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Epothilones – A fascinating family of microtubule stabilizing antitumor agents

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Abstract

In October 2007 the first epothilone derivative has received FDA approval for the treatment of metastatic breast cancer. This event forms the background of this review which tries to highlight the historical development of the epothilones, namely their discovery, biosynthesis, fermentation, total synthesis, semisynthetic derivatives and clinical trials. ***To cite this article: J. Mulzer et al., C. R. Chimie 11 (2008).***

Keywords: Antitumor agents; Multidrug resistance; Polyketides; Biosynthesis; Asymmetric synthesis; Phase I and Phase II clinical trials

Abbreviations: Ac, acetyl; AIBN, 2,2′-azabisisobutyronitrile; 9-BBN, 9-borobicyclo[3.3.1]nonane; Bn, benzyl; Bz, benzoyl; CSA, camphorsulfonic acid; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, dicyclohexylcarbodiimide; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; Dess–Martin-PI, Dess–Martin periodinane; DHP, dihydropyran; DIBAL-H, diisobutylaluminium hydride; DIC, *N*,*N*′-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMDO, dimethyldioxirane; DMF, *N*,*N*-dimethylformamide; DMPU, *N*,*N*′-dimethylpropylenurea; DMS, dimethylsulfide; DMSO, dimethylsulfoxide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HMPA, hexamethylsilylphosphoramide; Ipc, isopinocamphenyl; KHMDS, potassium hexamethylsilylazide; LAH, lithiumaluminiumhydride; LDA, lithium diisopropylamide; LiHMDS, lithium hexamethylsilylazide; *m*CPBA, *m*-chloroperoxybenzoic acid; MEM, 2-methoxyethoxymethyl; MMPP, magnesium monoperphthalate; MPM, *para*-methoxyphenylmethyl; MS, molecular sieves; Ms, methanesulfonyl; NaHMDS, sodium hexamethylsilylazide; NBS, *N*-bromosuccinimide; NIS, *N*-iodosuccinimide; NMO, *N*-methylmorpholine-*N*-oxide; PBS, phosphate-buffered saline; PCC, pyridinium chlorochromate; PDC, pyridinium dichromate; PG, protective group; Ph, phenyl; Piv, pivaloyl; PMB, *para*-methoxybenzyl; PPTS, pyridinium *p*-toluenesulfonate; py, pyridine; R, organic residue; RCM, ring closing metathesis; SAE, sharpless asymmetric epoxidation; SEM, 2-(trimethylsilyl)ethoxymethyl; SKR, sharpless kinetic resolution; TBAF, tetra-*n*-butylammonium fluoride; TBDPS, *tert*-butyldiphenylsilyl; TBHP, *tert*-butyl hydroperoxide; TBS, *tert*-butyldimethylsilyl; TCDI, thiocarbonyl diimidazole; TES, triethylsilyl; Tf, triflate; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; THP, tetrahydropyran; TIPS, triisopropylsilyl; TMS, trimethylsilyl; TPAP, tetra-*n*-propylammonium perruthenate; Tr, trityl; Troc, 2,2,2-trichloroethyl carbonate; Ts, tosyl; *p*TsOH, *p*-toluenesulfonic acid.

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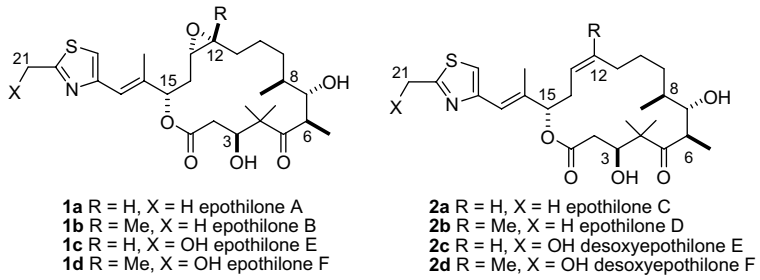


1a R = H, X = H epothilone A
1b R = Me, X = H epothilone B
1c R = H, X = OH epothilone E
1d R = Me, X = OH epothilone F

2a R = H, X = H epothilone C
2b R = Me, X = H epothilone D
2c R = H, X = OH desoxyepothilone E
2d R = Me, X = OH desoxyepothilone F

Fig. 1. Most important epothilones from fermentation.

1. Introduction and history

About 20 years ago, two metabolites with antifungal activity, later named epothilones A and B (**1a**, **1b**), were isolated at GBF (Gesellschaft für Biologisch-chemische Forschung at Braunschweig, Germany) from the soil bacterium *Sorangium cellulosum*, strain So ce90 (Fig. 1). Early application tests against plant pathogenic fungi failed due to phytotoxicity. The pharmaceutical industry, on the other hand, was not interested in a possible application as an anticancer agent, even though these compounds exhibited also significant toxicity in cell culture assays. Thus the compounds were abandoned by GBF in 1994 [1,2].

In 1993, MSD chemists set up a screening of natural products with taxane-like antitumor activity. They had one confirmed hit for an extract from an *S. cellulosum* strain coded SMP44. To their great surprise, the compounds responsible turned out to be **1a** and **1b** [3]. Compound **1b** was about 10 times more active than **1a** and even more active than taxol in the tubulin polymerisation assay; it replaced bound taxol from microtubules, and, most remarkably, its activity against cancer cells was hardly impaired by the resistance to taxol and other cytostatics. These exciting results immediately triggered a variety of activities in pharmaceutical companies and academia. The first total syntheses of **1a**, **1b** were published in 1996/1997 by the groups of Danishefsky, Nicolaou and Schinzer (see Section 5). Meanwhile GBF again improved the production strain, optimized nutrients and fermentation process, and streamlined the extraction process and large-scale purification by chromatography. From side fractions epothilones C and D (**2a**, **2b**) were isolated as major biosynthesis by-products along with 36 minor epothilones, for instance epothilones E and F (**1c**, **1d**) [4].

2. Mode of action

The observation that epothilones can displace tubulin-bound taxol [3] indicated a common pharmacophore. After the structure of the taxol–tubulin complex had been published, attempts were made to model epothilone within the taxol binding site. Thus, NMR experiments were performed with a soluble form of the epothilone A–tubulin complex [5]. The structure of epothilone A, bound to α,β-tubulin in zinc-stabilized sheets, was determined by a combination of electron crystallography at 2.89 Å resolution and nuclear magnetic resonance-based conformational analysis [6]. The complex explains both the broad-based epothilone structure–activity relationship and the known mutational resistance profile. Comparison with taxol shows that the longstanding expectation of a common pharmacophore is not met, because each ligand exploits the tubulin-binding pocket in a unique and independent manner.

However, no consistent pharmacophoric model could be derived from these controversial results so far.

3. Physical and chemical properties

Compounds **1a**, **1b** and **2b** have been crystallized. Epothilone A (**1**) is well soluble in polar organic solvents like methanol, ethyl acetate, acetone, diethyl ether and DMSO, whereas epothilone B is distinctly less soluble. Both are sparingly soluble in benzene, toluene and petroleum ether. A variety of melting points have been reported ranging from 76 °C to 128 °C depending on crystal form and nature of solvate. The X-ray crystal structures of **1b** from dichloromethane/petroleum ether and methanol/water show only small conformational changes in the orientation of the side chain, whereas the macrocyclic ring is virtually superimposable (Fig. 2) [1,7].[1]

[1] Crystallographic data of the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC-241333 and CCDC-241334. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

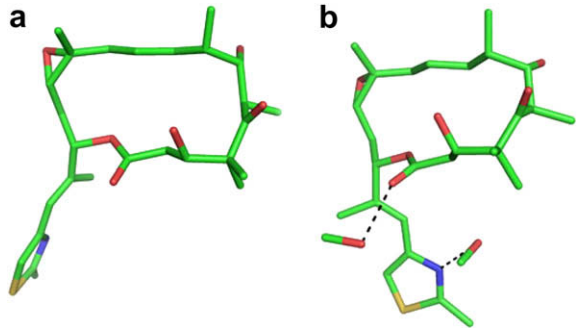


Fig. 2. (a) X-ray crystal structures of epothilone B (**1b**) from dichloromethane/petroleum ether and (b) methanol/water (modelling by W.-D. Schubert).

The preferred solution conformation of **1b**, derived from NMR studies in DMSO, was found to be very similar to that in Fig. 2a [1]. In a detailed conformational analysis, Taylor and Zajicek [8] detected a second minor conformer in which the 3-OH is rotated from a pseudo-axial to a pseudo-equatorial position.

Chemically, lactone and epoxide are the most reactive groups in **1a** and **1b** [9]. As expected, the lactone group is rapidly hydrolyzed in aqueous medium above pH 11; the epoxide is hydrolyzed or rearranged below pH 3 [10]. At neutral pH, pig liver esterase rapidly cleaves the lactone [9,11]. Otherwise, epothilones are perfectly stable under normal conditions.

4. Biosynthesis

It was originally speculated that the epothilones are assembled jointly by polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) multienzymes (Fig. 3). In such multimodular megasynthetases [12–14] the activated monomeric building blocks are selected as CoA esters in PKS by acyltransferase (AT) domains.

The activated acids are then covalently tethered to carrier proteins (acyl carrier proteins – ACPs – in PKS and peptidyl carrier proteins – PCPs – in NRPS) and then condensed with each other *via* the action of ketosynthase (KS) domains in a Claisen type reaction (PKS) or by condensation (C) domains in NRPS forming peptide bonds. Additional domains are ketoreductase (KR) domains, dehydratase (DH) domains, enoylreductase (ER) domains, and *O*-methyltransferase (O-MT) domains in PKS. In NRPS, heterocyclization (HC) domains forming thiazoline and oxazoline rings from cysteine and serine, respectively, can be employed. Additional oxidation (Ox) domains generate the thiazole and oxazole structures. Additionally, *N*-methyltransferase (N-MT) domains or epimerization (E) domains forming D-amino acids from the natural L-forms are found frequently. Typically, the fully extended intermediate is released from the final carrier protein by the action of a thioesterase domain forming free acids,

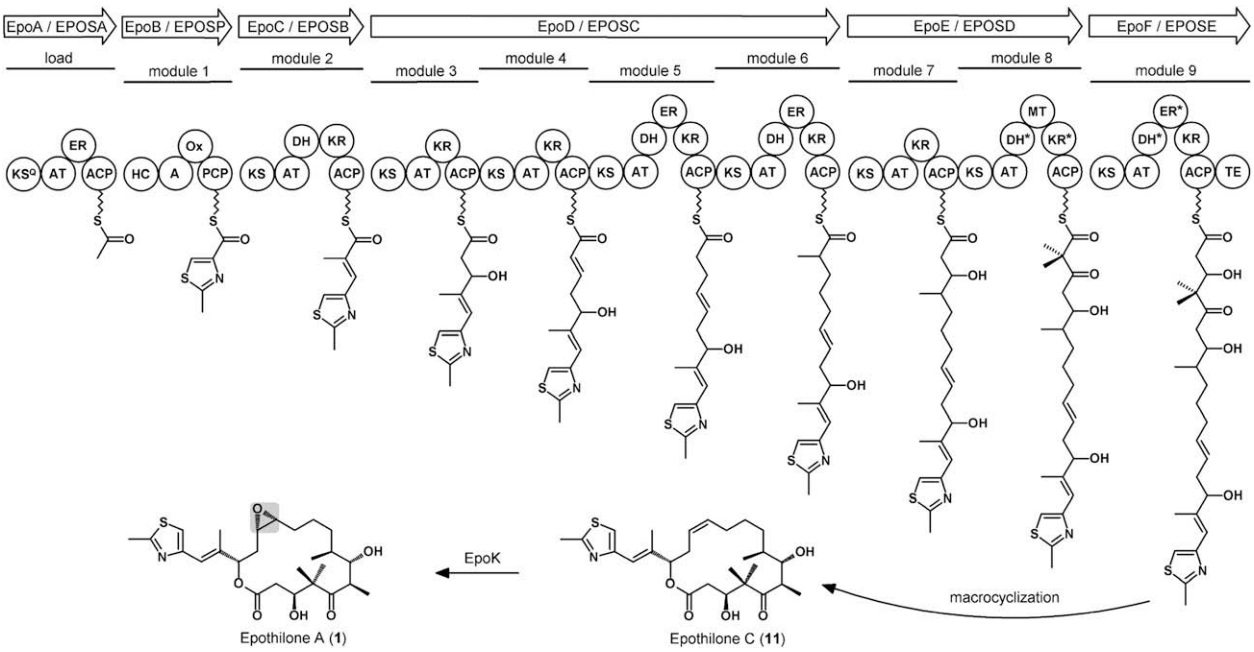


Fig. 3. Epothilone biosynthetic assembly line exemplified for the formation of epothilone A.

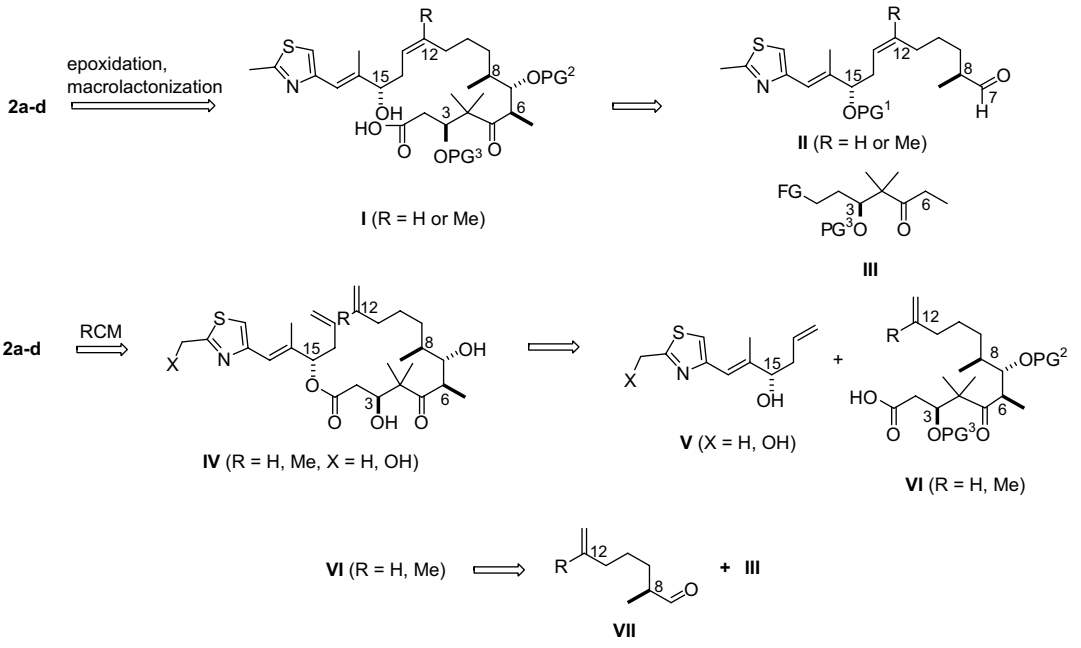
lactones, or lactams. In fact, feeding studies [15] in the natural host *S. cellulosum* So ce90 revealed that the carbon atoms in the epothilone backbone are derived from acetate (from malonyl-CoA; mCoA), propionate (from methylmalonyl-CoA; mmCoA), the methyl group of *S*-adenosyl-methionine (SAM), and cysteine (which also introduces the sulfur and nitrogen atoms). Compounds **1a** and **1b** are formed from the alternative incorporation of mCoA or mmCoA at position C11–C12. The epoxide is incorporated from molecular oxygen by a P450 type enzyme, EpoK, which was expressed in recombinant form. Its crystal structure has been solved [16,17].

It is noteworthy in this connection that Khosla et al. have synthesized epothilones using fermentation methods by cloning and heterologous expression of the epothilone gene cluster in *Myxococcus xanthus* [18].

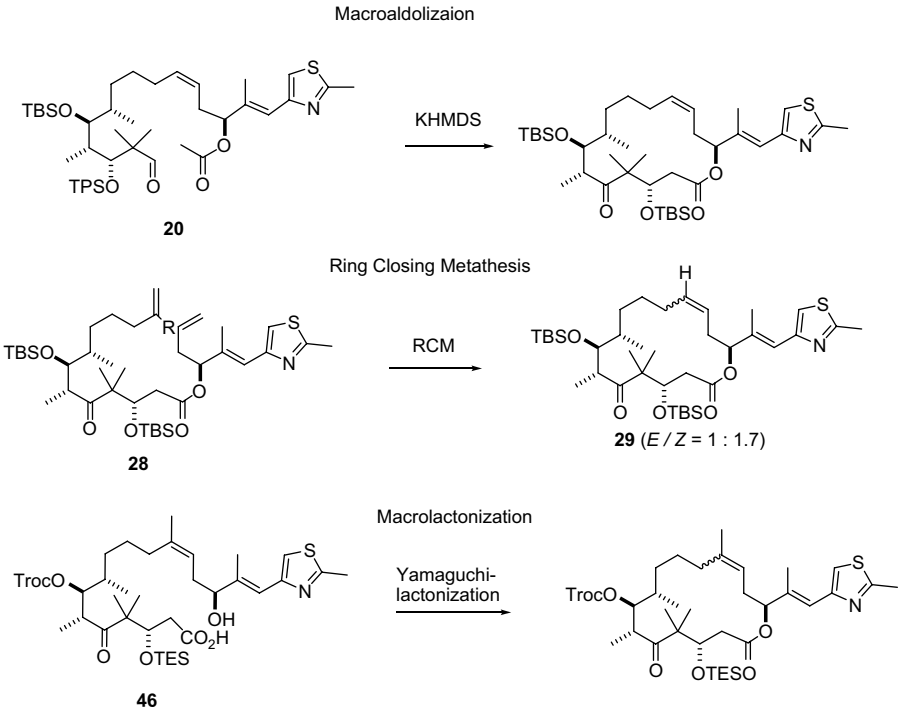
5. Total synthesis

The total synthesis of epothilones A–F is a worthwhile objective as their potential for broad refunctionalization and structural modification is rather limited. Moreover, the epothilones are structurally much less complex than, for instance, the taxanes. Altogether, 16 complete and formal approaches have been reported for **1a**, **2a** and 20 for **1b**, **2b** (for reviews, see Refs. [19,22]). In the following section, an arbitrarily chosen selection will be presented. It has to be emphasized that many of these approaches use the same disconnection strategies and key fragments, so that there is a limited number of truly independent syntheses. Typically, most sequences intercept one of the key intermediates (*vide infra*) from the Nicolaou, Danishefsky or Schinzer syntheses.

