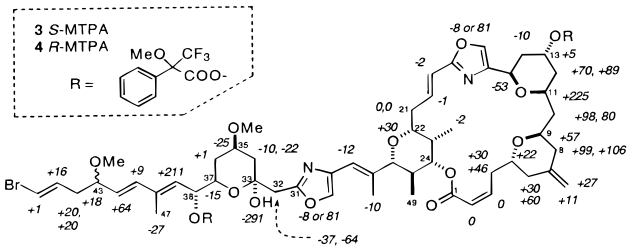

Figure 1. MTPA ester ^{1}H NMR analysis of **3** and **4**.[4] The units of $\Delta\delta = \delta_S - \delta_R$ (numbers in italics) are parts per billion (ppb). Oxazole proton assignments are interchangeable as are the corresponding $\Delta\delta$s (both negative).

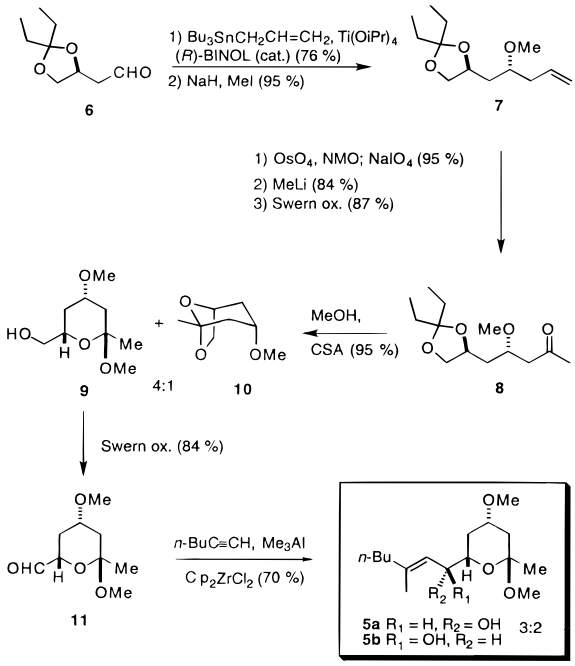

Figure 2. Synthesis of model compounds **5a** and **5b**.

in **1**. Compounds **5a** and **5b** were synthesized starting with aldehyde **6** (readily available from *S*-malic acid).[10] Asymmetric allylation of **6**[11] *via* Keck's protocol[12] gave the desired alcohol with excellent diastereoselectivity (10:1), which was methylated to afford **7**. Ketal **7** was subjected to oxidative cleavage, methylation, and oxidation to provide ketone **8**. Removal of the isopentylidene ketal in methanolic acid led to the desired oxane **9** along with a minor amount of bicyclic ketal **10**.[13] Oxidation of the remaining primary alcohol generated the aldehyde **11** which was combined with the product of carbometalation of 1-hexyne,[14] resulting in a 3:2 mixture of allylic alcohols *erythro*-**5a** and *threo*-**5b**.[15]

The ^{1}H NMR chemical shifts and vicinal couplings in **5a** and **5b** ($CDCl_3$) were assigned from extensive single-frequency homodecoupling experiments. The H38 signals (phorboxazole numbering) in **5a** and **5b** occurred at δ 4.44 (dd, J = 8.5, 3.5 Hz) and δ 4.28 (dd, J = 8.9, 8.0 Hz), respectively. Although the *threo*-isomer **5b** is antipodal (38*S*) to **1** at C33−38, it exhibits a vicinal H37,38 coupling (J = 8.0 Hz, $CDCl_3$) that matches that of **1** (J = 7.9 Hz),[1] proving that both molecules have the same *relative* configuration. Because the *threo* configuration has been established for **5b**,[15] phorboxazole A (**1**) must also have the C37,38 *threo* relative configuration. It follows from the independent determination of 38*R* in **1** (see above) that we can assign now the absolute configuration around the oxane hemiketal and macrolide rings in phorboxazole A as 5*S*, 9*R*, 11*S*, 13*R*, 15*R*, 22*R*, 23*S*, 24*S*, 25*R*, 26*R*, 33*S*, 35*R*, 37*R*, 38*R*. Phorboxazole B (**2**) had previously been determined[1] to be the C13 epimer of **1** by *J* analysis, so it was deduced that **2** has the same configuration as **1** at the other stereocenters.

In summary, the configurations of 14 out of 15 stereocenters in the cytostatic marine macrolides phorboxazoles A and B have been defined. This provides a foundation for total synthesis of these intriguing molecules by revealing the correct choice of starting materials from the chiral pool. It only remains to complete the more difficult determination of C43 configuration. This is the subject of ongoing studies in our laboratories and will be reported in due course.

