The Chemistry and Biology of Discodermolide

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Not only scientists but also the public closely monitors natural products that interfere with important cellular events since they are promising candidates to fight a variety of diseases such as cancer. Recently, much attention has been paid to paclitaxel (taxol) and epothilones, compounds that stop the proliferation of tumour cells. These compounds exert their biological activity by inhibiting microtubule depolymerization. The microtubule-stabilizing agent (+)-discodermolide is one of the recent compounds that scientists and the media have shown much interest in as a new chemotherapeutic agent for the treatment of cancer.

(+)-Discodermolide was isolated in 1990 by Gunasekera et al.[1] from the marine sponge *Discodermia dissoluta* and exhibits immunosuppressive activity at very low concentrations ($IC_{50} = 9$ nM) against purified murine T cell proliferation.[2] It has also been shown that discodermolide causes cell cycle arrest in the G2 or mitosis (M) phase in the range (IC_{50}) of 3 to 80 nM.[3] The Schreiber group initiated[4] the total syntheses of (+)- and (−)-discodermolide in order to find the cellular target of this natural product (the absolute stereochemistry was established by their total syntheses) and reported in 1996[5] that (+)-discodermolide arrests cells at a stage after entering mitosis. Both enantiomers exhibit antiproliferative activity, but at different stages of the cell cycle. The natural (+)-discodermolide blocks cells in the G2 or M phase, whereas the enantiomer inhibits the cell cycle in the S phase. Additionally, the two compounds unfold their biological activity at different concentrations. In a [^{3}H]thymidine incorporation assay with NIH3T3 cells (+)-discodermolide had an IC_{50} value of 7 nM, compared to 135 nM for the (−)-enantiomer. To further investigate the mode of action, Schreiber and coworkers studied the in vitro polymerization of tubulin. Paclitaxel induces this polymerization in the presence of 1 mM GTP at 37 °C. Under the same conditions discodermolide polymerizes tubulin[6] more potently, with a stoichiometry of one discodermolide molecule per tubulin dimer (Figure 1).

In competition experiments it could be shown that (+)-discodermolide has significantly higher affinity to the tubulin-binding site than paclitaxel (apparent $K_i = 0.4$ μM).[1, 5] These results suggest that both paclitaxel and (+)-discodermolide bind to the same or an overlapping site on microtubules. Furthermore, multidrug-resistant colon and ovarian carcinoma cells that are 900- and 2800-fold more resistant to paclitaxel, respectively, compared to the parental cell line, retain sensitivity to discodermolide (only 25- and 89-fold resistant, respectively).[4] Additionally, the aberrant aggregation of microtubules occurs more rapidly upon treatment with discodermolide than with paclitaxel. Schreiber et al. also identified positions at (+)-discodermolide that could be used for introducing binding probes.[5] These efforts led to the identification of two promising candidates (**2** and **3**) that can be used for identifying the exact binding site of discodermolide. Additionally, they found that the methyl group at C16 can be omitted without significant loss of activity (Figure 2). It was also found that the *R* configuration at C17 is absolutely essential for the biological activity. These results suggest that discodermolide is a promising candidate for the development of new chemotherapeutic compounds. A major obstacle in its potential medicinal use is the insufficient

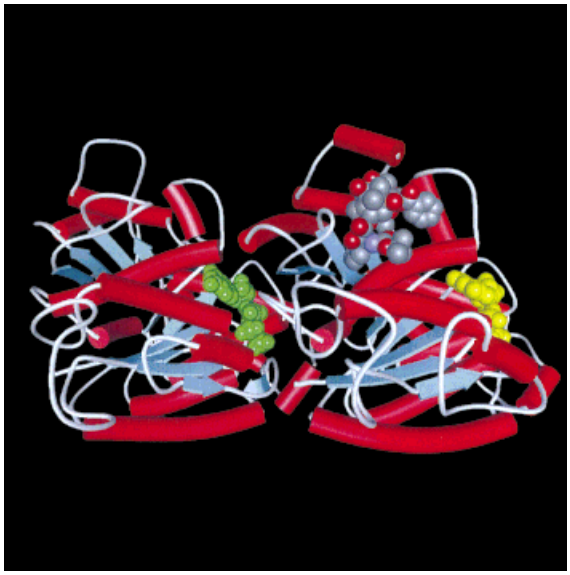

Figure 1. *Cow tubulin dimer in the presence of paclitaxel (PDB accession code: 1tub). The guanine-binding site on α tubulin is occupied with GTP (green). Paclitaxel as well as GDP (yellow) bind to the β-tubulin subunit. (+)-Discodermolide binds to the same or an overlapping site on microtubules.*

