PERSPECTIVE

OBC
www.rsc.org/obc

The merger of natural product synthesis and medicinal chemistry: on the chemistry and chemical biology of epothilones

Karl-Heinz Altmann*
Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH), Zurich

Received 19th April 2004, Accepted 7th June 2004
First published as an Advance Article on the web 14 July 2004

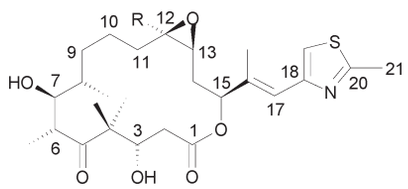
Based on epothilones as powerful natural product leads several promising new anticancer agents have emerged through concerted efforts in chemistry and biology.

Introduction and background

Cancer represents one of the most severe health problems worldwide and the search for novel anticancer drugs represents one of the most important, but also one of the most challenging areas in current drug discovery. Modern anticancer research encompasses a variety of molecular approaches, with a recent focus on "mechanism-based" strategies, which try to address targets related *e.g.* to cell cycle progression or signal transduction.[1] On the other hand, the search for new and improved cytotoxic agents directed towards ubiquitous cellular targets such as DNA or microtubules continues to be of significant importance and future clinical treatment strategies will likely involve combinations of cytotoxic drugs and new agents derived from mechanism-based approaches.[1] Within the former group, one of the most important clinical agents is the natural product Taxol® (paclitaxel), which inhibits human cancer cell growth through the stabilization of cellular microtubules and interference with microtubule dynamics.[2,3]

Members of the taxane family of natural products had long been the only known inhibitors of microtubule depolymerization, until it was discovered in 1995 that the bacteria-derived natural products epothilone A (**1**) and B (**2**) (first isolated two years earlier by the groups of Reichenbach and Höfle at the GBF;[4] Fig. 1) shared paclitaxel's ability to stabilize microtubules under otherwise destabilizing conditions.[5] Microtubule binding was shown to be competitive with paclitaxel, thus suggesting that epothilones and paclitaxel bind to the same, or at least largely overlapping, site(s) on β-tubulin.[5]

The structural and functional properties of microtubules are the subject of an excellent review by Linda Amos in this issue of OBC and shall thus not be discussed here in any detail. Suffice it to say that microtubule-stabilizing agents in general will also *promote* the polymerization of tubulin heterodimers into microtubule polymers and that the induction of tubulin polymerization, rather than microtubule stabilization itself, is commonly used as a biochemical readout for the interaction of microtubule stabilizers with tubulin/microtubules. This is illustrated by the data summarized in Table 1,



R = H: Epothilone A (**1**)

R = CH_3: Epothilone B (**2**)

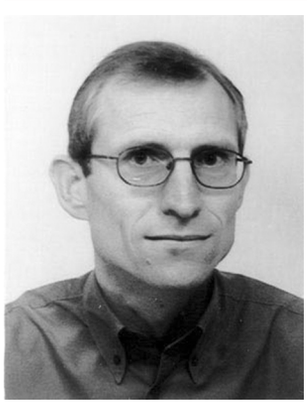
Fig. 1 Molecular structures of and numbering system for epothilones A and B.

which indicate that both **1** and **2** are more potent inducers of tubulin polymerization than paclitaxel, with **2** being the most active among the three compounds.[6]

At the cellular level interference with microtubule functionality during mitotic spindle formation causes cell cycle arrest in mitosis and induction of apoptosis (programmed cell death).[5,7–9] Accordingly, epothilones are potent antiproliferative agents, which inhibit the growth of a variety of human cancer cells *in vitro* with IC_{50}'s in the sub-nM to low nM range[5,7–9] (Table 2).

Epothilone B, **2** is a more potent inhibitor of human cancer cell growth than paclitaxel, which is in line with its more pronounced effects on microtubule stability *in vitro*. The antiproliferative activity of epothilone A, **1** is comparable with that of paclitaxel. Most significantly, and in contrast to paclitaxel (Taxol®), epothilones are also potent growth inhibitors of multidrug-resistant cancer cell lines (Table 2)[5,7–9] and they have been shown to be active against cell lines whose paclitaxel-resistance is derived from specific tubulin mutations.[7,10] **2** as well as a number of its analogs possess potent *in vivo* antitumor activity and at least five compounds of this class are currently undergoing clinical evaluation in humans. These include epothilone B itself, deoxyepothilone B (epothilone D, KOS-862), BMS-247550 (the lactam analog of epothilone B), BMS-310705 (C21-amino-epothilone B) and ABJ879 (C20-desmethyl-C20-methylsulfanyl-epothilone B) (*vide infra*).

Given their exceptional biological profile it is not surprising that epothilones have become important lead structures for the discovery



Karl-Heinz Altmann

Karl-Heinz Altmann was born in 1957 in Hochheim/Main, Germany. He studied chemistry at the Johannes-Gutenberg University in Mainz, from where he graduated with a diploma in 1983. His subsequent Ph. D. work in the area of peptide chemistry was performed at the University of Basel from 1984–1986. He then spent two and a half years as a post-doctoral associate at Cornell University, Ithaca, NY, which was followed by a one year stay as a master assistant at the University of Lausanne. In September 1990 Karl-Heinz Altmann joined Ciba-Geigy's Central Research Laboratories in Basel, where he worked on the design and synthesis of modified nucleosides as potential building blocks for antisense therapeutics until 1996. In 1997 he moved to Oncology Research within Novartis Pharma AG, where he was the Program Team Head for the epothilone program. In 2000 he was appointed the Novartis Senior Chemistry Expert and from January to June 2003 he was the acting Global Head of Chemistry of the Novartis Institutes for BioMedical Research. Since July 2003 Karl-Heinz Altmann is a professor of Pharmaceutical Biology at the Institute of Pharmaceutical Sciences of the ETH Zürich. In 1998 Karl-Heinz Altmann received the "Novartis Leading Scientist Award", an important internal science award of Novartis Pharma AG. Karl-Heinz Altmann's research interests are at the interface between chemistry and biology, with a particular focus on the chemical synthesis and the biological and pharmacological profiling of biologically active natural products and their synthetic and semi-synthetic analogs.

DOI: 10.1039/b405839a

Table 1 Induction of tubulin polymerization by epothilones and paclitaxel.

	Epo A (**1**)	Epo B (**2**)	Paclitaxel
Microtubule protein polymerization [% of control][a]	69[b]	90[b]	49[b]
EC_{50} (microtubule protein)/μM[c]	1.1[b]	0.7[b]	1.9[b]
EC_{50} (pure tubulin)/μM[d]	5.8[e]	1.9[e]	4.6[e]

[a] Induction of polymerization of porcine brain microtubule protein (tubulin with microtubule-associated proteins (MAPs)) by 2 μM of test compound relative to the effect of 25 μM of epothilone B (**2**), which gave maximal polymerization (85% of protein input). [b] Data from ref. 6. [c] Drug concentration required to achieve half-maximal polymerization of porcine brain microtubule protein. [d] Drug concentration required to achieve half-maximal polymerization of pure bovine brain tubulin. [e] M. Wartmann, unpublished data.

Table 2 IC_{50} values [nM] for net growth inhibition of human carcinoma cell lines by epothilones A and B in comparison with paclitaxel[a,b]

Cell line	Epo A (**1**)	Epo B (**2**)	Paclitaxel
A549 (lung)	2.67	0.23	3.19
HCT-116 (colon)	2.51	0.32	2.79
PC-3M (prostate)	4.27	0.52	4.77
MCF-7 (breast)	1.49	0.18	1.80
MCF-7/ADR[c]	27.5	2.92	9105
KB-31 (epidermoid)	2.1	0.19	2.31
KB-8511[d]	1.9	0.19	533

[a] Cells were exposed to drugs for 3–5 days, allowing for at least two population doublings. Cell numbers were estimated by quantification of protein content of fixed cells by methylene blue staining. Epo A, B = epothilone A, B. Data from ref. 6. [b] Multidrug-resistant cell lines are italic. [c] Multiple resistance mechanisms. [d] P-glycoprotein (Pgp) overexpression.

of new anticancer drugs and as such have been of major interest as targets for total synthesis. In contrast to paclitaxel, the more limited structural complexity of the epothilone scaffold immediately suggested that such structures should be accessible through total chemical synthesis in an efficient manner. Indeed, more than 30 total syntheses of epothilone A or B have been reported since the first disclosure of their absolute stereochemistry in 1996[11] (for reviews of work up to 2001 *cf*. refs. 12–15; for examples of more recent work *cf*. ref. 16). Interest in the total synthesis of the natural products has not ceased even today, in spite of the fact that the first successful syntheses of epothilone A were already accomplished six months after publication of its absolute stereochemistry.[17–20] Equally important as the total synthesis of the natural products themselves is the fact that the methodology developed in the course of those efforts could be exploited for the synthesis of a host of analogs (reviewed in refs. 12,13,21–23; more recent work will be referred to in the context of specific modifications). These synthetic efforts have allowed delineation of a rather comprehensive SAR picture for the epothilone class of natural products with a speed unprecedented in the area of natural product-based drug dicovery, thus attesting to the power of modern organic synthesis and its crucial role in the drug discovery process. The biological data generated for these analogs have in turn provided an important impetus and motivation for the design and synthesis of new structures, which has led to a continuous refinement of the chemistry employed to produce such molecules. Unlike the situation with paclitaxel, where a practical total synthesis is out of reach, hundreds of analogs of epothilones have been generated by chemical synthesis on a scale sufficient for extensive *in vitro* profiling and SAR studies and even the large-scale production of such compounds for clinical studies should be feasible.[13,24] It should also be remembered that in the absence of high-resolution structural information on complexes between epothilones and tubulin/microtubules or even the target protein alone,[25] progress in the understanding of the structural features critical for biological activity (which is a prerequisite for the design of improved analogs) hinges upon the efficient synthesis of new modified structures on a reasonable time-scale.

While total chemical synthesis so far has provided a wealth of important SAR data and may also be of relevance for the production of clinical material in specific cases, it is important to note that the majority of currently disclosed clinical development compounds is of semi-synthetic origin (at least 4 out of 5; *vide infra*). This bias towards semi-synthetic derivatives reflects the technical advantages (fewer chemical steps) still associated with natural product derivatization *vs*. total synthesis of fully synthetic analogs (provided an effcient process for the fermentation of starting materials is available!). Needless to say, however, that total synthesis allows the incorporation of structural modifications which are not acccessible through semi-synthesis and fully synthetic analogs may still be advanced to clinical trials in the near future.

