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Recent developments in the chemistry, biology and medicine of the epothilones†

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The epothilones have occupied center stage on the scenes of total synthesis, chemical biology and medicine for the last five years, no doubt because of their intriguing mode of action and unusually high potency against tumor cells, including multi-drug-resistant cell lines. This article highlights the most recent advances within this exciting field. Thus, an overview of recent synthetic endeavors culminating in a new generation of total syntheses and analogues, some with higher potencies than the naturally occurring substances, will be given, and the chemical biology, in particular the current understanding of structure–activity relationships of the epothilones, will also be discussed in light of the latest biological results. In addition, the recently elucidated biosynthetic machinery of the natural epothilone-producing myxobacterium *Sorangium cellulosum*, as it is now understood, will be described. Finally, some preclinical and clinical studies will be summarized.

† Dedicated to Drs Margaret Harris and Muriel Hall of Bedford College, London, for their dedicated and inspiring teachings and research contributions in celebration of their retirement.

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Introduction

Cancer claims approximately one death per minute in the United States alone. As such, this dreaded disease has stimulated enormous efforts directed at curative and preventive strategies to combat its menacing effects on society. Chemistry and biology provide most unique platforms for addressing this problem as evidenced by the several chemotherapeutic agents discovered and developed through endeavors in these disciplines. Prominent among them are Taxol® and Taxotere™, two tubulin binding anticancer drugs[1] whose combined sales exceed the 2 billion dollar mark. As a new class of potent tubulin polymerizing and microtubule stabilizing compounds, the epothilones[2] have received a great deal of attention over the last few years from chemists, biologists and clinicians. Research activities in this area span from isolation of natural products to genetic engineering of new producing organisms for fermentation purposes, from total synthesis to chemical synthesis of designed analogues, and from chemical biology to clinical studies. Despite the several reviews[3–6] covering the great strides made in the epothilone area, the fast pace of research surrounding these molecules necessitates this update which aims at summarizing and placing in perspective the latest developments in the field.

Recent total syntheses of epothilones and analogues thereof

Many groups have reported the total or partial syntheses of epothilone family members (Fig. 1) during the past years, and

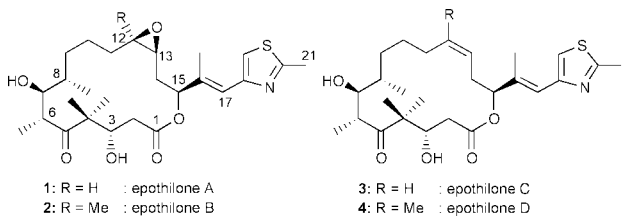
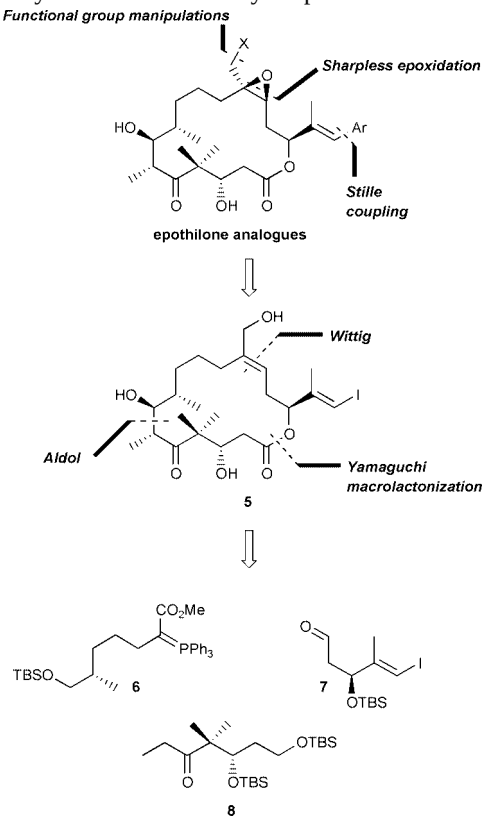


Fig. 1 The epothilones.

this subject has been extensively reviewed.[3–6] Within this section we will highlight the most recent developments with particular emphasis on the new generation total syntheses in the field of epothilones and analogues thereof.

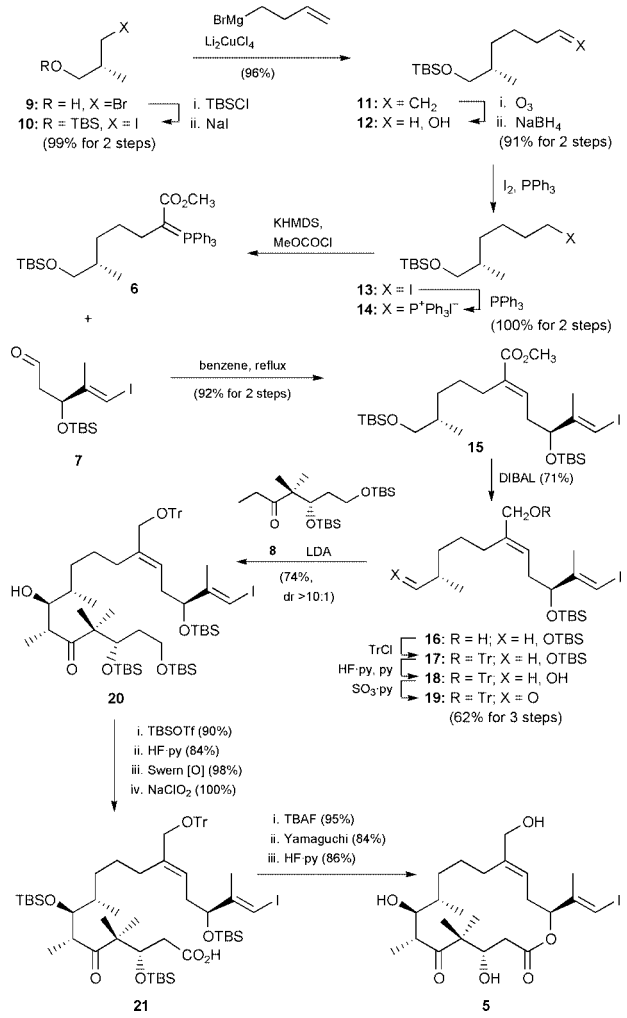
Although we had previously reported two different total synthesis strategies leading to epothilones and a large number of

analogues,[3] there was still room for improvements, particularly with regards to the stereoselectivity at some key points. Through our most recent work, these deficiencies have now been corrected, for the most part, so that a highly efficient, stereoselective, and flexible approach towards the epothilones has been established. Our general strategy is outlined retrosynthetically in Scheme 1. Key improvements include the introduction of the C12–C13 epoxide *via* a Sharpless asymmetric epoxidation directed by a C26 hydroxy group, and a highly efficient and reproducible protocol for the aldol coupling which creates the C6–C7 bond and simultaneously sets the stereochemistry at these centers with > 10 : 1 diastereoselectivity. A high degree of convergence was achieved, as the synthesis proceeds through the union of three fragments (**6**–**8**) of similar size and complexity, to afford the advanced intermediate **5**. This vinyl iodide (**5**) had already been proved to be highly versatile, as it allowed the synthesis of a large number of epothilones with aromatic side chains through our Stille coupling methodology,[7,8] and, in addition, enabled the synthesis of various C26-modified analogues.[7a]

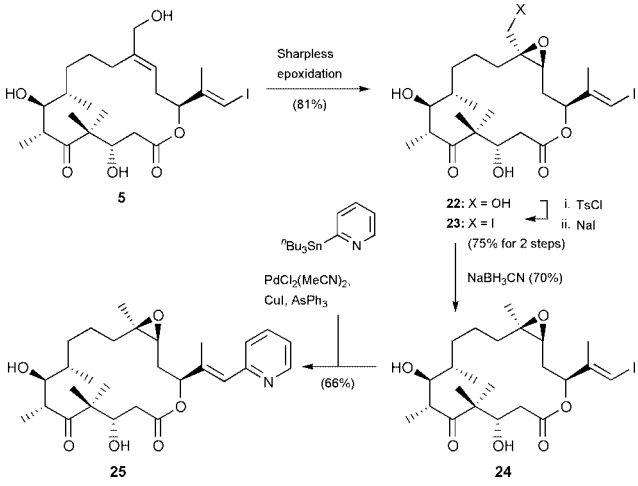


Scheme 1 Nicolaou's retrosynthetic analysis of epothilones.

The synthesis of vinyl iodide **5**[7b] is summarized in Scheme 2. Starting from the commercially available bromoalcohol **9** (optically active), protection and conversion to the iodide **10** was followed by an alkylation with but-4-enylmagnesium bromide to yield terminal olefin **11**. Through standard methodology, this was converted to the ylide **6**, which participated in a Wittig coupling with iodoaldehyde **7** to yield fragment **15**, which corresponds to the C7–C17 region of the epothilones. Functional group manipulations furnished aldehyde **19** in short order. When this derivative was subjected to our optimized aldol coupling protocol (enolate generation at −78 °C, then −40 °C for 1 h, then rapid addition of aldehyde at −78 °C followed by rapid quenching after 5 min with HOAc in THF)[7b,9] with ketone **8**,[10] a 74% yield of the desired aldol diastereomer **20** was realized. Moreover, the diastereoselectivity before purification was better than 10 : 1. With all the carbon atoms in place in **20**, straight-forward functional group manipulations and a Yamaguchi macrolactonization[10] afforded the desired vinyl iodide **5**.



Scheme 2 Nicolaou's synthesis of the advanced intermediate **5**.



Scheme 3 Nicolaou's synthesis of side chain-modified analogues.

The vinyl iodide **5** was converted into the epothilone B precursor **24** by a Sharpless epoxidation, followed by a mild deoxygenation protocol (Scheme 3). This vinyl iodide (**24**) was used to construct a series of aromatic side chain-modified epothilones (see Fig. 2). Notable among them are several pyridine derivatives (**25**–**28**, Fig. 2), some of which proved to possess remarkable biological activities (*vide infra*). Using analogous chemistry, 26-fluoroepothilone B (**37**)[11] and 16-desmethylepothilone B (**38**)[7b,9] were also constructed (see Fig. 2).

