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Total Synthesis and Stereochemical Reassignment of (+)-Dolastatin 19†

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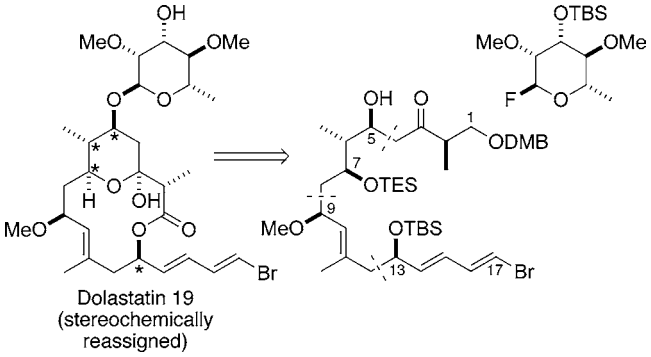
ABSTRACT



A revised configurational assignment for the cytotoxic marine macrolide dolastatin 19 is proposed and validated by total synthesis. Key features of the route include an asymmetric vinylogous aldol reaction to install the isolated C13 stereocenter and (*E*)-trisubstituted alkene, two sequential 1,4-syn boron-mediated aldol reactions, and a Mukaiyama glycosylation to append the L-rhamnose-derived pyranoside.

Dolastatin 19 is a polyketide of presumed cyanobacterial origin, first isolated in 2004 by Pettit and co-workers from the sea hare *Dolabella auricularia* collected in the Gulf of California.[1] Initial biological screening indicated significant cancer cell growth inhibitory activity (GI_{50} values of 0.72 μg/mL and 0.76 μg/mL for breast MCF-7 and colon KM20L2 cell lines, respectively). However, further biological evaluation of dolastatin 19, including elucidation of the mechanism of action, was precluded by the low isolation yield (0.5 mg was obtained from 600 kg of *D. auricularia*), inspiring our efforts toward the realization of a total synthesis.[2]

Following extensive spectroscopic analysis of dolastatin 19 by the Pettit group,[1] the structure was determined as a 14-membered glycosylated macrolide (**1**, Figure 1), containing a six-membered cyclic hemiacetal, appended with a bromine-substituted (*E*,*E*)-diene and a 2,4-di-*O*-methyl-L-rhamnopyranoside. It shares several structural features with the 14-membered macrolides auriside A (**2**) and B (**3**)[3] (also isolated from *D. auricularia*) as well as with callipeltoside A (**4**),[4] which have previously been synthesized in our laboratory.[2a,5,6] However, the assignment of relative stereochemistry for dolastatin 19, as shown in **1**, appears unusual on the basis of the anticipated common bacterial biogenesis of these related polyketides.[7] We now report the total synthesis and revised configurational assignment of dolastatin 19.

At the outset, we considered the preferred conformation of the related marine macrolides shown in Figure 1. Con-

† Dedicated to Professor K. C. Nicolaou on the occasion of his 60th birthday.

(1) Pettit, G. R.; Xu, J.-P.; Doubek, D. L.; Chapuis, J.-C.; Schmidt, J. M. *J. Nat. Prod.* **2004**, *67*, 1252.

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(4) Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085.

