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Synthesis and biological evaluation of (−)-laulimalide analogues

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Abstract—Analogues of the marine natural product (−)-laulimalide were prepared by total synthesis and evaluated in vitro for anticancer activity.
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The marine natural product (−)-laulimalide (**1**, Fig. 1) was first isolated in 1988 from several sources of marine sponge and shown to be highly cytotoxic in a number of human cancer cell lines.[1] Despite the interesting and challenging structural features of laulimalide, synthetic efforts were relatively few until the report that **1** induces microtubule polymerization and stabilization similar to paclitaxel, but retains activity in a P-glycoprotein (PgP) over-expressing multidrug resistant cell line.[2] It was recently reported that laulimalide binds to a different site of the tubulin polymer than other known microtubule stabilizers and retains activity against cell lines containing mutations in the β-tubulin gene.[3] These reports have generated an enormous amount of excitement about the therapeutic potential of this natural product and as a result, there has been a surge of synthetic efforts toward **1** resulting in total syntheses by eight separate groups,[4] as well as the syntheses of various fragments.[5] Despite these synthetic efforts, the biological evaluation of only a few laulimalide analogues have been reported to date.[3,6]

We began a program to explore the therapeutic potential of laulimalide and analogues. The initial goal was to design a total synthesis capable of producing gram quantities of **1** suitable for more in depth in vitro studies, and in vivo studies, with the flexibility to produce analogues for exploration of the structure–activity relationships. Herein we report the total syntheses and biological evaluation of **1** and some initial analogues.

The convergent strategy to synthesize **1** targets two major fragments: a C.2–C.14 bottom half (**3** or **4**) and C.15–C.27 upper half (**2**, Fig. 1). Indium mediated coupling to form the C.14–C.15 bond allows access to both diastereomers at C.15, as well as to the unnatural configuration of the epoxide. Ring closure via Horner–Wadsworth-Emmons (H-W-E) reaction[4a] from a C.3 aldehyde precursor (**3**) or Yamaguchi cyclization[4b] from

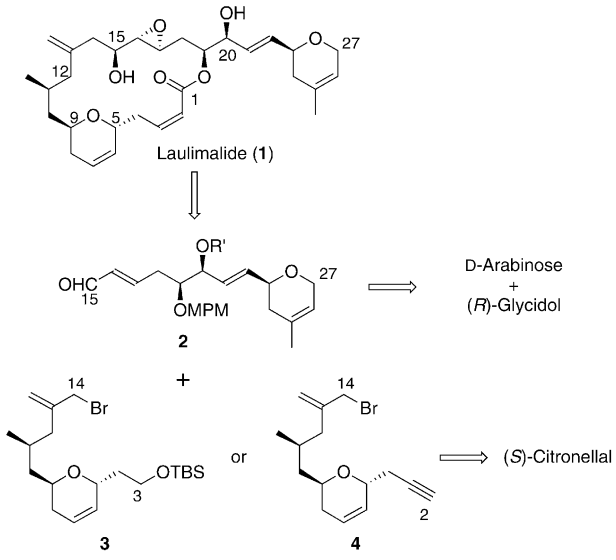

Figure 1. Synthetic strategy to access laulimalide.

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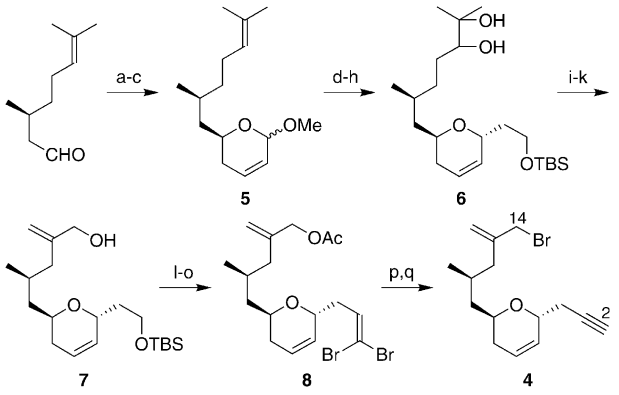
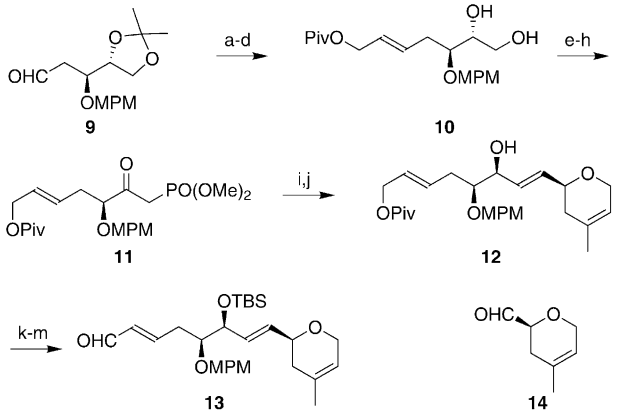
an alkynoic acid precursor (**4**) would allow for analogues with a modified C.2–C.3 region. Intermediates **3** and **4** are both derived from (*S*)-citronellal, and intermediate **2** is derived from D-arabinose and (*R*)-glycidol.

