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Design, Synthesis, and Evaluation of Analogues of (+)-14-Normethyldiscodermolide

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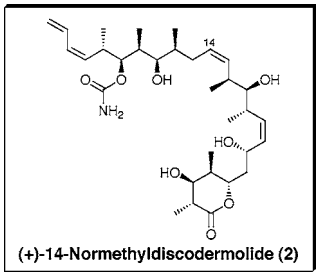
ABSTRACT

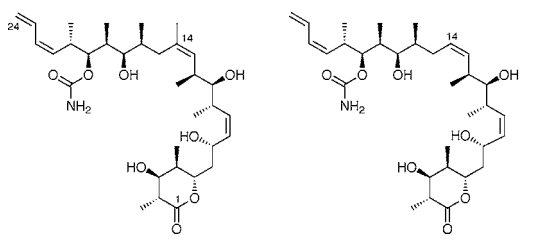


The design, syntheses, and biological evaluation of nine totally synthetic analogues of the microtubule-stabilizing agent (+)-14-normethyldiscodermolide (2) are reported. Simplification at the C(21)–C(24) terminal diene and at the C(1)–C(5) lactone moieties reveals significant structure–activity relationships.

(+)-Discodermolide (**1**, Scheme 1), a microtubule-binding antimitotic agent isolated in 1990 from the deep sea marine sponge *Discodermia dissoluta*,[1] was concurrently shown in 1996 by ter Haar and co-workers and Schreiber et al. to possess potent antimitotic activity, with a mechanism of action similar to the clinically proven anticancer agent Taxol (e.g., the binding and stabilization of microtubules).[2] To date, thirteen total syntheses have been published.[3] Additional effort in (+)-discodermolide has focused on the synthesis of structurally and thereby synthetically simplified analogues of this potentially important chemotherapeutic agent.[4]

In 2001, we reported the rational design and synthesis of (+)-14-normethyldiscodermolide (**2**),[4f] wherein replacement of the C(13)−C(14) (*Z*)-trisubstituted olefin found in (+)-discodermolide with a cis-disubstituted counterpart enabled a significant simplification to our initial synthetic route to this class of antitumor agents. Subsequent biological evaluation revealed that the tubulin polymerization and cytotoxicity of (+)-**2** were only slightly depressed relative to the

Scheme 1



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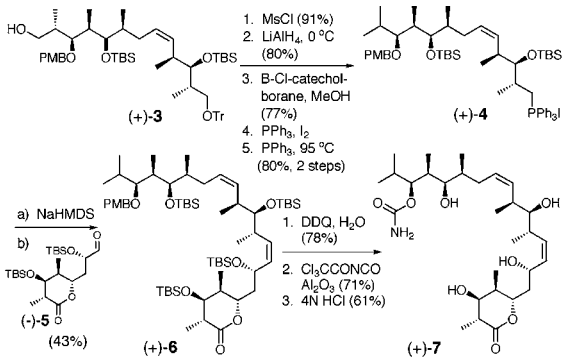
natural product. As part of our continuing program directed at the production of analogues designed to probe the structure−activity relationships, as well as to define the minimum structure element necessary for tumor cell growth inhibitory activity, we report herein the results of simplification of the C(1)−C(5) lactone and C(21)−C(24) dienyl subunits of (+)-14-normethyldiscodermolide (**2**).

Modifications of each of these regions of (+)-discodermolide have been reported previously. In 1996, Schreiber and co-workers disclosed that extension of the dienyl unit with long side chains led to analogues with potency only moderately diminished relative to the natural product and that substitution of the lactone carboxyl with either a thiophenyl *S*,*O*-acetal or a short-chain *O*,*O*-acetal produced compounds equipotent with the natural product.[4b] Subsequently, Gunasekera and co-workers revealed the isolation of three new naturally occurring congeners of (+)-discodermolide (**1**) bearing variations in the lactone region, namely, 2-*epi*-discodermolide, 2-normethyldiscodermolide, and the open-chain methyl ester 5-hydroxymethyldiscodermolate, all of which proved to be 3- to 6-fold less potent than discodermolide.[4i] Separately, via partial hydrogenation of the natural product, the Gunasekera group prepared 21,23-tetrahydrodiscodermolide.[4h] Importantly, the saturated variant proved to be *more active* than (+)-discodermolide when assayed against the P388 and A549 human tumor cell lines.[4h]