Lastly, it should be pointed out that recent progress in the elucidation of the epothilone gene cluster[26–29] has allowed the development of heterologous expression systems for the production of natural epothilones and also structurally modified variants.[29,30] The direct production of epothilone analogs through fermentation technologies may thus provide a future alternative to semi-synthesis or total synthesis.

The chemistry, biology, and SAR of epothilones have been comprehensively discussed in recent review articles[8,12,13,21–23] and it is not the purpose of this Perspective to provide an extensive repetition of the information compiled in those previous accounts. Rather, this article will focus on selected aspects of the synthetic chemistry of epothilones, in particular in the context of analog synthesis and SAR studies. The discussion will therefore be structured according to modification sites on the epothilone scaffold, but in contrast to common practice in other reviews in the area, the author has decided to integrate the discussion of synthetic aspects and biological data rather than discuss those in separate sections. Although at first glance this may appear less straightforward and clear than separating chemical and biological issues, it is perhaps a better reflection of the actual discovery process, which is characterized by a high degree of interactivity between chemistry and biology, or, more specifically, between analog design and synthesis and the pharmacological evaluation of new compounds.

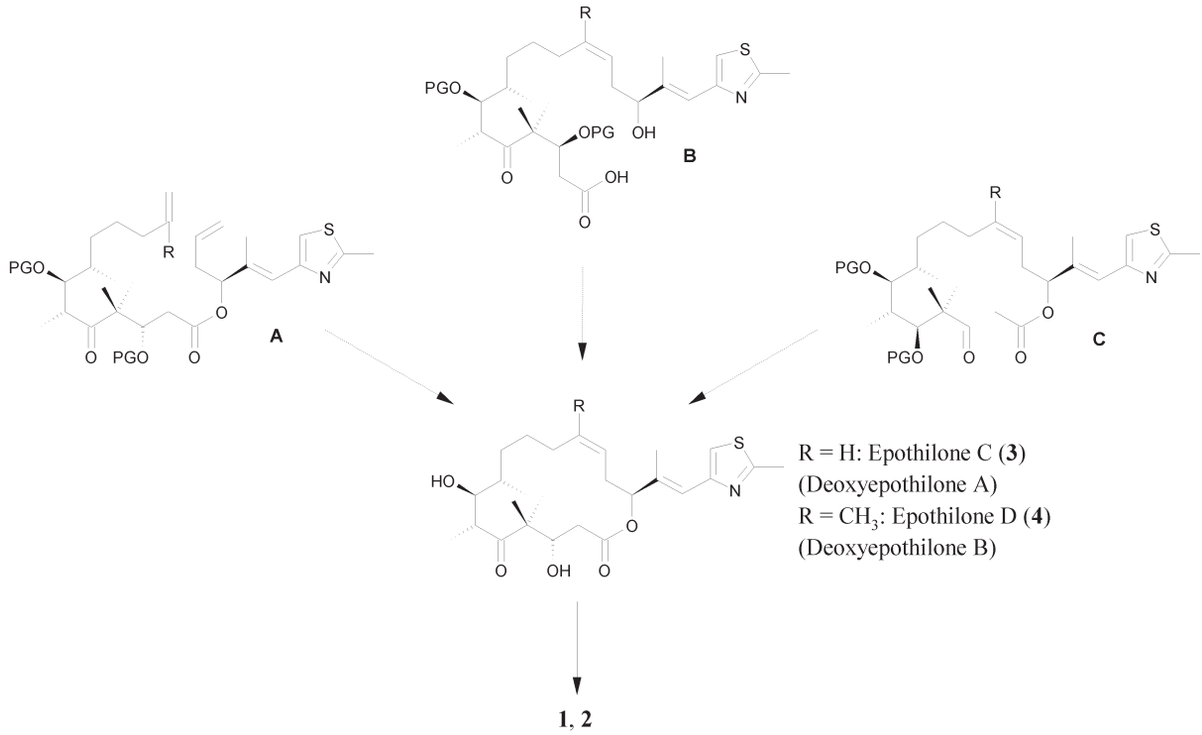
General approaches to the synthesis of epothilones

First generation approaches to the synthesis of epothilones were based on three different macrocyclization paradigms (Scheme 1), namely the formation of a C12–C13 double bond through ring-closing olefin metathesis (RCM), ring-closure through intramolecular ester bond formation, or the formation of the C2–C3 bond through intramolecular aldol reaction from precursors **A**, **B**, or **C**, respectively (reviewed in refs. 12,13).

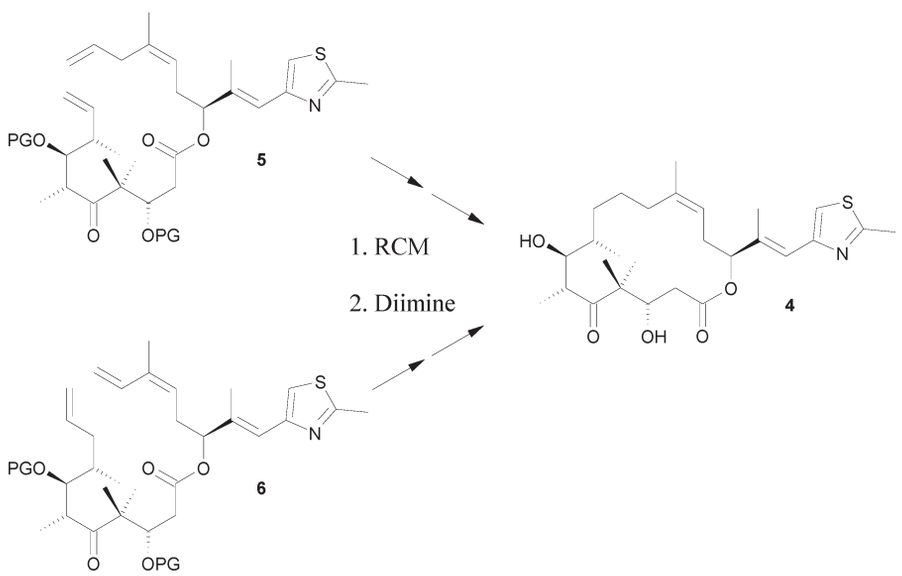
In all three paradigms ring closure was generally followed by protecting group removal from O3 and O7 and subsequent epoxidation of the olefinic double bond between C12 and C13. More recent deviations from this general theme are embodied by Mulzer's second generation synthesis of **2** (macrolactonization-based), which involves installment of the epoxide moiety in an acyclic precursor relatively early in the synthesis,[16g,31] as well as the work of Sun and Sinha, whose approach to **2** is based on the RCM of an epoxide-containing diene substrate.[16d]

Macrolactonization-based strategies continue to be an important element of epothilone chemistry, while precursors of type **A** and **C** have been used less frequently due to unsatisfactory E:Z ratios in the RCM and the lack of selectivity in the intramolecular aldol step, respectively. It should be noted, however, that an elegant and more efficient alternative to *olefin* metathesis at C12/C13 has been devised by Fürstner and coworkers, who have employed ring-closing *alkyne* metathesis to establish the C12/C13 linkage.[32] Ring-closure proceeded in excellent yield and the resulting C12/C13 alkyne could be selectively reduced to the required *Z*-olefin. As will be discussed in more detail below, more recent RCM-based cyclization strategies involve the formation of a C9–C10 or a C10–C11 double bond from precursors of type **5** or **6**, respectively (Scheme 2) and subsequent selective reduction of the disubstituted double bond with diimine.

Epoxidation of the C12–C13 double bond in deoxyepothilones A and B has been performed using a variety of reagents, including



Scheme 1 First generation approaches to the synthesis of epothilones. (PG = protecting group).

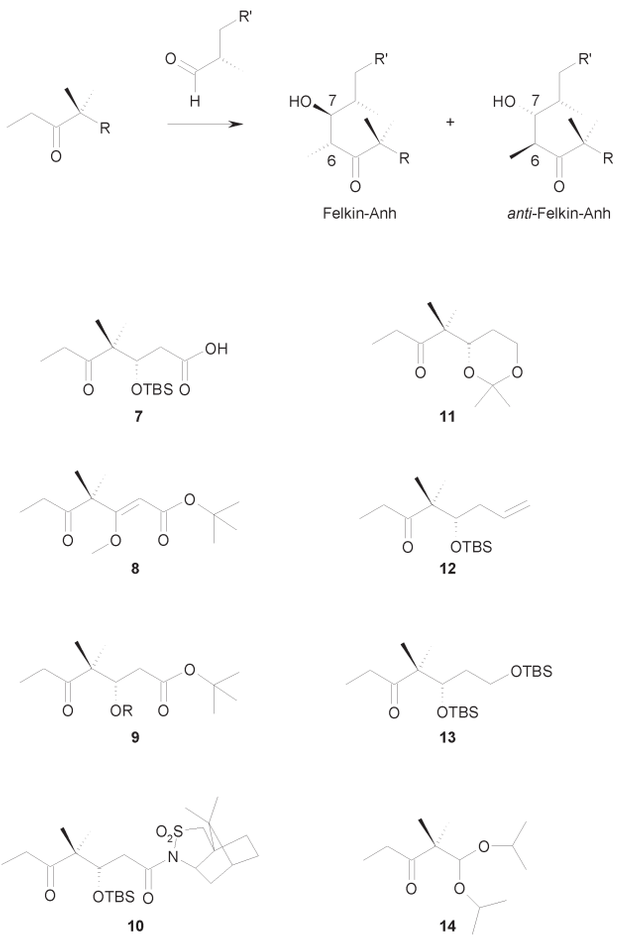


Scheme 2 Alternative RCM approaches to epothilones (PG = protecting group or H).

meta-chloroperbenzoic acid (MCPBA), dimethyldioxirane (DMDO), or trifluoromethyl-methyldioxirane (reviewed in refs. 12,13,15). Epoxidation selectivity is generally higher in the epothilone B series and also depends on the specific reagent employed. The most selective method reported so far involves the use of DMDO at −50 °C, which gave epothilone B **2** with ≥20 : 1 selectivity and 97% yield from deoxyepothilone B **4**.[33]

A variety of convergent approaches have been developed for the construction of cyclization precursors **A**, **B**, and **C**, with the key bond forming steps generally occurring between C1–O16 (ester bond formation) and C6–C7 (precursors of type **A**) or between C6–C7 and C11–C12 or C12–C13 (precursors of type **B**, **C**). One of the most critical steps in most epothilone syntheses thus consists in the stereoselective formation of the C6–C7 bond, a problem which is generally approached by aldol chemistry. A variety of ethyl ketones comprising C1–C6 of the epothilone framework have been employed in this step, the most important of which are depicted in Scheme 3.