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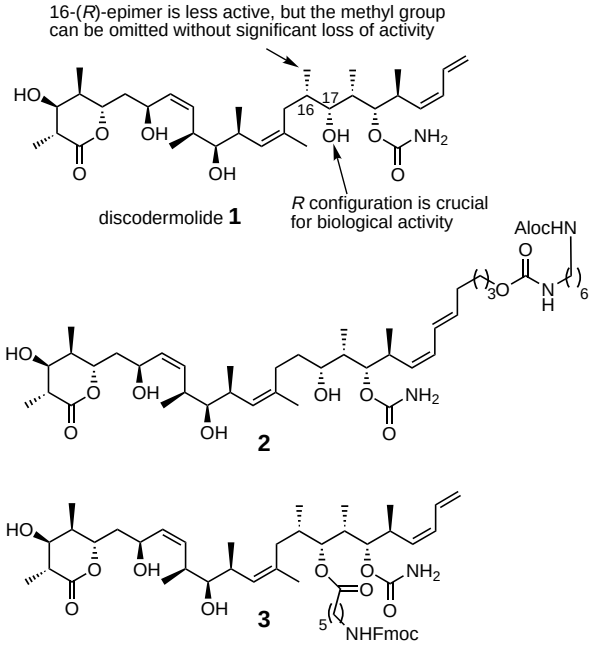


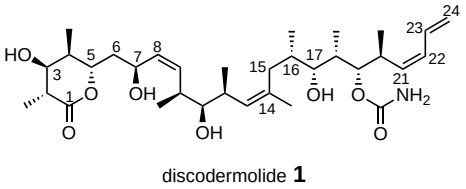
Figure 2. *(+)-Discodermolide (**1**) and derivatives as binding probes. Aloc = allyloxycarbonyl; Fmoc = 9-fluorenylmethoxycarbonyl.*

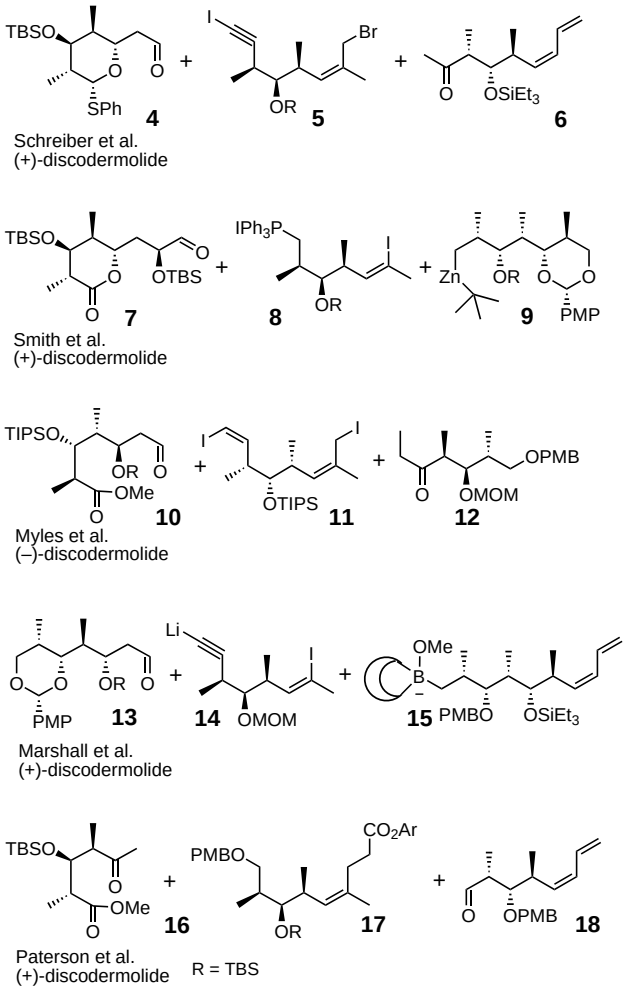
supply (0.002% (w/w) isolation yield) from natural sources.