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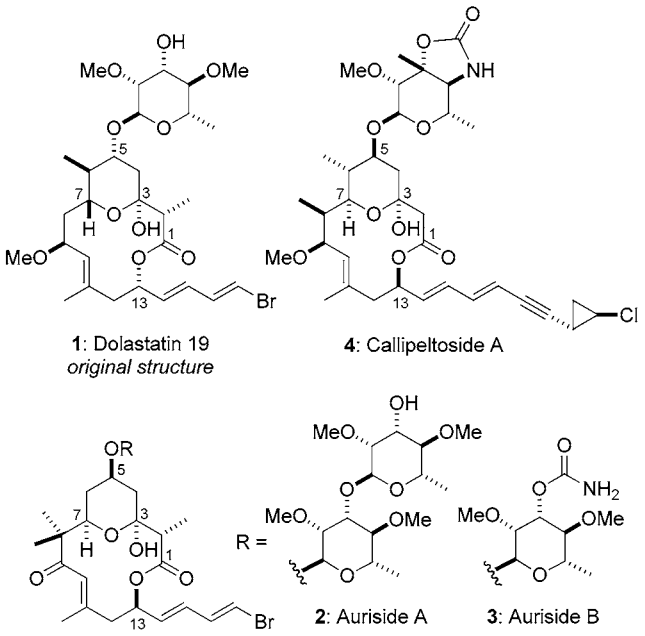
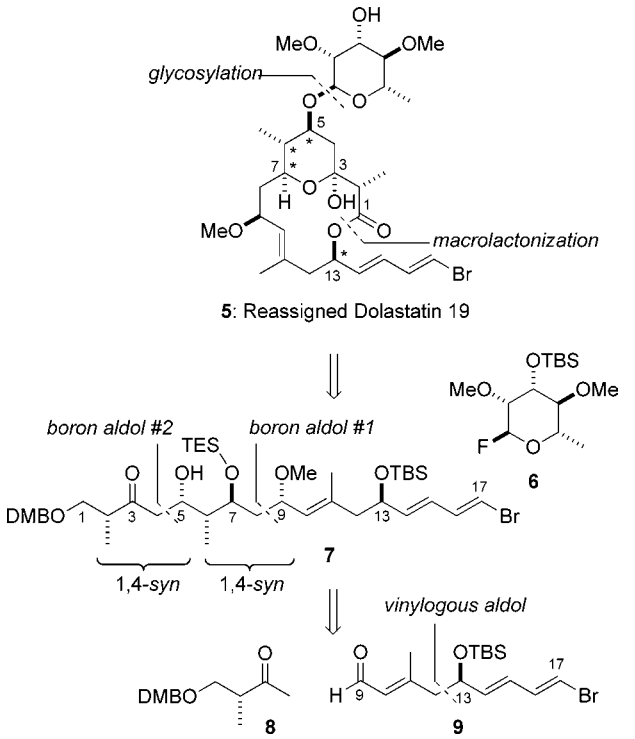


Figure 1. Dolastatin 19 and related marine-derived macrolides.

sistent with detailed ^{1}H NMR spectral analysis, callipeltoside and the aurisides share a similar diamond lattice arrangement for the macrolide with the six-membered hemiacetal ring adopting a chair conformation, facilitating a stabilizing hydrogen bond between the axial C3-OH and the lactone carbonyl oxygen and minimizing steric interactions. In contrast, the calculated lowest-energy conformation for **1**, the originally proposed structure for dolastatin 19, predicts a boat conformation for the pyran ring[8] and distortion of the macrolactone relative to that in callipeltoside and the aurisides. This conformational analysis prompted us to propose the stereochemical inversion of the C5−C7 array and the C13 carbinol in **1**, giving the putative structure **5** (Scheme 1) for dolastatin 19, which fits better with a common biogenesis for this family of cytotoxic polyketides.

As outlined in Scheme 1, our synthetic strategy for **5**, the proposed revised structure for dolastatin 19, envisaged an α-selective glycosylation of the aglycon with L-rhamnose-derived fluorosugar **6**, following suitable elaboration steps and macrolactonization of protected linear precursor **7**. The repeating 1,4-syn relationship found in **7** across the C2−C5 and C6−C9 arrays would, in turn, arise from two consecutive boron-mediated aldol reactions with α-chiral methyl ketone **8**[9] to chain extend aldehyde **9**. Application of an asymmetric

Scheme 1



*Denotes the stereocenter inverted in configuration relative to the original assignment, i.e., C5, C6, C7, and C13.

vinylogous Mukaiyama[10] aldol reaction would then allow for the simultaneous introduction of the remote C13 stereocenter and (*E*)-trisubstituted alkene in **9**.

