

Total synthesis of cytotoxic marine macrolides: Callipeltoside A, aurisides A and B, and dolastatin 19*

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Abstract: Synthetic studies pertaining to a novel class of structurally related glycosidic 14-membered macrolides of marine origin are reported. The evolution of a versatile aldol-based strategy that culminated in the total syntheses of callipeltoside A and aurisides A and B is detailed. Using a combination of biogenetic considerations and conformational analysis, a revised stereochemical assignment for the related polyketide dolastatin 19 was proposed and validated by total synthesis.

Keywords: natural products; macrolides; anticancer; stereocontrolled synthesis; aldol.

INTRODUCTION

Nature has long been recognized as a major reservoir of molecular diversity, with natural products making an indispensable contribution to the discovery and development of effective medicinal agents [1]. In particular, the marine environment has proven to be a rich source of bioactive compounds, many of which belong to novel chemotypes not found in terrestrial sources [2]. The harsh environment inhabited by marine organisms requires that they develop, or indeed sequester, effective chemical defenses to ward off potential predators, and, hence, the secondary metabolites associated with sponge invertebrates and sea hares possess intriguing biological profiles [3]. Unfortunately, owing to the low natural abundance of such compounds, further biological evaluation, including elucidation of the mechanism of action, is often precluded. Total synthesis can provide a powerful solution to this supply problem, and, furthermore, in cases where spectroscopic analysis may not have permitted a full assignment of structure, may be used to facilitate complete stereochemical determination [4]. Herein, we review our recent work directed toward the stereochemical assignment and total synthesis of a novel class of cytotoxic 14-membered macrolides, isolated from different marine sources, which includes the callipeltosides, the aurisides, and dolastatin 19.

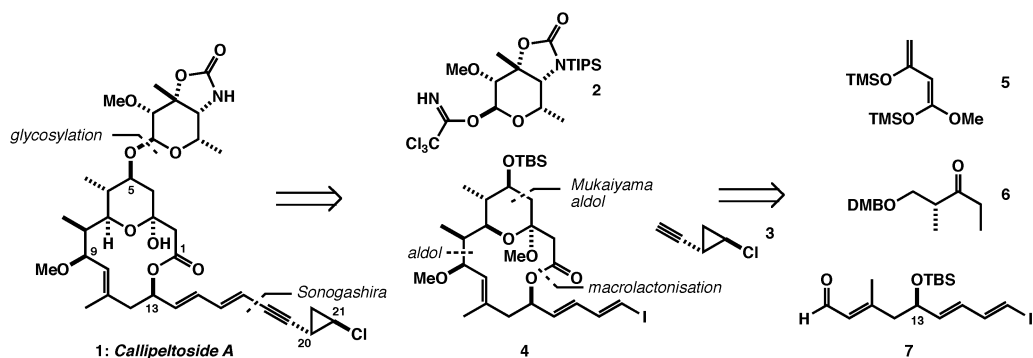
CALLIPELTOSIDE A

From collections of the shallow-water lithistid sponge *Callipelta sp.*, found off the coast of New Caledonia, D'Auria and coworkers reported in 1996 the isolation of a novel class of polyketide macrolides designated the callipeltosides (3.5 mg combined yield) [5]. Preliminary in vitro biological screening highlighted significant cytotoxicity, with the mechanism of action believed to involve the blocking of cell division in the G1 phase [5a]. From a structural perspective, callipeltoside A (**1**,

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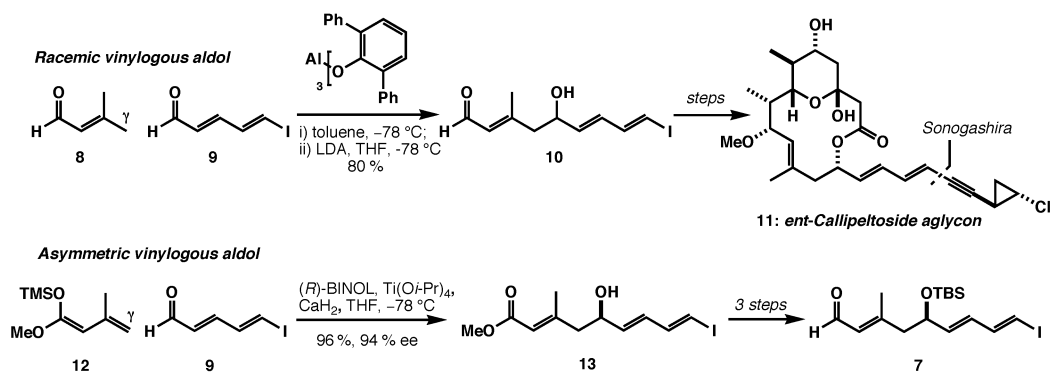
Scheme 1) was determined to be a 14-membered macrolactone, with a 6-membered hemiacetal ring linked glycosidically through C5 to an amino sugar moiety. Intriguingly, a highly unusual unsaturated side chain, comprising a dienyne linked through to a *trans*-chlorocyclopropane ring, is appended at C13. Captivated by this intriguing molecular architecture and promising biological properties, we were inspired to launch a synthetic effort toward callipeltoside A [6,7].



