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Design, Synthesis, and Biological Evaluation of Simplified Analogues of (+)-Discodermolide. Additional Insights on the Importance of the Diene, the C(7) Hydroxyl, and the Lactone

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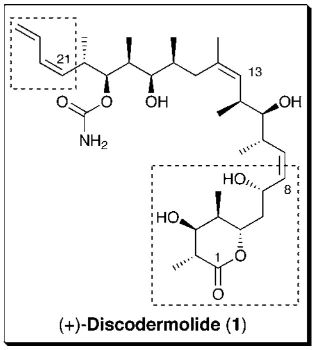
ABSTRACT



(+)-**Discodermolide** (**1**)

The design, synthesis, and biological evaluation of seven totally synthetic analogues of the antitumor agent (+)-discodermolide are reported. Saturation of the terminal diene system, alteration of the substituents on the lactone, and alkylation of the C(7)-hydroxyl group reveal significant structure–activity relationships.

(+)-Discodermolide (**1**), a polyketide natural product isolated from the marine sponge *Discodermia dissolute*,[1] displays both potent cytotoxic activity against a wide variety of human tumor cell lines and significant in vivo antitumor activity.[2] The mode of action, similar to that of paclitaxel, comprises binding and stabilization of microtubules leading to mitotic arrest and cell death.[3] In 2000, the Horwitz group in collaboration with our laboratory and that of Danishefsky demonstrated that discodermolide and paclitaxel display significant synergistic antiproliferative cooperativity.[4] Further collaboration with the Horwitz group led to the discovery that discodermolide, but not paclitaxel, processes a second significant mechanism of tumor cell growth inhibition, specifically induction of an accelerated senescence phenotype, which may play a role in the observed synergy.[5]

The remarkable biological profile and novel structure of (+)-discodermolide (**1**, Figure 1) make it a promising

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(1) (a) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912−4915. Additions and corrections: *J. Org. Chem.* **1991**, *56*, 1346. (b) Gunasekera, S. P.; Pomponi, S. A.; Longley, R. E. U.S. Patent No. US5840750, Nov 24, 1998.

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(3) (a) ter Harr, E.; Kowalski, R. J.; Hammel, E.; Lin, C. M.; Longley, R. E.; Gunaskera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243−250. (b) Hung, D. T.; Chen, J.; Schrieber, S. L. *Chem. Biol*. **1996**, *3*, 287−293.

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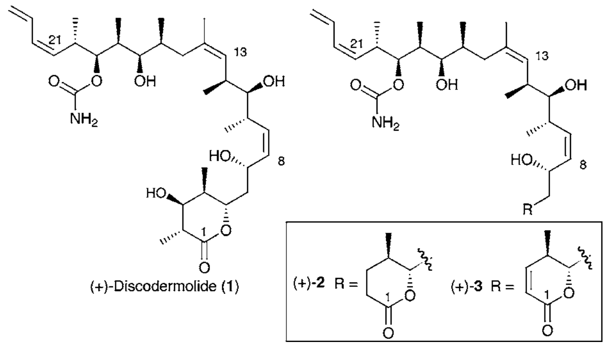


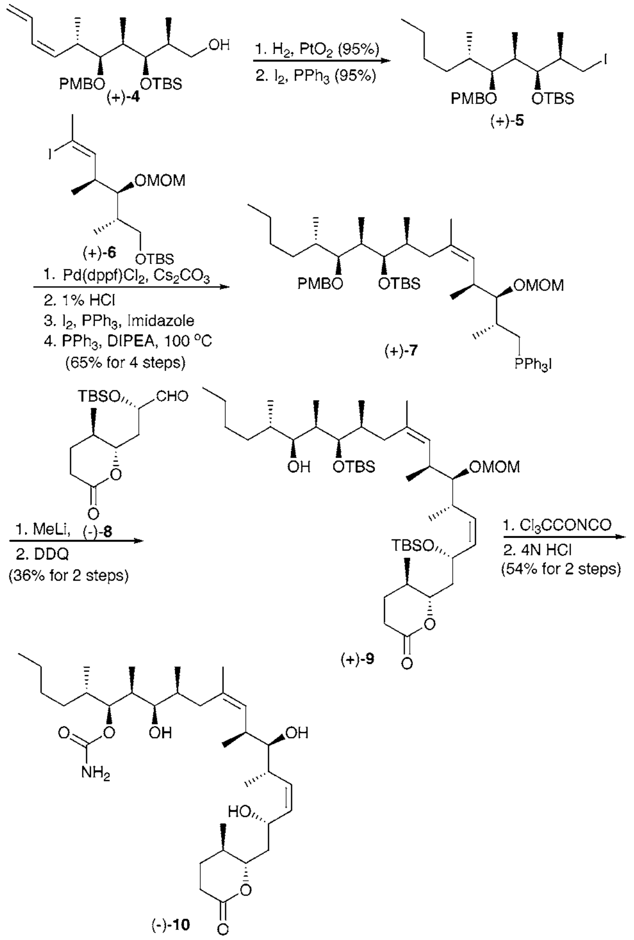
Figure 1. (+)-Discodermolide and simplified analogues.

