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Invited Review

Epothilone B and its Derivatives as Novel Antitumor Drugs: Total and Partial Synthesis and Biological Evaluation[a]

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Summary. Microtubule stabilizing natural products, as exemplified by paclitaxel (taxol®), are being considered as novel drugs against malignant therapy resistent solid tumors. Among these compounds, epothilone B and some of its derivatives have emerged as particularly promising candidates for industrial development. The total and partial syntheses of these compounds are described in detail, and some of the most important recent results on their biological activity are discussed.

Keywords. Epithilones; Macrolides; Antitumor drugs; Microtubule stabilizing agents; Total synthesis; Natural products.

General Background

The chemotherapy of cancer (Fig. 1) strongly depends on the nature of the individual tumor under consideration. For instance, tumors located in hormone dependent organs such as the ovaries, breasts, *etc*. are treated with antihormons, *e.g*. anti-estrogens or anti-androgens, which retard the growth of the individual tissue and thus also that of the tumor enclosed. In the regular case, however, the drug is intended to kill already existing tumor cells by damaging their *DNA* (mostly after intercalation) or by interrupting their mitotic cycle [1].

This aim is most efficiently achieved by addressing the so-called microtubles [2] (Fig. 2). Microtubules are dynamic structures within the cell that play a critical role in many cellular processes, one of their most important functions being the formation of the mitotic spindle which controls the movement of the chromatids throughout the cell division. Microtubules are made up of many individual protein subunits known as α- and β-tubulin (Fig. 2).

In the first step of the formation of microtubules, one α- and one β-subunit are joining to form heterodimers which polymerize to protofilaments. Subsequent

[a] Dedicated to Prof. Dr. *Günther Wulff* on the occasion of his 65th birthday

1. Hormone induced tumors: antihormones (tamoxifen, cyproterone acetate)
2. Cytotoxic drugs (chemicals of low molecular weight)
   2.1. Intercalation+*DNA*-damage (alkylation: CC-1065, double strand cross linking: *cis*-platin, oxidation: anthracyclinone, rupture: calicheamycin)
   2.2. Interference with mitotic cycle. Mitotic spindle cannot be formed due to:
      2.2.1. Destabilization of microtubules (colchicine, vinca alkaloids)
      2.2.2. Stabilization of microtubules (Paclitaxel)

Fig. 1. Chemotherapy of cancer

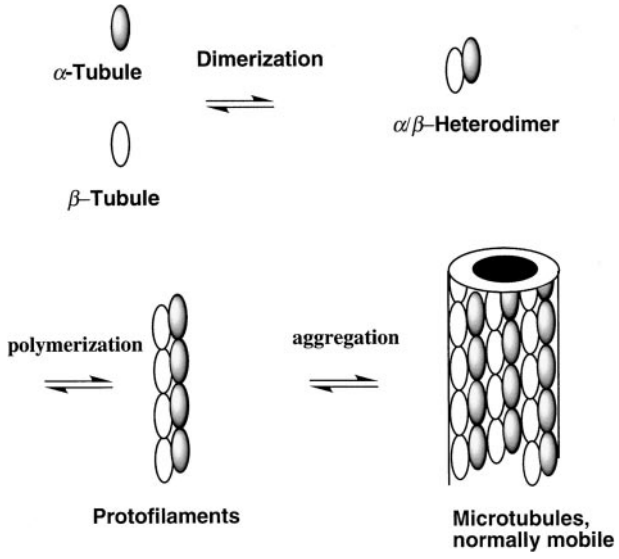
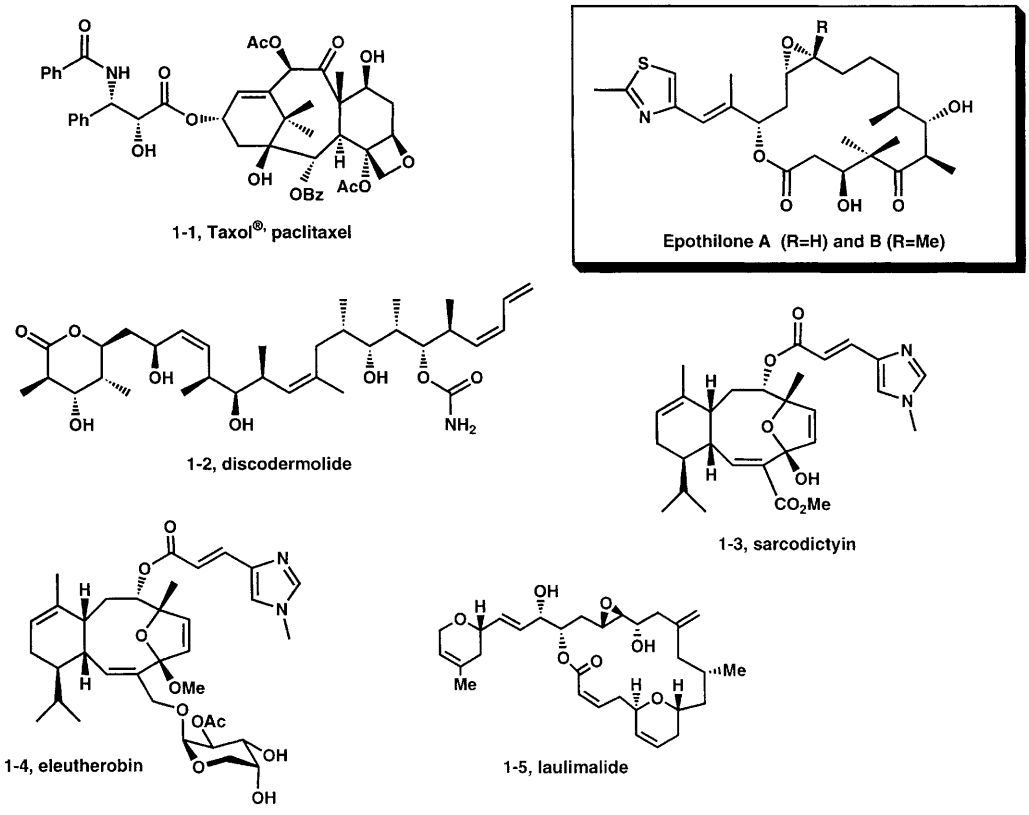


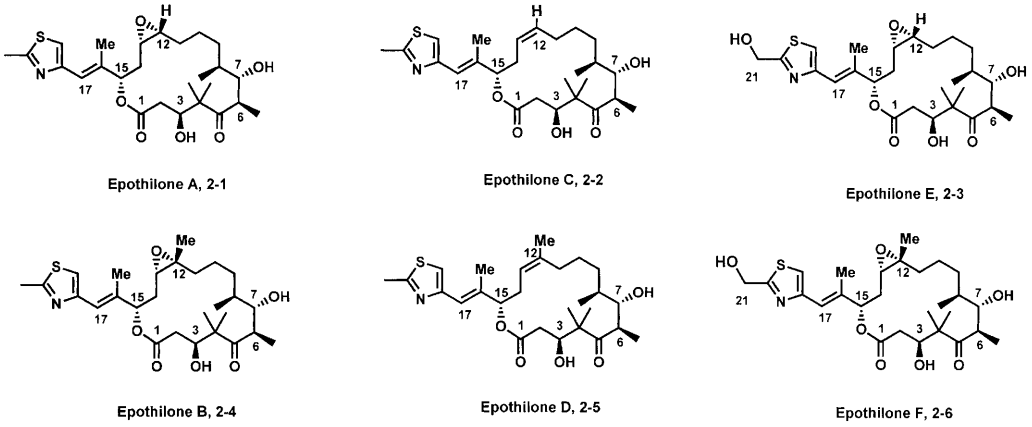
Fig. 2. Formation of microtubules

aggregation of these protofilaments leads to microtubules which are in a mobile equilibrium with the smaller fragments. If this equilibrium is disturbed, the mitotic cycle is interrupted. Alkaloids such as colchicine or vinblastine have been known for a long time to prevent the aggregation of the protofilaments. With the advent of paclitaxel (taxol®) (**1–1**, Scheme 1), however, a new mode of interaction was discovered. Paclitaxel stabilizes the microtubules by inhibiting their disaggregation. However, despite its impressive biological profile and its wide application (annual sales of about 1 billion USD), paclitaxel has turned out to be far from ideal for several reasons, *e.g.* multidrug resistance, poor bioavailability, and several serious side effects. However, its mode of action was considered as unique among all cytostatic drugs, until, quite sensationally, *Bollag* and coworkers discovered in 1995 [4a] that natural compound called epothilone not only binds to microtubules in a paclitaxel-like manner, but that it was much more active! Surprisingly, epothilone was not a new compound at that time; it had been isolated and structurally elucidated as a secondary metabolite from the soil myxobacterium



Scheme 1. Microtubule stabilizing natural products

Sorangium cellulosum by *Höfle* and *Reichenbach* and their coworkers at GBF in Braunschweig as early as 1987 [5]. Unfortunately, the screening at that time had been focused on pesticidal activity only. Epothilone, which turned out to be a mixture of epothilone A und B (**2**-**1** and **2**-**4**, Scheme 2), did show activity towards certain fungi, but was too toxic for any practical application. Another effect which was already detected at that time was the high cytotoxicity which, however, was not pursued any further. Epothilone thus shared the fate of Sleeping Beauty for about seven years, when, in the aftermath of the sensational success of paclitaxel, a general test for rapid detection of microtubule stabilizing substances (called "tubulin assay") was developed at Upjohn Company in 1994. About 140 000 compounds were screened with respect to their microtubule stabilizing effect, and they all proved inactive. It was in parallel tests at Merck, Sharp, and Dohme, however, that the above-mentioned hit [4a] was scored on testing an extract from *Sorangium cellulosum* containing epothilone A and B. In the tubulin assay, epothilone A turned out to be as active as paclitaxel, epothilone B was fifty times more active. These results were confirmed afterwards by a group from the National Cancer Institute [4b]. Later, several other natural products (**1**-**2** to **1**-**5**) were found with a similar microtubule stabilizing capability (Scheme 1) [6]. Nevertheless, however, the main interest continues to be concentrated on the epothilones, in particular epothilone B because it promises to have some significant advantages



Scheme 2. Naturally occurring epothilones

over paclitaxel and the other compounds depicted in Scheme 1. First of all, epothilone B is available in kg-quantities by fermentation. This is particularly important in considering that compounds **1**-**2** to **1**-**5** are all metabolites of marine organisms and can be isolated from the natural source only with great difficulty and in minute quantities. This excludes their practical applicability and limits the exploration of their bioprofile. Secondly, epothilone B is more soluble in water and has thus better galenic qualities than paclitaxel. Thirdly, it is still active on cells showing multidrug resistance. Fourthly, it exhibits similar toxicity towards various tumor cells (*e.g.* breast cancer, lung cancer), but acts more rapidly than paclitaxel does. Fifthly (and in our context most importantly), its molecular architecture is much simpler. This means that total synthesis of epothilone B and more potent unnatural derivatives is much more promising and practical than it is in the paclitaxel series. Meanwhile, the epothilone family comprises altogether six members (epothilones A-F, **2**-**1** to **2**-**6**, Scheme 2) which have all been isolated as metabolites from microbial sources.

On this general background, it was not surprising that soon after the exceptional biological properties of the epothilones were disclosed a race was started towards the first total synthesis, which mainly involved the well known US groups of *Danishefsky* and *Nicolaou* and the German team around *D. Schinzer.* All three competitors concentrated on the simpler epothilone A first. The first total synthesis of epothilone A was reported in late 1996 by *Danishefsky* [7], the other two groups followed a couple of weeks later [8,9]. The first total synthesis of epothilone B was completed by *Danishefsky* in 1997 [10]. Later on, several more syntheses of epothilone B were published [11].

It has become clear by a variety of biological tests (*vide infra*) that the epothilone B series (characterized by the 12-methyl group) contains the more promising candidates for further clinical development. This means that an efficient practical total synthesis of this class of compounds is essential to gain access to potent derivatives. Previous reviews [2,3] have dealt with many of the earlier aspects, in particular those regarding epothilone A and its derivatives. In the meantime great progress has been made with respect to epothilone B, which is now in the focus of

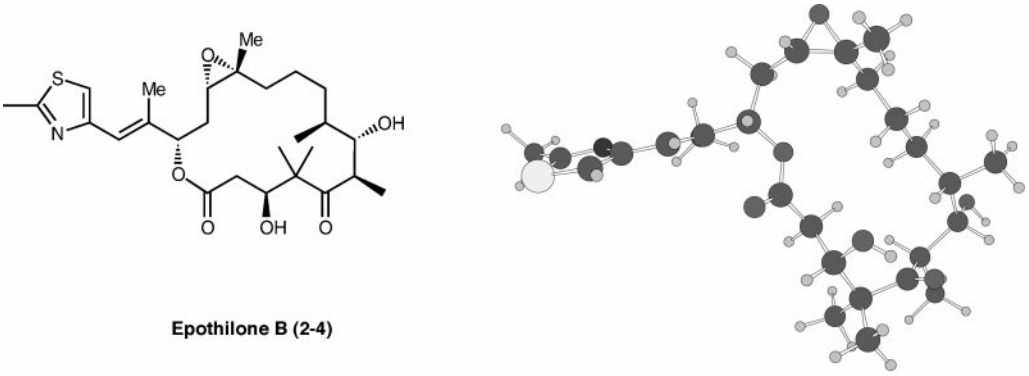


Fig. 3. Crystal structure of epothilone B

general interest. This review will therefore be restricted to the synthesis of the epothilone B family and the evaluation of their biological properties.