Scheme 1

At the outset of our work in 1999, the uncertainty over the full stereochemical assignment of callipeltoside A, including the configuration at C13 and the remote C20/C21 on the isolated cyclopropane ring, was factored into our synthetic planning (Scheme 1). Late-stage attachment of a suitable glycosyl donor, such as **2**, and the cyclopropyl alkyne **3** (or its enantiomer) to the advanced intermediate **4** was envisaged, following on from the hemiacetalization and macrolactonization of an appropriate linear precursor [6]. The macrolide core **4** would then be assembled by sequential aldol coupling of three key building blocks, namely, silyl dienolate **5**, ethyl ketone **6**, and aldehyde **7**. We initially prepared both C13 epimers, which enabled elucidation of the relative configuration within the macrocycle [6a] and provided a secure foundation to advance the total synthesis [6b].

A key objective was the early introduction of the (*E*)-trisubstituted alkene in aldehyde **7**, together with the potentially delicate (*E,E*)-iododiene. This was readily achieved in a racemic sense with a vinyllogous aldol reaction promoted by a bulky aluminum reagent (Scheme 2), using elegant chemistry developed by Yamamoto [8]. Addition of the extended enolate derived from enal **8** to aldehyde **9** generated the γ -adduct **10** with complete control over the alkene geometry. Notably, this tactic permitted the flexibility required at the C13 stereocenter and incorporated the iododiene precursor to the callipelto-