As shown in Scheme 2, preparation of the C9−C17 aldehyde **9** began with the vinylogous aldol reaction between the silyl dienolate **10**[11] and the (*E*,*E*)-bromodienal **11**.[12] Following our standard conditions,[5a,6] treatment of **11** with the chiral Lewis acid promoter (*R*)-BINOL-Ti(O^{i}Pr)$_2$ (generated in situ from (*R*)-BINOL and Ti(O^{i}Pr)$_4$) in THF at −78 °C, followed by addition of **10**, provided the adduct **12** in 93% yield and 94% ee. TBS ether formation, DIBAL reduction, and subsequent reoxidation of the resulting alcohol with MnO_2 gave aldehyde **9** (75%). The stage was now set for the first 1,4-syn aldol reaction with methyl ketone **8**. Building on experience gained in our synthesis of callipeltoside,[5] we chose the 3,4-dimethoxybenzyl (DMB) ether[13] in **8**[14] to alleviate later chemoselectivity complications arising from competitive C13 oxidation by DDQ. Enolization of **8** with (+)-Ipc$_2$BCl/Et$_3$N, followed by addition of aldehyde **9**, generated the expected 1,4-syn aldol adduct **13** in 88% yield and >95:5 dr.[9]

(7) Celmer's configurational model, as developed for macrolide antibiotics, may be usefully extended to predicting stereochemical homology in marine-derived secondary metabolites produced by polyketide synthases in genetically related bacteria. Celmer, W. D. *J. Am. Chem. Soc.* **1965**, *87*, 1801.

(8) The boat conformation of the pyran ring in dolastatin 19 was proposed by the Pettit group from interpretation of NOE correlations in 2D-ROESY experiments, leading to their stereochemical assignment in structure **1** (ref 1).

(9) (a) Paterson, I.; Goodman, J. M.; Isaka, M. *Tetrahedron Lett.* **1989**, *30*, 7121. (b) Paterson, I.; Oballa, R. M. *Tetrahedron Lett.* **1997**, *38*, 8241.

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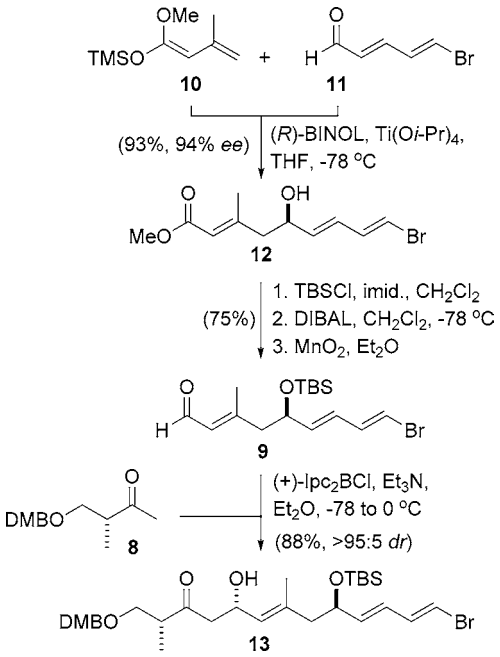
(12) (a) Becher, J. *Synthesis* **1980**, 589. (b) Soullez, D.; Plé, G.; Duhamel, L. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1639.

(13) Oikawa, Y.; Tanaka, T.; Horita, K.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1984**, *25*, 5393.

(14) Methyl ketone **8** was prepared from methyl (*R*)-3-hydroxy-2-methylpropionate: (i) DMBO(CCl$_3$)C=NH, PPTS, CH_2Cl_2; (ii) (MeO)NHMe·HCl, ^{i}PrMgCl, THF, −20 °C; (iii) MeMgCl, THF, 0 °C.