From these pioneering investigations, a standard retrosynthesis has emerged over the years (Scheme 1) [20–24]. The last step in the synthesis is the more or less stereocontrolled epoxidation of the C12,13-double bond. As the penultimate step, the macrolactonization of seco-acid **I** is performed in 10^{-3} M to 10^{-4} M solution *via* the Yamaguchi protocol [25]. Seco-acid **I** stems from a stereocontrolled aldol addition of the C21–C7 aldehyde **II** and the (*Z*)-enolate of ketone **III** [26], the asymmetric induction of which crucially hinges on the presence and the configuration of the stereocenter at C3 and the functionalization of the C1–C3-region in **III**. Aldehyde **II** has been prepared along a variety of routes which characteristically differ in the introduction of the C12,13-double bond. A second, less general access to **2a–d** is *via* RCM of di-olefin ester **IV** which is obtained from alcohol **V** and acid **VI**. For the preparation of **VI**, an aldol reaction between **III** and aldehyde **VII** is normally used, similar to the one between **III** and **II**.



Scheme 1. Retrosynthetic considerations.



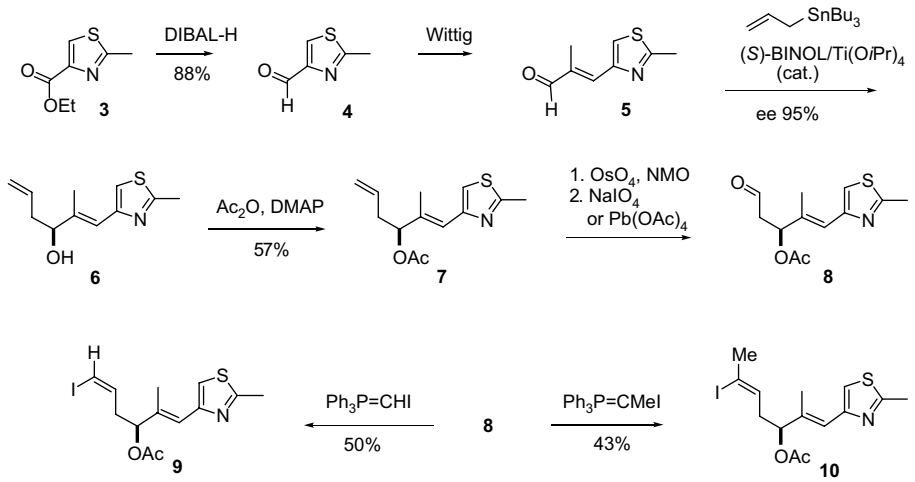
Scheme 2. Overview of Danishefsky's syntheses.

The first three syntheses (Danishefsky, Nicolaou, and Schinzer), though initially all aiming for **1a**, **2a**, were flexible enough to target also **1b**, **2b** without major alterations.

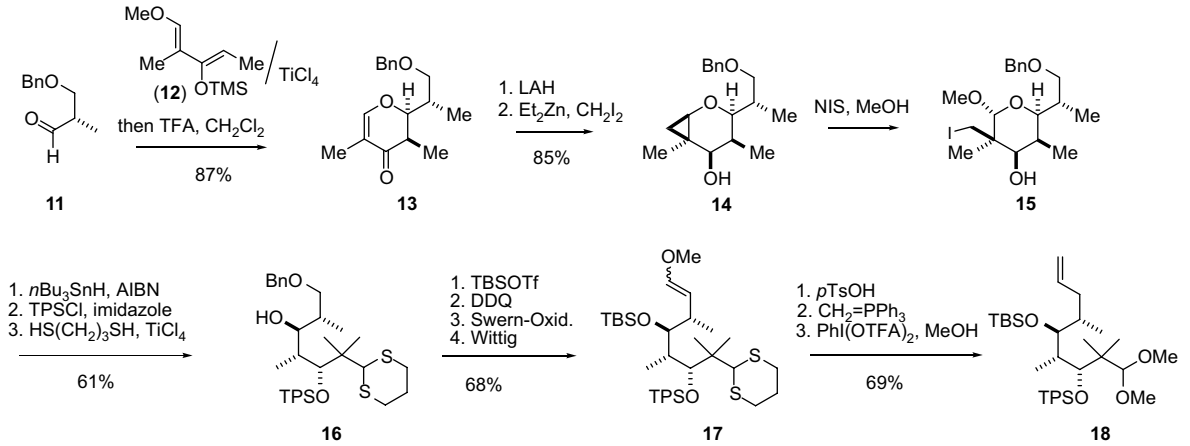
5.1. Danishefsky syntheses (Schemes 2–11)

The Danishefsky group first [27–29] has pursued three different ring closure strategies (Scheme 2). First, they started with the preparation of the C21–C12 segments **9** and **10** (Scheme 3) both easily available from thiazolyl ester **3**.

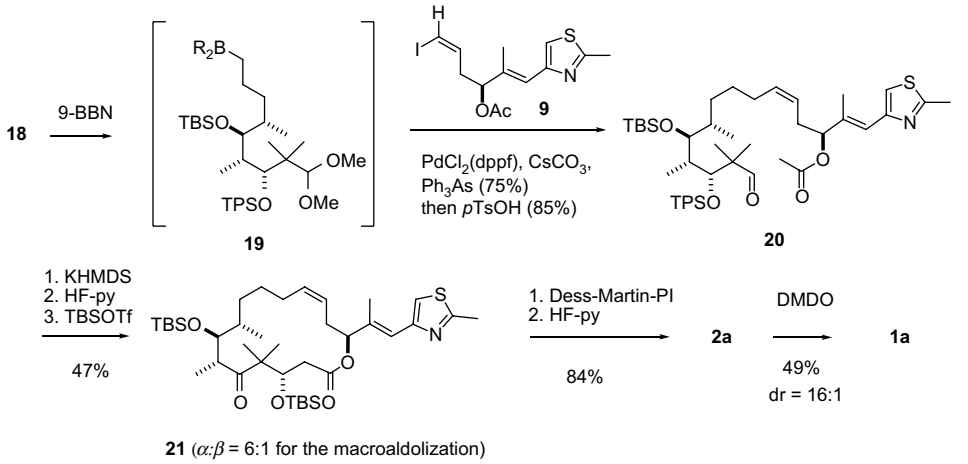
The C3–C11-segment **18** was obtained along a route (Scheme 4) that differs from the general strategy described in Scheme 1. A hetero-Diels–Alder reaction between chiral aldehyde **11** and Danishefsky diene **12** furnished dihydropyranone **13** with high Felkin–Anh selectivity. Cyclopropanation to **14** and



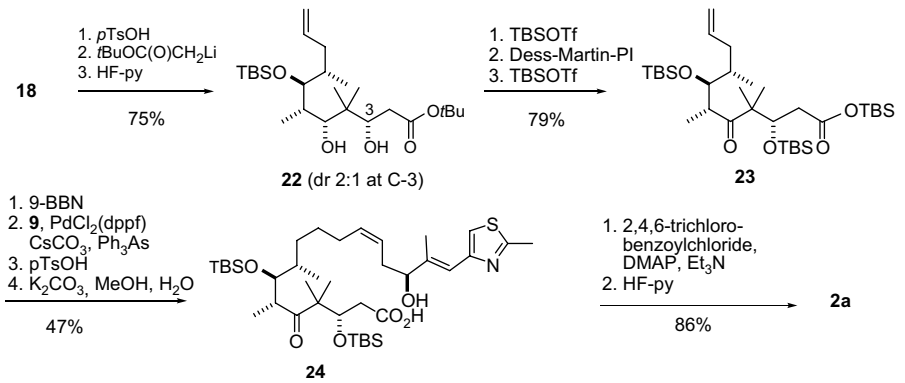
Scheme 3. Danishefsky's synthesis of epothilone building blocks.



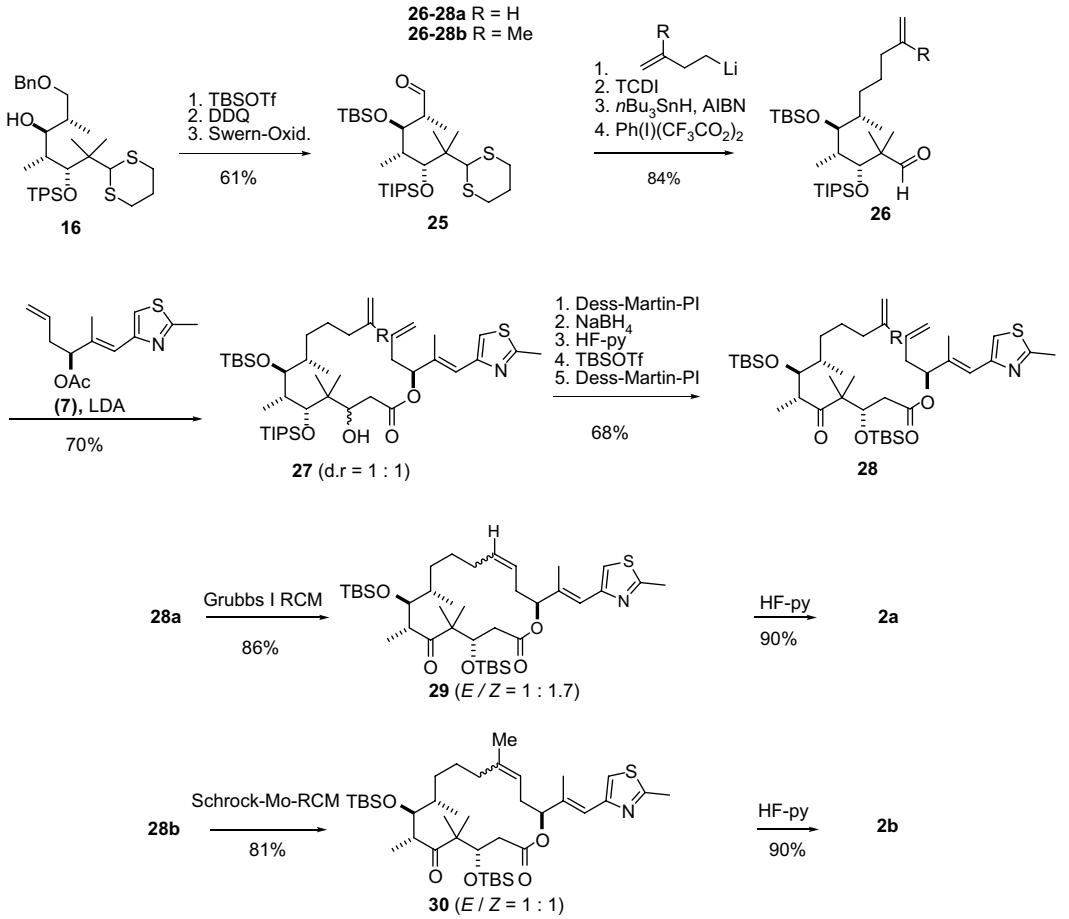
Scheme 4. Danishefsky's macroaldolization synthesis of epothilones A and C, part I.



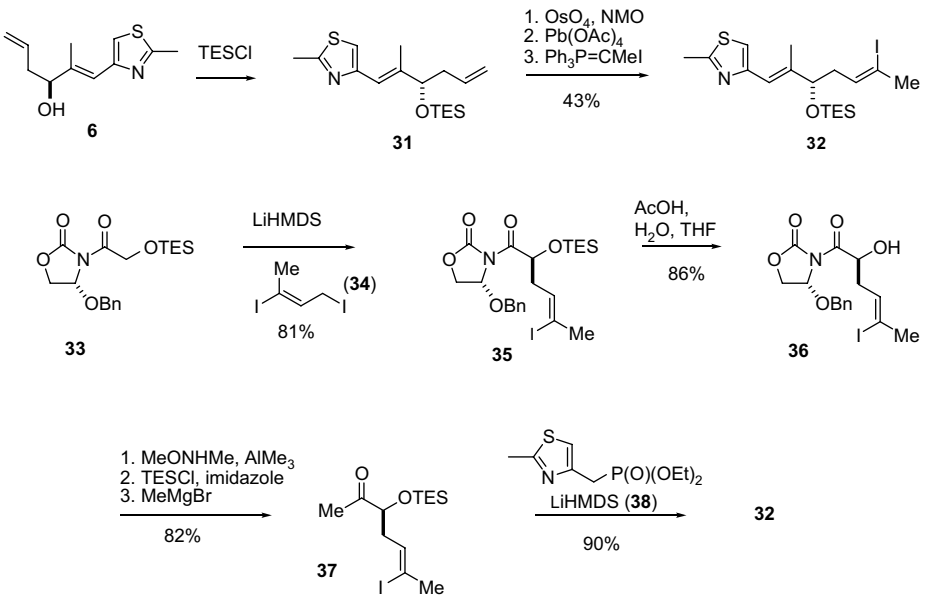
Scheme 5. Danishefsky's macroaldolization of epothilones A and C, part II.



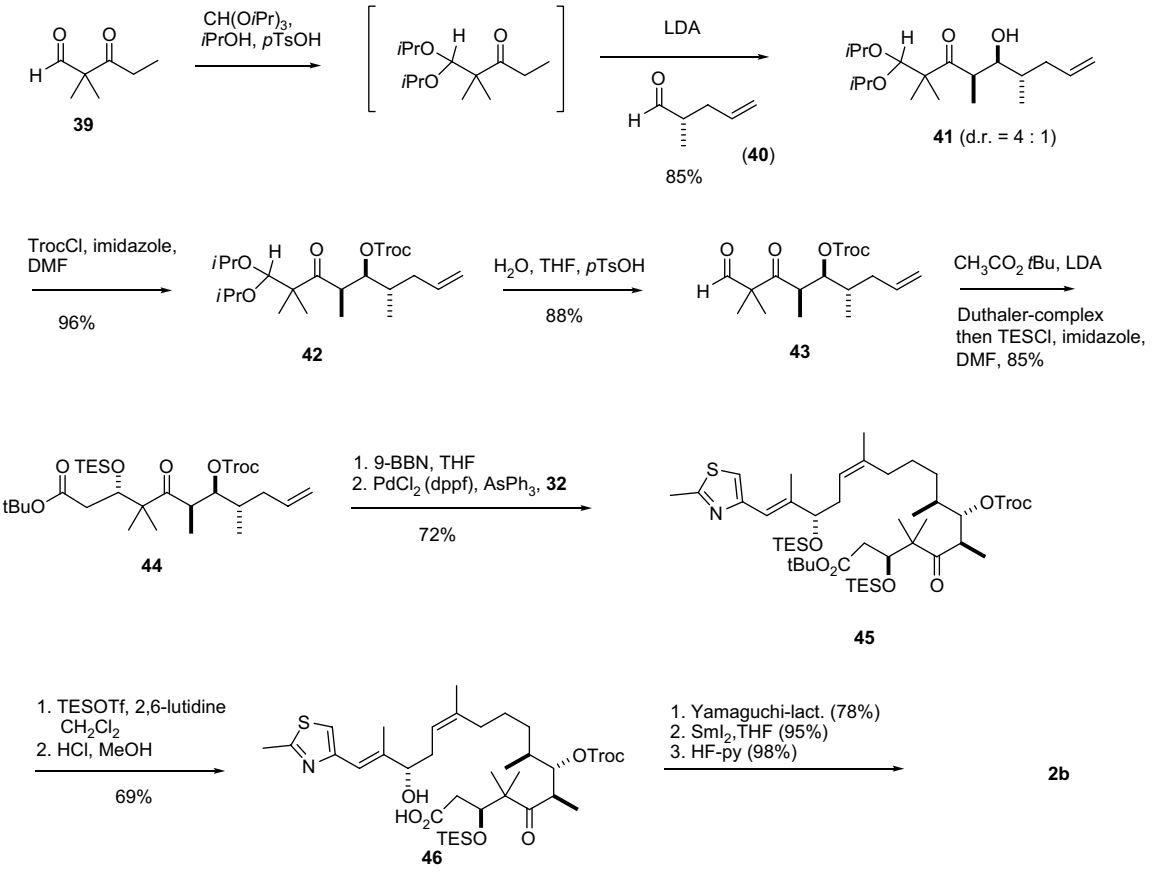
Scheme 6. Danishefsky's macrolactonization synthesis of epothilones A and C.



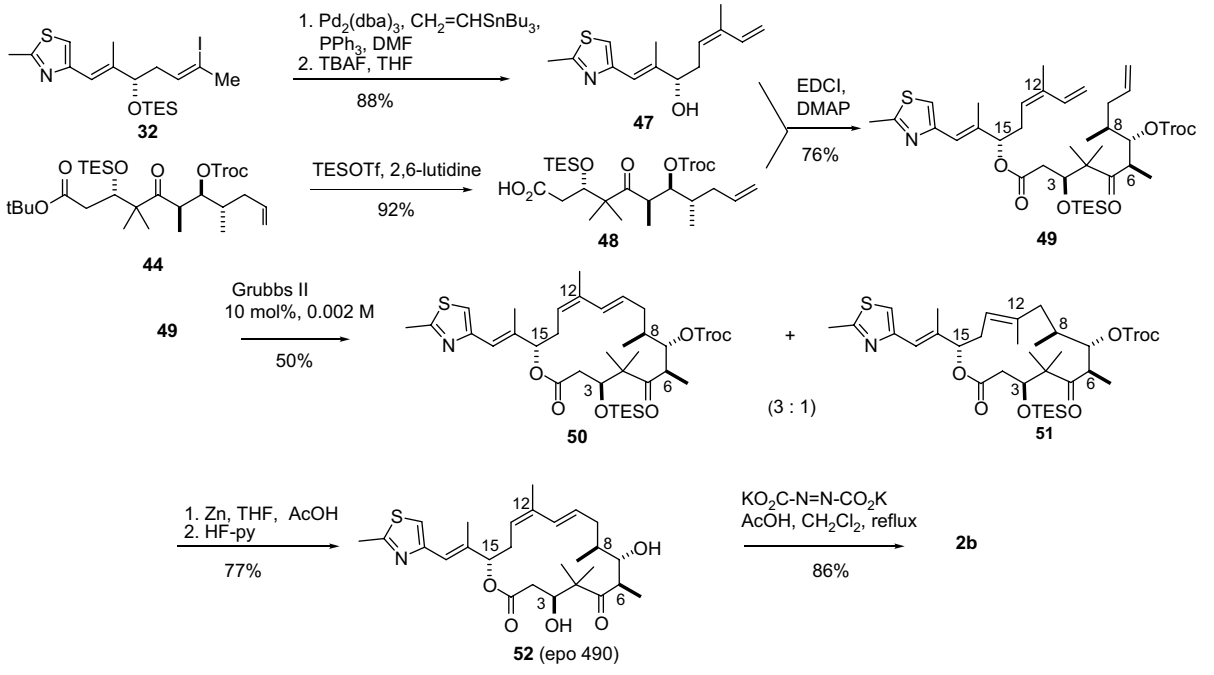
Scheme 7. Danishefsky's first RCM synthesis of epothilones A–D.