Due to the strong preference for *cis*-enolate formation in structures of this type, the C6–C7-*syn* aldol products are formed almost exclusively (Scheme 3). In general, the desired C6-*R*, C7-*S* isomer is the major product formed (when using α-substituted aldehydes with the absolute stereochemistry depicted in Scheme 3), but the degree of selectivity can vary substantially depending on the structure of the ketone and also the aldehyde. Thus, the dianion of carboxylic acid **7**, which was employed in Nicolaou and coworkers' first generation synthesis of epothilones,[34] generally gives low selectivity (2 : 1 to 3 : 1), whereas Schinzer and coworkers' ketone **11**[20,35] provides the desired aldol products with 20 : 1 selectivity or better. An interesting long range effect on aldol selectivity has been oberved by Danishefsky and coworkers,[36] who found that reactions of ketone **8** with aldehyde **15** (Fig. 2) provided the C6-*R*, C7-*S* aldol product with significantly higher selectivity than the related saturated aldehyde **16** (5.5 : 1 for **15** *vs*. 1.3 : 1 for **16**). It is believed that the enhanced degree of selectivity arises from favorable transition state interactions between the terminal double bond



Scheme 3 Examples of ethyl ketones employed for C6–C7-bond formation in epothilones through aldol chemistry.

and the aldehyde C=O group in **15**. This hypothesis is supported by the fact that the presence of an olefinic or aromatic double bond between positions 4 and 5 of α-methylated aldehydes related to **15** generally leads to increased aldol selectivity over aldehydes with a different location of (or which lack) the double bond. Another intriguing long-range effect on aldol selectivity has been observed by Mulzer *et al*. in the reaction between ketone **12** and aldehyde **17** (Fig. 2), which proceeded with > 95 : 5 selectivity in favor of the desired C6-*R*–C7-*S* isomer.[16g,31] The selectivity of this reaction thus is signficantly higher than what is usually observed for analogous reactions with the unsaturated aldehyde **18** (Fig. 2).

Although aldol chemistry has been used most frequently to achieve stereoselective bond construction between C6 and C7, alternative approaches have also been pursued to address this problem. Examples include the use of a stereoeselective hetero

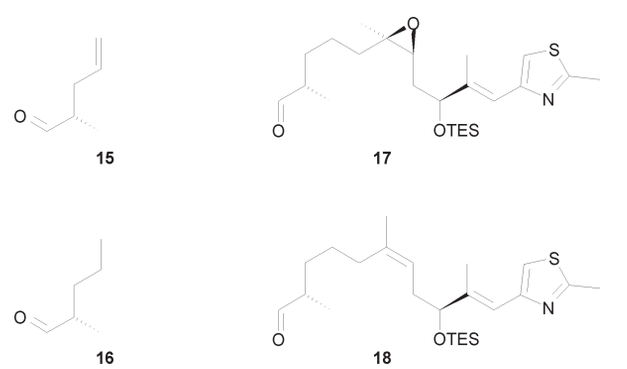


Fig. 2

Diels–Alder reaction as in Danishefsky's first generation synthesis of epothilones[19,33] or the use of the chiral crotylsilane **19**, which has been developed by Panek[16j] (Scheme 4).

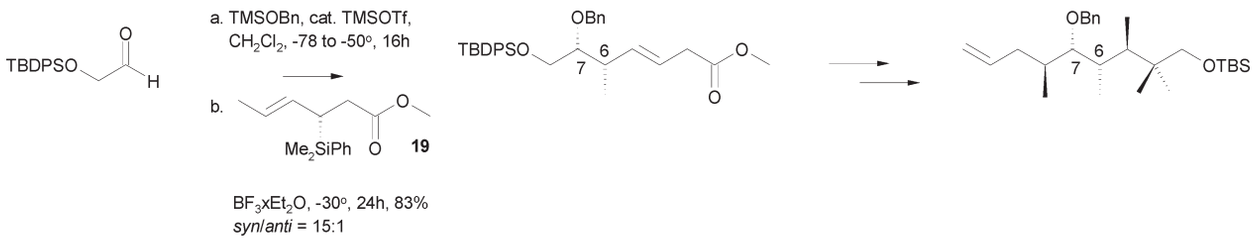
Synthesis of epothilone analogs and SAR studies

Modifications in the northern part of the macrocycle (C9–C12)

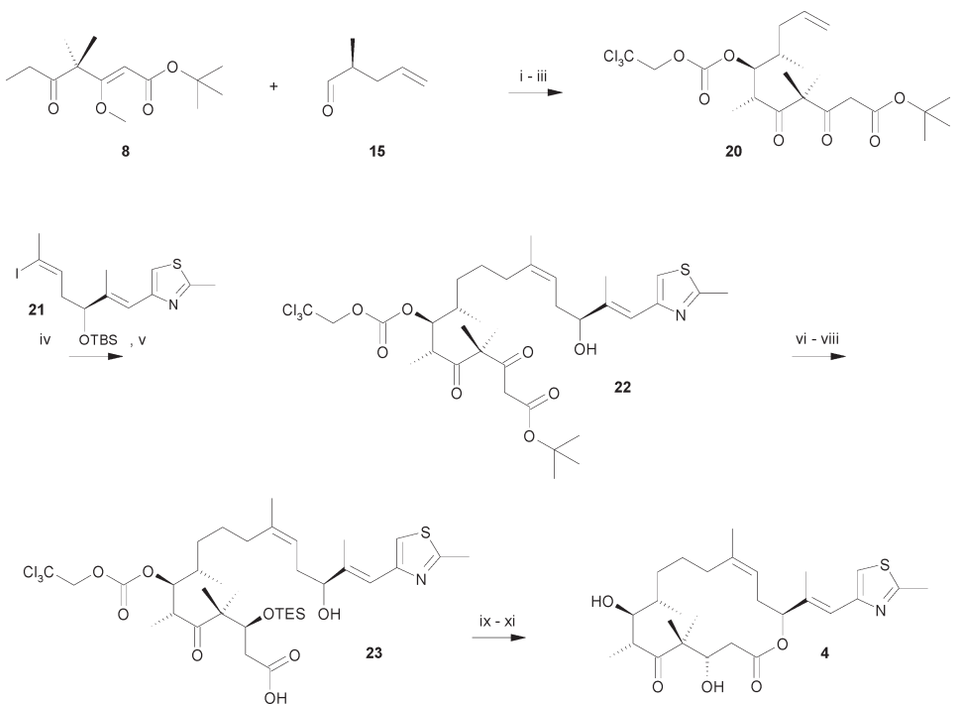
The northern part of the epothilone macrocycle has been subject to a variety of structural modifications, most of which involve changes around the epoxide moiety at C12 and C13 (reviewed in refs. 8,12,13,21–23. Early work in this area led to the discovery of the potent biological activity of "deoxyepothilones" (*i.e.* epothilones C(**3**) and D(**4**); Scheme 1) by the Danishefsky and Nicolaou groups,[37–41] which indicated that the potentially reactive epoxide moiety was not of critical importance for the antiproliferative activity of epothilone-type molecules. Thus, **4** is an equipotent inducer of tubulin polymerization as **2**, it inhibits the growth of human cancer cells with low nM IC_{50}'s (*i.e.* with only 5–30-fold lower potency than **2**) and, like **2**, it retains full activity against Pgp-overexpressing multidrug-resistant cells. Although **3** and **4** are also found as minor components in fermentation broths of myxobacateria,[42] it was only due to their availability as intermediates in the total synthesis of epothilones which allowed a fundamental feature of the epothilone SAR to be elucidated at a very early stage. Encouraged by its promising *in vitro* profile, Danishefsky and coworkers performed extensive *in vivo* studies with **4** in mouse models of human cancer and they found the compound to be a highly effective antitumor agent.[43,44] Based on these experiments they finally concluded that **4** was a more promising drug candidate than **2**. It remains to be seen whether this conclusion can be substantiated in human clinical trials, which are currently ongoing with both compounds.[45–47] However, the fact remains that **4** exhibits a very attractive *in vivo* pharmacological profile, which has provided the impetus for the Danishefsky group to work on the continuous improvement of their synthetic strategies towards this compound, in order to meet the increased demand of material for extensive *in vivo* studies and eventually clinical trials. These efforts resulted in a second generation approach to **4** (and implicitly also to **2**), which is summarized in Scheme 5.[36,48]

The key steps of this macrolactonization-based synthesis are the selective aldol reaction between ketone **8** and aldehyde **15** (*vide supra*) and the highly stereoselective reduction of the 3-keto group in β-keto ester **22**. This synthesis (including optimized approaches to the individual building blocks) is believed to be scalable to deliver sufficient material for clinical trials.[13,24] However, attempts to apply this strategy to the synthesis of deoxyepothilone F (dEpoF; 21-hydroxy-epothilone D) were thwarted by an unexpected solvolysis reaction in the hydrogenation step (Scheme 6), which lowered the yield of the desired compound **25** to a level unacceptable for practical applications.[49] This problem was overcome by further modifications to the second generation synthetic scheme, such that the C3–OH group was now established through aldol reaction between aldehyde **27** and *tert*-butyl acetate[50] (Scheme 7). Employing the glucose-based catalyst system developed by Duthaler and coworkers,[51] the desired aldol product could be obtained with ≥20 : 1 selectivity, thus giving access to dEpo F **29** (and also **4**) in a highly efficient manner. **29** was shown to possess *in vivo* antitumor activity similar to that of **4** in a breast tumor model, but due to its higher water solubility it may be a more viable drug candidate than **4**.[49]