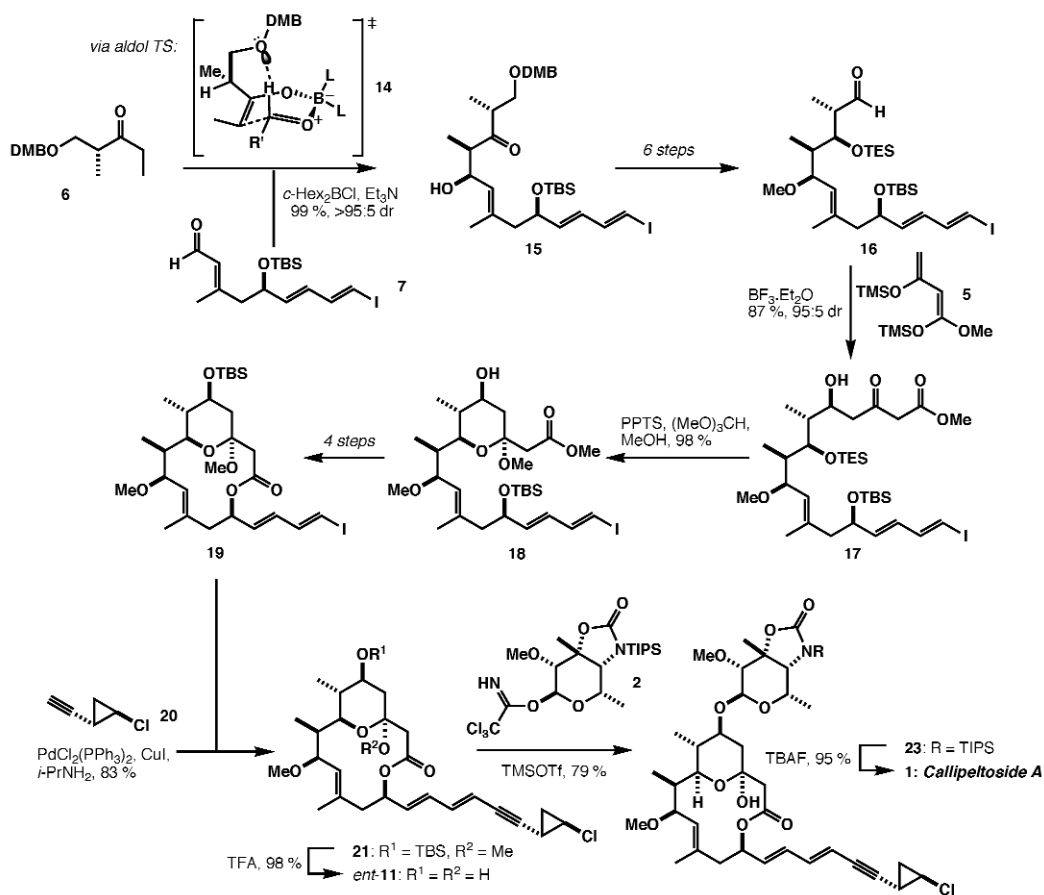


Scheme 2

side side chain. Initially, this intermediate **10** was progressed to *ent*-callipeltoside aglycon **11** which, taken with the findings reported by Trost [7a], enabled assignment of the full configuration of (–)-callipeltoside A.

Next, this vinylogous aldol process was extended to an asymmetric Mukaiyama-type variant by the use of silyl dienolate **12** and aldehyde **9** with a chiral Lewis acid promoter (Scheme 2). Initial experiments identified the (*R*)-BINOL-Ti(Oi-Pr)₂ system, formed in situ from (*R*)-BINOL and Ti(Oi-Pr)₄, as a promising catalyst, albeit requiring high loadings. The optimized conditions used CaH₂ as an additive (as a replacement for molecular sieves), which served to avoid isomerization of the iododiene functionality, and competing hydrolysis of **12** and generated the desired γ -adduct **13** cleanly in 96 % yield and 94 % ee on a multi-gram scale. From here, three steps were needed to provide the desired C9–C17 subunit **7**.

A highly stereocontrolled total synthesis of (–)-callipeltoside A (**1**) was accomplished based on several improvements to our earlier aglycon studies (Scheme 3). Chain extension of aldehyde **7** involved a boron-mediated aldol coupling with the (*E*)-enolate of Roche ester-derived ethyl ketone **6**. This efficient coupling proceeds through a bicyclic TS **14** involving a formyl hydrogen bond with the 3,4-dimethoxybenzyl (DMB) ether oxygen, to give the *anti,anti*-adduct in high yield and selectivity (99 %, >95:5 dr). A further six steps, including an Evans–Tischenko 1,3-*anti*-reduction, led to aldehyde **16** and were followed by a Mukaiyama-type aldol coupling with silyl dienolate **5** to generate the desired Felkin-Anh adduct **17** (85 %, 95:5 dr) as the fully extended linear precursor to the macrolide.



Scheme 3

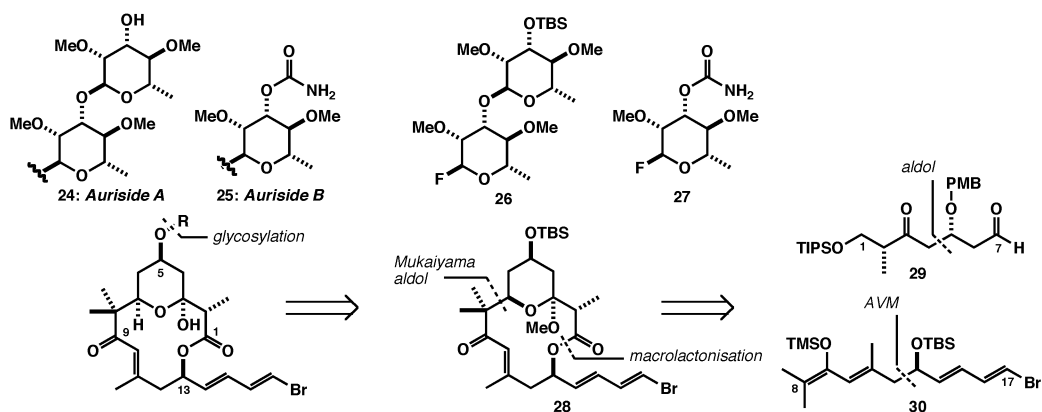
A five-step sequence, including cleavage of the triethylsilyl (TES) ether with concomitant methyl acetal formation to give **18**, generated the corresponding *seco*-acid, which underwent Yamaguchi macrolactonization to provide the macrolide core **19**.