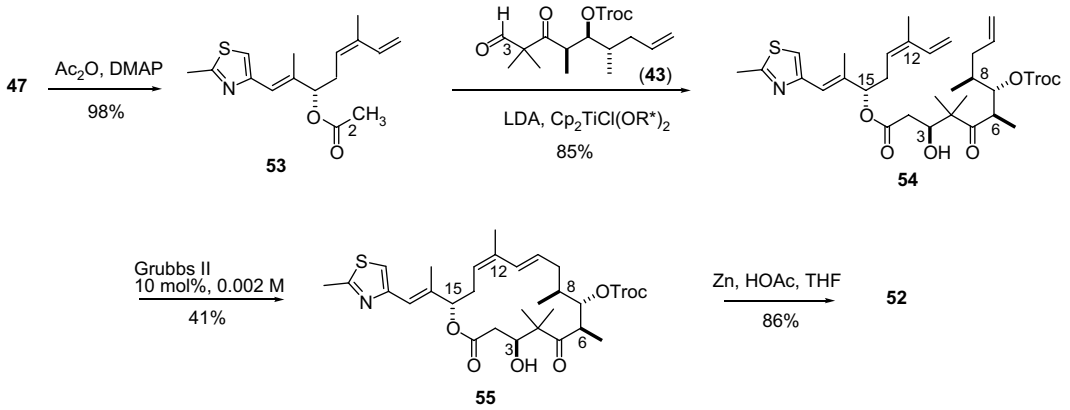
Scheme 8. Danishefsky's B-alkyl-Suzuki approach to epothilone B, part I.



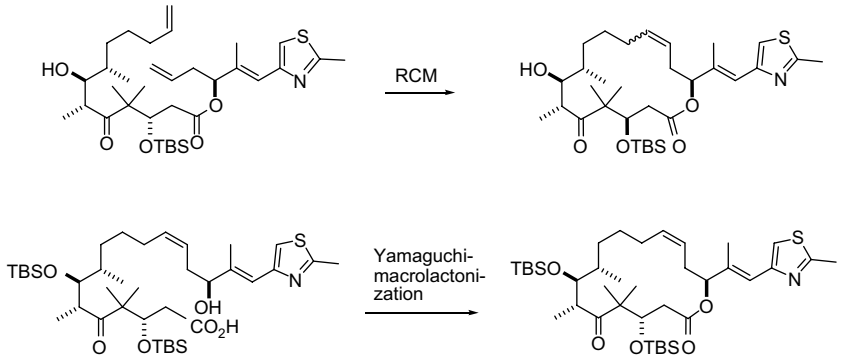
Scheme 9. Danishefsky's B-alkyl-Suzuki approach to epothilone B, part II.



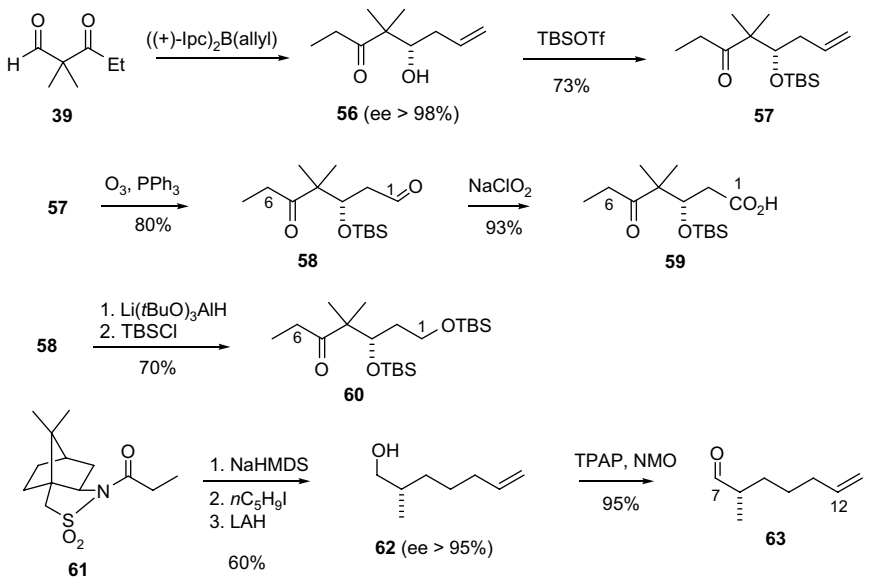
Scheme 10. Danishefsky's second RCM synthesis of epothilone D.



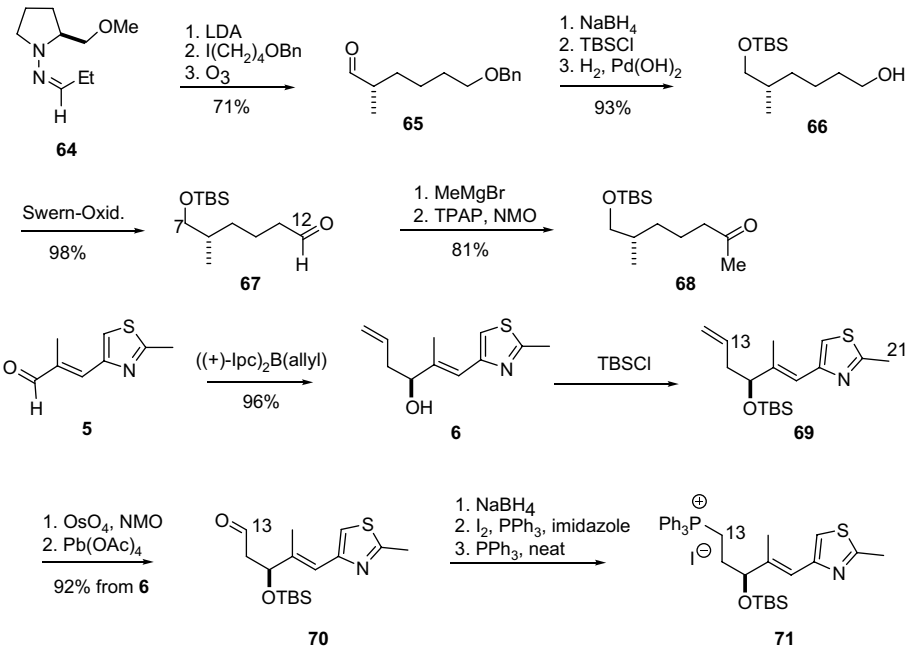
Scheme 11. Danishefsky's third RCM synthesis of epothilone D.



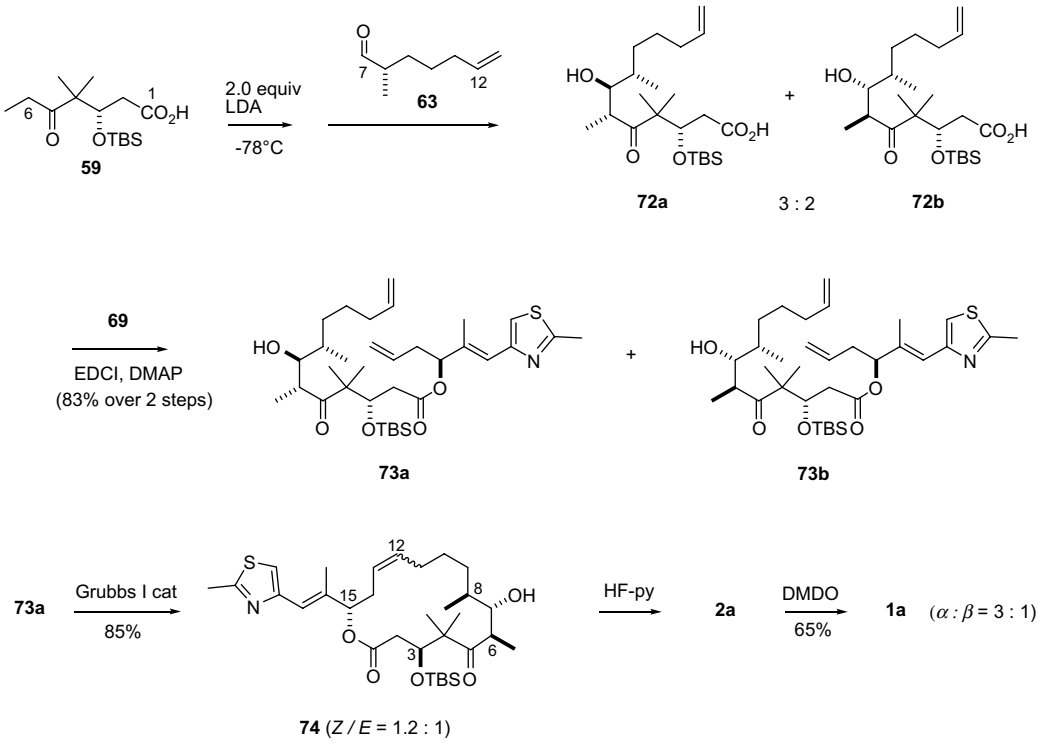
Scheme 12. Overview of Nicolaou's syntheses.



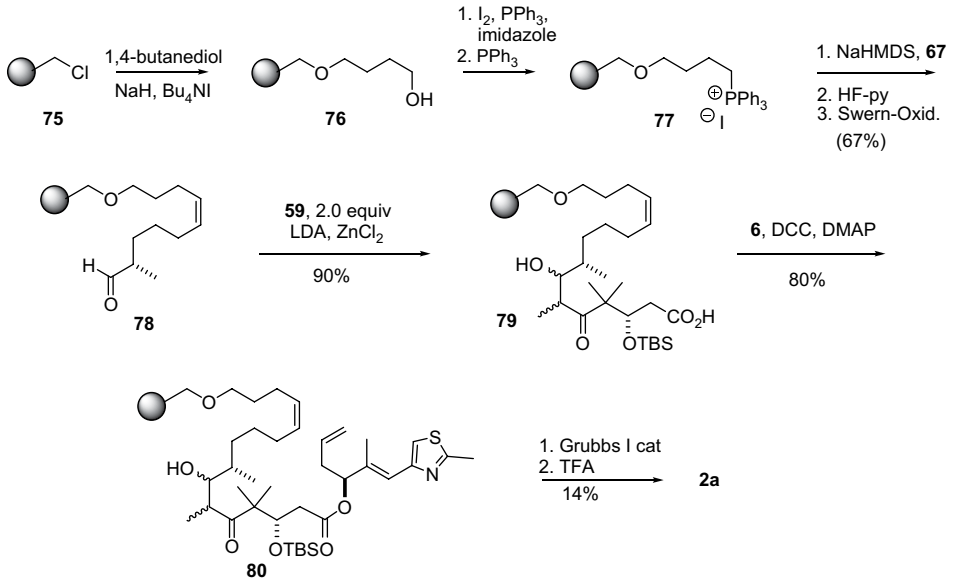
Scheme 13. Nicolaou's synthesis of epothilone building blocks.



Scheme 14. Nicolaou's synthesis of epothilone fragment **71**.



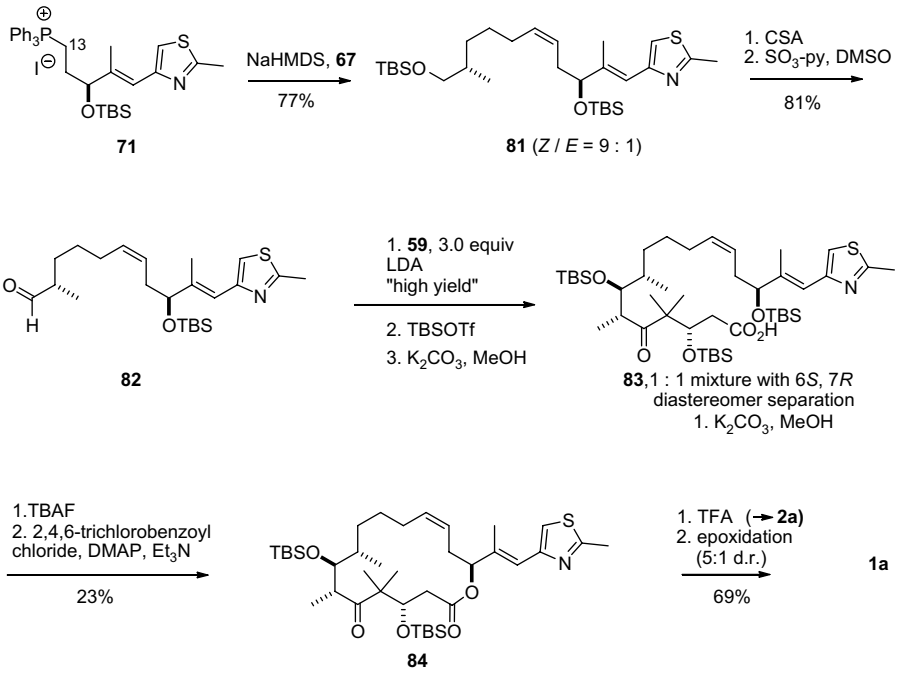
Scheme 15. Nicolaou's RCM synthesis of epothilones A and C.



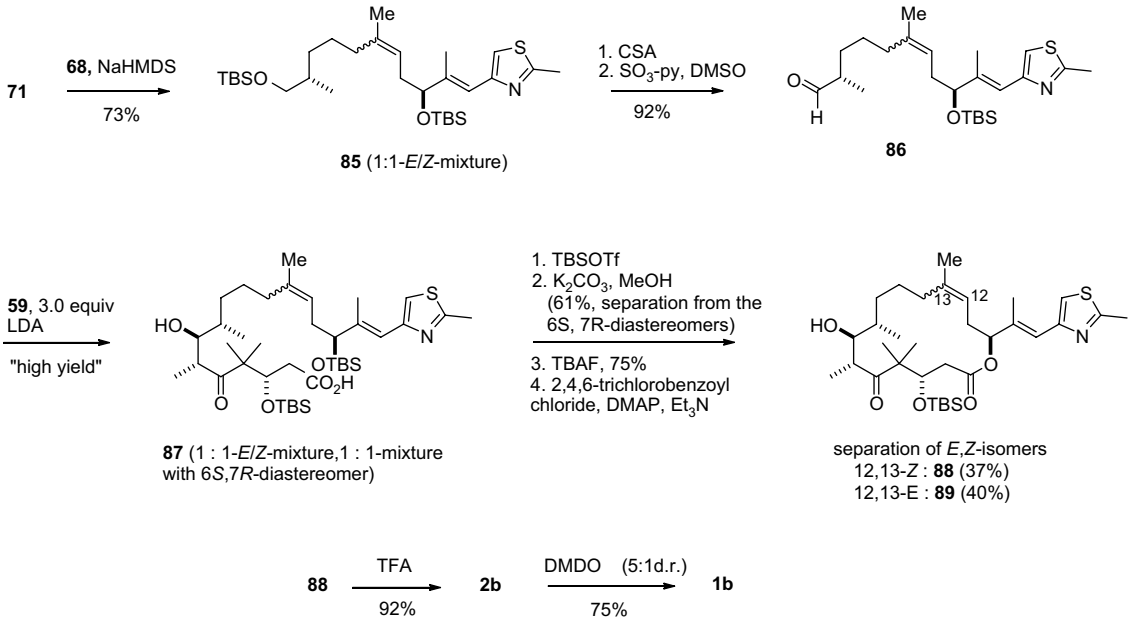
Scheme 16. Nicolaou's solid phase synthesis of epothilone C.

ring opening to iodide **15** gave the acyclic C3–C9-fragment **16** after deiodination. Elaboration into the C3–C11-fragment **18** and B-alkyl-Suzuki coupling with vinyl iodide **9** completed the epothilone A/C seco-intermediate **20** (Scheme 5). Macroaldolization gave **21** with a 6:1 diastereomeric ratio at C3. Oxidation at C3 and desilylation led to **2a**. The regio- and stereoselective epoxidation of the C12,13-double bond to **1a** was achieved with DMDO [30].

A second approach to **2a** [31] used macrolactonization as the ring closing step (Scheme 6). Hence, acetal **18** was converted into the aldehyde, which was subjected to an aldol addition with *t*-Bu-acetate to give, after deprotection, compound **22** as a separable 2:1



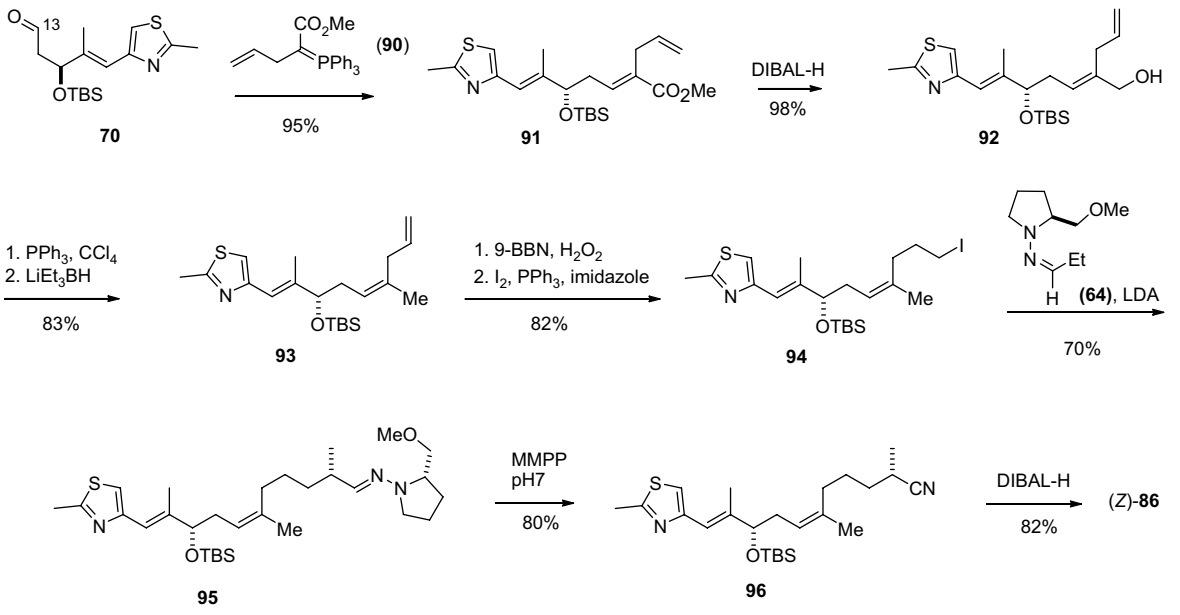
Scheme 17. Nicolaou's macrolactonization approach to epothilones A and C.