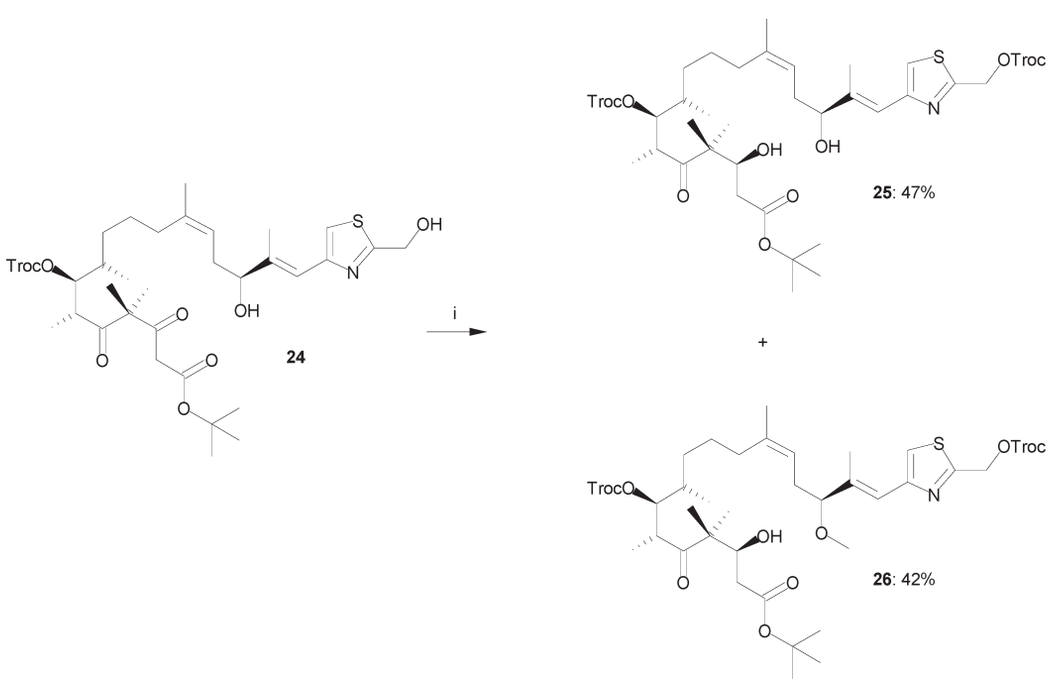
In addition to total synthesis, **4** can also be accessed through semisynthesis from **2** and the BMS group has developed an efficient one-step process for this conversion[52] (Scheme 8). **4** could be further transformed into cyclopropane analog **30** by means of CH_2Br_2–NaOH and subsequent reduction of the resulting dibromocyclopropane, albeit in moderate yield (Scheme 8). Cyclopropylepothilone B **30** proved to be essentially indistinguishable from **2** with regard to induction of tubulin polymerization and inhibition of human cancer cell proliferation.[52] This finding has reconfirmed the earlier conclusion derived from studies with **3** and **4** that the biological activity of epothilonens does not depend on the presence of an



Scheme 4 Ref. 16*j*: establishment of the C6–C7-bond of the epothilone macrocycle through stereoselective crotylation reaction.



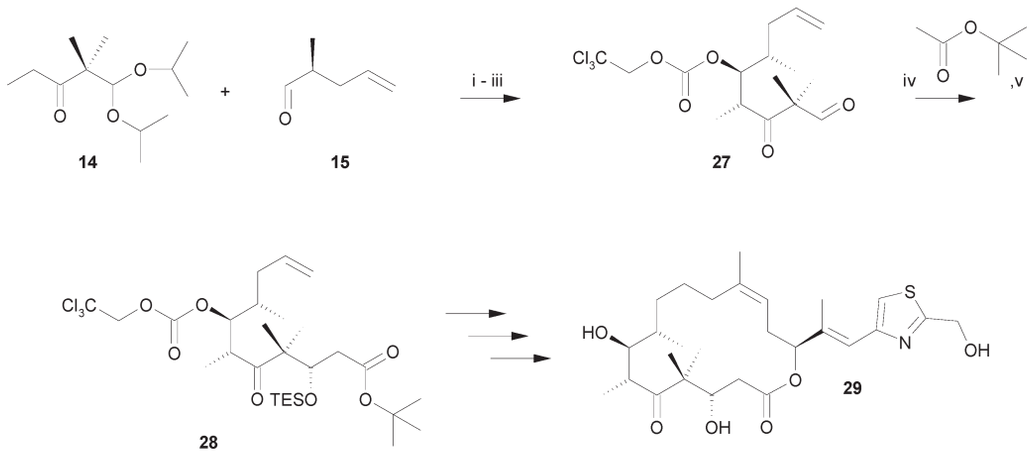
Scheme 5 Ref. 36: i: LDA, −120 °C, 50–60%. ii: Troc-Cl, pyridine, 0 °C. iii: *p*-TSA, acetone, 87% (2 steps). iv: 9-BBN; Cs_2CO_3, $Pd(dppf)_2Cl_2$, Ph_3As, DMF, H_2O, *ca*. 75%. v: 0.5 M HCl–MeOH, 85%. vi: $[RuCl_2((R)\text{-BINAP})_2][Et_3N]$, H_2, 1200 psi, MeOH, HCl, 82–88% (> 95% de). vii: TESOTf, 2,6-lutidine, −78 °C→RT. viii: 0.1 M HCl–MeOH, 70–77% (2 steps). ix: 2,4,6-trichlorobenzoylchloride, Et_3N, DMAP, 78%. x: SmI_2, cat. NiI_2, −78 °C, 90–95%. xi: HFxpyridine, 0 °C, 98%.



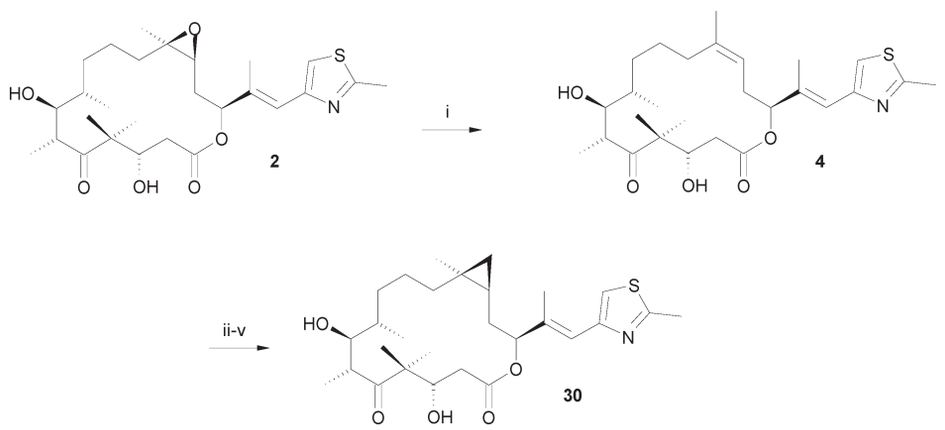
Scheme 6 Ref. 49: i: 5% $Et_2NH_2[(R)\text{-(BINAP)RuCl)}_2Cl_3]$, HCl–MeOH, H_2 (120 psi), RT, 8 h.

epoxide moiety. It thus appears that rather than acting as a reactive electrophile or a hydrogen bond acceptor, the oxirane ring system merely serves to stabilize the proper bioactive conformation of the epothilone macrocycle.

An improved second generation approach to analogs of **2** and **4** has also been elaborated by Nicolaou and coworkers[53,54] (Scheme 9). Key improvements of this strategy over their original first generation synthesis were (i) the highly selective installment of



Scheme 7 Ref. 50: i. LDA, −78 °C, 85%, dr = 4 : 1. ii. TrocCl, pyridine, CH_2Cl_2, 0 °C, 99%. iii. H_2O–THF, cat. TsOH, 88%. iv. $CpTiCl(OR)_2$ (R = 1,2 : 5,6-di-*O*-isopropylidine-α-L-glucofuranos-3-*O*-yl), THF, −78 °C, 89%, dr > 20 : 1. v. TESCl, imidazole, DMF, 96%.



Scheme 8 Ref. 52: i: WCl_6, *n*-BuLi, 78%. ii: TBS–OTf, 2,6-lutidine, CH_2Cl_2, 0 °C, iii. Benzyltriethylammonium chloride, 50% NaOH (aq), $CHBr_3$, 45 °C (*ca*. 30%), iv. Bu_3SnH, AIBN, 70 °C, v. 20% CF_3COOH–CH_2Cl_2, −15 °C. (No yields are reported in ref. 52 for steps ii, iv, and v).

the tri-substituted *cis*-double bond between C12 and C13 (through the use of stabilized ylide **32**), (ii) a substantially increased selectivity in the aldol step, which involves the use of the protected keto-diol **13** rather than keto acid acid **7**,[54] and (iii) the stereoselective epoxidation of the C12/C13 (now allylic) double bond in **38** under Sharpless conditions (Scheme 9). Intermediate **39** thus obtained has been elaborated into different types of C26-modified analogs of **2** (*cf*., *e.g.* Scheme 10) and in principle could also be reduced to deliver **4** (although this specific transformation has not been explicitly described in the literature; *cf*., however, ref. 54).