Acknowledgment. Support for this research was provided by the National Institutes of Health (AI 31660 to T.F.M.), the National Science Foundation (CHE-9502149 to J.W.L.) and Eli Lilly (predoctoral fellowship to L.J.B.). The 500 MHz NMR spectrometer was partially funded through NIH ISIO-RR04795 and NSF BBS88-04739. T.F.M. gratefully acknowledges the receipt of an American Cyanamid Faculty Award. J.W.L. is a 1995 Cottrell Scholar and thanks the Research Corporation for this award.

Supporting Information Available: ^{1}H NMR (500 MHz), DQF-COSY, complete ^{1}H assignments, and HRESIMS spectra for **3** and **4**, experimental procedures and spectral data for the synthesis of **3**−**11**, Mosher's ester analysis for **5a** and **5b** (18 pages). See any current masthead page for ordering and Internet access instructions.

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(9) The difficulties of assigning *erythro* or threo stereochemistry at centers α to oxane rings by ^{1}H NMR without recourse to models is illustrated by the elucidation of palytoxin stereochemistry. See the following citations and references cited within: (a) Moore, R. E.; Bartolini, G.; Barchi, J. *J. Am. Chem. Soc*. **1982**, *104*, 3776−3779. (b) Klein, L. L.; McWhorter, W. W., Jr.; Ko, S. S.; Pfaff, K.-P.; Kishi, Y.; Uemura, D.; Hirata, Y. *J. Am. Chem. Soc*. **1982**, *104*, 7362−7364. (c) Ko, S. S.; Finan, J. M.; Yonaga, M.; Kishi, Y.; Uemura, D.; Hirata, Y. *J. Am. Chem. Soc*. **1982**, *104*, 7364−7367. (d) Fujioka, H.; Christ, W. J.; Cha, J. K.; Leder, J.; Kishi, Y.; Uemura, D.; Hirata, Y. *J. Am. Chem. Soc*. **1982**, *104*, 7367−7369. (e) Cha, J. K.; Christ, W. J.; Finan, J. M.; Fujioka, H.; Kishi, Y.; Klein, L. L.; Ko, S. S.; Leder, J.; McWhorter Jr, W. W.; Pfaff, K.-P.; Yonaga, M.; Uemura, D.; Hirata, Y. *J. Am. Chem. Soc*. **1982**, *104*, 7369−7371.

(10) Clive, D. L. J.; Murthy, K. S. K.; Wee, A. G. H.; Prasad, J. S.; da Silva, G. V. J.; Majewski, M.; Anderson, P. C.; Evans, C. F.; Haugen, R. D.; Heerze, L. D.; Barrie, J. R. *J. Am. Chem. Soc*. **1990**, *112*, 3018. The product *S*-aldehyde **6** was >99% ee.

(11) All new synthetic compounds gave satisfactory ^{1}H, ^{13}C NMR spectra and elemental analyses.

(12) Keck, G. E.; Tarbet, K. H.; Geraci, L. S. *J. Am. Chem. Soc*. **1993**, *115*, 8467.

(13) Compound **9** was assigned an axial OMe group, as expected from the anomeric effect and by analogy with compound number **13** reported in the total synthesis of acutiphycin. Smith, A. B.; Chen, S. S. Y.; Nelson, F. C.; Reichert, J. M.; Salvatore, B. A. *J. Am. Chem. Soc*. **1995**, *117*, 12013−12014. The oxane hemiketal OH group in acutiphycin is also axial, as is C33 OH in **1**. Barchi, J. J.; Moore, R. E.; Patterson, G. M. L. *J. Am. Chem. Soc*. **1984**, *106*, 8193−8197. Bicyclic ketal **10** should, in theory, be transformed into **9** under the reaction conditions but thus far has proved to be remarkably stable.

(14) Van Horn, D. E.; Negishi, E. *J. Am. Chem. Soc*. **1978**, *100*, 2252.

(15) The relative configurations of **5a** and **5b** were secured from analysis of the corresponding *R* and *S* Mosher's esters together with the known configuration of **6** (see supporting information). Interestingly, the MTPA esters of *threo*-isomer **5b** (but not *erythro*-**5a**) also exhibited an anomalous $\Delta\delta$ for the vinyl methyl ^{1}H NMR signal, although this time positive ($\Delta\delta$ + 20 ppb) because of its antipodal relationship to **1** (see footnote 7). We were surprised to find that the major product of this addition, **5a**, was the isomer expected from non-chelation-controlled addition. Efforts are underway to improve stereocontrol in the generation of this center. Nevertheless, both diastereomers of **5** were made available for comparison with **1**.