Tubulin-Binding Agents

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The Chemical Synthesis of Discodermolide

I. Paterson and G.J. Florence

Abstract The marine sponge-derived polyketide discodermolide is a potent antimitotic agent that represents a promising natural product lead structure in the treatment of cancer. Discodermolide shares the same microtubule-stabilising mechanism of action as Taxol®, inhibits the growth of solid tumours in animal models and shows synergy with Taxol. The pronounced cytotoxicity of discodermolide, which is maintained against cancer cell lines that display resistance to Taxol and other drugs, combined with its scarce availability from its natural source, has fuelled significant academic and industrial interest in devising a practical total synthesis as a means of ensuring a sustainable supply for drug development. This chapter surveys the various total syntheses of discodermolide that have been completed over the period 1993–2007, focusing on the strategies employed for introduction of the multiple stereocentres and achieving control over the alkene geometry, along with the various methods used for realising the pivotal fragment couplings to assemble progressively the full carbon skeleton. This dedicated synthetic effort has triumphed in removing the supply problem for discodermolide, providing sufficient material for extensive biological studies and enabling its early stage clinical development, as well as facilitating SAR studies for lead optimisation.

Keywords Anticancer, Natural products, Polyketides, Stereocontrol, Tubulin

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1 Introduction

The marine ecosystem provides an extensive source of structurally diverse natural products isolated from numerous organisms including algae, molluscs, corals, sponges, tunicates and phytoplankton, as well as associated microorganisms [1–4]. The biological activity of these compounds is often startling, demonstrating, for example, potent cytotoxic, immunosuppressive and antibiotic properties making them of interest to the pharmaceutical industry [5–8]. However, the low natural abundance of many of these lead structures means that realistic and practical synthetic routes are required to provide material to investigate and exploit further their biological activity [8, 9]. These factors, combined with the exquisite molecular architectures that many of these compounds possess, offer demanding challenges to the modern synthetic chemist. In particular, the marine polyketide discodermolide (I, Fig.1) has inspired intense synthetic activity, due to its biological profile and potential as a new generation anticancer agent given the onset of multidrug resistance in established cancer chemotherapies (see [10–13] for previous reviews on the synthesis of discodermolide; see [14] for a recent review on the synthesis of bioactive marine polyketides; see [15, 16] for an excellent recent review of microtubule-stabilising natural products.

1.1 Isolation and Structure of Discodermolide

Discodermolide (I) is a unique polyketide isolated by Gunasekera and co-workers at the Harbor Branch Oceanographic Institution in 1990 from the Caribbean deep-sea sponge Discodermia Dissoluta [17, 18]. This sponge was collected at a depth of 33m and several other new Discodermia species containing discodermolide were collected by manned submersibles at depths between 185 and 220m. These samples were exhaustively extracted and purified to provide discodermolide in an initial isolation yield of 0.002wt% from frozen sponge. Its gross structure was determined by extensive spectroscopic studies and the relative configuration was established by single crystal X-ray crystallography as shown in Fig.1. Structurally, discodermolide bears 13 stereogenic centres, a tetrasubstituted δ-lactone (C1–C5), one di- and one
tri-substituted (Z)-double bond, a carbamate moiety (C19) and a terminal (Z)-diene (C21–C24). Discodermolide adopts a U-shaped conformation in the solid state, where the (Z)-olefins at C13–C14 and C8–C9 act as conformational locks by minimising A(1,3) strain between their respective substituents. The δ-lactone adopts a boat-like conformation with H-bonding between the lactone moiety and the C7-OH group, which is retained in solution [19].

1.2 Biology of Discodermolide

Discodermolide was initially reported to be a potent immunosuppressive agent, both in vivo and in vitro, comparable with FK-506 and rapamycin, as well as displaying antifungal activity. It inhibited T-cell proliferation with an IC₅₀ of 9nM in the mixed leukocyte reaction and graft vs host disease in transplanted mice [20, 21]. Further biological screening revealed pronounced cytotoxicity, causing cell cycle arrest at the G2/M phase in a variety of human and murine cell lines, with
IC$_{50}$ values ranging from 3 to 80nM. In a similar fashion to Taxol, discodermolide functions by microtubule stabilisation, halting mitosis and causing cell death by apoptosis [22]. Discodermolide is an important member of a unique group of secondary metabolites (Fig.2) that act as microtubule-stabilising agents and mitotic spindle poisons, which currently include Taxol (paclitaxel) (2) [23], epothilones A (3) and B (4) [24], sarcodecytin A (5) [25], eleutherobin (6) [26], laulimalide (7) [27], FR182877 (cyclostreptin) (8) [28], peloruside A (9) [29], dictyostatin (10) [30–32] and the semi-synthetic Taxol analogue, Taxotere (11) [33]. Despite the lack of structural similarities, the cytotoxicity and microtubule stabilising properties of discodermolide are comparable to Taxol. Moreover, the growth of Taxol-resistant ovarian and colon carcinoma cells are inhibited by discodermolide (IC$_{50}$<2.5nM) [34]. Initial investigations into determining the discodermolide binding site through competition studies with radiolabelled Taxol suggested it occupied an identical or similar binding site on β-tubulin [35]. In comparative studies with the Taxol-dependent human lung carcinoma cell line A549-T12, discodermolide was unable to act as a substitute for Taxol, whereas the epothilones and eleutherobin were able to maintain the viability of the cell line [36]. Significantly, a synergistic potentiation of discodermolide’s cytotoxicity was observed, when used in combination with Taxol, suggesting that discodermolide may bind to tubulin at a distinct site from Taxol. More recent in vivo studies on ovarian tumour xenograft bearing mice have also shown this synergy, inducing tumour regression without toxicity to the mouse [37]. Notably, these studies suggest that the synergistic combination of Taxol and discodermolide may offer a useful therapy, minimising the plethora of

Fig. 2  Structures of other microtubule-stabilizing natural products
toxicity related side-effects that are observed in the clinic with high doses of Taxol. In 2006, the group of Carlomagno elucidated the bioactive conformation of discodermolide bound to soluble tubulin by using elegant NMR studies and proposed a common pharmacophore with the epothilones [38].

