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Total Synthesis and Configurational Validation of (+)-Phorbaside A

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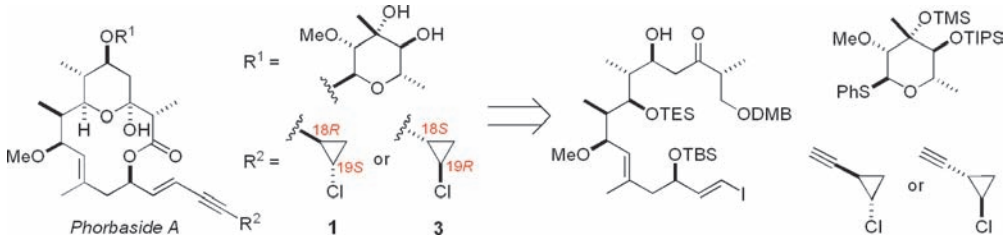
ABSTRACT



The configurational assignment of the cytotoxic marine macrolide phorbaside A has been verified by the stereodefined synthesis of the proposed structure 1 and its (18*S*,19*R*)-diastereomer 3, followed by correlation using circular dichroism spectroscopy. This first total synthesis, which proceeds in 8.2% yield over 23 steps, features two 1,4-*syn* boron aldol reactions, a Sonogashira coupling, and an α-glycosylation to append the L-evalose sugar moiety.

In recent years, marine macrolides have gained prominence as potent disrupters of cell cycle events and promising lead structures for the development of anticancer agents, provided the supply issue can be resolved.[1] Phorbaside A is a cytotoxic marine macrolide, isolated in 2007 by Molinski and co-workers from the sponge *Phorbas* sp. collected off the Western Australian coastline.[2] Together with the congeneric phorbasides B−E, this rare series of glycosylated macrolides, differing only in their respective sugar moieties, exhibited significant levels of cytotoxicity (IC_{50} = 2−62 μM) against the HCT 116 (human colon cancer) cell line. Such sensitivity to relatively minor structural variation renders the phorbasides prime candidates for further mode of action and structure−activity relationship studies.

Extensive spectroscopic analysis of phorbaside A (**1**, Figure 1) highlighted its structural relationship to callipeltoside A (**2**),[3,4] previously isolated from a lithistid sponge, *Callipelta* sp., found in New Caledonia. Both 14-membered glycosylated macrolides contain a six-membered cyclic hemiacetal, an (*E*)-trisubstituted alkene, and a distinctive unsaturated side chain terminating in a *trans*-chlorocyclopropane ring. Within the macrolide ring, a high degree of structural homology is apparent between the phorbasides and the callipeltosides, which also extends to the aurisides[5] and dolastatin 19,[6] related cytotoxic polyketides isolated from the sea hare *Dolabella auricularia*, suggesting a common bacterial biogenesis.[4] Despite these compelling similarities, the assigned (18*R*,19*S*) configuration of the cyclopropane ring of the phorbasides, as defined by elegant semiquantitative circular dichroism (CD) studies using model fragments, surprisingly was determined to be *opposite* to that found in the callipeltosides and, more recently, muironolide A.[7]

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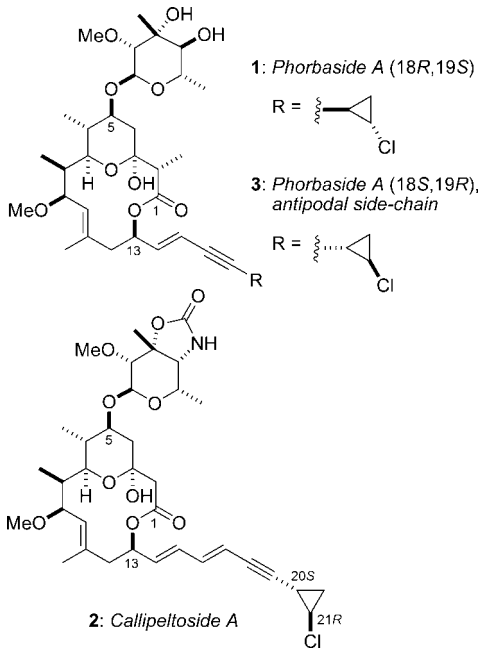


