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Design, Synthesis, and Biological Evaluation of Potent Discodermolide Fluorescent and Photoaffinity Molecular Probes

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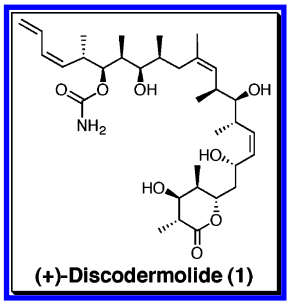
ABSTRACT



The design, synthesis, and biological evaluation of a series of (+)-discodermolide molecular probes possessing photoaffinity and fluorescent appendages has been achieved. Stereoselective olefin cross-metathesis comprised a key tactic for construction of two of the molecular probes. Three photoaffinity probes were radiolabeled with tritium.

The mechanism of action of (+)-discodermolide (**1**, Figure 1), similar to that of the clinically proven anti-cancer agent paclitaxel (Taxol, **2**), entails the binding and stabilization of microtubules,[1] which results in potent inhibition of cell proliferation in a number of human cancer cell lines.[2,3a] Importantly, discodermolide retains activity against paclitaxel-resistant cell lines.[2a]

Despite the shared mechanism, the Horwitz, Smith, and Danishefsky groups demonstrated that discodermolide and paclitaxel exhibit a highly synergistic antiproliferative cooperativity.[3] Similar synergism was not observed with other combinations of microtubule-stabilizing agents, such as paclitaxel and epothilone B, which display only an additive effect on tumor cell growth inhibition. Further collaboration

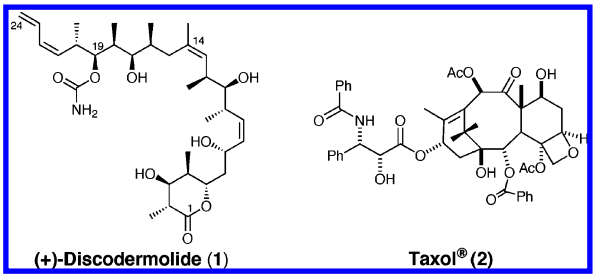


Figure 1. (+)-Discodermolide and Taxol.

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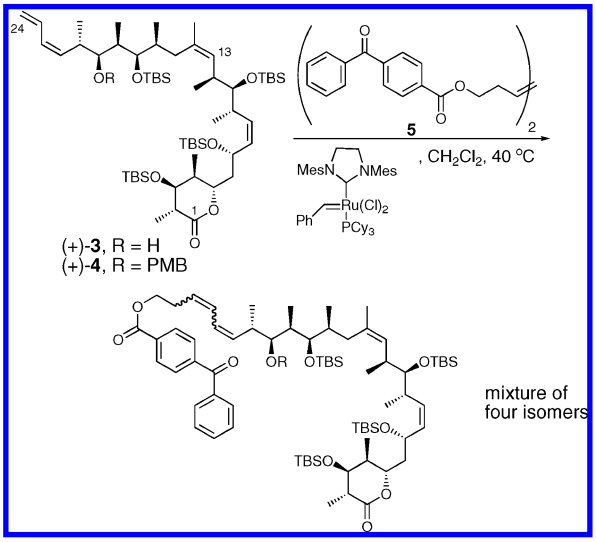
with the Horwitz group revealed that discodermolide, but not paclitaxel, possesses another mechanism of tumor cell growth inhibition, specifically the powerful induction of an accelerated senescence phenotype,[4] which may play a role in the observed synergy.

That paclitaxel and discodermolide share a common or overlapping tubulin binding site was shown early on by Schreiber et al.[1] via displacement studies. However, unlike the definitive structural information on the Taxol-tubulin binding site, defined by Horwitz and Orr in an elegant series of photoaffinity labeling studies beginning in 1994[5] and subsequently shown by Nogales and co-workers by electron diffraction studies,[6] the precise binding site and orientation of discodermolide at the molecular level remains largely speculative, despite extensive NMR and computational studies.[7a,8] To address this issue, we have prepared five discodermolide photoaffinity probes possessing benzophenone[9] appendages.