The highly encouraging biological profile of discodermolide led it to become a promising candidate for clinical development as a chemotherapeutic agent for breast cancer and other multi-drug-resistant cancers. This potential was recognised by Novartis Pharma AG, who licensed discodermolide from the Harbor Branch Oceanographic Institution and launched an impressive large-scale total synthesis campaign to supply material for clinical trials for the treatment of advanced solid malignancies. However, these trials have since been halted due to toxicity associated with the chemotherapy [39].

2 Total Synthesis

While the early clinical development of Taxol was severely hampered by the supply problem, this was eventually resolved by semi-synthesis from 10-deacetyl baccatin III, obtained from the needles of the common European yew tree. In comparison, the epothilones, which are currently in advanced clinical trials as anticancer agents, are obtained by fermentation [40]. Ixempra®, the lactam analogue of epothilone B which is obtained by semi-synthesis, has recently been approved by the FDA in the United States for the treatment of advanced breast cancer [41]. Unfortunately, a fermentation process is not as yet possible for discodermolide, even though as a polyketide it is presumably produced by a symbiotic microorganism associated with the sponge source (see [42] for a review on symbiotic bacteria as a source of marine natural products). Therefore, the supply problem for discodermolide could only be resolved via total synthesis. Consequently, there has been considerable synthetic effort directed towards discodermolide, culminating in 13 completed total syntheses reported by 8 groups [43–55] and numerous fragment syntheses [55–97]. This chapter highlights the total syntheses of discodermolide by ourselves and the groups of Schreiber, Smith, Myles, Marshall, Panek, Ardisson, and the Novartis process chemistry team in Basel led by Mickel. The key carbon-carbon bond disconnection strategies employed in all these approaches are highlighted in Fig. 3. In particular, we focus on the strategies employed to configure the multiple stereogenic centres, the methods employed to introduce the synthetically challenging (Z)-olefins and the pivotal fragment coupling steps.

2.1 Schreiber Total Synthesis

Schreiber and co-workers reported the first total synthesis of (–)-discodermolide (ent-1) in 1993, confirming the relative stereochemistry and establishing the absolute configuration [43, 44]. In 1996, they reported the first synthesis of the natural
antipode (+)-discodermolide (1), following an almost identical route performed in the correct enantiomeric series (Scheme 1), along with several analogues designed to study the mode of tubulin binding and microtubule stabilizing properties [44].

Their synthetic strategy towards (+)-discodermolide involved key fragment couplings via Nozaki-Kishi addition [98–101] at C7–C8 and enolate alkylation at C15–C16, requiring the three key subunits 12 (C1–C7), 13 (C8–C15) and 14 (C16–C24). In turn, the homoallylic alcohols 15 and 16 served as building blocks for the subunit synthesis, accessible via Roush crotylation reactions with the aldehyde 17 [102], derived from Roche ester 18 which serves as a starting point in all of the synthetic endeavours reported to date.

The C1–C7 aldehyde 12 was prepared in eight steps starting with the Roush crotylation of aldehyde 17 (Scheme 2) [102]. Ozonolysis and chain extension of 15 provided (E)-enoate 19; the C5 stereocentre was then introduced via an Evans-Prunet hetero-Michael addition [103]. Transformation of 20 into aldehyde 12 was completed in six steps. The C16–C24 methyl ketone 14 was obtained in seven steps from homoallylic alcohol 15. Protection, ozonolysis and Stork-Wittig olefination [104] of the intermediate aldehyde was followed by a palladium-catalysed Negishi coupling [105] of vinyl iodide 21 with vinyl zinc bromide to introduce the terminal (Z)-diene unit in 14. The synthetically demanding C13–C14 trisubstituted (Z)-alkene found in 13 was introduced by Still-Gennari HWE olefination (Z:E ≥ 20:1) [106], following silyl protection and ozonolysis of 16. Protecting group manipulations with the resulting (Z)-enoate 22 and homologation to iodoacetylene 13 via oxidation, alkylation and iodination at C8 completed the final subunit.

Assembly of the subunits began with the Nozaki-Kishi coupling reaction of iodoacetylene 13 and aldehyde 12, in the presence of CrCl₂/NiCl₂, to provide propargylic alcohol 23, with 3:1 dr at C7 (Scheme 3). The four-step conversion to bromide 24 was followed by the enolate alkylation of methyl ketone 14.

Fig. 3  Key disconnections employed in completed syntheses of discodermolide
Further alkylation of the lithium (Z)-enolate of 25 with methyl iodide gave 26, introducing the C16 stereocentre (3:1 dr) and completing the carbon backbone. Oxidation at C1 and carbamate formation gave 27 which underwent a chelation-controlled reduction at C17 (30:1 dr). Finally, global deprotection completed the synthesis of discodermolide (1), with an overall yield of 4.3% achieved over 24 steps in the longest linear sequence.

The Schreiber synthesis is particularly noteworthy in that the absolute configuration of discodermolide was assigned unambiguously, and through the preparation of numerous analogues the first structure-activity relationship study was possible [35, 44]. Their synthesis of the unnatural antipode (ent-1) also led to the unexpected discovery that it causes cell cycle arrest in the S-phase [107].

## 2.2 Smith Total Syntheses

### 2.2.1 First Generation (1995)

Smith and co-workers reported their initial synthesis of (−)-discodermolide (ent-1) in 1995 [45]. They invoked key fragment unions at C8–C9 and C14–C15 via Wittig
Scheme 2 Schreiber’s synthesis of C1–C7, C8–C15 and C16–C24 subunits
Scheme 3: Schreiber's subunit assembly and completion of discodermolide
olefination and Negishi cross-coupling [105], respectively (Scheme 4), dividing the carbon skeleton into three key subunits 28 (C1–C8), 29 (C9–C14) and 30 (C15–C21). Smith recognized the repeating stereotriad found in all three subunits and took advantage of a common precursor 31 for fragment synthesis. For clarity of presentation, the Smith first-generation synthesis is shown here in the correct enantiomeric series corresponding to natural (+)-1.