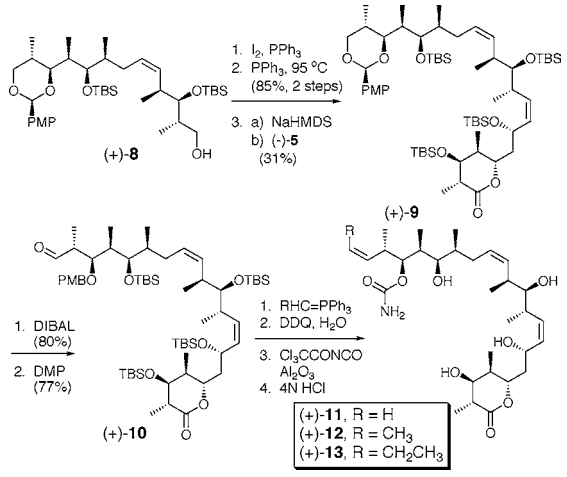
Utilizing the synthetically simplified 14-normethyldiscodermolide[4f] framework for our studies, we first sought to explore the effects of saturation and truncation of the terminal diene. To begin, we undertook the synthesis of the diene-deletion congener (+)-**7** (Scheme 2), which proved to be readily accessible via an advanced intermediate in our synthesis of (+)-14-normethyldiscodermolide. Toward this end, mesylation of known alcohol (+)-**3**,[4f] followed in turn by displacement with lithium aluminum hydride, removal of the primary trityl ether, iodination, and treatment with triphenylphosphine, provided Wittig salt (+)-**4**. Subsequent Wittig coupling with known aldehyde (−)-**5**,[3e] DDQ-mediated removal of the secondary *p*-methoxybenzyl ether, carbamate installation, and global deprotection completed construction of (+)-**7**, in which the C(21) through C(24) substructure had been removed.

We next turned attention to the construction of the highly elaborated aldehyde (+)-**10** (Scheme 3), from which we envisioned diene replacement analogues would readily emerge. Iodination of the previously reported alcohol (+)-

Scheme 2



Scheme 3



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8[4f] and treatment with triphenylphosphine yielded the corresponding Wittig salt. Union with known aldehyde (−)-**5**,[3e] reductive opening of the PMP acetal (with concomitant reduction of the lactone to a mixture of lactols), and Dess−Martin oxidation furnished (+)-**10**. Parallel Wittig coupling with methyl, ethyl, and *n*-propyl triphenylphosphonium bromide, respectively, followed by deprotection, carbamate installation, and global deprotection, produced analogues (+)-**11**, (+)-**12**, and (+)-**13**.

In vitro evaluation of the tumor cell growth inhibitory activity in the multidrug-resistant cell line NCI/ADR revealed that the 14-normethyl analogues of (+)-discodermolide (**7**, **11**−**13**) are essentially inactive at submicromolar levels (Table 1). Likewise, the most truncated analogues, (+)-**7** and (+)-**11**, display no appreciable cytotoxicity in any of the cell lines tested. Reincorporation of carbon C(23) in the form of a monoene [(+)-**12**] revealed moderate cytotoxicity in MCF-7 and SKOV-3 cells, while (+)-**13**, which retains the full carbon skeleton of (+)-**2**, but with a saturated terminus, displayed potency rivaling that of the natural product. Cell growth inhibition of congeners (+)-**12** and (+)-**13** proved to be somewhat lower in the A549 cell line.

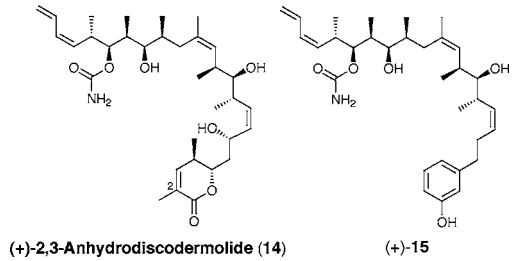
Table 1. Cytotoxicity Observed for Analogues **7** and **11**−**13**

	cytotoxicity IC_{50}, nM			
	MCF-7	NCI/ADR	A549	SKOV-3
(+)-**1**	28	240	22	21
(+)-**2**	46	8200	50	35
(+)-**7**	>1000	>1000	>1000	>1000
(+)-**11**	>1000	>1000	>1000	>1000
(+)-**12**	72	>1000	570	130
(+)-**13**	28	>1000	100	20