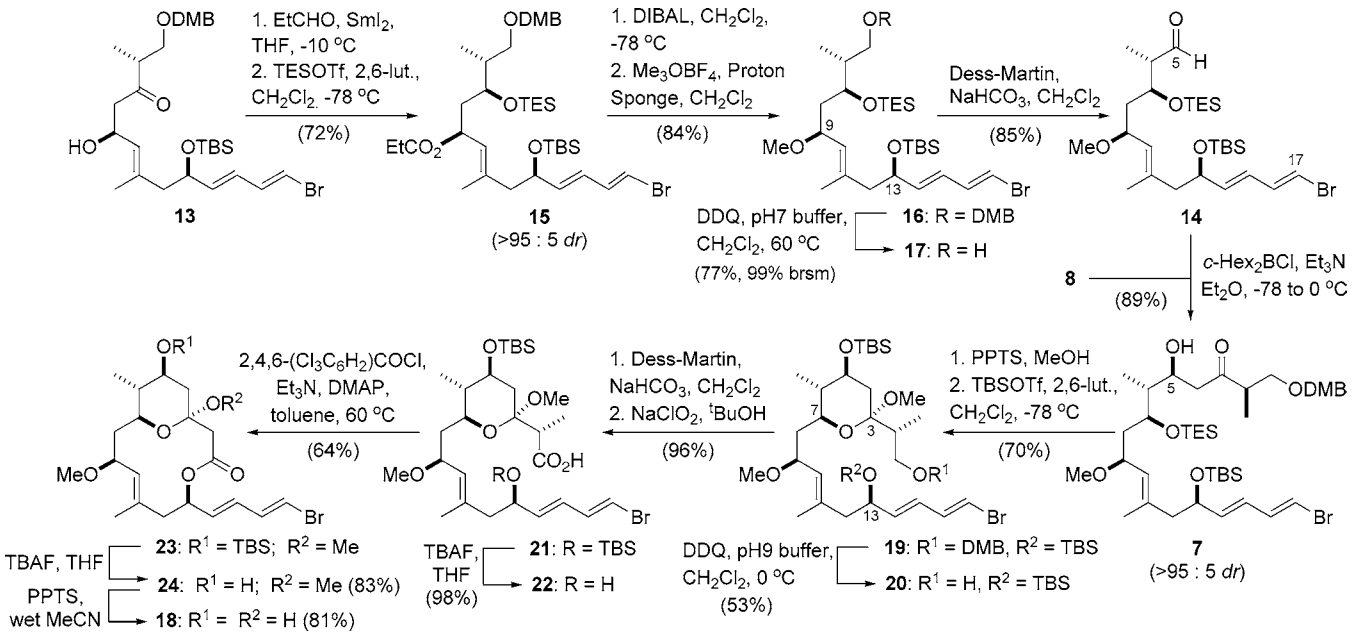
Scheme 2



With aldol adduct **13** in hand, elaboration to C5−C17 aldehyde **14** was required (Scheme 3). Evans−Tischenko 1,3-anti reduction[15] of β-hydroxy ketone **13** using SmI_2 and propionaldehyde provided the intermediate hydroxy ester, which was readily converted into TES ether **15** (72% over two steps, >95:5 dr). Reductive cleavage of the propionate ester in **15** by DIBAL and *O*-methylation of the resulting C9 alcohol with Meerwein's salt provided **16** (84%). Oxidative cleavage of the primary DMB ether was now required in the presence of the potentially labile allylic TBS ether at C13. Employing the optimized conditions from our callipeltoside synthesis,[5] treatment of DMB ether **16** with DDQ in CH_2Cl_2 and pH 7 buffer (60 °C, 10 min) gave alcohol **17** in 77% yield (99% based on recovered **16**). Conversion into the C5−C17 aldehyde **14** was then achieved by Dess−Martin oxidation of **17**, in preparation for the second 1,4-syn aldol reaction with **8**. Enolization of methyl ketone **8** with *c*-Hex_2BCl/Et_3N and reaction with **14** proceeded under efficient substrate control, imparted from both aldol partners, to give the expected Felkin−Anh adduct **7** in excellent yield and selectivity (89%, >95:5 dr).

With the full C1−C17 carbon backbone in place, attention was now directed to assembling the putative aglycon **18** of dolastatin 19. Treatment of **7** with PPTS and trimethyl orthoformate in MeOH led to removal of the TES ether at C7, with concomitant cyclization and methyl acetal formation, giving **19** (70%) after TBS protection of the C5 hydroxyl (TBSOTf/2,6-lutidine).[16] Cleavage of the DMB ether in **19** was now required. However, employing the conditions used successfully for **16** led only to poor conversion, and prolonged exposure of **19** to DDQ led to hydrolysis of the methyl acetal and C13 oxidation. After extensive optimization, treatment of **19** with DDQ (5 equiv) in pH 9 buffer and CH_2Cl_2 (0 °C, 10 min) provided the desired primary alcohol **20** in 53% yield. Next, oxidation of **20** with Dess−Martin periodinane gave the intermediate aldehyde, which was readily oxidized with $NaClO_2$ to carboxylic acid **21** (96%). Selective cleavage of the allylic TBS ether at C13 with TBAF then provided the required *seco*-acid **22** (98%), in preparation for the key macrolactonization step. Under Yamaguchi conditions,[17] **22** was converted into the 14-membered macrolide **23** in 64% yield. Cleavage of the remaining silyl group at C5 with TBAF, followed by acid hydrolysis of the methyl acetal **24**, provided the putative

