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Review Article

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COMPREHENSIVE REVIEW

Diversity through semisynthesis: the chemistry and biological activity of semisynthetic epothilone derivatives

Karl-Heinz Altmann · Fabienne Z. Gaugaz · Raphael Schiess

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Abstract Epothilones are myxobacterial natural products that inhibit human cancer cell growth through the stabilization of cellular microtubules (i.e., a "taxol-like" mechanism of action). They have proven to be highly productive lead structures for anticancer drug discovery, with at least seven epothilone-type agents having entered clinical trials in humans over the last several years. SAR studies on epothilones have included a large number of fully synthetic analogs and semisynthetic derivatives. Previous reviews on the chemistry and biology of epothilones have mostly focused on analogs that were obtained by de novo chemical synthesis. In contrast, the current review provides a comprehensive overview on the chemical transformations that have been investigated for the major epothilones A and B as starting materials, and it discusses the biological activity of the resulting products. Many semisynthetic epothilone derivatives have been found to exhibit potent effects on human cancer cell growth and several of these have been advanced to the stage of clinical development. This includes the epothilone B lactam ixabepilone (Ixempra®), which has been approved by the FDA for the treatment of advanced and metastatic breast cancer.

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Introduction

Microtubule-interacting agents (MSA) are an important class of antitumor agents [1], which are in use for the treatment of a variety of cancers, either as single agents or as part of combination chemotherapy [2,3]. MSA can be divided into two distinct functional classes, namely compounds that inhibit the assembly of soluble tubulin into microtubule polymers ("tubulin polymerization inhibitors") and those that *promote* the assembly of tubulin heterodimers into microtubule polymers and stabilize microtubules ("microtubule stabilizers") [4]. Among microtubule stabilizers the natural product taxol (paclitaxel; Taxol®) and its semisynthetic analog docetaxel (Taxotere®) (Fig. 1) are an important part of today's armamentarium for the pharmacotherapy of cancer [5].

After the elucidation of taxol's mode of action in 1979 [6], it took more than a decade before other microtubule-stabilizing agents with non-taxol-like structures were discovered. Most prominent among these new microtubule stabilizers are the epothilones, which are bacteria-derived macrolides whose microtubule-stabilizing properties were discovered in 1996 by a group at Merck Research Laboratories [7]; the compounds themselves, however, had been first isolated 9 years earlier from the myxobacterium *Sorangium cellulosum Sc 90* by Reichenbach and Höfle (Fig. 2) [8,9].

The major products originally isolated by Reichenbach and Höfle were epothilone A and epothilone B (Epo A and B), but numerous other members of this natural products family have subsequently been obtained as minor components from fermentations of myxobacteria [10]. Based on their unusual

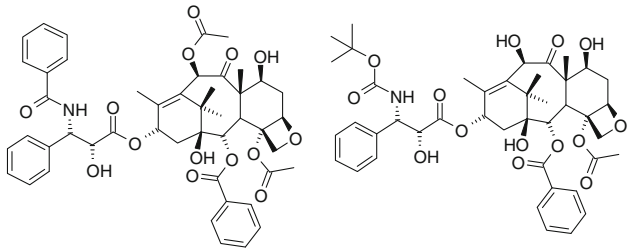


Fig. 1 Molecular structures of taxol and docetaxel

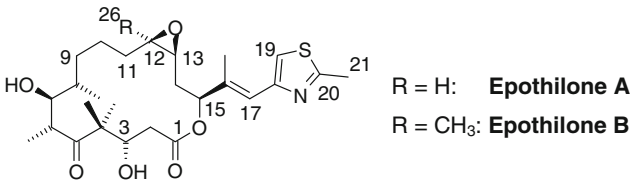


Fig. 2 Molecular structures of epothilones A and B

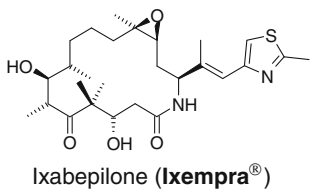


Fig. 3 Molecular structure of the anticancer drug ixabepilone

mechanism of action (at the time of its discovery), Epo A and B were quickly adopted as attractive targets for total synthesis and, more importantly, as lead structures for anticancer drug discovery.

Epothilone-based drug discovery research was additionally triggered by the fact that epothilones, in contrast to taxol, can inhibit the growth of multidrug-resistant cancer cell lines at concentrations similar to or only slightly higher than those required against drug-sensitive cancer cells, [7,11–13] including cells whose taxol resistance is mediated by specific tubulin mutations [13,14]. Epo B and a number of its analogs have been demonstrated to possess potent in vivo antitumor activity and at least seven epothilone-type compounds have entered clinical evaluation in humans (although several of these are not anymore under development). One of these compounds, the epothilone B lactam BMS-247550, was approved for clinical use in humans in 2007 (ixabepilone, Ixempra®), (Fig. 3) [15].

As indicated above, epothilones have been attractive targets for total chemical synthesis and numerous syntheses of Epo A and B have been successfully completed (for reviews cf. [16–22]). At the same time, the methodology developed in the course of those studies has been exploited for the synthesis of a host of synthetic analogs for SAR studies (reviewed in [16,17,20,23–27]), which highlights the difference in structural complexity (and, consequently, in synthetic accessibility) between epothilone-type structures and taxol. Beyond the investigation of fully synthetic analogs, however, important aspects of the epothilone SAR (structure–activity relationship) have also been derived from numerous semisynthetic epothilone analogs and ixabepilone (Fig. 3), the only epothilone that has reached the approval stage so far, in fact is a semisynthetic derivative of Epo B.

Semisynthetic derivatives of natural products hold a prominent place in natural product-based drug discovery in virtually all disease areas [28,29]; due to the structural complexity of many biologically active natural products [30], the chemical derivatization of material isolated from natural sources often represents the only practical means to explore structure–activity relationships and to produce analogs with improved biological and/or pharmaceutical properties. In cancer treatment, important natural product derivatives include compounds such as etoposide or teniposide (derived from podophyllotoxin) [31–33], irinotecan and topotecan (derived from camptothecin) [34–36], or docetaxel (derived from 10-deacetylbaccatin III) [5,37]. Even for the natural product taxol [38], the sustained supply of sufficient quantities of drug material for clinical use could only be secured for some time through the development of a semisynthetic production process from another natural product, namely 10-deacetylbaccatin III [39,40]. Thus, it is not surprising that semisynthetic approaches have also featured prominently in the elucidation of the SAR of epothilones and in the discovery of a number of clinical development candidates. In fact, out of the seven epothilones that have entered clinical evaluation in humans so far (including the natural product Epo B), only one is produced by total chemical synthesis. This bias towards semisynthesis reflects the technical (fewer chemical steps) and economic (cost of goods) advantages still associated with natural product derivatization.

Obviously, the most fundamental provision for the generation of semisynthetic derivatives of a natural product is a sufficient supply of the natural product starting material itself. Fermentation processes are characterized by their own complexities; thus, in the case of epothilones, only few groups have had access to fermentatively produced starting materials to perform semisynthetic work. Thus, most semisynthetic epothilone derivatives reported in the literature originate either from the Höfle group at the former "Gesellschaft für Biotechnologische Forschung" in Braunschweig, Germany (GBF, now "Helmholtz Centre for Infection Research"), one of the discoverers of epothilones, or the group at Bristol–Myers–Squibb (BMS), either by themselves or in collaboration [8,41]. Semisynthetic work on epothilones, although more limited in scope, has also been reported by groups at Novartis, Kosan, and, most recently, our own group at the ETH Zürich.

This review will provide an overview on the semisynthetic work that has been reported for epothilones in the public literature over the last 15 years. The discussion will address both, the organic chemistry of the system, as well as the most important aspects of the biological activity and SAR of these derivatives, and it will be structured according to the location of groups of modifications in the overall epothilone structural framework. This will facilitate the comparison of the biological effects of related structural changes. However, it is important to note that biological data (i.e., tubulin polymerization, in vitro and in vivo antiproliferative activity) may not always be directly comparable when originating from different laboratories, due to differences in the experimental conditions employed.

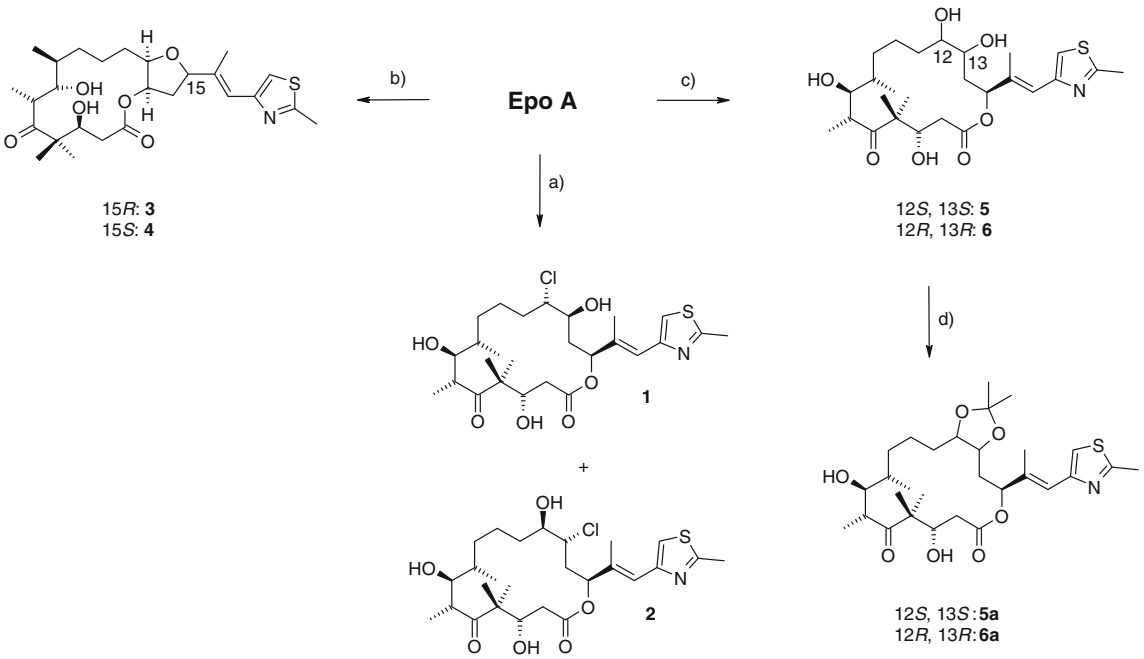
Semisynthesis and SAR studies

Modifications of the epoxide moiety

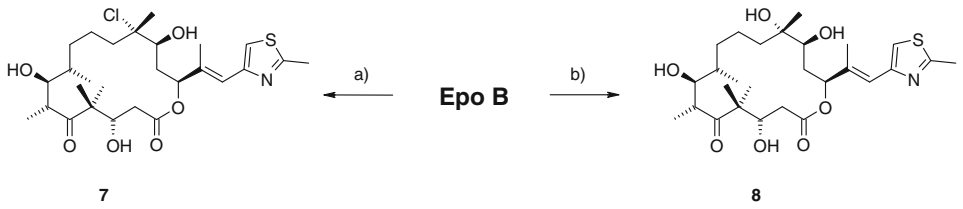
Modifications of the epoxide moiety have been an important trait of the semisynthetic work on epothilones from the very beginning, which is unsurprising in light of the multitude of transformations that are conceivable for an oxirane ring and the potential for further elaboration of the initial reaction products. The earliest contributions to this area stem from the GBF group and involved the transformation of epothilones A, B, and C (*vide infra*) into a variety of C12/C13-modified derivatives [23]. Thus, treatment of Epo A with HCl in THF (aq) or with 1M HCl gave chlorohydrins **1** and **2** in 60–80% overall yield in a ca. 2–4:1 ratio (in favor of the C12-chloro isomer **1**; Scheme 1) [42].

