Communication

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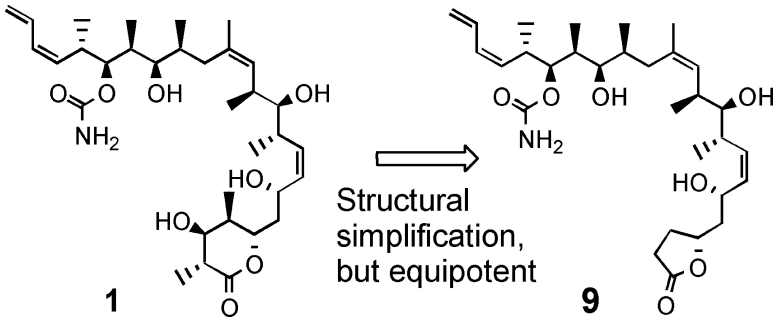

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Toward Understanding How the Lactone Moiety of Discodermolide Affects Activity

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Discodermolide **1**, a marine sponge metabolite from *Discodermia dissoluta*,[1] displays potent growth inhibitory activity against human cell lines. The mechanism of action has been demonstrated to be similar to that of paclitaxel, namely the binding and stabilization of microtubules, leading to mitotic arrest and cell death.[2]

Currently, large quantities of this material cannot be obtained from natural sources. As such, the majority of discodermolide has been produced by total synthesis.[3] Indeed, discodermolide has recently been advanced into an early stage clinical trial using synthetic material supplied by a hybrid of the Smith and Paterson approaches.[4,5]

While total synthesis of (+)-discodermolide has been successful, the 13 stereocenters make it a formidable undertaking. Any means to simplify the molecule's synthetic complexity while maintaining the potent cytotoxicity would be valuable for the development of future clinical candidates. Contributions in this field have come from Novartis,[6] Harbor Branch,[6d] Curran and Day,[7] Paterson,[8] and our laboratories.[9] In that regard, we recently reported a series of compounds in which the lactone moiety was replaced with an aromatic group.[9b] While these compounds show high nanomolar cytotoxicity they nevertheless are 2 orders of magnitude weaker than the natural product, suggesting that the lactone may play a key role in activity. In this study, we set out to define the critical requirements of the lactone ring region necessary to maintain low nanomolar cytotoxicity.

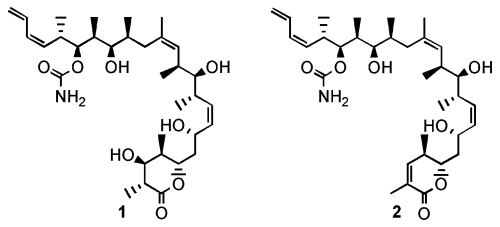
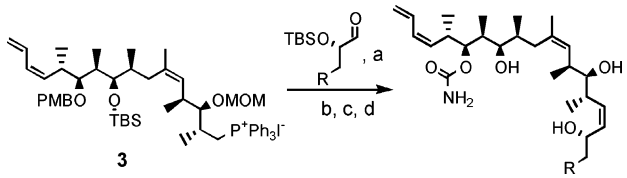


Figure 1. Discodermolide **1** and 2,3-anhydrodiscodermolide **2**.

As a starting point, we were drawn to the 2,3-anhydro compound (**2**) reported by Smith et al.[9a] This compound shows improved in vitro activity over the natural product despite the loss of the 3-hydroxyl, suggesting that this is not necessary for activity (Figure 1).

A series of six-membered ring analogues was therefore synthesized using the Wittig coupling strategy developed by the Smith group[3j] (Scheme 1). The required aldehydes for this initial survey were tailored in accord to a previous disclosure[9c,d] and coupled to phosphonium salt **3**. The 2-normethyl-2,3-anhydro compound **4** and 2,4-normethyl-2,3-anhydro compound **5** show improved activity over discodermolide **1**, suggesting that both the 2- and 4-methyl and 3-hydroxyl groups do not play a critical role in potent

Scheme 1 [a]



[a] (a) MeLi−LiBr, THF. (b) DDQ, CH_2Cl_2, H_2O. (c) i. $Cl_3C(O)NCO$, CH_2Cl_2. ii. K_2CO_3, MeOH. (d) HCl, MeOH.

cytotoxicity against selected cancer cell lines (Table 1). The potential reactivity of the α,β-unsaturated carbonyl of **2**, **4**, and **5** was removed in **6** without loss of activity.