At the outset of probe design and synthesis, we set four goals. First, the labeled probe must possess potent biological activity similar to the natural product; second, the number of chemical transformations after incorporation of the label should be minimal; third, the synthetic transformations must be efficient; and finally, a single radiolabeled precursor, in this case a benzophenone moiety possessing tritium, would be ideal. Based on these criteria, and earlier structure−activity relationship studies, we selected three sites for benzophenone incorporation: the C(24) terminus, the C(19) carbamate, and the C(1)−C(7) lactone.

To attach a benzophenone moiety at the C(24) terminus, we initially explored an olefin cross-metathesis tactic with readily available, protected discodermolide congeners (Scheme 1). Unfortunately, treatment of either (+)-**3** or (+)-**4**, late-stage intermediates in our gram-scale synthesis of discodermolide,[10] with the benzophenone dimer **5**, in the presence of the Grubbs second-generation ruthenium catalyst, afforded

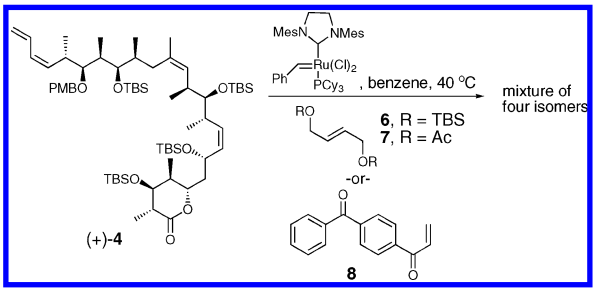
Scheme 1



a mixture of several cross-metathesis adducts. Analysis of the ^{1}H NMR spectrum revealed that not only was the nascent olefin generated as a mixture of *cis* and *trans* isomers, but that the C(21)−C(22) *cis* olefin had also undergone significant isomerization.

Seeking to remediate the olefin geometry problem, we examined the cross-metathesis tactic employing protected butenediol derivatives **6** and **7**,[11] as well as α,β-unsaturated ketone **8** (Scheme 2). Again, only complex mixtures of isomers resulted.

Scheme 2



Undeterred, we next evaluated 2-butenediol as a cross-metathesis coupling partner. In the event, treatment of *trans*-2-butenediol and alcohol (+)-**3** as a mixture (25:1 molar ratio) with the Grubbs second-generation catalyst in benzene at 45 °C furnished the desired diol (+)-**9** in 71% yield *as a single trans isomer* (Scheme 3). A mechanistic rationale for this stereochemical outcome is under active investigation in our laboratory.

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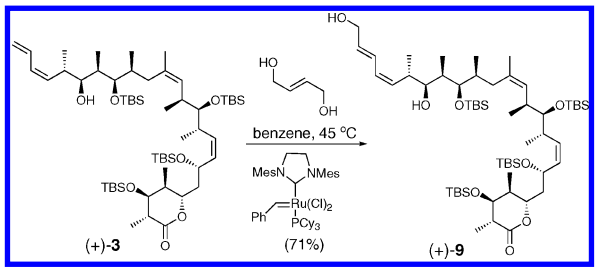
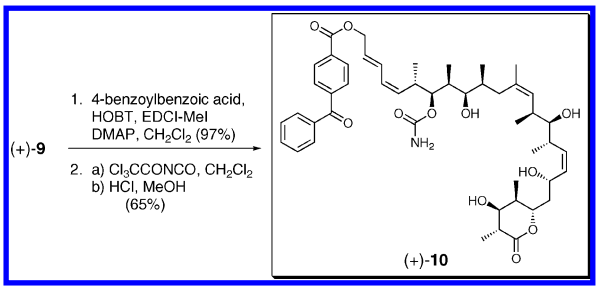
Scheme 3



With an efficient method for diene functionalization in hand, we turned to incorporation of a prospective benzophenone photoaffinity tag. Toward this end, chemoselective esterification of the primary hydroxyl in diol (+)-**9** with commercially available 4-benzoylbenzoic acid in the presence of 1-hydroxylbenzotriazole (HOBT) and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide methyl iodide salt (EDCI-MeI) furnished the corresponding ester in excellent yield (Scheme 4). Introduction of the requisite discodermolide

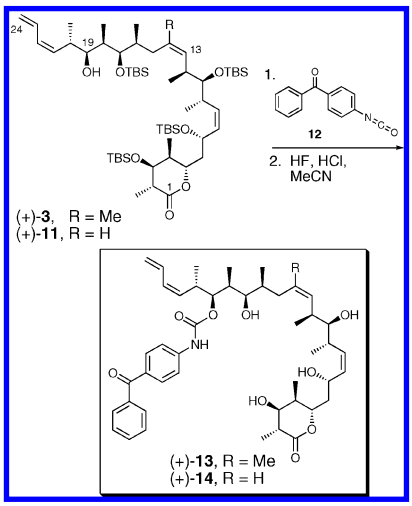
Scheme 4



carbamate moiety via the Kocovsky protocol,[12] followed by global deprotection, then furnished the target ester (+)-**10**.