The corresponding bromo- and iodohydrins were obtained with bromine or iodine in $CCl_4/CHCl_3$, respectively, with a ca. 3:1 preference for the C12-halo regioisomer in both cases [42]. Preferential (but not completely selective) opening of the epoxide moiety at position 12 upon treatment of Epo A with different nucleophiles (HCl, $MgBr_2 \cdot Et_2O$, NaI/TMS-I, LiN_3, $Mg(OMe)_2$) has also been reported by the Novartis group [43]; in contrast, the reaction of Epo A with $MgBr_2 \cdot Et_2O$ in CH_2Cl_2 at −20°C to −5°C leads to the C13-bromo isomer preferentially with less than 2% of the C12-regioisomer being formed [44,45] (*vide infra*). Due to the greater stability of the C12 over the C13 carbocation in S_N1-type reactions, treatment of Epo B with HCl gave chlorohydrin **7** as the *only* regioisomer in >80% yield (Scheme 2) [42].

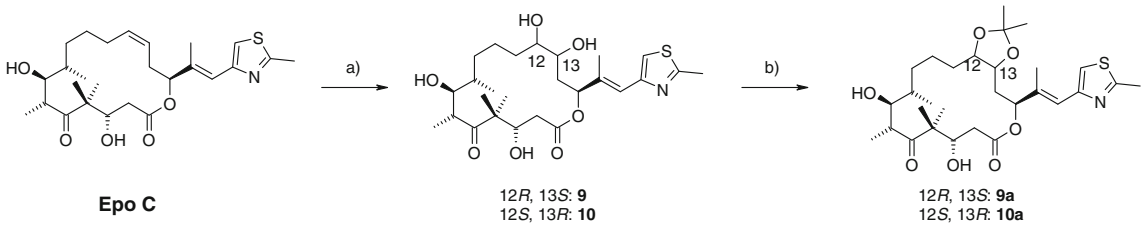
Treatment of Epo A with a non-nucleophilic Brønsted acid such as TFA led to rearranged products **3** and **4** exclusively (85% combined yield), when acetone was used as the solvent (Scheme 1) [42]. In contrast, exposure of Epo A or B to non-nucleophilic acids in the presence of water gave diols **5/6** (Scheme 1) and **8** (Scheme 2), respectively. As for halohydrin formation, nucleophilic attack of the epoxide moiety in Epo A occurs at position 12 preferentially, leading to isomer



Scheme 1 *a* THF/HCl (aq) or 1 M HCl, RT, 20 min, 60–80%, **1**:**2**, 2:1–4:1. *b* 0.65 M CF_3COOH, acetone, 50°C, 10 h, 50% (**3**) and 35% (**4**). *c* 0.65 M CF_3COOH, H_2O, 23°C, 48 h, 55% (**5**) and 15% (**6**). *d* *p*-TsOH, acetone, 28% (**5a**) and 15% (**6a**). (Yields for **5a** and **6a** are from [43])



Scheme 2 *a* THF/HCl (aq) or 1 M HCl, RT, 20 min, >80%. *b* CF_3COOH/H_2O, 23°C or H_2SO_4/H_2O/THF, 60°C, 75% (H_2SO_4) or 45% (CF_3COOH)



Scheme 3 *a* OsO_4 cat., NMO, *t*-BuOH, THF/H_2O, RT, 75 min, 62%, **9**:**10**, 2:1 (inseparable mixture). *b* acetone, *p*-TsOH, RT, 2 h, 90% (for separable mixture of isomers)

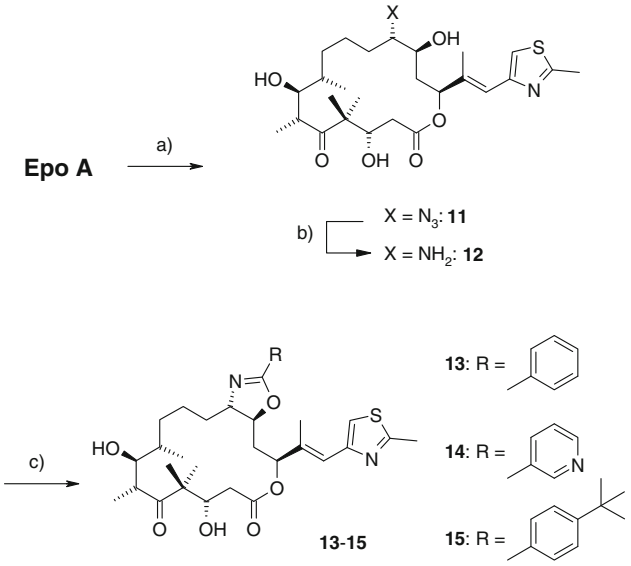
5 as the major (but not the only) product; with Epo B diol **8** is the only isomer formed. The rearranged products **3** and **4** show substantially lower antiproliferative activity against human cancer cells than Epo A [42].

The GBF group has also used OsO_4-catalyzed dihydroxylation of fermentatively produced Epo C (12,13-deoxyepothilone A) to prepare *cis*-diols **9** and **10** (Scheme 3); these compounds were subsequently converted into acetonides **9a** and **10a** (as were diols **5** and **6**; Scheme 1) [42] (see also [43]). Acetonides **5a/6a** and **9a/10a** have been independently reported by the Novartis group [43], which has also investigated the biological activity of these analogs.

Interestingly, the acetonides derived from 13*S* diols **5** and **9** (i.e., **5a** and **9a**) proved to be only 10–15-fold less potent antiproliferative agents than Epo A against the human cervical carcinoma cell line KB-31 and its P-gp-expressing KB-8511 subline (IC_{50} values of 23 nM (10 nM) and 30 nM (17 nM), respectively), while the respective diastereoisomers **6a** and **10a** were found to be 30–100-fold less potent [43]; likewise, Sefkow et al. [42] have reported **5a** to have similar antiproliferative activity as Epo C against the L929 mouse fibroblast cell line. These data suggest that for a tetrahedral geometry at C12 and C13 the size of the ring fused to the C12–C13 single bond can be significantly increased without substantial loss in biological potency (which does not seem to be the case for analogs with a planar geometry of the C12–C13 bond [43,46]). In addition, the data for **9a** and **5a** also illustrate that, given the proper absolute stereochemistry at C12 and C13, activity is retained even upon moving from a *cis*- to a *trans*-fused system; this is in line with data obtained for a number of synthetic C12,C13-*trans* epothilones A [27]. It should be noted, however, that the absolute configuration of compounds **5a** and **9a** (or the respective diastereoisomers) has not been rigorously established in the literature, and it is simply inferred from a comparison of the biological data with those obtained for Epo A/*epi*-Epo A (the inactive 12*S*,13*R*-isomer of Epo A) and 12*S*,13*S*/12*R*,13*R*-*trans*-Epo A, respectively.

In contrast to the above acetonides, *cis* and *trans* diols **9** and **5** did not show any appreciable biological activity (IC_{50}'s for cancer cell growth inhibition >1 μM) [42,43]. Interestingly, however, the azido alcohol obtained through epoxide ring opening with NaN_3 at position C12 (i.e., (12*R*, 13*S*)-12-azido-13-hydroxy-12,13-dihydro-Epo C (**11**), Scheme 4) was found to be significantly more potent (e.g., IC_{50}'s of **11** against the human cervix cancer cell lines KB-31 and KB-8511 are 61 and 64 nM, respectively) [43]. This indicates that the loss in activity for the above diols cannot be simply ascribed to increased conformational flexibility. However, the interpretation of changes in cellular activity is not straightforward, as they may be caused by a combination of changes in target affinity, cellular penetration, and metabolic stability.

Building on the above findings on the potent activity of acetonides **5a** and **9a**, we have recently studied bicyclic epothilones **13**–**15** (Scheme 4) and a series of related analogs [48], in order to delineate the biological effects of other 5-membered heterocycles fused to C12–C13 in a non-planar arrangement and, in particular, to assess the impact of substituents on the 5-membered ring. The synthesis of Epo A-derived oxazolines **13**–**15** was based on amino alcohol **12** as the central intermediate (Scheme 4). As illustrated in Scheme 4, **12** was obtained through nucleophilic ring-opening of the epoxide moiety in Epo A with azide anion



Scheme 4 *a* LiN_3, NH_4Cl, DMF, 85°C, 24 h, 38%. *b* Ph_3P, THF/H_2O 15/1, RT, 88 h, 50%. *c* **13**: $PhC(OC_2H_5)_3$, DCE, 90°C, 69%. **14**: $C_5H_4NC(=NH)OC_2H_5$, DCE, 90°C, 3 h, 33%. **15**: p-$C_4H_9PhC(=NH)OC_2H_5$, DCE, 90°C, 1 h, 54%

(to produce **11**) and subsequent reduction of the azide group under Staudinger conditions (Ph_3P/H_2O) [48]. The structure of azido alcohol **11** (with a 12-azido group) as the major product of the reaction between Epo A and LiN_3 in DMF in the presence of NH_4Cl (the conditions employed in [48]) was firmly established by means of NMR spectroscopy. The regiochemical course of the epoxide opening reaction is thus identical with that reported for the reaction of 12,13-bis-*epi*-Epo A with NaN_3 in EtOH [45].

The elaboration of amino alcohol **12** into oxazolines **13-15** involved reaction of **12** with (commercially available) triethyl-*ortho*-benzoate (**13**) or crude imino esters (**14/15**) (Scheme 4); the latter were prepared from the corresponding nitriles by treatment with HCl EtOH.

The phenyl-substituted oxazoline **13** was found to inhibit human cancer cell growth in vitro with IC_{50} values around 20 nM [48]; thus, the activity of this analog is within a 10-fold range of the activity of Epo A and it is comparable with the activity of cyclic acetals **5a** and **9a** (*vide supra*). Quite intriguingly, the cellular activity of pyridyl derivative **14** is even >10-fold higher than for **13** and the IC_{50} values of this compound for cancer cell growth inhibition are now comparable with those of Epo A. Likewise, **14** is also a potent inducer of tubulin assembly, but the difference in polymerization efficiency between **14** and **13** is less pronounced than the difference in antiproliferative activity [48]. It is, therefore, unclear to what extent (if at all) the enhanced cellular activity of **14** (over **13**) is a result of higher affinity interactions with the tubulin/microtubule system (possibly through H-bond formation between the pyridine nitrogen and a donor group on the protein).

In contrast to the unsubstituted phenyl and pyridyl moieties in **13** and **14**, respectively, the presence of a *p-tert*-butyl-phenyl substituent in the 2-position of the oxazoline ring leads to a dramatic loss in potency; the corresponding analog **15** no longer exhibits any meaningful antiproliferative activity ($IC_{50} > 10\,\mu M$) [48]. The lack of activity for **15** represents the culmination of a general SAR trend that points to an inverse relationship between the size of a *para*-substituent on an aryl moiety at the 2-position of the oxazoline ring and antiproliferative activity.

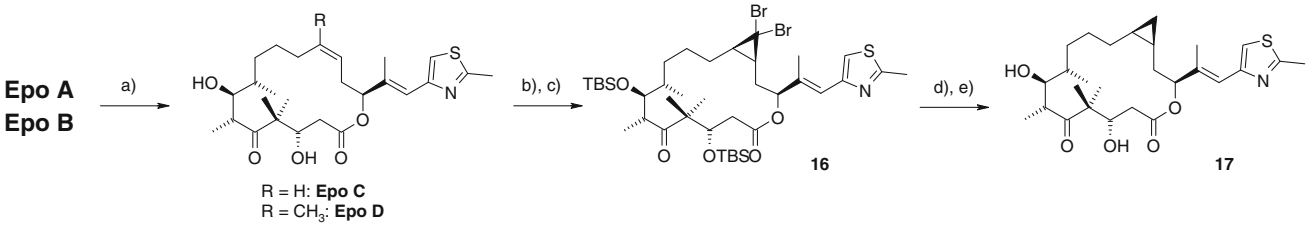
Epo A or B can be deoxygenated and thus chemically converted into Epo C and D (deoxyepothilones A and B), respectively. In the case of Epo A, this transformation can be achieved either with 3-methyl-2-selenoxo-benzothiazole in CH_2Cl_2/CF_3COOH (40%) [43] or with $TiCp_2Cl_2/Mg$ in THF (80%, Scheme 5) [49].

The Epo B → Epo D conversion seems to be best accomplished with WCl_6 and *n*-BuLi in THF, which gave Epo D in 78% yield (Scheme 5) [49].