The common precursor 31 was configured using the Evans syn aldol reaction of propionimide 33 with the aldehyde 32, available in three steps from Roche ester 18 (Scheme 5) [108–111]. The (Z)-alkenyl iodide at C14 was introduced by a Zhao-Wittig olefination on aldehyde 34 in moderate yield with variable selectivity (Z:E = 8–17:1) [112, 113]. The synthesis of the C15–C21 segment 30 utilized a second Evans aldol reaction to configure the C16–C17 syn-relationship to provide 35 which was transformed into iodide 30. A palladium-catalysed Negishi cross-coupling of the corresponding zincate from the C14 vinyl iodide 29 with iodide 30 then gave the C9–C21 segment 36 [105]. The synthesis of the C1–C8 thioacetal aldehyde 28 began with the six-step elaboration of 31 to dithiane 37, coupling with epoxide 38 was then followed by dithiane cleavage and Evans-Saksena 1,3-anti reduction of the intermediate β-hydroxy ketone to provide 39 [114], and installing the C7 stereocentre. A further 5 steps were then required to access the β-ethylthioacetal aldehyde 28, giving a total of 14 steps from 31.

As shown in Scheme 6, the elaboration of the C9–C21 fragment 36 to phosphonium salt 40 proved problematic, requiring the treatment of the intermediate iodide 41 with triphenylphosphine at ultrahigh pressure (12.8kbar) for six days. The (Z)-selective Wittig coupling of aldehyde 28 and phosphonium salt 40 then gave the C1–C21 intermediate 42.
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(Z : E = 49:1). At this stage, the (Z)-diene unit was introduced utilizing the Yamamoto olefination protocol to give 43 [115]. A further five steps were then required to complete Smith’s first-generation synthesis of (–)-discodermolide (ent-1), in 2.2% overall yield over 28 steps in the longest linear sequence based on the C1–C8 aldehyde 28.

### 2.2.2 Second Generation (1999)

Smith and co-workers reported their second-generation approach to discodermolide in 1999, which was now performed in the correct enantiomeric series [46–48]. By carefully redesigning the route, the overall number of steps was significantly reduced and, importantly, it enabled a gram-scale synthesis. As outlined in Scheme 7, the key bond constructions at C8–C9 and C14–C15 were retained, while strategic modifications involved the earlier introduction of the terminal (Z)-diene unit in 44 and a revised C1–C8 subunit 45.

As shown in Scheme 8, the synthesis of aldehyde 45 was achieved in eight steps utilizing the common precursor 31 [46–48]. Remarkably, the Mukaiyama aldol addition of silyl enol ether 46 to aldehyde 47 proceeded with anti-Felkin selectivity, which was attributed to involvement of the Weinreb amide and aldehyde carbonyl...
Scheme 6  Smith’s first-generation assembly of discodermolide

Scheme 7  Smith’s second-generation strategy
oxygens in forming an eight-membered chelate with the titanium Lewis acid, and after work-up and acid-catalysed δ-lactonisation with TFA this gave the ketone 48. K-Selectride reduction at C7 (9:1 dr), followed by TBS protection and ozonolysis provided 45, in 13 steps and 21% overall yield from 18 [70–97].

Starting from the C9–C21 fragment 36, the installation of the terminal Z-diene unit was achieved in a five-step sequence, in which the Yamatoto olefination gave a Z:E ratio of 8–14:1 (Scheme 9) [115]. The phosphonium salt 49 was prepared from 44 in two steps, again requiring ultrahigh pressure conditions. The Wittig coupling of phosphonium salt 49 and aldehyde 45 then gave 50 (Z:E = 15–24:1). A further three steps were required to complete Smith’s second-generation synthesis of discodermolide, which proceeded in an improved 6% yield over 24 steps (longest linear sequence). The Smith second-generation synthesis is notable in that it provided an impressive 1.04 g of discodermolide, enabling extensive biological and preclinical evaluation. However, further scale up of this route would pose significant technical challenges due, in particular, to the limited availability of suitable ultrahigh pressure reactors for performing large-scale preparations of the pivotal phosphonium salt 49. This limiting factor was then addressed in a third-generation approach [49].

2.2.3 Third Generation (2003)

Although the Smith second-generation synthesis offered several improvements over their initial route, the use of the ultrahigh pressure over extended time periods in the formation of the key Wittig salt 49 severely limited the possibility of further
scale-up. Thus, the Smith group’s third-generation synthesis addressed this limiting step [49], through the simple replacement of the sterically demanding TBS group at C11 with a MOM group in 51, while retaining the successful C14–C15 and C8–C9 coupling strategy (Scheme 10).

As detailed in Scheme 11, a Negishi-type coupling of 30 and the revised C9–C14 subunit 52, readily derived from 31, followed by an established sequence gave alcohol 53 [46–48], in preparation for phosphonium salt formation. Conversion into the intermediate iodide and reaction with triphenylphosphine at ambient pressure proceeded smoothly to give 51 in 70% yield. However, Wittig olefination of aldehyde 45 with 51 using methyl lithium/lithium bromide for deprotonation generated the C8–C9 (Z)-olefin with substantially reduced selectivity (third generation = 4:1, cf second generation = 24:1). Conversion into discodermolide then required three steps, giving a 1.9% overall yield over 24 steps in the longest linear sequence.

This revised strategy demonstrates that a simple change in hydroxyl protecting groups can markedly influence reactivity and selectivity. While formation of the Wittig salt 51 could now be achieved at atmospheric pressure, its coupling with aldehyde 45 only proceeded in moderate yield and selectivity.