In order to probe the significance of the epoxide oxygen atom, a novel class of epothilones in which the epoxide was replaced by a cyclopropyl or cyclobutyl moiety was targeted for

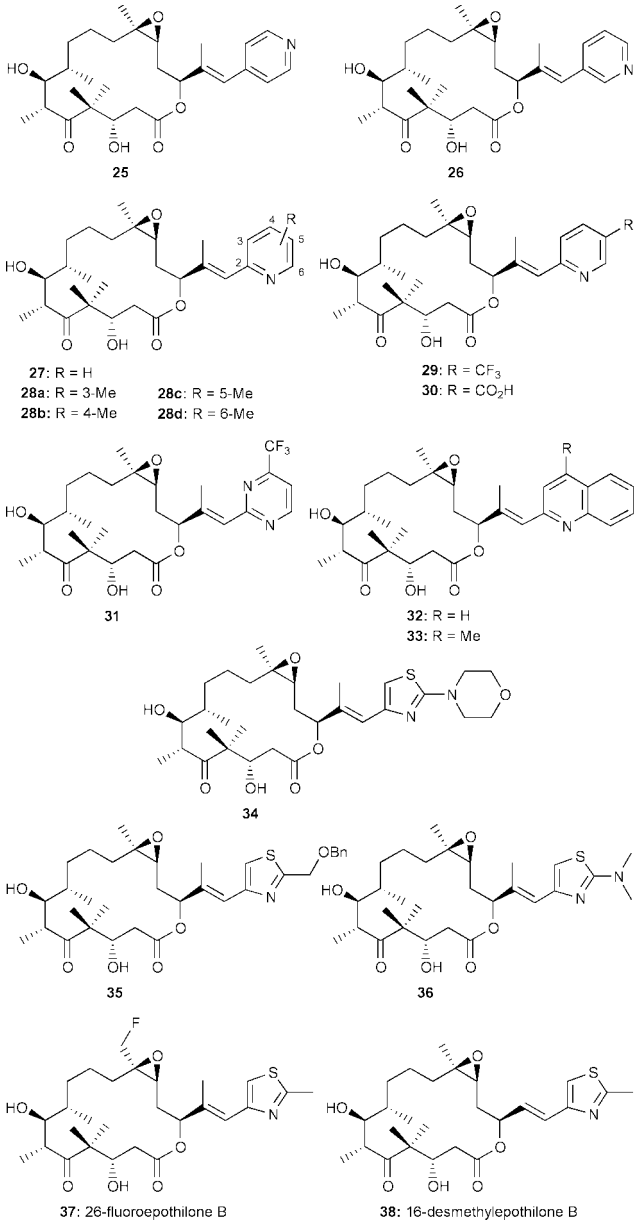
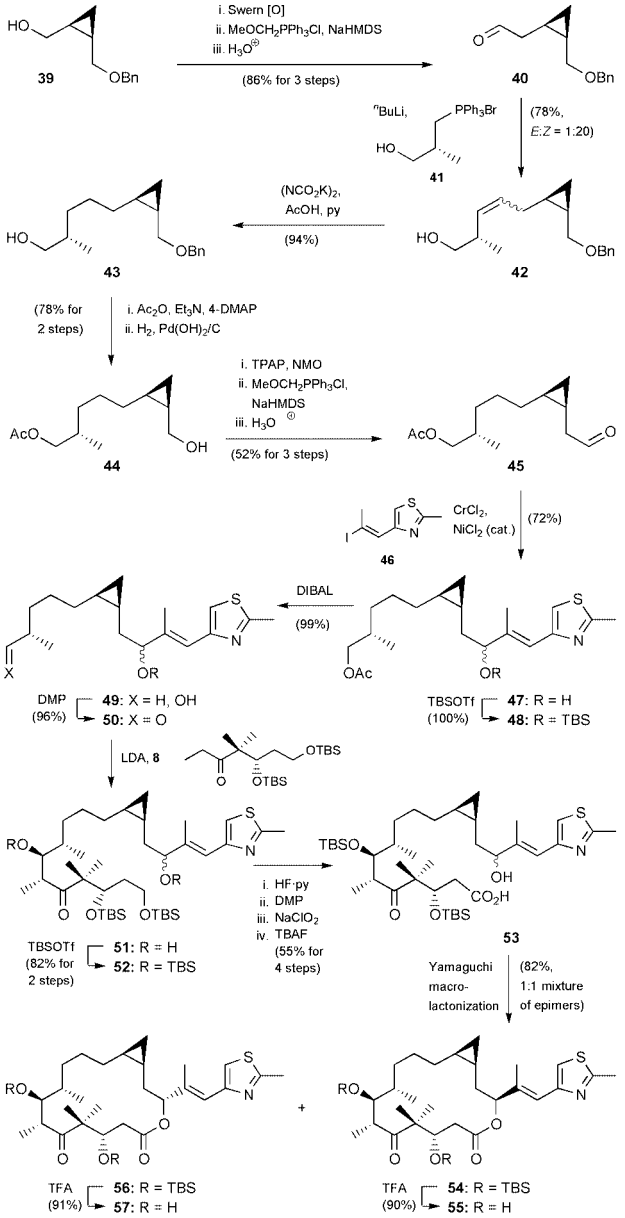


Fig. 2 Selected epothilone analogues.



Scheme 4 Nicolaou's synthesis of cyclopropylepothilone A (**55**).

synthesis. Our original studies[12] with cyclopropyl epothilones probing this question were plagued by stereochemical ambiguities. Specifically, our original assignments[12] of the C12–C13 stereochemistries of cyclopropyl epothilones A were proven incorrect[13,14*a*] (the correct structures of these compounds are shown in Fig. 3, structures **58** and **59**). We therefore embarked on a new program directed at clarifying this issue. To this end, a new total synthesis had to be developed, which allowed for the incorporation of the requisite cycloalkyl moieties in a stereoselective manner at an early stage, in order to assure that no stereochemical ambiguity would exist in the final products.

The total synthesis of 12,13-cyclopropylepothilone A (**55**) serves to exemplify the approach to these compounds (Scheme 4).[14] This synthesis starts from the known cyclopropyl alcohol **39**, readily obtained by enantioselective Charette cyclopropanation[15] of *cis*-4-benzyloxybut-2-enol in 94% ee. Homologation and Wittig olefination with the commercially available chiral phosphonium bromide **41** afforded alkene **42**, predominantly as the *cis* isomer. Diimide reduction followed by hydrogenolysis of the benzyl ether and a second homologation yielded aldehyde **45**. At this point, a Nozaki-Kishi coupling with vinyl iodide **46**[14] afforded a 1:1 mixture of C15 epimers of the C7–C21 fragment **47**. Standard manipulations afforded aldehyde **50**, which smoothly underwent aldol coupling with ketone **8** to yield open-chain derivative **52** as a single diastereomer after TBS protection of the secondary alcohol. The same sequence of operations, *i.e.* protecting group manipulations, oxidation at C1 and Yamaguchi macrolactonization, as was described in Scheme 2, was then applied to complete the synthesis. After macrolactonization, the C15 epimers were separated and individually desilylated to afford the desired *cis*-12,13-cyclopropylepothilones **55** and **57**. This synthetic route also allowed the preparation of several other cycloalkylepothilone derivatives (**60**–**67**, Fig. 3) of various stereochemistries.[14*b*]

The Danishefsky group has made major contributions in the epothilone field, not only with regards to their total synthesis and their analogues, but also by extensive *in vitro* and *in vivo* studies.[4] A recent development from this camp is a new and highly efficient synthesis of epothilone D (**4**, Schemes 5 and 6),[16] and a closely related total synthesis of 12,13-desoxyepothilone F (**90**, Scheme 7),[17] which promise to secure sufficient quantities of these interesting compounds for ongoing biological and clinical investigations.

Danishefsky's 'new-generation synthesis' of epothilone D (**4**) is summarized in Scheme 6.[16,18] This new venture into the epothilone class features a newly developed *anti*-Felkin–Ahn selective aldol coupling of the (*Z*)-lithium enolate of **72**, an

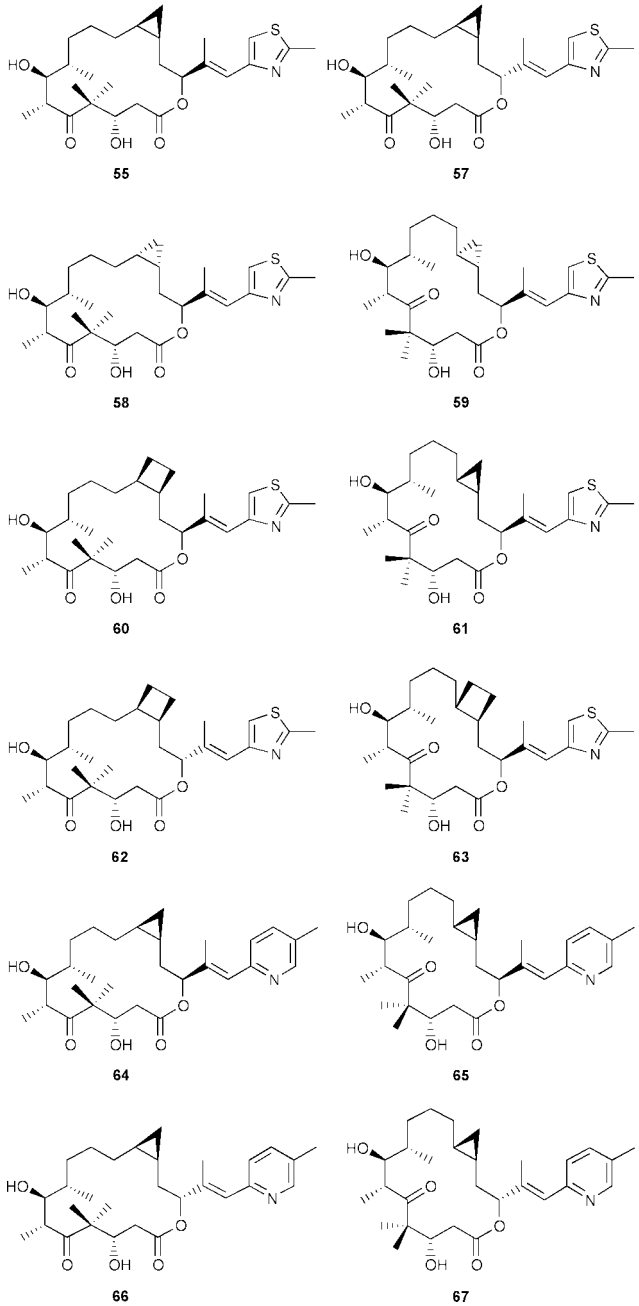
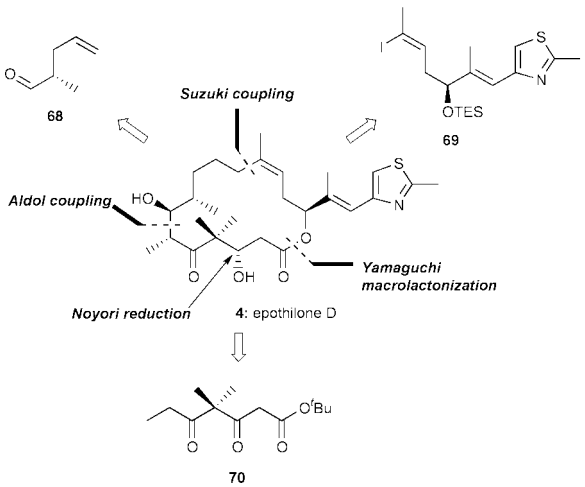


Fig. 3 Cyclopropyl and cyclobutylepothilones.