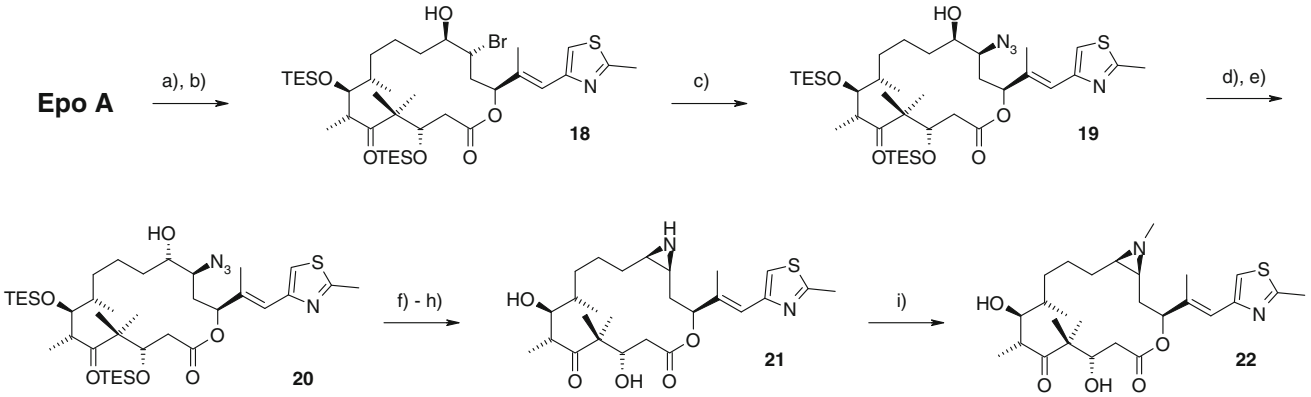
Biological studies with deoxyepothilones were first conducted with fully synthetic material (Epo C and D are advanced precursors in the total synthesis of Epo A and B, respectively). These studies showed, at a very early stage, that the oxirane ring is not an absolute structural requirement for highly potent biological activity of epothilone-type molecules [50–53]. Thus, Epo C and D are virtually equipotent inducers of tubulin polymerization as Epo A and B, respectively; they are also potent inhibitors of human cancer cell growth in vitro, including multidrug-resistant cells, although compared to the corresponding parent epoxides their overall activity is somewhat reduced. For example, Epo D inhibits the growth of the human cervix cancer cell line KB-31 and the leukemia cell line CCRF-CEM with IC_{50} values of 2.7 nM [43] and 9.5 nM [54], respectively, versus IC_{50}'s of 0.19 and 0.35 nM for Epo B. The reduced antiproliferative activity of Epo D compared to Epo B may be related to differences in cellular uptake between the two compounds [55].

The C12–C13 double bond in Epo C can be selectively reduced (over the C16–C17 double bond) with in situ generated diimide to provide an analog with a completely saturated macrocyclic core structure in 60% yield (98% based on recovered starting material) [42]. This compound proved to be several hundred-fold less active than Epo A [42].

The above methodology for the reduction of Epo A and B to *cis* olefins Epo C and D, respectively, which was largely developed by the BMS group, provided the basis for the semisynthesis of cyclopropane-based analogs of epothilones, which is exemplified in Scheme 5 for 12,13-cyclopropyl-Epo A **17** [49]. The synthesis of **17** from Epo C proved to be highly challenging, as the direct cyclopropanation of the 12,13-double bond under Simmons–Smith conditions did not produce any practical amounts of the desired product. In contrast, treatment of TBS-protected Epo C with $CHBr_3$/50% NaOH



Scheme 5 *a* Epo A: $TiCp_2Cl_2$, Mg(s), THF, 80%; Epo B: WCl_6, *n*-BuLi, THF, 78%. *b* Epo C: TBSOTf, CH_2Cl_2, 2,6-lutidine, 0°C, 69%. *c* $BnEt_3N^+Cl^-$, 50% NaOH (aq), $CHBr_3$, 45°C, 12%. *d* Bu_3SnH, AIBN, C_6H_{12}, 70°C, 76%. *e* 20% CF_3COOH/CH_2Cl_2, −15°C, 90%



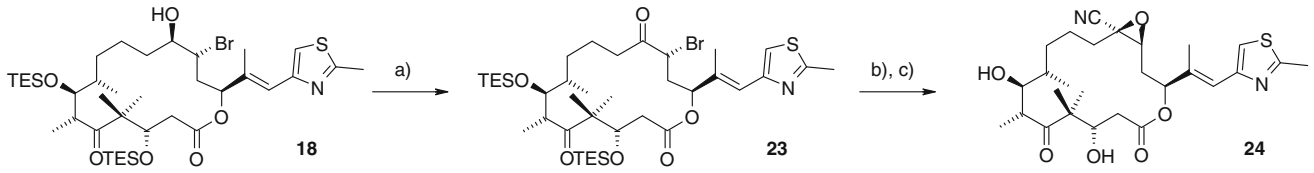
Scheme 6 *a* TESCl, DIEA, DMF, 90%. *b* $MgBr_2 \cdot Et_2O$, CH_2Cl_2, −20 → −5°C, 45% (<2% of the C12-Br, C13*R*-OH regioisomer). *c* NaN_3, DMF, 48 h, 42°C, 60%. *d* *p*-NBA, DEAD, Ph_3P, 99%. *e* NH_3, MeOH, 88%. *f* MsCl, Et_3N, CH_2Cl_2, 98%. *g* Me_3P, THF/H_2O, 98%. *h* 10% CF_3COOH/CH_2Cl_2, −10°C, 90%. *i* Me_2SO_4, proton sponge, THF, 31%

in the presence of benzyltriethylammmonium chloride at 45°C gave dibromocyclopropane **16** as a single diastereoisomer, albeit in a very low yield of only 12%; nevertheless, this process gave access to sizable quantities of the target structure **17** (after reduction and deprotection, Scheme 5). Cyclopropyl-Epo A **17** and the corresponding Epo B analog were found to be equipotent with Epo A and B; e.g., IC_{50}'s for **17** and cyclopropyl-Epo B against the human colon carcinoma cell line HCT-116 are 1.4 and 0.7 nM, respectively [49]. These findings re-confirmed the notion that the oxirane ring system in epothilones merely serves to stabilize the proper bioactive conformation of the macrocyclic skeleton rather than acting as a reactive electrophile or a hydrogen bond acceptor. As shown in a later study by Buey et al. [56], the replacement of the epoxide moiety by a cyclopropane ring also produces enhanced binding to stabilized microtubules in vitro. Bis-substitution of the oxygen-replacing carbon in cyclopropyl-Epo B by bulky bromine substituents leads to reduced activity, but the resulting analog is still more potent than Epo D against the HCT-116 cell line (IC_{50} against HCT-116 of 3.8 nM versus 6.5 nM for Epo D [49]).

In addition to cyclopropyl-epothilones, the BMS group has also devised a strategy for the conversion of Epo A to a whole range of analogs incorporating a (substituted) aziridine ring in place of the epoxide moiety [45].

As illustrated in Scheme 6, the optimized route to these compounds involves regioselective epoxide ring-opening with $MgBr_2 \cdot Et_2O$ (at C13!) followed by bromide displacement with azide ion, Mitsunobu-based inversion of configuration at C12, activation of the 12-hydroxyl group as a mesylate and finally aziridine ring formation through azide reduction under Staudinger conditions. The resulting *N*-unsubstituted 12,13-aziridinyl-Epo A **21** has been converted into a series of *N*-substituted derivatives *via* alkylation, acylation, carbamoylation, or sulfonylation. Several of these derivatives show antiproliferative activities that are comparable with or even superior to that of Epo A [45]. (For example, IC_{50}'s for **21** and **22** against the human colon carcinoma cell line HCT-116 are 2.7 and 0.13 nM, respectively). Most recently, a folate conjugate of the *N*-(hydroxyethyl)-derivative of **21** (epofolate, BMS-753493) has been advanced by BMS to two Phase I/II clinical studies; these studies were terminated as of July 2010 [57].

Bromohydrin **18** (Scheme 6) has also been elaborated into 12-cyano-Epo A **24** which was expected to be more acid-stable than the natural product Epo B (Scheme 7) [44]. As outlined in Scheme 7, oxidation of **18** with pyridinium chlorochromate (PCC) furnished bromo ketone **23**, whose treatment with KCN led to cyanohydrin formation and concomitant epoxide ring closure. Deprotection with CF_3COOH



Scheme 7 *a* PCC, pyridine, CH_2Cl_2, 92%. *b* KCN, 18-C-6, THF, 49%. *c* 10% CF_3COOH/CH_2Cl_2, −10°C, 98%

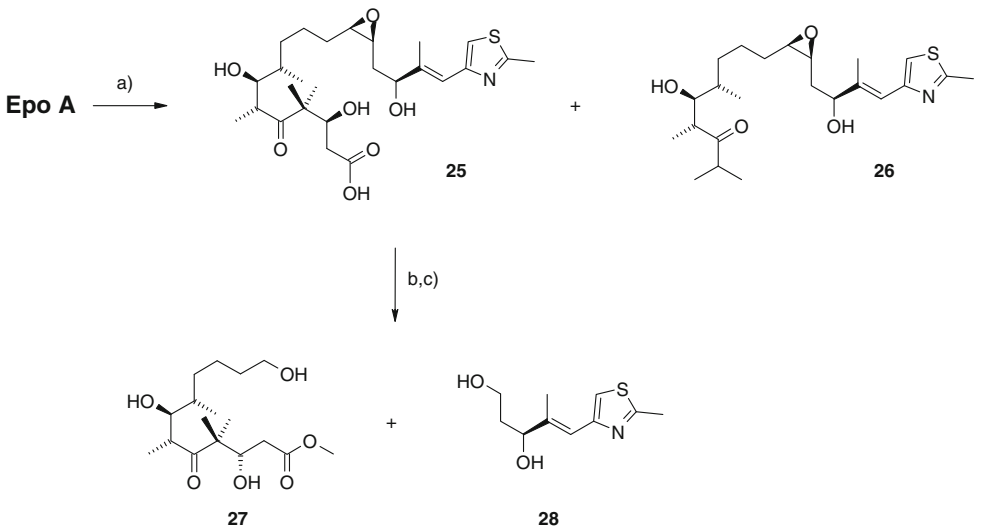
then provided the target structure **24**. Interestingly, chlorohydrins **1**, **2**, and **7** (Schemes 1 and 2) have been reported *not* to undergo epoxide formation even under strongly basic conditions [42], in contrast to the facile formation of the epoxide ring from the cyanohydrin of ketone **23**. The antiproliferative activity of 12-cyano-Epo A (**24**) is comparable with that of Epo A (i.e., the compound is highly potent, but the activity is lower than that of its (C12-methyl) parent compound Epo B).

Modifications of the ester moiety

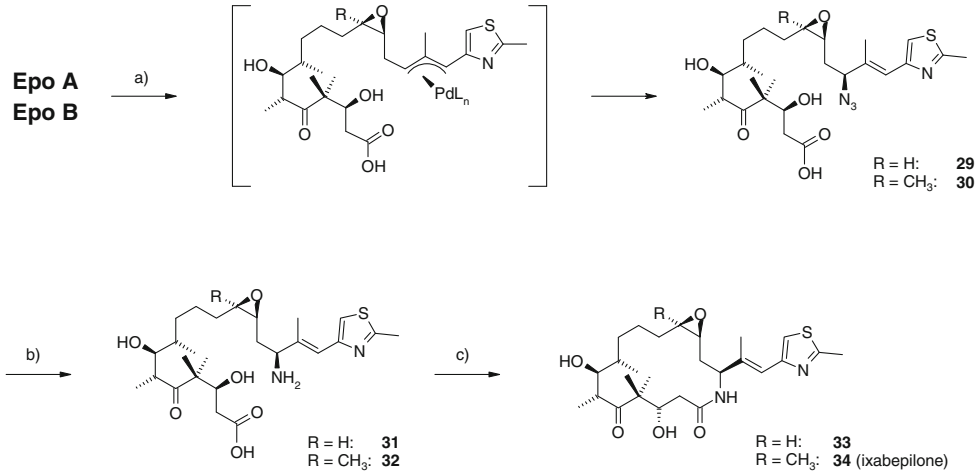
One of the most obvious modifications of the epothilone scaffold is the hydrolysis of the ester bond to produce the corresponding seco acid.