These figures set the background for the recent efforts in the syntheses of discodermolides. Beside the intellectual challenge of synthesizing a molecule of that complexity, much energy has been devoted to establish a synthesis that can provide gram quantities of (+)-discodermolide. Remarkably, in all total syntheses a common stereochemical triad with a *syn,anti* relationship[7] was identified in three regions, and 3-hydroxy-2-methylpropionic acid methyl ester was always the starting material for these three fragments. Scheme 1 shows the retrosynthetic disconnections within these five total syntheses of discodermolide.[8]

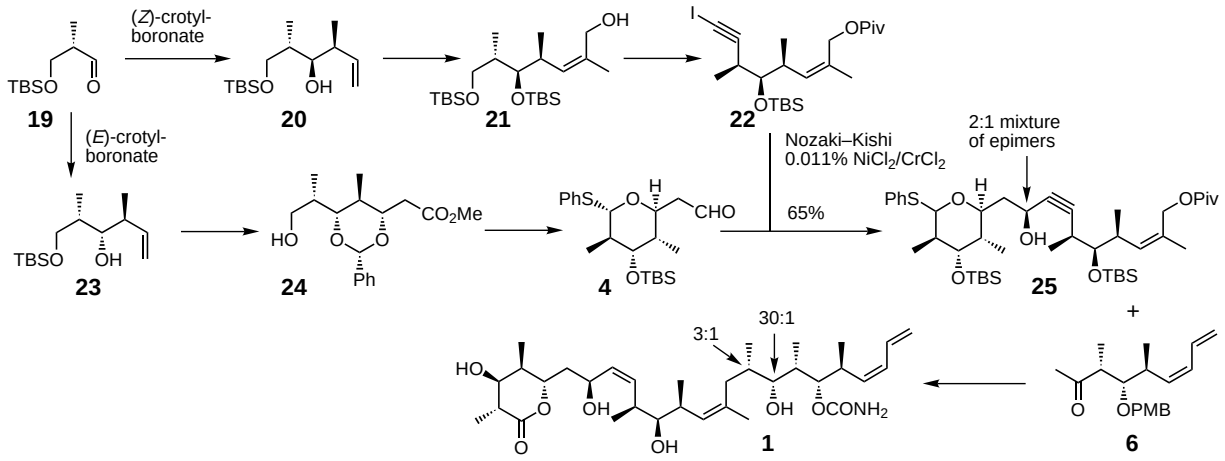
Schreiber et al.[9] (Scheme 2) used the separate addition of (*E*)- and (*Z*)-crotylboronate to aldehyde **19** for the generation of the stereochemical triad as described by Roush.[10] Alcohol **23**, obtained from the addition of (*E*)-crotylboronate was converted to the thioacetal **4**. Alcohol **20**, derived from the addition of (*Z*)-crotylboronate to **19**, was transformed into the *Z*-trisubstituted olefin **21** by the Still–Gennari method. Its transformation into the iodoacetylene **22** followed by Nozaki–Kishi coupling with aldehyde **4** established compound **25** as a 2:1 mixture of diastereomers. Alkylation of methyl ketone **6** with the corresponding bromide established the backbone of discodermolide. Subsequent methylation and selective reduction with $LiAlH(OtBu)_3$ followed by acidic removal of the protecting groups completed the synthesis of (+)-discodermolide (**1**) in 36 steps (24 steps in the longest linear sequence) with 4.3% overall yield.

Myles and co-workers[11] synthesized (−)-discodermolide (Scheme 3) by using **31** in a hetero-Diels–Alder reaction that led to fragment **35**[12] with the trisubstituted double bond established by the Diels–Alder reaction. Chelation-controlled alkylation (LiHMDS, TMEDA, 45% *n*-hexane in THF, −78 °C) of ketone **12**[13] with vinyl iodide **36** established compound **37** as a 6:1 mixture with the desired C16 *R*-isomer as the major product. It turned out that following these





Scheme 1. *Retrosynthetic disconnections of the discodermolide syntheses. MOM = methoxymethyl; PMB = para-methoxybenzyl; PMP = para-methoxyphenyl; TBS = tert-butyldimethylsilyl; TIPS = triisopropylsilyl.*