At this stage, the required elaboration included installation of the chlorocyclopropyl-containing side chain (obtained by using a Charette asymmetric Simmons-Smith reaction) and glycosylation of the C5 hydroxyl with an appropriate *L*-rhamnose-derived sugar unit. Firstly, the fully elaborated side chain was introduced via a Sonogashira cross-coupling between macrocyclic dienyl iodide **19** and alkyne **20**, generating the desired protected aglycon product **21** (83 %) with complete retention of double-bond geometry. Acid-mediated silyl ether cleavage and hydrolysis of the methyl acetal gave callipeltoside aglycon (*ent*-**11**), and was followed by coupling with the activated sugar unit **2** (obtained from *L*-rhamnose by adaptation of the sequence reported by Guilano and coworkers [9]) under Schmidt conditions. Final desilylation of **23** gave (–)-callipeltoside A (**1**), which was found to display similar cytotoxic activity to natural material, with an IC₅₀ value of 7.75 µg/mL against 1A9 human ovarian adenocarcinoma cells. Overall, this highly stereoselective sequence efficiently delivered (–)-callipeltoside A in 23 steps and 4.8 % yield [7]. It also highlighted the potential utility of this novel asymmetric vinylogous Mukaiyama aldol (AVM) reaction, used in tandem with our boron-mediated aldol methodology, to provide a general entry to other marine macrolides possessing this unusual molecular framework.

AURISIDES A AND B

The aurisides are 14-membered glycosylated macrolides obtained from *Dolabella auricularia*, a sea hare from the *aplysiidae* family of marine opisthobranchs, that were first reported by Yamada and coworkers in 1996 [10]. *D. auricularia*, typically found in shallow circumtropical seas, is a generalist herbivore that feeds on microalgae and cyanobacteria which has proven to be a rich source of novel bioactive agents. However, the true origin of such structurally intriguing metabolites is likely to be cyanobacteria, sequestered through diet or symbiosis [11]. Extracts of Japanese specimens of *D. auricularia* (138 kg) led to the isolation of aurisides A (**24**, 0.8 mg) and B (**25**, 0.7 mg), which differ only in the sugar moiety appended at C5 (Scheme 4). In common with the callipeltosides, the aurisides are 14-membered macrolides, containing a cyclic hemiacetal moiety and unusual pyranoside sugar units, but now feature an (*E,E*)-bromodiene in the side chain. Both aurisides A and B were found to be cytotoxic against HeLa S₃ cell lines, with IC₅₀ values of 0.17 and 1.2 µg/mL, respectively.