This transformation has been achieved by treatment of Epo A with NaOH/MeOH, which gave seco acid **25** in 65% yield (Scheme 8); however, ester hydrolysis was also accompanied by the retro aldol cleavage of the C3–C4 bond, thus leading to 23% of the retro aldol product **26** (Scheme 8) [58]. Ester hydrolysis is also possible with pig liver esterase (PLE) as a catalyst and this provides for a more selective course of the reaction [8].

Seco acid **25** can be further degraded to ester **27** and diol **28** via acid-catalyzed epoxide hydrolysis followed by esterification with TMS-diazomethane, periodate cleavage of the 12,13-diol, and finally borohydride reduction of the mixture of cleavage products. Compounds **27** and **28** may be used as chiral building blocks for the synthesis of other complex target structures. No primary data on the biological activity of **25** have appeared in the literature, but the compound has been reported by the Novartis group to be several 1000-fold less active in cell proliferation assays than Epo B in a number of presentations. These reports are in line with observations by the BMS group on the significantly diminished in vitro cytotoxicity of epothilones when preincubated with mouse plasma, which leads to rapid ester cleavage [59]. While the situation is more complex in vivo and the stability of Epo B is much higher in human than in rodent plasma, their findings on the reduced activity of epothilones in the presence of mouse plasma have led the BMS group to pursue lactam-based epothilone analogs as metabolically more stable alternatives to the natural macrolactones [41,59]. In the course of this work, the group has developed a very elegant approach for the preparation of the macrolactam analogs of Epo A and B. The process exploits the allylic nature of the ester group in epothilones and involves Pd(0)-catalyzed ring opening and



Scheme 8 *a* NaOH/MeOH, RT, 5 min, 65% (**25**) and 24% (**26**). *b* 1. H_2SO_4, THF/H_2O, RT, 5 h. 2. $TMSCHN_2$, 2.5 h, 54%. *c* 1. $NaIO_4$, MeOH/H_2O, RT, 45 min; 2. BH_4^--ion exchange resin, 41% (**27**) and 49% (**28**)



Scheme 9 *a* $Pd(PPh_3)_4$, NaN_3, THF/H_2O, 45°C, 1 h, **29**: 60%, **30**: 70%. *b* PtO_2, H_2, 20 h, **31**: 89%, **32**: 53% or Me_3P, THF, 45°C, 14 h, 70% (**32**). *c* DPPA, $NaHCO_3$, DMF, 4°C, 24 h, 43% (**34**) *or* EDCI, HOBt, MeCN/DMF, ca. 65% (**34**)

trapping of the ensuing Pd-π complex with a nitrogen nucleophile. Thus, the treatment of Epo A or B with NaN_3 in the presence of $Pd(PPh_3)_4$ led to azido acids **29** and **30**, respectively, with full retention of configuration at C15 (Scheme 9).

Reduction of the azide group either through catalytic hydrogenation or with trimethyl phosphine led to amino acids **31** and **32**, respectively, which could be ring-closed to furnish the lactam analogs of Epo A and B, **33** and **34**, respectively. Using this chemistry, the BMS group has also developed an efficient one-pot process for the conversion of Epo B into lactam derivative **34**, which involves the above Pd(0)-catalyzed ring-opening reaction, reduction of the azide with trimethyl phosphine and macrolactam formation with EDCI/HOBt [59]; this provides the desired lactam in 23% overall yield.

Epo B lactam **34** (BMS-247550, ixabepilone) is a potent inducer of tubulin polymerization, but its antiproliferative activity is ca. one order of magnitude lower than that of Epo B [23,59] (e.g., IC_{50} values against the human colon carcinoma cell line HCT-116 are 3.6 and 0.42 nM, respectively, for **34** and Epo B [59]). Similar differences have been reported between the lactam analogs of Epo A (**33**), Epo C and Epo D and the respective parent macrolactones; methylation of the lactam nitrogen in **33** results in a substantial loss in potency [60].

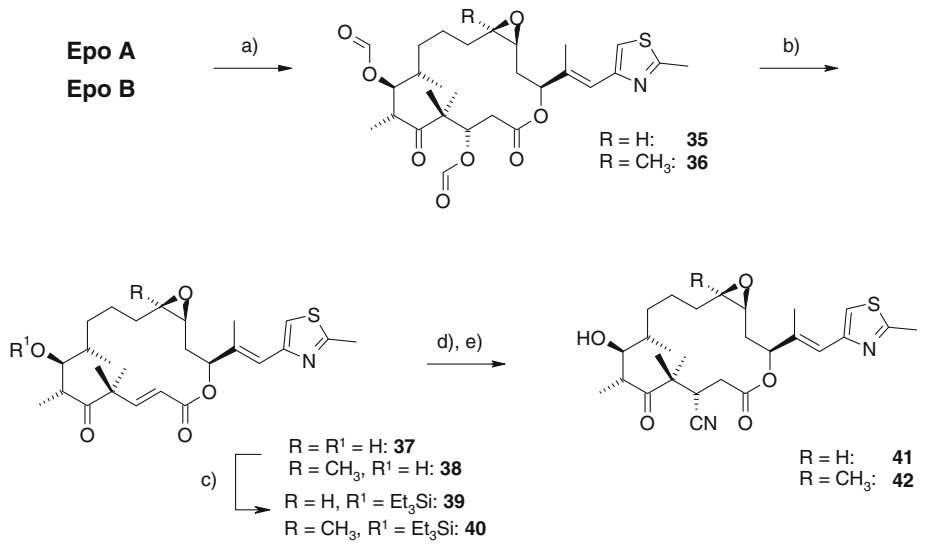
In contrast to natural epothilones, **34** is significantly less active against the highly P-gp overexpressing, multidrug-resistant KB-8511 variant of the KB-31 cell line than the drug-sensitive KB-31 parental line (IC_{50}'s of 2.85 and 128 nM against KB-31 and KB-8511 cells, respectively [23]). This indicates that **34** is at least a moderate P-gp substrate, a conclusion which is also supported by data obtained for other lactam-based epothilone analogs [61]. In vivo **34** exhibits antitumor activity similar to that of Taxol® in Taxol®-sensitive tumor models (i.e., A2780 human ovarian carcinoma, HCT-116 and LS174T human colon carcinomas) [62]. In spite of its limited effects against highly multidrug-resistant cell lines in vitro, intriguingly, the compound has also been found to be superior to Taxol® in Taxol®-resistant tumor models [62].

Based on its highly compelling preclinical profile Epo B lactam **34** was selected by BMS for clinical development (as BMS-247550 or ixabepilone) and in 2007 was approved by the FDA for the treatment of metastatic or advanced breast cancer, either as single agent or in combination with capecitabine [15]; the drug is marketed in the US by Bristol-Myers Squibb (BMS) under the trade name Ixempra®.

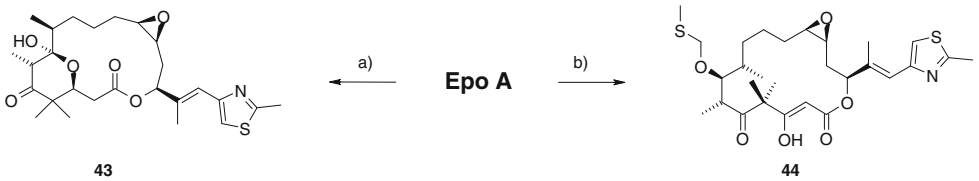
Modifications in the C2–C8 region

Semisynthesis-based modifications in the C2–C8 region have involved transformations of all functional groups present in this sector of the epothilone structure, including the hydroxyl groups at C3 and C7 as well as the keto group at C5. Epo A and B can be readily converted to the corresponding C3/C7-bis-formyloxy derivatives **35** and **36** (Scheme 10), which can then be further transformed into the α, β-unsaturated lactones **37** and **38**, respectively, by treatment with NH_3/MeOH, which results in selective based-induced elimination of formic acid across the C2–C3 bond and concurrent cleavage of the formate ester at C7 [44].

After TES protection of **37** and **38**, to give **39** and **40**, 1,4-addition of cyanide ion produced a ca. 1/1 mixture of 3*S* and 3*R* isomers, which were separated and then deprotected with acetic acid individually to provide **41** and **42** (as well as their corresponding 3*R* isomers). The in vitro activity of analogs **37/41** and **38/42** is less than one order of magnitude lower than that of Epo A and B, respectively; for **38** and **42** their in vitro antiproliferative activity is thus comparable with



Scheme 10 *a* HCO_2H, Et_3N, DMAP, H_2O. *b* 1. DBU, CH_2Cl_2; then NH_3, MeOH, **37**: 73%, **38**: 95%. *c* Et_3SiCl, Et_3N, DMAP, CH_2Cl_2, 45°C, **39**: 96%, **40**: 90%. *d* KCN, 18-C-6, DMF, R=H: 38%, R=CH_3: 65%. *e* AcOH/THF/H_2O, **41**: 83%, **42**: 44%



Scheme 11 *a* PDC, CH_2Cl_2, RT, 36 h, 80%. *b* $(CH_3)_2S$, $(PhCO_2)_2$, MeCN, 0°C → RT, 24 h, 30%

taxol [44]. In contrast, the 3*R* isomers of **41** and **42** exhibit significantly reduced activity [44].

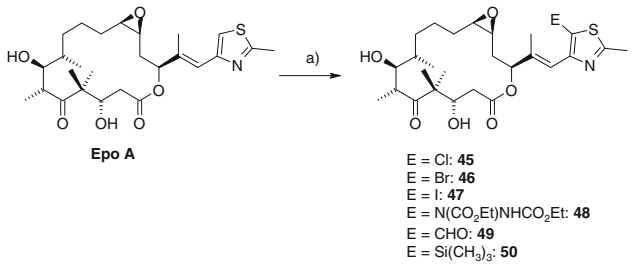
The GBF group has also investigated oxidative and reductive transformations in the C2–C8 region of Epo A, using a variety of different conditions [47]. Thus, treatment of Epo A with neutral pyridinium dichromate (PDC) in CH_2Cl_2 or DMF provided acetal **43** in 80% yield (Scheme 11), while oxidation under Swern conditions or with acidic PDC was either less selective or produced complex reaction mixtures, respectively [47]. Selective oxidation of the hydroxyl group on C3 in Epo A is more difficult and could only be accomplished in very moderate yield with a mixture of dimethylsulfide and dibenzoylperoxide [47]. No oxidation of the 7-OH group takes place under these conditions, but the oxidized product is obtained as its C7-methylthiomethyl (MTM) ether **44** (30% yield; Scheme 11). The compound is present as a 2/3 mixture of keto/enol isomers [47]. In addition to **44**, the reaction also produced the C7-mono-MTM and the C3,C7-bis-MTM ethers of Epo A, each in about 30% yield.

The C5-keto group in Epo A can be selectively reduced with $NaBH_4$ in a THF/pH 7 buffer system, to give a 1:1 mixture of diastereomeric C5-alcohols (ca. 70%). Interestingly, unbuffered reaction conditions led to rapid cleavage of the ester bond [47]. Treatment of **43** with $NaBH_4$ in MeOH resulted in the selective reduction of the C5-keto group without any effect on the C7-ketone, although **43** has been reported to exist in a 97/3 equilibrium mixture between the cyclic acetal and the keto alcohol form (which, in principle, should also make it susceptible to reduction at C7).

Acetal **43** retains some activity against the mouse leukemia cell line L929 (IC_{50} of ca. 400 nM [47] versus 8 nM for Epo A [63]), while oxidation at C3 or reduction at C5 lead to IC_{50} values of $> 1\,\mu M$ [47].

Side chain modifications

The C15 side chain of epothilones has been targeted for semisynthesis in a number of different ways that include modifications (or replacement) of the heterocycle, the vinyl linker between the heterocycle and the macrolactone ring, or both. Quite intriguingly, given the type functionality present in the macrolactone core, the treatment of Epo A with an excess of *n*-BuLi in THF at −90°C and subsequent quenching with different electrophiles has allowed the preparation of a number of C19-substituted Epo A derivatives in modest yields (Scheme 12) [64]. As an unfortunate exception, C19-fluoro Epo A could not be obtained with a range of fluorinating agents.