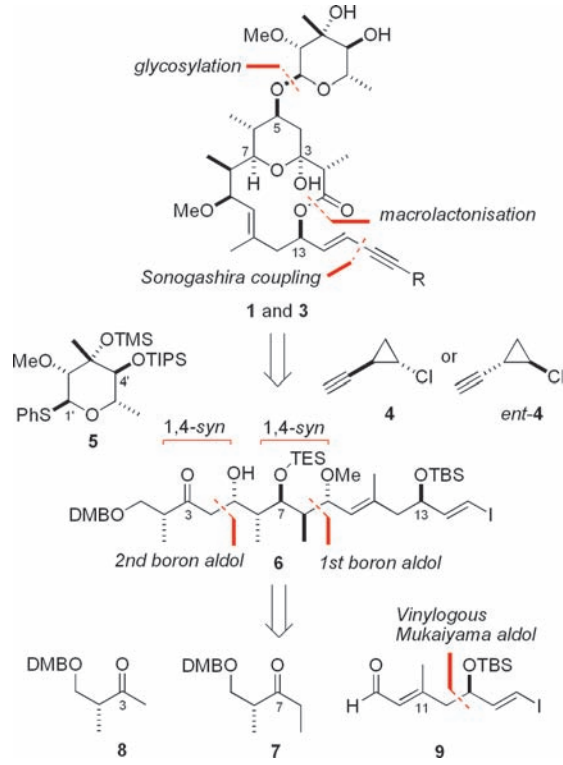
Figure 1. Phorbaside and callipeltoside marine macrolides.

As part of our ongoing interest in these structurally intriguing marine macrolides,[4,8] we sought to apply our versatile aldol-based strategy to achieving an efficient synthesis of the phorbasides. We now report the first total synthesis of (+)-phorbaside A (**1**), together with its (18*S*,19*R*)-diastereomer **3** having the antipodal cyclopropane ring as found in the callipeltosides, thus validating the Molinski configurational assignment for the phorbasides by CD spectroscopic correlation.

To allow for appropriate late-stage diversification to assemble **1** and **3**, our strategy involved the installation of the *trans*-chlorocyclopropyl enyne side chain through Sonogashira couplings of alkynes **4** and *ent*-**4**, respectively, with a preformed macrolactone, followed by α-selective glycosylation at the C5 hydroxyl using thioglycoside **5** (Scheme 1). This pivotal macrocyclic intermediate would be obtained by elaboration of linear precursor **6** containing a terminal vinyl iodide. Recognition of the indicated 1,4-*syn* relationships in **6** guided the use of two boron-mediated aldol couplings using ketones **7** and **8** to construct the carbon backbone of the macrocycle. An asymmetric vinylogous Mukaiyama aldol reaction would be used to set the (13*R*)-hydroxyl configuration and simultaneously install the (*E*)-geometry in enal **9**.[8b]

Accordingly, treatment of **10**[9] with (*R*)-BINOL-Ti(O*i*-Pr)$_4$,[8b] followed by addition of **11**[10] at −78 °C, afforded aldol

Scheme 1



adduct **12** (87%, >95% ee, Scheme 2). TBS protection, ester reduction, and subsequent allylic oxidation using MnO_2 proceeded smoothly to form aldehyde **9** (97% over 3 steps), primed for further chain elongation using the first boron-mediated aldol addition.