candidate for clinical development as a cancer chemotherapeutic agent. Along with the scarcity of the natural material, these properties have stimulated intensive synthetic effort, including the gram-scale syntheses achieved by our laboratory and that of the Novartis group.[6] Additional endeavors have focused on the design and synthesis of structurally simplified analogues of this potentially important chemotherapeutic agent.[7]

Recently, in collaboration with Kosan Bioscience, Inc., we reported a series of analogues in which the lactone moiety was significantly simplified.[8] Among the analogues prepared, the 2-normethyl-3-deoxy congener (+)-**2** and the 2-normethyl-2,3-anhydro counterpart (+)-**3** (Figure 1) revealed improved activity over (+)-discodermolide (**1**), despite the removal of two stereogenic centers, suggesting that the 2-methyl and 3-hydroxyl groups do not play a critical role in the observed cytotoxicity. Given the enhanced cytotoxicity and simplification in structure, analogues (+)-**2** and (+)-**3** were selected as leads for further modification to explore additional structure activity relationships (SAR). Herein we describe the synthesis and biological evaluation of a series of seven potent congeners of (+)-**2** and (+)-**3**,[9] several of which display equal or enhanced cytotoxicity over (+)-discodermolide employing a range of drug-sensitive human tumor cell lines, as well as the NCI/ADR drug-resistant cell line. Importantly, access to the seven analogues was only possible due to the wide variety of advanced intermediates available from our gram-scale synthesis.[6a]

We first explored the effect of saturation of the terminal diene system (Scheme 1). Early results by Gunasekera et al. demonstrated that the saturated C(21)−C(24) variant of (+)-discodermolide (**1**) is more active than the parent molecule, when assayed against the P388 and A549 human tumor cell lines.[10] Toward this end, hydrogenation of known alcohol (+)-**4**[6h] followed by iodination at the primary hydroxyl provided (+)-**5**. Subsequent Suzuki coupling with known vinyl iodide (+)-**6**,[6h] followed by removal of the primary

Scheme 1



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(9) Previous results from our collaboration with Kosan Biosciences (ref 8) also revealed that butyrolactone derivatives of discodermolide exhibit improved activity. Similar SAR study employing butyrolactone analogues as leads is currently undergoing. Results will be reported elsewhere.

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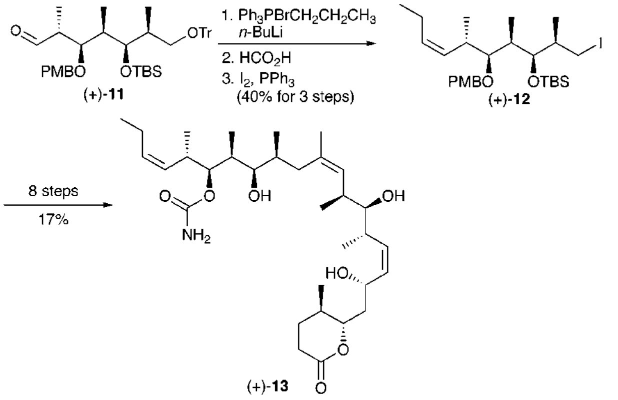
TBS silyl ether, iodination, and treatment with triphenylphosphine, led to Wittig salt (+)-**7**. Union with known aldehyde (−)-**8**,[8] and in turn removal of the PMB moiety, installation of the carbamate moiety, and global deprotection, then furnished the fully saturated diene analogue (−)-**10**.

