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Total Synthesis and Biological Evaluation of 11-Desmethyllaulimalide, a Highly Potent Simplified Laulimalide Analogue

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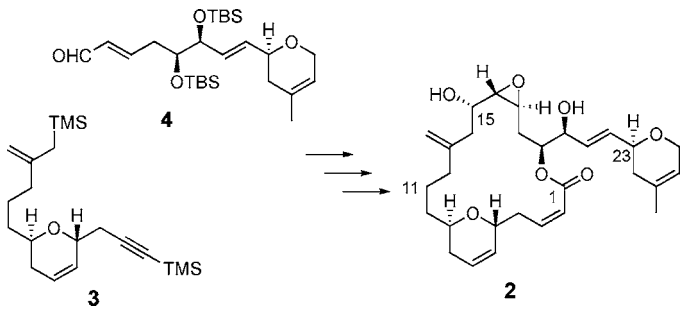
ABSTRACT



A step-economical synthesis of 11-desmethyllaulimalide (2) is reported. This simplified analogue is available through an improved second-generation synthetic approach to the laulimalides, in a shorter step count and from much less expensive starting material than the parent compound. This new lead retains the anticancer function of laulimalide.

Laulimalide[1] (a.k.a. fijianolide, **1**, Scheme 1) is a complex marine macrolide that was initially found to be a potent inhibitor of cancer cell proliferation. Subsequent studies revealed that it is a microtubule stabilizer with a mode of action similar to that of Taxol.[2] Laulimalide stimulates the polymerization of purified tubulin and in cells causes mitotic arrest and initiation of apoptosis.[2] Significantly, it offers a distinct potential therapeutic advantage over Taxol due to its capacity to inhibit the proliferation of multidrug-resistant cell lines.[2,3] Of special therapeutic importance, laulimalide does not bind to microtubules at the same site as Taxol,[3] and the two have been shown to synergistically stimulate the polymerization of tubulin in vitro.[4] These findings coupled with the scarce natural abundance of laulimalide have prompted a flurry of activity in the chemical community, resulting in several reports of the total synthesis of laulimalide itself[5] and of various non-natural analogues,[6,7] some of which have been shown to stabilize microtubules in the same manner as **1**.[7]

A major goal associated with the advancement of this therapeutic lead is the identification of stable analogues that

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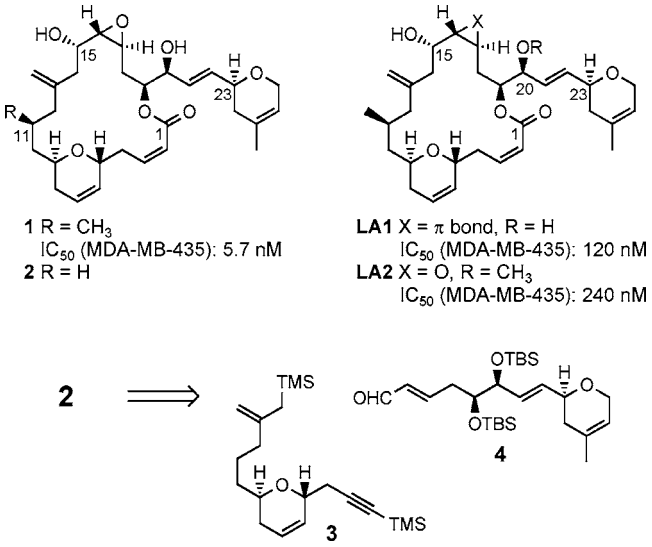
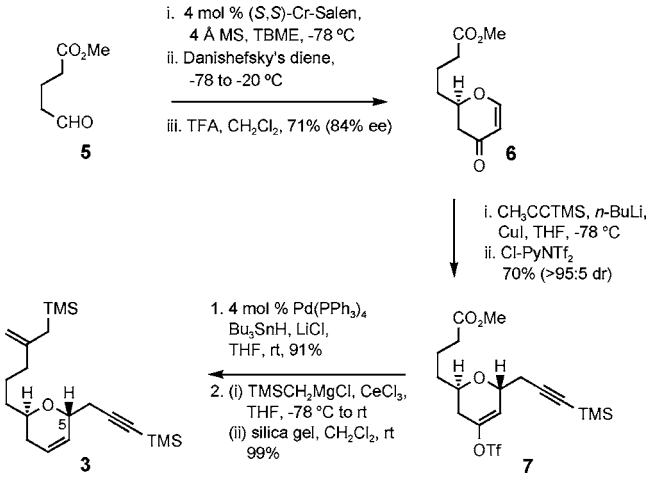
Scheme 1. Retrosynthesis of 11-Desmethyllaulimalide