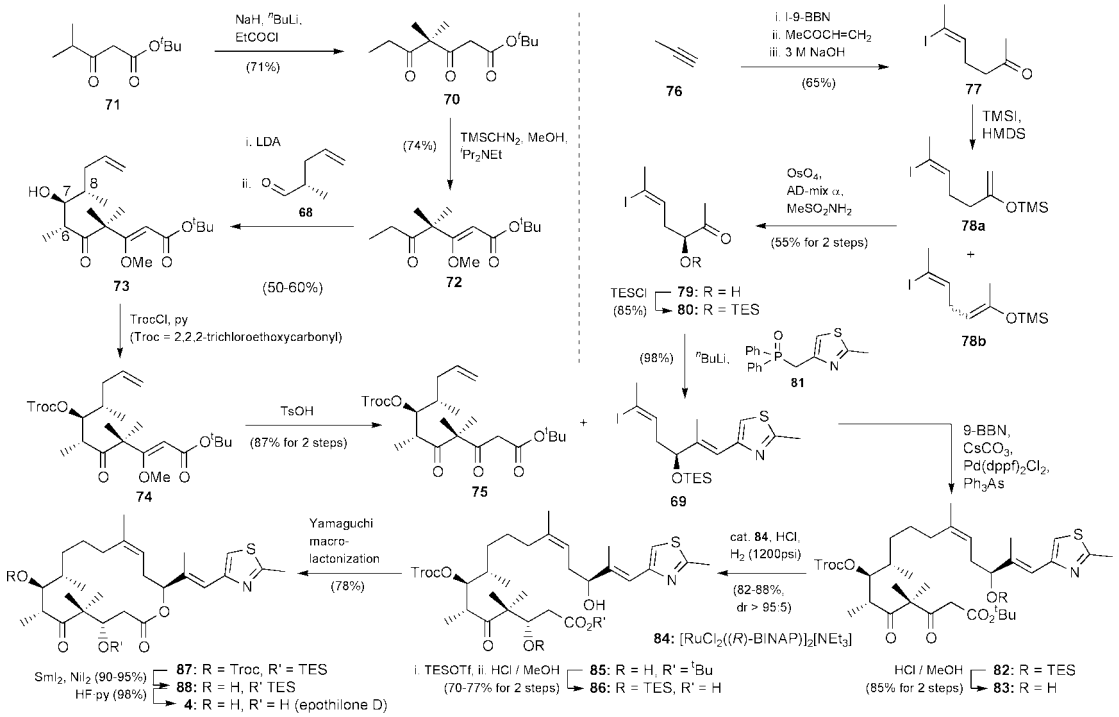


Scheme 5 Danishefsky's new retrosynthetic analysis of epothilones.

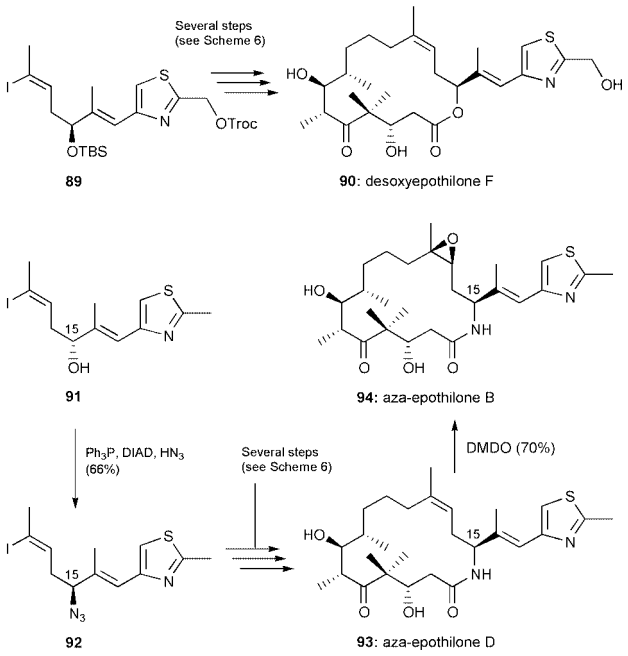
alkylborane Suzuki coupling of advanced intermediates **75** and **69**, and a stereoselective Noyori reduction of the C3 carbonyl group at a late stage of the synthesis. To this end, the requisite aldol coupling precursor **72** was assembled from the known β-ketoester **71** in two steps, and the chiral aldehyde **68** was derived from isoprene *via* asymmetric epoxidation. After extensive experimentation, good diastereoselectivity, 5–6 : 1 with the *syn*-(C6,C7)/*anti*-(C7,C8) compound **73** as the major isomer, was achieved in the aldol coupling between **68** and **72**. This outcome was rationalized by postulating neighboring-group participation by the olefinic moiety of **68**. Straightforward manipulations converted aldol **73** into olefin **75**. The vinyl iodide **69**, derived from propyne (**76**) *via* vinyl iodide **77** by an efficient protocol,[18] was joined with alkene **75** *via* the alkylborane Suzuki coupling, which proceeded smoothly to afford the open-chain ester **82**. After deprotection leading to alcohol **83**, the crucial Noyori reduction afforded the C3 alcohol **85** in > 95 : 5 diastereoselectivity, and in 82–88% yield. Protecting group manipulations and Yamaguchi macrolactonization, followed by global deprotection, finally afforded the desired epothilone D (**4**). By closely analogous routes, see Scheme 7, the Danishefsky group also prepared 12,13-deoxyepothilone F (**90**),[17] aza-epothilone D (**93**)[19] (both C15 epimers of **93** were prepared), and aza-epothilone B (**94**),[19b] the latter of which had previously been synthesized and shown to be very promising by the Bristol-Myers Squibb company (BMS), *vide infra*.

Using their reactive immunization technology,[20] the Lerner–Sinha group set out to devise a new synthesis of epothilones, where key intermediates would be generated by aldolase-like antibody catalysts. These efforts culminated in the syntheses of epothilones A–F,[21–23] as shown in Scheme 8. Thus, monoclonal antibodies, generated by reactive immunization against a β-diketone hapten, were used to prepare enantiomerically enriched key intermediates for epothilone A synthesis, either by kinetic resolution of racemic substrates through a retro-aldol reaction, or by enantioselective aldol reactions with prochiral substrates. The so obtained building blocks were elaborated to the target molecules, epothilones A–F. Remarkably, in their most recent work,[22,23] these authors report aldolase-type antibodies capable of resolving racemic aldols with 95–99% ee at 50% conversion, and with as little as 0.003 mol % of the antibody catalyst.

The C15 stereocenter of epothilones is arguably one of the most challenging to install, and many different solutions to this problem have been reported. In their first-generation synthesis,[21] the Lerner–Sinha group used their 38C2 antibody to catalyze the addition of acetone to aldehyde **95** (see Scheme 8a). At 51% conversion, an ee of 75% for **96** was realized for this tranformation. Using a new hapten, more efficient aldolase antibodies were raised, which allowed a retro-aldol kinetic resolution of (±)-**96**, affording **96** in > 97% ee and 45% isolated yield.[22,23] Moreover, the aldehyde **95** could be recovered and recycled back to (±)-**96**. Furthermore, it was possible to generate a host of closely related thiazoles, including **97**, which was subsequently elaborated into epothilone E. The team's total synthesis of epothilones started with a retro-aldol kinetic resolution of (±)-**98** to afford (–)-**98** in 98% ee. This operation set the stereocenters at C6 and C7 of epothilones. Hydrogenation afforded a 1:1 mixture of C8 epimers (**99**), and the desired epimer **99b** was taken on to the aldols **101a** and **101b**, obtained as a 1:2 mixture of diastereomers favoring the desired isomer **101b**. Further standard manipulations afforded acid **102**, which was esterified with alcohol **104**, obtained from **96** *via* a seven-step sequence. Ring-closing metathesis and deprotection afforded epothilone C (**3**) which was epoxidized to epothilone A (**1**) using a sequence of steps analogous to those previously reported in related works.[3–5] The Lerner-Sinha group also reported a related synthesis of epothilones A (**1**) and C (**3**)[21] *via* the macrolactonization approach,[10] as well as syntheses of epothilones B (**2**), D (**4**), E (**184** in Scheme 16) and F (**185** in Scheme 16).[21–23]



Scheme 6 Danishefsky's synthesis of epothilone D (**4**).