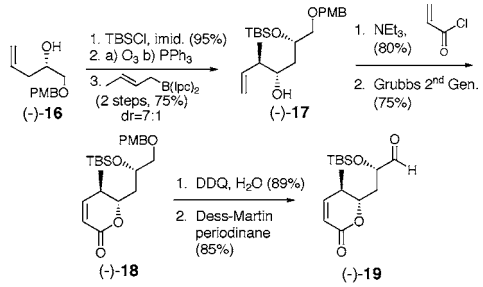
In the discodermolide series [i.e., intact C(14) methyl group], simplification of the lactone moiety previously resulted in enhanced cytotoxicity. For example, in 2001, we reported that (+)-2,3-anhydrodiscodermolide (**14**, Scheme 4) displays in vitro activity superior to that of the natural product.[4f] Subsequently, studies from our laboratory revealed that (+)-**15** and several related congeners retained significant cell growth inhibitory activity despite removal of *five stereogenic centers*![4o]

To extend these promising results to the 14-normethyldiscodermolide scaffold, we designed and synthesized an analogous series of 14-normethyl lactone-replacement congeners. To this end, α,β-unsaturated lactone aldehyde (−)-**19** was constructed from known alcohol **16**[5] (Scheme 5).

Scheme 4

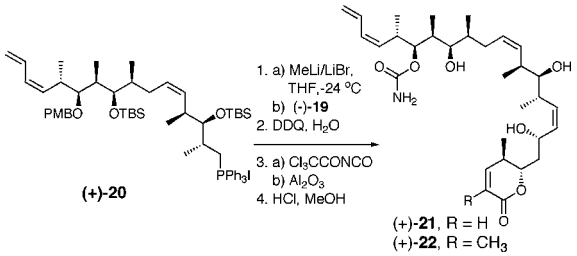


Scheme 5



Protection of the secondary alcohol in (−)-**16** as the *tert*-butyldimethylsilyl ether, followed by ozonolysis and Brown crotylation, furnished (−)-**17** in both good yield and diastereoselectivity.[6] Acylation of the resultant secondary hydroxyl with acryloyl chloride, followed by ring-closing metathesis,[7] led to lactone (−)-**18**. DDQ-mediated removal of the *p*-methoxybenzyl ether and Dess−Martin periodinane oxidation furnished the desired aldehyde (−)-**19**. Wittig union with known phosphonium salt (+)-**20**[4f] (Scheme 6), followed by oxidative removal of the PMB ether, carbamate installation, and global deprotection, ultimately produced lactone congener (+)-**21**. The C(2) methyl derivative (+)-**22** was accessed via base-promoted β-elimination from the corresponding tetra-TBS lactone, followed by global deprotection. The results of biological evaluation are presented in Table 2.

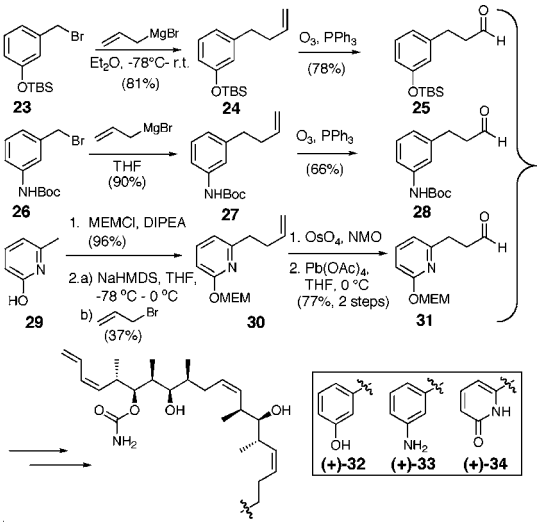
Scheme 6



Encouraged also by the potency of the previously reported (+)-discodermolide analogue (+)-**15** (Scheme 4, Table 2), in which the entire C(1)−C(7) region had been replaced with a *m*-hydroxyphenylethylene unit, we turned to the construction of additional simplified C(1)−C(7) congeners employing the (+)-14-normethyldiscodermolide skeleton (**2**). Synthetic routes to the requisite aldehydes for this venture are presented in Scheme 7. Known benzyl bromide[8] **23** was subjected to allylmagnesiumbromide, followed by ozonolysis to provide aldehyde **25**, which incorporates a protected *m*-phenol as the carbocycle. In similar fashion, treatment of the known aniline bromide **26**[9] with allylmagnesium bromide followed by ozonolysis afforded aldehyde **28**. Finally, aldehyde **31**,