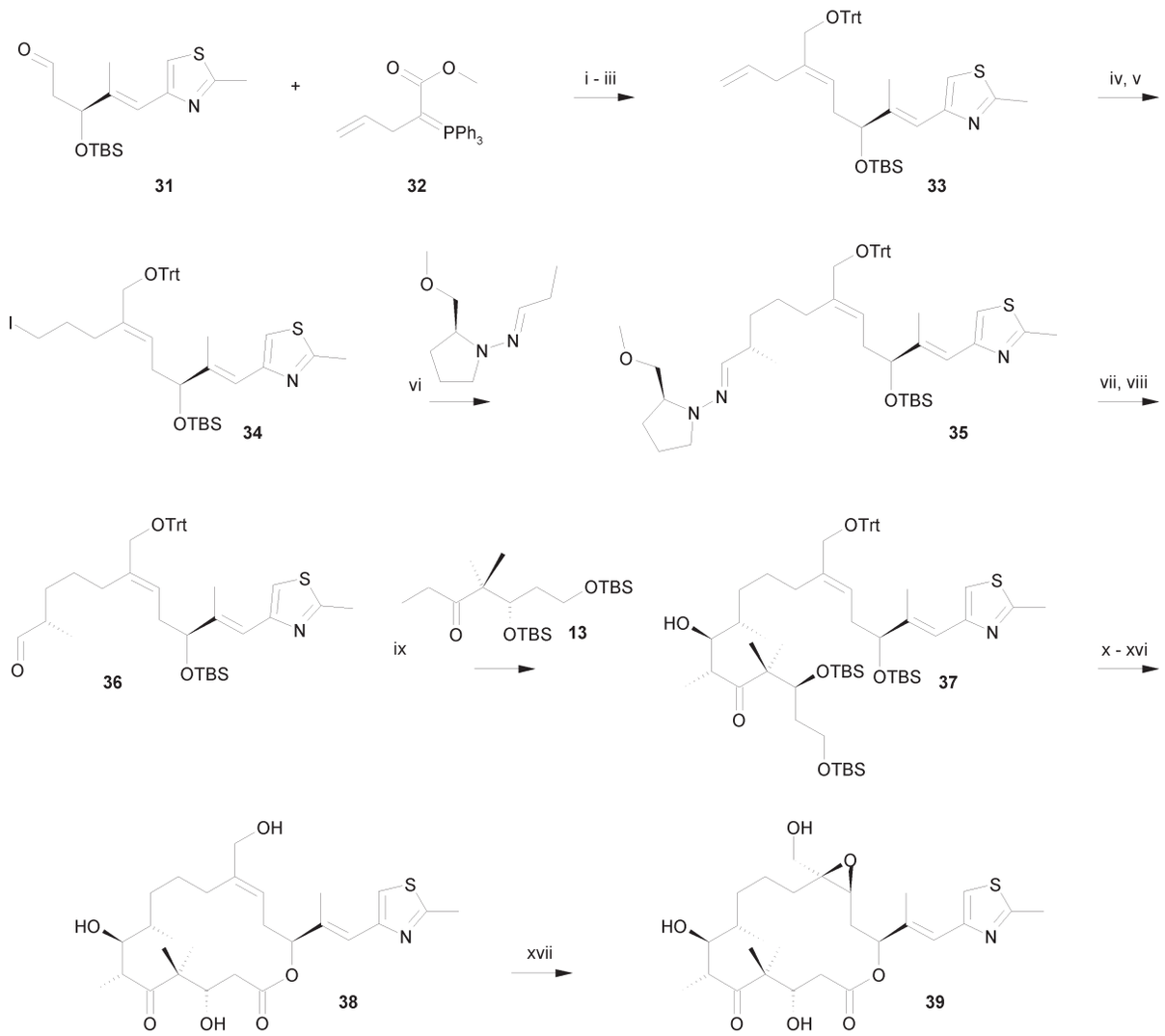
Analogs of **2** with modifications at C26 have been shown to exhibit potent biological activity (for C26-substituents of limited size) and the same is true for the the corresponding analogs of **4**[55] (*cf*. also ref. 39). 26-Fluoro-epothilone B **41** (Scheme 10) possesses *in vitro* antiproliferative activity which is equivalent to that of **2**[55] and in a human prostate xenograft model in nude mice the compound was found to have significantly better antitumor activity than paclitaxel when both agents were administered at equitoxic doses.[56] No comparison with **2** was included in this work, but data from our own laboratory indicate that the *in vivo* profile of **41** is similar to that of **2**.[57]

Apart from the discovery of the potent biological activity of deoxyepothilones, a second intriguing observation made in the course of early SAR studies was the retention of significant potency by the non-natural C12–C13 *trans*-analogs of epothilones.[37,39–41] Thus, *trans*-deoxyepothilone A was found to be only slightly less active than **3** and *trans*-epothilone A was reported by Nicolaou *et al*. to be virtually equipotent with **1** on an ovarian (1A9) and a breast cancer (MCF-7) cell line.[40] However, rather than being the product of a directed synthetic effort, these *trans*-isomers were merely obtained as undesired by-products in the synthesis of **1** or **2** and the absolute stereochemistry of the active epoxide isomer of *trans*-epothilone A was not determined. In view of the interesting biological properties of *trans*-(deoxy)epothilones and in order to address the problem of the absolute stereochemistry of the active epoxide isomer of *trans*-epothilone A, our own laboratory has subsequently devised a stereoselective synthesis of such analogs[58] (Scheme 11).

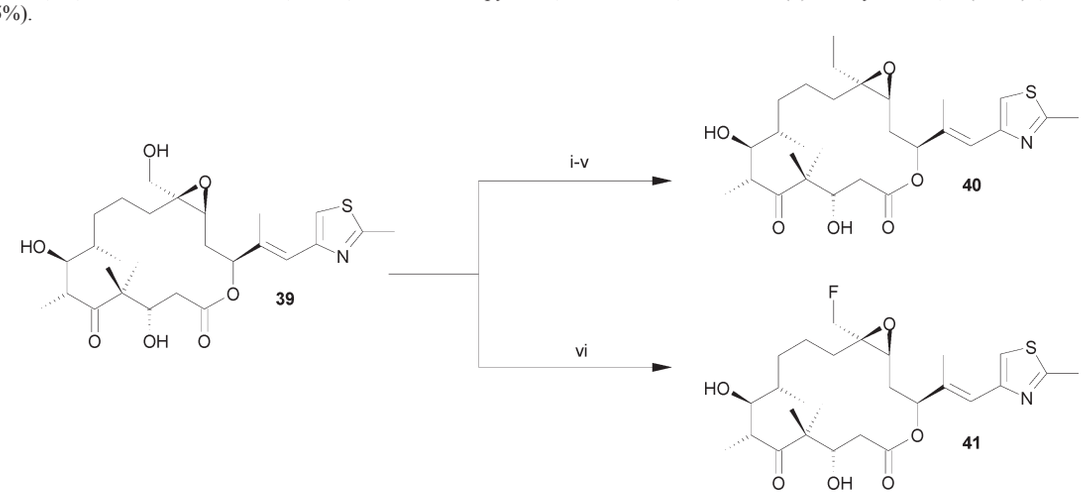
The key steps for our construction of the macrocyclic carbon framework of *trans*-epothilones A consist of (i) the highly stereoselective aldol reaction of the Schinzer ketone **11** with aldehyde **42** (> 20 : 1 selectivity) and (ii) the *B*-alkyl Suzuki coupling between olefin **44** and *trans* vinyl iodide **45** to produce **46** in 61% yield. While conversion of **46** into *trans*-deoxyepothilone A **49** proved to be straightforward (38% overall yield for 4 steps), the most critical step in the preparation of *trans*-epothilones A was the stereo- and regioselective epoxidation of the C12–C13 *trans* double bond in **49**. Even though this problem could not be solved to our full satisfaction even after extensive optimization efforts, the use of fructose-based epoxidation catalyst **50**[59] at least enabled the preparation of (12*S*,13*S*) *trans*-epothilone A **51** with *ca*. 8 : 1–10 : 1 selectivity (Scheme 11), albeit only in moderate yield (27% for an 8 : 1 mixture of isomers (54% based on recoverd starting material); 11% of stereochemically pure **51** after HPLC purification).[58]

(12*S*,13*S*) *trans*-epothilone A **51** is a strong inducer of tubulin polymerization *in vitro* and exhibits potent antiproliferative activity, whereas its (12*R*,13*R*)-isomer is at least 500-fold less active. **51** in fact shows slightly higher growth inhibitory activity than **1** and we have observed this rank order of activity across a wide range of human cancer cell lines (*e.g.* IC_{50}-values against the human epidermoid carcinoma cell lines KB-31/KB-8511 are 2.0 nM/1.8 nM for **1** and 1.0 nM/0.8 nM for **51**, respectively).[58]

In a collaborative effort proceeding in parallel with our own evaluation of **51** at Novartis, the Nicoalou group at TSRI has developed a synthesis of the cyclopropyl analog of **51** as well as a number of side-chain modified derivatives thereof.[60] As illustrated in Scheme 12, Nicolaou's synthesis of *trans*-cyclopropyl-epothilone A **63**