retain laulimalide's activity and that could be supplied through total synthesis. We previously reported that the cause of laulimalide's instability, an internal nucleophilic substitution reaction leading to isolaulimalide, can be disabled by removal of either the electrophilic or nucleophilic functionalities involved in the process.[6b] The most potent analogues of this group, **LA1** and **LA2** (Scheme 1), are more stable than laulimalide and retain its mode of action.[7] In an effort to identify simpler analogues that could be supplied with greater step economy, we have targeted 11-desmethyllaulimalide (**2**, Scheme 1) as the starting scaffold for a new analogue series. This selection was made with the expectation that this analogue and, therefore, desmethyl derivatives related to **LA1** and **LA2** could be readily accessed from less costly starting materials using our previously reported synthesis of laulimalide. Justifying this target selection further were favorable comparisons of the global minimum conformations of **2** and **1**,[8] suggesting that the absence of the C11 methyl substituent would not have a detrimental effect on potency. The Paterson group has independently and contemporaneously produced a synthesis of this target.[6d] Herein we report a uniquely short and selective synthesis of **2** via our second-generation synthetic approach to the laulimalides and a preliminary evaluation of its biological activity. This report was held back to address incomplete spectroscopic correlations with the Paterson report which have now been resolved.[9]

Our convergent synthetic strategy draws on the disconnection of **2** into two simplified fragments, allyl silane **3** and enal **4** (Scheme 1), each containing 14 carbons of the target. The absence of the C11 methyl group in **3** allows its synthesis from the readily available aldehyde **5**,[10] thereby offering a cost and step advantage over the synthesis of the C11 methyl analogue that rested on citronellic acid as a starting material. Treatment of aldehyde **5** with Danishefsky's diene and Jacobsen's (*S*,*S*)-Cr-Salen catalyst[11] yielded pyranone **6** (71% yield, 84% ee). Cuprate addition of the anion of 1-trimethylsilyl-1-propyne[12] and in situ trapping of the resulting enolate with Comins' reagent afforded enol triflate **7** with high diastereoselectivity (70%, >95:5 dr). Reduction of this enol triflate followed by treatment of the resulting product with excess $TMSCH_2MgCl$ and $CeCl_3$ and subsequent Peterson olefination provided the desired allyl silane **3** in four steps from **5** or six steps from δ-valerolactone (Scheme 2). In addition to the cost and step advantages associated with the use of **5**,

Scheme 2. Four-Step Preparation of 11-Desmethyl Allyl Silane **3**



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(8) As determined using the MacroModel program, MM2 force field in chloroform, 10000-step Monte Carlo search.

(9) Delaying our report of the synthesis of this analogue was an incomplete correlation between the ^1H and ^{13}C NMR spectra of **2** and those appearing in the Supporting Information of the Paterson publication (ref 6d). A similar incomplete correlation was seen for the penultimate intermediate **18**. The published spectra were subsequently found to be incorrectly submitted. Spectra later provided by the Paterson group (Supplementary Information) are in agreement with our own and consistent with the structure of **2**.

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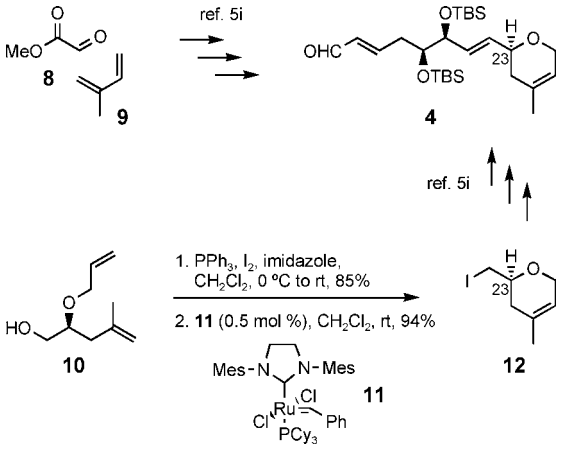
the direct introduction of a propargyl group through a conjugate addition (**6** to **7**) saves three steps relative to our original synthesis, which involved introduction of a vinyl group and subsequent conversion into the propargyl system.