required for the pyridol-derived δ-lactone mimic, was prepared from commercially available 2-hydroxy-6-methylpyridine **29**. Protection of **29** as the methoxyethoxymethyl (MEM) ether followed by deprotonation and alkylation at the benzylic position afforded olefin **30**. Two-step oxidative cleavage then furnished the desired aldehyde **31**. Wittig reaction of the three aldehydes with salt (+)-**20**, followed by deprotection, carbamate installation, and acid-mediated deprotection, yielded phenol (+)-**32**, aniline (+)-**33**, and pyridone (+)-**34**, respectively. In general, the synthetic sequences proved to be efficient.

Scheme 7



This series of analogues again demonstrated the impotency of the 14-normethyl variants of (+)-discodermolide (**1**) in the multidrug-resistant NCI/ADR cell line (Table 2). Interestingly, while dehydration of the lactone of (+)-discodermolide (**1**) to form (+)-**14** leads to a more potent compound in the three drug-sensitive cell lines, identical modification to the 14-normethyl scaffold, as in (+)-**22**, results in a significant *decrease* in tumor cell growth inhibition. Further simplification by removal of the C(2) methyl group [(+)-**21**] restores potency to the levels displayed by the parent (+)-14-normethyldiscodermolide (**2**) in the MCF-7 and SKOV-3 lines, while activity in the A549 cell line is relatively depressed, a trend also seen in the diene congeners (+)-**12** and (+)-**13**. An even more striking contrast between the discodermolide and the 14-normethyl scaffolds appears when the hydroxyphenylethylene unit replaces the southern lactone. While the 14-methylated congener of this compound [(+)-**15**] displays significant potency, no cytotoxicity is observed with the 14-normethyl variant (+)-**32** at levels up to 1 μM. Replacement of the phenol with an aniline moiety, as in (+)-**33**, likewise leads to an inactive congener, as does wholesale substitution of the lactone with a pyridone ring [(+)-**34**].

Table 2. Cytotoxicity for Analogues **21**, **22**, and **32**−**34**

	cytotoxicity IC_{50}, nM			
	MCF-7	NCI/ADR	A549	SKOV-3
(+)-**1**	28	240	22	21
(+)-**2**	46	8200	50	35
(+)-**14**	5.6	463	8.6	3.4
(+)-**15**	130	390	190	40
(+)-**21**	45	>1000	300	50
(+)-**22**	185	>1000	536	82
(+)-**32**	>1000	>1000	>1000	>1000
(+)-**33**	>1000	>1000	>1000	790
(+)-**34**	>1000	>1000	>1000	>1000

In summary, nine new totally synthetic analogues of (+)-14-normethyldiscodermolide (**2**) have been prepared and evaluated for tumor cell growth inhibitory activity in four human tumor cell lines. The results clearly exhibit a trend of decreasing activity as the C(24) terminus is truncated, while isosteric replacement of the terminal olefin with a saturated counterpart preserves cell growth inhibitory activity. Unlike the highly potent discodermolide congener (+)-2,3-anhydrodiscodermolide (**14**), (+)-2,3-anhydro-14-normethyldiscodermolide (**22**) exhibits significantly reduced cytotoxicity, especially in the A549 human tumor cell line. Interestingly, removing the C(2) methyl group from **22** restores potency in the drug-sensitive cell lines, but further simplification (**32**−**34**) proves quite detrimental. Finally, the 14-normethyl congeners of (+)-discodermolide demonstrate a general trend of relative inactivity against the NCI/ADR multidrug-resistant cell line. The underlying nature of these results and the subtle interplay between structure and function in these systems continues to be the subject of active investigation in our laboratories.

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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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