### 2.2.4 Fourth Generation (2005)

In 2005, Smith and co-workers reported their fourth-generation synthesis of discodermolide [50]. Using the Suzuki-coupling at C13–C14 developed by Marshall [53, 54,
a revised strategy was devised based on reversing the order of the key fragment couplings. Thus, a Wittig olefination at C8–C9 between a modified C1–C8 aldehyde and phosphonium salt was followed by C14–C15 bond formation with the C15–C24 subunit, as shown in Scheme 12. These modifications were designed to further increase the overall efficiency and convergency of their approach.
As outlined in Scheme 13, the synthesis of the C15–C24 subunit 56 started with an Evans aldol reaction between the aldehyde 57 and 58 [108–111]. Transformation into aldehyde 59 and a Brown crotylation then gave 60 [117], which was converted into 56 in five steps.

The C1–C8 aldehyde 54 and the C9–C14 Wittig salt 55 were accessed from previously reported intermediates 48 and 52 (Scheme 14). A Wittig olefination of aldehyde 54 with 55 gave the C1–C14 intermediate 61. In turn, a Suzuki coupling of the boronate derived from 56 with 61 provided 62 [116]. Following their established endgame, the synthesis of discodermolide was now achieved in 9.0% overall yield with 17 steps in the longest linear sequence.
Myles and co-workers reported the synthesis of (−)-discodermolide (ent-1) in 1997 [51]. This was followed by their synthesis of the natural antipode in 2003, exploiting an analogous coupling strategy with improved subunit syntheses, the details of which are presented here [52]. The Myles approach to discodermolide relied on key bond unions at C7–C8 (Nozaki-Kishi) and C15–C16 (enolate alkylation) giving rise to the subunits 63 (C1–C7), 64 (C9–C15), and 65 (C16–C21) (Scheme 15). The synthesis of these subunits utilized a combination of both substrate and reagent controlled reactions, including a novel solution to the installation of the C13–C14 (Z)-trisubstituted olefin using the cycloadduct 66 [118].

The synthesis of the C9–C15 subunit 64 commenced with the cyclocondensation of aldehyde 67 and diene 68 to provide dihydropyrone 66 (Scheme 16) [118]. A Luche reduction [119, 120] and acid-mediated Ferrier rearrangement gave lactol 69 [121], installing the C13–C14 (Z)-trisubstituted olefin. A further five steps
were then required to access 64, involving the reductive opening of 69 followed by a series of protecting and functional group manipulations. The C16–C21 subunit 65 was conveniently accessed from the Smith common precursor 33. Myles’ improved synthesis of the C1–C7 subunit 63 began with the tin-mediated allylation [122–124] of 70 under chelation control (>20:1dr), which after silyl protection and debenzylation provided 71. The C2 and C3 stereocentres were configured via an Evans aldol reaction [108–111] and the resulting adduct was converted into 63 over four steps.

As shown in Scheme 17, Myles’ fragment assembly began with the demanding enolate alkylation of ketone 65 with iodide 64 to form the C15–C16 bond, a tactic previously explored by both Schreiber and Heathcock with little success [44, 71]. The combination of MOM protection at C19 in 65 and lithium amide base in a mixed hexanes/THF solvent system proved essential to the success of this alkylation (6:1dr at C16) [51, 52]. A chelation directed reduction of 72 afforded 8:1 selectivity at C17, which after a series of protecting group manipulations provided 73. A Stork-Wittig olefination ($Z:E = 20:1$) [104] and elaboration into aldehyde 74 was followed by the introduction of the terminal ($Z$)-diene unit via addition of allylboronate 75 and Peterson-type elimination of the resulting 1,2-anti hydroxy-silane to give 76 ($Z:E \geq 20:1$) [125, 126]. Following formation of carbamate 77, the stage was set for

Scheme 15 Myles’ synthetic strategy
the Nozaki-Kishi coupling with 63. In Myles’ initial synthesis of ent-1 employing 1mol% NiCl2/CrCl3 in DMSO, this coupling gave low yields and selectivity at C7 (20–40%, 2.5:1dr). However, employing Kishi’s chiral bispyridinyl ligand with 20 mol% NiCl2/CrCl3 in THF provided adduct 78 in more reliable, albeit moderate, yields with slightly improved selectivity at C7 (3:1dr) [127]. Finally, global deprotection with concomitant δ-lactonization gave discodermolide in 1.1% overall yield over 22 steps in the longest linear sequence from 67. The Myles synthesis, akin to the Schreiber approach, highlighted the limited utility of the Nozaki-Kishi bond disconnection at C7–C8 in terms of both yield and control over the C7 stereocentre.

2.4 Marshall Total Synthesis

In 1998, Marshall and co-workers demonstrated the utility of allenyl metal addition methodology for the synthesis of the polypropionate subunits contained in discodermolide [53, 54]. As outlined in Scheme 18, Marshall divided the carbon backbone...
of 1 into three segments 79 (C1–C7), 80 (C8–C13) and 81 (C15–C24), reliant on lithium acetylide addition to an aldehyde at C7–C8 and Suzuki cross-coupling at C14–C15 [116]. This latter bond construction appears to have significant advantages over Smith’s Negishi-type coupling due to its greater robustness and scalability, and was subsequently adopted by Novartis in their process development scale-up effort (see Sect. 2.8) [65–69].
The synthesis of the C1–C7 and C8–C13 subunits 79 and 80 began with the addition of the chiral allenylzinc species, generated in situ from the treatment of propargylic mesylate 82 with Et$_2$Zn/catalytic Pd(0), to aldehyde 83 (Scheme 19) [53, 54]. Completion of the C1–C7 subunit 79 required ten further steps in which 84 was converted into propargylic alcohol 85 and subsequently underwent alkyne reduction, Sharpless epoxidation and directed hydride opening of the resulting epoxide to introduce the C5-OH stereocentre [54]. Stereoselective addition of the lithium anion of 80–79 (6:1dr at C7), followed by a Lindlar hydrogenation and protecting group adjustments gave 86. Oxidation and a Zhao-Wittig olefination provided the C1–C14 intermediate 87 in moderate yields with variable Z:E ratios ranging from 1.3 to 9:1.