Table 1. Cytotoxicities of Analogues **1**, **2**, and **4**−**8**

Compound	IC_{50} (nM) MCF-7	NCI/ADR	A549	CCRF-CEM
1	28	240	22	16
2	5.6	463	8.6	3.0
 **4**	2.1	95	3.7	2.7
 **5**	3.2	630	7.9	3.8
 **6**	2.7	150	6.0	1.5
 **7**	4.6	350	7.8	4.0
 **8**	8.4	>1000	36	2.9

A critical discovery in our investigation was the synthesis of the 4,5-epi-2,3-anhydrodiscodermolide **7**. It is often the case that the inversion of stereocenters leads to significantly reduced activity (cf. 7,5-epi-discodermolide);[3g] however, in this case single digit nanomolar potency was maintained. To understand these results, we considered the solution structures published by the Smith[10] and Snyder groups.[11] Both structures suggest that the lactone ring adopts a planar twist boat conformation with all the substituents in pseudoequatorial positions. This observation led us to the hypothesis that the functionality about the ring might act to set the lactone ring conformation, allowing the lactone moiety to make a critical interaction. Thus, the 4,5-bis-epi compound is able to maintain a similar conformation with the lactone position maintained. On the basis of these observations, the unsubstituted lactone **8** was synthesized. While this compound is extremely potent in several cell lines tested, given the removal of all the substituents on the ring, it does show lowered activity over the natural product in the

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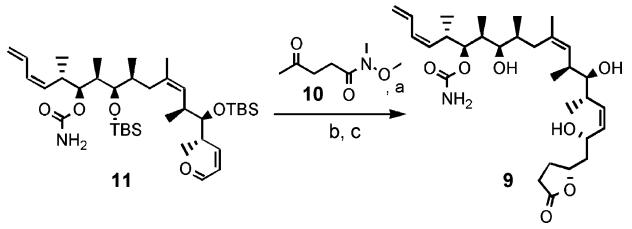
multi-drug-resistant NCI/ADR cell line, which overexpresses the P-glycoprotein efflux pump. This suggests that **8** may be a better substrate for the pump.

We next considered that a conformationally more rigid five-membered ring might be an effective scaffold. The unsubstituted butyrolactone **9** was particularly attractive since the fragment required for the lactone **10** can be readily generated from leuvilinic acid, via formation of the Weinreb amide, and then employed in the Paterson first-generation aldol approach to discodermolide[3g] with aldehyde **11**. In this way, butyrolactone **9** was synthesized along with the 7,5-bis-epimer **12** (discodermolide numbering, Scheme 2) after 1,3-anti reduction[12] and deprotection.

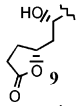
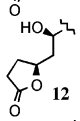
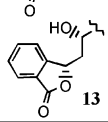
Scheme 2 [a]



[a] (a) (+)-Ipc$_2$BCl, Et$_3$N, Et$_2$O (ratio 7*S*−7*R* 7:3). (b) NaB(OAc)$_3$H, CH$_3$CN, AcOH 68% (over two steps). (c) HCl, MeOH 64%.

Importantly, butyrolactone **9** displays a 10-fold improvement over discodermolide in sensitive cell lines, while maintaining potency against the multi-drug-resistant cell line (Table 2). By contrast, the 7,5-epimer **12** is significantly less potent. In comparison with **8** (the six-membered ring variant), butyrolactone **9** possesses improved cytotoxicity in sensitive cell lines, while maintaining activity against the resistant cell line. We attempted to investigate reintroduction of steric bulk onto the ring by generating the 2,3-benzobutyrolactone **13**. The requisite aldehyde can again be generated in one step and used in the Paterson approach. The benzo-analogue is also potent, though the cytotoxicity is diminished over both the butyrolactone **9** and **1**.

Table 2. Cytotoxicities of Analogues **9**, **12**, and **13**

Compound	IC_{50} (nM)			
	MCF-7	*NCI-ADR*	*A549*	*CCRF-CEM*
1	28	240	22	16
2	5.6	463	8.6	3.0
 **9**	2.9	350	4.9	2.3
 **12**	390	>10000	2000	310
 **13**	38	4000	100	nd

Due to the enhanced cytotoxicity and synthetic simplification, compounds **4**, **6**, and **9** were selected for further in vivo study. The results will be reported in due course.

In conclusion, the potent discodermolide analogues generated in this study have led to a hypothesis that the six-membered ring of discodermolide positions the lactone moiety for a critical interaction within the tubulin binding site. Credence for this hypothesis derives from the sequential removal of functionality from the lactone moiety. On the basis of these observations, a novel butyrolactone analogue has been generated which exhibits improved activity over discodermolide and comprises a significant simplification of the structure. Taken together, these observations could have a significant impact on both the cost and ease of synthesizing a discodermolide derivative as a pharmaceutical.

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Supporting Information Available: Spectral characterization of compounds **4**−**9**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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