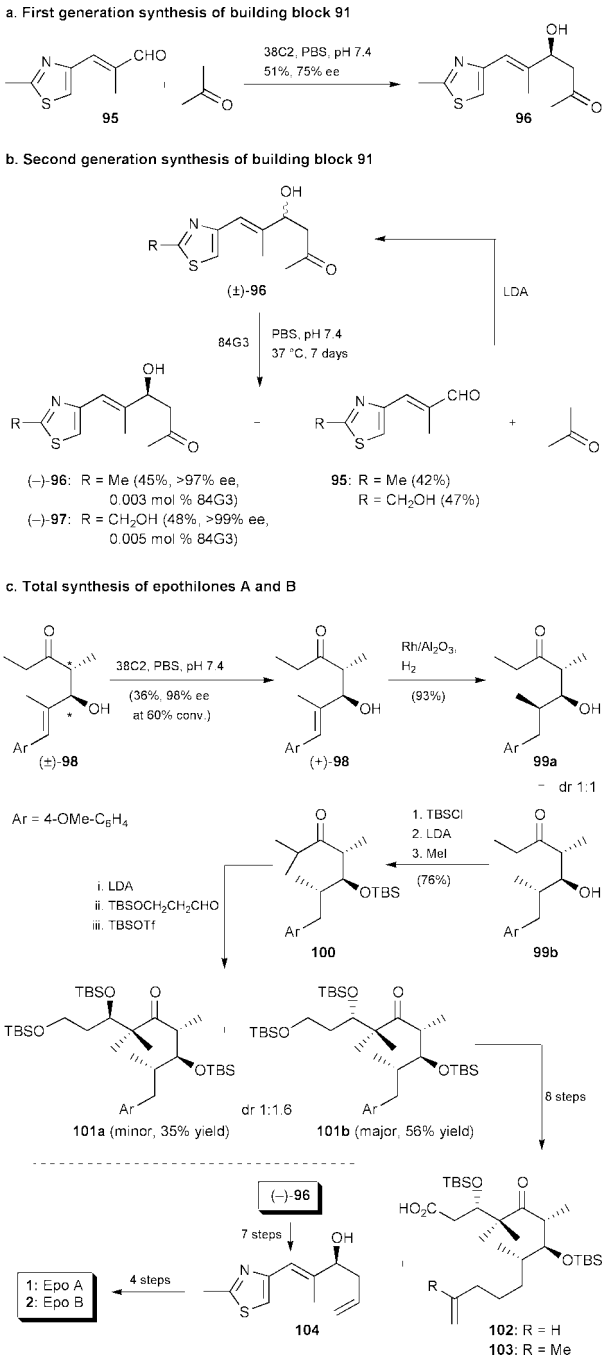


Scheme 7 Danishefsky's synthesis of selected epothilone analogues.

The Shibasaki group's syntheses of epothilones A and B addressed the stereochemical challenges posed by these target molecules by employing their recently developed Lewis acid–Lewis base bifunctional asymmetric polyheterometallic catalysts for cyanosilylation, aldol coupling and asymmetric protonation.[24,25] Thus, in their preparation of the C12–C21 fragment **111** (see Scheme 9), a catalytic asymmetric cyanosilylation using a bis(phosphine oxide)binaphthol–aluminum complex (**106**) as a chiral Lewis acid–Lewis base catalyst was successfully applied to the α,β-unsaturated aldehyde **105** to furnish, after acidic workup, the corresponding chiral cyanohydrin **107** in 97% yield and 99% ee. Straight-forward manipulations, including homologation, Wittig olefination and iodination, afforded the desired vinyl iodide **111**.

Two other multifunctional asymmetric catalysts, previously developed by the Shibasaki group, were applied to the preparation of the C1–C11 fragment **120** (see Scheme 10). In the first-generation approach,[24] the oxime ether epoxide **114** was constructed by a short sequence of steps from diol **112** using standard methodology. A cyanocuprate-based addition of a methyl group to **114** yielded the racemic *anti*-aldol **115**, by a process representing a new general approach to *anti*-aldols. Reductive cleavage of the O–N bond followed by regioselective alkylation of the resulting ketone furnished ketone **116**. The latter compound was reduced with good diastereoselectivity, and the resulting 1,3-diol was subjected to acetonide formation and other manipulations, ultimately leading to racemic aldehyde **118**. At this point, resolution by enantioselective aldol addition of acetophenone, catalyzed by the heteropolymetallic catalyst (*R*)-$LaLi_3$tris(binaphthoxide), afforded the desired aldol **119a** in 30% yield and 89% ee, together with its diastereomer **119b** (29%, 88% ee), which could be removed chromatographically. Standard transformations eventually afforded the desired fragment **120**. Noting the low efficiency of their resolution-based scheme, the Shibasaki group proceeded to develop a second generation synthesis,[25] starting with a catalytic asymmetric protonation of the enolate of thioester **121** (Scheme 10b). In the event, treatment of **121** with 5 mol % of $SmNa_3$tris(binaphthoxide) in the presence of 4-*tert*-butylthiophenol afforded the Michael adduct **122** in 92% yield and 88% ee. This intermediate was then elaborated into aldehyde **123**, which smoothly underwent an aldol coupling with the required ketone **124** to afford aldol **125** (dr = 4:1). Standard chemistry finally furnished the advanced intermediate **120** *via* **126**. The total synthesis of epothilone B (**2**) was completed by the joining of fragments **111** and **120** through a Suzuki coupling, macrolactonization and protecting group removal. The Shibasaki group also completed the synthesis of epothilone A (**1**) using closely related chemistry.[25]

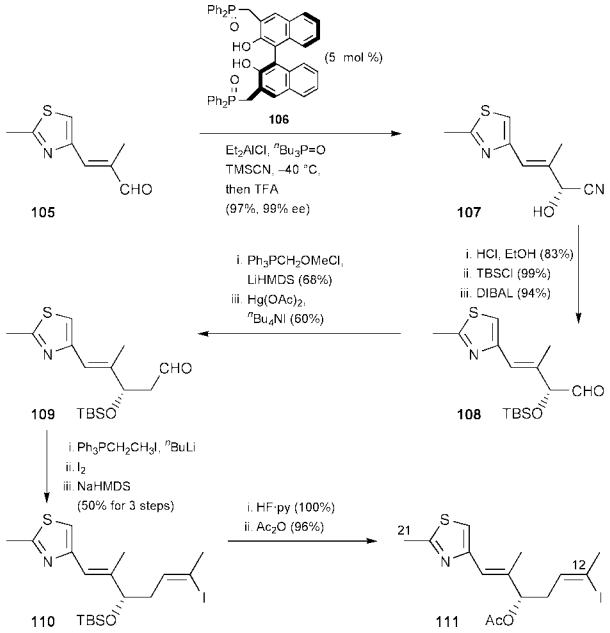
Although several of the reported epothilone syntheses form the macrocycle by ring-closing metathesis, poor *E/Z* selectivities are generally observed.[3–5] To circumvent this problem, the Kalesse group's formal total synthesis[26] of epothilone A (**1**) employed the metathesis ring closure to form a smaller, 10-membered lactone, which would be expected to favor the *Z* geometry on the basis of lower ring strain as opposed to the *E* arrangement. To this end (see Scheme 11), chiral aldehyde **127** was allylated and the product was esterified with hept-6-enoic acid to afford the cyclization precursor **129**. Ring-closing



Scheme 8 Lerner–Sinha's synthesis of epothilones A (**1**) and B (**2**).

metathesis of this diene system yielded 63% of the desired lactone **130**, with an *E*:*Z* ratio of 1 : 12; and furthermore, the *E* isomer could be separated and recycled, in 60% yield, to afford additional amounts of the desired *Z* lactone (*Z*)-**130** (see Scheme 11). Alkylation of the lactone enolate derived from (*Z*)-**130** with methyl iodide then afforded methylated lactone **131** (as a single diastereomer), the configuration of which was confirmed by independent synthesis using Evans' oxazilidinone technology. Elaboration of this intermediate (**131**), including installation of the thiazole side chain, ultimately yielded the aldehyde **136**. Since this aldehyde had previously been converted to epothilone A (**1**) by Nicolaou *et al.*[10] *via* coupling to ketoacid **137**, the formal total synthesis was complete. The Kalesse group also reported an independent synthesis of carboxylic acid **137**.[26]

The Panek group's synthesis of epothilone A (see Scheme 12) utilizes a lipase resolution to establish the stereochemistry at C15 of key fragment **111**, a Suzuki coupling between **111** and olefin **138** to form the C11–C12 carbon–carbon bond, a



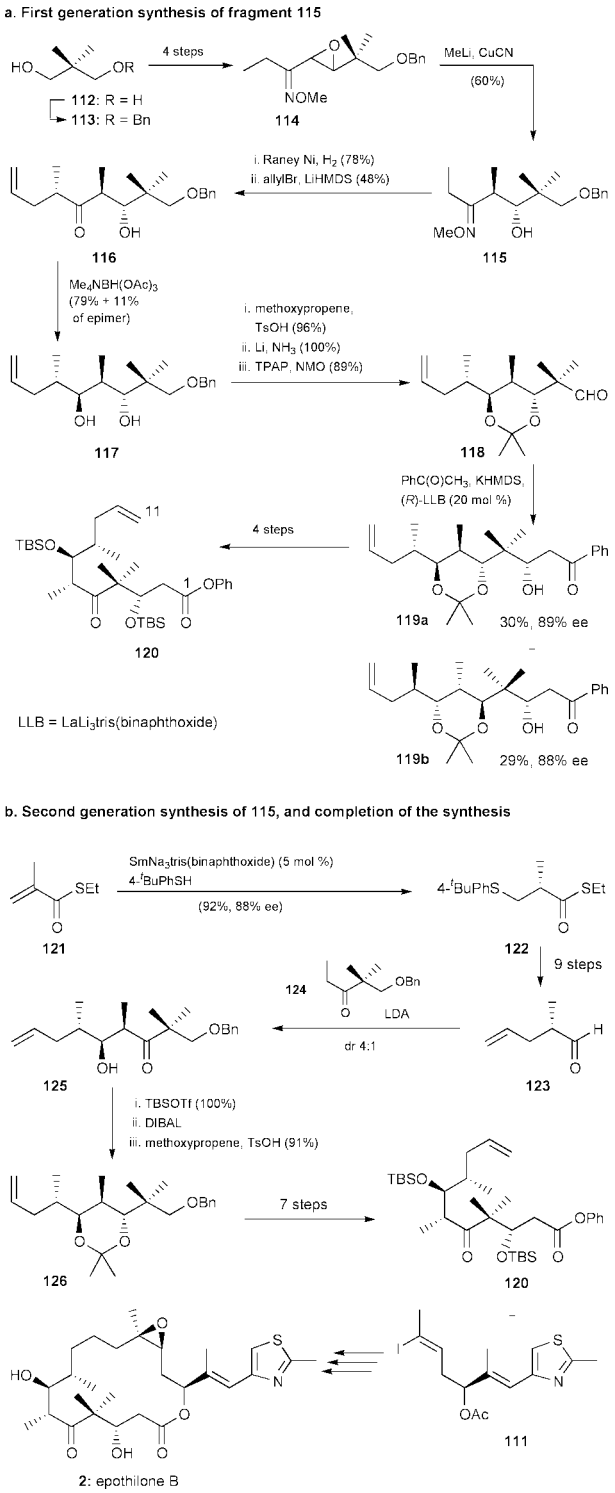
Scheme 9 Shibasaki's synthesis of vinyl iodide **111**.