Scheme 12 *a* *n*-BuLi (5 equ), THF, −90°C, electrophile (CCl_4, NBS, I_2, DEAD, $HCONMe_2$, Me_3SiCl). **45**: 50%; **46**: 15%; **47**: 25%; **48**: 50%; **49**: 30%; **50**: 35%

In some cases, deprotonation also occurred at C21 (in addition to C19), albeit to a lower extent. Only with EtI as the electrophile reaction took place exclusively at C21, leading to C20-desmethyl-C20-propyl-Epo A as the only isolable product in 8% yield (40% based on recovered starting material). All of the C19-modified Epo A analogs were found to be significantly less active than the parent natural product in cancer cell proliferation assays [64].

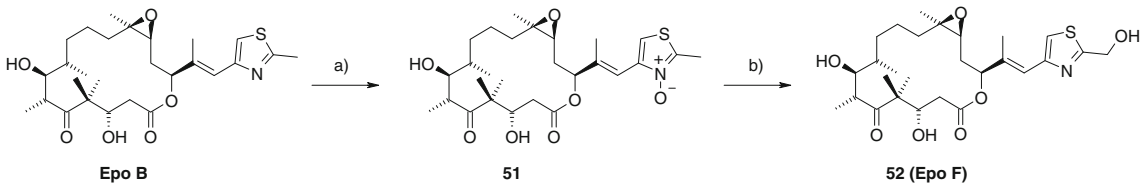
While not accessible by base treatment and subsequent electrophilic quenching, C21-substituted epothilone derivatives can nevertheless be obtained through semisynthesis in a very efficient manner. As illustrated in Scheme 13 for Epo B as the starting material, Epo A and B can be converted to the corresponding C21-hydroxyl derivatives Epo E and F, respectively, through a Polonovski-type [65] rearrangement [63].

Thus, treatment of Epo B with *m*-chloroperbenzoic acid (*m*-CPBA) produced the thiazole *N*-oxide **51**, which rearranged to C21-trifluoroacetoxy-Epo B upon reaction with trifluoroacetic anhydride. Treatment of this intermediate with NH_3 (aq) in THF then gave Epo F (**52**) in 38% overall yield (for the 3-step sequence from Epo B). In a completely analogous way, Epo A could be converted into Epo E [63].

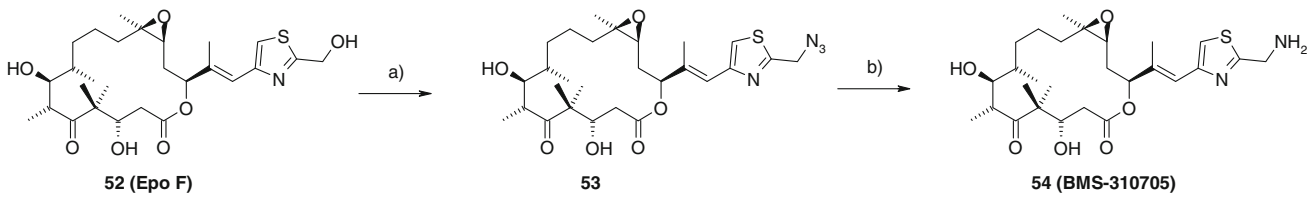
Epo E and F, which have also been isolated from fermentations of myxobacteria, exhibit similar antiproliferative activity as Epo A and B, respectively, against drug-sensitive cell lines, but are somewhat less potent against P-gp-overexpressing multidrug-resistant lines. (For example, IC_{50} values of 0.23 and 0.94 nM have been reported for Epo F against the drug-sensitive and drug-resistant human cervix carcinoma cell lines KB-31 and KB-8511, respectively [23]). Quite intriguingly, the activity of *N*-oxide **51** is also comparable with that of Epo B [63].

Both Epo E and F have been elaborated into different C21-modified derivatives [66] and C20 substituents of limited size (but still larger than the natural methyl group) have been found compatible with potent antiproliferative activity; more bulky substituents result in a substantial loss in potency. Most of the corresponding analogs have been obtained by total synthesis rather than semisynthetic transformations or were isolated from fermentations of myxobacteria (including Epo E and F, *vide supra*) [10]. However, semisynthesis from Epo B (*via* Epo F (**52**)) has served to prepare C21-amino-Epo B (**54**), which has been advanced to Phase I clinical studies by BMS (as BMS-310705 [25]). As outlined in Scheme 14, BMS-310705 (**54**) could be prepared from Epo F via azide **53**, which was obtained by treatment of **52** with diphenylphosphoryl azide (DPPA); Staudinger reduction of **53** with PMe_3 then gave the desired **54** [25].

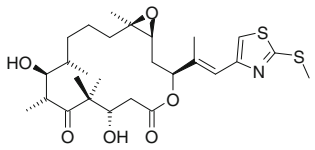
Only limited biological data have been disclosed for **54** so far. Based on a patent application [67], the compound shows an IC_{50} value of 0.8 nM against the human cervix cancer cell line KB.31 (versus 1.2 nM for Epo B under comparable experimental conditions [63]); it was also demonstrated to induce substantial apoptosis in early passage, taxol- and platinum-refractive ovarian cancer cells (OC-2) at a concentration of 50 nM [68]. BMS-310705 (**54**) exhibits improved water-solubility over BMS-247550 (**34**), which



Scheme 13 *a* *m*-CPBA, CH_2Cl_2, RT, 3 h, 48%. *b* 1. $(CF_3CO)_2O$, 2,6-lutidine, CH_2Cl_2, 75°C, 10 min; 2. THF/25% NH_3, 45°C, 10 min, 78%



Scheme 14 *a* DPPA, DBU, THF, 94%. *b* $P(CH_3)_3$, THF/H_2O, 91%



ABJ879 (55)

Fig. 4 Molecular structure of ABJ879

enables the use of clinical formulations not containing Cremophor-EL® [25].

Phase I clinical development has also been reported for C20-desmethyl-C20-methylsulfanyl-Epo B (**55**, ABJ879; Novartis) (Fig. 4).

Like BMS-310705 (**54**), **55** is a semisynthetic Epo B derivative [69], but the route for its preparation has not been disclosed in the literature. ABJ879 (**55**) is a markedly more potent antiproliferative agent than Epo B, with an average IC_{50} for growth inhibition across a panel of drug-sensitive human cancer cell lines of 0.09 nM (vs. 0.24 nM for Epo B and 4.7 nM for taxol) [69]. The compound retains full activity against cancer cells overexpressing the drug efflux pump P-gp or harboring tubulin mutations.

ABJ879 (**55**) has demonstrated potent antitumor activity in experimental animal models [69], where it produced transient regressions and inhibition of tumor growth of slow-growing (NCI H-596 lung adenocarcinomas, HT-29 colon tumors) as well as fast-growing, difficult-to-treat tumors (NCI H-460 large cell lung tumors). In addition, single dose administration of ABJ879 (**55**) produced long-lasting regressions and cures in a Taxol®-resistant KB-8511 cervix carcinoma model [69]. In spite of these favorable preclinical data, the clinical development of ABJ879 (**55**) was discontinued after Phase I for undisclosed reasons [70]; the same appears to be true for BMS-310705 (**54**).

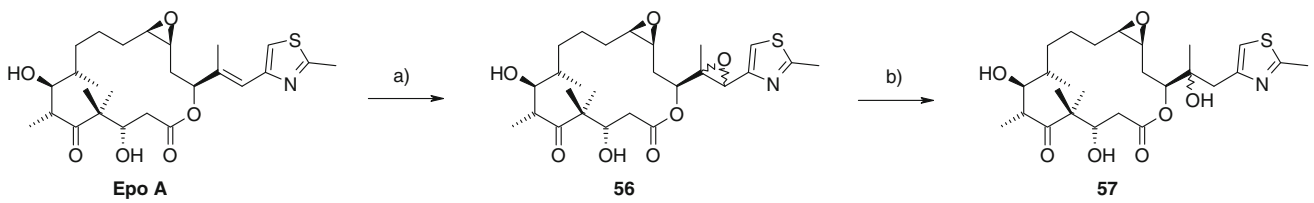
As indicated above, semisynthetic modifications of the epothilone side chain are not confined to variations in heterocycle substituents, but also include manipulation of the C16-C17 double bond. The latter can be epoxidized with *m*-CPBA or dimethyl dioxirane (DMDO) in low to moderate yield to furnish **56** as a 3/2 mixture of diastereoisomers (Scheme 15). Subsequent hydrogenolysis over Pd/C in EtOH gave a mixture of epimeric C16-alcohols in quantitative yield (i.e., hydrogenation occurred selectively at the position α to the thiazole ring, Scheme 15) [47].

The C16–C17 double bond can be completely cleaved by means of ozonolysis (O_3, CH_2Cl_2, −70°C, 10 min, then (Me_2S) [47]; the resulting ketone **58** (in the case of Epo A) was immediately reacted with trimethylsilyl (TMS) chloride to provide bis-TMS ether **59** in 70% overall yield (Scheme 16). Ketone **58** could be readily transformed into O-substituted oximes **61** and **62** [47] and it was also elaborated into imidazole-containing analog **63** through base-catalyzed aldol condensation [71,75] (Scheme 16). In contrast, olefination reactions involving the C16 keto group proved to be highly problematic. While **59** could be converted to methylene derivative **60** in a modest overall yield of 15% (after deprotection), all attempts to re-introduce the natural thiazole side chain or to create a phenyl-based Epo A analog using Wittig-type chemistry were unsuccessful [47,71,75]. However, the successful and unproblematic instalment of the thiazole side chain through Wittig olefination has been reported for a methyl ketone formally derived from *E*-9,10-didehydroEpo D; the latter was prepared by total synthesis [77].

The reasons for this discrepancy have not been investigated. All analogs depicted in Scheme 16 were found to be significantly less active in proliferation or tubulin assays than Epo A [47,71,75].

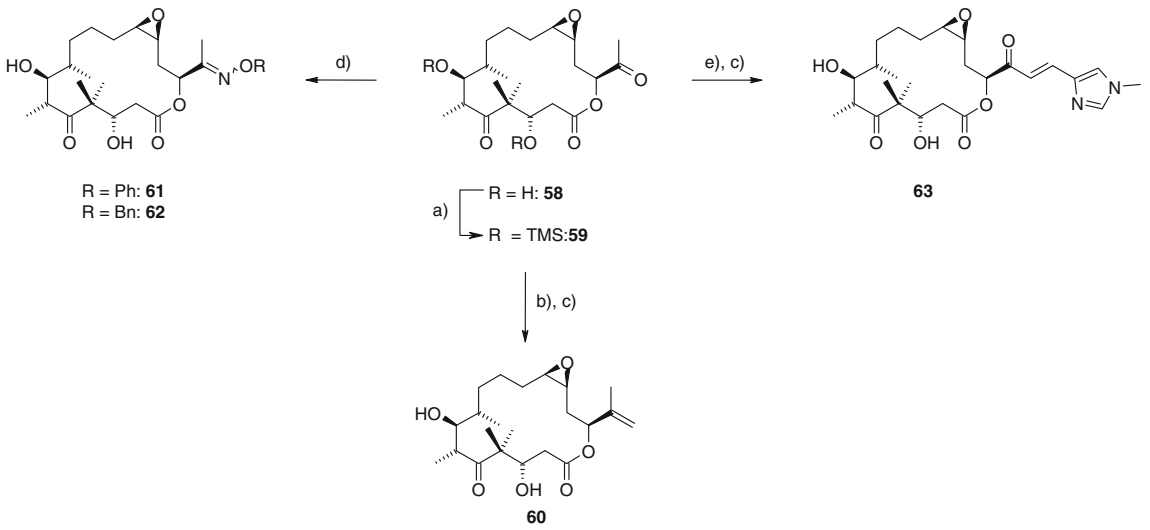
In order to enable the replacement of the thiazole ring by other (hetero)aryl moieties, Höfle and co-workers have developed an alternative strategy for the construction of the aryl-vinyl part of the epothilone side chain from ketone **59** (Scheme 17).