Structure and Conformational Behaviour of Epothilone B

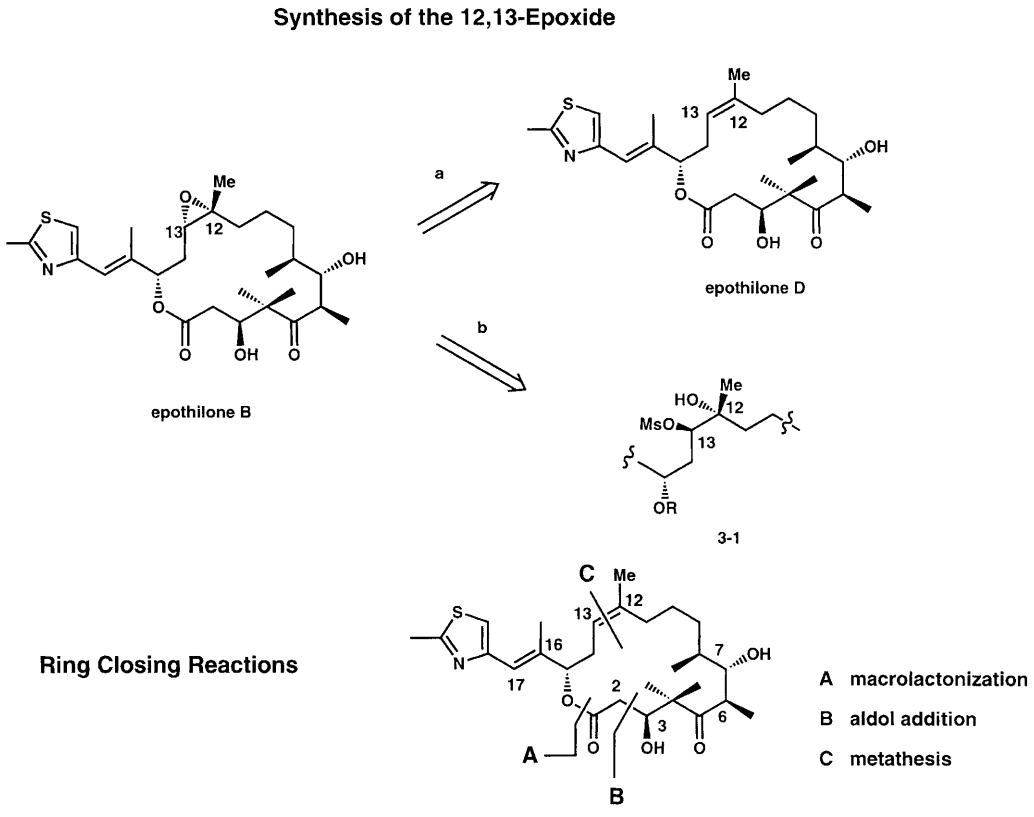
The structure of epothilone B in the solid state [5d] (Fig. 3) has been elucidated by single crystal diffraction. It reveals the structure of a 16-membered lactone, whose C1-C4-segment shows a zig-zag antiparallel arrangement including one of the 5-methyl groups. A second similarly zig-zag oriented segment is found at C7-C12. These two segments are arranged roughly parallel to each other and are crosslinked by the C13-C15-and the C5-C6-chains. From this box-like macrolide, three structural moieties project away and may thus act as anchor groups for the receptor: the C16-C17-thiazolalkylidene moiety, the 12,13-epoxide, and the 8-methyl group, and indeed these moieties are responsible for a high biological activity. Regarding the conformational behaviour in solution so far only epothilone A has been analyzed in detail [12] by NMR techniques and computational methods. According to these studies the C1-O15-C15-C14-C13-C12- and the C1-C5-parts are relatively rigid, whereas the C6-C11-section is flexible. Two stable conformers are found in a ratio of 4:1, the main conformer being very similar to the solid state conformation.

General Retrosynthetic Considerations

At first sight epothilone B does not look structurally complicated, in particular when compared to paclitaxel and the other compounds with microtubule stabilizing effects (Scheme 1). However, some considerations are important in designing a suitable strategy (Scheme 3).

1. Introduction of the 12,13-epoxide

This is the central issue of the entire synthesis. There are two basic options: *1.1*. The epoxide is generated by epoxidation of the corresponding olefin (epothilone D). This



Scheme 3. Basic transformations in epothilone B total synthesis; epoxide formation and ring closure

strategy has to cope with the problem of chemoselectivity (the 16,17-olefin must not be affected) and stereoselectivity (a (*Z*)-double bond has to be generated and to be epoxidized diastereoselectively from the α-face). In fact, the solid state structure of epothilone B indicates that in epothilone D the α-face should be more accessible that the β-face. Additionally, a 12,13-olefin is a suitable functional entity for connecting two major fragments (C1-C12 and C13-21) by *Wittig* type olefinations.

1.2. A second option is to generate the epoxide from a diol intermediate **3-1** by an intramolecular S_N2-displacement reaction of a 13-mesylate. The regioselectivity of the mesylation is controlled by the substitution of the OH-functions (13-OH is tertiary and 12-OH is secondary). The more difficult problem, however, is the stereocontrolled generation of a 12(*R*),13(*R*)-diol moiety, such as in intermediate **3-1**.

2. Question of ring closure

The most obvious ring closure method is that achieved by macrolactonization. In view of the highly sensitive allylic and homoallylic 15-OH-function, a carboxyl group activated lactonization is indicated which proceeds under retention of the configuration at C15. Other plausible ring closures would imply ring closing metathesis (RCM) [13] (12,13-double bond), aldol-type connections (between C2-C3,

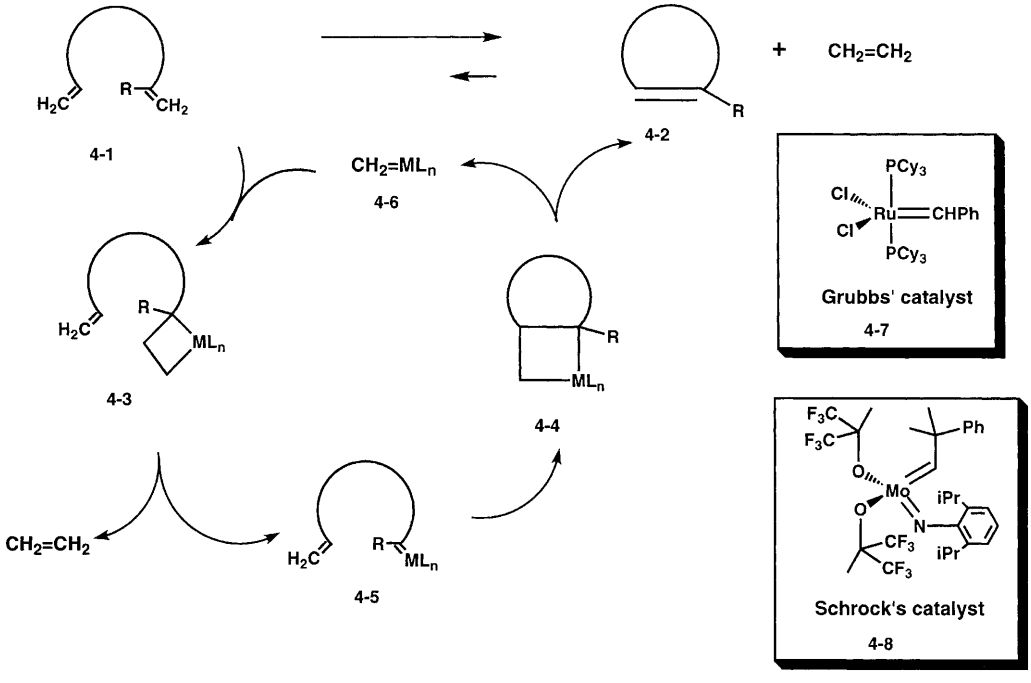
C3-C4, or C6-C7) or *Wurtz* type sp^3-sp^3-coupling (*e.g.* between C-9-10). So far only the ring closures A–C shown in Scheme 3 have actually been performed. RCM has received particular attention, the currently accepted mechanism of which [13] is outlined in Scheme 4; the crucial intermediates are the metal carbenes **4-3** and **4-6** from which the metallacyclobutanes **4-3** and **4-4** are generated. The driving force to shift the equilibrium towards the product is the evolution of gaseous ethylene. Two catalysts (**4-7** [14] and **4-8** [15]) have been introduced; the *Grubbs* catalyst is more stable than the *Schrock* catalyst; however, for the formation of trisubstituted olefins such as in epothilone D the much more active *Schrock* catalyst has to be used.

3. Stereocontrol

Altogether 7 stereogenic centers have to be installed with defined configuration, in addition to two double bonds. To achieve this there are two basic options [16]: *3.1.* asymmetric induction by chirality transfer from chiral auxiliaries or chiral catalysts or *3.2.* incorporation of stereogenic centers from the chiral carbon pool. The latter option has the advantage that the absolute configuration and the optical purity are guaranteed.

4. Linear or convergent synthesis, number of steps

A convergent approach appears indicated, especially as the disconnections are obvious (aldol-type additions between C6-C7, C3-C4, and C2-C3. *Wittig* olefinations between C12-C13 and C16-C17, addition of a C14-C13-C12-allylmetal to a C15-



Scheme 4. Mechanism of ring closing metathesis (RCM)

aldehyde). The fragments should be so chosen that the total number of steps should not exceed 20–25 (as a rule of thumb, a good synthesis should require no more than 2–3 steps for each stereogenic center and/or functional group in the molecule.

Individual Syntheses

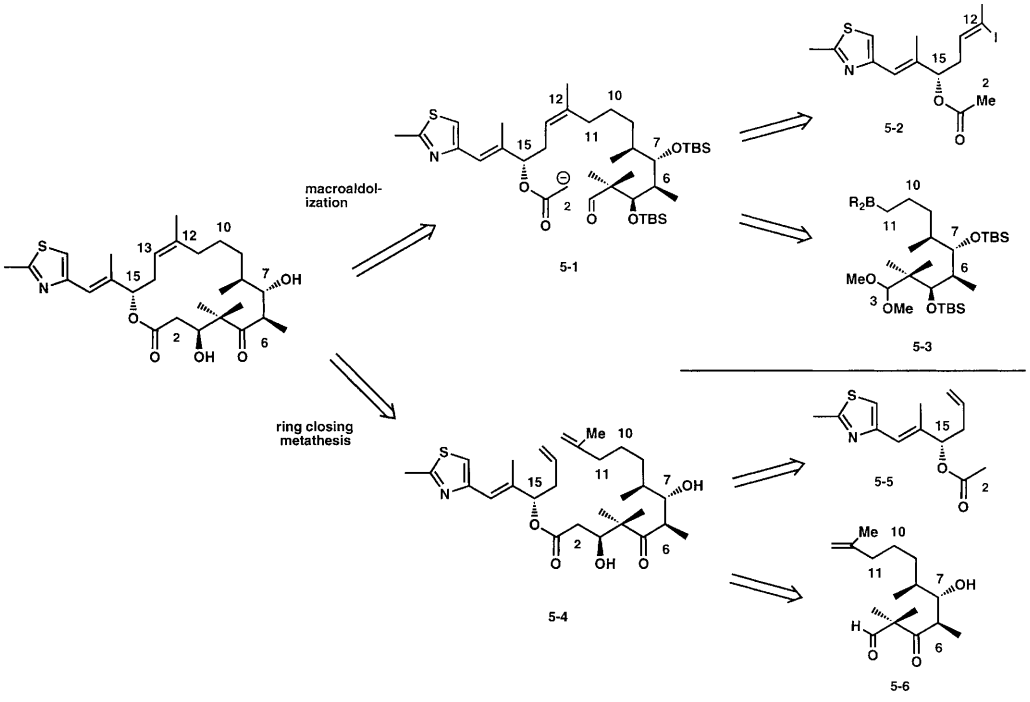
Syntheses Nos. 1 and 2 (Danishefsky)

Danishefsky and his group were the only ones to test all three ring closing processes shown in Scheme 3. Their first two syntheses [7,10,11b] were based on RCM and macroaldolization as outlined in Scheme 5.

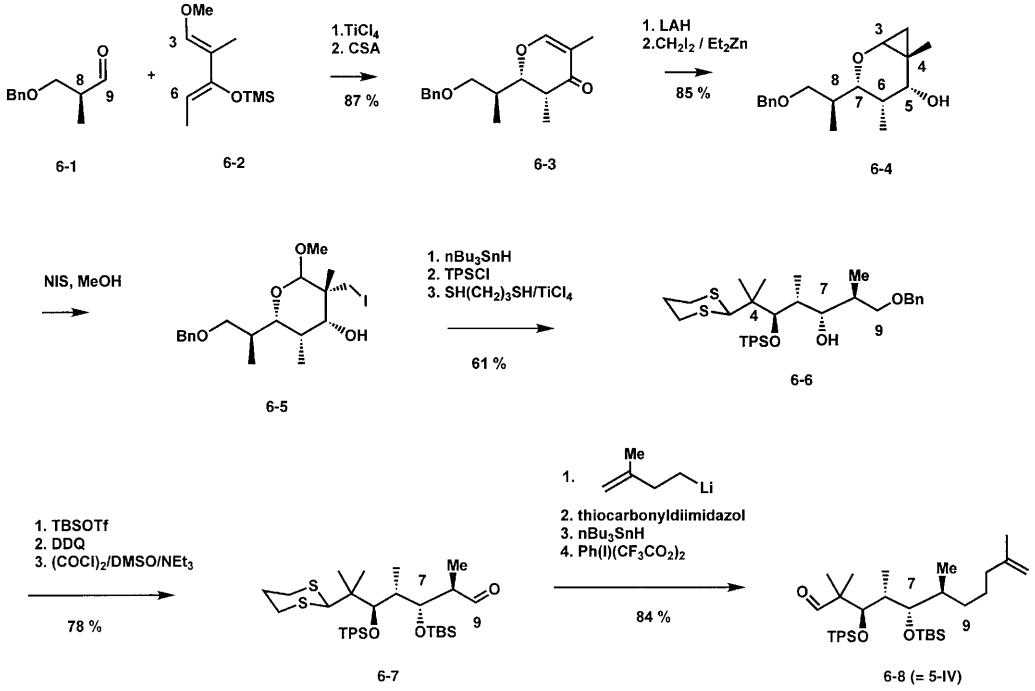
The macroaldolization was designed to close the C2-C3-bond *via* intermediate **5-1** by generating a C2-ester enolate in the presence of a nonenolizable sterically hindered C3-aldehyde.

Intermediate **5**-1 is assembled *via* a C11-C12 *Suzuki* coupling [17] of the vinyl iodide **5-2** with organoborane **5-3**. On the other hand, the RCM was performed with a diolefin **5-4** which was prepared by the same aldol-type addition as described before, however in an intermolecular fashion, using ester **5-5** and aldehyde **5-6**. This makes clear that the molecular fragments used in both approaches are quite similar and, hence, exchangeable. Thus, **5-3** and **5-6** are prepared by analogous methodology (Scheme 6).