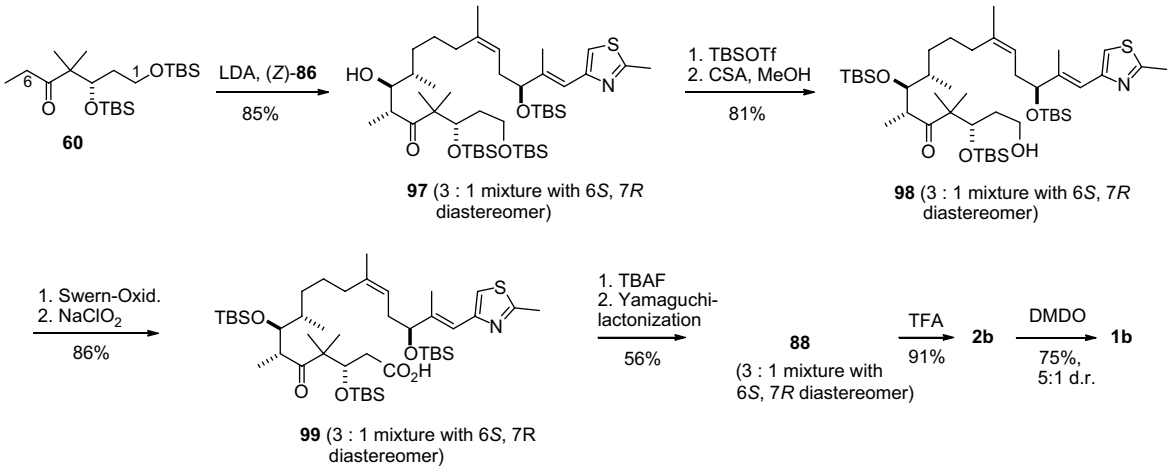
Scheme 18. Nicolaou's first synthesis of epothilones B and D.

diastereomeric mixture at C3. After oxidation and silylation **23** was obtained which was hydroborated and subjected to a Suzuki coupling with **9** to furnish seco-acid **24**. Yamaguchi lactonization and desilylation gave **2a**. Although this second route is four steps longer, the overall yield is about the same (23% *vs* 25%).

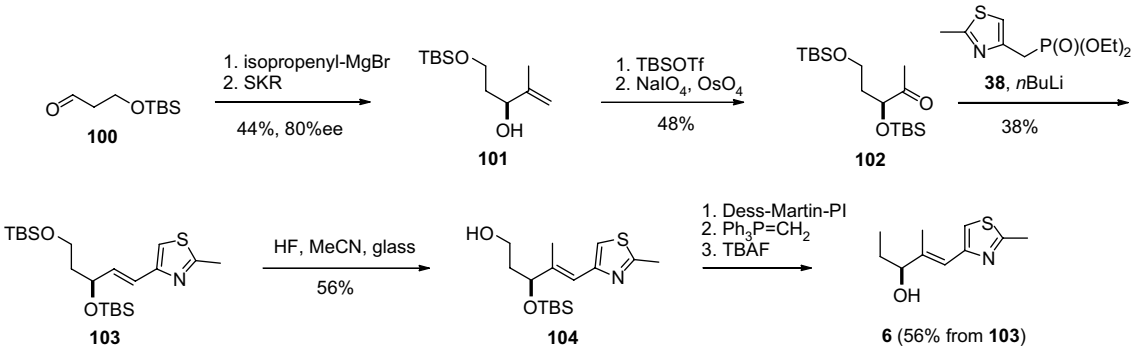
Ring closing metathesis (RCM) was tested as a third mode of macrocyclization [32], this time for both **2a** and **2b** (Scheme 7). In fact, the epothilones were the first relatively complex substrates for an RCM cyclization. In the event, intermediate **16** was converted into C9-aldehyde **25**, which after chain elongation gave olefinic aldehydes **26a**, **26b**. Aldol addition with **7** furnished **27a**, **27b** as 1:1 diastereomeric mixtures at C3. The configuration at C3 was rectified to (3*S*) *via* an oxidation–reduction sequence after which some



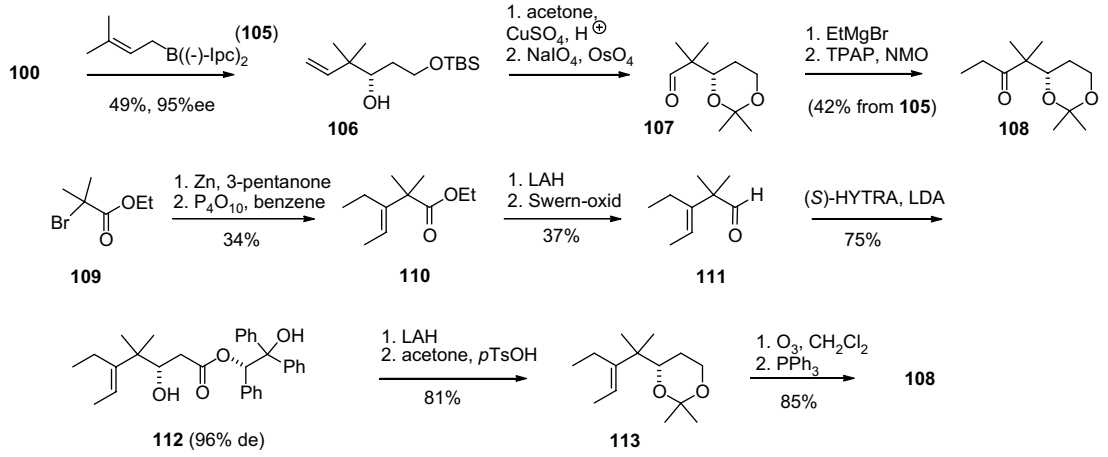
Scheme 19. Nicolaou's synthesis of key intermediate (*Z*)-**86**.



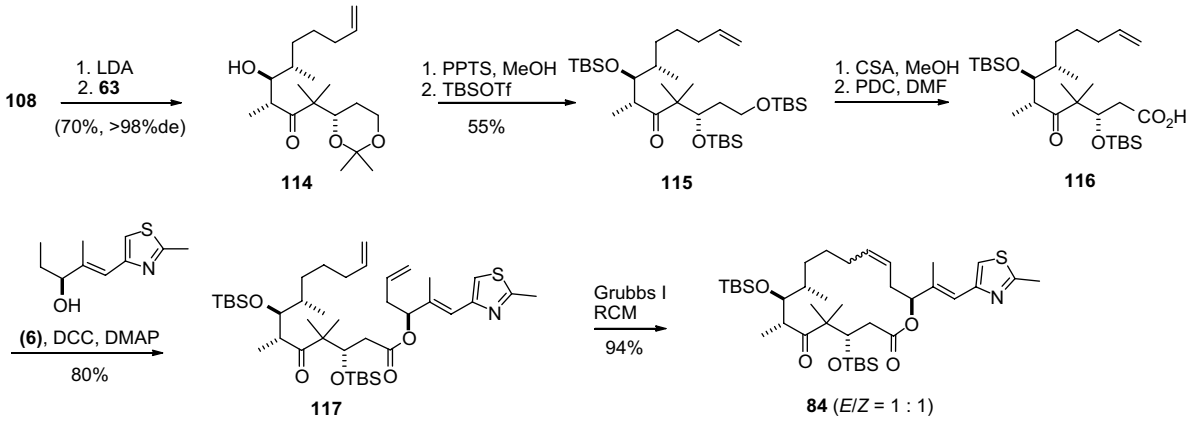
Scheme 20. Nicolaou's second synthesis of epothilones B and D.



Scheme 21. Schinzer's synthesis of epothilone A, part I.



Scheme 22. Schinzer's synthesis of epothilone A, part II.

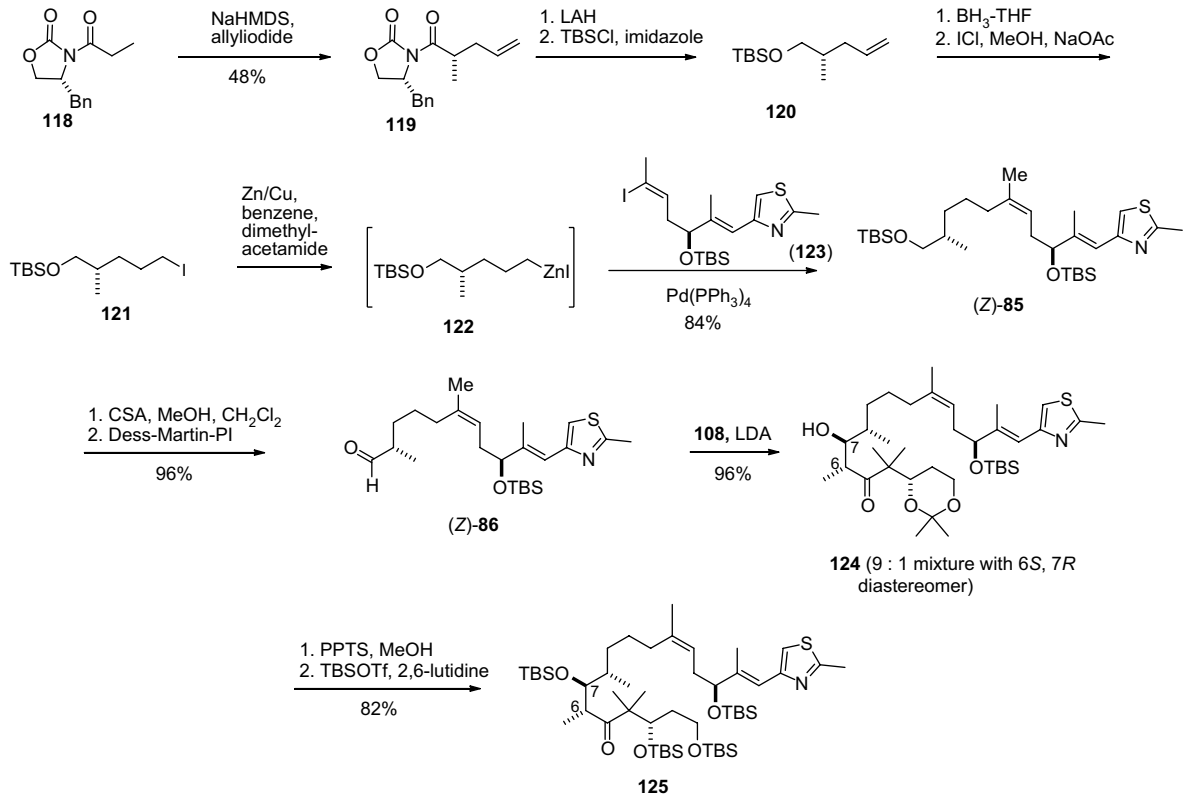


Scheme 23. Schinzer's synthesis of epothilone A, part III.

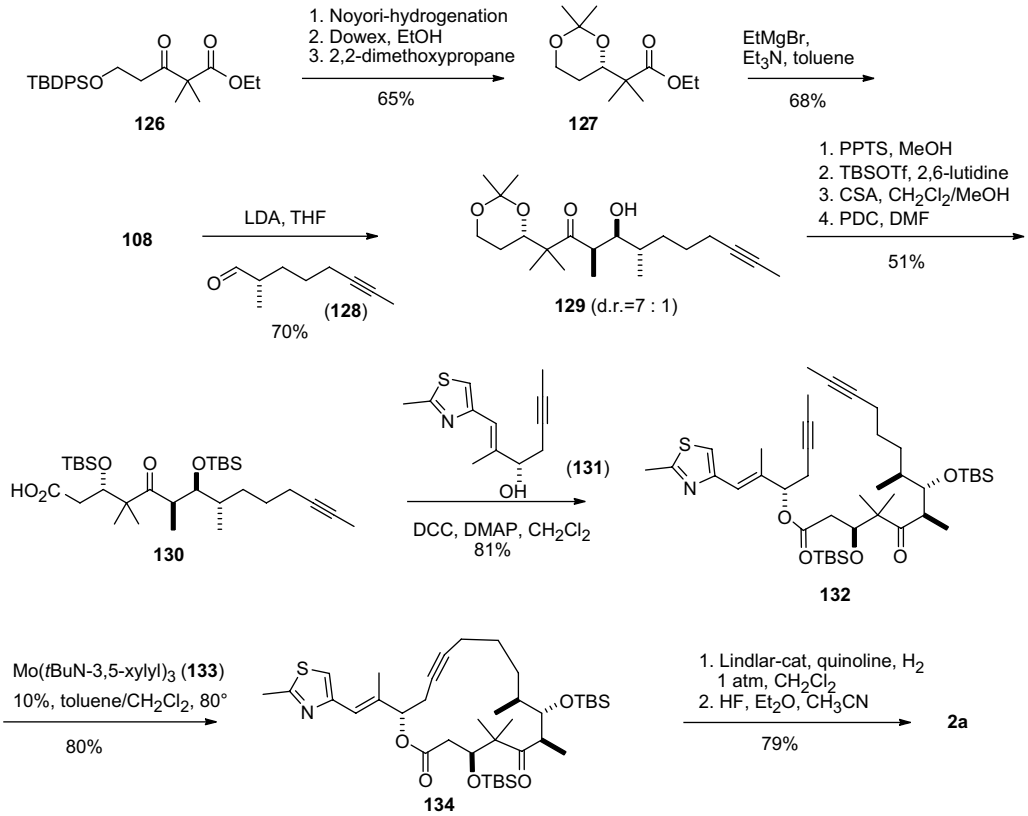
further modifications led to the diastereomerically pure ketones **28a**, **28b**. Epothilone A precursor **28a** was cyclized with Grubbs' first generation RCM catalyst to **29** as a 1:1.7-*E*/*Z*-mixture which was separated and desilylated to give **2a**. In contrast, the RCM of epothilone B intermediate **28b** had to be performed with Schrock's catalyst to furnish **30** as a 1:1-*E*/*Z*-mixture. Separation and desilylation gave **2b**.

In an updated approach, OTES-protected vinyl iodide **32** was prepared from **31** (Scheme 8) [33] or, better, via Evans' allylation of **33** with di-iodide **34**.

For the B-alkyl-Suzuki coupling olefinic ester **44** was prepared *via* an aldol addition between aldehyde **40** and the isopropyl acetal of ketone **39**, which gave **41** with moderate diastereoselectivity (Scheme 9). Isomer **41** was separated, converted into aldehyde **43**,



Scheme 24. Schinzer's synthesis of epothilone B.



Scheme 25. Fürstner's alkyne metathesis approach to epothilone C.

which was subjected to a Duthaler aldol addition to give **44** with high stereocontrol. Suzuki coupling of **44** with vinyl iodide **32** furnished seco-intermediate **45**. Deprotection gave the seco-acid **46** which was macrolactonized and deprotected to **2b**.

A variation of the original RCM approach (Scheme 10) [33] was initiated by preparing the terminal olefins **47** and **48** from **32** and **44**, respectively, and connecting them *via* esterification to the seco-intermediate **49** which on RCM with Grubbs' second generation catalyst gave a 3:1 mixture of the desired macrocycle **50** and the ring-contracted derivative **51**. After separation, **50** was deprotected to give epothilone 490 (**52**) as a highly active non-natural epothilone derivative. Selective hydrogenation of the C10,11-double bond with diimide led to **2b**.

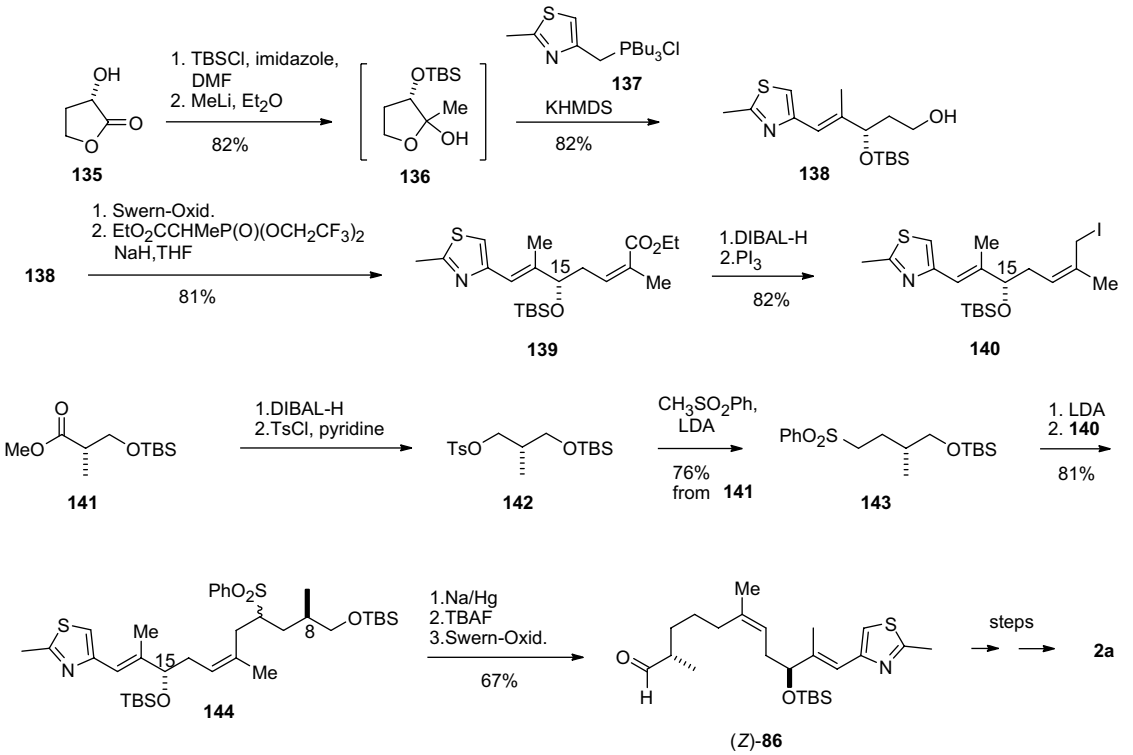
In a second approach to **52** (Scheme 11) the Duthaler aldol addition of acetate **53** to aldehyde **43** was used. Compound **54** was obtained stereoselectively and macrocyclized by RCM to furnish **55**. In this case, no ring contraction was observed. After removal of the Troc-group **52** was obtained.