Mukaiyama–type aldol coupling with **139** to construct the C2–C3 bond, and finally Yamaguchi macrolactonization and epoxidation.[27]

The Carreira group's synthesis of epothilones A (**1**) and B (**2**) features a unique nitrile oxide cycloaddition approach to the solution of the stereochemical problems at C12, C13 and C15 (see Scheme 13).[28] The highly diastereoselective cycloaddition of allylic alcohol **141** or **143** and nitrile oxide **145**, generated from phosphonate **140**, gave a single isoxazoline diastereomer (**142** and **146**, respectively), thus creating the C14–C15 bond while simultaneously establishing the correct stereochemistry at C12 and C13. A Horner–Emmons olefination with aldehyde **147** next installed the side chain, while reduction of the isoxazoline moiety of the resulting compound **148** yielded diol **149** stereoselectively. This intermediate was then conveniently converted to the key epoxide **150** *via* 1,3-cyclic sulfite formation and TBAF-mediated C12 desilylation. The latter compound (**150**) was then elaborated into epothilone A (**1**) following Mulzer's general strategy.[5] In a similar manner, the isoxazoline **142** was employed to synthesize epothilone B (**2**).

The Fürstner group's total synthesis of epothilone C (**3**), and formal total synthesis of epothilone A (**1**), features a novel ring-closing alkyne metathesis reaction as the key step (see Scheme 14).[29] The C1–C6 fragment **154** was prepared from ketone **151** through a Noyori asymmetric hydrogenation, protection and Grignard addition of an ethyl group to ester **153**. An aldol coupling was then employed to join the ketone **154** with the aldehyde **155** in a stereoselective manner to yield intermediate **156**. Straight-forward manipulations yielded carboxylic acid **157**, which was esterified with the side chain alcohol **158**, derived from the aldehyde **95** *via* a Brown asymmetric allylboration. The so obtained cyclisation precursor **159** was treated with 10 mol % of the metathesis catalyst **161** to afford the desired macrocycle **160**. Lindlar reduction and deprotection finally afforded the desired epothilone C (**3**).

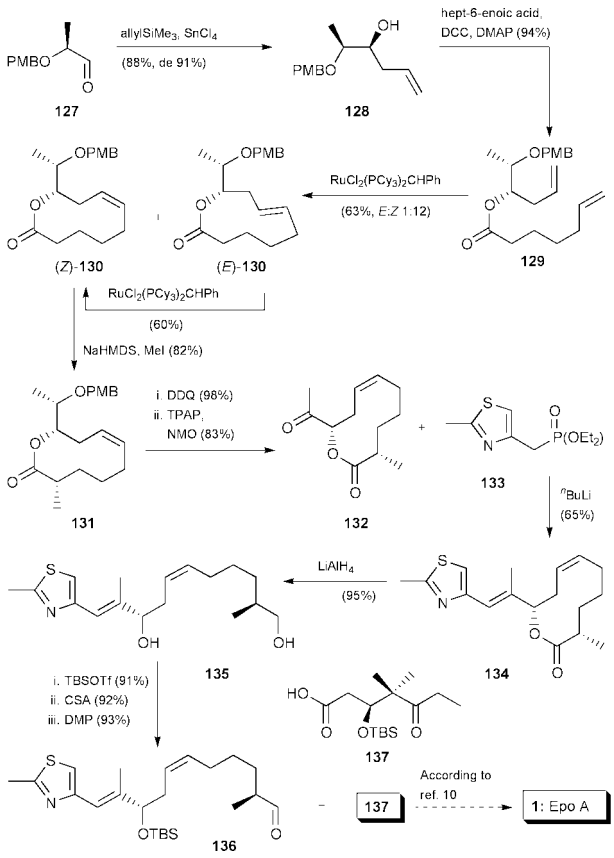
An ongoing research program at Novartis Pharma AG, led by Altmann, has resulted in a number of highly potent epothilone derivatives.[30,31] Thus, following a convergent route, several heterocyclic epothilone derivatives were prepared (see Scheme 15).[31] The required C1–C11 fragment **165** was reached by a short sequence of steps from **162** and **163**. This olefin was coupled to a series of vinyl iodides (**169**), which were synthesized using the Oppolzer sultam aldol reaction starting with sultam **166** and aldehyde **167**. Stille coupling of **169** with **165** followed by straight-forward manipulations and macro-



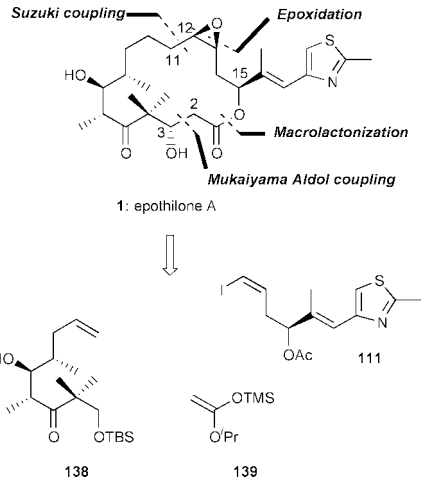
Scheme 10 Shibasaki's synthesis of epothilone B (**2**).

cyclization yielded the epothilone D derivatives **170**–**173**. Epoxidation of the latter compounds afforded epothilone B analogues **174**–**177**. Using similar methodology, a number of C12–C13 modified epothilones were also prepared.[30]

Many other groups, in particular those of Grieco,[32] Schinzer,[33] Mulzer[34] and White,[35] also made significant contributions to the epothilone field, and these efforts have been reviewed recently.[3,5] The Schinzer group, together with Altmann's group at Novartis, also reported a total synthesis of two 'aza-epothilone C' derivatives (epothilone lactams) using a ring-closing metathesis strategy.[36] An interesting biocatalytic method for the generation of intermediates for epothilone synthesis has been reported by Wong.[37]



Scheme 11 Kalesse's formal synthesis of epothilone A (**1**).

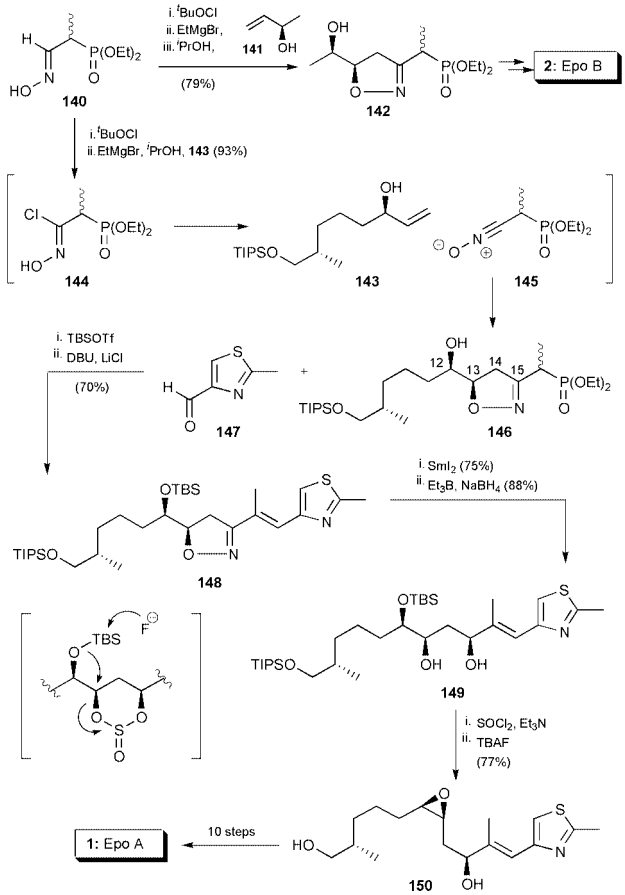


Scheme 12 Panek's retrosynthetic analysis of epothilone A (**1**).

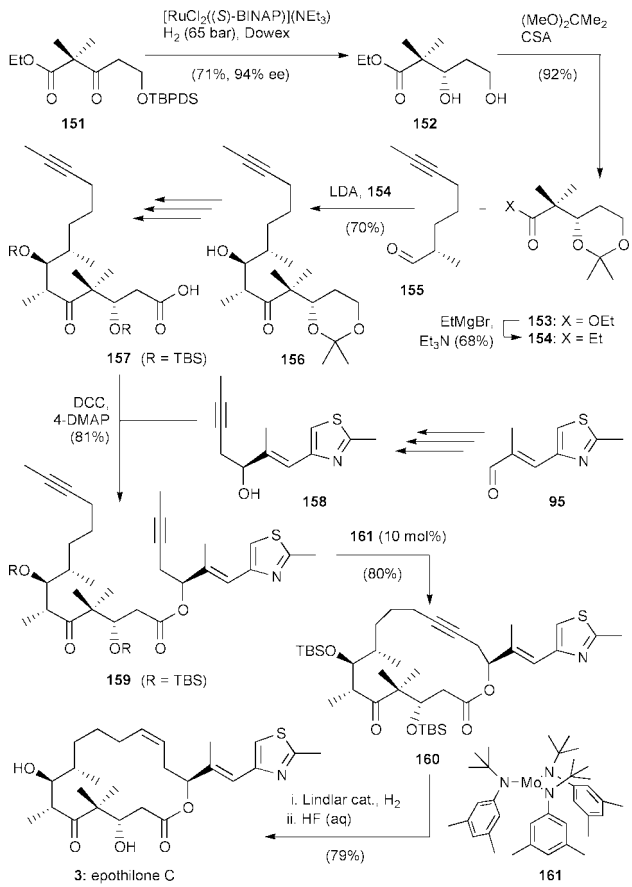
Partial syntheses of epothilone analogues

From an industrial standpoint, and despite the progress outlined above, fermentation followed by partial synthesis may still hold certain advantages over total synthesis. With such advantages in mind, a number of groups initiated programs directed at semisynthesis of epothilone analogues. Particularly notable are the reports from the Höfle[38] and Bristol-Myers Squibb[13,39] groups.