Modification of the route described by Davidson[5i] (Scheme 1) provided access to intermediate **7**, which could be converted to **3** for the H-W-E ring closing strategy (not shown). However, the most efficient approach to **1** involved incorporation of the C.2–C.3 alkyne in **4** prior to coupling with the top fragment. This route produced tens of grams of the allyl bromide **4**.



Scheme 1. Synthesis of the C.2–C.14 fragment.[7] Reagents and conditions: (a) (i) $Ipc_2BCH_2CH=CH_2$, Et_2O, −78 °C; (ii) 3 M NaOH, 30% H_2O_2, 75%; (b) 1-methoxy-1,2-propadiene, $Pd(OAc)_2$, Et_3N, CH_3CN, 80 °C, 65%; (c) $Cl_2(PCy_3)_2Ru=CHPh$, CH_2Cl_2, 40 °C, 85%; (d) $CH_2CHOTBS$, $LiClO_4$, Et_2O, 0 °C; (e) $NaBH_4$, THF/H_2O (100/1), 0 °C, 50%; (f) *m*CPBA, CH_2Cl_2, satd $NaHCO_3$, 0 °C; (g) 0.1 M H_2SO_4, THF; (h) TBSCl, Et_3N, DMAP, 0 °C, 70%; (i) $Pb(OAc)_4$, $PhCH_3$, 95%; (j) (i) $H_2CN(CH_3)_2I$, CH_2Cl_2; (ii) Et_3N; (k) $NaBH_4$, $CeCl_3$, MeOH, −78 °C, 55%; (l) Ac_2O, DMAP, pyridine, 95%; (m) H_2SiF_6, CH_3CN, 80%; (n) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 80%; (o) Ph_3P, CBr_4, CH_2Cl_2, −78 °C, 70%; (p) *n*-BuLi, THF, −78 °C, 80%; (q) Ph_3P, NBS, CH_2Cl_2, 0 °C, 80%.

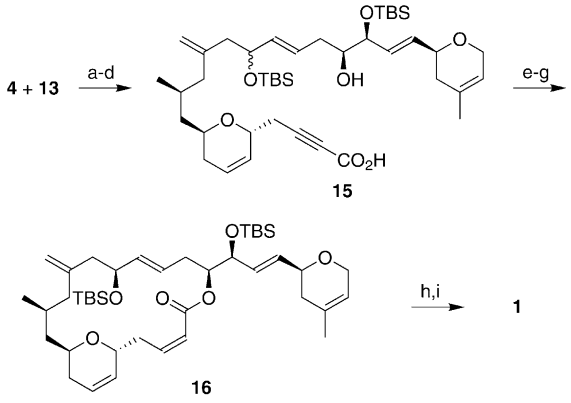


Scheme 2. Synthesis of the C.15–C.27 fragment.[7] Reagents and conditions: (a) (i) $(MeO)_2POCH_2CO_2Me$, NaH, THF, 0 °C; (ii) Bu_3P, CH_3CN, 60 °C, 85%; (b) DIBAL, CH_2Cl_2, −78 °C; (c) PivCl, pyridine, CH_2Cl_2, 85%; (d) 1 M HCl/THF (1/1); (e) $NaIO_4$, THF/H_2O, 0 °C; (f) $NaClO_2$, H_2NSO_3H, *t*-BuOH/H_2O, 0 °C; (g) $TMSCHN_2$, $PhCH_3$/MeOH (3/1), 50%; (h) (i) $(MeO)_2POCH_2Li$, THF, −78 °C; (ii) PivCl, pyridine, CH_2Cl_2, 90%; (i) Et_3N, LiCl, **14**, THF, 0 °C to rt, 70%; (j) L-Selectride, THF, −78 °C, 70%; (k) TBSOTf, imidazole, DMF, 0 °C, 90%; (l) NaOMe, MeOH, 0 °C, 80%; (m) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 85%.