5.2. *Nicolaou syntheses (Schemes 12–20)*

The Nicolaou group was second to complete total syntheses of both **1a** and **1b**. Their strategy (cf. Scheme 12) was focused on an aldol addition between a C6 ketone enolate (as in **57**, **59**) and a C7 aldehyde **63**. The C12,13-double bond was formed *via* metathesis in the A series [34] and *via* Wittig olefination in the B series [35].

The preparation of the building blocks was different from the Danishefsky approach. Thus (Scheme 13), the C1–C6 fragment **59** was prepared *via* a regio- and enantiocontrolled Brown allylation of aldehyde **39** to form **57** after silylation. Ozonolysis led to **58** which was either oxidized to carboxylic acid **59** or reduced to diol **60**. The C7–C12-aldehyde **63** was obtained *via* alcohol **62** by an alkylation with Oppolzer's sultam **61**.

An alternative route to the C7–C12-fragment (Scheme 14) started with an Enders alkylation of **64** to form **65** enantioselectively, which was converted into aldehyde **67** (epothilone A series) and ketone **68**



Scheme 26. Mulzer's synthesis of epothilone D.

(epothilone B series), respectively. The C13–C21-phosphonium iodide **71** was prepared *via* a Brown allylation of aldehyde **5** to **6**, from which **71** was obtained in five steps.

Nicolaou's first route to **1a** [34] started with a non-stereoselective aldol type addition of the dianion of **59** to aldehyde **63** (Scheme 13). RCM of olefin **73a** with Grubbs' first generation catalyst furnished an *E*/*Z*-mixture of the macrolides **74** which was separated and desilylated to give **2a**. Epoxidation with DMDO as before led to **1a**.

The RCM approach was also used for a solid phase supported synthesis of **2a** (Scheme 16) [36]. In this modification, the RCM served to disconnect substrate **80** from the solid support by a cyclorelease process.

As an alternative to the RCM, Yamaguchi macrolactonization was also used for the synthesis of **1a**, **1c** (Scheme 17) [34]. Thus, Wittig reaction of phosphonium salt **71** with aldehyde **67** gave a 9:1-*Z*/*E*-mixture of olefins **81**. Conversion into aldehyde **82** followed by aldol addition with keto acid **59** led to a 1:1 diastereomeric mixture of aldol adducts **83**, which were separated, deprotected and cyclized to **84** by Yamaguchi lactonization.

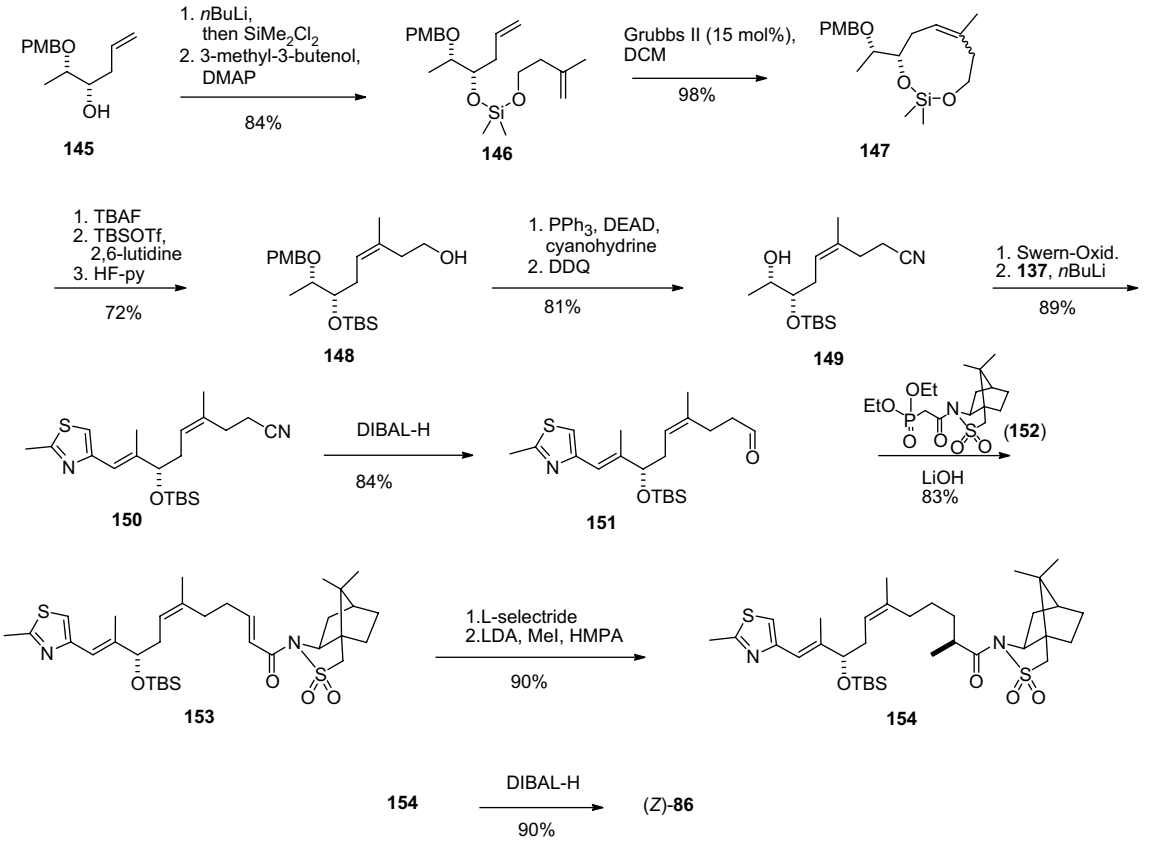
A related protocol was also applied to the synthesis of **1b**, **2b** (Scheme 18) [35]. The only modification was the unselective Wittig reaction of **71** with ketone **68** to give **85** as a 1:1-*E*/*Z*-mixture which was converted to aldehyde **86**.

To improve the stereocontrol of this sequence [37,38] aldehyde **86** was prepared as a pure (*Z*)-olefin (Scheme 10) *via* an *E*-selective Wittig olefination of aldehyde **70** with phosphorane **90**.

Aldol addition of (*Z*)-**86** with ketone **60** (Scheme 20) furnished a 3:1 diastereomeric mixture of **97** in favor of the desired 6*R*,7*S*-diastereomer. Without separation, the 7-OH group was silylated, the primary alcohol at C1 was deprotected selectively and oxidized to the acid **99**. Selective deprotection of the 15-OTBS group followed by Yamaguchi lactonization gave **88** which was separated into the diastereomers. Deprotection gave **2b** and epoxidation led to **1b** eventually.

5.3. Schinzer synthesis

Schinzer's group was chronologically third to complete syntheses of both **1a**, **1b** and **2a**, **2b**. The preparation of the key fragments was different from the Danishefsky and Nicolaou approaches [39]. For instance the C21–C13-fragment **6** was synthesized from aldehyde **100** (Scheme 21). The C1–C5-acetonide **108** was available from **100** *via* Brown allylation or from



Scheme 27. Mulzer's silicon tethered RCM approach to aldehyde (*Z*)-**86**.

a Reformatsky addition of bromoester **109** to 3-pentanone (Scheme 22).

A major innovation was the acetonide moiety in **108** which turned out to be beneficial for the aldol addition to aldehyde **63** (Scheme 23). In contrast to Nicolaou's procedure a high selectivity in favor of adduct **114** was achieved. The rest of the synthesis [40] closely resembles Nicolaou's RCM approach.

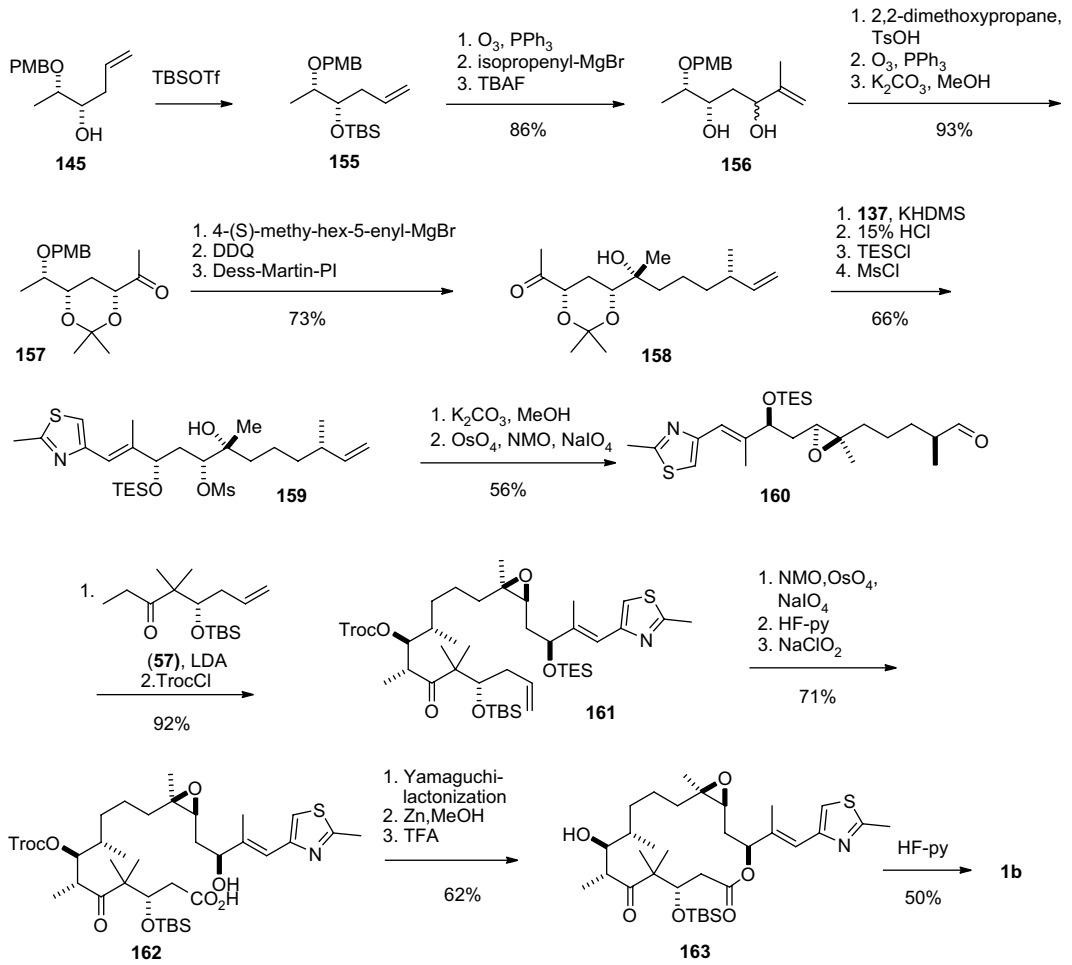
Schinzer's synthesis of **1b/2b** (Scheme 24) [41] was based on a Zn-modification of Danishefsky's B-alkyl-Suzuki coupling to prepare pure (*Z*)-aldehyde **86**. The aldol addition of (*Z*)-**86** to ketone **108** led to adduct **124** with a d.r. of 9:1. Acetonide hydrolysis followed by global *O*-silylation delivered Nicolaou's intermediate **125**.

5.4. Fürstner's alkyne RCM [42]

The early RCM approaches to **1** by Danishefsky, Nicolaou and Schinzer were all flawed by the missing *E*/*Z*-selectivity. In their synthesis of **2a**, Fürstner et al. (Scheme 25) applied an alkyne RCM approach to generate macrolide **134** which was converted into the (*Z*)-olefin exclusively *via* Lindlar hydrogenation. The synthesis started with Schinzer's ketone **108**, which was used for an aldol addition with aldehyde **128**, to give adduct **129** with good selectivity. Esterification of acid **130** with alcohol **131**, prepared from Nicolaou's intermediate **6** *via* Corey–Fuchs homologation to introduce the alkyne moiety, gave seco-intermediate **132**. The RCM was achieved in good yield with Mo–catalyst **133**.

5.5. Mulzer syntheses

The Mulzer group has published three different approaches to **1b**, **2b**. The first one (Scheme 26) [43], although it was carried through to the final target, is a formal one and aims for an easier access to Nicolaou's compounds **60** and (*Z*)-**86** (Scheme 19). Mulzer's contribution lies in a facile introduction of the stereogenic center at C3 in ketone **60** via a Kiyooka type aldol addition [44], and a stereocontrolled synthesis of the (*Z*)-C12,13-olefin moiety in aldehyde (*Z*)-**86**.

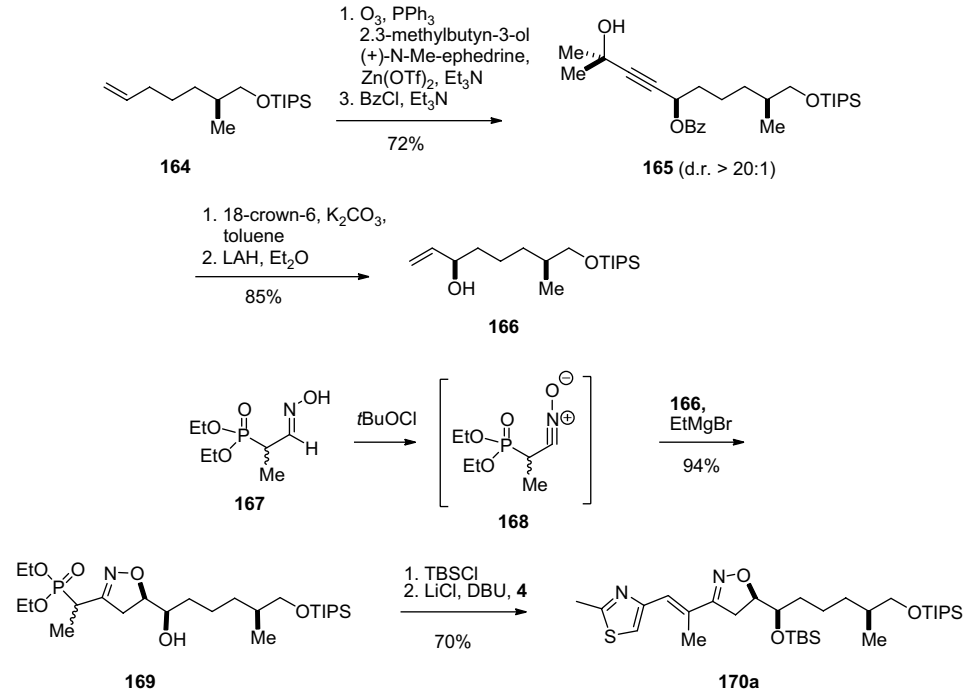


Scheme 28. Mulzer's early epoxide approach to epothilone B.

Thus, commercially available hydroxyl lactone **135** *via* hemiketal **136** and Wittig olefination with **137** gave alcohol **138**. These steps have subsequently also been used by Ley [45] (Scheme 43). For the introduction of the (*Z*)-olefin a Still-Gennari olefination was employed to give enoate **139** which was converted to iodide **140**. Sulfone **143** was obtained in good yield from Roche's ester **141** *via* tosylate **142**. Alkylation of **143** with iodide **140** furnished **144** which was desulfonylated and converted to (*Z*)-**86**, in which both stereogenic centers at C15 and C8 have thus been derived from the chiral carbon pool.

In an alternative approach to (*Z*)-**86** [46] a silicon tethered RCM reaction of di-olefin **146**, easily available from known alcohol **145** was employed (Scheme 27). Cycloolefin **147** was obtained as an easily separable 5:1-*Z*/*E*-mixture which was converted to aldehyde (*Z*)-**86** as shown.

Whereas these two approaches led to **2b** first, the third approach furnished **1b** directly (early epoxide approach, Scheme 28) [47]. By this concept, which was later adopted in Carreira's synthesis, the low-yielding nonselective and potentially hazardous epoxidation of **2b** with peroxides was avoided. The synthesis started with the chain elongation of intermediate **155** to **156**, and its conversion into ketone **157** which originally was obtained as an epimeric mixture. Base catalyzed epimerization gave diastereomerically pure **157**. A chelate-Cram induced Grignard addition, followed by PMB deprotection and oxidation furnished tertiary alcohol **158** selectively from which mesylate **159** was easily available. Base induced cyclization gave the desired epoxide from which aldehyde **160** was obtained after ozonolysis. The conversion into **1b** follows the established aldol addition–macrolactonization strategy.

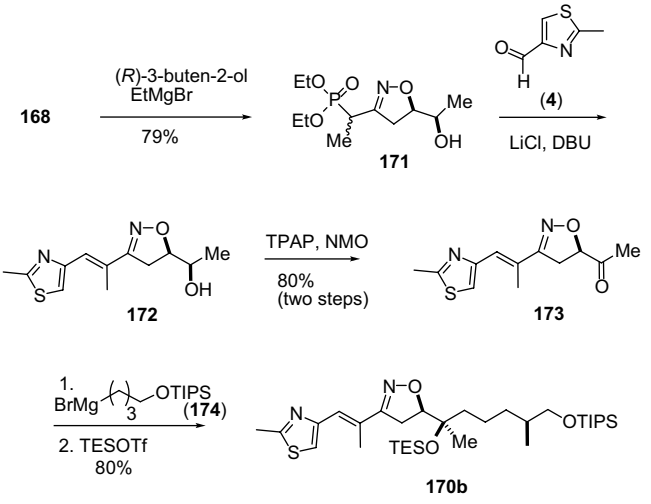


Scheme 29. Carreira's synthesis of epothilones A and B, part I.