We next examined substitution of the discodermolide C(19) carbamate with a carbamate bearing a benzophenone moiety (Scheme 5). Here, alcohol (+)-**3** and the 14-normethyl

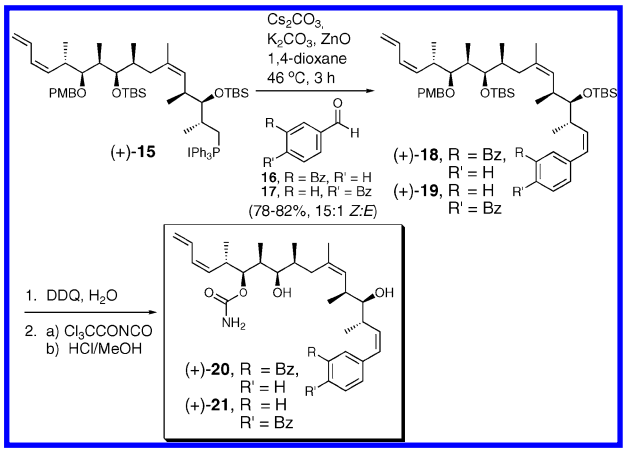
Scheme 5



variant (+)-**11** were separately subjected to isocyanate **12**, readily available in one step from 4-benzoylbenzoic acid. Global desilylation furnished benzophenone carbamate (+)-**13** and the 14-normethyl counterpart (+)-**14**,[7e] respectively.

Two additional discodermolide photoaffinity probes, comprising replacement of the C(1)−C(7) lactone with a benzophenone moiety, were designed and synthesized. For this effort, Wittig union of known phosphonium salt (+)-**15**[10a] with *m*- or *p*-benzoylbenzaldehyde (**16** and **17**, respectively) exploiting zinc-oxide chelation[13] efficiently furnished the desired olefins (+)-**18** and (+)-**19** both in good yield and with a high degree of *cis* selectivity (Scheme 6). Removal of the PMB group under oxidative conditions, followed by carbamate installation and global desilylation, led to the *m*- and *p*-benzophenone analogues (+)-**20** and (+)-**21**, respectively.

Scheme 6



Evaluation of the antiproliferative activity as well as the tubulin polymerization profile (as measured by a turbidity assay)[14] for each of the potential photoaffinity probes was carried out (Table 1). Based on these results, three com-

Table 1. Bioassay Data for (+)-Discodermolide (**1**) and for the Designed Molecular Probes

	tubulin polymerization (%)	A549 IC_{50} (nM)		tubulin polymerization (%)	A549 IC_{50} (nM)
(+)-**1**	100	19.7	(+)-**20**	10	259
(+)-**10**	52	18	(+)-**21**	8	92
(+)-**13**	70	7.2	(+)-**28**	94	16
(+)-**14**	5	40	(+)-**31**	58	6.2

pounds [(+)-**10**, (+)-**13**, and (+)-**14**] were selected as prospective photoaffinity probes, requiring incorporation of a radiolabel.

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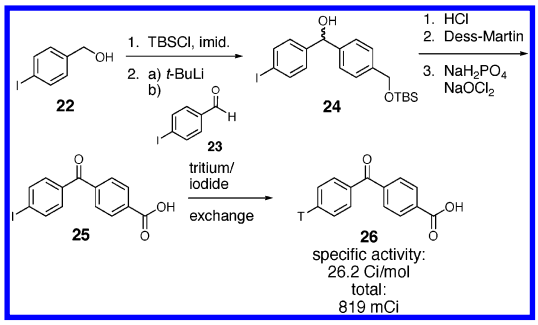
By design, the radiolabeled compounds were each constructed employing the same [^{3}H]-4-benzoylbenzoic acid intermediate **26**, the synthesis of which is detailed in Scheme 7. The iodine/tritium exchange was outsourced to Perkin-Elmer Life Sciences, New England Nuclear Division.