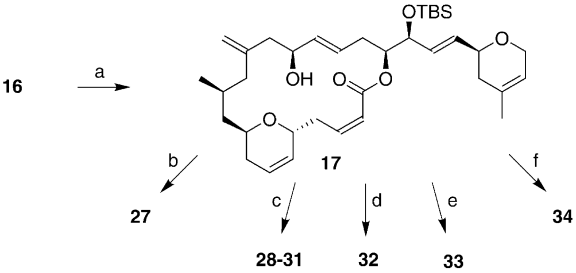
The enal **13** was constructed starting from the known aldehyde **9**[8] (Scheme 2), available in six steps from D-arabinose. A number of different approaches to attach the side chain were explored, but a modification of the previously reported H-W-E reaction[5l] utilizing phosphonate **11** proved to be the most efficient and flexible. The known aldehyde **14** was most efficiently prepared from *R*-glycidol.[5f,g,j,n] This approach produced tens of grams of the coupling partner enal **13**.

Indium mediated coupling of **4** with **13** produced a ~1:1 mixture at C.15 (Scheme 3). Macrolactonization[4b] of alkynoic acid **15** proved to be the most efficient strategy for the synthesis of **1**. The C.15 diastereomers were separated by chiral preparative HPLC (Chiralpak® AD) following Lindlar reduction. Global deprotection and Sharpless epoxidation provided **1**.[4e] This strategy proved capable of producing gram quantities of **1** for evaluation and derivatization.

This route provided the requisite flexibility to produce analogues of interest. Analogues lacking the epoxide



Scheme 3. Synthesis of laulimalide (**1**).[7] Reagents and conditions: (a) In, THF/H_2O (3/1), HCl (cat.), 90%; (b) TBSOTf, pyridine, DMAP, CH_2Cl_2, 90%; (c) (i) *n*-BuLi, THF, −78 °C; (ii) CO_2 (s); (d) DDQ, CH_2Cl_2/pH 7 buffer (1/1); (e) 2,4,6-trichlorobenzoyl chloride, DMAP, $PhCH_3$, 50%; (f) H_2, Lindlar cat., quinoline, hexane/CH_2Cl_2 (3/1), 95%; (g) chiral HPLC; (h) H_2SiF_6, CH_3CN; (i) (+)-DIPT, $Ti(OiPr)_4$, *t*-BuOOH, CH_2Cl_2, −20 °C, 80%.

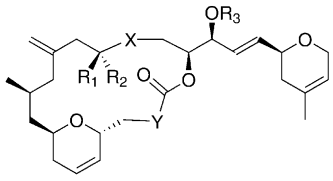


Scheme 4. Synthesis of analogues **27**–**34**. Reagents: (a) H_2SiF_6, CH_2Cl_2/CH_3CN/THF (2/2/1), 70%; (b) (+)-DIPT, $Ti(OiPr)_4$, *t*-BuOOH; (c) (i) (+)-DIPT, $Ti(OiPr)_4$, *t*-BuOOH; (ii) RCOCl; (iii) Et_3N-3HF, CH_3CN; (d) (i) (+)-DIPT, $Ti(OiPr)_4$, *t*-BuOOH; (ii) NaH, MeI; (iii) Et_3N-3HF, CH_3CN; (e) (i) Dess–Martin; (ii) Et_3N-3HF, CH_3CN; (f) (i) Dess–Martin; (ii) $MeONH_3Cl$; (iii) Et_3N-3HF, CH_3CN.

(e.g., **18** and **19**) were prepared by deprotecting **16** and the corresponding C.15 *epimeric* diastereomer. Fragment **3** was coupled to **13** in a sequence analogous to Scheme 3, which enabled the synthesis of analogues **20** and **21** via a H-W-E ring closure.[4a] The C.20 methoxy derivatives **22**–**24** were synthesized by methylating intermediate **12** under standard conditions (NaH, MeI). Compound **24** was a minor by-product from the Lindlar reduction. Acylation (Ac_2O, pyridine) of **1** was reasonably selective for the C.20 alcohol to produce **25**, but also yielded some of the bis-acylated derivative **26**. The C.20 TBS ether (**27**) was synthesized as shown in Scheme 4.

Analogues at the C.15 position (**28**–**34**) were synthesized as shown in Scheme 4. Mono-deprotection of **16** occurred selectively at C.15 using H_2SiF_6. Following epoxidation and/or derivatization of C.15, the C.20 TBS ether was removed using Et_3N-3HF.[4i] C.16-C.17 *epimeric* epoxide analogues (**35** and **36**) were made in an

Table 1. In vitro growth inhibition activities of laulimalide (**1**) and analogues **18**–**36**