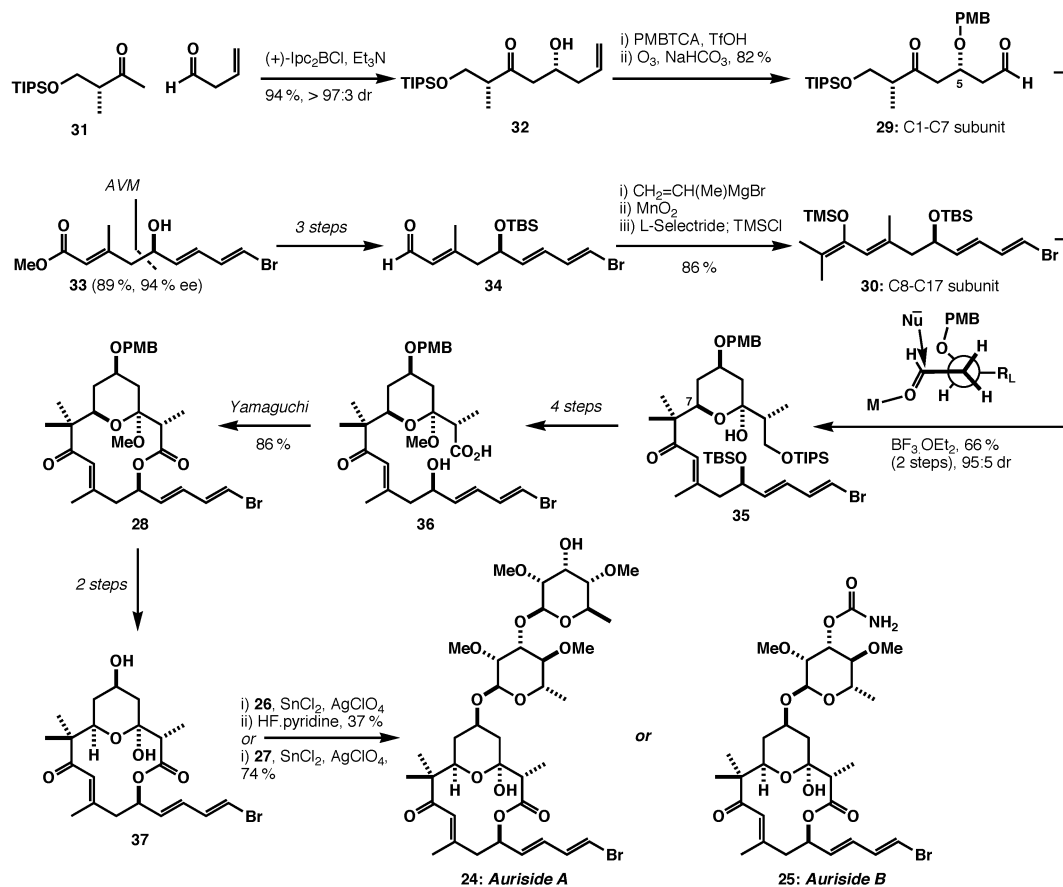
Our total synthesis of these natural products relied upon late-stage α -selective glycosylation of the equatorial C5 alcohol, using either fluoro disaccharide **26** for auriside A or fluoro saccharide **27** for auriside B (Scheme 4) [12,13]. The macrolide core **28** was constructed via Yamaguchi macrolactoniza-



Scheme 4

tion, with the neopentyl C7 stereocenter installed using an adventurous Mukaiyama aldol coupling between aldehyde **29** and the highly unsaturated silyl enol ether **30**. Employing the efficient AVM reaction used previously in our callipeltoside work, the remote C13 stereocenter and (*E*)-trisubstituted double bond would be introduced in a single step, while the C5 stereocenter would be secured using an analogous methyl ketone aldol reaction.

As outlined in Scheme 5, preparation of the C1–C7 subunit **29** was readily achieved by enolization [(+)-Ipc₂BCl/Et₃N] of methyl ketone **31** and addition of 3-butenal, leading to the 1,4-*syn* adduct **32** (94 %, >97:3 dr). Following *p*-methoxybenzyl (PMB) ether formation, careful ozonolysis generated 1,5 ketoaldehyde **29**. For the C8–C17 fragment **30**, bromodiene **33** was prepared in an analogous manner to iododiene **7**, again employing the efficient AVM reaction of silyl dienolate **12**. A 3-step sequence gave aldehyde **34**, which was elaborated to the desired coupling partner **30**, involving a crucial regioselective 1,4-reduction of the less sterically encumbered enone.



Scheme 5

A Mukaiyama aldol coupling of **29** and **30** generated the corresponding aldol adduct, which cyclized to its hemiacetal tautomer **35**, with efficient control over the C7 stereocenter (95:5 dr). The 1,3-*anti*-induction from the C5 PMB ether is rationalized by operation of the Evans polar model in the acyclic transition state [14]. From here, the derived *seco*-acid **36** underwent Yamaguchi macrocyclization to produce the 14-membered macrolactone **28**. A further 2 steps then completed the preparation of the aglycon **37**. Finally, the Mukaiyama protocol [15] was adapted for the coupling of aglycon

37 with either **26** or **27** to achieve clean α -glycosylation and, following desilylation, (–)-aurisides A (**24**) and B (**25**) were obtained. Overall, a highly stereocontrolled synthesis of both members of the auriside family was achieved, proceeding in 18 steps (1.7 %) for (–)-auriside A and 17 steps (3.5 %) for (–)-auriside B [13].

DOLASTATIN 19

The Pettit group extracted a collection of the sea hare *D. auricularia* from the Gulf of California, which led in 2004 to the reported isolation of a novel 14-membered macrolide, designated dolastatin 19 [16]. Initial biological screening indicated significant cancer cell growth inhibitory activity (GI_{50} values of 0.72 and 0.76 $\mu\text{g/mL}$ for breast MCF-7 and colon KM20L2 cell lines, respectively). However, further biological evaluation was precluded by its scarce availability from the natural source (0.5 mg was obtained from 600 kg of *D. auricularia*), inspiring our efforts towards the realization of a total synthesis [17].