The common intermediate is **6-7** which is procured *via Danishefsky*'s general polyketide strategy [18] centered around a hetero-*Diels-Alder* addition of the chiral



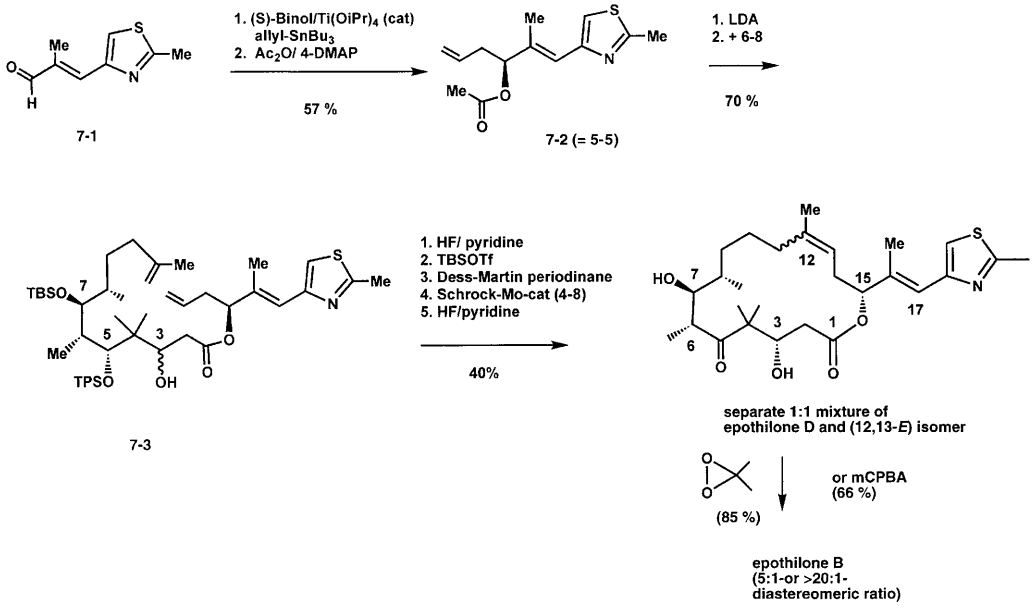
Scheme 5. Overview of *Danishefsky*'s first total syntheses of epothilone B



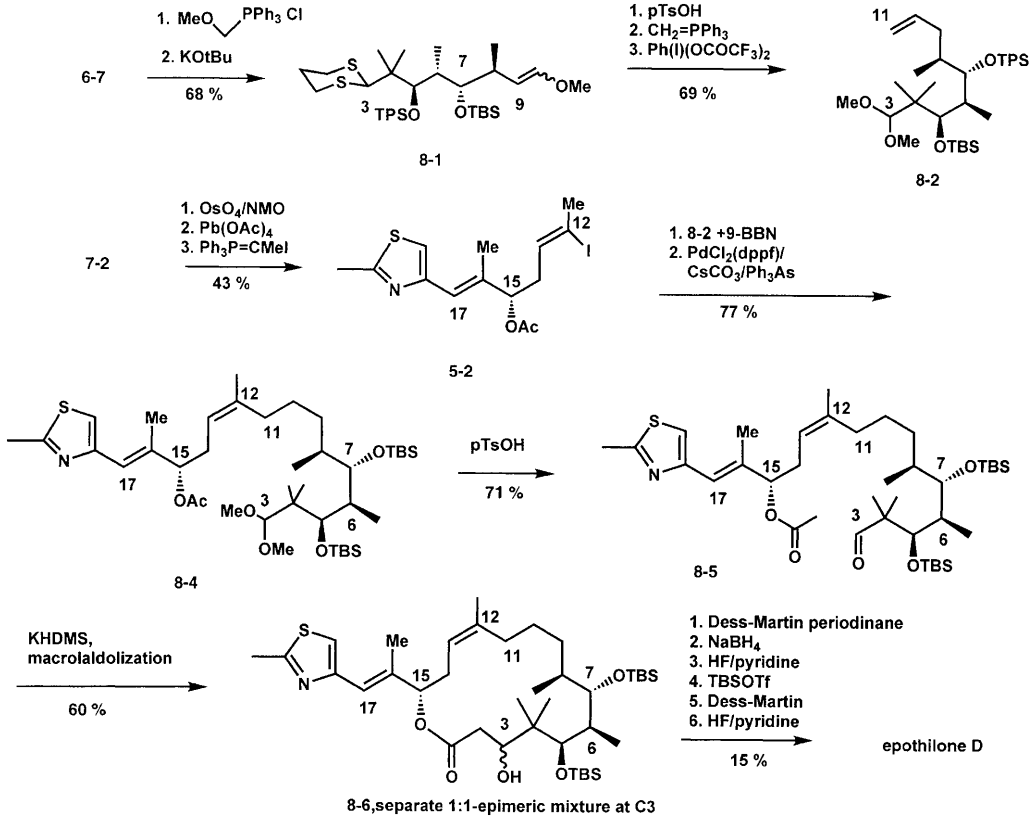
Scheme 6. *Danishefsky*'s hetero-*Diels-Alder* approach to the C3-C12 fragment

aldehyde **6-1** to diene **6-2**. With good stereoselectivity adduct **6-3** is formed which *via* routine steps furnishes the C3-C9-precursor **6-7**. The metathesis chain elongation is achieved by organometallic addition to the C9-aldehyde and subsequent reduction of the 9-hydroxyl function to yield the olefinic aldehyde **6-8**. The synthesis of fragments **5-2** and **5-5** starts with the aldehyde **7-1** (Scheme 7) which is subjected to an asymmetric *Keck-Umani-Ronchi*-allylation [10] to form, after acylation, **7-2** which is connected with aldehyde **6-8** *via* an aldoltype addition to generate a 1:1-C3-epimeric mixture of diolefin **7-3**. Functional group adjustment generates the 5-carbonyl group, and this substrate is subjected to RCM with the *Schrock* catalyst **4-8** to furnish a 1:1-mixture of epothilone D and its (*E*)-isomer after desilylation. The chemoselective epoxidation of the 12,13-double bond is achieved by dimethyldioxirane to generate the desired α-epoxide (= epothilone B) with α/β-stereoselectivity of > 20:1 (*Nicolaou et al.* report a 5:1-α/β-ratio under the same conditions [11a]). *m*-Chloroperbenzoic acid is similarly chemoselective at $-15°C$, but inferior with respect to stereoselectivity. If applied at room temperature it also generates the N-oxide (Scheme 32). The epoxidation of epothilone D as the last step in an epothilone B synthesis has been adopted by all syntheses cited in Ref. [11].

For the macroaldolization approach (Scheme 8), aldehyde **6-7** is converted to the enol ether **8-1** *via* a *Wittig* reaction. Further functional group manipulation leads to olefin **8-2** which is the precursor of the *in-situ*-organoborane **5-3**. The second component of the *Suzuki* coupling [17], vinyl iodide **5-2**, is prepared (*Z*)-selectively from aldehyde **7-2** in three steps. The coupling of **5-2** and **5-3** furnishes **8-4** under retention of the (*Z*)-olefin geometry. Deketalization gives the aldehyde **8-**



Scheme 7. *Danishefsky*'s RCM approach to epothilone B



Scheme 8. *Danishefsky*'s macroaldolization approach to epothilone B

5, whose macroaldolization furnishes a 1:1-C3-epimeric mixture of macrolide **8-6** which is converted into epothilone D (and eventually epothilone B).

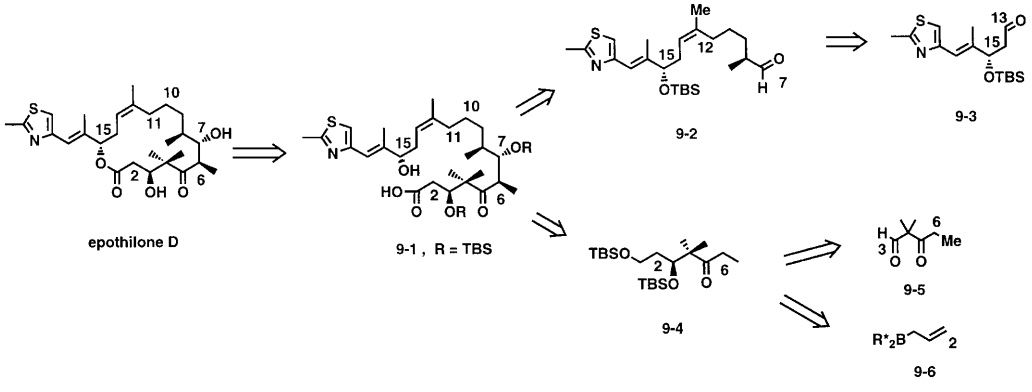
Synthesis No. 3 (Nicolaou)

Very soon after *Danishefsky* the *Nicolaou* group was successful with an entirely different strategy centered around a macrolactonization as the ring forming step [8,11a]. A C6-C7-aldol-type addition was used as the key CC-connecting operation of the synthesis. This aldol-type reaction has been first described by our group [23b] (Scheme 19) and has been applied in a related form both by *Nicolaou* and *Schinzer* [10] in their syntheses of epothilones D and B. In the meantime, all syntheses of epothilones utilize this aldol-type addition for connecting the C6-C7-bond. *Nicolaou*'s retrosynthetic planning is shown in Scheme 9.

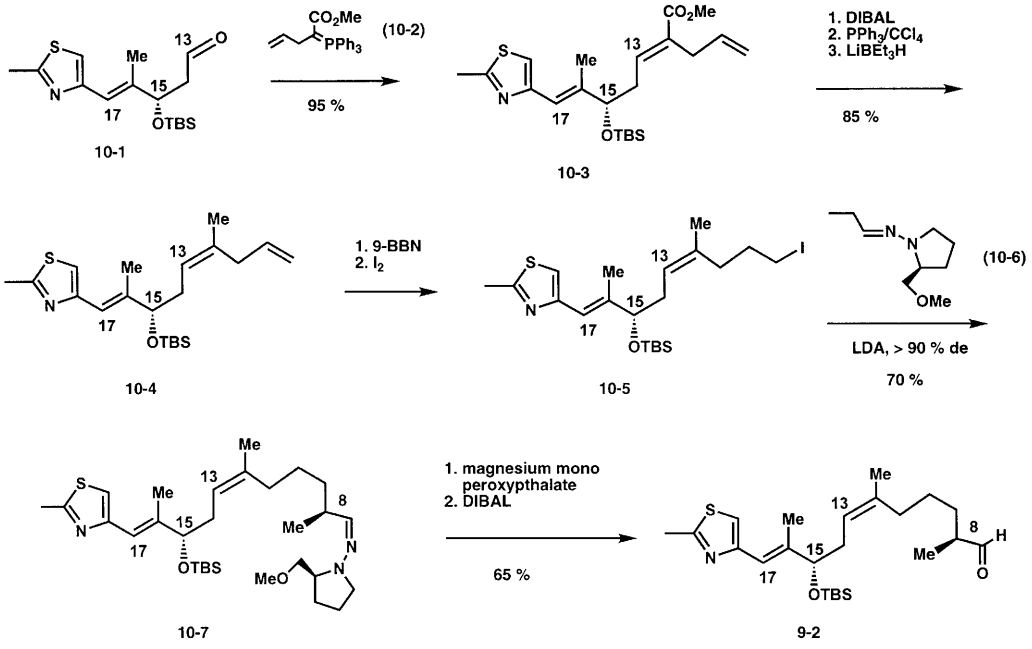
Macrolactonization is performed on seco acid **9-1**, which is assembled by aldol-type addition of ketone **9-4** to aldehyde **9-2**. Quite straightforwardly, **9-4** is prepared *via* a *Brown* allylation [20] of aldehyde **9-5**, whereas **9-2** is generated from aldehyde **9-3** *via* an (*E*)-selective *Wittig* olefination. The synthesis of aldehyde **9-2** is outlined in Scheme 10.

It starts from aldehyde **10-1** which is prepared analogously to Danishefsky's intermediate **7-2** except for a *TBS* ether instead of an acetyl as the C15-O-protective group. The ensuing *Wittig* reaction has to be performed with the stabilized ylide **10-2** in order to exert stereocontrol on the olefin formation. With unstabilized ylides the yield is much lower and (*Z*/*E*)-mixtures are obtained. The ester group in **10-3** is converted into a methyl group, and the terminal olefinic unit in triolefin **10-4** undergoes a chemoselective hydroboration-iodination sequence to give iodide **10-5** which is subjected to an *Enders* alkylation [21] with the *SAMP*-hydrazone **10-6**. Highly diastereoselective CC-elongation is achieved to afford **10-7** which is converted into the desired aldehyde **9-2** *via* the nitrile.

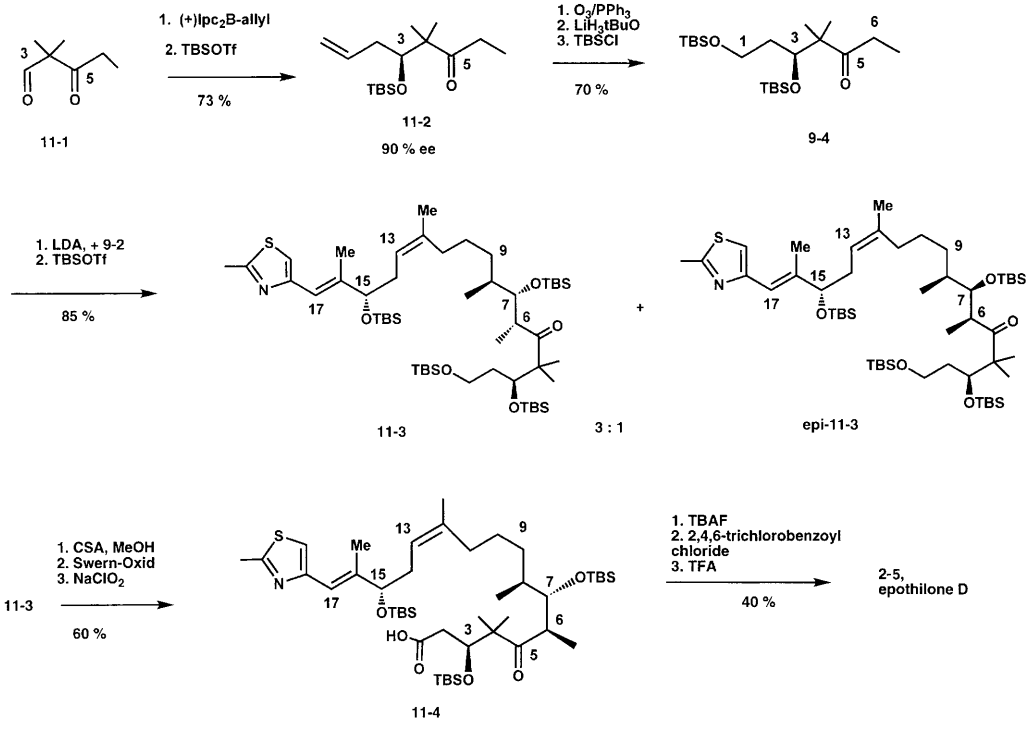
The preparation of ketone **9-4** (Scheme 11) starts with a *Brown* allylation [20] of the known ketoaldehyde **11-1** to form **11-2** which is converted into **9-4** in three simple steps. The aldol addition with aldehyde **9-2** leads to a 3:1-mixture of **11-3** and its (6*S*,7*R*)-epimer. The major diastereomer is converted into seco acid **11-4**



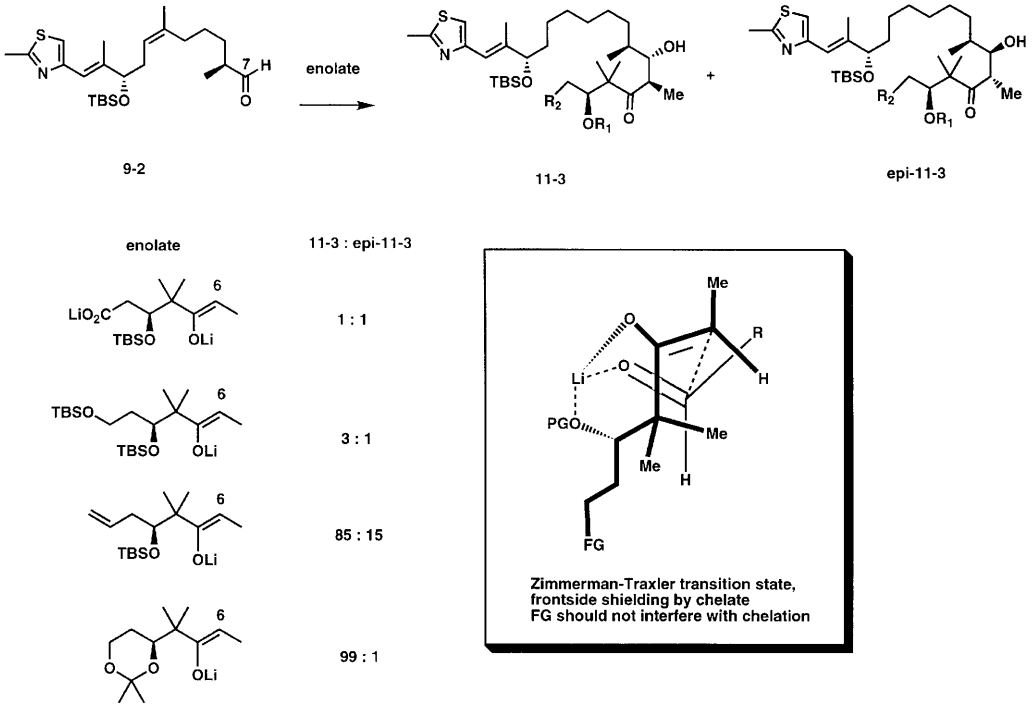
Scheme 9. Overview of *Nicolaou*'s macrolactonization approach to epothilone B