In the event, treatment of the (*E*)-boron enolate of ethyl ketone **7**[8b] with aldehyde **9** at −78 °C gave the expected 1,2-*anti*,1,4-*syn* adduct **13** in excellent yield and diastereoselectivity (98%, >95:5 dr).[11] The newly formed hydroxyl stereocenter was then relayed to set the C7 configuration through Evans−Tishchenko reduction,[12] which also allowed for diol differentiation. TES protection followed by ester cleavage then provided **14** in 87% yield. Methylation of the C9 hydroxyl was then achieved using Meerwein's salt. Following conditions developed previously,[8b] treatment with DDQ in CH_2Cl_2/pH 7 buffer at 60 °C led to rapid (10 min) and clean removal of the DMB ether. Dess−Martin oxidation then afforded aldehyde **15** (95% over 3 steps), set for the second boron aldol reaction. In this case, the dicyclohexylboron enolate of methyl ketone **8**[8d] was treated with **15** at −78 °C to afford adduct **6** (97%, >95:5 dr). Here the dominant 1,4-*syn* influence from ketone **8** is matched with the Felkin−Anh bias of aldehyde **15**.[8d]

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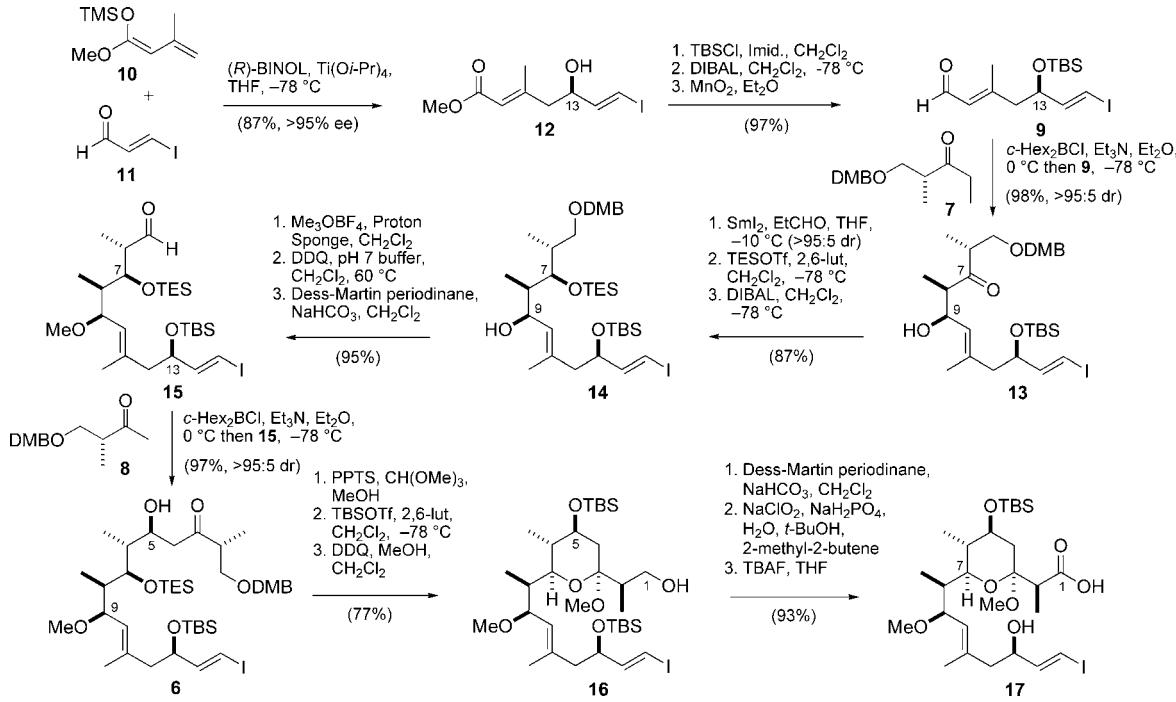
Scheme 2



With the linear precursor **6** in hand, attention was directed toward formation of the macrolactone. Selective cleavage of the TES ether in **6** occurred with cyclization to the six-membered methyl acetal upon treatment with catalytic PPTS and $(MeO)_3CH$ in MeOH. The remaining hydroxyl at C5 was then converted into the TBS ether, after which treatment[13] with DDQ in $MeOH/CH_2Cl_2$ removed the DMB group cleanly to give **16** (77% over 3 steps). Sequential oxidation then provided the corresponding carboxylic acid, which functioned as an internal buffer during the selective removal of the allylic C13 silyl ether using TBAF. This sequence of reactions afforded *seco*-acid **17** in excellent yield (93%).