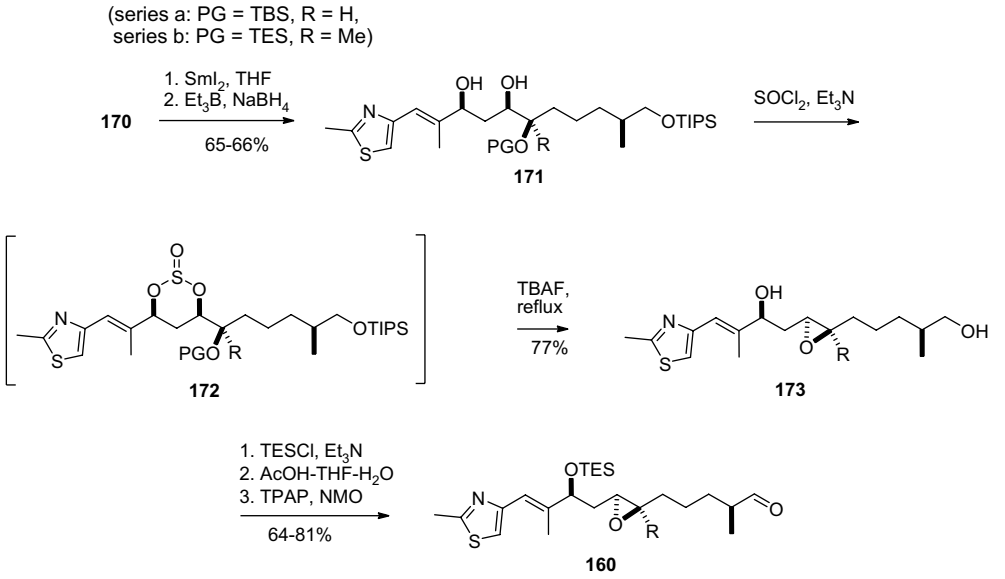
5.6. *Carreira's synthesis [48,49]*

The Carreira group has also opted for Mulzer's "early epoxide" approach [47]. Moreover, they developed a highly innovative nitrile–oxide–olefin cycloaddition to establish the C12–C15 section in both the epothilone A and B series. Thus (Scheme 29), olefin **164** was oxidized to the aldehyde and treated with 3-methylbutyn-3-ol under asymmetric catalysis to give **165** with high selectivity. Additions of this type have been developed earlier in the group. Removal of the terminal tertiary alcohol and reduction gave the vinyl alcohol **166** which was subjected to a stereocontrolled 1,3-dipolar cycloaddition with nitriloxide **168**, prepared in situ from oxime **167**. The resulting isoxazoline **169** was olefinated with aldehyde **4** to give the C21–C7-epothilone A fragment **170a**.

The corresponding epothilone B intermediate **170b** (Scheme 30) was obtained starting from the addition



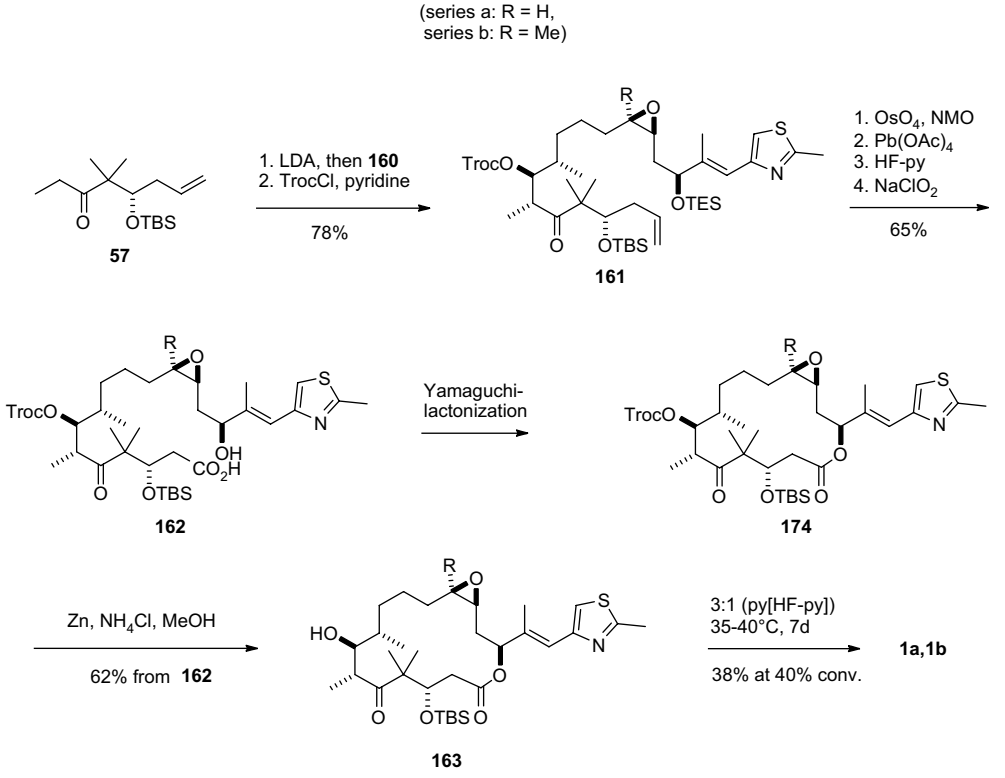
Scheme 30. Carreira's synthesis of epothilone B, part II.



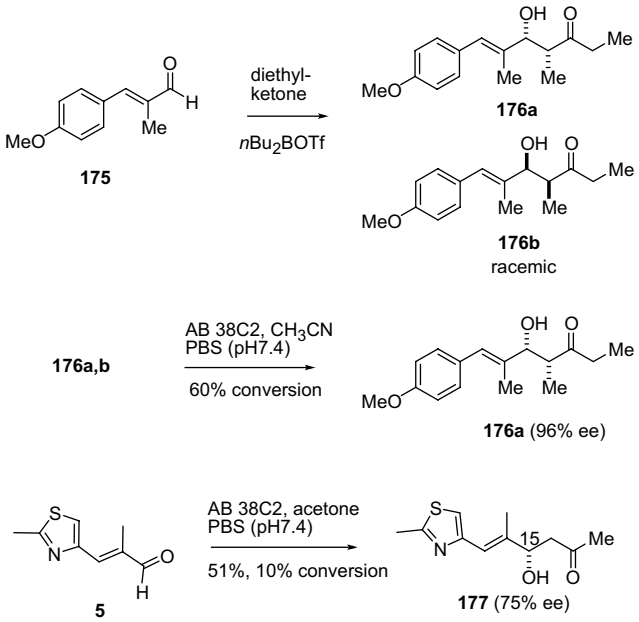
Scheme 31. Carreira' synthesis of epothilones A and B, part III.

of **168** to (*R*)-3-buten-2-ol. Adduct **171** was converted to ketone **173**. Chelate-Cram induced addition of Grignard derivative **174** gave **170b** selectively.

In parallel sequences (Schemes 31 and 32) intermediates **170a**, **170b** were processed towards the formation of **1a**, **1b**. Specifically, the isoxazoline ring was cleaved reductively to give triol **171**, which was converted into epoxide **173** *via* the cyclic sulfite **172**. Ditesylation followed by selective mono-detesylation and oxidation of the primary OH-function gave



Scheme 32. Carreira's synthesis of epothilones A and B, part IV.

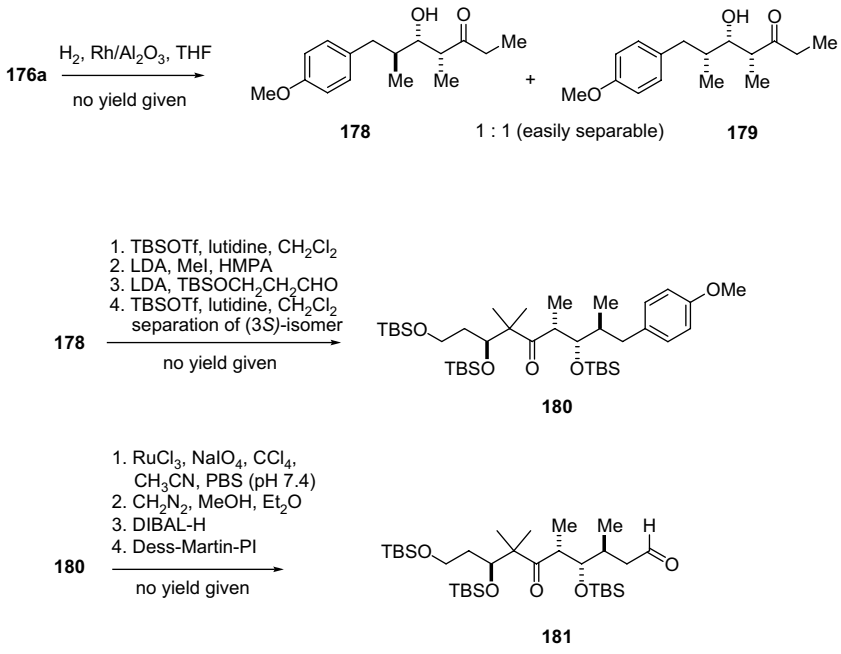


Scheme 33. Sinha's synthesis of epothilone B, part I.

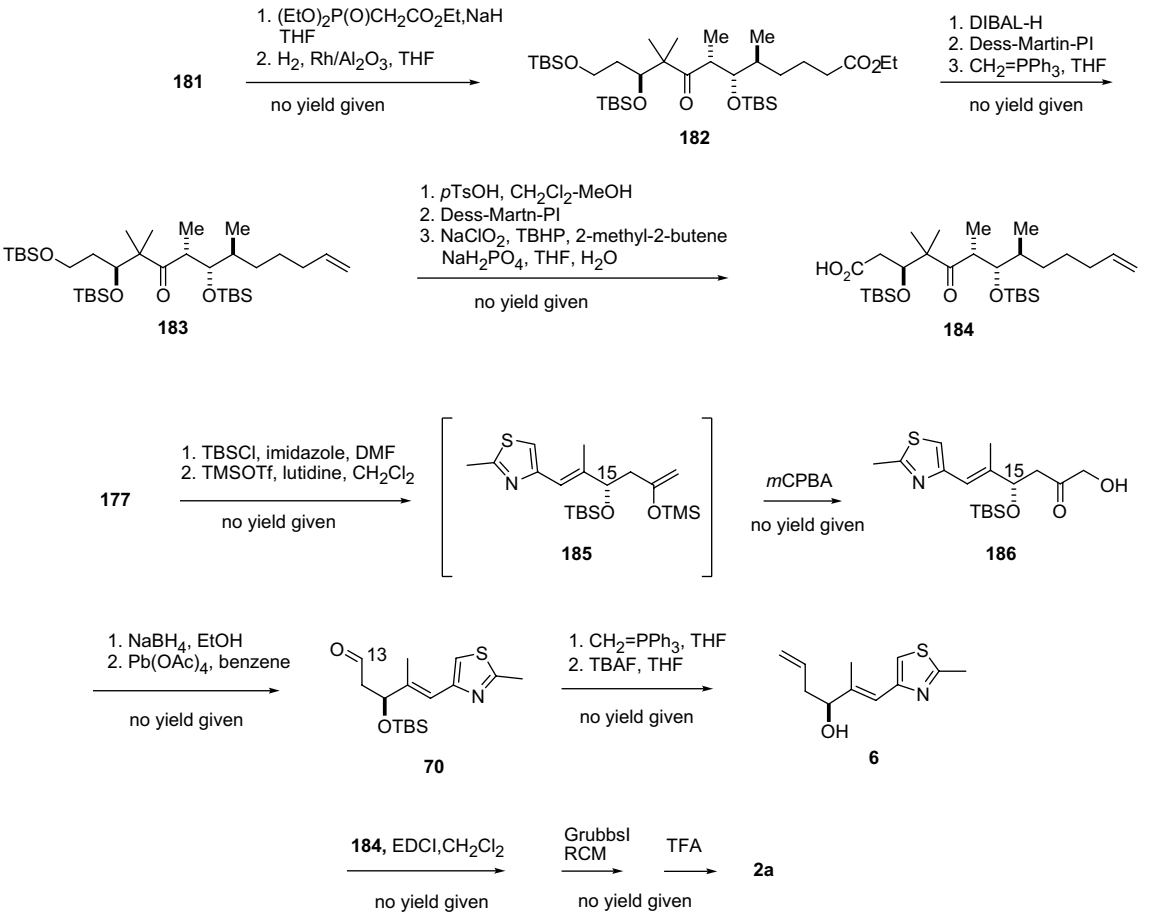
aldehyde **160**, which underwent a highly selective aldol addition with ketone **57** to give aldol adduct **161** after Troc-protection of the resulting C7-alcohol function. The endgame was modelled after Mulzer's synthesis (Scheme 28) [47] to provide **163** *via* seco-acid **162** and macrolactone **174**.

5.7. Sinha syntheses [50–53]

In their synthesis of **2a**, which incorporates modifications of both Nicolaou's RCM and Danishefsky's macrolactonization approach, the Sinha group has made use of "in-house" catalytic antibody



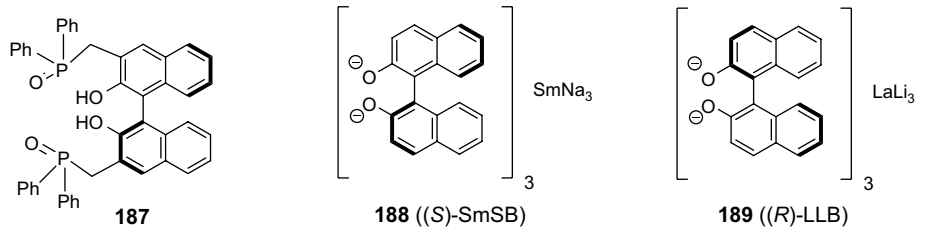
Scheme 34. Sinha's synthesis of epothilone B, part II.



Scheme 35. Sinha's synthesis of epothilone B, part III.

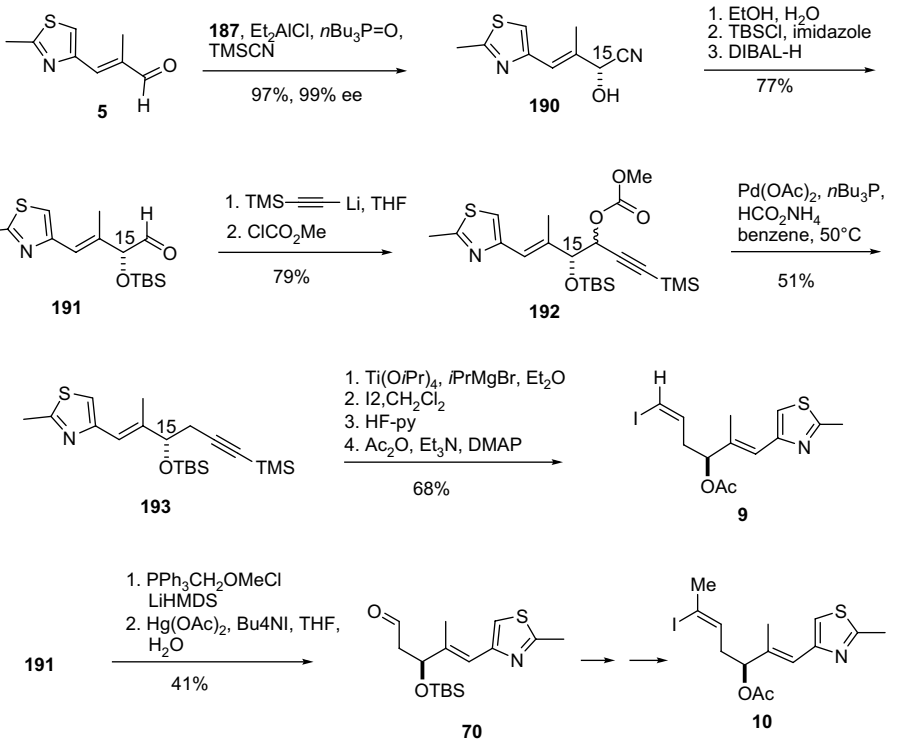
methodology (Schemes 33 and 34) [50]. Thus, a racemic mixture of aldol adducts **176a**, **176b** was prepared and subjected to retro-aldol cleavage catalyzed by antibody AB 38C2. Enantiomer **176a** was obtained in 96% ee. Conversely, aldehyde **7** was converted into adduct **177** *via* antibody catalyzed aldol addition with acetone, however, in low conversion and only 75% ee. Compound **177a** was converted into the C1–C10-segment of **1** *via* catalytic hydrogenation to give a separable mixture of diastereomers **178**, **179**. Pure **178**, after *O*-silylation and methylation, was subjected to an aldol addition to give after silylation and diastereomer separation intermediate **180** in unreported yield. The phenol ring was oxidized to the carboxylic acid and then converted into C1–C10-aldehyde **181** (Scheme 33).