In the synthesis of the C15–C24 subunit 81, Marshall utilized the Lewis-acid promoted addition of the allenylstannane 88 to aldehyde 17 to configure the syn stereotriad in 89 (Scheme 20) [128, 129]. A five-step sequence was then required to configure the C19–C20 stereocentres, involving hydroalumination, Sharpless epoxidation and epoxide opening with lithium dimethylcyanocuprate. Conversion of 90 into aldehyde 91 was followed by (Z)-diene installation using the Paterson and Schlapbach protocol [75], involving sequential Nozaki-Hiyama addition of allylbromosilane 92 and Peterson-type elimination. Following manipulation of the C15-terminus, iodide 81 was transformed into the intermediate C15–C24 boronate 93 for the Suzuki cross-coupling reaction with 87, thus assembling the C1–C24 intermediate 94. A further nine steps involving a series of protecting group manipu-
lations and oxidation state adjustments then completed Marshall’s synthesis of discodermolide in 2.2% overall yield over 29 steps (longest linear sequence).

The Marshall synthesis clearly demonstrated both the versatility of allenyl metal methodology for the preparation of polypropionate arrays and the utility of the Suzuki cross-coupling in complex fragment assembly.
Scheme 20  Marshall’s synthesis of the C15–C24 subunit and completion of discodermolide
2.5 *Paterson Total Synthesis*

2.5.1 *First Generation (2000)*

In 2000, Paterson and co-workers reported their first-generation synthesis of discodermolide [55–57]. As shown in Scheme 21, their novel construction of the carbon skeleton of discodermolide relied on an ambitious boron aldol coupling reaction at C6–C7 between methyl ketone 95 and the advanced (Z)-enal 96, and an equally challenging lithium-mediated aldol reaction at C16–C17 between aryl ester 97 and aldehyde 98, thus installing three new stereogenic centres in the fragment union steps. In turn, the anti aldol reactions of the respective chiral ethyl ketones 100–102 were used to configure the stereochemical motifs in the three subunits 95 (C1–C6) [130], 97 (C9–C16) [131] and 98 (C17–C24) [131–138] (see [139] for a review). In addressing the installation of the trisubstituted (Z)-alkene in 97, the application of Holmes’ Claisen rearrangement methodology provided a further novel solution [140–144], which also introduced the correct oxidation state for the planned C16–C17 aldol coupling.

As outlined in Scheme 22, the synthesis of the C1–C6 subunit 95 commenced with the anti aldol reaction of the ethyl ketone 100, prepared in three steps from Roche ester 18, and acetaldehyde, with in situ reduction to give diol 103 (>30:1dr) [130, 132–136, 145, 146]. Completion of 103 then required a series of protecting group manipulations

Scheme 21  Paterson’s first-generation strategy
and oxidation state adjustments. The synthesis of the C9–C16 aryl ester 97 began with the anti aldol reaction of ethyl ketone 101 and methacrolein to provide 104 (>30:1 dr) [130, 132–136]. A 1,3-anti reduction provided diol 105 (>30:1 dr) [114], which then underwent transacetalisation with 106. Following the Holmes protocol [140–144], oxidation of 107 followed by thermal selenoxide elimination gave the transient ketene acetal which underwent Claisen [6, 6] rearrangement to afford the 8-membered lactone 108, introducing the trisubstituted C13–C14 (Z)-alkene cleanly. Opening of 108, esterification and TBS protection then completed 97. The synthesis of the C17–C24 aldehyde 98 began with an anti aldol reaction between the lactate-derived ketone 102 and the aldehyde 17 to provide 109 (>30:1 dr) [131, 137, 138]. Transformation to aldehyde 110 was followed by the Paterson and Schlapbach diene installation, involving sequential Nozaki-Hiyama allylation with bromide 92 and Peterson elimination [75]. Conversion of 111 into aldehyde 98 then paved the way for the first of the key fragment unions.

As shown in Scheme 23, the lithium-mediated anti aldol reaction of the aryl ester 97 and aldehyde 98 gave the expected Felkin-Anh adduct 112 (30:1 dr) [147, 148].
Scheme 23  Paterson's subunit assembly and completion of discodermolide

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Following ester reduction, either in situ or upon isolation of 112, the superfluous oxygen functionality at C16 in diol 113 was removed and converted into intermediate 114 in four steps. Selective primary oxidation with TEMPO [149], followed by a Still-Gennari HWE olefination [106] installed the C8–C9 (Z)-olefin in 115 (Z:E => 30:1). Transformation into enal 96 required three further steps, prior to the final C6–C7 aldol coupling. Considerable effort was required to secure the desired adduct 116 bearing the correct C7 configuration [56, 57]. Using the achiral boron reagent c Hex₂BCl for enolization of 95 gave the undesired (7R)-adduct 117 (7:1 dr). This unprecedented selectivity arose from high levels of remote 1,4-stereoinduction imparted by the aldehyde component. In order to overturn the π-facial bias of aldehyde 96, the chiral (+)-Ipc₂BCl reagent was employed and the desired (7S)-adduct 116 was now obtained with 5:1 dr (see [139] for a review) [150–152]. An Evans 1,3-anti reduction of 116 introduced the final stereogenic centre at C5 (>30:1 dr) [114], which following acid-mediated desilylation and δ-lactonization gave discodermolide, completing the Paterson group’s first-generation synthesis in 10.3% yield over 23 steps (longest linear sequence).