In contrast to our previous experience with this class of 14-membered macrolides,[4,8] the crucial macrolactonization of **17** proved difficult to achieve. While mixed anhydride formation proceeded cleanly, commonly this intermediate could not be coaxed into undergoing a productive cyclization. Presumably the additional methyl substituent present at C2 in the phorbaside system, relative to that in the callipeltosides, sterically hinders lactonization. After extensive investigation, this challenging macrocyclization was performed under modified Yamaguchi conditions[14] to provide **18** in 42% yield (Scheme 3). At this stage, the route diverged to target **1**, having the proposed (18*R*,19*S*) stereostructure of phorbaside A, as well as the diastereomeric side-chain analogue **3**. Sonogashira $sp-sp^2$ coupling[15] of iodide **18** with (18*R*,19*S*)-alkyne **4**[8a] or *ent*-**4**,[8a,b] using $(Ph_3P)_2PdCl_2$ and CuI, introduced the appropriate enyne *trans*-chlorocyclopropane side chain. Treatment with TFA in wet THF then hydrolyzed the methyl acetal and cleaved the TBS group at C5 to provide the diastereomeric phorbaside aglycons **19** and **20**, in readiness for sugar attachment.

Synthesis of the appropriate thioglycoside **5** commenced from the known L-evalose scaffold **21**[2b] (Scheme 4). After silylation of the C4′ hydroxyl group with TIPSOTf, the acetonide was cleaved using aqueous AcOH to yield diol **22**. Selective methylation of the secondary alcohol was achieved using Meerwein's salt to provide **23** (84%), from which thioglycoside **5** was obtained on treatment with TMSSPh in the presence of ZnI_2 and Bu_4NI.[16] This latter transformation also resulted in silylation of the C3′ tertiary hydroxyl group.

Completion of phorbasides **1** and **3** relied on the α-selective glycosylation of aglycons **19** and **20** with thioglycoside **5**. This was achieved using NIS in combination with catalytic TfOH and 2,6-di-*tert*-butyl-4-methylpyridine in CH_2Cl_2.[17] Finally, cleavage of the silylethers within the sugar moiety with TBAF in THF gave the targeted phorbasides **1** and **3**, obtained in 23 steps and 8.2% and 5.4% overall yield, respectively, from **10**.

With synthetic samples of **1** and **3** now in hand, it proved possible to verify the structure of phorbaside A. As expected from the callipeltosides,[8a,17c,18] these diastereomers proved to be indistinguishable by ^1H and ^{13}C NMR spectroscopy, although both correlated with the NMR data reported[19] for

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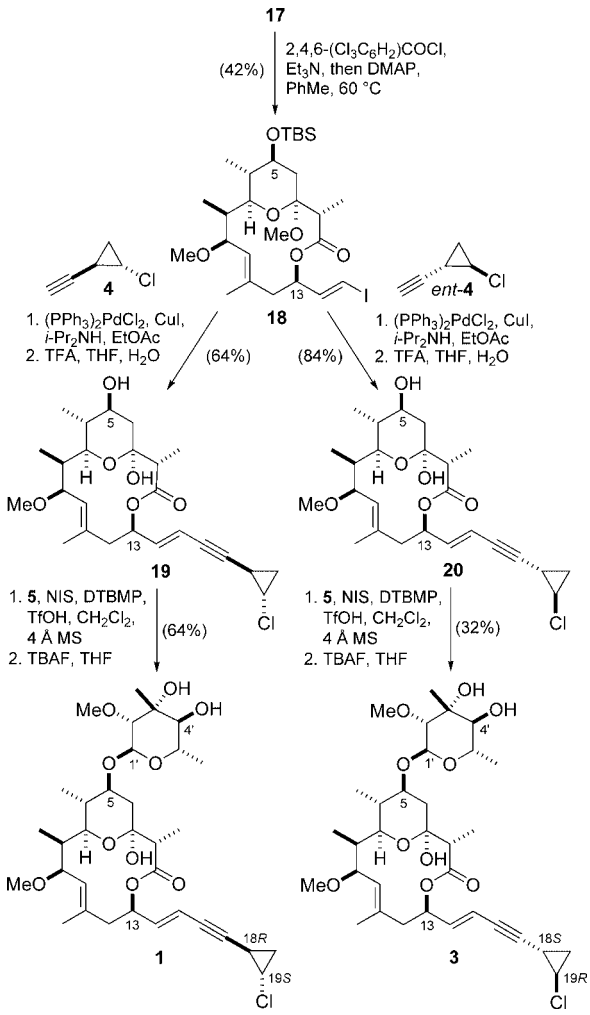
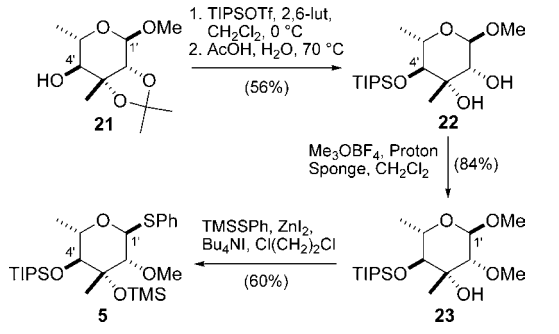
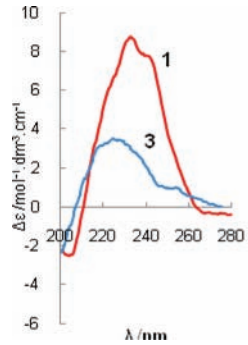
Scheme 3