Following extensive spectroscopic analysis by Pettit and coworkers, the full stereostructure of dolastatin 19 was originally proposed as **38** (Fig. 1). As a 14-membered macrolide containing a 6-membered cyclic hemiacetal and appended with an (*E,E*)-diene and a 2,4-di-*O*-methyl-L-rhamnopyranoside, it bears a strong resemblance to both the aurisides and the callipeltosides. Examination of the respective linear *seco*-acids **39** and **40** (Fig. 1) serves to highlight the structural similarities. On careful inspection, the assignment of the configuration of dolastatin 19 across C5–C7 and at C13 in the corresponding *seco*-acid **41** appeared to us to be inconsistent with the anticipated common bacterial biogenesis of these polyketides.

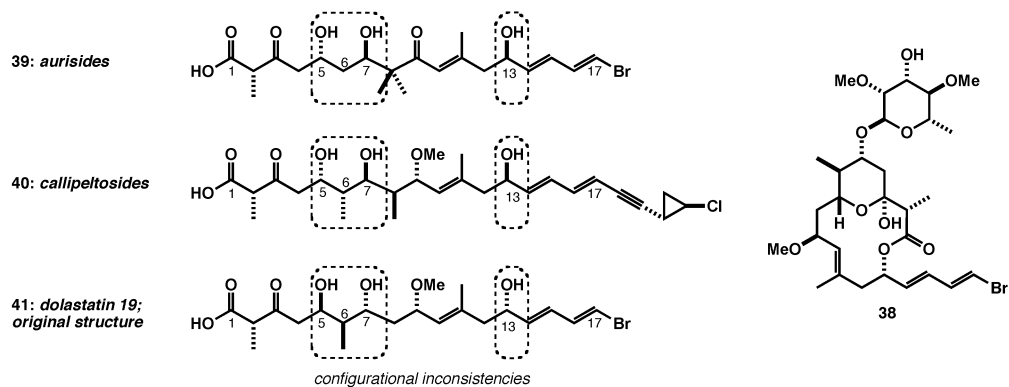
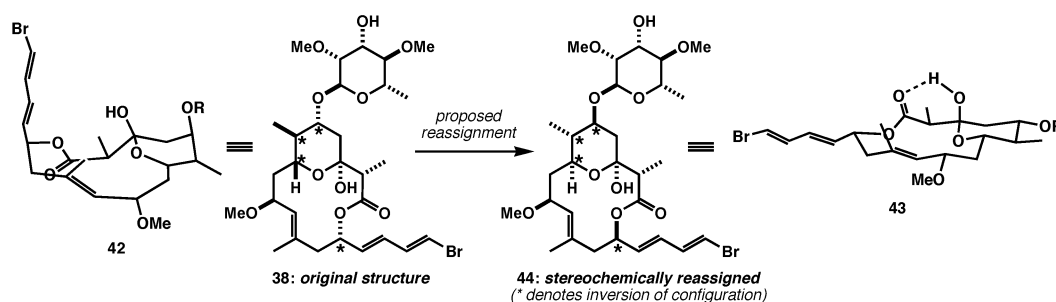


Fig. 1

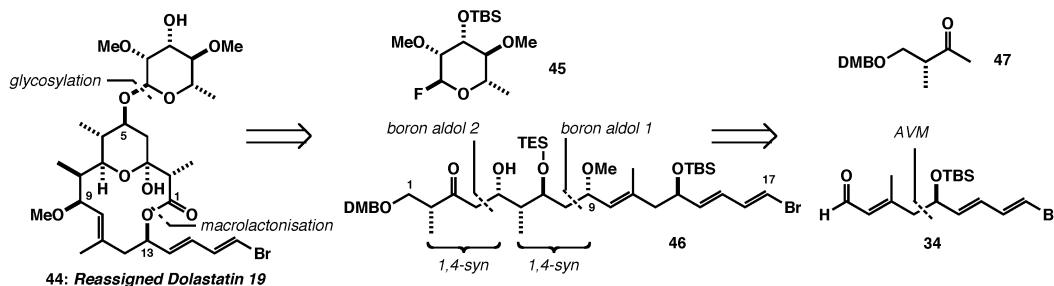
Prior to the onset of a synthetic campaign toward dolastatin 19, we considered the preferred conformation of such related macrolides (Scheme 6). Reassuringly, both the auriside and callipeltoside aglycons share a similar diamond lattice arrangement of the macrolide ring. The six-membered hemiacetal ring adopts a chair conformation, in which all the substituents are equatorially disposed, and anomeric stabilization is achieved at C3. This preferred conformation also facilitates a stabilizing hydrogen bond between the C3-OH and the oxygen of the lactone carbonyl, and minimizes steric interactions throughout the carbon framework. By contrast, the calculated lowest energy conformation **42** corresponding to the originally proposed structure for dolastatin 19 indicates the pyran ring adopts a boat conformation, with the macrolactone distorted relative to the common, and presumably favorable, diamond lattice arrangement. This analysis, together with the assumption of a common biogenesis, prompted us to propose the configurational inversion of both the C5–C7 stereotriad and the isolated C13