Via Horner olefination and hydrogenation **181** was transformed into ester **182**, which was then elaborated *via* **183** into the olefinic carboxylic acid **184** (Scheme 35). Aldehyde **6** was prepared from **177** *via* enol ether **185** and hydroxyl ketone **186** and olefinated

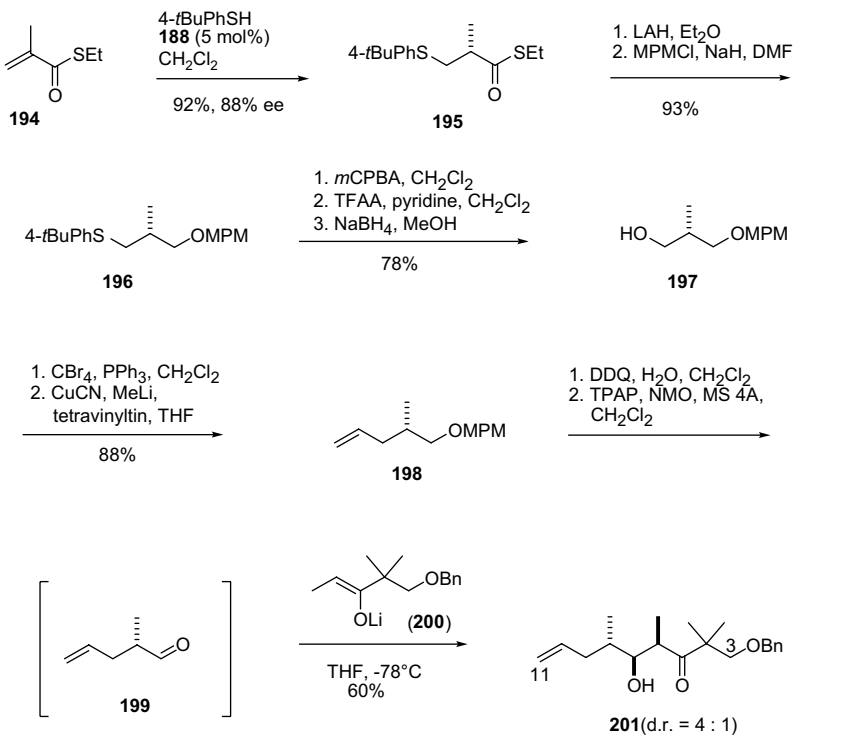


187 **188** ((*S*)-SmSB) **189** ((*R*)-LLB)

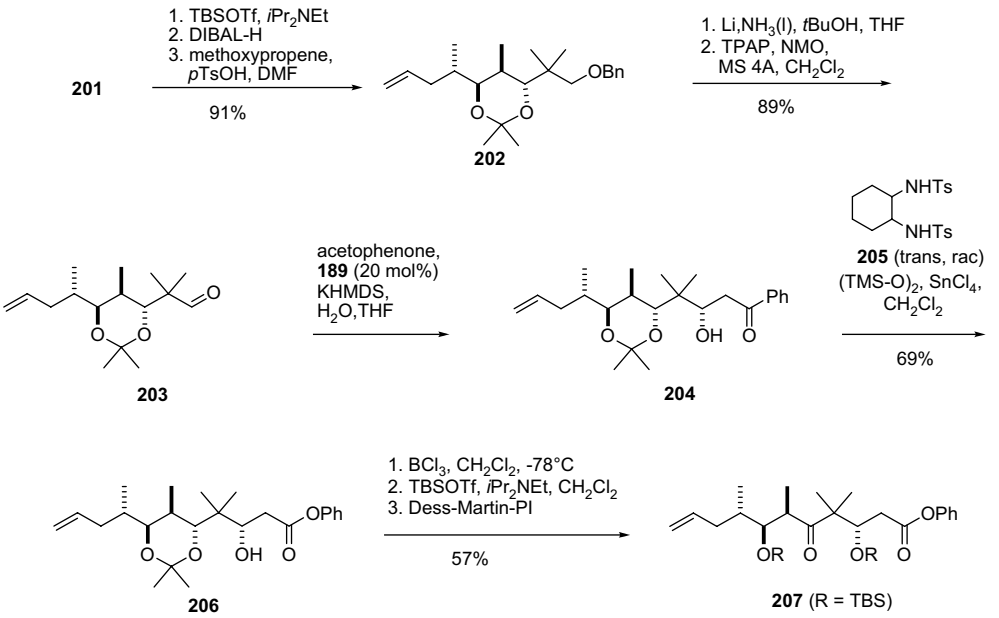
Fig. 4. Asymmetric catalysts from the Shibasaki group.



Scheme 36. Shibasaki's synthesis of epothilones C and D, part I.



Scheme 37. Shibasaki's synthesis of epothilones C and D, part II.

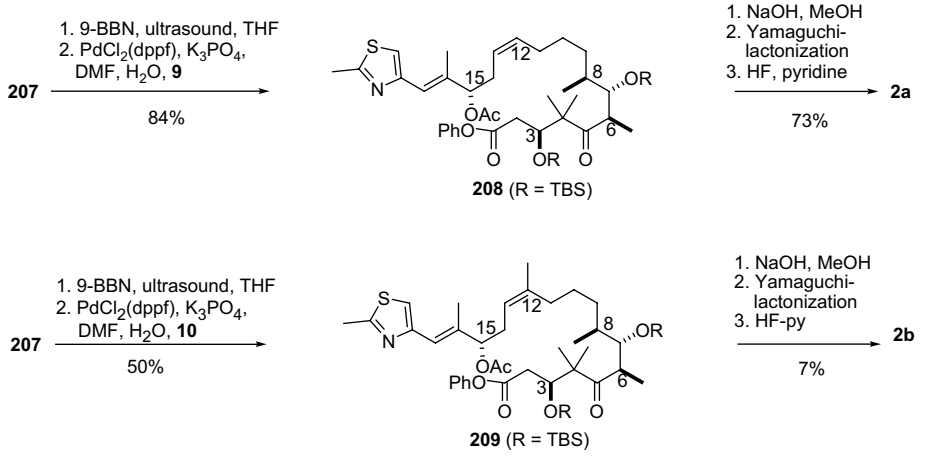


Scheme 38. Shibasaki's synthesis of epothilones C and D, part III.

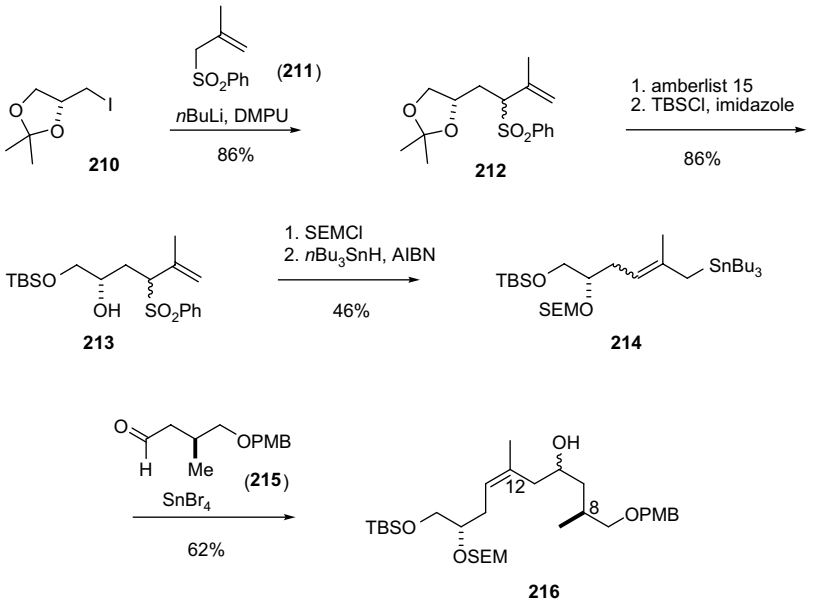
to alcohol **69**. Esterification with acid **184** followed by RCM and desilylation led to **2a**, in analogy to Nicolaou's earlier approach. Additionally, the Sinha group reported a synthesis of **1b** along an ''early epoxide approach'' [53].

5.8. Shibasaki approach [54–57]

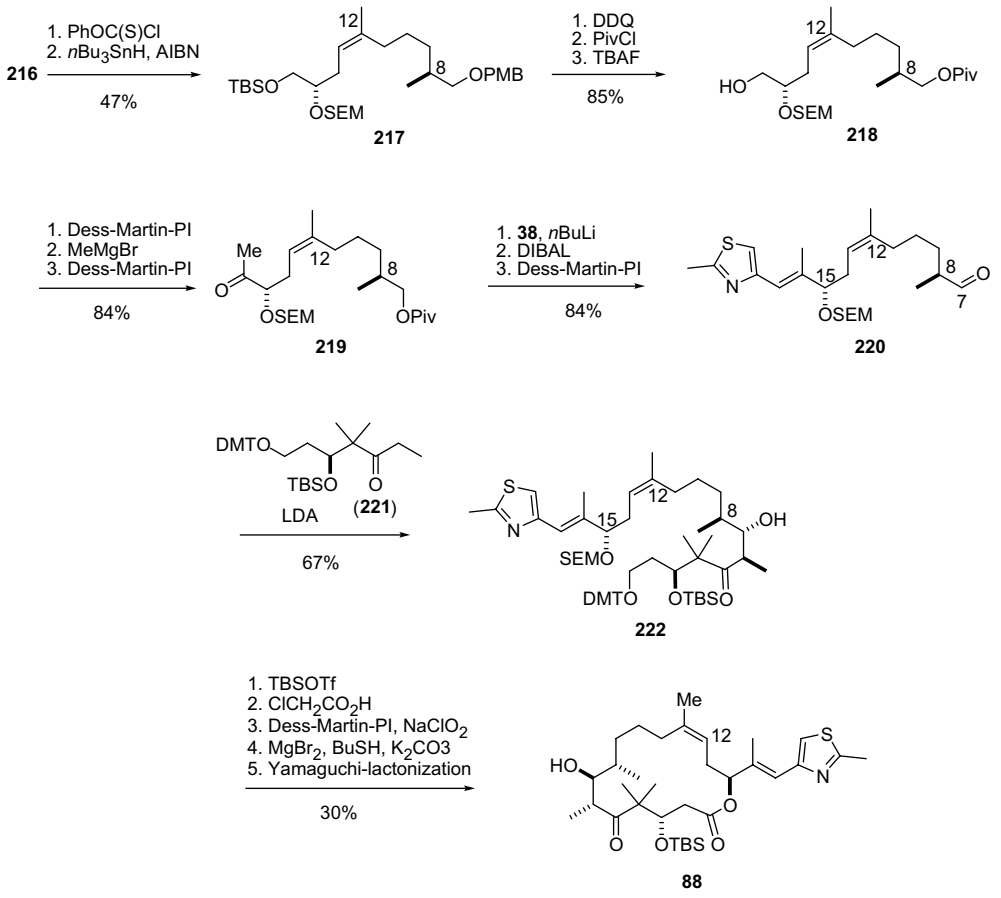
The Shibasaki group has developed a variety of chiral multifunctional catalysts (e.g. **187–189**, Fig. 4) which they apply to natural product synthesis [54]. Their epothilone A/B synthesis (Schemes 36–39) is an adaptation of Danishefsky's B-alkyl-Suzuki reaction of vinyl iodides **9**, **10** and terminal olefin **207** [55]. Thus (Scheme 36), aldehyde **5** underwent a highly enantioselective cyanosilylation with catalyst **187** [56] to form **190** which was converted into aldehyde **191** and then alkyne **193**. Hydromagnesiation–iodination of **193** was used to generate Danishefsky's epothilone A vinyl iodide **9** stereoselectively. The preparation of the epothilone B vinyl iodide **10** [57] involves a homologation of aldehyde **191** to Schinzer's



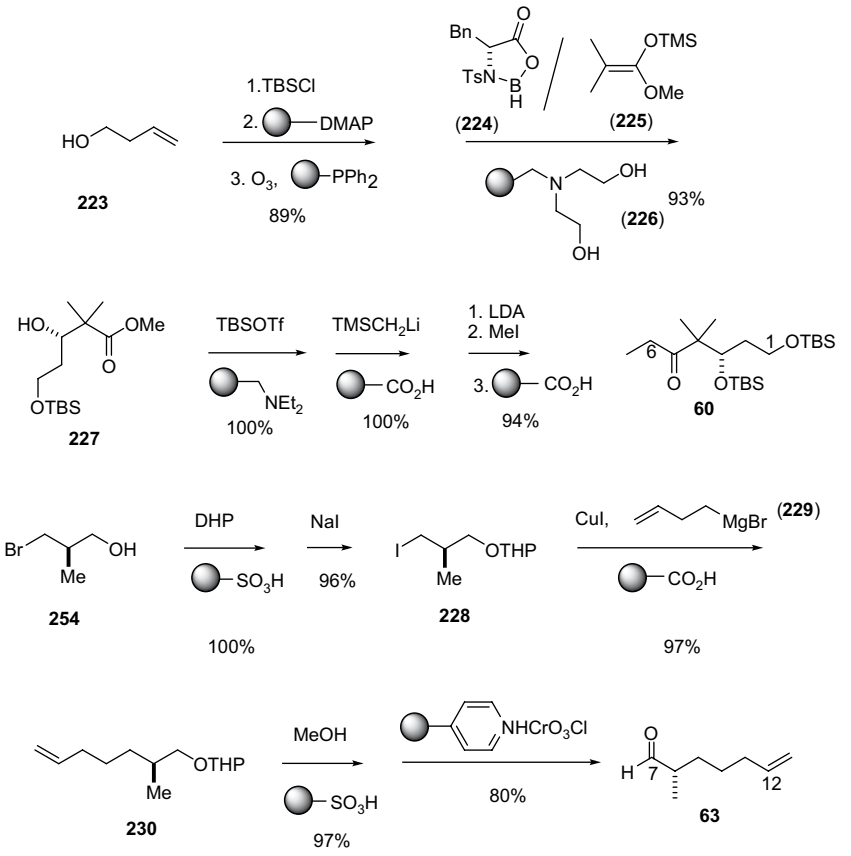
Scheme 39. Shibasaki's synthesis of epothilones C and D, part IV.



Scheme 40. E.J. Thomas's synthesis of epothilone D, part I.



Scheme 41. E.J. Thomas's synthesis of epothilone D, part II.



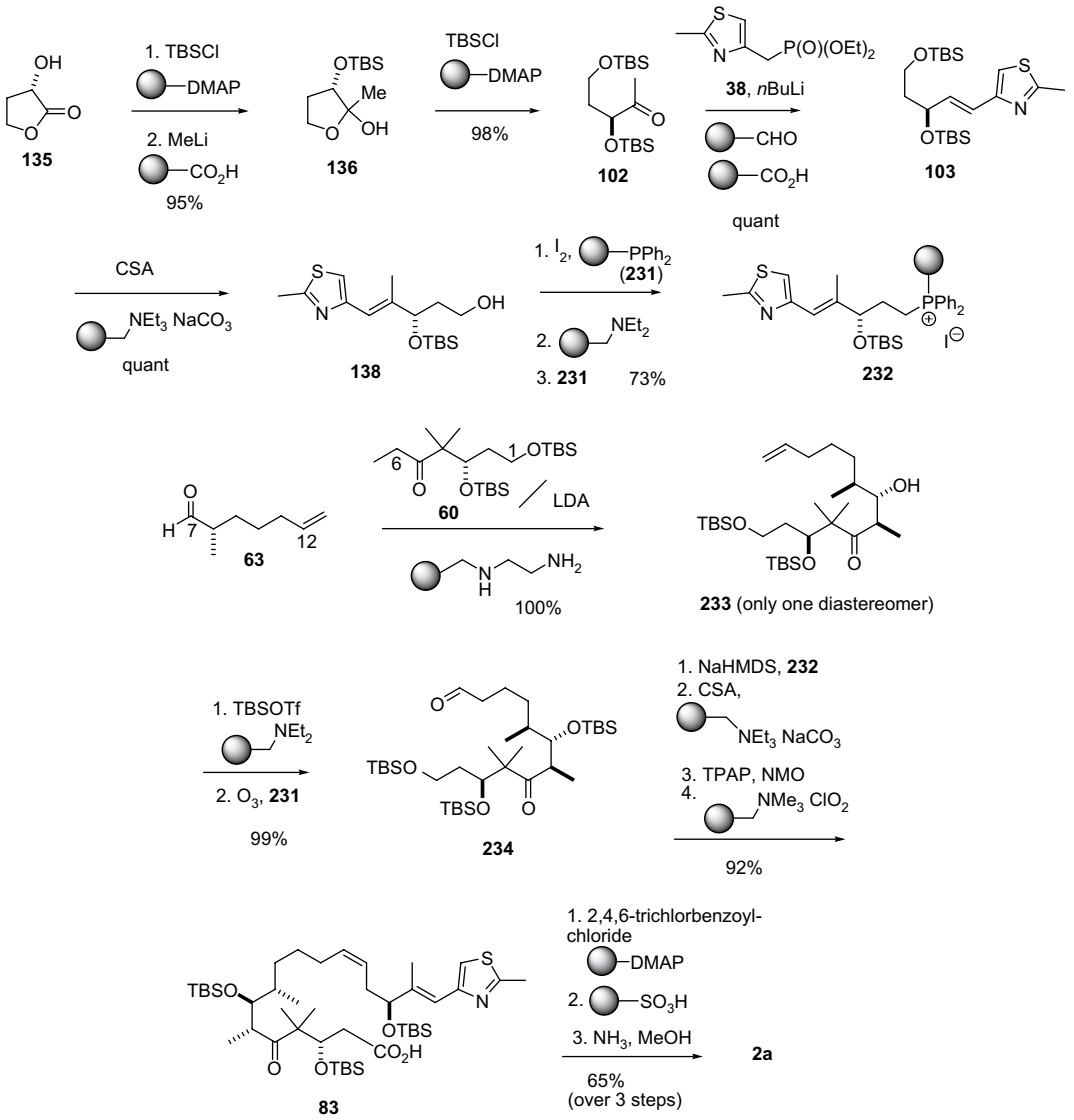
Scheme 42. Ley's synthesis of epothilones A and C using immobilized reagent and scavengers, part I.

aldehyde **70** which was then elaborated into **10** following the Danishefsky/Schinzer precedence.

The synthesis of **207** (Schemes 37 and 38) started with a 1,4-addition of 4-*t*-BuPh–SH to thiol ester **194** under the enantiocatalysis of **188** to give **195**. Reduction of the thiol ester and *O*-protection gave thioether **196**, which was converted into **197** *via* Pummerer oxidation–reduction. Chain elongation to olefin **198** was followed by MPM deprotection and oxidation to the labile aldehyde **199** which was added to enolate **200** to give the epothilone A/B C3–C11-fragment **201** (Scheme 37). Standard methodology was used to convert **201** into acetonide **202** and then aldehyde **203** (Scheme 38). Aldol addition with acetophenone under the catalysis of **189** gave phenyl ketone **204**, which was converted to phenyl ester **206** *via* a novel Baeyer–Villiger oxidation, catalyzed by diamide **205**. In three additional steps ketone **207** was prepared, which was the coupling partner of **9** and **10** to give the **2a**-precursor **208** and the **2b**-precursor **209**, respectively (Scheme 39).