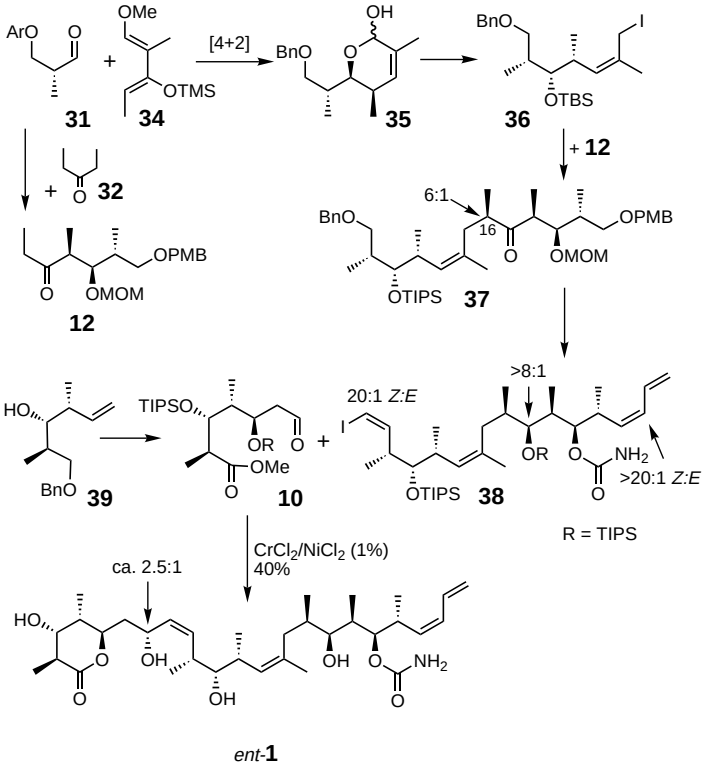


Scheme 2. *The Schreiber synthesis.[9] Piv = pivaloyl.*

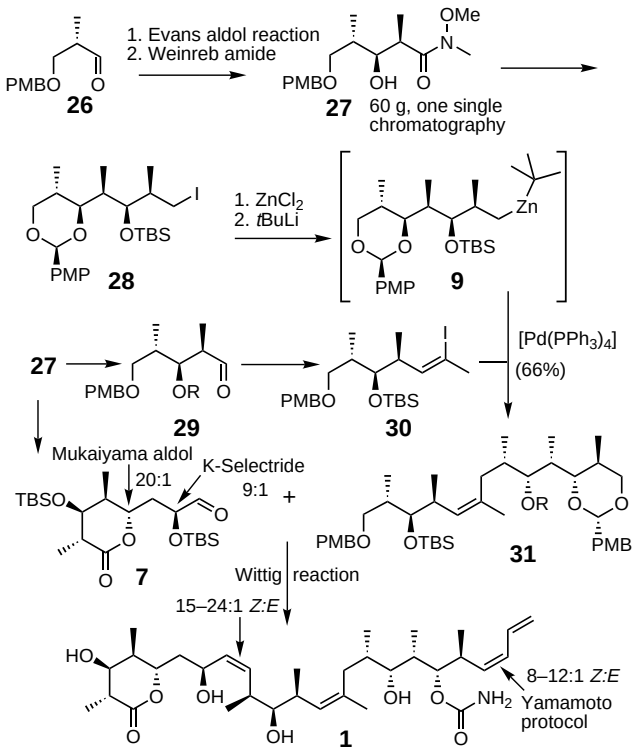
exact reaction conditions was crucial for obtaining the observed selectivity. Subsequent reduction (chelation control) of the C17 carbonyl group with $LiAlH_4$/LiI occurred with $>8{:}1$ diastereoselectivity. After routine functional group transformations, the terminal diene was introduced through a modified Peterson olefination. As in the Schreiber synthesis, the final C7–C8 bond formation was achieved by a nucleophilic attack at the carbonyl group of aldehyde **10** ($CrCl_2$/$NiCl_2$). Subsequent acidic deprotection gave (−)-discodermolide.

The great appeal of the synthesis by Smith et al.[14] (Scheme 4) is the fact that all three subunits of discodermolide are derived from the same intermediate **27**. It is noteworthy that the reaction sequence leading to **27** which uses Evan's aldol chemistry only involves one chromatography step. Lactone **7** was constructed using a Mukaiyama reaction and the C7 alcohol configuration was established by K-selectride reduction. Coupling of **9** and **30** was achieved through a modified palladium-catalyzed ([$Pd(PPh_3)_4$]) Negishi cross-coupling. The *Z*-disubstituted double bond between C8 and C9 was introduced through a Wittig reaction. Applying this strategy, Smith and co-workers could synthesize 1.043 g of (+)-discodermolide in 6% overall yield.

Marshall and co-workers[15] used their strategy of chiral allenyl metal reagents (from chiral propargylic mesylate **45**) to