The Paterson first-generation synthesis of discodermolide provides a clear demonstration of the utility of complex boron aldol reactions in the context of polyketide natural product synthesis. Recently, Paton and Goodman reported theoretical DFT studies of related boron aldol transition states to rationalise the very high degrees of stereoselectivity obtained in these powerful reactions [153]. In contrast to previous syntheses, essentially complete control was now achieved over the double bond geometries and the only step proceeding with less than optimal stereocontrol was the final mismatched aldol coupling. However, it is notable that the Novartis process group chose to adopt the key C6–C7 aldol disconnection in their large-scale synthesis of discodermolide for clinical trials (see Sect. 2.8).

2.5.2 Second Generation (2003)

In 2003, Paterson and co-workers reported a second-generation strategy for the synthesis of discodermolide, which aimed to eliminate the use of all chiral reagents and auxiliaries, and reduce the total number of synthetic steps (Scheme 24) [58, 59]. These specific aims were achieved by employing an unprecedented aldol coupling at C5–C6 between C1–C5 aldehyde 118 and the advanced C6–C24 methyl ketone 119 and utilising diol 120 as a common precursor for the synthesis of the three subunits 118, 121 (C9–C16) and 98 (C17–C24).

The common precursor 120 was prepared in two steps from ethyl ketone 101 on a multigram scale (Scheme 25), where the 1,3-anti methyl groups were configured by a boron aldol reaction with formaldehyde (20:1 dr) [130, 132–136]. Aldol adduct 122 then underwent a hydroxyl-directed reduction to provide diol 120 (10:1 dr). The C1–C5 subunit 118 was prepared in six steps from diol 120, starting with the selective TEMPO oxidation of the C1-OH to give aldehyde 123, and five further steps involving oxidation state adjustments and protecting group manipulations followed. Aldehyde 123 was also used in the five-step preparation of the C9–C16 subunit 121.
The trisubstituted (Z)-olefin was introduced by Still-Gennari HWE olefination, as preceded by Schreiber [43, 44, 106], and following silyl protection provided 124. Conversion into the iodide 125 was followed by alkylation with the lithium enolate of aryl ester 126, to complete the C9–C16 subunit 121. The synthesis of the C17–C24 subunit 98 from 120 began with a four-step sequence involving protecting group manipulations and oxidation at C21 to provide aldehyde 127, converging with the earlier route to 98 [55–57].

As outlined in Scheme 26, assembly of the subunits began with the lithium-mediated aldol coupling of 121 and 98 to provide 129 (6:1dr) [147, 148], which was converted into the diol 114, following the first-generation route [55–57]. Primary oxidation of 114 and modified Still-Gennari olefination introduced the (Z)-enone moiety in 119 (Z:E = 12:1) [106, 154]. The boron aldol reaction of methyl ketone 119 and aldehyde 118 gave the desired (5S)-adduct 130 with 20:1dr, arising from long-range 1,6-asymmetric induction from the remote C10 γ-stereocentre in the ketone component. Acid-promoted δ-lactonisation of 130, K-Selectride reduction of the C7 ketone (>30:1dr) [46–48] and global deprotection then completed the second-generation synthesis of discodermolide in 5.1% overall yield, with 24 steps in the longest linear sequence.
Scheme 25 Paterson’s synthesis of C1–C5, C9–C16 and C17–C24 subunits from common precursor diol

The Paterson second-generation approach substantially reduced the total number of steps required to complete discodermolide. Notably, the use of chiral reagents and auxiliaries was completely eliminated, relying solely on substrate control to configure all the remaining stereocentres from the ubiquitous Roche ester (18), achieving a more cost-effective route.

2.5.3 Third Generation (2004)

In 2004, the Paterson group implemented a further revision to their strategy designed to overcome the perceived technical difficulties of performing late-stage boron aldol couplings on an industrial scale (Scheme 27) [60, 61]. Building on the experience already gained and their contemporary synthesis of dictyostatin (2), a third-generation approach to discodermolide involved the application of a Still-Gennari-type HWE olefination in the final fragment coupling step using the highly functionalized C1–C8 β-ketophosphonate 131 and the C9–C24 aldehyde 132.
As outlined in Scheme 28, the synthesis of the β-ketophosphonate 131 began with a one-pot anti-aldol/reduction step between ethyl ketone 101 and aldehyde 133, giving the 1,3-syn diol 134 (>30:1 dr) [130, 132–136, 145, 146]. The diol 134 was then converted into the carboxylic acid 135 in six steps. Completion of the subunit 131 required conversion into the acid chloride and reaction with the lithium anion of methyl-(di-1,1,1-trifluoroethyl)-phosphonate. The C9–C24 aldehyde 132 was prepared in two steps from 136, an intermediate from previous routes [55–58]. The Still-Gennari-type coupling of 131 and 132 was readily achieved via treatment with
Scheme 27  Paterson’s third-generation strategy

Scheme 28  Paterson’s synthesis of C1–C8 phosphonate and completion of third-generation synthesis
NaH to give the advanced enone 137 (Z:E = 10:1). A further four steps were then required to complete the third-generation synthesis, beginning with C19 carbamate installation, followed by K-Selectride reduction to introduce the requisite (7S)-stereocentre. Finally, global deprotection with concomitant δ-lactonisation gave discodermolide (1) in 11.1% overall yield over 21 steps (longest linear sequence).

In summary, the Paterson group’s third-generation synthesis demonstrated the versatility of the Still-Gennari HWE coupling of advanced polypropionate subunits. This revised endgame gave improved levels of efficiency and, importantly, the overall linear sequence was shortened with increased yields. The experimentally undemanding conditions of the Still-Gennari-type HWE coupling protocol offers distinct advantages over previous routes using boron aldol reactions, offering a viable alternative for industrial scale-up.

### 2.6 Panek Total Synthesis

Panek and co-workers reported their synthesis of discodermolide in 2005 [62, 63], relying on the application of their crotylsilane methodology for the synthesis of polypropionate motifs [155, 156] (see [157] for a review) [158, 159, 182]. Their strategy involved key bond disconnections at C6–C7 and C14–C15, based on an aldol coupling and the established Suzuki cross-coupling respectively, employing the key subunits 137 (C1–C6), 138 (C7–C14) and 81 (C15–C24) (Scheme 29).