Scheme 6

carbinol stereocenter. This led to the calculated preferred conformation **43** (now analogous to that of the aurisides and callipeltoside) and putative revised structure **44** for dolastatin 19.

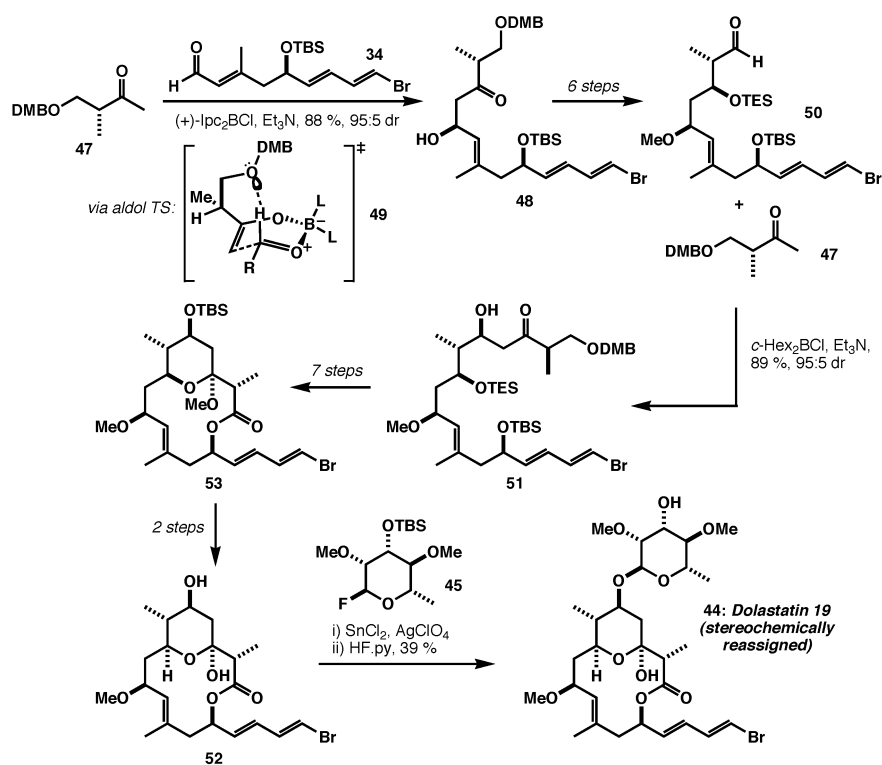
The resulting synthetic plan for dolastatin 19, outlined in Scheme 7, built upon the expertise and knowledge gained during our callipeltoside and auriside studies. We again envisaged a late-stage glycosylation, with L-rhamnose-derived fluorosugar **45**, following hemiacetal formation at C3 and macrolactonization of the C1–C17 linear precursor **46**. Inspection of the complete aglycon framework revealed two 1,4-*syn* relationships that could be selectively installed using iterative boron-mediated aldol reactions, both involving methyl ketone **47** [18]. The first aldol coupling, between aldehyde **34** and ketone **47**, would introduce the requisite C9 stereocenter. Aldehyde **34** was already available using the AVM aldol reaction used in our auriside work.