Scheme 3. *The Myles synthesis.[11] Bn = benzyl; TMS = trimethylsilyl.*

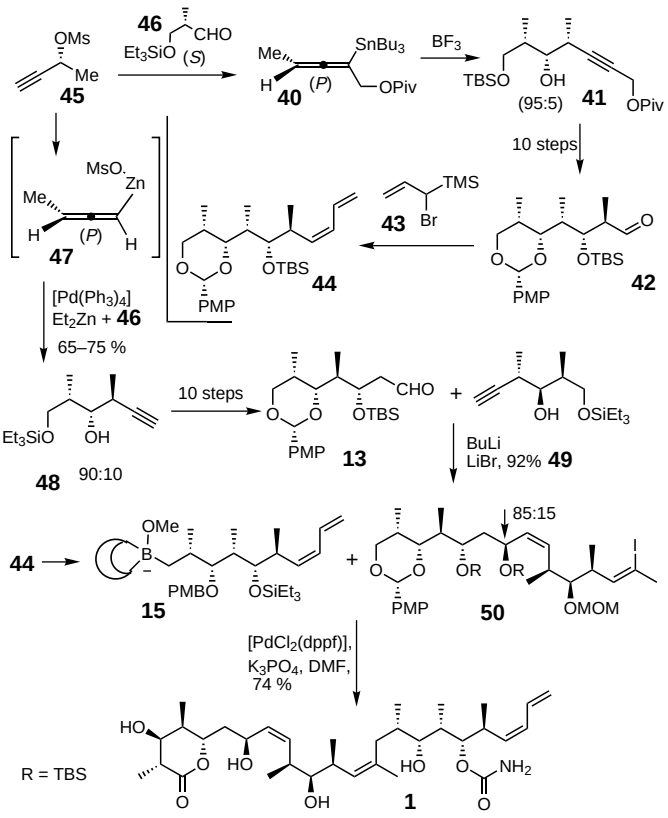


Scheme 4. *The Smith synthesis.[14]*

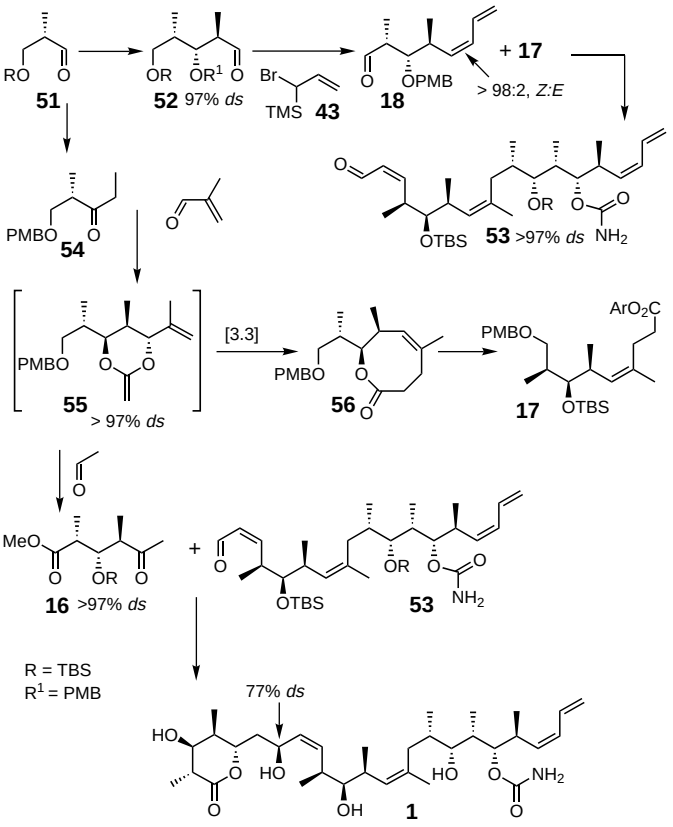
establish the alkyne fragments **41** and **48** (Scheme 5). By changing the reaction conditions, which generated either the allenyl stannane **40** or the corresponding zinc species **47**, the *syn*,*syn* (**41**) and the *syn*,*anti* (**48**) products were formed, respectively. Compound **48** was transformed into **13** and coupled with **49** by nucleophilic attack at the carbonyl group. As in the syntheses by Paterson and Myles, the terminal *Z*-diene subunit was introduced through a modified Peterson olefination. Suzuki coupling ([$PdCl_2$(dppf)]) with **15**, followed by routine functional group transformations, established (+)-discodermolide.

Paterson's strategy[16] was to build up all three intermediates through boron-mediated *anti*-selective aldol reactions of chiral ketones (Scheme 6). A Peterson olefination established compound **18** starting from **52**. The central fragment with the trisubstituted double bond was generated from **55** by [3.3] sigmatropic rearrangement. Ring opening of lactone **56** and aldol reaction with **18** gave compound **53**. The final aldol coupling was achieved with methyl ketone **16** and completed their synthesis of (+)-discodermolide in 7.7% yield and 27 steps.

These syntheses together now open access to large quantities of discodermolides and therefore provide a variety of possibilities for molecular probes and analogues in order to further evaluate the biological target or structure – activity relationships.



Scheme 5. *The Marshall synthesis.[15] dppf = 1,1′-bis(diphenylphosphanyl)ferrocene.*



Scheme 6. *The Paterson synthesis.[16]*

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