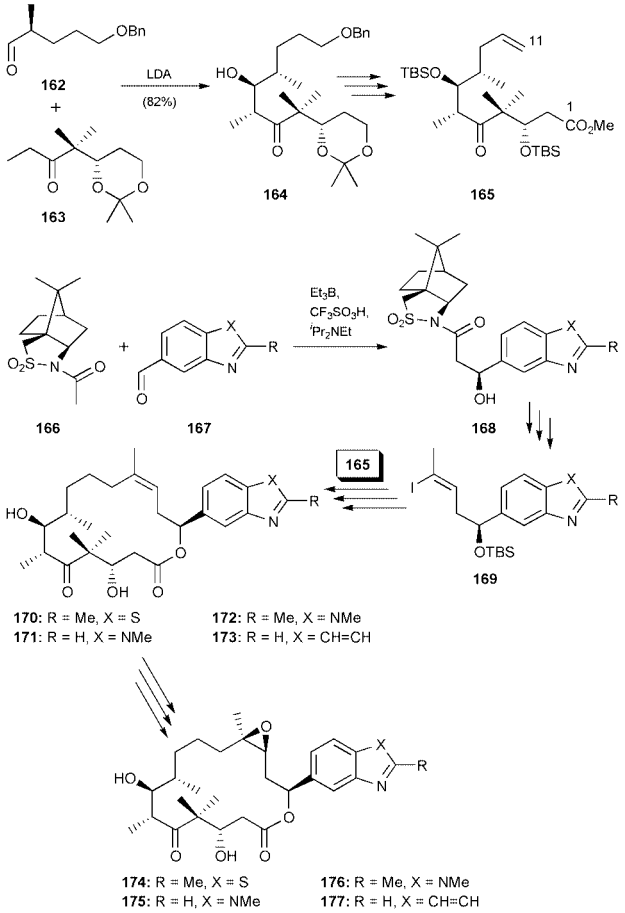
Thus, several interesting epothilone tranformations have been carried out by Höfle (see Scheme 16).[38] Ozonolysis and silylation of epothilone A (**1**) or B (**2**) afforded the versatile methyl ketone **178** (R = H, Me). Although Wittig-type reactions with this substrate were largely unsuccessful, probably due to enolization of the methyl ketone, aldol condensations with aromatic aldehydes produced the side chain modified epothilones **179**, none of which, however, exhibited any tubulin polymerization activity or cytotoxicity. Alternatively, ketone **178** could be converted into the vinyl boronic acid **180** (*E*:*Z* =



Scheme 13 Carreira's synthesis of epothilones A (**1**) and B (**2**).



Scheme 14 Fürstner's synthesis of epothilone C (**3**).



Scheme 15 Altmann's synthesis of side chain-modified analogues.

7:3), the *E* isomer of which could be transformed into vinyl iodide **181**, which bears considerable similarity to the highly versatile intermediate **24** (see Scheme 3) used by the Nicolaou group to generate a number of analogues *via* Stille coupling methodology.[3] Höfle also discovered that epothilones could be *N*-oxidized in fair yields by MCPBA. Upon acetylation, the *N*-oxides underwent a Polonovsky-type rearrangement to yield, after hydrolysis, the side chain oxidized epothilones E (**184**) and F (**185**) (see Scheme 16).[38]

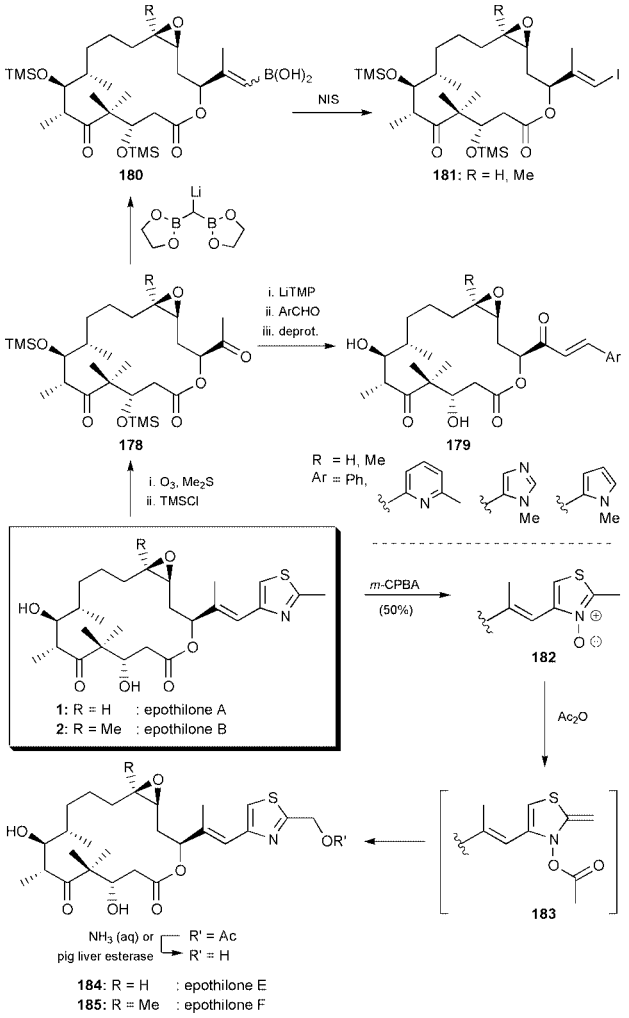
A partial synthesis of a number of cyclopropylepothilones has been disclosed by a Bristol-Myers Squibb group[13] (see Scheme 17). Thus, deoxygenation protocols to convert epothilones A (**1**) and B (**2**) into the C12–C13 olefinic epothilones C (**3**) and D (**4**) were developed. Epothilone C (**3**) could be cyclopropanated in 12% yield using dibromocarbene, generated from bromoform and aqueous base under phase-transfer conditions, to form the protected derivative **186**. Dehalogenation with $^{n}Bu_3SnH$ afforded silyl ether **187** which was deprotected to yield cyclopropylepothilone A (**55**). Using similar chemistry, cyclopropylepothilones **188**, **189** and **190** were also prepared (Scheme 17).

The Bristol-Myers Squibb (BMS) group developed a very convenient three-step, one-pot procedure for the conversion of epothilones into the corresponding macrolactams (aza-epothilones)[39] (see Scheme 17). To this end, treatment of epothilone B (**1**) with $Pd(PPh_3)_4$ in the presence of NaN_3 afforded the corresponding azide (**192**) with complete retention of stereochemistry at C15. Reduction of this azide with PMe_3 followed by macrolactamization afforded aza-epothilone B (**94**, BMS-247550) in moderate yield, and without the need to isolate any intermediates.

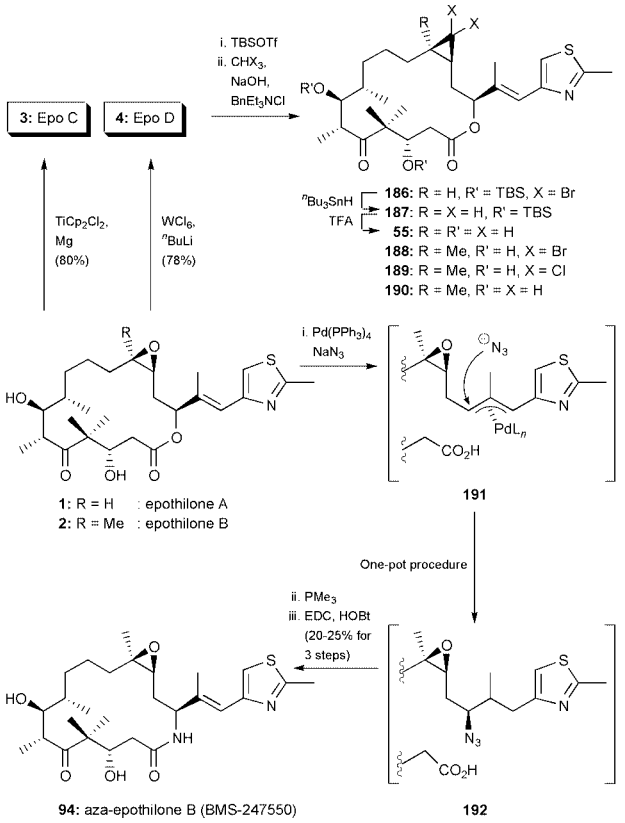
Chemical biology of epothilones

Biological evaluation

As more and more experimental data have accumulated, a fairly good understanding of what modifications to the epothilone



Scheme 16 Höfle's partial syntheses of side chain-modified analogues.



Scheme 17 BMS's partial syntheses of cyclopropyl and aza analogues.

structure might produce active analogues has developed. This section aims to survey some of the most recent progress in this area. Several excellent reviews have appeared,[3–6] and most of the data published in these works will not be repeated here.

Some difficulty in evaluating the results from different studies do arise as a result of the use of many different cell lines, and even differences in experimental protocols may lead to considerable variability between seemingly identical experiments. The *in vitro* experimental data generally fall into two categories, namely tubulin polymerization assays using purified tubulin, and cytotoxicity assays employing various cancer cell lines. Although there is generally some degree of correlation between the results from these two assays, factors such as uptake into and retention by cells obviously play a part in determining the observed effect on cell proliferation, in addition to the effects caused by the tubulin polymerization properties of a given agent. In fact, the concentrations needed to induce tubulin polymerization are two to three orders of magnitude higher than the medium concentrations which will induce cell death. This observation is rationalized by the fact that epothilones, like Taxol®, readily accumulate in cells, so that the relatively high concentrations necessary for tubulin polymerization are finally reached.[6] Because of differences in protocols and cell lines, it will be convenient to first discuss the contributions from the different research groups separately, and then compare the results in order to draw some general conclusions.

We have recently reported two new classes of epothilone analogues with potent biological activities, namely epothilones with pyridine or related side chains (Fig. 2, Table 1),[8] and derivatives of epothilone A (**1**) where the epoxide group has been replaced by a cyclopropyl or a cyclobutyl moiety (Fig. 3, Table 2).[14] A systematic substitution study[8] of pyridine epothilones where the nitrogen atom was "walked" around the ring (**25**–**27**) revealed its crucial position adjacent to the macrocycle attachment site in **27** (Fig. 2, Table 1). A second study "walked" a methyl group around the most active pyridine epothilone analogue (**27**) revealing the C4 and C5 methyl derivatives (**28b**, **28c**, Fig. 2, Table 1) as the most active. Interestingly, the latter compounds (**28b**,**c)** are more active than natural epothilone B (**2**) itself (see Table 1), and they are in fact among the most active derivatives reported to date, with IC_{50} values on the order of 10^{-10} M.