The approach is based on the conversion of **59** to boronic acid **64**, which was accomplished in 83% yield with the bisboryl methyl lithium reagent **65**. While **64** was obtained as a 7/3 mixture of *E*/*Z* double bond isomers, these could be separated by preparative HPLC and the pure *E*-isomer was converted to the protected phenyl-based Epo A analog **66** through Suzuki coupling [71,75]. Alternatively, reaction of *E*-**64** with *N*-iodosuccinimide (NIS) gave the corresponding vinyl iodide, which underwent smooth Stille coupling with stannane **67** to give **68** [71,75]. No activity data have been reported for the (deprotected version of) thiophene

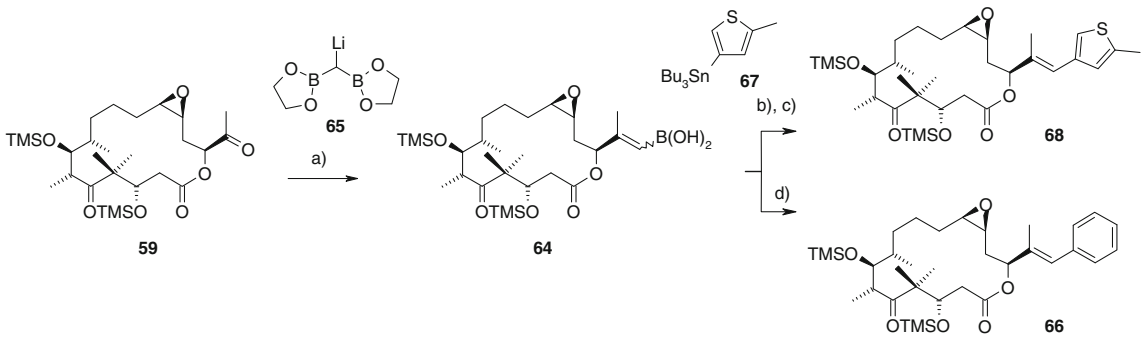


Epo A **56** **57**

Scheme 15 *a m*-CPBA, CH_2Cl_2, RT, 1 h, 10% or DMDO, acetone, 0°C → RT, 5 h, 25% (3/2 mixture of isomers). b) H_2, 10% Pd-C, EtOH, RT, 90 h, quant



Scheme 16 *a* TMSCl, Et_3N, DMAP, CH_2Cl_2, RT, 18 h, 70% (2 steps). *b* $Ph_3P{=}CH_2$, THF. *c* citric acid, MeOH, 65°C, 1 h, 15% (2 steps). *d* **58**, $RONH_3Cl$, EtOH, pyridine, RT, 18 h, 50–60%. e) 1. LiTMP, THF, −70°C; 2. N^1-methyl-imidazole-4-carboxaldehyde (no yields reported for step *e* in [71])



Scheme 17 *a* 1. **65**, CH_2Cl_2/THF; 2. hydrolysis, 83% (*E*/*Z*, 7/3). *b* *N*-iodo succinimide. *c* **67**, $Pd(PPh_3)_4$. *d* PhI, $Pd(PPh_3)_4$, TlOEt, EtOH. No yields are reported in [70] for steps *b*, *c*, and *d*; likewise, the deprotection of **66** and **68** has not been described

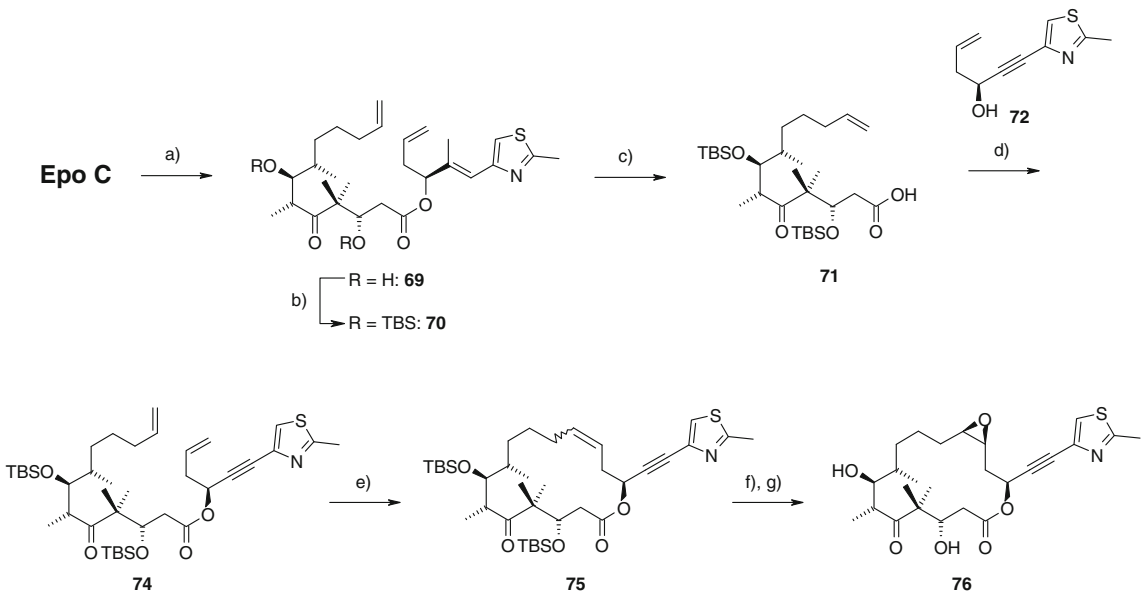
derivative **68**; the phenyl analogs of Epo C and Epo B have been reported by others to retain most of the activity of the thiazole-containing parent compounds [77].

Replacement of the C13-O16 segment

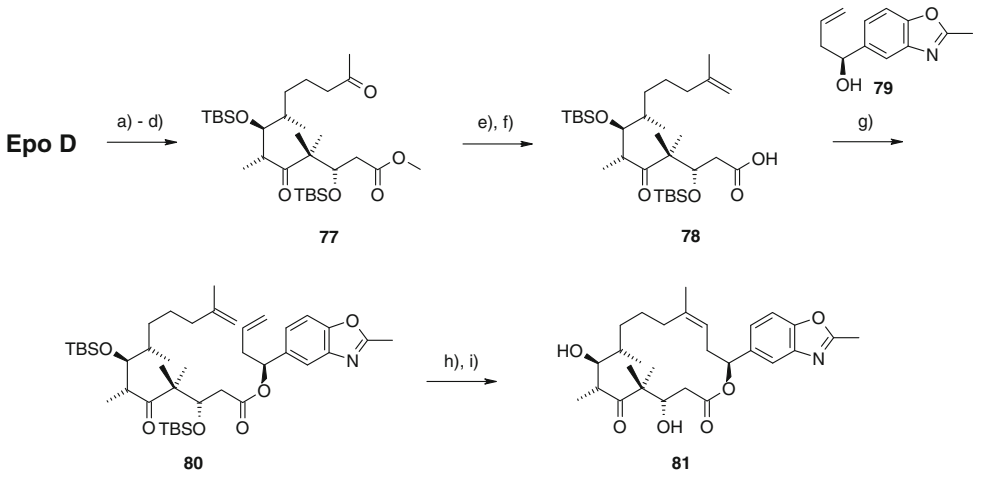
Semisynthetic epothilone analogs have also been prepared *via* intermediates that were obtained through the degradative removal of the entire C13-O16 segment (including the pendant side chain), which were then (re)elaborated into modified versions of the original structure. The first implementation of this concept was reported by the GBF group, who used ring-opening olefin metathesis (ROM) with ethylene for the conversion of Epo C into the ring-opened product **69** in 73% yield (employing Grubbs II catalyst) [73] (Scheme 18).

Diene **69** was then further elaborated into protected acid **71** through TBS-protection (at −20°C) and selective cleavage of the TBS-*ester* with an excess of TBSOTf (at 30°C). Esterification of **71** with alcohol **72** provided yne-diene **74**, which underwent smooth ring-closing olefin metathesis (RCM) with Grubbs I catalyst to reconstitute the macrocyclic epothilone framework in the form of 12/13-deoxy analog(s) **75** (obtained as a 1/1 mixture of *E*/*Z*-isomers in 52% yield). After deprotection of **75** with CF_3COOH the *E*- and *Z*-isomers could be separated and were isolated in 43% yield each. Epoxidation of the *Z*-isomer with DMDO gave Epo A analog **76** in 61% yield (together with 33% of its 12*S*,13*R* isomer). **76** (as well as its 12,13-deoxy derivative) showed significantly reduced antiproliferative activity against the mouse fibroblast cell line L929, relative to Epo A (IC_{50} values > 1 μM versus 8 nM for Epo A [63]) [73].

More recently, an approach similar to the one depicted in Scheme 18 has been developed by Dong et al. at Kosan, who have converted fermentatively produced Epo D into keto ester **77** (Scheme 19) [74]. In contrast to Höfle's approach, cleavage of the 12,13-double bond in this case involved stoichiometric OsO_4-mediated dihydroxylation followed by



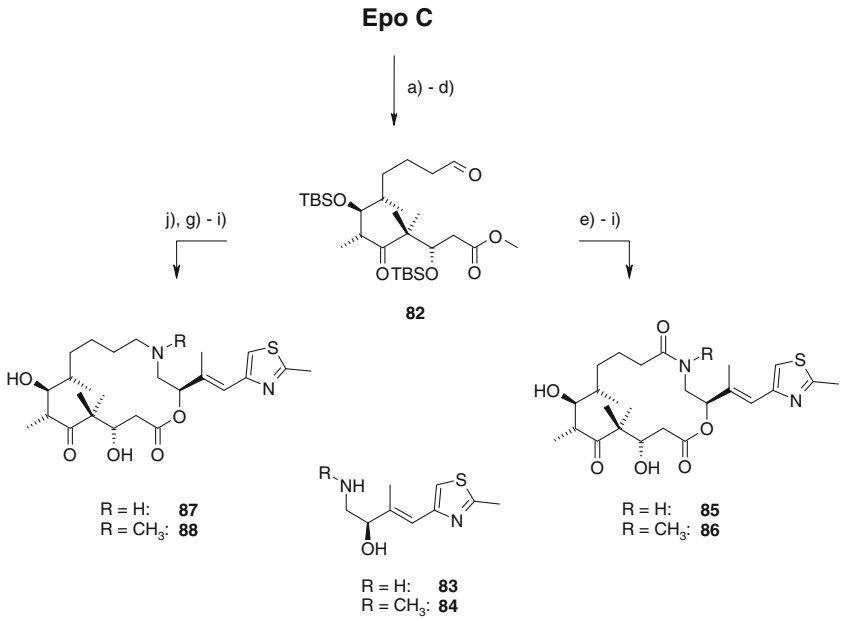
Scheme 18 *a* CH_2=CH_2, Grubbs II catalyst (0.15 equ), 44 h, 73% (83% based on recovered starting material). *b* TBSOTf, 2,6-lutidine, CH_2Cl_2, −20°C, 24 h, 75%. c) TBSOTf, 2,6-lutidine, 30°C, 2 h, 94%. *d* DCC, DMAP, **72**, 0°C → RT, 16 h, 91%. e) Grubbs I catalyst (0.2 equ), CH_2Cl_2, RT, 48 h, 52% (1/1 mixture of *E*/*Z*-isomers). *f* CF_3COOH/CH_2Cl_2 6/1, 0°C, 2 h, 43% (+ 43% of 12,13*E*-isomer). *g* DMDO, CH_2Cl_2/acetone, −20°C, 2 h, 61% (+32.5% 12*S*,13*R* isomer)



Scheme 19 *a* TBSOTf, Et_3N, CH_2Cl_2, −78°C, 85%. *b* OsO_4, TMEDA, −78°C, then $NaHSO_3$, 65°C, 75%. *c* $Pb(OAc)_4$, benzene, then K_2CO_3, MeOH. *d* $TMSCHN_2$, MeOH, toluene, 81% (2 steps). *e* Cp_2TiMe_2, toluene, 80°C, 74%. *f* LiOH, *i*-PrOH/H_2O, 86%. *g* **79**, EDC, DMAP, CH_2Cl_2, 54–82%. *h* CH_2Cl_2, Grubbs II catalyst, refl., 8 h, 60% (1/1 mixture of *E*/*Z*-isomers). *i* CF_3COOH, CH_2Cl_2, 0°C → RT or HF·pyridine, THF, 0°C → RT, 32–59%

oxidative cleavage of the resulting 12,13-diol with $Pb(OAc)_4$ (rather than ROM).