Compd	R_1	R_2	R_3	X	Y	MDA-MB-435[a]	HT-29[a]
1	OH	H	H			2.3±0.2 (3)	6.9 (1)
18	OH	H	H			289±2 (2)	960 (1)
19	H	OH	H			790±10 (2)	2700 (1)
20	OH	H	H			2760±20 (2)	8500 (1)
21	H	OH	H			4295±295 (2)	9600 (1)
22	OH	H	Me			242 (1)	590 (1)
23	OH	H	Me			>1000 (1)	>1000 (1)
24	OH	H	Me			>1000 (1)	>1000 (1)
25	OH	H	Ac			91±27 (2)	ND[b]
26	OAc	H	Ac			289±17 (2)	ND[b]
27	OH	H	TBS			>1000 (2)	ND[b]
28	*p*-$NO_2(C_6H_4)CO_2$	H	H			37±4 (3)	ND[b]
29	OAc	H	H			23±2 (3)	ND[b]
30	$MeOCO_2$	H	H			422±41 (2)	ND[b]
31	Me_2NCO_2	H	H			601 (1)	ND[b]
32	OMe	H	H			>1000 (1)	ND[b]
33	$R_1=R_2=O$		H			>1000 (2)	ND[b]
34	$R_1=R_2=N(OMe)$		H			>1000 (2)	ND[b]
35	H	OH	H			176±15 (2)	ND[b]
36	*p*-$NO_2(C_6H_4)CO_2$	H	H			>1000 (2)	ND[b]

[a] Cell growth inhibition under continuous exposure for 3–4 days, IC_{50}±range nM (*n*) for $n=2$ or IC_{50}±SEM nM (*n*) for $n=3$.
[b] Not determined.

analogous manner to **27**–**34** starting from the C.15 *epi* diastereomer of **16** (not shown) utilizing (−)-DIPT for the epoxidation. The C.15 stereochemistry of **36** was set under standard Mitsunobu conditions (Ph_3P, DEAD) in the presence of the epoxide.

All final compounds were evaluated in the MDA-MB-435 human breast cancer cell line, and in some cases the HT-29 human colon cancer cell line, for growth inhibitory activities under continuous exposure conditions.[9] The results are summarized in Table 1.

The tendency for the epoxide to be opened by the C.20 alcohol[1b,c] presents a potential liability for drug development. To address this, a series of analogues were prepared to explore the possibility of eliminating this isomerization. Replacement of the epoxide of **1** with an alkene (**18**) resulted in a loss in potency of two orders of magnitude, indicating that this functionality is either mechanistically or conformationally important for activity. Capping the C.20 alcohol with a methyl group (**22**) resulted in a similar loss of potency. Interestingly, the C.20 acetoxy analogue (**25**) was more potent than **22**, and the C.20 TBS ether (**27**) was inactive up to 1 μM. This may indicate the importance of the C.20 alcohol to participate in H-bonding interactions or that steric bulk at this position is not tolerated. The reduction in potencies of C.20 derivatives relative to **1** may also be due to conformational changes in the side-chain and/or the macrocycle. Regardless, these analogues indicate that the C.20 alcohol plays an important role in biological activity.

Inversion of the alcohol stereochemistry at C.15 resulted in only a minor reduction in potency (cf. **18** versus **19** and **20** versus **21**) indicating some tolerance for modification at this position. To explore this further a series of C.15 alcohol derivatives were prepared (**28**–**32**), but all the analogues exhibit a loss in potency relative to **1**. Replacement of the C.15–C.17 part of the molecule with a Michael accepting substructure (cf. **33** and **34**) resulted in inactive compounds up to 1 μM. Interestingly, the C.15–C.17 tri-*epi* analogue (**35**) still remained fairly potent. Thus while the absolute stereochemistry of C.15 may be of minor importance, the alcohol at this position appears to contribute to the potency.

The C.2–C.3 *Z* enoate also plays an important role in potency. The C.2–C.3 *E* enoate results in an approximate 5- to 10-fold loss in potency (cf. **20** versus **18** and **21** versus **19**), and the C.2–C.3 alkynoate **23** or saturated compound **24** are inactive. Whether this part of the molecule is part of the pharmacophore or simply necessary for macrocycle conformation is unclear at present.

In summary, a route to laulimalide was identified, which enabled the synthesis of significant quantities of **1** and related analogues to begin to explore the SAR. The key steps include an indium mediated coupling to form the C.14–C.15 bond, and a Yamaguchi macrolactonization or H-W-E ring closure. All the analogues prepared exhibited decreased potencies relative to **1**. The C.16–C.17 epoxide, the C.20 alcohol, and the C.2–C.3 enoate all appear to be important for activity. Also, there appears to be some flexibility in the stereochemistry at C.15, but an alcohol or isostere at this position may be important. Derivatizations of the C.21–C.27 side–chain and C.5–C.9 dihydropyran were not explored in the current strategy, but may lead to analogues with increased potencies. The results of these efforts will be reported in due course.

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