Scheme 7

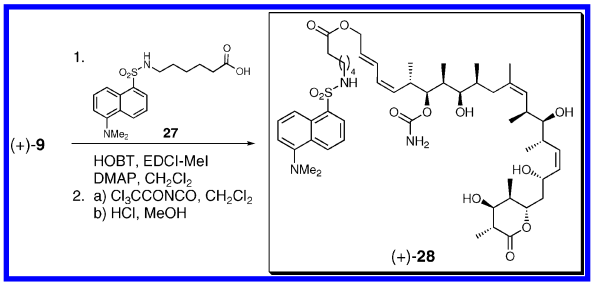


The [^{3}H]-4-benzoylbenzoic acid thus obtained was employed, after dilution with nonradiolabeled material, exactly as described above for production of radiolabeled photoaffinity probes [^{3}H]-**10**, [^{3}H]-**13**, and [^{3}H]-**14**.[15] Importantly, only two transformations were required with the radiolabeled material. Studies directed toward tubulin photoincorporation, digestion, and analysis are ongoing in the Horwitz Laboratory and will be reported in due course.

In addition to the discodermolide photoaffinity probes outlined above, two molecular probes each incorporating a fluorescent dansyl tag were designed and synthesized. Such probes hold the promise of localization of discodermolide in treated cells and in turn the possibility of more precise information on the cellular mechanism(s) of action and possibly on the nature of the synergistic relationship with paclitaxel.

For the first fluorescent probe we opted to append the diene, exploiting the same protocol that proved successful for the benzophenone counterpart (Scheme 8). Thus, chemose-

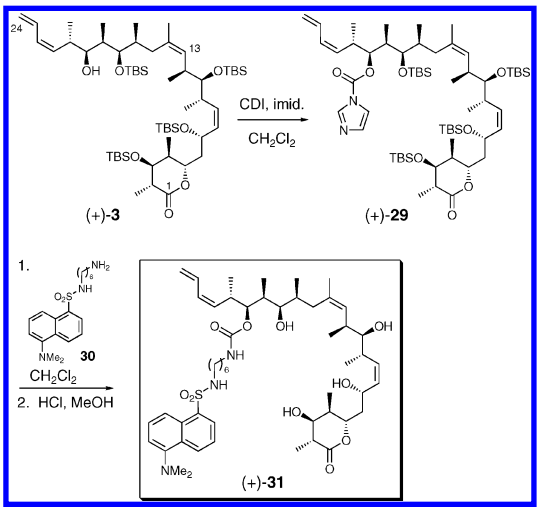
Scheme 8



lective esterification of the primary hydroxyl in diol (+)-**9** with acid **27**,[16] followed by carbamate installation and global deprotection, furnished (+)-**28**.

For the second fluorescent probe, attachment via the C(19) carbamate was selected (Scheme 9). To this end, desymmetrization of carbonyl diimidazole (CDI)[17] by treatment with alcohol (+)-**3** in CH_2Cl_2 provided imidazole carbamate (+)-**29**. Subsequent displacement of imidazole by dansyl sulfonamide **30**, followed by global desilylation, furnished the dansyl-substituted carbamate derivative (+)-**31**. Pleasingly,

Scheme 9



fluorescent probes (+)-**28** and (+)-**31** each exhibited potent cell growth inhibitory activity and tubulin binding properties (Table 1).

In conclusion, five prospective discodermolide photoaffinity probes possessing a benzophenone moiety have been designed, synthesized, and evaluated for tubulin polymerization and cytotoxicity. Three of these probes, (+)-**10**, (+)-**13**, and (+)-**14**, were then prepared in tritiated form and submitted for tubulin photoincorporation, digestion, and analysis experiments in the Horwitz Laboratory. In addition, two discodermolide fluorescent probes that hold the promise for elucidation of the cellular location and the mechanism of action of (+)-discodermolide have been designed and synthesized. The results of biological investigations exploiting both the photoaffinity and the fluorescent probes will be reported.

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

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(15) Detailed experimental procedures for the production of the radiolabeled compounds are presented in the Supporting Information.

(16) Acid **27** is generated via amination of dansyl chloride with 6-aminohexanoic acid.

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