Scheme 7

Treatment of DMB-protected methyl ketone **47** with (+)- Ipc_2BCl and Et_3N , followed by addition of aldehyde **34**, generated the expected 1,4-*syn* aldol adduct **48** (88 %, >95:5 dr) (Scheme 8). Recent *in silico* studies regarding the origin of stereoinduction in the boron-mediated aldol reactions of β -alkoxy ketones have shown such processes to proceed via a boat-like transition state [19]. The high level of enolate π -facial selectivity observed is governed by the formation of a stabilizing formyl hydrogen bond with the oxygen of the DMB ether, acting in unison with the minimization of steric interactions between the α -stereocenter of the enolate and the aldehyde. In this case, the Ipc ligand reinforces this substrate induction in the favored bicyclic TS **49**.

Elaboration to C5–C17 aldehyde **50** was performed by a straightforward 6-step sequence, in readiness for the second 1,4-*syn* aldol reaction. Enolization of methyl ketone **47**, now using *c*-Hex₂BCl/ Et_3N , and reaction with **50** proceeded under efficient substrate control, imparted in a matched sense from both coupling partners, to give the expected Felkin-Anh adduct **51** (89 %, 95:5 dr). With the full C1–C17 carbon backbone in place, attention was directed to assembling the putative aglycon **52**. A robust series of reactions were performed, including acid-mediated cleavage of the TES ether with concomitant hemiacetal formation, to generate the corresponding *seco*-acid which underwent



Scheme 8

Yamaguchi macrolactonization to give the 14-membered macrocycle **53**. From here, a further two steps generated the aglycon **52**, where comparison of ^1H and ^{13}C NMR spectra 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 was achieved by the controlled glycosylation of **52** with fluorosugar **45**, previously used for auriside A. Cleavage of the residual *tert*-butyldimethylsilyl (TBS) ether afforded macrolide **44** with complete α -selectivity. Gratifyingly, the spectroscopic and chiroptical data obtained for **44**, along with the biological activity [GI_{50} values of 0.89, 1.04, and 1.20 $\mu\text{g}/\text{mL}$ against HT-29 (colon), NSCLC (lung), and MDA-MB-231 (breast) cell lines, respectively] displayed, correlated fully with that of natural (+)-dolastatin 19, thus validating our structural reassignment. Overall, the total synthesis of (+)-dolastatin 19 was achieved in 23 steps and 1.7 % yield.

CONCLUSION AND OUTLOOK

Highly stereocontrolled routes to various members of a class of cytotoxic 14-membered macrolides isolated from sponges and sea hares have been developed, enabling completion of several total syntheses. A key feature of this work was the evolution of an AVM aldol process, to simultaneously install the remote C13 stereocenter and the (*E*)-trisubstituted olefin. This was used to good effect in conjunction with our versatile boron aldol methodology to complete the stereocontrolled total syntheses of callipeltoside A and aurisides A and B. A combination of biogenetic considerations and molecular modeling subsequently prompted us to propose the configurational reassignment of another related macrolide, dolastatin 19, which was validated by the first total synthesis. Recent additions to this intriguing class of marine natural products include phorbosides A (**54**, Fig. 2) and B (**55**), isolated by Molinski and

coworkers from the sponge *Phorbas* sp. [20]. Owing to the remote location of the enyne chlorocyclopropane in the side chain relative to the macrolide core, configurational assignment proved challenging and required the use of a semi-quantitative circular dichroism (CD) method. Surprisingly, the configuration of the *trans*-chlorocyclopropane proved to be opposite to that of its nearest relative callipeltoside A. Our general synthetic strategy should be readily applicable to these new members of nature's diverse polyketide library that continues to provide an important source of new leads for drug discovery and development.

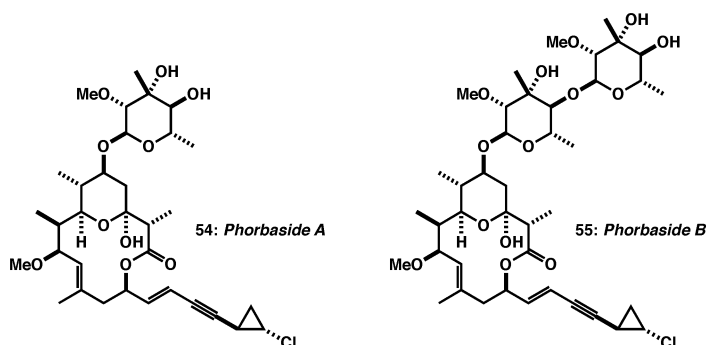


Fig. 2

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