Treatment of the crude keto aldehyde thus obtained with K_2CO_3 led to facile elimination of the C1-C12 segment as the free carboxylic acid, which was converted to ester **77** with TMS-diazomethane. Petasis olefination followed by ester saponification and re-esterification of the resulting acid **78** with alcohol **79** then provided diene **80**. The latter underwent RCM in the presence of Grubbs II catalyst, furnishing the desired macrolactone as a 1/1 mixture of *E*/*Z* isomers about the C12/C13 bond in 60% total yield. Isomer separation was possible after deprotection of the mixture with CF_3COOH. Analog **81** was found to be 2-3-fold less potent than Epo D in cancer cell proliferation assays [74]; in contrast, fully synthetic Epo B and D analogs incorporating benzothiazole-, quinoline-, or benzimidazole side chains had been shown previously to be more potent than the corresponding parent compounds [27]. In fact, one of the epothilone-type clinical development compounds (ZK-Epo, sagopilone) is an analog of Epo B with a benzothiazole side chain (analogous to the benzoxazole side chain in analog **81**) [75]; however, sagopilone could not be prepared



Scheme 20 *a* PLE. *b* $TMSCHN_2$, 25% (2 steps, plus recovered starting material). *c* TBSOTf, 44%. *d* O_3, Ph_3P, 86%. *e* $NaClO_2$, NaH_2PO_4, 43%. *f* EDC, HOBt, Et_3N, 79%. *g* LiOH, 86%. *h* 2,4,6-trichlorobenzoyl chloride, 37%. *i* *CF_3COOH, 56%. j $NaBH(OAc)_3$, 74% (with amine* **84***). (Yields for steps f–i are for intermediates leading to* **86***)*

according to the approach shown in Scheme 19, as it also includes a C6-allyl substituent in place of the natural methyl group [75].

Employing PLE-catalyzed hydrolysis of the lactone group and subsequent cleavage of the C12/C13 double bond by ozonolysis, the BMS group was able to establish the controlled degradation of Epo C into ester **82** (Scheme 20).

Oxidation of **82** with $NaClO_2$ gave the mono-ester of a dicarboxylic acid, which could be coupled with amines **83** or **84**, to give the C12–C13 amide-based analogs **85** and **86**, respectively, after ester saponification, Yamaguchi-type macrolactonization and final deprotection with CF_3COOH (Scheme 20) [41]. Alternatively, **82** and amines **83/84** were submitted to reductive amination and the resulting secondary and tertiary amines were elaborated into 13-aza epothilones **87** and **88**, respectively (Scheme 20). Unfortunately, none of the derivatives **85**–**88** showed any significant tubulin-polymerizing or growth-inhibitory activity. In contrast, different (fully synthetic) N12-acyl- or carbamoyl-substituted 12-aza-epothilones have been shown to exhibit very potent biological effects [76].

Conclusions

The work discussed in this review article has defined the chemistry associated with the epothilone molecular framework in significant detail. This chemistry has enabled the preparation of a large number of structurally modified, semisynthetic epothilone derivatives, which have contributed significantly to our current understanding of the epothilone SAR by complementing the results obtained with fully synthetic analogs. Most importantly, these efforts have led to the discovery of three derivatives that have been advanced to clinical studies in humans. Thus, notwithstanding the wealth of fully synthetic analogs that have been prepared over the last 15 years and many of which exhibit very attractive biological profiles (at least in vitro), semisynthesis so far has had the more profound impact on the clinical advancement of the epothilone class of microtubule stabilizers than total synthesis.

At the same time, it is clear that the potential of semisynthesis for the creation of new structurally unique epothilone analogs is far from being exhausted. Many additional derivatives with a diverse range of structural features are conceivable that should be chemically accessible from natural epothilones as starting materials and that might exhibit interesting biological properties. However, the further exploration of semisynthetic approaches towards new epothilones analogs is limited by access to the fermentatively produced starting materials. It is unclear at this point, whether those groups with a sufficient supply of natural epothilones (BMS, GBF (now Helmholtz Centre for Infection Research), Novartis) continue to be active in the area of epothilone semisynthesis.

References

1. Lu MC (1995) Antimitotic agents. In: Foye WO (ed) Cancer chemotherapeutic agents. American Chemical Society, Washington, pp 345–368

2. Mekhail TM, Markman M (2002) Paclitaxel in cancer therapy. Expert Opin Pharmacother3:755–766. doi:10.1517/14656566.3.6.755
3. Obasaju C, Hudes GR (2001) Paclitaxel and docetaxel in prostate cancer. Hematol oncol Clin North Am 15:525–545. doi:10.1016/S0889-8588(05)70230-6
4. Hamel E (1996) Antimitotic natural products and their interaction with tubulin. Med Res Rev 16:207–231. doi:10.1002/chin.199628329
5. Rowinsky EK (1997) The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. Annu Rev Med 48:353–374. doi:10.1146/annurev.med.48.1.353
6. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667. doi:10.1038/277665a0
7. Bollag DM, McQueney PA, Zhu J, Hensens O, Koupal L, Liesch J, Goetz M, Lazarides E, Woods CM (1995) Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. Cancer Res 55:2325–2333
8. Höfle G, Reichenbach H (2005) Epothilone, a myxobacterial metabolite with promising antitumor activity. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products. Taylor & Francis, Boca Raton pp 413–450
9. Gerth K, Bedorf N, Höfle G, Irschik H, Reichenbach H (1996) Epothilons A and B: antifungal and cytotoxic compounds from *Sorangium cellulosum* (Myxobacteria). Production, physico-chemical and biological properties. J Antibiot 49:560–563. doi:10.1002/chin.199647256
10. Hardt IH, Steinmetz H, Gerth K, Sasse F, Reichenbach H, Höfle G (2001) New natural epothilones from *Sorangium cellulosum*, strains So ce90/B2 and So ce90/D13: isolation, structure elucidation, and SAR studies. J Nat Prod 64:847–856. doi:10.1021/np000629f
11. Kowalski RJ, Giannakakou P, Hamel E (1997) Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel (Taxol). J Biol Chem 272:2534–2541. doi:10.1074/jbc.272.4.2534
12. Altmann K-H, Wartmann M, O'Reilly T (2000) Epothilones and related structures—a new class of microtubule inhibitors with potent in vivo antitumor activity. Biochim Biophys Acta 1470:M79–M91. doi:10.1016/S0304-419X(00)00009-3
13. Wolff A, Technau A, Brandner G (1997) Epothilone A induces apoptosis in neuroblastoma cells with multiple mechanisms of drug resistance. Int J Oncol 11:123–126
14. Giannakakou P, Sackett DL, Kang YK, Zhan Z, Buters JT, Fojo T, Poruchynsky MS (1997) Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. J Biol Chem 272:17118–17125. doi:10.1074/jbc.272.27.17118
15. Lechleider RJ, Kaminskas E, Jiang X, Aziz R, Bullock J, Kasliwal R, Harapanhalli R, Pope S, Sridhara R, Leighton J, Booth B, Dagher R, Justice R, Pazdur R (2008) Ixabepilone in combination with capecitabine and as monotherapy for treatment of advanced breast cancer refractory to previous chemotherapies. Clin Cancer Res 14:4378–4384. doi:10.1158/1078-0432.CCR-08-0015
16. Nicolaou KC, Roschangar F, Vourloumis D (1998) Chemical biology of epothilones. Angew Chem Int Ed 37:2014–2045. doi:10.1002/(SICI)1521-3773(19980817)37:15<2014::AID-ANIE2014>3.3.CO;2-U
17. Harris CR, Danishefsky SJ (1999) Complex target-oriented synthesis in the drug discovery process: a case history in the dEpoB series. J Org Chem 64:8434–8456. doi:10.1021/jo991006d
18. Mulzer J, Martin HJ, Berger M (1999) Progress in the synthesis of chiral heterocyclic natural products: epothilone B and tartrolon B. J Heterocycl Chem 36:1421–1436. doi:10.1002/chin.200019288
19. Nicolaou KC, Ritzen A, Namoto K (2001) Recent developments in the chemistry, biology and medicine of the epothilones. Chem Commun 1523–1535. doi:10.1039/B104949F
20. Altmann K-H (2004) The merger of natural product synthesis and medicinal chemistry: on the chemistry and chemical biology of epothilones. Org Biomol Chem 2:2137–2152. doi:10.1039/B405839A
21. Watkins EB, Chittiboyina AG, Jung JC, Avery MA (2005) The epothilones and related analogues-A review of their syntheses and anti-cancer activities. Curr Pharm Des 11:1615–1653. doi:10.2174/1381612053764742
22. Watkins EB, Chittiboyina AG, Avery MA (2006) Recent developments in the syntheses of the epothilones and related analogues. Eur J Org Chem 18:4071–4084. doi:10.1002/ejoc.200600149
23. Wartmann M, Altmann K-H (2002) The biology and medicinal chemistry of epothilones. Curr Med Chem Anti-Cancer Agents 2:123–148. doi:10.2174/1568011023354489
24. Altmann K-H (2003) Epothilone B and its analogs—a new family of anticancer agents. Mini-Rev Med Chem 3:149–158. doi:10.2174/1389557033405269
25. Borzilleri RM, Vite GD (2002) Epothilones: new tubulin polymerization agents in preclinical and clinical development. Drugs Future 27:1149–1164. doi:10.1358/dof.2002.027.12.711728
26. Altmann K-H (2005) Recent developments in the chemistry and biology of epothilones. Curr Pharm Des 11:1595–1613. doi:10.2174/1381612053764715
27. Altmann K-H, Pfeiffer B, Arseniyadis S, Pratt BA, Nicolaou KC (2007) The chemistry and biology of epothilones—the wheel keeps turning. ChemMedChem 2:397–423. doi:10.1002/cmdc.200600206
28. Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67:2141–2153. doi:10.1021/np040106y
29. Hamann MT (2003) Enhancing marine natural product structural diversity and bioactivity through semisynthesis and biocatalysis. Curr Pharm Design 9:879–889. doi:10.2174/1381612033455297
30. Henkel T, Brunne RM, Müller H, Reichel F (1999) Statistical investigation into the structural complementarity of natural products and synthetic compounds. Angew Chem Int Ed 38:643–647. doi:10.1002/(SICI)1521-3773(19990301)38:5<643::AID-ANIE643>3.0.CO;2-G
31. Kuhn M, Keller-Juslén C, von Wartburg A (1969) Communication on mitosis-inhibiting natural substances. 22. Partial synthesis of 4'-demethylepipodophyllotoxin. Helv Chim Acta 52:944–947. doi:10.1002/hlca.19690520410
32. Keller-Juslén C, Kuhn M, Wartburg A, Stähelin H (1971) Mitosis-inhibiting natural products. 24. Synthesis and antimitotic activity of glycosidic lignan derivatives related to podophyllotoxin. J Med Chem 14: 936–940. doi:10.1021/jm00292a012
33. You Y (2005) Podophyllotoxin derivatives: current synthetic approaches for new anticancer agents. Curr Pharm Design 11:1695–1717. doi:10.2174/1381612053764724
34. Sawada S, Yokokura T, Miyasaka T (1995) Synthesis and antitumor activity of A-ring or E-lactone modified water-soluble prodrugs of 20(S)-camptothecin, including development of irinotecan hydrochloride trihydrate (CPT-11). Curr Pharm Design 1:113–132
35. Kingsbury WD, Boehm JC, Jakas DR, Holden KG, Hecht SM, Gallagher G, Caranfa MJ, McCabe FL, Faucette L, Johnson RK, Hertzberg RP (1991) Synthesis of water-soluble (aminoalkyl) camptothecin analogs: inhibition of topoisomerase I and antitumor activity. J Med Chem 34:98–107. doi:10.1021/jm00105a017
36. Mathijssen RHJ, Loos WJ, Verweij J, Sparreboom A (2002) Pharmacology of topoisomerase I inhibitors irinotecan (CPT-11) and