Scheme 10. *Nicolaou*'s synthesis of key aldehyde **9-2**



Scheme 11. *Nicolaou*'s aldol addition-macrolactonization approach to epothilone B



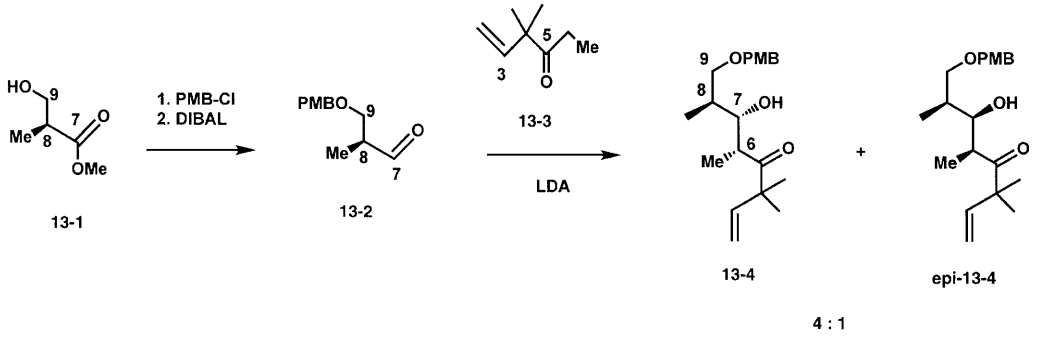
Scheme 12. Rationalization of the stereochemical course of the key aldol addition

which is converted into epothilone D by *Yamaguchi* macrolactonization [22]. The endgame of this synthesis is notable for the successful differentiation between the four O*TBS* protective groups in **11-3**. The primary 1-O*TBS* group is removed first with camphorsulfonic acid, the O-15-*TBS* group is removed with tetrabutylammonium fluoride after the generation of the C1-carboxylic acid. On the way from **11-4** to epothilone D the O-7-*TBS* group is removed frist with trifluoroacetic acid, whereas the removal of the O-3-*TBS* group requires prolonged treatment.

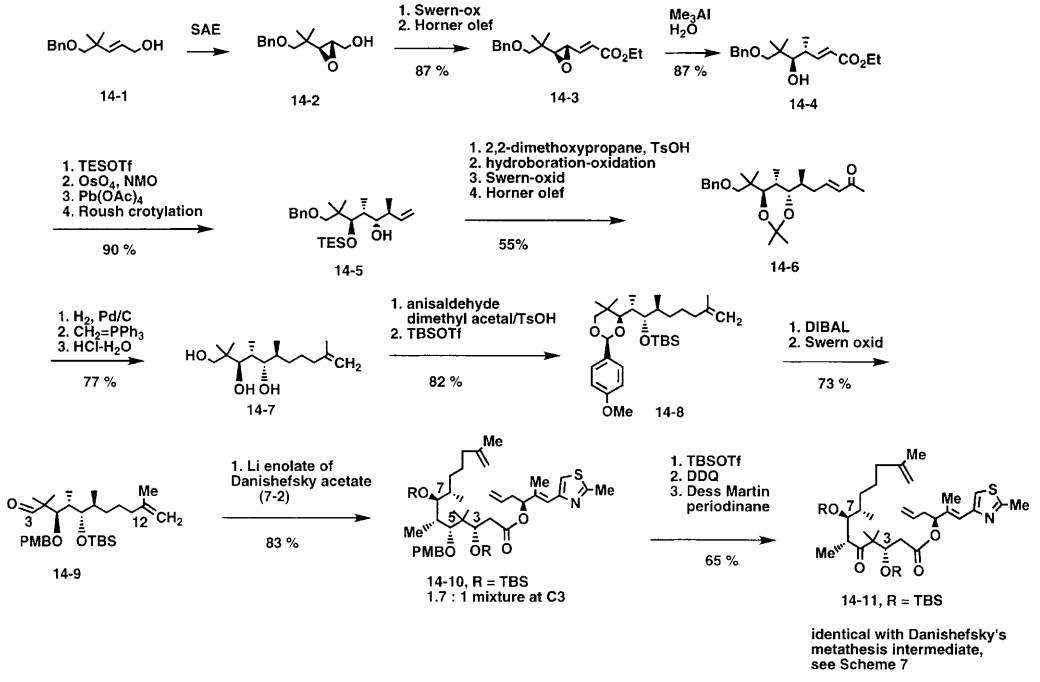
The stereoselectivity of the aldol-type addition has traditionally been interpreted [23a] in terms of the *Zimmerman-Traxler* transition state model shown in Scheme 12 which also demonstrates that the facial selectivity, *i.e.* the ratio of **11-3** to *epi*-**11-3**, strongly depends on the substituents of the keto component, in particular those at C1 and C3. A chelate has been postulated to explain this. However, our initial study [23b] (Scheme 13) was performed with the achiral nonchelating keto component **13-3**. In this case a 4:1-ratio of **13-4** and *epi*-**13-4** was obtained. It thus appears that the chelate postulated in Scheme 12 is not so important; rather, it may be the 6-methyl substituent in the aldehyde component that is primarily responsible for the facial selectivity.

Synthesis No. 4 (Grieco)

Grieco [11c] (Scheme 14) presented a formal total synthesis of epothilone B based on *Danishefsky*'s RCM strategy (Scheme 7).

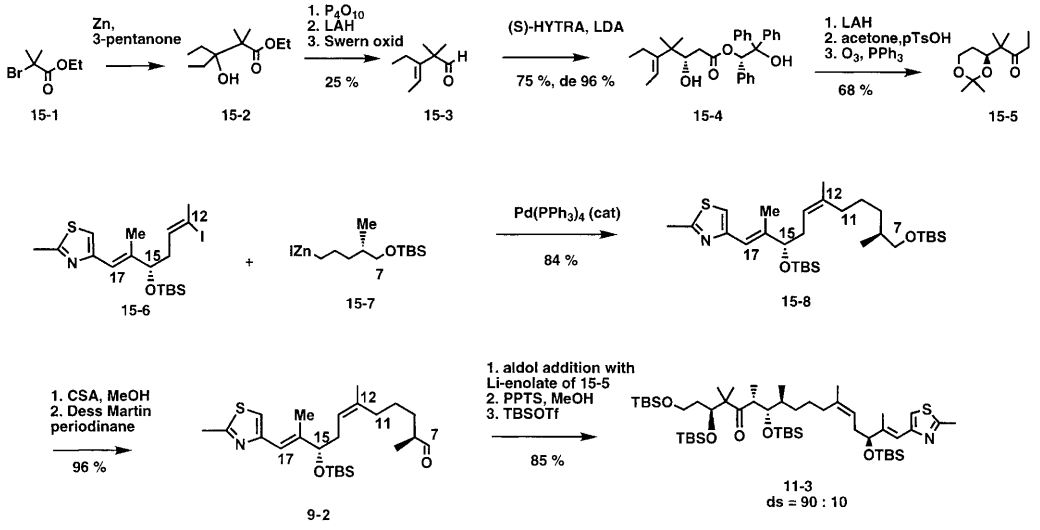


Scheme 13. Aldol addition by *Mulzer-Mantoulidis* [23]



Scheme 14. *Grieco*'s formal RCM synthesis of epothilone B

The innovation lies in the preparation of the C3-C13 fragment **14-9** which is achieved in a straightforward stereoselective manner starting out with the Sharpless asymmetric epoxidation (SAE) [24] of the known allylic alcohol **14-1** to give **14-2** with unspecified optical purity. After chain elongation to enoate **14-3** an interesting regio- and stereocontrolled epoxide opening with trimethylaluminum furnishes adduct **14-4** which after oxidation to the aldehyde is subjected to a *Roush* crotylation [25] to give isomer **14-5** as the sole product (matched case in the *Roush* procedure!). Acetonide protection of the 1,3-diol and chain elongation furnishes the unsaturated ketone **14-6** which is transformed into olefin **14-7**. Protective group manipulations are required to generate aldehyde **14-9** which is subjected to an aldol addition with *Danishefsky*'s acetate **7-2** to give adduct **14-10** as an epimeric mixture at C3. Further adjustment of the functional group furnishes diolefin **14-11**



Scheme 15. *Schinzer*'s synthesis of *Nicolaou*'s macrolactonization key intermediate

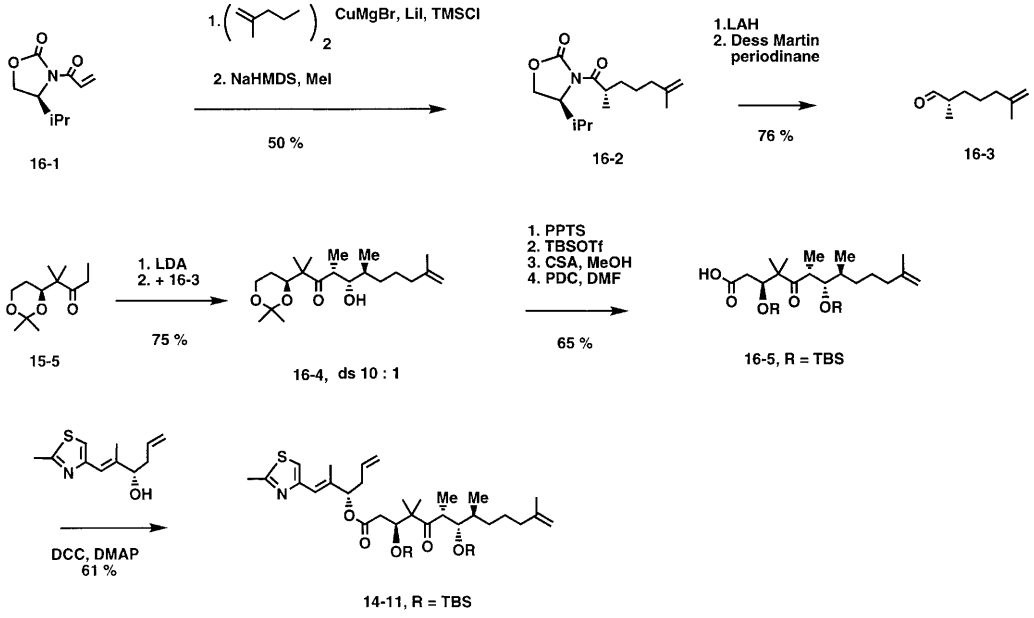
which is identical with the intermediate in *Danishefsky*'s synthesis (Scheme 7). Altogether, *Grieco*'s synthesis of **14-11** requires 23 steps compared to 19 steps in *Danishefsky*'s route.

Synthesis Nos. 5 and 6 (Schinzer)

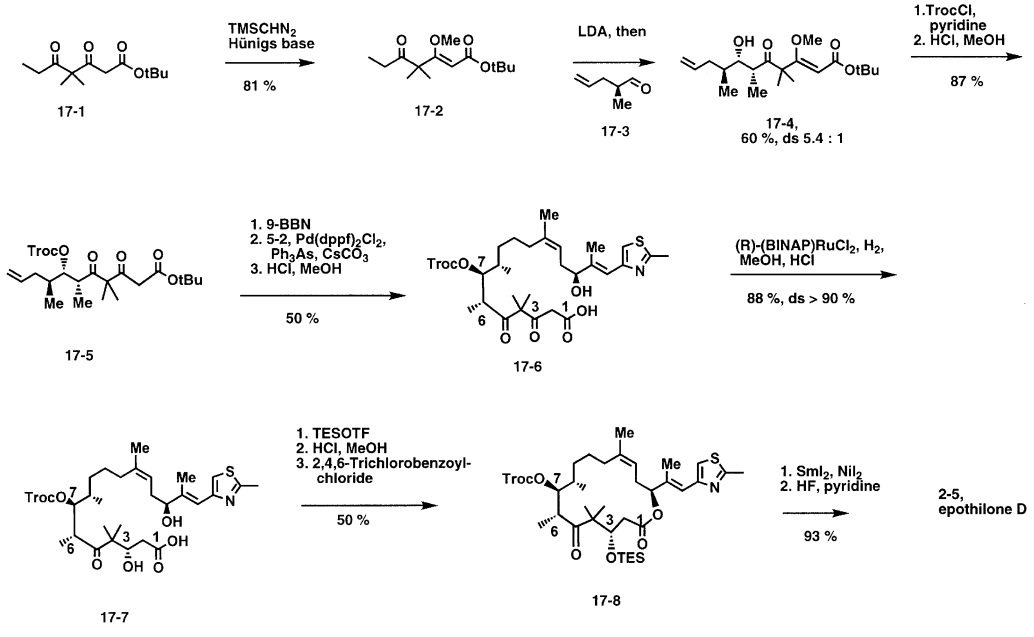
Schinzer [11d] described a macrolactonization (Scheme 15) and a RCM (Scheme 16) approach to epothilone B. The crucial CC-connecting step in both approaches is the familar aldol-type addition of a C6-enolate to a C7-aldehyde. The C1-C6-component **15-5** is prepared from bromoester **15-1** which is converted into the olefinic aldehyde **15-3** *via* a *Reformatsky* reaction. The stereogenic center at C3 is generated *via Braun*'s HYTRA methodology [26] which furnishes hydroxyester **15-4** in high optical purity. Three straightforward steps lead to **15-5**. The aldehyde component **9-2** is identical with that used by *Nicolaou* and is prepared in close analogy to *Danishefsky*'s procedure, using an organometallic CC-coupling of the vinyl iodide **15-6** with the organozinc component **15-7**. Aldol coupling proceeds with an exceptionally high diastereocontrol to furnish *Nicolaou*'s intermediate **11-3** which has already been converted into epothilone B by macrolactonization. In the RCM variation, **15-5** is connected with aldehyde **16-3** by a highly diastereoselective aldol addition to give **16-4** which is concerted into acid **16-5**. Esterification with the known [10] chiral alcohol **16-6** furnishes the *Danishefsky-Grieco* RCM intermediate **14-11**.