The second key fragment in our synthesis, enal **4**, can be efficiently prepared as reported in our earlier communication.[5i] However, our original construction of the chiral C23 dihydropyran ring relied on the asymmetric hetero-Diels−Alder reaction of methyl glyoxylate and isoprene. Difficulty in consistently obtaining high yields for this key step eventually led us to develop a more robust and repeatable synthesis of this fragment. Thus, starting from known alcohol **10**,[13] a two-step iodination/ring-closing metathesis[14] sequence allowed for the intersection of our original route at iodide **12** in consistently high overall yield (55%) from the commercially available (*R*)-glycidol (Scheme 3). This modification allowed

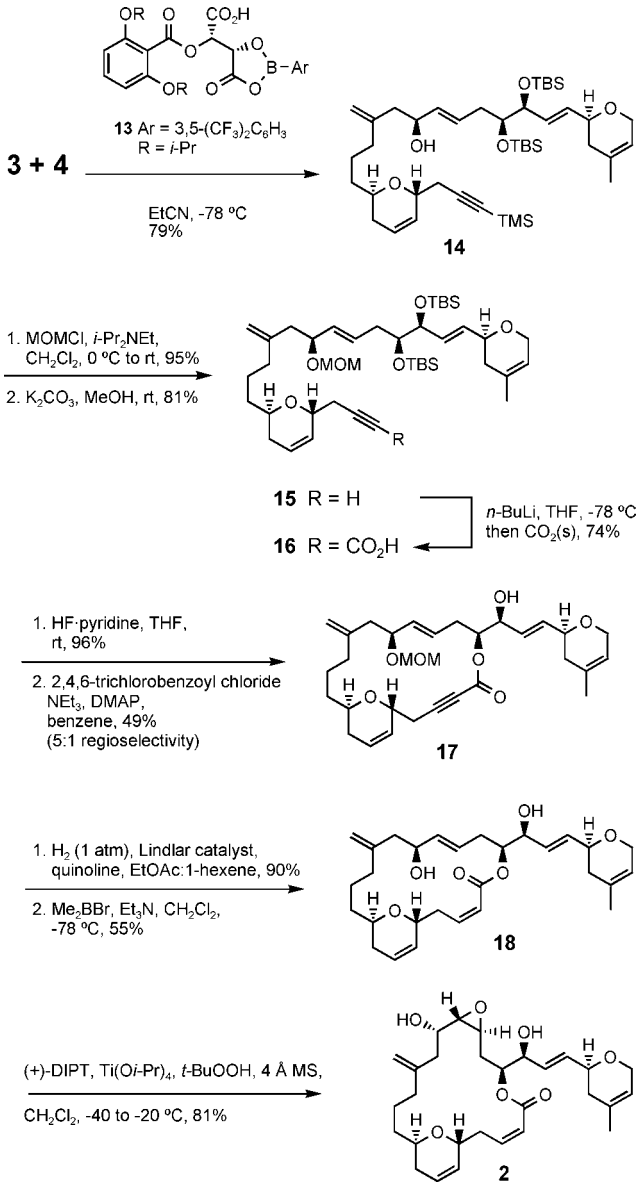
Scheme 3. Improved Preparation of the C23 Dihydopyran



for a more scalable route to fragment **4**.