As outlined in Scheme 30, the synthesis of the C1–C6 subunit 137 began with the crotylation of aldehyde 139 with the (S)-allylsilane 140, which following silyl depro-
Scheme 30  Panek’s synthesis of C1–C6, C7–C14 and C15–C24 subunits

detection gave diol 141 (dr > 30:1) [81, 168, 170, 171] (see [157] for a review). Subsequent anisylidene acetal formation and ozonolysis completed 137. The (S)-crotylsilane 142 was used as the starting point for the synthesis of the C7–C14 subunit with its addition to aldehyde 70. A four-step sequence on 143 was then used to access the silylacetylene 144, which upon treatment with Schwartz’s reagent [160, 161] and quenching with iodine gave (Z)-iodovinylsilane 145. A palladium-mediated
coupling of 145 with methylzinc chloride gave the (Z)-vinylsilane 146 exclusively, providing a further solution for the installation of the C13–C14 trisubstituted olefin. The subunit 138 was then completed by the Corey-Fuchs olefination at C9, treatment of the intermediate vinyl dibromide with BuLi and quenching with ethyl formate. The synthesis of the C17–C24 subunit 81 started with the crotylation of aldehyde 139 with (R)-crotylsilane 147 to configure the all-syn stereotriad in 148. Conversion into the aldehyde 149 was followed by a second crotylation, now with (S)-crotylsilane 142, to configure the C19–C20 stereocentres in 150, which was then transformed into the aldehyde 151 in four steps. Treatment of 151 with a Roush-type allylationonate reagent and subsequent Peterson elimination selectively introduced the terminal (Z)-diene [51, 52, 125], which was readily transformed into iodide 81.

As outlined in Scheme 31, Panek’s assembly of subunits began with the boron-mediated aldol reaction of ketone 137 and aldehyde 138, which gave the expected 1,4-syn-1,5-anti aldol product 152 [162, 163]. An Evans-Tischenko reduction efficiently introduced the C5-stereocentre in 153 [164]. Methanolysis, acid-mediated migration of the PMP acetal and primary oxidation with Oshima’s reagent [165] gave aldehyde 154, which was converted into 155 in three steps. Lindlar reduction of the C8–C9 alkyne, and conversion of the C14-vinyl silane into the vinyl iodide 156, then set the stage for the C14–C15 bond formation. The success of both Suzuki- and Negishi-type couplings required exchange of the protecting group at C13 from TBS in 156 to MOM in 157, which then underwent cross-coupling with iodide 81 to provide 158 [53, 54, 105, 116]. The completion of discodermolide from 159 then required an additional four steps. Overall, Panek’s synthesis proceeded in 2.1% yield based on 27 steps (longest linear sequence).

2.7 Ardisson Total Synthesis

In 2007, Betzer, Ardisson and co-workers reported their synthesis of discodermolide [64] following the Marshall disconnection strategy of C7–C8 acetylide addition and Suzuki cross-coupling at C14–C15 (Scheme 32) [53, 54]. The synthesis of the key subunits 160 (C1–C7), 161 (C8–C14) and 162 (C15–C24) demonstrated the versatility of the Hoppe crotyltitanation reaction [166–169] in the synthesis of polypropionate motifs, using the incorporated (Z)-O-enecarbamate to configure the requisite alkene substitution patterns [170, 171].

As shown in Scheme 33, the synthesis of the C1–C7 amide 160 began with a Hoppe crotyltitanation reaction between the aldehyde 17 and the (R)-crotyltitanium 163, prepared in situ (crotyl diisopropylcarbamate with sBuLi/(−)-sparteine/Ti(OiPr)₄), to give O-enecarbamate 164 (>30:1 dr) [166–169]. Ozonolysis and HWE chain extension was followed by an Evans-Prunet 1,4-addition to install the C5-stereocentre to complete 160 [103]. The synthesis of the C8–C14 subunit 161 started with an elegant installation of the C13–C14 (Z)-olefin. Deprotonation of the dihydrofuran 165, available in three steps from bromo alcohol 166, with tBuLi and transmetallation with Me₂CuLi-LiCN, and subsequent 1,2-cuprate transfer gave the
Scheme 31  Panek’s subunit assembly and completion of discodermolide
intermediate 167, which was trapped with tributyltin chloride to provide the (Z)-vinyl stannane 168 exclusively [172–175]. Oxidation of 168 was followed by a second Hoppe reaction with 163 to give the intermediate (Z)-O-enecarbamate, which underwent Fritsch-Buttenberg-Wiechell rearrangement to give, after MOM protection, alkyne 161 (see [176] for review) [177]. The synthesis of the C15–C24 subunit 162 commenced with the diastereoselective crotylation of aldehyde 32 with (E)-crotylstannane to give 169. Conversion into aldehyde 170 was followed by a third crotylatitanation reaction with 163 (>30:1dr). A four-step sequence was then required to complete 162, in which the terminal (Z)-diene was installed via a nickel-catalysed cross coupling of the C22-carbamate with vinylmagnesium bromide.

The assembly of the subunits began with the addition of the lithium anion of acetylene 161 to Weinreb amide 160, followed by CBS reduction to give 171 installing the C7 stereocentre with > 20:1dr (Scheme 34) [178]. Following elaboration to vinyl iodide 157, an intermediate shared with the Panek route [62, 63], Suzuki cross-coupling with the boronate derived from iodide 162 gave the advanced C1–C24 intermediate 172, which following a three-step sequence provided discodermolide in 1.6% yield over 21 steps (longest linear sequence).