natural phorbaside A. Ultimately, CD studies confirmed Molinski's assignment of the (18*R*,19*S*) configuration of the cyclopropane ring in phorbaside A (Figure 2).[2] The peak shape and intensity of the CD spectrum of **1** correlates with that reported for the natural product, whereas that of **3** shows a drop in intensity and reaches a maximum at a lower wavelength. Together with the respective NMR data, the CD

Scheme 4





compound	λ_1 (Δε)	λ_2 (Δε)
phorbaside A	232 (+9.1)	241 (+8.1)
1	233 (+8.7)	242 (+7.7)
3	225 (+3.6)	241 (+2.4)

Figure 2. CD spectra in MeOH of synthetic compounds **1**, $[\alpha]_D$ = +53.5 (*c* = 0.2, MeOH) cf. lit. +38 (*c* = 0.06, MeOH), and **3**, with peak maxima compared to that of natural (+)-phorbaside A.

spectra provide convincing evidence that the structure of (+)-phorbaside A is indeed as depicted in **1**.

In conclusion, we have completed the first total synthesis of the rare marine macrolide (+)-phorbaside A, thereby verifying its full configurational assignment and providing further material for evaluation of its anticancer properties. Notably, the cyclopropane configuration found in the phorbasides is established by CD correlation to be *opposite* to that found in the callipeltosides, suggesting antipodal stereopreferences in the biosynthesis of the starter unit required for chain extension by the polyketide synthase in these otherwise analogous natural products.[2a] Finally, the present work further demonstrates the effectiveness of our boron aldol methodology to efficiently construct such complex marine macrolides.

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Note Added after ASAP Publication. The Abstract text read "3 steps" instead of "23 steps" in the version published ASAP April 13, 2010; the corrected version reposted April 15, 2010.

Supporting Information Available: Experimental details and spectroscopic data for new compounds, copies of ^{1}H and ^{13}C NMR spectra for synthetic phorbasides **1** and **3**, and comparison with natural phorbaside A. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Trost, B. M.; Gunzner, J. L.; Dirat, O.; Rhee, Y. H. *J. Am. Chem. Soc.* **2002**, *124*, 10396.
(19) The chemical shift of the C19 chloromethine carbon in natural phorbaside A appears to be incorrectly tabulated as 36.9 ppm in ref 2a. Inspection of the HSQC spectrum shows that H19 correlates instead to a signal at around 34.3 ppm, which is consistent with the C19 shifts measured for both synthetic **1** and **3**, as well as the analogous C21 chloromethine carbon in the callipeltosides. We thank Professor Molinski for confirming our proposed correction.