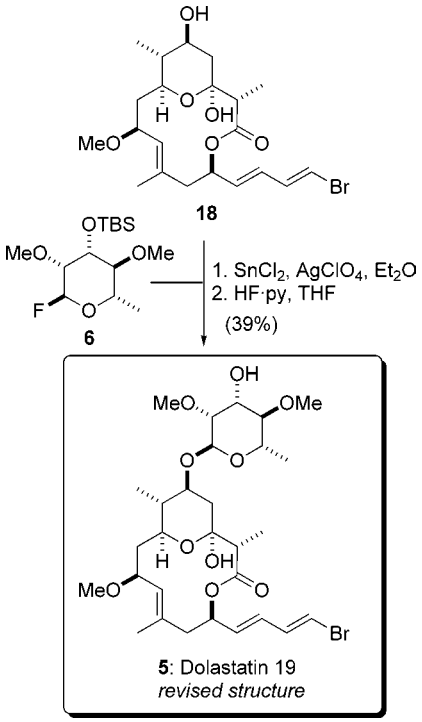
Scheme 3



aglycon **18** (67%). At this stage, comparison of the ^{1}H and ^{13}C NMR spectra of aglycon **18**[18] with the reported data for the macrolide region of dolastatin 19 showed close agreement, providing an early indication of the likely validity of our proposed stereochemical reassignment.

Completion of the synthesis of dolastatin 19 (Scheme 4) required the stereocontrolled glycosylation of the aglycon

Scheme 4



18 with rhamnose-derived fluorosugar **6**, previously utilized for auriside A.[6] Coupling of **18** and **6** under Mukaiyama conditions ($SnCl_2/AgClO_4$),[19] followed by cleavage of the residual TBS ether with HF·pyridine, afforded **5** in 39% yield with complete α-selectivity. Gratifyingly, the spectroscopic data[18] obtained for **5** (^{1}H and ^{13}C NMR, MS) and the measured specific rotation, $[\alpha]_D^{20}$ +2.2 (*c* 0.18, MeOH) cf. +7.5 (*c* 0.04, MeOH), correlated fully with that of natural dolastatin 19.[1] Together with the biological data obtained,[20] this provided convincing evidence that the relative and absolute configuration of (+)-dolastatin 19 is that indicated in structure **5** (i.e., 2*S*, 3*S*, 5*S*, 6*R*, 7*S*, 9*S*, 13*R* in the aglycon).

In conclusion, we have resolved the stereochemical ambiguities surrounding the structure of the cytotoxic macrolide (+)-dolastatin 19, isolated from the sea hare *Dolabella auricularia*, by completing the first total synthesis (23 steps, 1.7% overall yield), and have enabled further biological studies.[20] The present work further demonstrates the versatility of our aldol methodology for providing synthetic access to rare bioactive marine polyketides, as well as for establishing their full stereochemistry.[21]

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Supporting Information Available: Representative experimental details and spectroscopic data for all new compounds and copies of ^{1}H and ^{13}C NMR spectra for synthetic and natural dolastatin 19 with full assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

OL060609Q

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(16) The stereochemistry of **19** was confirmed by ^{1}H NMR analysis, with irradiation of H5 providing diagnostic NOE enhancements with the C3-OMe and C6-Me, consistent with their 1,3-diaxial and cis orientations, respectively.
(17) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
(18) See the Supporting Information.
(19) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431.
(20) GI_{50} values measured for **5** against representative cancer cell lines: 0.88 μM for HT29 (colon), 1.04 μM for NSCLC (lung), 1.20 μM for MDA-MB-231 (breast). These biological results are consistent with that reported for dolastatin 19 (ref 1).
(21) For a review on the role of total synthesis in the reassignment of natural product structures, see: Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012.