topotecan. Curr Cancer Drug Targets 2:103–123. doi:10.2174/1568009023333890
37. Guenard D, Gueritte-Voegelein F, Potier P (1993) Taxol and taxotere: discovery, chemistry, and structure-activity relationships. Acc Chem Res 26:160–167. doi:10.1021/ar00028a005
38. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327. doi:10.1021/ja00738a045
39. Holton RA, Biediger RJ, Boatman D (1995) Semisynthesis of taxol and taxotere. In: Suffness M (ed) Taxol: science and applications. CRC, Boca Raton pp 97–121. doi:10.1002/chin.199552275
40. Wuts PGM (1998) Semisynthesis of Taxol. Curr Opin Drug Discov Dev 1:329–337
41. Vite GD, Borzilleri RM, Kim SH, Regueiro-Ren A, Humphreys WG, Lee FYF (2001) Epothilones A and B: springboards for semisynthesis of promising antimitotic agents. In: Ojima I, Vite GD, Altmann K-H (eds) Anticancer agents—frontiers in cancer chemotherapy. ACS Symposium Series 796. American Chemical Society, Washington, pp 148–170
42. Sefkow M, Kiffe M, Höfle G (1998) Derivatization of the C12–C13 functional groups of epothilones A, B and C. Bioorg Med Chem Lett 8:3031–3036. doi:10.1016/S0960-894X(98)00546-0
43. Altmann K-H, Bold G, Caravatti G, End N, Flörsheimer A, Guagnano V, O'Reilly T, Wartmann M (2000) Epothilones and their analogs—potential new weapons in the fight against cancer. Chimia 54:612–621
44. Regueiro-Ren A, Leavitt K, Kim SH, Höfle G, Kiffe M, Gougoutas JZ, DiMarco JD, Lee FYF, Fairchild CR, Long BH, Vite GD (2002) SAR and pH stability of cyano-substituted epothilones. Org Lett 4:3815–3818. doi:10.1021/ol026589j
45. Regueiro-Ren A, Borzilleri RM, Zheng X, Kim SH, Johnson JA, Fairchild CR, Lee FY, Long BH, Vite GD (2001) Synthesis and biological activity of novel epothilone aziridines. Org Lett 3:2693–2696. doi:10.1021/ol016273w
46. Glunz PW, He L, Horwitz SB, Chakravarty S, Ojima I, Chou TC, Danishefsky SJ (1999) The synthesis and evaluation of 12,13-benzodesoxyepothilone B: a highly convergent route. Tetrahedron Lett 40:6895–6898. doi:10.1016/S0040-4039(99)01433-1
47. Sefkow M, Kiffe M, Schummer D, Höfle G (1998) Oxidative and reductive transformations of epothilone A. Bioorg Med Chem Lett 8:3025–3030. doi:10.1016/S0960-894X(98)00545-9
48. Pfeiffer B, Hauenstein K, Merz P, Gertsch J, Altmann K-H (2009) Synthesis and SAR of C12–C13-oxazoline derivatives of epothilone A. Bioorg Med Chem Lett 19:3760–3763. doi:10.1016/j.bmcl.2009.04.112
49. Johnson J, Kim SH, Bifano M, DiMarco J, Fairchild C, Gougoutas J, Lee F, Long B, Tokarski J, Vite GD (2000) Synthesis, structure proof, and biological activity of epothilone cyclopropanes. Org Lett 2:1537–1540. doi:10.1021/ol0058240
50. Su DS, Balog A, Meng D, Bertinato P, Danishefsky SJ, Zheng YH, Chou TC, He L, Horwitz SB (1997) Structure-activity relationships of the epothilones and the first in vivo comparison with paclitaxel. Angew Chem Int Ed 36:2093–2096. doi:10.1002/anie.199720931
51. Meng D, Su DS, Balog A, Bertinato P, Sorensen EJ, Danishefsky SJ, Zheng YH, Chou T-C, He L, Horwitz SB (1997) Remote effects in macrolide formation through ring-forming metathesis: an application to the synthesis of fully active epothilone congeners. J Am Chem Soc 119:2733–2734. doi:10.1021/ja964275j
52. Su DS, Meng D, Bertinato P, Balog A, Sorensen EJ, Danishefsky SJ, Zheng YH, Chou T-C, He L, Horwitz SB (1997) Total synthesis of (-)-epothilone B: an extension of the Suzuki coupling method and insights into structure-activity relationships of the epothilones. Angew Chem Int Ed 36:757–759. doi:10.1002/anie.199707571
53. Nicolaou KC, Winssinger N, Pastor J, Ninkovic S, Sarabia F, He Y, Vourloumis D, Yang Z, Li T, Giannakakou P, Hamel E (1997) Synthesis of epothilones A and B in solid and solution phase. Nature 387:268–272. doi:10.1038/387268a0
54. Chou TC, Zhang XG, Balog A, Su DS, Meng D, Savin K, Bertino JR, Danishefsky SJ (1998) Desoxyepothilone B: an efficacious microtubule-targeted antitumor agent with a promising in vivo profile relative to epothilone B. Proc Natl Acad Sci USA 95:9642–9647. doi:10.1073/pnas.95.16.9642
55. Lichtner RB, Rotgeri A, Bunte T, Buchmann B, Hoffmann J, Schwede W, Skuballa W, Klar U (2001) Subcellular distribution of epothilones in human tumor cells. Proc Natl Acad Sci USA 98:11743–11748. doi:10.1073/pnas.171023398
56. Buey RM, Diaz JF, Andreu JM, O'Brate A, Giannakakou P, Nicolaou KC, Sasmal PK, Ritzén A, Namoto K (2004) Interactions of epothilone analogs with the paclitaxel binding site: relationship between binding affinity, microtubule stabilization, and cytotoxicity. Chem Biol 11:225–236. doi:10.1016/j.chembiol.2004.01.014
57. http://clinicaltrials.gov/ct2/results?intr=%22Epofolate%22
58. Niggemann J, Michaelis K, Frank R, Zander N, Höfle G (2002) Natural product-derived building blocks for combinatorial synthesis. Part 1. Fragmentation of natural products from myxobacteria. JCS Perkin Trans 1:2490–2503. doi:10.1039/b206953a
59. Borzilleri RM, Zheng X, Schmidt RJ, Johnson JA, Kim SH, DiMarco JD, Fairchild CR, Gougoutas JZ, Lee FYF, Long BH, Vite GD (2000) A novel application of a Pd(0)-catalyzed nucleophilic substitution reaction to the regio- and stereoselective synthesis of lactam analogues of the epothilone natural products. J Am Chem Soc 122:8890–8897. doi:10.1021/ja001899n
60. Schinzer D, Altmann K-H, Stuhlmann F, Bauer A, Wartmann M (2000) Synthesis and biological evaluation of aza-epothilones. ChemBioChem 1: 67–70. doi:10.1002/1439-7633(20000703)1:1<67::AID-CBIC67>3.0.CO;2-I
61. Stachel SJ, Lee CB, Spassova M, Chappell MD, Bornmann WG, Danishefsky SJ, Chou T-C, Guan Y (2001) On the interactivity of complex synthesis and tumor pharmacology in the drug discovery process: total synthesis and comparative in vivo evaluations of the 15-Aza epothilones. J Org Chem 66:4369–4378. doi:10.1021/jo010275c
62. Lee FY, Borzilleri R, Fairchild CR, Kim SH, Long BH, Reventos-Suarez C, Vite GD, Rose WC, Kramer RA (2001) BMS-247550: a novel epothilone analog with a mode of action similar to paclitaxel but possessing superior antitumor efficacy. Clin Cancer Res 7:1429–1437
63. Höfle G, Glaser N, Kiffe M, Hecht H-J, Sasse F, Reichenbach H (1999) *N*-oxidation of epothilone A-C and O-acyl rearrangement to C-19- and C21-substituted epothilones. Angew Chem Int Ed 38: 1971–1974. doi:10.1002/(SICI)1521-3773(19990712)38:13/14<1971::AID-ANIE1971>3.0.CO;2-X
64. Sefkow M, Höfle G (1998) Substitutions at the thiazole moiety of epothilone. Heterocycles 48:2485–2488. doi:10.3987/COM-98-8351
65. Huisgen R, Kolbeck W (1965) *N*-Acyloxyammonium salts. Tetrahedron Lett 6:783–787. doi:10.1016/S0040-4039(01)83985-X
66. Höfle G, Glaser N, Leibold T, Karama U, Sasse F, Steinmetz H (2003) Semisynthesis and degradation of the tubulin inhibitors epothilone and tubulysin. Pure Appl Chem 75:167–178. doi:10.1351/pac200375020167
67. Höfle G, Glaser N, Leibold T (2000) Synthesis and cytotoxicity of C-21 modified epothilones. Ger Offen DE 19907588
68. Uyar D, Takigawa N, Mekhail T, Grabowski D, Markman M, Lee F, Canetta R, Peck R, Bukowski R, Ganapathi R (2003) Apoptotic pathways of epothilone BMS 310705. Gynecol Oncol 91:173–178. doi:10.1016/S0090-8258(03)00481-5

69. Wartmann M, Loretan J, Reuter R, Hattenberger M, Muller M, Vaxelaire J, Maira S-M, Flörsheimer A, O'Reilly T, Nicolaou KC, Altmann K-H (2004) Preclinical pharmacological profile of ABJ879, a novel epothilone B analog with potent and protracted anti-tumor activity. Proceedings of the American Association for Cancer Research 45. Abstract #5440
70. http://sec.edgar-online.com/2007/01/31/0001047469-07-000510/Section5.asp
71. Höfle G, Glaser N, Leibold T, Sefkow M (1999) Epothilone A-D and their thiazole-modified analogs as novel anticancer agents. Pure Appl Chem 71:2019–2024. doi:10.1351./pac199971112019. See also: Glaser N (2001) Semisynthese Seitenketten-Modifizierter Epothilone. Doctoral Thesis, Technical University of Braunschweig.
72. Altmann K-H, Blommers MJJ, Caravatti G, Flörsheimer A, Nicolaou KC, OReilly T, Schmidt A, Schinzer D, Wartmann M (2001) Synthetic and semisynthetic analogs of epothilones: chemistry and biological activity. In: Ojima I, Vite GD, Altmann K-H (eds): Anticancer agents—frontiers in cancer chemotherapy. ACS Symposium Series 796. American Chemical Society, Washington, pp 112–130. doi:10.1021/bk-2001-0796.ch007
73. Karama U, Höfle G (2003) Synthesis of epothilone 16,17-alkyne analogs by replacement of the C13-C15(O)-ring segment of natural epothilone C. Eur J Org Chem:1042–1049. doi:10.1002/ejoc.200390146
74. Dong SD, Sundermann K, Smith KMJ, Petryka J, Liu F, Myles DC (2004) Rapid access to epothilone analogs via semisynthetic degradation and reconstruction of epothilone D. Tetrahedron Lett 45:1945–1947. doi:10.1016/j.tetlet.2003.12.123
75. Klar U, Buchmann B, Schwede W, Skuballa W, Hoffmann J, Lichtner RB (2006) Total synthesis and antitumor activity of ZK-EPO: The first fully synthetic epothilone in clinical development. Angew Chem Int Ed 45:7942–7948. doi:10.1002/anie.200602785
76. Feyen F, Cachoux F, Gertsch J, Wartmann M, Altmann K-H (2008) Epothilones as Lead Structures for the Synthesis-Based Discovery of New Chemotypes for Microtubule Stabilization. Acc Chem Res 41:21–31. doi:10.1021/ar700157x
77. Rivkin A, Yoshimura F, Gabarda AE, Cho YS, Chou T-C, Dong H, Danishefsky SJ (2004) Discovery of (E)-9,10-dehydroepothilones through chemical synthesis: on the emergence of 26-trifluoro-(E)-9,10-dehydro-12,13-desoxyepothilone B as a promising anticancer drug candidate. J Am Chem Soc 126:10913–10922. doi:10.1021/ja046992gx