Remarkably, some of the prepared epothilone A cyclopropanes and cyclobutanes (**55**, **57**–**67**) were also active (Fig. 3, Table 1), in some cases even more active than the parent epoxide epothilone A (**1**).[14] In particular, the hybrid epothilones **64** and **65** with pyridine side chains and cyclopropyl moieties at C12–C13 (Fig. 3) were found to be highly active. On the other hand, all derivatives with the unnatural C15 (*R*) configuration (**57**, **62**, **66**, **67**, Fig. 3, Table 1) were essentially inactive. In addition, we have previously reported that *cis*- and *trans*-cyclopropyl epothilones **58** and **59** (Fig. 3, Table 1), with the configuration at C13 opposite to that of the natural series, were inactive.[12,14] From these results, it became clear that in the epothilone A series the stereochemistry at C13 and C15 is critical to the activity. Specifically, the configurations at these centers must match those of the natural compound. On the other hand, the C12 stereocenter appears to play a minor role, as both *cis*- and *trans*-cyclopropanes exhibited similar activity. Several other epothilone B derivatives with heteroaromatic side chains were also prepared (Fig. 2, Table 2; unpublished results). For example, the quinoline derivatives **32a** and **32b** retain significant activity, indicative of the fact that relatively large side chains are tolerated, as long as the basic nitrogen atom is present at the right position.

The Novartis group has also made major contributions to the field, and an excellent review on the biology of epothilones reporting up to the end of 1999 has been published by Altmann.[6] Notable among the many analogues reported by the Novartis group are a series of bicyclic aromatic side chain analogues of epothilones D (**170**–**173**) and B (**174**–**177**) where the aromatic moiety replaces the C16–C17 double bond in the native

Table 1 Induction of tubulin polymerization[a] and cytotoxicity[b] towards human cancer cell lines of selected epothilone analogues from the Nicolaou group and Novartis

Cpd.	% TP	KB-31	KB-8511	1A9	A8	PTX10	PTX22	Ref.
2	85	0.18	0.18	0.2	5.4	0.6	0.2	8
25	35	11.8	34.7	5.75	38	180	25	8
26	42	4.32	16.5	1.7	23	35	8	8
27	80	0.30	0.3	0.1	3	1	0.15	8
28a	12	39.3	50.5	> 300	> 300	> 300	> 300	8
28b	90	0.16	0.16	0.1	2.5	0.36	0.1	8
28c	89	0.11	0.1	0.15	1.5	0.6	0.15	8
28d	30	9.05	10.6	9	180	72	18	8
37	93	*nd*	*nd*	0.2	*nd*	0.4	0.2	3
1	69[c]	2.15[c]	1.91[c]	2.37	117	23.4	5.21	14*b*
2	90[c]	0.19[c]	0.18[c]	0.095	2.14	0.55	0.16	14*b*
Txl	49[c]	2.92[c]	626[c]	1.77	18.0	52.8	28.5	14*b*
55	83	0.84	0.41	1.60	23.4	10.9	2.6	14*b*
57	26	160	66.7	> 300	> 300	> 300	> 300	14*b*
58	2	*nd*	*nd*	> 100	*nd*	> 100	> 100	12
59	2	*nd*	*nd*	> 100	*nd*	> 100	> 100	12
60	79	60.7	29.7	8.8	196	62	20	14*b*
61	29	378	156	> 300	> 300	> 300	*nd*	14*b*
62	100	0.97	0.64	2.7	48	14.4	3.7	14*b*
63	82	23.1	11.5	25.5	> 300	146	63	14*b*
64	100	0.62	0.45	1.40	53.5	8.15	1.17	14*b*
65	94	0.84	0.68	0.63	9.5	3.49	0.39	14*b*
66	6	> 10^3	> 10^3	> 300	> 300	> 300	> 300	14*b*
67	< 10	930	> 10^3	> 300	> 300	> 300	> 300	14*b*

Abbreviations: Cpd. = compound, *nd* = not determined, Txl = Taxol®. [a] %TP = percent tubulin polymerized after incubation of tubulin with a known concentration of compound (typically 3 μM). [b] Cytotoxicity (nM) towards human cancer cell lines. KB-31: epidermoid Taxol®-sensitive, KB-8511: epidermoid Taxol®-resistant (due to P-gp overexpression), 1A9: ovarian Taxol® sensitive, A8: ovarian epothilone-resistant (due to β-tubulin mutations), PTX10 and PTX22: ovarian Taxol®-resistant (due to β-tubulin mutations). [c] Data from ref. 31.

Table 2 Induction of tubulin polymerization[a] and cytotoxicity[b] towards human epidermoid cancer cell lines of selected epothilone analogues from the Nicolaou group and Novartis

Compound	% TP	KB-31	KB-8511	Ref.
1: EpoA	69	2.15	1.91	31
2: EpoB	90	0.19	0.18	31
4: EpoD	83	2.70	1.44	31
Taxol®	49	2.92	626	31
29	77	32	31	[c]
30	49	184	351	[c]
31	70	25	25	[c]
32	78	0.58	0.47	[c]
33	80	0.49	0.37	[c]
34	75	123	302	[c]
35	74	64	132	[c]
36	82	38	41	[c]
38	84	0.45	0.53	[c]
170	76	0.45	0.23	31
171	86	0.46	0.91	31
172	94	0.21	0.73	31
173	90	0.59	0.38	31
174	83	0.13	0.09	31
175	97	0.13	0.46	31
176	99	0.14	0.38	31
177	78	0.11	0.10	31

[a] %TP = percent tubulin polymerized after incubation of tubulin with a known concentration of compound (typically 3 μM). [b] Cytotxicity (nM) towards human cancer cell lines. KB-31: epidermoid Taxol®-sensitive, KB-8511: epidermoid Taxol®-resistant (due to P-gp overexpression). [c] Unpublished results.

epothilones, while retaining the overall shape and, most importantly, the position of the crucial nitrogen atom (see Scheme 15).[31] These compounds are also among the most active analogues reported to date (see Table 2), and they are indeed of comparable activity to our pyridine analogues **28b** and **28c** (*vide supra*).

The BMS group has reported an interesting, one-pot transformation which converts epothilones into their potentially more metabolically stable lactam congeners (*vide supra*).[39] While most of these lactams were found to be significantly less active than their parent lactones,[19,36,39] one compound, 'aza-epothilone B' (**94**: BMS-247550), with good activity was selected as a drug candidate and is currently in clinical trials (see Table 3).[39] The BMS group also reported the partial synthesis of cyclopropyl epothilones A (**55**) and B (**190**) and their potent biological activities (see Table 3).[13]

Table 3 Induction of tubulin polymerization[a] and cytotxicity towards human colon carcinoma cells of selected epothilone analogues from BMS

Compound	Tubulin $EC_{0.01}$ (μM)	HCT-116 IC_{50} (nM)	Ref.
1: EpoA	2.0	4.4	13
2: EpoB	1.8	0.8	13
Taxol®	4.6	2.3	13
3: EpoC	3.9	63	13
4: EpoD	0.6	6.0	13
55: cpEpoA	1.4	1.4	13
188: Br_2cpEpoB	1.6	3.8	13
189: Cl_2cpEpoB	1.7	1.9	13
190: cpEpoB	2.1	0.7	13
1: EpoA	2.3	3.2	39
2: EpoB	1.4	0.42	39
Taxol®	5.0	2.3	39
184: EpoE	17	6.0	39
185: EpoF	1.8	0.77	39
94: azaEpoB	3.8	3.6	39

[a] Tubulin $EC_{0.01}$ (effective concentration) is defined as the interpolated concentration of compound capable of inducing an initial slope of 0.01 OD min^{-1} rate of polymerization.

The Danishefsky group has pursued an extensive synthesis and screening program, which led to a number of interesting results.[4] These authors reported that initial animal studies using the highly potent epothilone B (**2**) were plagued by the high toxicity of this compound. Specifically, it was difficult to achieve high enough doses to effect tumor regression in mice with human tumor xenografts without simultaneously causing lethal toxicity. This led the group to propose epothilone D (**4**), and later desoxyepothilone F (**90**),[17] as more viable drug candidates. Treatment efficacy of these compounds was demonstrated *in vivo*, again using mouse xenograft models.[17,40] The potency of these epothilones is about one order of

magnitude less than that of epothilone B (**2**), but this is apparently more than compensated for by their much lower toxicities to the animals used. Table 4 summarizes the observed

Table 4 Cytotoxicity[a] towards human leukemia cell lines of selected epothilone analogues from the Danishefsky group

Compound	CCRF-CEM	CCRF-CEM/VBL_{100}	CCRF-CEM/VM_1	CCRF-CEM/Taxol®	Ref.
1: EpoA	3.0	200	*nd*	*nd*	17
2: EpoB	0.2	1.0	*nd*	*nd*	17
Taxol®	2.1	4140	6.6	120	17
3: EpoC	22	12	*nd*	*nd*	17
4: EpoD	9.5	17	14	16	17
90: dEpoF	2.7	47	4.9	5.3	17
93: azaEpoD	27.8	997	*nd*	791	19*b*
94: azaEpoB	2.1	2990	39	171	19*b*

Abbreviations: *nd* = not determined. [a] Cytotxicity (nM) towards human T-cell acute lymphoblastic leukemia cell lines. CCRF-CEM: parental cell line, CCRF-CEM/VBL_{100}: vinblastin resisistant, multidrug-resistant (due to P-gp overexpression), CCRF-CEM/VM_1: teniposide-resistant (due to mutated topoisomerase II), CCRF-CEM/Taxol®: Taxol®-resistant.

cytotoxicities for these compounds against some sensitive and multidrug-resistant human leukemia cell lines.

Structure–activity relationships

As a result of the extensive chemical synthesis–chemical biology studies of hundreds of epothilone analogues, structure–activity relationships could be established quite rapidly.[3] An electron crystallographic structure of the tubulin αβ dimer with bound Taxotere™ at 3.7 Å resolution has been disclosed, and although the resolution is too low to pinpoint the exact conformation of bound ligand, it clearly identifies its binding site.[41] The solution conformations of epothilones have been investigated by NMR methods,[42] and by purely computational techniques.[43] Several pharmacophore models have been advanced,[44–48] generally incorporating not only epothilones, but also taxoids and other tubulin binding molecules, under the assumption that they all bind to a common binding site on the tubulin dimer. This assumption was based on the similar biological effects of these various substances, the competitive and mutually exclusive binding of different compounds to tubulin, and the partial cross-resistance acquired by tubulin mutants with amino acid replacements at the proposed binding site. Although there are many differences between these studies, particularly as to the proposed binding conformations, there seems to be some level of consensus as to what features are of importance to binding (see Fig. 4).