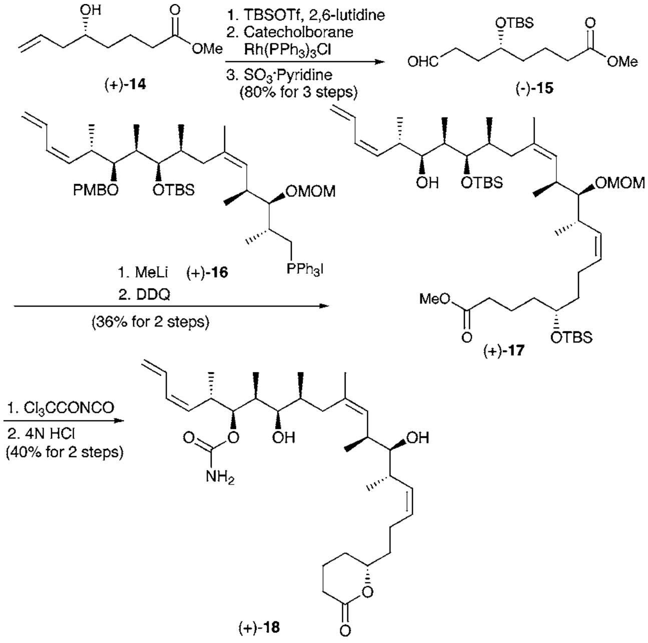
The partially saturated C(23)−C(24) analogue (+)-**13** (Scheme 2) was next prepared from the corresponding alkyl iodide (+)-**12** via the same synthetic sequence, with a similar overall efficiency. In this case, iodide (+)-**12** was obtained from known aldehyde (+)-**11**[6h] in three steps: Wittig reaction, removal of the trityl group, and iodination.

Scheme 2



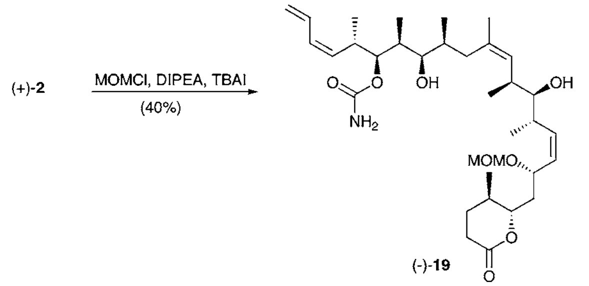
Early results from both our and the Kosan laboratories suggested that the C(4)-methyl group does not play a critical role for potent cytotoxicity against selected cancer cell lines.[8] We therefore turned to the construction of the C(4)-normethyl analogue **18** (Scheme 3). Protection of the secondary hydroxyl as the TBS ether in known ester (+)-**14**,[11] followed by rhodium-catalyzed hydroboration[12] and oxidation with SO_3·pyridine,[13] efficiently furnished aldehyde (−)-**15** as a precursor for the unsubstituted lactone moiety of **18**. Wittig union with known phosphonium salt (+)-**16**,[6h] followed by removal of the PMB moiety, carbamate installation, and global deprotection with concomitant lactone ring formation, produced analogue (+)-**18**.

Scheme 3



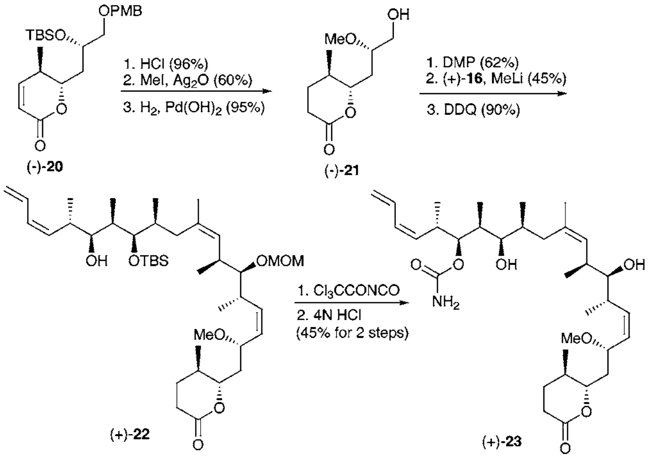
The Gunasekera group had also prepared the C(7)-acetate analogues of discodermolide which proved to be almost 10-fold more potent than the parent compound against P388 leukemia cells.[14] Based on this observation, we reasoned that the C(7)-hydroxyl group on (+)-**2** (Scheme 4) might prove to be an attractive site for further modification. Toward this end, the C(7)-MOM analogue (−)-**19** was prepared chemoselectively via treatment of (+)-**2** with MOMCl.