2.8 Novartis Process Chemistry Group Synthesis

Early preclinical development of discodermolide had shown its clear potential as a new generation anticancer agent for the treatment of a range of multidrug-resistant human cancers. This attracted the attention of Novartis Pharma AG, who with a
Scheme 33 Ardisson’s synthesis of C1–C7, C8–C14 and C15–C24 subunits
license agreement from the Harbor Branch Oceanographic Institution, took on the
task of progressing discodermolide into clinical trials. This required a practical
synthetic solution [65–69, 90, 92, 95], as eco-harvesting the rare natural source was
clearly not an option, either economically or ecologically. Rising to this challenge,
the Novartis Process Chemistry group in Basel led by Mickel reported in 2004 the
synthesis of 64 g of discodermolide.[65–69]. Following careful consideration of the
successful approaches to discodermolide at the time, Mickel and co-workers
adopted a hybrid synthesis incorporating features of the Smith [45, 46] and Paterson
[55–57] routes (Sects. 2.2 and 2.5, respectively), providing valuable insights into
the complexities of performing a multistep “academic” synthesis in an industrial
setting. As outlined in Scheme 35, the Novartis strategy opted for Paterson’s aldol
disconnection at C6–C7 and the Smith/Marshall cross-coupling at C14–C15. In
turn, the three key subunits 173 (C1–C6), 29 (C9–C14) and 30 (C15–C24) would
be accessed on a kilogram-scale from Smith’s common precursor 31 [46–48].

As shown in Scheme 36, the Novartis group’s large-scale (20–25kg) preparation
of Smith’s common precursor 31 began with the established Evans aldol reaction
between the Roche ester-derived aldehyde 32 and the propionimide 33 [65].
Scheme 35  Novartis synthetic strategy

Scheme 36  Novartis large-scale synthesis of C1–C6, C9–C14 and C15–C21 subunits
Transformation of 174 into the common precursor 31 could not be achieved directly due to safety considerations regarding the use of Me₃Al on pilot plant scale. Thus, hydrolysis of 174 and isolation of the intermediate acid salt, was followed by the formation of a mixed anhydride and subsequent amide formation to provide 29 kg of 31 [179, 180]. Having achieved access to the common precursor, the synthesis of the C1–C6 subunit 142 was completed in five steps in 66% yield on scales of several kilograms, only requiring chromatographic purification at the final stage [66]. The synthesis of the C9–C14 subunit 31 essentially followed an analogous route to Smith [46–48, 66]. Following TBS protection of 31, Red-Al was used in preference to DIBAL-H for the reduction to aldehyde 34. At this stage, introduction of the C13–C14 (Z)-vinyl iodide was required and, as expected from the work of Smith and Marshall, the Zhao-Wittig olefination gave only moderate yields of 29, limiting the scale of the reaction to a maximum of 2.5 kg substrate. The synthesis of the C15–C21 subunit 30 followed the Smith route [46–48, 67], beginning with the conversion of 31 into the intermediate aldehyde for the Evans aldol reaction with 33. In order to minimise the formation of by-products in this reaction, it was necessary to maintain the reaction below –10°C at all times and required a “rapid workup and isolation” to prevent epimerization at C16 which was readily observed at ambient temperatures [67]. The problems associated with epimerisation were negated by the subsequent silyl protection of 35. However, the reductive removal of the auxiliary with LiBH₄ led to the formation of numerous by-products requiring careful chromatographic purification of ca. 1-kg batches of crude material, prior to the formation of iodide 30.

As outlined in Scheme 37, the Novartis assembly of discodermolide began with the Marshall Suzuki-type cross-coupling of vinyl iodide 29 and iodide 30 to give 36 on a kilogram scale [68]. At this point, the crossover of routes required the introduction of the terminal (Z)-diene to give the Paterson intermediate 175 [55–57]. Following an analogous six-step sequence to that reported by the Paterson group, 810 g of the C7–C24 (Z)-enal 96 was prepared from 175. Notably, this only required two chromatographic purifications – after the Still-Gennari HWE reaction to introduce the (Z)-olefin at C8–C9 and following the final oxidation step. With substantial quantities of 96 available, the reagent-controlled boron aldol coupling with ketone 173 posed the largest challenge of the entire scale-up campaign [55–57, 69, 181]. In order to obtain reproducible yields of the desired aldol adduct 176, a number of limiting factors were identified, namely reagent quality and handling, work-up and isolation procedures. It was found that a commercial solution of (+)-Ipc₂BCl in hexane proved to be more reliable in terms of quality than the highly hygroscopic solid form and also conveniently negated the issue of handling the solid reagent. It was found that an oxidative work-up procedure should be avoided in favour of the direct purification of the crude product mixture by reverse phase chromatography, leading to the formation of 176 in 50–55% yield. In contrast, the introduction of the C7-stereocentre by Evans-Saksena reduction and global deprotection proved uneventful. Finally, reverse phase chromatography and crystallisation provided a 64-g batch of pure discodermolide monohydrate [69].
The Chemical Synthesis of Discodermolide

The Novartis Process Chemistry group’s preparation of discodermolide represents a landmark in the industrial synthesis of complex natural products and pharmaceutical development. Having taken the bold decision to pursue the clinical development of discodermolide under tight time constraints, they met the challenge of combining the approaches of Smith and Paterson and delivering sufficient quantities of active pharmaceutical ingredient to enable clinical trials.

Scheme 37  Novartis fragment assembly and completion of discodermolide
Summary

The various synthetic approaches developed to date have served to eliminate the supply problem for discodermolide, enabling extensive biological evaluation and early stage clinical development of this remarkable microtubule-stabilising anticancer agent. Discodermolide has inspired many creative and elegant total syntheses based on the application of contemporary methods of substrate and reagent based stereocontrol; these approaches are summarized chronologically in Table 1. Importantly, this shows that natural products with challenging molecular architectures can be accessed synthetically in an efficient and timely manner, and further underlines their resurgence as serious candidates for drug development [8]. Thanks to the power of modern organic synthesis, one can contemplate the practical synthesis of other such complex natural products where the natural supply is insufficient for detailed biological evaluation, let alone potential clinical application.

References

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