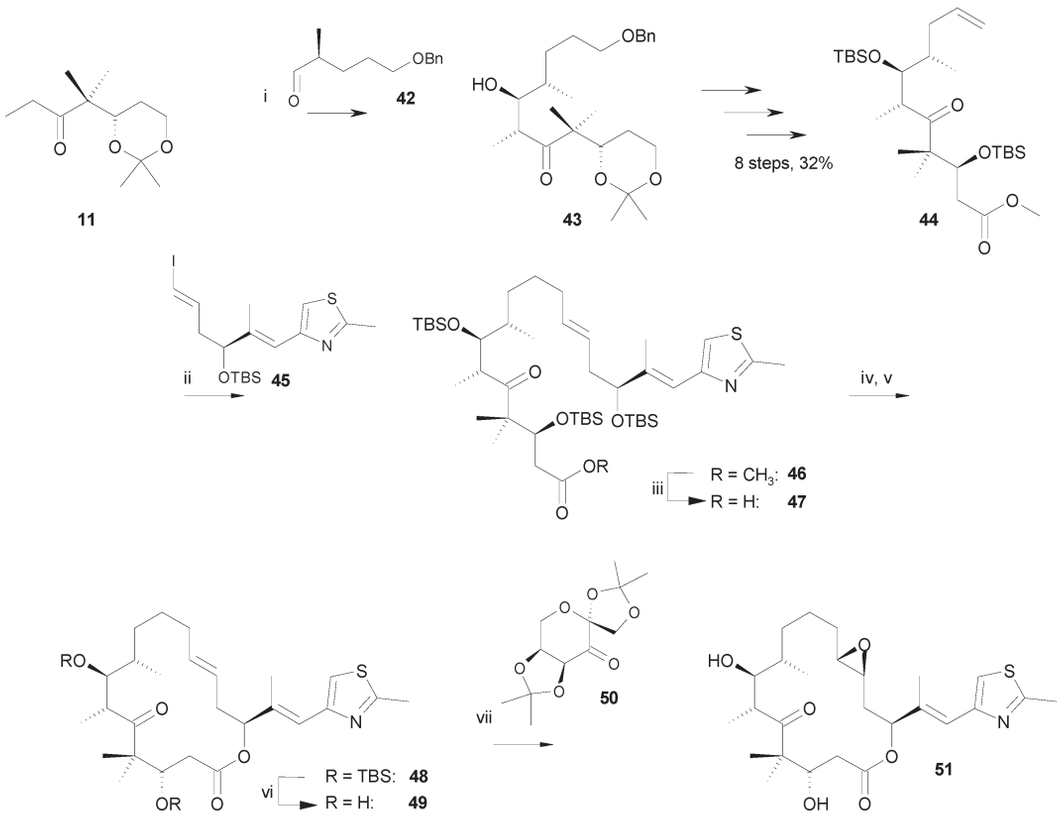


Scheme 9 Ref. 53: i: benzene, refl., 95%. ii: DIBAL, −78 °C, 98%. iii: Trt-Cl, DMAP, 70 °C, 99%. iv: a. 9-BBN, 0 °C; b. NaOH, H_2O_2, 94%. v. I_2, imidazole, Ph_3P, 0 °C, 90%. vi: a. LDA, 0 °C, 14 h; b. **34** in THF, −100 °C→−20 °C, 66%. vii: Monoperoxyphthalic acid Mg-salt, 0 °C, 91%. viii: DIBAL, −78 °C, 97%. ix: a. LDA, 0 °C; b. **36**, −78 °C, 85% (3 : 1 mixture of diastereoisomers; *cf*., however, ref. 54). x: TBSOTf, 2,6-lutidine, 92%. xi: HF x pyridine, 0 °C→25 °C, 74%. xii: $(COCl)_2$, DMSO, Et_3N, −78 °C→0 °C, 99%. xiii: $NaOCl_2$, 2-methyl-2-butene, 25 °C, 99%. xiv: TBAF, 25 °C, 89%. xv: 2,4,6-trichlorobenzoylchloride, Et_3N, 0°, then addition of DMAP, 75 °C, 75%. xvi: HFxpyridine, 0 °C→25 °C, 78%. xvii: (+)-Diethyltartrate, Ti(*i*-OPr)4, *t*-BuOOH, −30 °C, 76% (de > 95%).



Scheme 10 Ref. 53: i. (a) TMSCl, DMF, 25 °C; (b) silica gel, CH_2Cl_2, 25 °C, 98% (two steps). ii. 10% TPAP, NMO, CH_2Cl_2, 25 °C, 90%. iii. $Ph_3P^+CH_3Br^-$ (mixture with $NaNH_2$), THF, −5 °C, 65%. iv. H_2NNH_2, H_2O_2, EtOH, 0 °C. v. HFxpyridine, THF, 0 °C→25 °C, 75% (two steps). vi. DAST, −78 °C, 65%. (TPAP = tetrapropylammonium perruthenate; TMS = trimethylsilyl).

relies on the early introduction of the cyclopropane moiety through stereoselective cyclopropanation of allylic alcohol **52** and a high degree of selectivity in the crucial aldol step (**61→62**) was again ensured through the use of ketone **13**. In contrast to all prior epothilone syntheses, the side-chain of **63** was introduced in its entirety in a single step through Nozaki–Kishi coupling between aldehyde **58** and vinyl iodide **59**. While the coupling reaction was non-selective, this deficiency could be corrected through oxidation of the diastereomeric mixture to the C15-ketone and subsequent stereoselective reduction to the desired (*S*) alcohol. As for cyclopropane-based analogs of **1** and **2**, compound **63** exhibits similar *in vitro* activity as the corresponding epoxide **51**. In contrast, *trans*-analogs of **2** (epoxide



Scheme 11 Ref. 58: i. LDA, THF, −78 °C, 82%. ii. a. Olefin **44**, 9-BBN, THF, RT; b. Cs_2CO_3, $PdCl_2(dppf)_2$, Ph_3As, vinyl iodide **45**, DMF, −10 °C→RT, 63%. iii. LiOH (6 eq.), *i*-PrOH–H_2O 4 : 1, 50 °C, 85%. iv. TBAF, THF, 64%. v. 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, DMAP, THF–toluene, 61%. vi. CF_3COOH–CH_2Cl_2, 91%. vii. Oxone®, **50** (30 mol%), Bu_4NHSO_4 (cat.), K_2CO_3, CH_3CN–DME–0.05 M $Na_2B_4O_7{\cdot}10H_2O$ in 4×10^{-4} M Na_2EDTA 2 : 1 : 2, RT, 1 h, 27% (dr *ca*. 8 : 1; 50% recovered starting material).

or cyclopropane-based) or **4** generally appear to be less potent than the corresponding *cis*-isomers[61] (*cf*., however, ref. 41).

In contrast to the extensive early modification efforts around the epoxide moiety, structural changes in the C9–C11 region of the northern part of the epothilone scaffold have become a subject of interest only relatively recently. Early work in this area had shown that analogs with enlarged or reduced ring size (based on the incorporation or removal of methylene groups in the C9–C11 trimethylene fragment) exhibit greatly reduced biological activity.[39,62] More recently, however, a number of new epothilones with structural variations in the C9–C11 region have emerged, which not only exhibit potent antiproliferative activity *in vitro*, but are also characterized by improved pharmacological properties over the corresponding parent compounds **2** or **4**. These analogs were obtained through a number of different approaches, including heterologous expression of the modified epothilone polyketide synthase in Myccococus xanthus,[63] total chemical synthesis,[64–69] or biotransformation of **2**.[70] Perhaps most significantly, epothilone 490 **68** (Scheme 13) which was first obtained as a minor fermentation product from a modified strain of M. xanthus, was found to be only 3–4-fold less active than **4** against the MCF7 breast, SF268 glioma, NCI-H460 lung cancer, and HL60 promyelocytic leukemia cell lines, while being equipotent even with **2** against the human T-cell leukemia cell lines CCRM-VEM and CCRM-VEM/VBL.[63] This observation has triggered a substantial synthetic effort by the Danishefsky group directed at the synthesis of epothilone analogs with structural modifications in the C9–C11 region. As illustrated in Scheme 13 these efforts have resulted in an efficient total synthesis of **68**, which also provides a new alternative access to **4** and other epothilone analogs.[64] The key strategic element of Danishefsky's approach to **68** is a RCM with diene **65** to establish the C10–C11 double bond. Ring closure between C10 and C11 exclusively led to the formation of a *trans* double bond, but in intial experiments with a fully protected substrate (C3-OTES analog of **65**) in CH_2Cl_2 as solvent formation of the desired 16-membered ring was accompanied by substantial amounts of a by-product arising from ring-closure between C10 and C13 (epothilone numbering applied to the acyclic precursor C3-OTES-**65**). This side-reaction could be suppressed almost completely by using the partially protected diene **65** or its *fully* deprotected version (OH-groups on C3 and C7 both unprotected). RCM with the latter compound in CH_2Cl_2 (catalyst **66**, 35 °C, 5.5 h) or toluene (catalyst **66**, 110 °C, 25 min) directly gave **68** in 64% and 55% yield, respectively, with none of the 14-membered lactone side-product being observed.[64]

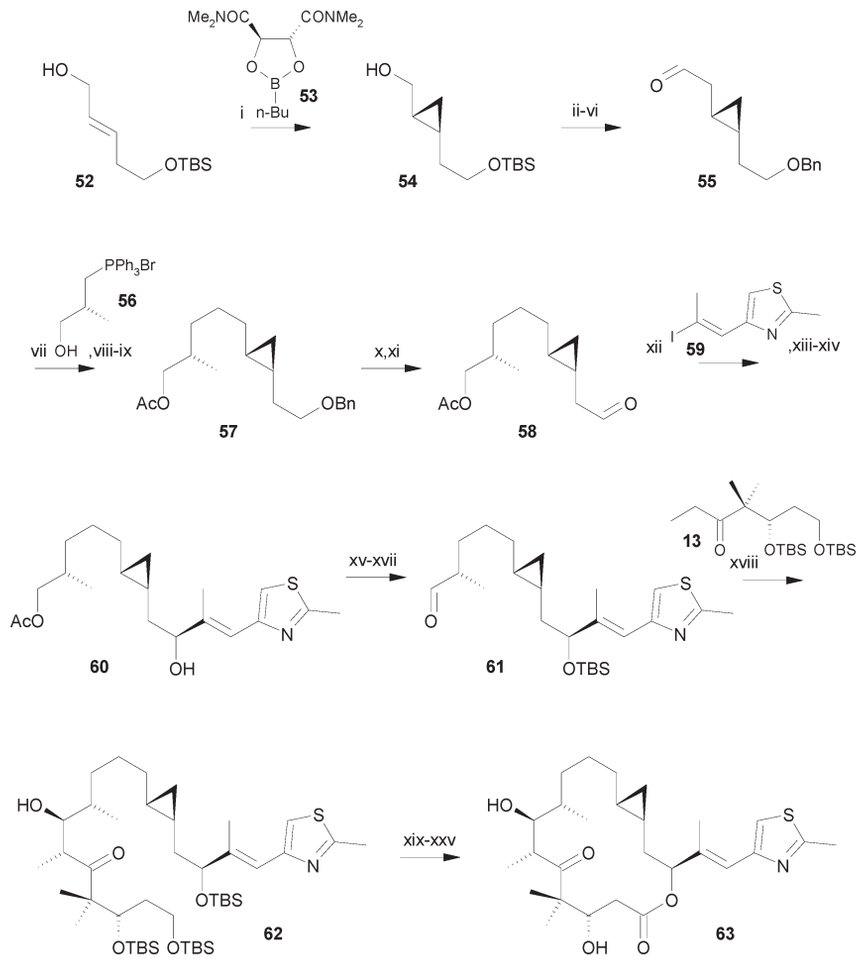
As an extension of their work on **68**, Danishefsky's group has also prepared *trans*-C9,C10-didehydro-epothilone D **76**, again employing a RCM-based cyclization strategy (Scheme 14).[67]

As observed for the cyclization of **65**, RCM with diene **73** was highly *trans* selective and gave the desired C9–C10-*trans* isomer **74** in 78% yield. As **78** can be converted into **4** in high yield (Scheme 15), the route depicted in Scheme 14 also embodies a highly efficient new strategy for the synthesis of **4** and **2**. A similar approach has been independently pursued by Sun and Sinha, whose recent synthesis of **2** is based on RCM with epoxide-containing diene **77**, employing the second generation Grubbs catalyst **66**[16d] (Fig. 3).