Synthesis No. 7 (Danishefsky)

In their latest approach [11e] the *Danishefsky* group have also used the aldol-type connection between C6 and C7 (Scheme 17) in a very direct approach. The ketoester **17-1** serves as an achiral version of the C1-C6-fragment. After conversion into the enol ether **17-2**, an aldol addition is performed with aldehyde **17-3** to give adduct **17-**



Scheme 16. *Schinzer*'s synthesis of *Danishefsky*'s RCM key intermediate

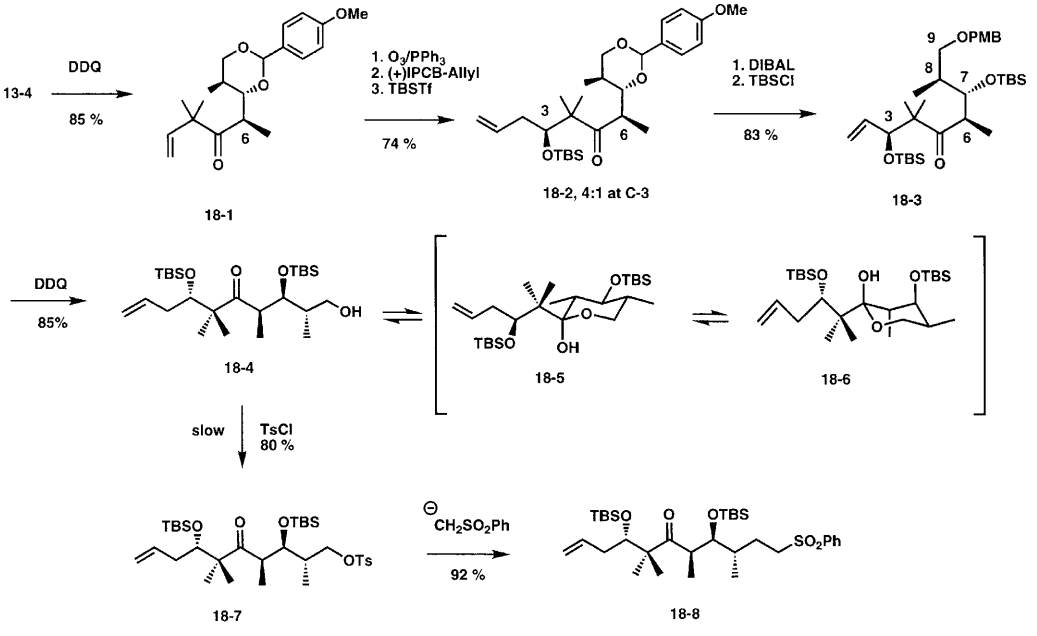


Scheme 17. *Danishefsky*'s aldol addition-macrolactonization approach to epothilone B

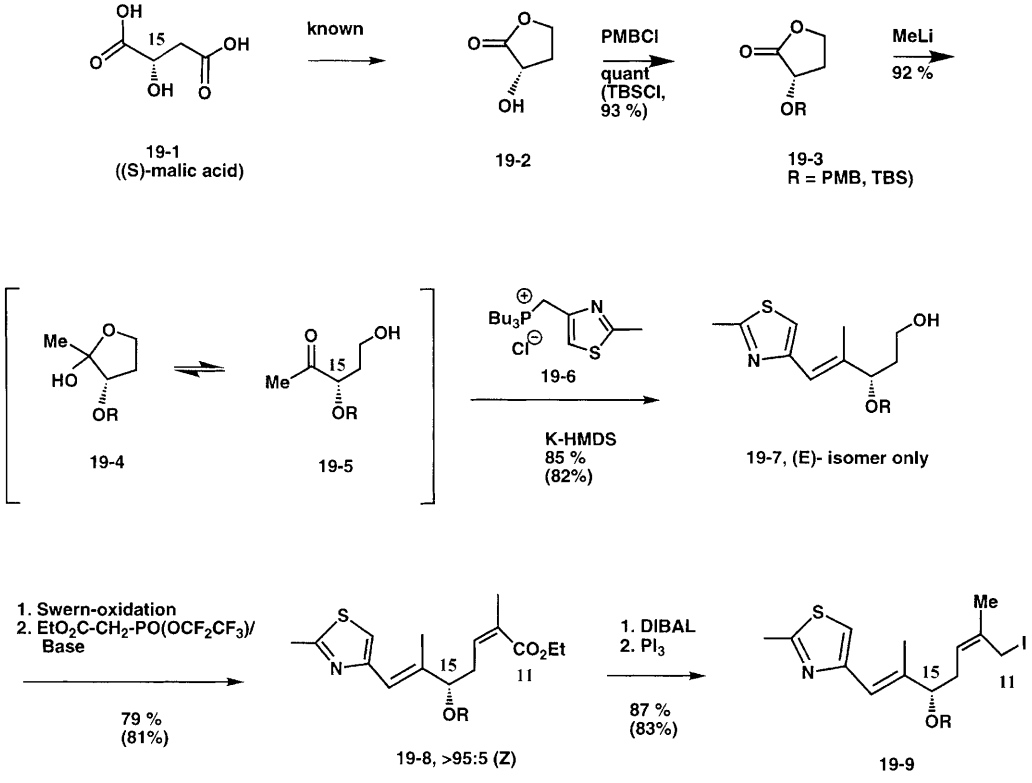
4 with acceptable stereoselectivity. 7-OH-protection and chain elongation with vinyl iodide **15**-**6** as before furnishes the tricarbonyl compound **17**-**6** which is subjected to a chemo- and stereoselective *Noyori* hydrogenation [27] of the C3-carbonyl to give **17**-**17** which is O-silylated and cyclized to macrolide **17**-**8**. Deprotection leads to epothilone D.

Synthesis No. 8 (Mulzer 1)

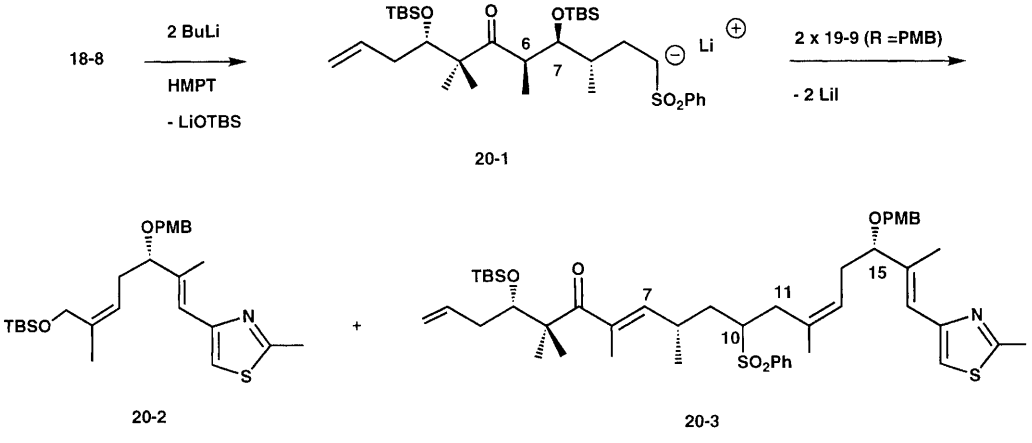
Mulzer's first approach [28] (Schemes 18–22) is based on the alkylation of sulfone **18-8** with allyl iodide **19-9** to form the carbon skeleton of epothilone D. The preparation of **18-8** starts from the C3–C9-intermediate **13-4** [23] which is converted into the cyclic acetal **18-1** and oxidized to the C3-aldehyde. *Brown* allylation gives **18-2** with 4:1-diastereoselectivity at C3. Readjustment of the protective groups leads to hydoxy ketone **18-4** which is in equilibrium with the cyclic acetals **18-5** and **18-6**. Tosylation of the primary hydroxyl function in **18-4** proceeds slowly to furnish tosylate **18-7** which is alkylated with the anion of methylphenyl sulfone to give **18-8**. The allyl iodide **19-9** is prepared from (*S*)-malic acid (**19-1**) *via* the lactone **19-3** and the methyl ketone **19-5** *Wittig* reaction with the tributylphosphonium ylide **19-6** furnishes the (*E*)-olefin **19-7** selectively. *Swern* oxidation, followed by a (*Z*)-selective *Still-Gennari-Horner* olefination [29] generates ester **19-8** which is converted into **19-9** eventually. On deprotonation with butyllithium, **19-8** presumably undergoes an elimination of LiO*TBS* from the 6,7-positions to form **20-1**. After a second deprotonation with butyllithium **20-1** adds **19-9** to form **20-3**. A second equivalent of **19-9** is consumed by an S_N2 reaction with LiO*TBS* to form **20-2**. This failure obviously results from the 1,5-arrangement of the carbanion and an acidic 6-H in **20-1**. So the plan was changed to the effect to alkylate **19-9** with the smaller carbonyl free sulfonyl fragment **21-2** which is generated from the *TBDPS*-protected *Roche* ester. Alkylation with **19-9** gives **21-3** in good yield. Reductive desulfonation and oxidation of the terminal primary alcohol furnishes aldehyde **21-4**, identical with Nicolaou's aldehyde **9-2** except for the *PMB* protective group. Aldol addition with the enolate of ketone **9-4** affords adduct **22-1** which is oxidized to carboxylic acid **22-2**. However, all



Scheme 18. *Mulzer*'s unsuccessful sulfone alkylation approach



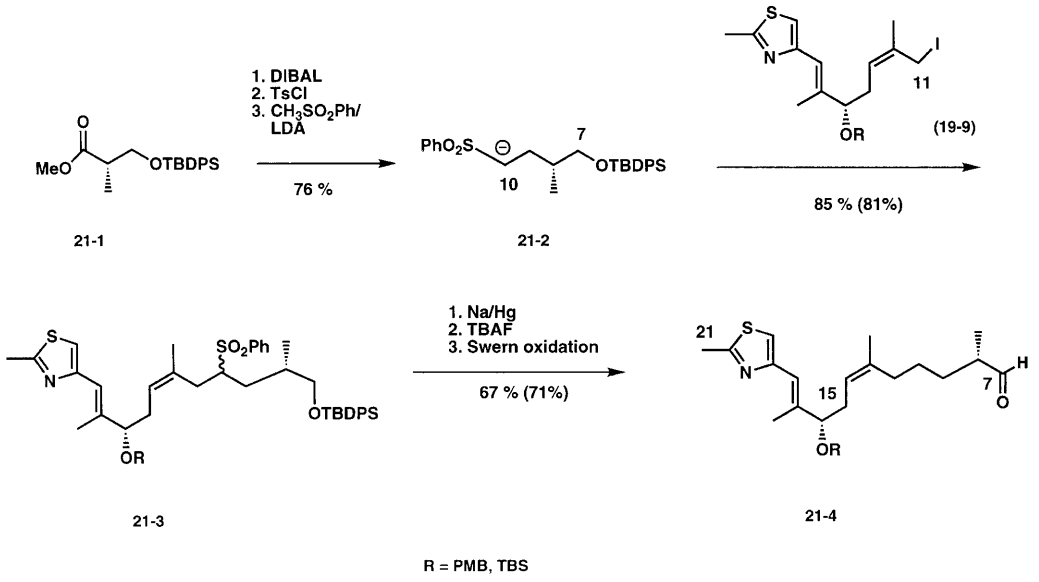
Scheme 19. *Mulzer*'s synthesis of the key C21-C11-allyliodide fragment



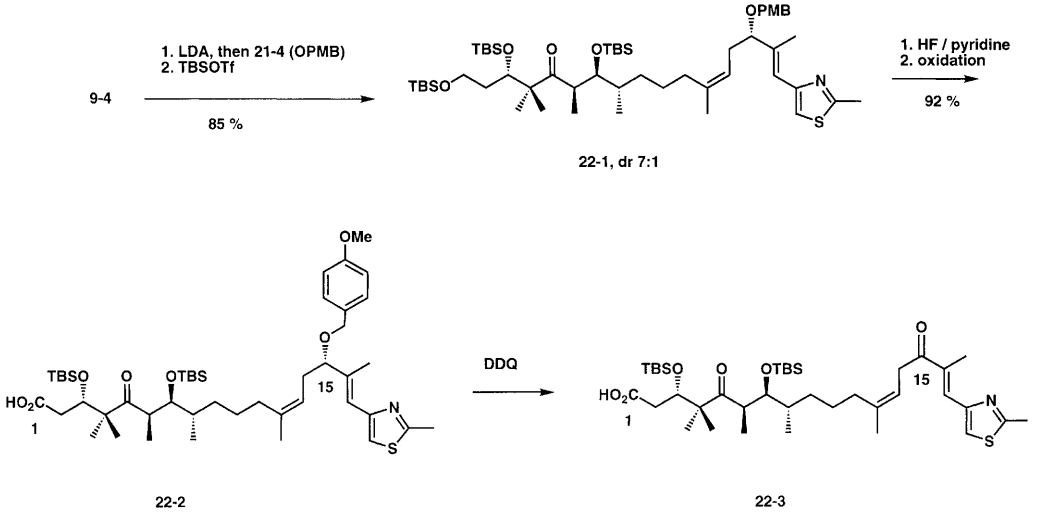
Scheme 20. Unsuccessful Sulfone Coupling

attempts to remove the 15-O-*PMP* group from **22-2** to get the seco acid failed. *DDQ* oxidation, for instance, leads to the 15-ketone **23-3**.

Thus, the overall sequence was repeated [11f] with a 15-O*TBS* instead of the unsuitable *PMB* protective group *via* the same intermediates as described previously (Schemes 19 and 21, yields in parentheses). Additionally, a novel synthesis of the C1-C6-fragment **9-4** was developed following a procedure described by *Kiyooka et al.* [30] (Scheme 23).

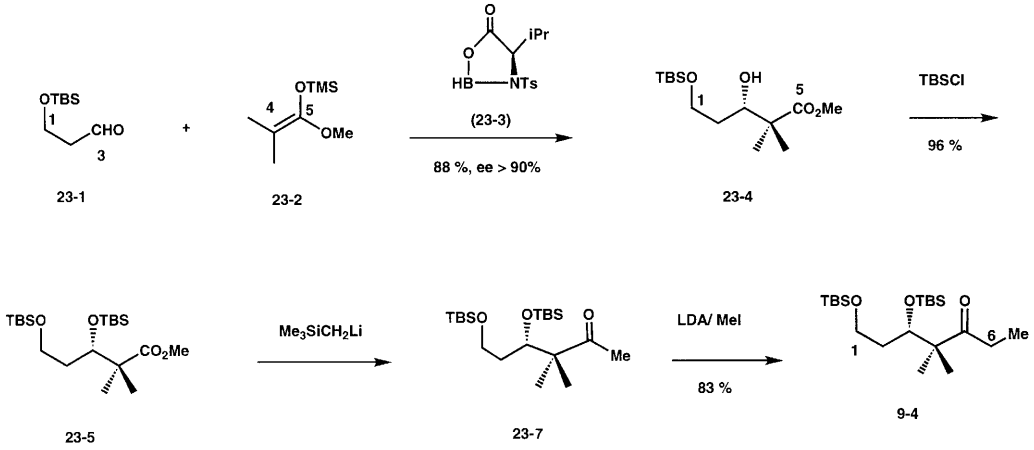


Scheme 21. Successful sulfone coupling generates the C21-C7-key aldehyde **21-4**