Treatment of a solution of allyl silane **3** and enal **4** with Yamamoto's (acyloxy)borane **13** (Scheme 4)[15] allowed for the convergent assembly of the two fragments through an asymmetric Sakurai reaction,[16] providing the desired allylic alcohol **14** in 79% yield. This result provides yet another example of the high diasteroselectivity that can be achieved with this process even with complex fragments. The expected (*S*) stereochemistry of the Sakurai product was confirmed[17] by analysis using the advanced Mosher method[18] of the diastereomeric MTPA esters formed from **14**. Protection of the allylic hydroxyl as a MOM ether followed by TMS cleavage gave terminal alkyne **15**, which was deprotonated and trapped with CO_2 to yield carboxylic acid **16**. A high-yielding cleavage of both TBS protecting groups (96%)

Scheme 4. Completion of 11-Desmethyllaulimalide



followed by macrolactonization using the Yamaguchi procedure[19] gave macrolactone **17** as a 5:1 mixture favoring the desired 18-membered macrocycle over the 19-membered macrocycle. Lindlar reduction was followed by cleavage of the C15 MOM ether using dimethylbromoborane. Finally, regio- and stereoselective epoxidation of penultimate intermediate **18** using the Sharpless procedure[5i] provided 11-desmethyllaulimalide in 81% yield (Scheme 4).

Notably, this second-generation synthetic strategy for the construction of laulimalide and its analogues, making use of an allyl silane fragment **3** bearing a 1-TMS-1-propyne substituent at the C5 position, resulted in a savings of three steps (total and longest linear) in the synthesis of macrolactone **17** over the synthesis of the corresponding intermediate in our total synthesis of laulimalide. Also very significantly,

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the synthesis of allyl silane **3** absent the C11 stereocenter provided a further step savings as well as a 22-fold cost reduction in the starting material for that fragment. The improved approach to **4** offers greater throughput. Finally, the ready incorporation of the new substrates into our previous plan bodes well for its further use in the synthesis of additional analogue series.[20]

The anti-proliferative activity of analogue **2** was evaluated in two drug-sensitive cell lines, HeLa and MDA-MB-435, using the SRB assay.[21] The IC_{50} values observed are shown in Table 1.[22] Consistent with our hypothesis regarding the structural basis for laulimalide's activity, **2** retains the biological function of the parent compound and much of its potency. The ability of **2** to circumvent Pgp-mediated drug resistance was also evaluated using NCI/ADR cells, which express high levels of Pgp. The relative resistance values shown in parentheses represent the IC_{50} of the resistant cell line (NCI/ADR) divided by the IC_{50} of the sensitive cell line (MDA-MB-435). These values indicate that the resistant cells are roughly 350-fold more sensitive to our analogue than to Taxol, suggesting that like laulimalide analogue **2** is a poor substrate for Pgp. Finally, initial investigations indicate that **2** demonstrates laulimalide-like ability to induce tubulin polymerization.[23]

Table 1. IC_{50} Values[a] of Laulimalide, Analogue **2**, and Taxol

compd	HeLa	MDA-MB-435	NCI/ADR[b]
1		5.7 ± 0.6[24]	
2	38.5 ± 4.3	49.0 ± 2.3	292 ± 20 (6.0)
Taxol		1.15	2454 ± 307 (2134)

[a] IC_{50} values are given in nM. [b] Resistance factors are given in parentheses.

In summary, we have achieved an efficient synthesis of a potent, structurally simplified analogue of laulimalide, 11-desmethyllaulimalide **2**. This success illustrates the generality of our original synthetic plan. At the same time, this second-generation effort has led to some significant improvements in our approach. The new analogue is obtainable via a more cost- and step-economical synthetic route than laulimalide itself and is the most potent laulimalide analogue yet reported. Collectively, these factors make the 11-desmethyl analogue a more attractive platform for the design of new microtubule-stabilizing small molecules than laulimalide itself.

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Supporting Information Available: Experimental conditions and spectral data for all new compounds, as well as comparisons of spectral data of **2** with previously reported spectra and additional data supplied by the authors of ref 6d. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) A modified version of this new strategy has also been amenable to the preparation of laulimalide itself, representing a significant improvement over our total synthesis of **1**. Full details will be reported elsewhere.

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(22) Cells were treated with a range of concentrations of **2** and Taxol for 48 h. The IC_{50} values represent the means of three experiments $\pm$ SD.

(23) Full details will be reported elsewhere.

(24) This value is from ref 2.