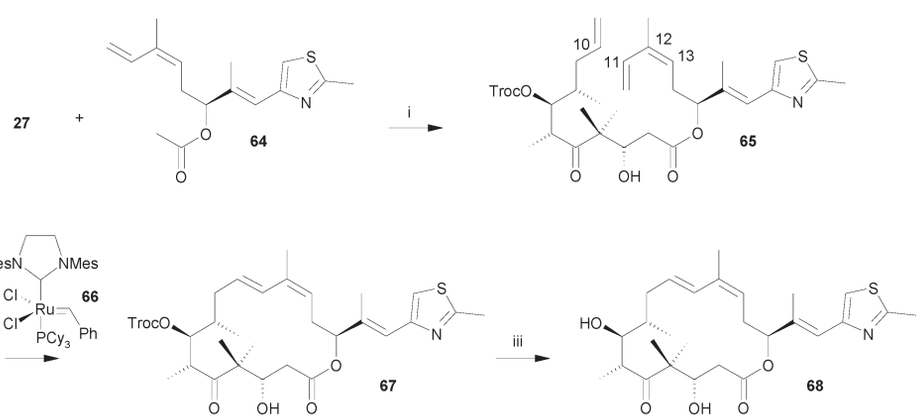
The presence of a *trans* double bond between C9 and C10 in **76** results in a marked *increase* in antiproliferative activity over **4** against the human leukemia cell line CCRF-CEM (IC_{50} of 0.9 nM for **76** *vs*. 3.6 nM for **4**).[67] Likewise, the C12–C13 epoxide corresponding to **76**, *i.e.* **78** (Scheme 15) was found to be 3–4-fold more potent than **2**.[68] Interestingly, the selectivity of the epoxidation of **76** with DMDO is much lower than is the case for the epoxidation of **4** under identical conditions and the major epoxidation product in fact is the undesired α-isomer **79** (Scheme 15).

This change in epoxidation selectivity has been ascribed to the specific conformational preferences imposed upon the macrocycle by the presence of the second double bond between C9 and C10.[68]

In contrast to **76**, the corresponding *cis* analog **80** (Fig. 3) has been reported by White *et al*. to be *ca*. 30-fold less active against the human epidermoid cancer cell line KB-31 than **4**.[71a] (Note that the compound assumed to be *trans* analog **76** in[71a] later was found to be *cis* isomer **80**[71b] (Fig. 3)). The above data support findings from recent spectroscopic studies,[72] which suggest that the bioactive conformation of epothilones is characterized by *anti*-periplanar



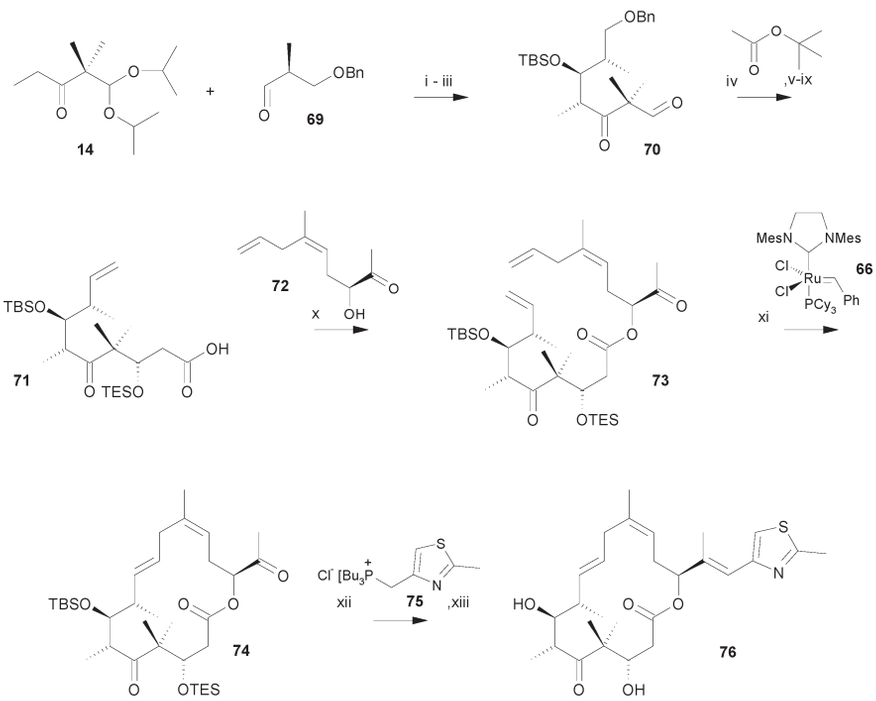
Scheme 12 i. DME, Et_2Zn, CH_2I_2, CH_2Cl_2, 98% (>90% ee). ii. Et_3N, SO_3xpy, CH_2Cl_2–DMSO 4 : 1, 0 °C. iii. $MeOCH_2PPh_3Cl$, NaHMDS, THF, −40 °C→ 25 °C, 81% (2 steps). iv. TBAF, THF, 25 °C. v. NaH, BnBr, THF–DMF 5 : 1, 0 °C→25 °C. vi. HCl (cat.), acetone–water 9 : 1, 50 °C. vii. NaHMDS, TMSCl, THF, 58% (4 steps). viii. $(NCO_2K)_2$, HOAc, MeOH, py, 25 °C. ix. Ac_2O, Et_3N, 4-DMAP, CH_2Cl_2, 0 °C. x. 20% $Pd(OH)_2$/C, H_2 (1 atm), EtOAc–EtOH 1 : 1 25 °C, 98% (3 steps). xi. DMP, CH_2Cl_2, 0 °C→25 °C. xii. $CrCl_2$, $NiCl_2$ (cat.), DMSO, 25 °C, 91% (2 steps). xiii. DMP, CH_2Cl_2, 0 °C→25 °C, 83%. xiv. (−)-DIPCl, Et_2O, −15 °C→25 °C, 84%. xv. TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 91–100%. xvi. DIBAL, CH_2Cl_2, −78 °C, 93–96%. xvii. DMP, CH_2Cl_2, 25 °C. xviii. LDA, THF, −78 °C, 4 min, 70%. xix. TBSOTf, 2,6-lutidine, CH_2Cl_2, −25 °C→25 °C, 94%. xx. HFxpy, py, 0 °C→25 °C. xxi. DMP, $NaHCO_3$, CH_2Cl_2, 25 °C. xxii. $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*-BuOH–H_2O 4 : 1, 25 °C. xxiii. TBAF, THF, 25 °C. xxiv. 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, 0 °C, then 4-DMAP, toluene, 75 °C, 53% (5 steps). xxv. 25% TFA in CH_2Cl_2, 25 °C, 73%. (DIBAL, diisobutylaluminum hydride; DIPCl, diisopinocampheyl chloroborane; 4-DMAP, 4-(dimethylamino)pyridine; DMP, Dess–Martin periodinane; LDA, lithium diisopropylamide; NaHMDS, sodium hexamethyldisilazide, py, pyridine; TBAF, tetrabutylammonium fluoride; TMSCl, chlorotrimethylsilane).



Scheme 13 Ref. 64: i. **64**, LDA, Et_2O, −78 °C, then $CpTiCl(OR)_2$ (R = 1,2:5,6-di-*O*-isopropylidine-α-L-glucofuranos- 3-*O*-yl), −78 °C→−30 °C, then **27**, −78 °C, 85%. ii. CH_2Cl_2 (0.002 M), 35 °C, 41%. iii. Zn, THF, AcOH, 86%.

conformations about the C9–C10 and C10–C11 bonds. Olefin **76** and epoxide **78** (Schemes 14 and 15) are potent inhibitors of tumor growth in the human breast cancer model MX-1[67,68] and in both cases *in vivo* potency is markedly improved over their respective parent structures **4** and **2**. For **76** this effect has been specifically ascribed to a combination of enhanced antiproliferative activity and improved plasma stability in mice,[69] but, unfortunately, the compound is also associated with significantly enhanced toxicity.[69] In contrast, excellent *in vivo* antitumor activity has been observed for the corresponding C26-trifluoro derivative **86** (Scheme 16) in the absence of lethalities or irreversible toxicity.[69]

The synthesis of **86** proceeded through diene **85** as a key intermediate (Scheme 16), for which an efficient 6-step sequence had been developed starting from keto ester **81**.[69] **85** Was then elaborated into **86** through the same sequence of reactions as depicted in Scheme 14 for the preparation of **76**. Ring-closure *via* RCM in this case proceeded in 71% yield even for the fully protected substrate (C3-OTES, C7-OTBS) to give the desired 16-membered lactone



Scheme 14 Ref. 67: i: LDA, THF, −90 °C, 78% (based on aldehyde), dr = 85 : 15. ii: TBSOTf, 2,6-lutidine, CH_2Cl_2, −40 °C→−20 °C, 97%. iii. *p*-TosOH (cat.), THF–H_2O 4 : 1, 64 °C, 98%. iv. LDA, $CpTiCl(OR)_2$ (R = 1,2:5,6-di-*O*-isopropylidine-α-L-glucofuranos-3-*O*-yl), Et_2O, −78 °C, 86% (dr > 20 : 1). v. TESCl, DMF, 0 °C→RT, 98%. vi. H_2, Pd–C (10%), EtOH, 83%. vii. TPAP, NMO, CH_2Cl_2, 95%. viii. $MePPh_3I$, *n*-BuLi, THF, −78 °C→−5 °C, 78%. ix. TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C→RT. x. EDCI, DMAP, CH_2Cl_2, 0 °C→RT (81%, 2 steps). xi. toluene, 110 °C, 20 min, 78%. xii. KHMDS, THF, −78 °C→−20 °C, 76%. xiii. HFxpyridine, THF, 97%.