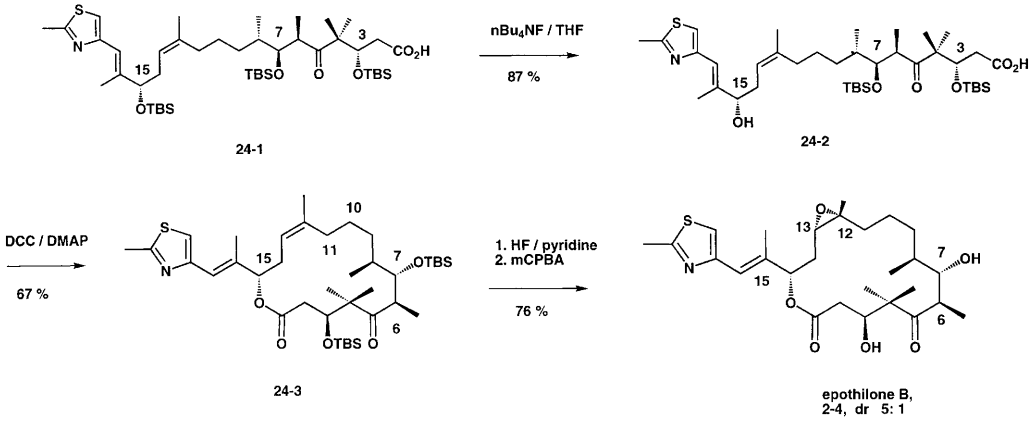


Scheme 22. Endgame fails due to Unsuitable 15-O*PMB* protecting group

To this end, aldehyde **23-1** is treated with ketene acetal **23-2** in the presence of borane **23-3**, easily prepared *in situ* from N-*Ts-D*-valine and diborane. Hydroxy ester **23-4** is formed in high optical purity. Unfortunately, the addition of ethyl magnesium bromide to ester **23-5** does lead to ketone **9-4**, but larger amounts of the diethyl alcohol are also produced. Reagent **23-6**, however, exclusively furnishes the methyl ketone **23-7** after methanolysis, which after C-methylation of the enolate gives **9-4** eventually. With Nicolaou's fragments **9-4** and **9-2** at hand, seco acid **24-2** is prepared Macrolactonization *via Keck*'s method [31] furnishes macrolide **24-3** which is deprotected and epoxidized to give epothilone B in an unprecedentedly



Scheme 23. Novel aldol addition approach to the C1-C6-ketone fragment

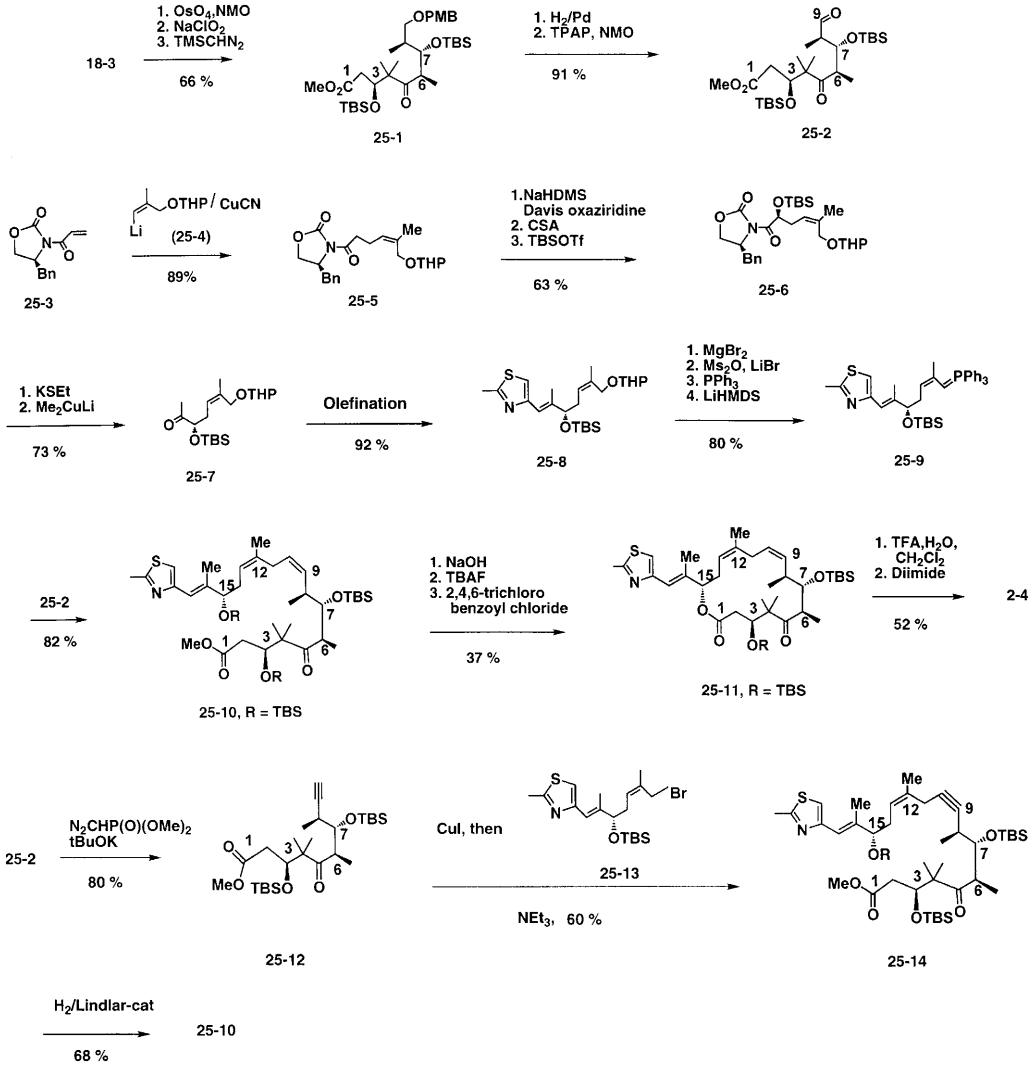


Scheme 24. Successful sulfone coupling and macrolactonization approach to epothilone B

short and stereoselective sequence. The main advantage over Nicolaou's synthesis is the concise access to ketone **9-4** and the (*E*)-selective preparation of aldehyde **9-2**. Additionally, the stereogenic centers at C15 and C8 are adapted from the chiral carbon pool instead of using chiral auxiliaries.

Synthesis No. 9 (White)

J. D. White reported a synthesis of epothilone B [11g] which is based on our fragment **18-3**. The carbon skeleton is constructed by forming a C9-C10-double bond *via* a *Wittig* reaction between the C1-C9-aldehyde **25-2** and the C10-C21-phosphonium ylide **25-9** (Scheme 25). More specifically, compound **18-3** is oxidized to the carboxylic ester **25-1** and then converted into aldehyde **25-2**. The phosphonium ylide **25-9** is prepared from the *Evans* oxazolidinone **25-3** which is converted into **25-5** *via* a conjugate addition of cuprate **25-4**. The introduction of the 15-hydroxyl function is achieved *via* enolate hydroxylation with *Davis*' reagent [32] to furnish **25-6** after O-silylation. Oxazolidinone removal with mercaptide and

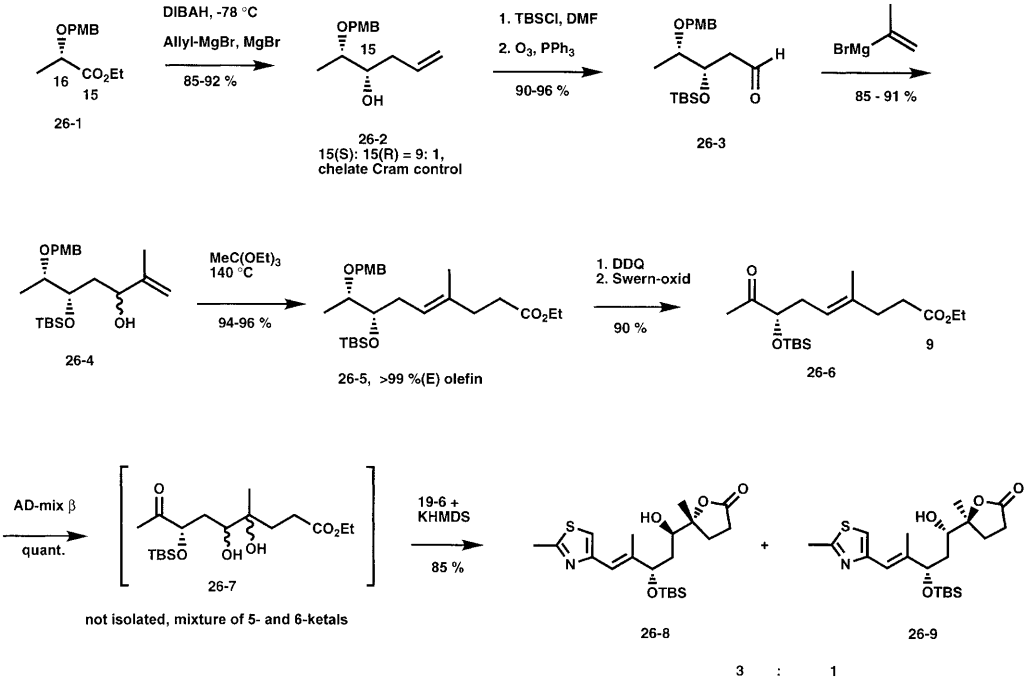


Scheme 25. *J. D. White*'s epothilone B synthesis

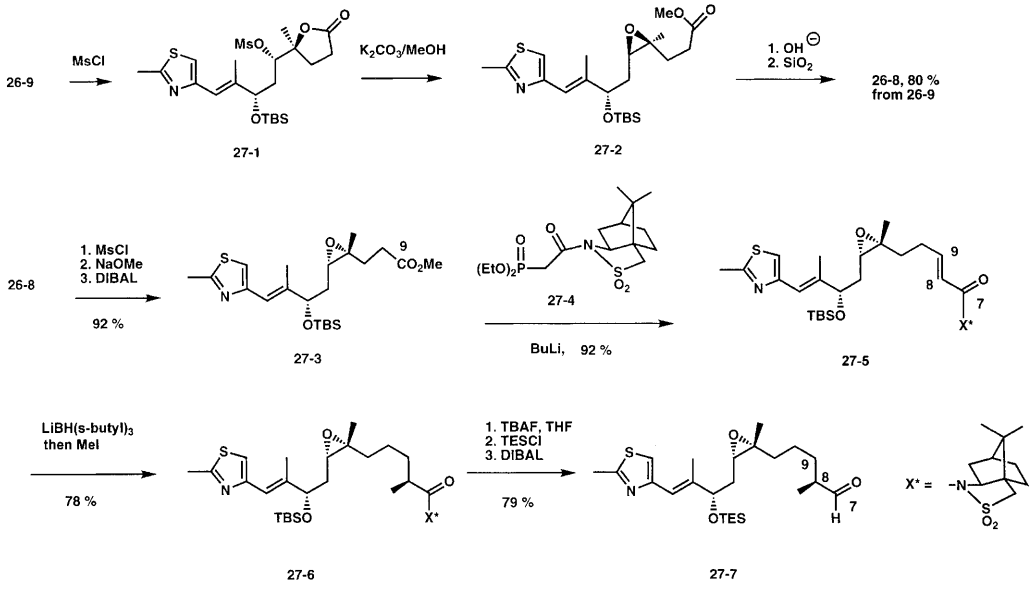
methyl cuprate addition to the thioester leads to the methyl ketone **25-7** which is converted into **25-8** *via* olefination. Four additional steps are required to generate phosphorane **25-9** which is treated with aldehyde **25-2** to give the (*Z*)-olefin **25-10** selectively. Macrolactonization is achieved in low overall yield from **25-10**, and after deprotection the superfluous 9,10-double bond is selectively hydrogenated with diimide to give epothilone D. *White*'s synthesis is distinguished by the high stereocontrol exerted on all stereogenic units (double bonds and stereogenic centers); however, it requires altogether more than 30 steps when starting from commercially available materials. In a later version [11h] the crucial CC coupling was performed with alkyne **25-12** and the allylic bromide **25-13** to give diene-yne ester **25-14** which was selectively hydrogenated over *Lindlar*'s catalyst to furnish **25-10**. In this way the obvious disadvantages of the *Wittig* reaction were circumvented.

Synthesis No. 10 (Mulzer 2)

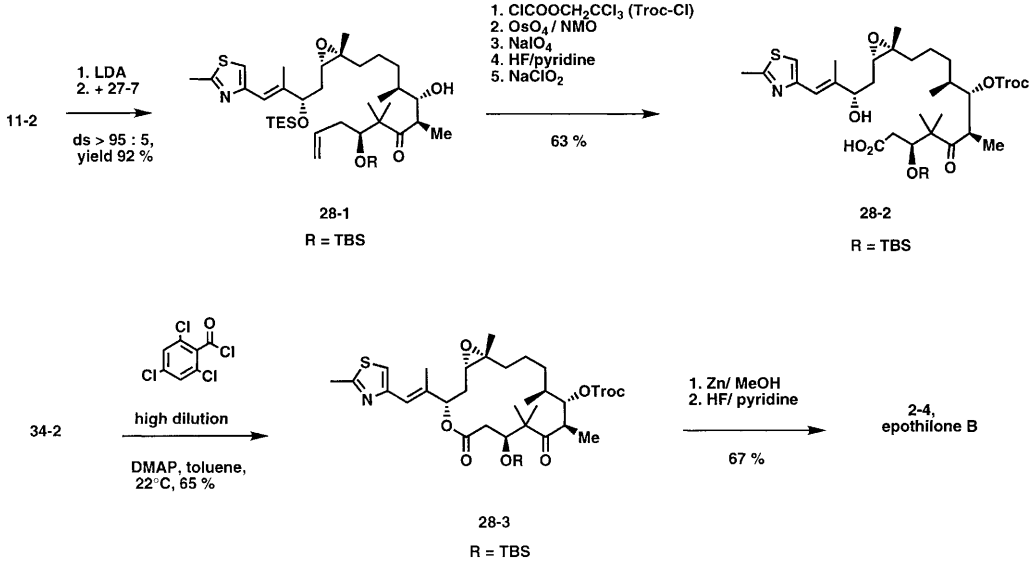
So far, all syntheses of epothilone B have involved the (more or less) stereoselective epoxidation of epothilone D in the last step, obviously to avoid undesired side reactions of the presumably labile 12,13-epoxide. In their second approach to epothilone B (Scheme 26) the *Mulzer group* wanted to test this lability by generating the 12,13-epoxide relatively early in the synthesis and exposing it deliberately to the hardships of a long sequence [33]. The synthesis starts from protected lactic ester **26-1** which in a one-pot operation is reduced to the aldehyde and treated with allylmagnesium bromide to furnish the chelate *Cram* adduct **26-2** selectively. Oxidation to the aldehyde and addition of isopropenyl magnesium bromide gives allylic alcohol **26-4** as a 1:1-epimeric mixture which is subjected to a *Claisen-Johnson* rearrangement to give ester **26-5**. After elaboration of **26-5** to the methyl ketone **26-6** the double bond is dihydroxylated according to *Sharpless*' procedure [34] to give the diol **26-7** which is subjected to a *Wittig* reaction with **19-6** to furnish a 3:1-mixture of the lactones **26-8** and **26-9** easily separable by chromatography. NMR analysis clarified that the *Wittig* reaction has been >98% (*E*)-selective, whereas the dihydroxylation has only furnished a 3:1-diastereomeric mixture. To avoid the loss of one third of the material, the undesired stereoisomer **26-9** is transformed into the desired one, **26-8**, by double inversion at C12 and C13 *via* the intermediates **27-1** and **27-2**. The synthesis is continued with the conversion of diol **26-8** into the epoxide **27-3**. The ester is reduced to the aldehyde which is



Scheme 26. *Mulzer*'s second epothilone B synthesis: The early epoxide approach part I: *Claisen* Rearrangement-Dihydroxylation



Scheme 27. Chain elongation *via Oppolzer*'s sultam



Scheme 28. Completion of the synthesis: Crucial role of a 7-O*Troc*-15-O*TES* protecting group strategy

subjected to a *Horner* olefination with *Oppolzer*'s phosphonate **27-4** [35] to generate the enoyl sultam **27-5**. 1,4-Hydride addition to the 8,9-double bond and stereoselective methylation of the resulting 7,8-enolate furnishes **27-6**. Next, the 15-O*TBS* group is exchanged for a O*TES* group, and this compound is reduced to the aldehyde **27-7**. Aldol addition with ketone **11-2** produces **28-1** with high stereoselectivity. After transformation of **28-1** into the seco acid **28-2**, *Yamaguchi-*

macrolactonization gives a satisfactory yield of **28**-**3** which is smoothly deprotected to form epothilone B. It thus turns out that the epoxide is rather stable and tolerates a number of oxidizing, reducing and anionic reagents. The only epoxide opening process found was the desired conversion of **27**-**2** into **26**-**8**. The early introduction of the epoxide has been of advantage in the overall synthesis. For instance, the formation of thiazol-N-oxides previously observed in the *mCPBA* epoxidation of the 12-13-double bond [37] has been avoided as well as the formation of the (12*S*,13*R*)-epoxide which is hard to separate from the correct stereoisomer. Additionally, the diastereocontrol of the aldol addition with the epoxyaldehyde is significantly better than it is with the olefinic aldehyde [11]. The use of ester **26**-**5** as an intermediate allows application of the *Claisen* rearrangement as an efficient chain elongation procedure. Also, it is possible to introduce additional double bonds after the epoxide has been generated which may be useful for the preparation of novel derivatives.