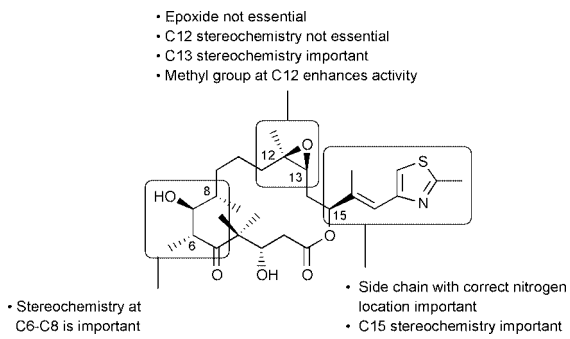


Fig. 4 Structure–activity relationships for the epothilones.

The configurations at C6–C8 are vital for the biological activity, probably because this region strongly influences the overall conformation of the macrocycle through steric and/or stereoelectronic effects.[42] There was initially some speculation that the epoxide oxygen of epothilone played a role as a hydrogen bond acceptor, but after independent reports by several groups[13,14,40*b*] it became clear that the epoxide moiety is not essential for biological activity. However, a substituent at C12, particularly a methyl group, consistently enhances the activity. Interestingly, both *cis*- and *trans*-epoxides and cyclopropanes are of comparable activity, so that as long as the configuration at C13 agrees with that of the native epothilones, the C12 stereochemistry is of relatively little importance. This is most probably due to the flexible C9–C11 trimethylene element, which allows both stereoisomers to be accomodated within the binding site. The side chain is also highly important for biological activity, with 4- or 5-methylpyridine or related derivatives being the optimum choice so far, about two-fold more active than the native 2-methylthiazole analogues.[8] Even quinoline side chains resulted in very active analogues, indicative of the fact that considerable steric bulk is tolerated in the side chain. Finally, the stereochemistry at C15 is very important, with C15 epimers being largely devoid of any biological activity.[14,19]

Biosynthesis

The biosynthetic pathway leading to the epothilones has been elucidated in some detail (Fig 5). Two independent reports, originating from Novartis[49] and KOSAN Biosciences,[50] on the gene cluster responsible for epothilone production in different *Sorangium cellulosum* strains have appeared with essentially identical results and conclusions. Both epothilones A and B are produced by the same polyketide synthase (PKS), which includes a non-ribosomal peptide synthase (NRPS) domain for the formation of the thiazole side chain from cysteine. One of the C4 gem-dimethyl groups is introduced by an (*S*)-adenosylmethionine-dependent methyltranferase domain which is also part of the PKS. It appears that the acyltransferase domain responsible for the installation of the C11–C12 fragment is rather unselective for malonyl-CoA *vs.* methylmalonyl-CoA, and can incorporate either of these units, ultimately giving rise to epothilones C and D, respectively. Both of these latter compounds are the end products of the same PKS, and the epothilones A and B are formed by post-PKS oxidation by a cytochrome P450 oxygenase, which is also part of the epothilone gene cluster. Additional biosynthetic studies have been performed by the Höfle group, and their results confirm the findings discussed above.[51,52] Using labeling techniques, it was confirmed that the epothilones are indeed synthesized from acetate and propionate units, one cysteine unit (C17–C19 of the thiazole side chain), and the methyl group of methionine (incorporated as one of the C4 methyls). It was further shown that epothilones C and D are the final products of the same PKS, and these are oxidized by a separate enzyme to epothilones A and B. Although only trace amounts of epothilones C and D are produced by the native strain of *S. cellulosum*, mutants with defects which render the oxygenase enzyme inactive have been created and shown to produce only epothilones C and D.[52] It has also been suggested that by replacing the C11–C12 acyltransferase domain with a methylmalonyl-CoA-specific one should lead to a PKS specific for epothilone D.[50] Impressively, it has already been possible to produce a mixture of epothilones A (**1**) and B (**2**) by cloning of the entire epothilone gene cluster and expressing it in *Streptomyces coelicolor*, a much better understood organism, and with a ten-fold faster rate of growth as compared to *S. cellulosum*.[50]

Preclinical and clinical studies

In contrast to the extensive chemistry and *in vitro* biological studies discussed above, relatively scarce data have been disclosed on the *in vivo* efficacy of the epothilones. To date, published results only exist for natural epothilones B (**2**)[6,40] and D (**4**),[40] aza-epothilone B (**94**: BMS-247550, Scheme 7),[19*b*,53] desoxyepothilone F (**90**, Scheme 7),[17] and 26-fluoroepothilone B (**37**, Fig. 2).[54] Danishefsky's group initially reported promising activity against subcutaneously implanted tumors in SCID mice for epothilone B (**2**),[40*a*] but they later encountered severe toxicity problems with this compound.[40*b*] On the other hand, it was claimed that epothilone D (**4**) was much less toxic, and this compound was found to be superior to both epothilone B (**2**) and Taxol® in a variety of mouse tumor models.[40*b*,40*c*] In some cases, epothilone D (**4**) was found to be curative against

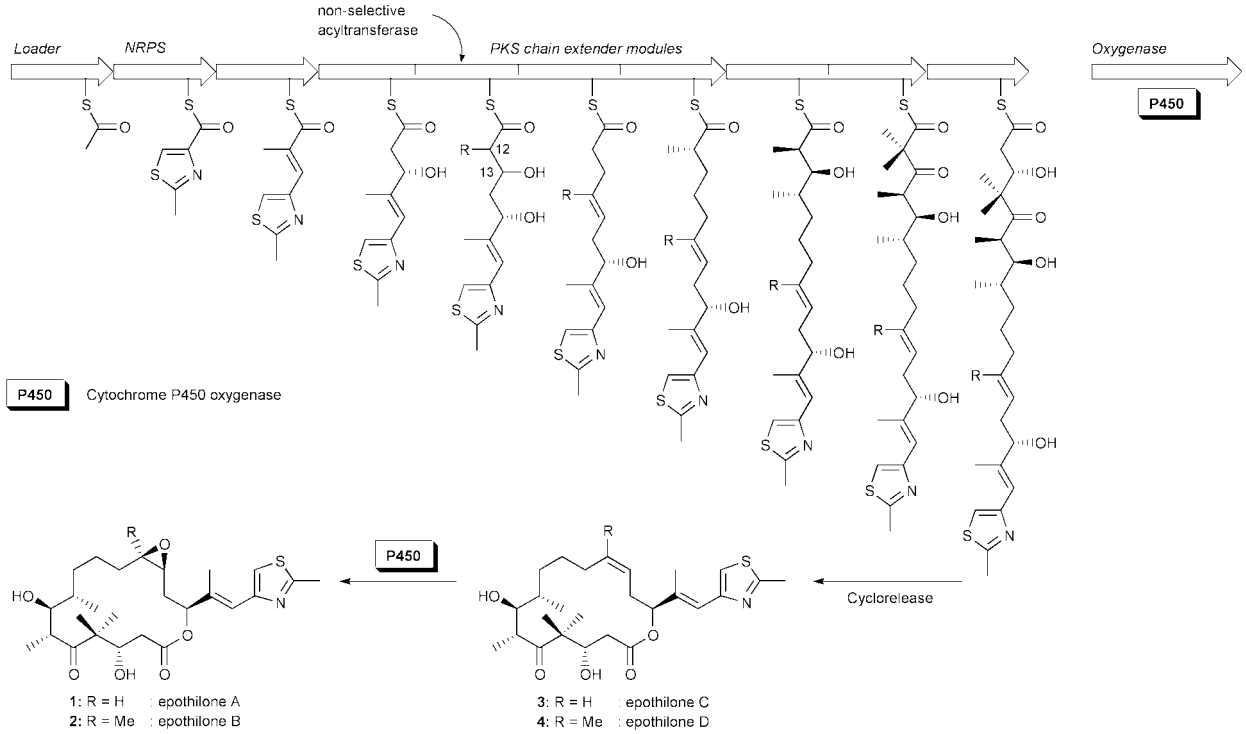


Fig. 5 Structure of the epothilone biosynthetic gene cluster from *Sorangium cellulosum*. NRPS = non-ribosomal peptide synthase, PKS = polyketide synthase.

human tumor xenografts, even when these were unresponsive to Taxol®.[40c] Preliminary results for desoxyepothilone F (**90**)[17] showed that this compound is highly potent in the mouse tumor models employed, while preliminary data for aza-epothilone B (**94**) showed that this compound appeared to be less effective in reducing tumor growth.

The BMS team also encountered difficulties with natural epothilone B (**2**) not only due to its toxicity in mice and lower primates, but also because of its low metabolic stability towards various esterases. To ameliorate these problems, aza-epothilone B (**94**: BMS-247550) was targeted and it was found to possess a very promising pharmacological profile, despite its lower *in vitro* activity compared to epothilone B (**2**).[53] In fact, even when orally administered, **94** was found to be highly effective against a range of human tumor xenografts in mice and rats, including taxol-resistant tumors.[53]

Phase I clinical trials with this compound have been conducted by BMS, and the results are so far very promising.[55] It was found that although the toxicity of **94** was similar to that of Taxol®, **94** did show evidence of being effective in patients with taxane-resistant tumors. Clinical trials with this compound are currently entering phase II.

Contrary to the results above, through tumor graft studies in mice, the Novartis group found epothilone B (**2**) itself to be a viable drug candidate,[6] and this compound is also currently in phase II clinical trials, having successfully been evaluated in clinical phase I trials.[56] The Novartis group has also carried out extensive preclinical studies with a number of our designed epothilone analogues. In collaboration with the Logothesis–Navone group, we have also carried out comparative *in vivo* studies with Taxol®, epothilone B (**2**) and 26-fluoroepothilone B (**37**),[54] and we found that the latter compound was more active than Taxol® in inhibiting growth of human prostate cancer xenografts in mice, and the tolerated dose of this agent was higher than that for either Taxol® or epothilone B (**2**). These observations were attributed to lower overall toxicity of the fluoroepothilone analogue **37**.

Conclusion

With a number of epothilones (from both the natural and designed categories) in clinical trials as potential anticancer agents, the anticipation regarding this class of compounds is climaxing. Indeed their emergence as top candidates for cancer chemotherapy was rapid, being greatly facilitated by chemical synthesis and chemical biology studies. Carried out by many groups around the world, these investigations ensured the availability of not only the naturally occurring substances, but also of thousands of analogues which allowed elucidation of structure–activity relationships. While more results from the clinical trials of this first generation epothilone drug candidates are eagerly awaited, the basic research efforts that brought these molecules thus far will no doubt continue unabated for some time to come.

Addendum

Since the submission of this manuscript relevant publications have appeared in the literature.[57–65]

Acknowledgements

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Notes and references

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