Scheme 4



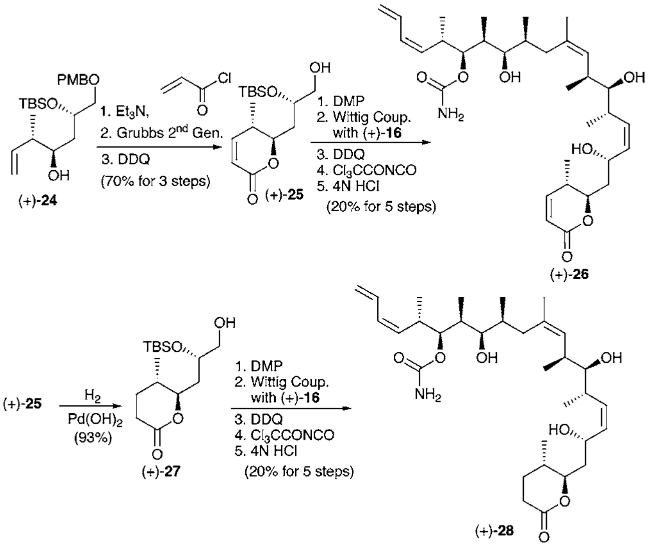
The C(7)-methoxy analogue (+)-**23** was also prepared, albeit begining with lactone (−)-**20**,[7d] as outlined in Scheme 5. In this case, removal of the TBS silyl ether, followed by methylation and hydrogenation yielded alcohol (−)-**21**, which upon Dess−Martin oxidation, union with Wittig salt (+)-**16**, and removal of the PMB group furnished (+)-**22**. Carbamate installation followed by global deprotection completed construction of the C(7)-methoxy analogue (+)-**23**.

Scheme 5



To explore the effect of stereogenicity on the lactone ring,[12] the 4,5-epimers of (+)-**2** and (+)-**3** were constructed. For this goal, the secondary alcohol (+)-**24**, isolated as a minor diastereomeric product en route to lactone (−)-**20**,[7d] was acylated with acryloyl chloride (Scheme 6). Ring-closing metathesis employing the second-generation Grubbs catalyst,[15] followed by removal the PMB ether furnished alcohol (+)-**25**; hydrogenation then readily provided the corresponding saturated alcohol (+)-**27**. Alcohols (+)-**25** and (+)-**27** in turn were subjected to our now standard five-step endgame synthesis to furnish congeners (+)-**26** and (+)-**28**, respectively.

Scheme 6



The synthetic analogues were evaluated for tumor cell growth inhibitory activity against three drug sensitive cancer cell lines (MCF-7, A549, and CCRF-CEM), as well as against the multidrug resistant NCI/ADR cell line. The results, presented in Table 1, clearly demonstrate that further simplification of analogue (+)-**2** is possible with retention and in some cases enhanced tumor cell growth inhibitory activity. For example, although saturation of the terminal diene [analogues (−)-**10** and (+)-**13**] resulted in a loss of activity against the NCI/ADR cell line, these congeners retained comparable cytotoxicity to that of discodermolide (+)-**1** against the drug-sensitive cell lines MCF-7 and A549. Further simplification of (+)-**2** by removal of the C(4)-methyl and C(7)-hydroxyl groups [(+)-**18**] restores potency nearly to the levels displayed by discodermolide (+)-**1** in the MCF-7, CCRF-CEM, and most importantly in the NCI/ADR cell line, while activity in the A549 cell line is somewhat depressed.

Table 1. Cytotoxicity Observed for Synthetic Analogues

	cytotoxicity IC_{50}, nM			
	MCF-7	NCI/ADR	A549	CCRF-CEM
(+)-**1**	28	240	22	16
(+)-**2**	2.7	150	6.0	1.5
(+)-**3**	2.1	95	3.7	2.7
(−)-**10**	17	2000	71	
(+)-**13**	3.9	650	13	
(+)-**18**	23	330	160	6.3
(−)-**19**	3.4	130	5.8	3.6
(+)-**23**	1.3	29	4.0	
(+)-**26**	130	>2000	430	57
(+)-**28**	4.7	1000	13	

The cytotoxicity of the C(7)-modified analogues (−)-**19** and (+)-**23** also proved highly informative. As stated above the Gunasekera group had observed that acetylation of the C(7)-hydroxyl group significantly enhanced the cytotoxicity over (+)-discodermolide (**1**).[14] Similar introduction of the MOM or methoxyl group on the C(7)-hydroxyl of (+)-**2** resulted in analogues [(−)-**19** and (+)-**23**], which also displayed enhanced activity over the parent compound, especially in the multi-drug resistant NCI/ADR cell line. Indeed, analogue (+)-**23** is currently our most potent congener. Finally, inversion of the stereogenicity at C(4) and C(5) of (+)-**2**, as in (+)-**28**, led only to a slight loss of potency in the MCF-7 and A549 cell lines, but to significant loss of activity in the NCI/ADR cell line. Interestingly, identical modification of the unsaturated congener (+)-**3** as with (+)-**26** resulted in a significant loss of activity (ca. average of 2 orders of magnitude).

In summary, seven new, totally synthetic analogues of (+)-discodermolide (**1**) have been prepared and evaluated for tumor cell growth inhibitory activity in four human tumor cell lines. The results indicate that the terminal diene system does not contribute significantly to the activity. Additionally, substitution on the C(7)-hydroxyl group appears to have a very positive impact on cytotoxicity.

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Supporting Information Available: Representative procedures, spectral data, and analytical data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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