In Table 1 the total syntheses of epothilone B reported so far are compared in terms of (1) total number of the synthetic operations involved, (2) total number of the steps in the longest linear sequence, (3) overall yield along the longest linear sequence, and (4) stereocontrol over each stereogenic center. No evaluation, however, will be tried in this review, as all syntheses are viable and have been successfully applied and all syntheses certainly have merits and disadvantages.

Table 1. Comparison of the known total syntheses of epothilone B

Synthesis No.	Author (year)	Total Number of steps[a]	Total yield (%)	*ee* at C-3	*ee* at C-6	*ee* at C-7	*ee* at C-8	*ee* at C-12/13	*ee* at C-15
1	*Danishefsky* (1997)	27/21	4–6	40	?	?	?	0/70	>95
2	*Danishefsky* (1997)	30/26	ca 1	34	?	?	?	70	>95
3	*Nicolaou* (1997)	28/24	3	>98	50	50	>98	70	>97
4	*Grieco* (1998)	30/26	4	20	>95	>95	>95	0/70	>95
5	*Schinzer* (1998)	34	0.6	96	80	80	>95	70	100[b]
6	*Schinzer* (1998)	25	ca 1	96	84	84	80	0/70	100[b]
7	*Danishefsky* (1998)	27/17	5–7	80	>95	68	68	70	70
8	*Mulzer* (1998)	23	8	95	60	60	100[b]	66	100[b]
9	*White* (1998)	29	2	80	>95	>95	100[b]	70	?
10	*Mulzer* (1998)	32/26	3–5	>98	>90	>90	>98	>98	80

[a] Total number of steps/number of steps in the longest linear sequence; [b]taken from the chiral carbon pool

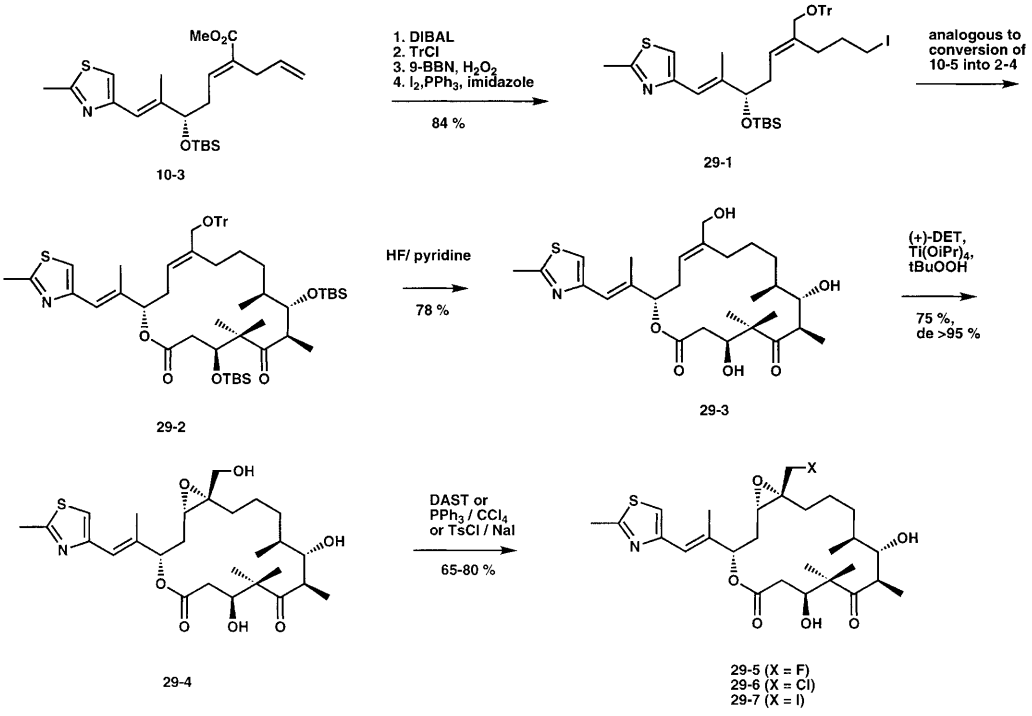
Synthesis of Derivatives

Nicolaou made extensive use of his epothilone synthesis [11a] for preparing derivatives in the hope of improving the biological activity. A host of epothilone A analogues were produced using combinatorial chemistry especially by applying the *Wittig* reaction to construct the C12, C13-olefinic bond [3]. In the epothilone B series, however, non-combinatorial methods were applied. For instance, intermediate **10-3** (Scheme 29) served as the starting material for the preparation of epothilones **29-5** to **29-7**, with a halo-methylene unit in position 12 [36].

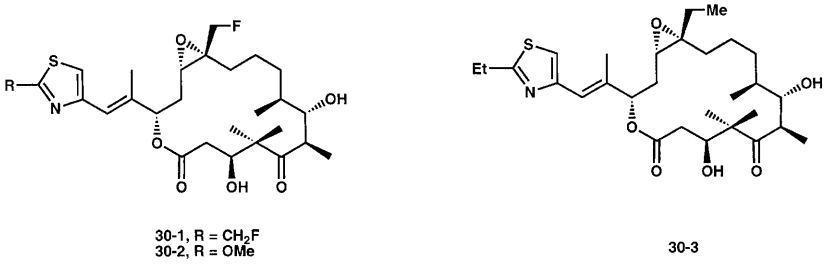
The synthesis takes advantage of the 12-hydroxymethylene unit which allows the application of *Sharpless*' AE reaction [24] to generate the epoxide in **29-4** from **29-3** with high stereoselectivity. The hydroxyl-halogen exchange takes place without affecting the epoxide. Analogues **30-1** to **30-3** are prepared similarly using the appropriate olefinating component (Scheme 30).

The 12,13-*trans*-epothilones C and D (**31-1** and **31-2**) are available as side products from the (*E/Z*)-12,13-olefin mixtures generated from unstereoselective Wittig or RCM reactions in *Nicolaou*'s, *Danishefsky*'s, and *Schinzer*'s total syntheses [10, 11a-d]. The epoxides **31-3** and **31-4** are obtained, along with the β-epoxides, from the (unstereoselective) epoxidation of **31-1** and **31-2** (Scheme 31).

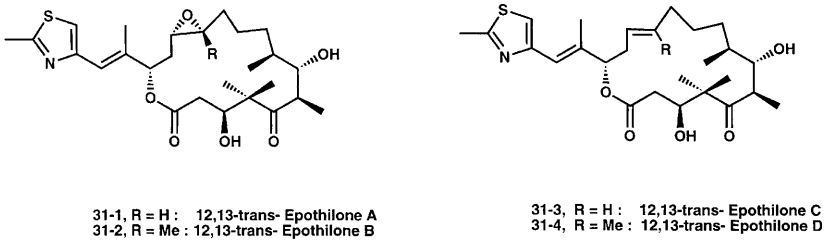
Interesting analogues have also been prepared by partial synthesis from the naturally occuring epothilones. For instance, *Höfle et al.* [37] reported that epothilone B (**2-4**) is converted into the N-oxide **32-1** with *m*-chloroperbenzoic acid (Scheme 32). On heating with acetic anhydride, **32-1** undergoes a *Polonovsky*-



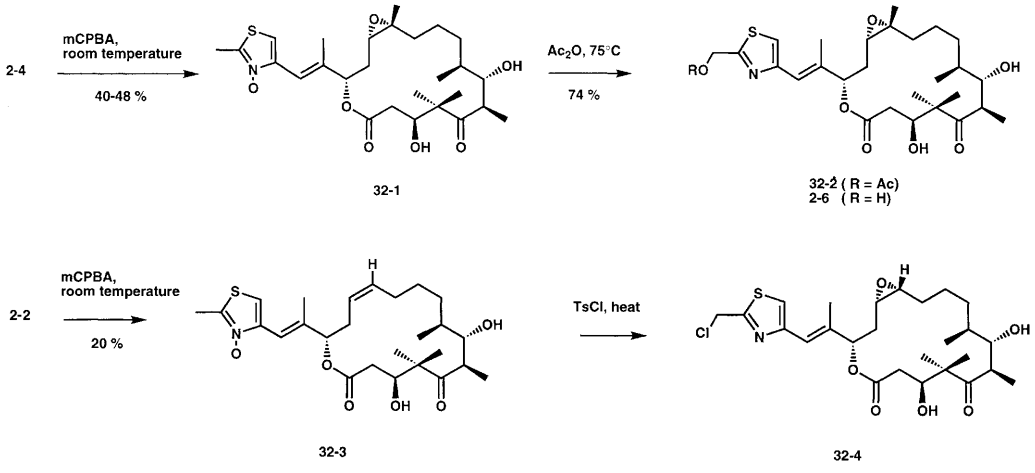
Scheme 29. *Nicolaou*'s synthesis of epothilone B derivatives



Scheme 30. Additional epothilone B derivatives from the *Nicolaou* group

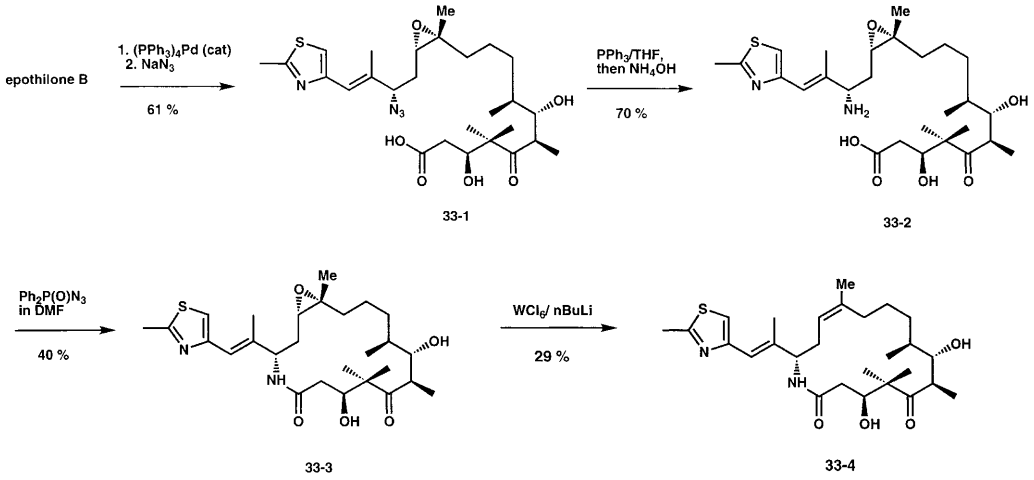


Scheme 31. Epothilone B derivatives with 12,13-*trans*-stereochemistry



Scheme 32. *Höfle*'s partial synthesis of new epothilone derivatives

like rearrangement to form the 21-acetate **32-2** whose saponification leads to epothilone F (**2-6**) which has also been detected as a natural metabolite. An analogous sequence can be performed with epothilone C (**2-2**). Interestingly, the oxidation with peracid leads to the N-oxide **32-3** chemoselectively without touching the 12,13-double bond. *Polonovsky*-type rearrangements also occur with anhydrides or sulfonyl chlorides to furnish 21-substituted derivatives, *e.g.*, **32-4**.



Scheme 33. Partial synthesis of epothilone B lactams

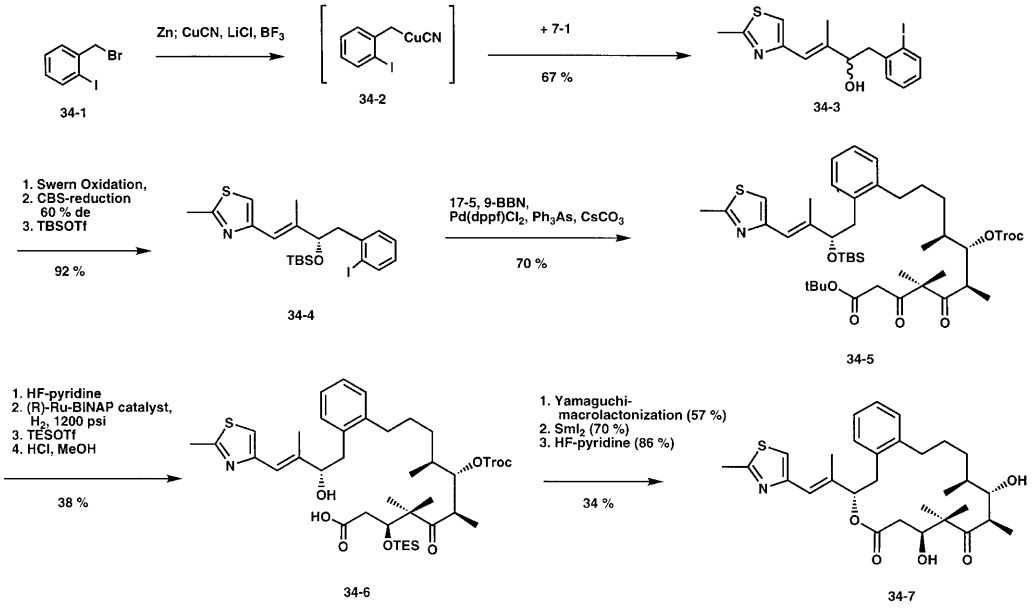
The epothilone lactam analogues (aza-epothilones) **33-3** and **33-4** have been prepared by an industrial research group (Scheme 33) [38]. They use a palladium catalyzed S_N2-type ring opening of the macrolide [39] at the C15-17-allylic ester moiety with sodium azide as the nucleophile (Scheme 33). Azide **33-1** is formed unter retention of configuration at C15. Reduction of the azide furnishes the amino acid **33-2** which is cyclized to the lactam **33-3** under carboxyl group activation. The epoxide can be deoxygenated to the olefin **33-4** by a tungsten hydride species generated *in situ* from tungsten chloride and *n*-butyl lithium. This deoxygenation can also be applied to the conversion of epothilone B into epothilone D or of epothilone A into epothilone C under retention of the olefin configuration.