5.9. Synthesis by E.J. Thomas [58]

Thomas' contribution lies in a novel stereoselective approach to the (*Z*)-12,13-double bond (Schemes 40 and 41). The sequence started with an alkylation of sulfone **211** with iodide **210** to give **212** as a mixture of diastereomers. Deprotection and reprotection *via* **213** followed by an S_R2' addition of tributylstannane gave **214**, which was added to aldehyde **215** under twofold allylic Thomas rearrangement to give the homoallylic alcohol **216** as a diastereomeric mixture, however, with purely (*Z*)-olefin geometry. From **216**, the superfluous OH-function was removed in a Barton–McCombie sequence to furnish **217**, which was converted into alcohol **218** and then ketone **219** by conventional methodology. Usual Horner olefination with **38** followed by deprotection and oxidation gave aldehyde **220**, which is identical with Nicolaou's intermediate (*Z*)-**86** except for the 15-OSEM protecting group. Aldol addition with ketone **221**, prepared from pantolactone analogously to Schering's synthesis of

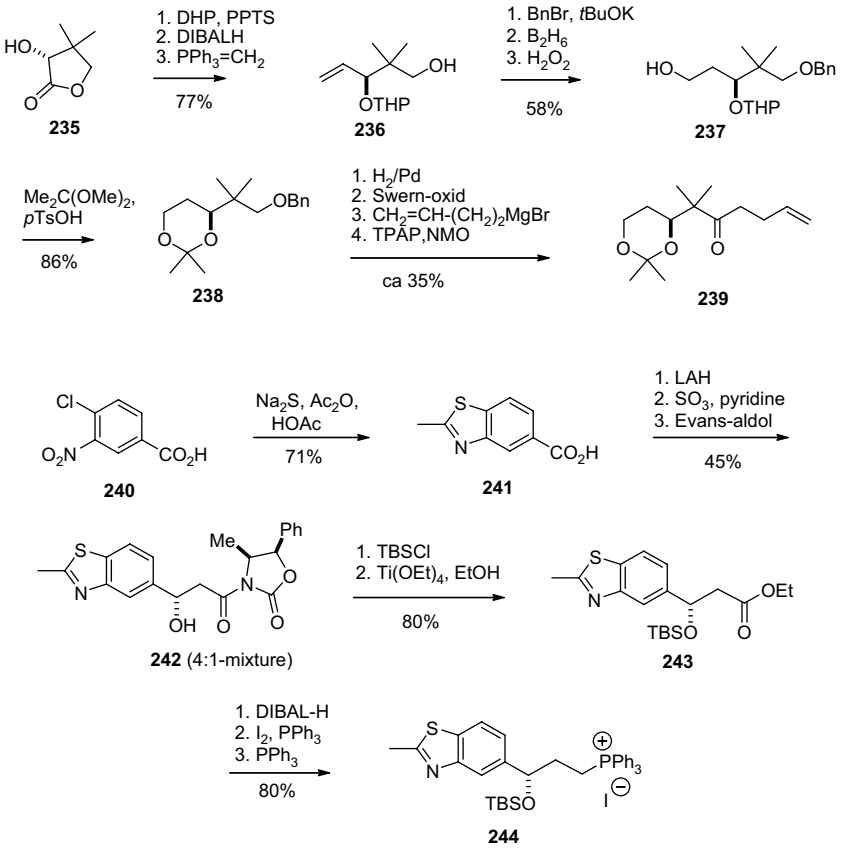


Scheme 43. Ley's synthesis of epothilones A and C using immobilized reagent and scavengers, part II.

sagopilone (Schemes 44 and 45) gave adduct **222** with high diastereoselectivity. The rest of the synthesis is essentially identical to Nicolaou's precedence (Scheme 18).

5.10. Ley's approach [45]

In pioneering studies to improve large-scale preparations of complex natural products, the Ley group focused on the use of immobilized reagents and scavengers. This philosophy is reflected in an approach to **1a** in which (Schemes 42 and 43) the key steps are the aldol addition of ketone **60** to aldehyde **63** to form **233** and the (*Z*)-selective Wittig reaction of aldehyde **234** and the ylide generated from the immobilized phosphonium salt **232**. The preparation of ketone **60** and fragment **103** is adapted from Mulzer's synthesis of **1b** [43] and the Wittig reaction between **232** and **234** stems from Nicolaou's synthesis of **1a** (Scheme 17). Ley's achievement lies in the fact that the acids, bases and the diphenyl phosphine previously required for catalyzing reactions, destroying intermediates and isolating reaction products are fixed to a solid support. In this way, aqueous workup, filtration or chromatography are largely avoided and the yields are extremely high, in most steps nearly quantitative.



Scheme 44. Schering synthesis of ZK-Epo (sagopilone), part I.

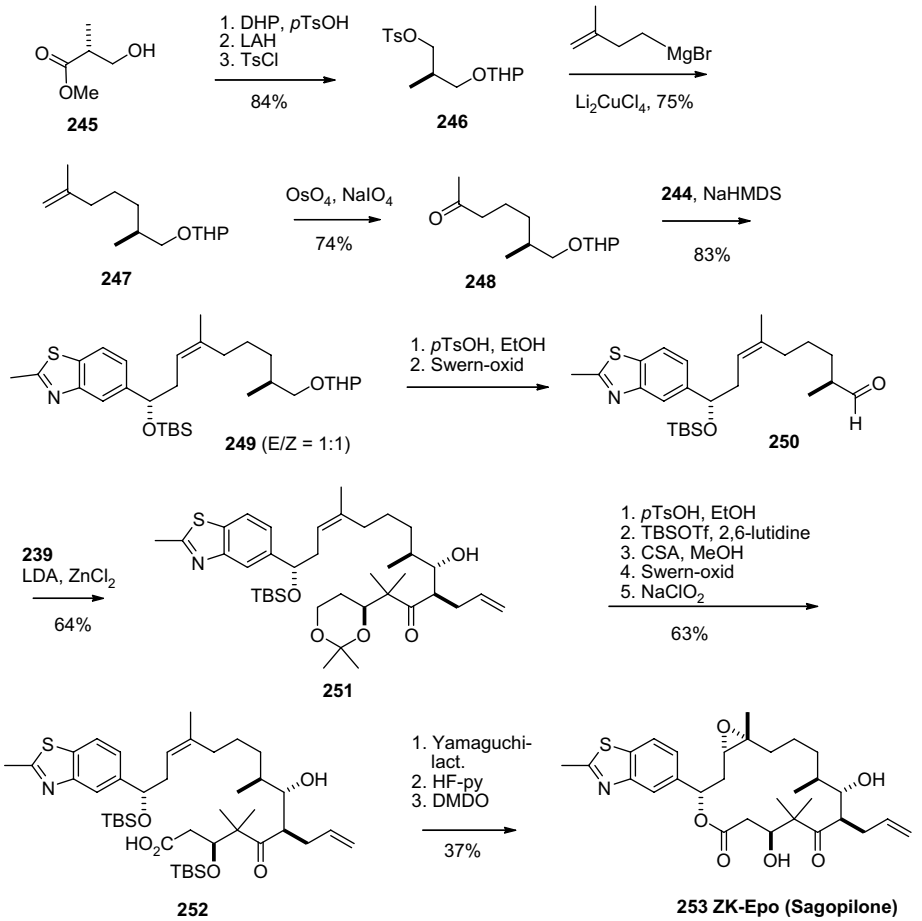
5.11. Synthesis of epothilone analogs

For SAR studies (see Section 6) a broad variety of epothilone derivatives were desirable. From early on, the Nicolaou group, together with the Danishefsky group, applied their synthetic approach for structural variations in essentially all sections of the epothilone skeleton. From the methodological viewpoint basically little new was developed. Thus it may suffice to mention two representative examples, one from the Nicolaou group [59] which was used to generate highly active epothilone B analogs [60], and one from the Danishefsky manifold [61] (trifluorodehydroEpoB, fludelone).

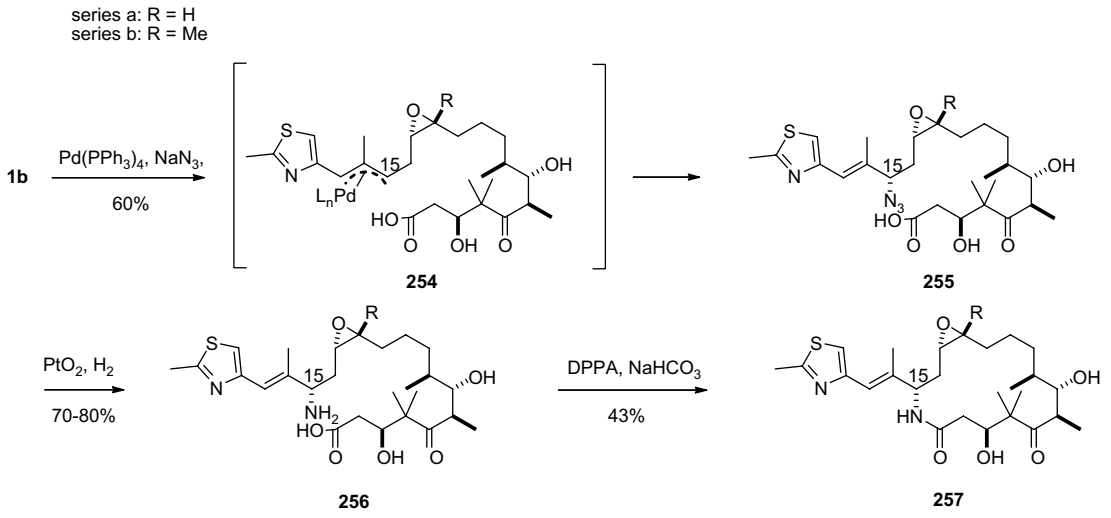
5.12. Conclusion and an industrial application (ZK-Epo (sagopilone)) [62]

The total synthesis of the epothilones has undoubtedly been one of the largest projects ever in the history of organic chemistry. At first sight relatively little new methodology has emanated from these huge worldwide efforts. However, several useful novelties may be gleaned: application of RCM reactions to a rather complex substrate, all kinds of stereoselective approaches to generate a (*Z*)-tri-substituted double bond, long range effects on stereoselective aldol additions that connect oxygenated fragments, the robustness and generality of Yamaguchi macrolactonization, and last but not least stereo- and regioselective epoxidation with DMDO and the stability of epoxides towards a variety of aggressive reagents.

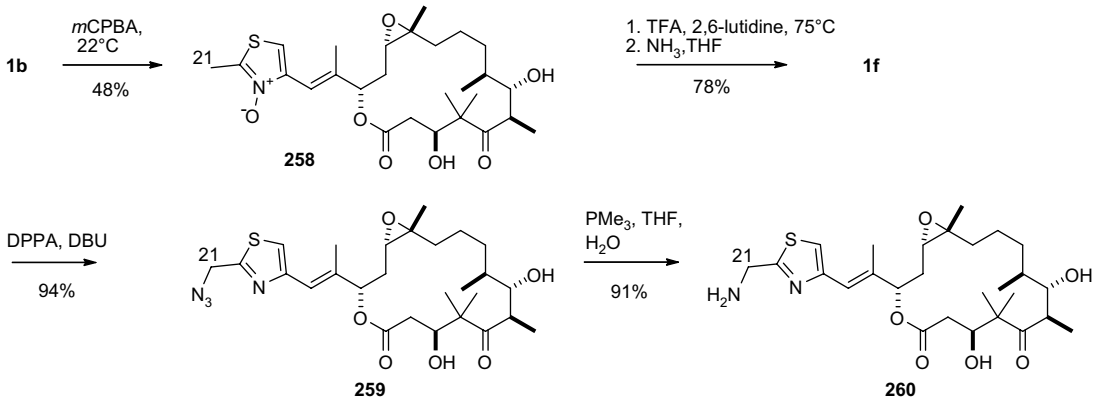
In fact, many of the academic approaches have been transferred to the kilogram scale industrial synthesis of ZK-Epo (**253**, sagopilone) [62], a highly potent unnatural analog of **2a**, which is in Phase II clinical trial (see Section 7.6) (Schemes 44 and 45). The synthesis essentially is a hybrid of Schinzer and Nicolaou components based on three major fragments **239**, **244**, and **248**. The Wittig olefination of ketone **250** and phosphonium salt **244** furnishes a 1:1-*E*/*Z*-mixture of **249**. After chromatographic separation the undesired *E*-olefin is recycled by photochemical *cis*–*trans*-equilibration. The aldol addition of Schinzer's ketone **239** and aldehyde



Scheme 45. Schering synthesis of ZK-Epo (sagopilone), part II.



Scheme 46. BMS synthesis of Aza-EpoB.

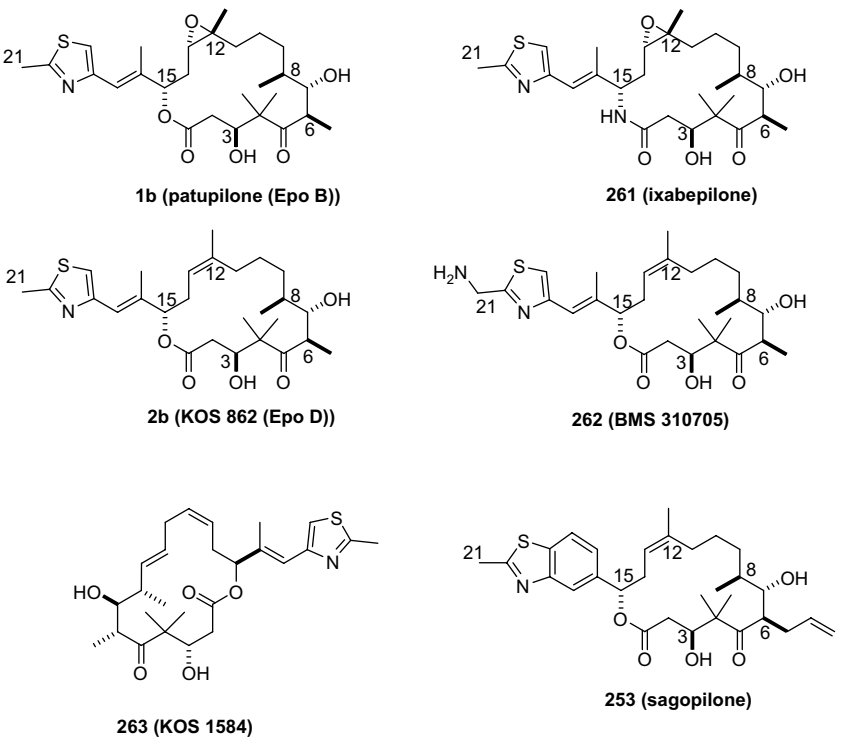


Scheme 47. Modification of C21.

250 gives adduct **251** with ''good selectivity'', yet only 64% yield. The endgame follows the routine Yamaguchi lactonization, desilylation, and DMDO epoxidation (selectivity 7:1) protocol. The overall sequence consists of no less than 44 steps, all told. In this respect, the synthesis is an impressive achievement and emphatically demonstrates that multistep synthesis is not a purely academic playground, but can be performed on an industrial scale if so desired.

6. Semisynthetic derivatives

For biologically active structurally complex natural products the chemical derivatization of material isolated from natural sources often represents the only feasible means to explore structure–activity relationships (SARs) and to produce analogs with more favorable pharmacokinetic and pharmacological properties [63–65]. Typically, six out of seven epothilones (see



Scheme 48. Epothilones in clinical evaluation.

Section 7) that have entered clinical evaluation in humans so far (including the natural product epothilone B (**1b**)) are made by derivatization, and only one is produced by total chemical synthesis (**253**, sagopilone; see previous section).

Lactam-based epothilone analogs are metabolically more stable than the parent natural macrolactones [66,67]. Therefore, the BMS (Bristol–Myers–Sqibb) group has developed a route capitalizing on the reaction of an allylic Pd–π complex with a nitrogen nucleophile. Thus, the treatment of **1a**, **1b** with NaN_3 in the presence of $Pd(PPh_3)_4$ leads to azido acids **255a** and **255b**, respectively, with full retention of configuration at C15 (Scheme 46).

Reduction of the azide group followed by cyclization furnished the lactam analogs of EpoA and B, **257a** and **257b**.

A second modification refers to the C21 position through Polonovski rearrangement [68] of *N*-oxide **258** and further transformation of the ensuing C21-trifluoroacetoxymethyl group [69,70] to **259** and **260** (Scheme 47).

7. Clinical data

For seven epothilone-based drugs (Scheme 48) clinical trials have been performed [71–73].

7.1. Patupilone (EPO906, *1b*)

Patupilone was the first epothilone to enter clinical trials [74]. In Phase II the most promising data have been obtained in prostate, ovarian, and NSCL (non-small-cell lung cancer) patients. In particular, good results were observed for platinum- and taxane-resistant ovarian carcinomas and platinum-pretreated NSCLC [75].

7.2. Ixabepilone (*261*)

The BMS–epothilone B-lactam **261**, now called ixabepilone, received FDA approval for the treatment of metastatic or advanced breast cancer on October 16, 2007 [76]. Tumor types that have been investigated with **261** include breast, prostate, colorectal, non-small-cell lung, gastric, hepatobiliary, gynaecological, and pancreatic cancers. In addition, studies have been conducted for the treatment of sarcoma, melanoma, and non-Hodgkin's lymphoma [77].

7.3. KOS-862 (EpoD, *2b*)

Several Phase I trials have been reported for KOS-862 (R1492, **2d**, deoxyepothilone B) [78,79]. Although tumor shrinkage was observed in these studies in two patients with large cell and mediastinal B-cell lymphomat the clinical development has been terminated [80]. Lately, Phase II trials have been reported [81].

7.4. BMS-310705 (*262*)

As a back-up for **261**, two Phase I studies with BMS-310705, which is more water-soluble than **261**, have been reported in abstract form [82–84]; no Phase II data are (publicly) available for the compound at this point. Overall, the toxicities associated with BMS-310705 treatment are similar to those observed for **261** [85,86].

7.5. KOS-1584 (*263*)

Interim results of ongoing Phase I trials with KOS-1584 (R1645) have been reported [87–89]. No recommended Phase II dose has yet been defined.

7.6. Sagopilone (ZK-Epo (sagopilone), *253*)

Sagopilone, from Bayer–Schering, is the only fully synthetic epothilone analog so far in Phase I/II clinical development [90,91]. As communicated by the company, **253** is currently evaluated "in an extensive program of clinical Phase II studies in various oncological indications"… At the time of reporting 63 patients had entered the study, with data being available on 30 patients. The trial was still ongoing at the time of reporting.

8. Conclusions

As yet, data from more than 20 Phase II trials with four different epothilones (patupilone, ixabepilone, KOS-862, sagopilone) have been reported. Measurable antitumor activity has been observed in metastatic breast cancer, ovarian cancer, and hormone-resistant prostate cancer, with sporadic activity in colon cancer. So far, however, only ixabepilone has received FDA approval for the treatment of metastatic or advanced breast cancer.

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