Danishefsky prepared the promising 12,13-benzo-analogue **34-7** of epothilone B (Scheme 34) [40] essentially along the same route as that one used for epothilone D (Scheme 17).

Biological Activity

As mentioned above, the initial test for biological activity of the epothilones was the microtubule aggregation test ('tubulin assay') developed for paclitaxel. At that time, it was taken for granted that high microtubule aggreation would always go along with high antitumor activity. Very soon, however, it turned out that – at least for paclitaxel analogues – high activity in terms of microtubule aggreagation is not always accompanied by high cytostatic activity [41]. However, this could not be confirmed for the epothilones, where a high activity in the tubulin assay does indeed normally lead to high cytotoxicity (Table 2) [42,43].

This is particularly true for epothilones B and D and also the derivatives **30-1**, **30-2** and **30-3** [36]. Although there are significant differences with respect to the individual epothilones they are apparently about as active or even more active than paclitaxel both in the tubulin assay and in the cytotoxicity tests (Table 3).



Scheme 34. *Danishefsky*'s synthesis of a benzo analogue of epothilone D

Table 2. Relative biological activities of epothilones and derivatives and paclitaxel [42,37]

Paclitaxel	15[a]	2.0[cI]	50[cII]	43[cIII]	>100[cIV]
Epothilone A (**2**-**1**)	14[a]	2.0[cI]	19[cII]	4.2[cIII]	2.4[cIV]
Epothilone B (**2**-**4**)	4.0[a]	0.040[cI]	0.035[cII]	0.045[cIII]	0.040[cIV]
Epothilone D (**2**-**5**)	3.3[a]	2.0[cI]	33[cII]	3.5[cIII]	1.5[cIV]
Epothilone C (**2**-**2**)	25[a]	25[cI]	>100[cII]	75[cIII]	22[cIV]
12,13-*trans*-Epothilon D (**31**-**4**)	39[a]	48[cI]	>100[cII]	75[cIII]	24[cIV]
12,13-*trans*-Epothilone B (**31**-**2**)	22[a]	3.5[cI]	30[cII]	5.5[cIII]	3.0[cIV]
Epothilone E (**2**-**3**)	–	>100[cI]	50[cII]	20[cIII]	–
30-**1**	Highly active[b]	0.54[cI]	2.8[cII]	1.5[cIII]	–
30-**2**	Highly active[b]	0.40[cI]	1.2[cII]	2.5[cIII]	–
30-**3**	Highly active[b]	0.12[cI]	0.35[cII]	0.14[cIII]	–

[a] EC_{50} (μg) = quantity required for causing 50% of the tubulin to assemble in polymers; [b]induction of tubulin assembly; [c]inhibition of human ovarian carcinoma cell growth, IC_{50} (ng); I = cell line 1A9, II = cell line 1A9PTX10, III = cell line 1A9PTX22, IV = MDR line A2780AD

Table 3. *In vitro* cytotoxicity of epothilone derivatives and paclitaxel [43]

Compound	Cytotoxicity I[a]	Cytotoxicity II	Cytotoxicity III
Paclitaxel	80	12	4
Epothilone A	8	2	1.4
Epothilone B	1.4	1.2	0.2
Epothilone C	100	40	60
Epothilone D	20	24	20
Epothilone E	40	10	6
Epothilone F	3	1.0	0.2
32-1	4	2	1.5
32-2	400	130	20
32-3	1400	200	600
32-4	1100	600	1100

[a] Cytotoxicity was determined as IC_{50} (n*M*) with the following cell lines: I = mouse fibroblasts (ATCC CCL 1), II = human cervix carcinoma (DSM ACC 158), III = human lung carcinoma (DSM ACC 107)

Preliminary SAR results

Using a broad selection of epothilone B analogues, SAR studies have been performed by *Nicolaou* [3a] and, more recently by *Danishefsky* [3b]. Roughly the molecule may be subdivided into three zones 1–3 (Fig. 4) whose modifications have very different effects on the microtubule stabilization and cytotoxicity. Zone 1, *i.e.*, the polyketide section, is the most intolerant one with respect to structural modifications. For example, inversion of the stereochemistry at C3 or reduction of the C5 carbonyl resulted in serious arrest of activity. Similarly, analogues with functionalities at C3, C5, C6, C7, and C8 removed or modified demonstrated reduced tubulin binding and cytotoxicity. Zone 2 is remarkably tolerant to modification. For instance, the (*Z*)-deoxy derivative (epothilone B) and also the (*E*)-deoxy derivative show significant activity, and substitution at C12 with ethyl or propyl and other alkyl residues was well tolerated both in the epoxy and the desoxy series. Also, the configuration of C15 appears not crucial for biological activity. Polar substituents in the 13-appendage enhance the susceptibility to succumb to MDR. Zone 3 eventually, *i.e.* the aryl substituents must have an aryl group and the correct olefin spacer, but within these limits variations are tolerated.

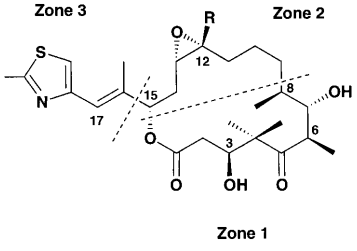


Fig. 4. Pharmocophoric zones in epothilone B

Second generation biodata of epothilones; comparison with other antitumor drugs

An extremely important aspect is the comparison of the epothilones with other antitumor drugs with respect to multidrug resistance (MDR) [3b, 44] (Table 4). A comparison of the epothilones, paclitaxel, and two classical antitumor drugs (actinomycin D and adriamycin) shows that although all these compounds do exhibit extremely high cytotoxicity the epothilones are significantly superior in terms of multidrug resistance towards several tumor cell lines. Quite interestingly epothilone D (**2**-**5**) now emerges as the top candidate of all compounds tested so far, as **2**-**5** combines high antitumor activity with high multidrug resistance. It is clearly superior to epothilone B (**2**-**4**).

This is in clear contrast to the data of Table 1 where epothilone D shows a significantly lower cytotoxicity than epothilone B, although the activity in the tubulin assay is about the same for both compounds. The superiority of epothilone D over epothilone B is more impressively borne out when discussing the results of in vivo tests with normal athymic nude mice bearing human mammary adenocarcinoma MX-1 xenografts (Table 5).

When a daily dose of 0.6 mg/kg of epothilone B was applied to normal nude mice intraperitonally (i.p.), all mice were dead after seven days. When, however 25 mg/kg of epothilone D was applied i.p., all mice survived. More important, epothilone B had only a marginal therapeutic effect, whereas epothilone D led to a drastic reduction of the tumor size, so that one out of six mice was without a detectable tumor after 35 days. When considering tumor therapy combined with low toxicity, epothilone D is superior not only to epothilone B, but also to paclitaxel and other antitumor drugs (Table 4). It has also been demonstrated that, again in the nude mice test, epothilone D is curative against human tumor

Table 4. Comparison of *in vitro* growth inhibition potency of epothilone derivatives against various parent and drug-resistant tumor cell lines [44]

Compound	DC-3F	DC-3F/ADX	P338/0	P338/Adr	SK-N-As	SK-N-FI	MCF-7	MCF-7/Adr
2-**1**	0.0037	0.053 (14.5 x)	0.0018	0.0010 (5.3 x)	0.012	0.023 (1.9 x)	0.0030	0.0094 (3.1 x)
2-**4**	0.0006	0.017 (28 x)	0.00029	0.0016 (5.5 x)	0.004	0.010 (25 x)	0.0005	0.0027 (5.4 x)
2-**2**	0.011	0.042 (3.9 x)	0.0213	0.0125 (0.59 x)	0.073	0.223 (3.1 x)	0.032	0.144 (4.5 x)
2-**5**	0.00097	0.00091 (0.9 x)	0.0068	0.0042 (0.62 x)	0.021	0.046 (2.2 x)	0.0029	0.0071 (2.4 x)
Paclitaxel	0.095	32.0 (338 x)	0.0029	0.326 (111 x)	0.0016	0.130 (80 x)	0.0033	0.150 (46 x)
Actinomycin D	0.00044	0.572 (13000 x)	0.00015	0.0012 (8 x)	0.00085	0.0119 (14 x)	0.00068	0.00167 (2.5 x)
Adriamycin	0.018	2.236 (124 x)	0.0055	2.65 (482 x)	0.077	1.42 (18.4 x)	0.057	0.216 (3.8 x)

Numbers are IC_{50} (μg); numbers in parentheses are fold of resistance based on the IC_{50} ratio when compared with the corresponding parent cell lines except for P388/0 and P388/Adr, and XTT assay was used

Table 5. Therapeutic effect of epothilone D (**2**-**5**), epothiline B (**2**-**4**), paclitaxel (taxol), vinblastine, and camptothecin in nude mice bearing human MX-1 xenograft [44]

Drug	Dose (mg/kg)	7[a]	11[a]	13[a]	15[a]	17[a]	11[b]	13[b]	15[b]	17[b]	Toxicity/ death	*n*
Control		27.2	+0.8	+1.1	+1.9	+0.6	1.00	1.00	1.00	1.00	0/8	8
2–5	15	27.1	+0.8	+1.1	+1.6	+1.5	0.65	0.46	0.49	0.41	0/6	6
	25	27.0	+0.4	+0.7	+1.0	+0.7	0.35	0.11	0.05	0.04	0/6	6
2–4	0.3	26.9	+0.5	+0.4	−0.3	−1.2	1.00	0.71	0.71	0.84	0/7	7
	0.6	27.4	−0.3	−1.3	−2.1	−2.1	1.08	0.73	0.81	0.74	3/7	7
Paclitaxel	5	26.9	−0.1	+0.4	+1.1	+1.2	0.54	0.46	0.40	0.45	0/7	7
	10	27.6	−2.7	−1.1	−0.3	+2.2	0.43	0.37	0.12	0.11	4/7	7
Vinblastine	0.2	25.7	+0.6	+1.4	+2.3	+2.9	0.65	0.54	0.56	0.88	0/7	7
	0.4	26.4	+0.8	+0.5	+1.9	+2.1	0.80	0.56	0.83	0.88	1/7	7
Camptothecin	1.5	27.4	−0.9	−0.7	−0.4	+1.0	0.61	0.45	0.32	0.36	0/7	7

[a] Average body weight change (g) on day (indicated); [b]average tumor size, T/C (test *vs*. control) on day (indicated)

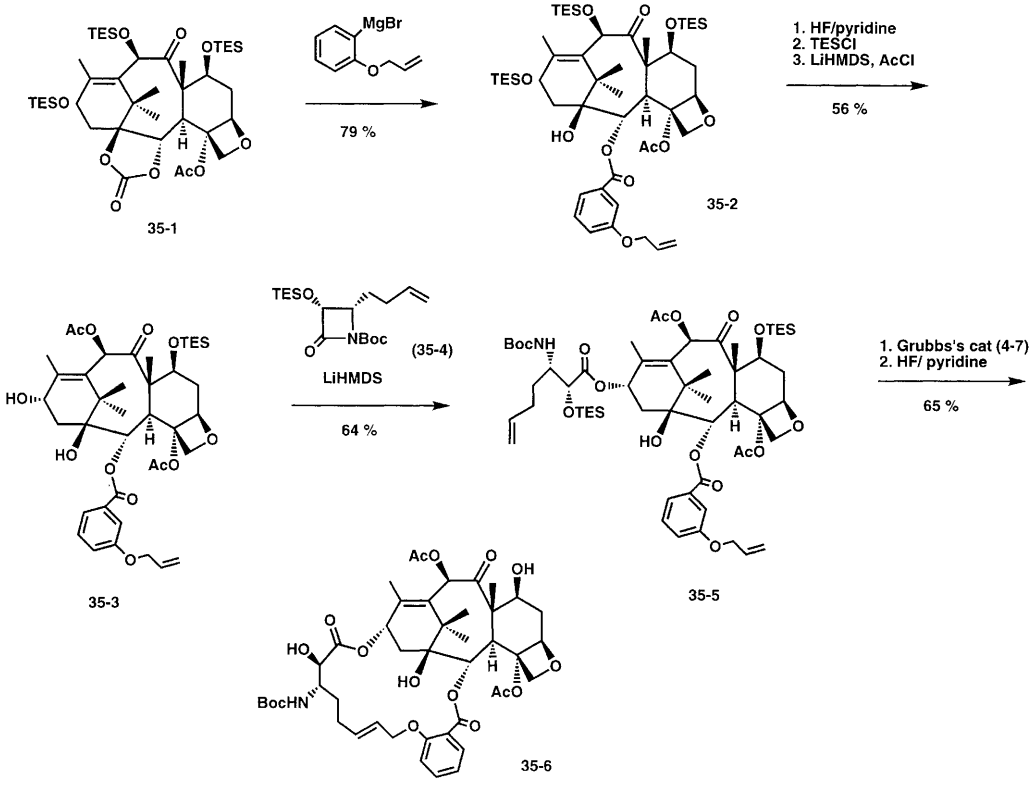
xenografts that are refractory to paclitaxel [45]. However, in all these *in vivo* tests the absolute number of test animals appears far too low to allow final conclusions.

The promising qualities of epothilone D (**2**-**5**) were the reason for synthesizing its benzo analogue (**34**-**7**) (Scheme 34) [39]. In the tubulin assay **34**-**7** showed 40% of the activity of **2**-**5** and the cytotoxicity activity of **34**-7 was lower than that of **2**-**5** by a factor of 500. So far no *in vivo* tests have been described.

Thus, in conclusion, it thus appears that the biological tests are getting more and more confusing, and former statements will have to be reconsidered. The tubulin assay is of rather limited value, as are the *in vivo* cytotoxicity tests using tumor cell lines, and in future much more emphasis has to be put on *in vivo* tests with nude mice and other animals (*e.g.* dogs).

Common pharmacophore

Paclitaxel remains the standard against which the epothilones are measured, and, thus, there is an ongoing discussion whether or not a common pharmacophore can be detected in paclitaxel, the epothilones, and also in eleutherobine and discodermolide. This pharmacophore could be considered as a common binding site in the drug-microtubule interaction. In fact, several authors have postulated [46,47] such common pharmacophores from conformational analysis of the molecules by molecular dynamics studies, but so far, conclusive evidence has not been provided. A hybrid structure has been synthesized [47] combining the terpenoid core and the amino alcohol side chain of paclitaxel with the macrolide olefin structure of epothilone D. The synthesis (Scheme 35) starts from the baccatin derivative **35**-**1** which is converted into the benzoate **35**-**2**. Protective group adjustment leads to the alcohol **35**-**3** which is acylated with the β-lactam **35**-**4** to give the diolefin **35**-**5**. RCM and deprotection affords the desired paclitaxel-epothilone hybrid structure **35**-**6**. The compound exhibits submicromolar level IC_{50} values (0.39 µg against the human breast cancer cell line MDA-435/LCC6-WT) and 37% activity in the tubulin assay as compared to paclitaxel.



Scheme 35. *Danishefsky-Ojima* Synthesis of a paclitaxel-epothilone hybrid construct (**35**-**6**)

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