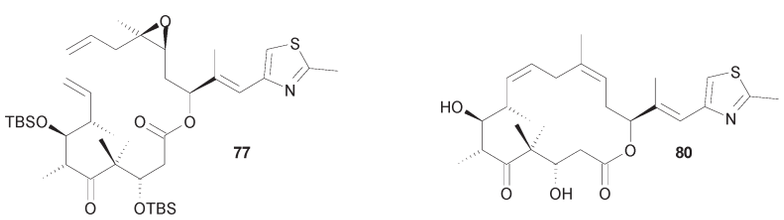
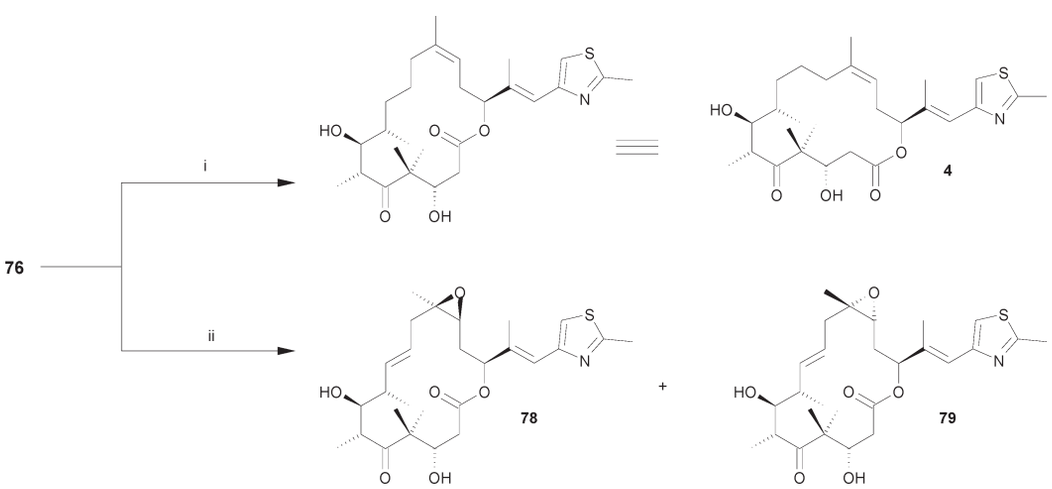


Fig. 3 Molecular structures of Sun and Sinha's RCM substrate in their synthesis of **2** (**77**)[16d] and of *cis*-9,10-didehydro-epothilone D (**80**).[71a]



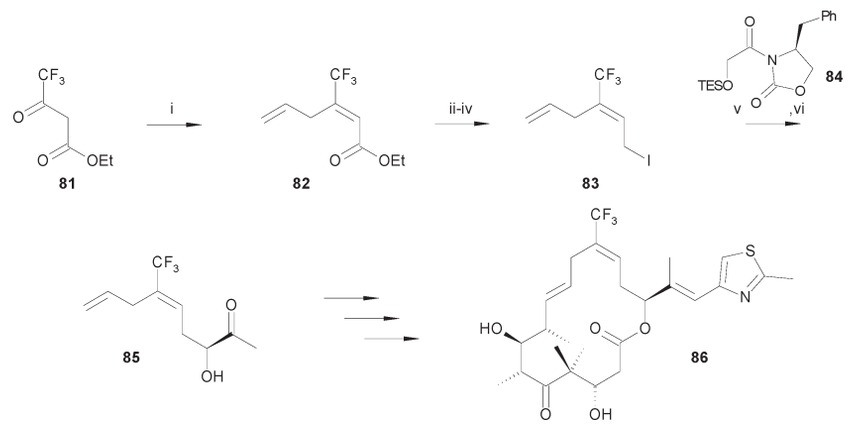
Scheme 15 Ref. 68: i: $TrisNH_2NH_2$, Et_3N, $ClCH_2CH_2Cl$, 50 °C, 91%. ii. DMDO, CH_2Cl_2, −78 °C→−50 °C, 87%, **78** : **79** = 1 : 2.6.

with a C9–C1 *trans* double bond as the only observable isomer.[69] The *in vitro* antiproliferative of **86** is comparable with that of **2** and the enhanced *in vivo* activity of the compound, as for the non-fluorinated analog **76**, may be a consequence of improved pharmacokinetic properties.

Apart from the discovery of the potent *in vivo* activity of **76, 78**, and **86**, perhaps one of the most intriguing findings that has emerged from the recent work of the Danishefsky laboratory is the fact that the presence of a *trans* double bond between C10 and C11 allows the insertion of an additional methylene group between C11 and C12 (thus creating a 17-membered ring) without substantial loss in antiproliferative activity.[65] Compound **87** (Fig. 4) thus is only 4-fold less active against the human leukemia cell line CCRF-CEM than the parent compound **4**.[65] In contrast, the simple incorporation of an additional methylene group in the C9–C11 region of **1** or **3** had previously been found to result in a significantly more pronounced loss in potency.[62]

Modification of the ester group

Modification of the ester moiety in epothilones so far has been limited to the replacement of the lactone oxygen by (substituted) nitrogen. This strategy has emerged as an important element of epothilone-based anticancer drug discovery, which has produced



Scheme 16 Ref. 69: i. Allyl bromide, In, THF–H_2O 3 : 1, 48 °C, 85%. ii. $SOCl_2$, pyridine, 55 °C, 77%. iii. DIBAL-H, CH_2Cl_2, −78 °C→RT, 99%. iv. I_2, Ph_3P, imidazole, CH_2Cl_2, 74%. v. (a) LiHDMS, THF, −78 °C→RT; (b) HOAc–THF–H_2O 3 : 1 : 1, 81% (2 steps). vi. (a) $Al(CH_3)_3$, $MeONHCH_3$, THF, 0 °C→RT; (b) CH_3MgBr, THF, 0 °C, 53%.

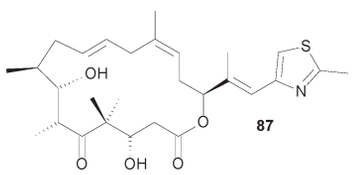


Fig. 4 Molecular structure of ring-enlarged epothilone D analog **87**.[65]

the most advanced clinical development compound identified to date (**90**, BMS-247550; Scheme 17). Lactam-based analogs of epothilones were first pursued by the BMS group in order to address the limited metabolic stability of **1** and **2** in rodent plasma, which was presumed to foreshadow similar problems in humans.[73]

The relevance of this rationale (not of the compounds as such), however, appears at least questionable, as both **2** as well as **4** exhibit very potent *in vivo* antitumor activity in mice (in spite of their limited plasma stability in rodents; *vide supra*). Furthermore, **4** has subsequently been shown to be significantly more stable in human than in rodent plasma.[75]

After initial unsatisfactory attempts to produce lactam analogs of **1** and **2** by total synthesis,[73] the BMS group has devised a highly original approach to the preparation of such compounds through semi-synthesis. This strategy exploits the fact that the ester group in epothilones in fact is allylic in nature, which has allowed the development of a higly effective three-step sequence for the semi-synthesis of **90** from **2**[73] (Scheme 17). Thus, Pd(0)-catalyzed lactone opening of **2** and concomitant introduction of an azide group at C15 produces azide **88** with complete retention of configuration at C15. Reduction of the azide to an amino group and subsequent EDCI–HOBt mediated macrolactamization then furnishes the desired **90**. All three steps can also be carried out in a single reactor in 20–25% yield.[73] The total synthesis of **90** has been reported by Danishefsky and coworkers[76] (*cf*. also ref. 77).

90 Is a potent inducer of tubulin polymerization, but its antiproliferative activity is *ca*. one order of magnitude lower than that of **2**[21,73] (*e.g.*, IC_{50}-values against the human colon carcinoma cell line HCT-116 are 3.6 nM and 0.42 nM, respectively, for **90** and **2**[73]). Our own studies with **90** have also indicated that the compound exhibits a significant activity differential between the drug-sensitive human epidermoid cancer cell line KB-31 and its P-gp overexpressing multidrug-resistant KB-8511 variant (IC_{50}s of 2.85 nM and 128 nM against KB-31 and KB-8511 cells, respectively;[21] for data for **2** *cf*. Table 2), thus indicating that **90** is a substrate for the P-gp efflux pump. Similar observations have been reported by the Sloan–Kettering group.[76] On the other hand, **90** exhibits very potent *in vivo* antitumor activity (in animal models), including tumor types which are non-responsive to treatment with Taxol® (paclitaxel).[74] It is thus unclear to what extent the susceptibility of the compound to P-gp-mediated drug efflux in highly P-gp overexpressing cell lines *in vitro* is of relevance for its potential clinical utility. In fact, phase II clinical trials with **90** have produced promising results, including objective repsonses in tumors which had been refractory to treatment with platinum-based drugs or taxanes.[23,78] Based on these data **90** has been advanced to phase III studies, which are currently ongoing (in parallel with a number of phase II trials, including combination studies).[23]

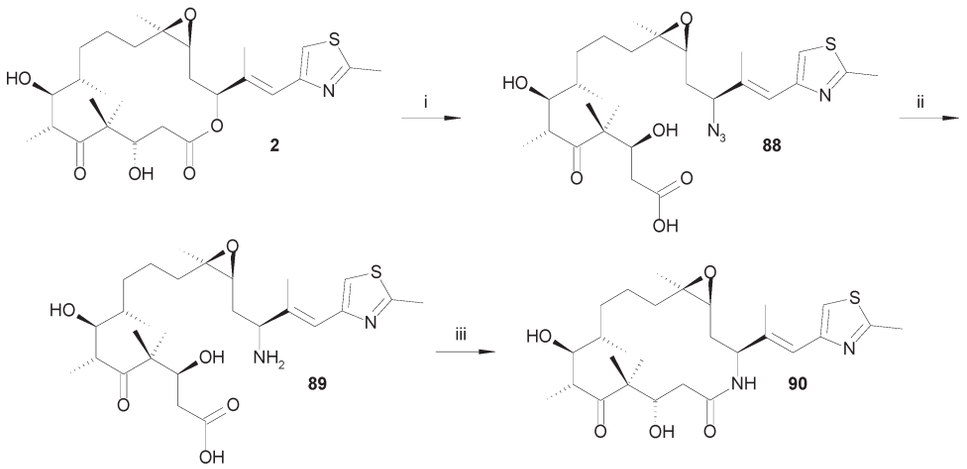
Side-chain modifications

The heterocylce-bearing side-chain of epothilones has been the subject of extensive SAR studies, which have involved modifications of the thiazole moiety at the 2- and 4-position,[41,61,79–81] the replacement of the thiazole ring by other heterocyclic structures[39,41,82] or a simple phenyl group,[39,41,54*b*] and the synthesis of C16-desmethyl epothilone B.[54] Most likely, much of this research (at least in our own group) was initially driven by the idea that modifications in heterocycle structure represent the most probable approach to potent analogs with an altered overall biopharmaceutical profile (*e.g.* solubility, pharmacokinetic properties). These studies, *e.g.*, have shown that the substitution of oxygen for sulfur in the heterocycle (to produce oxazole-derived epothilone analogs) does not affect biological potency;[39,41] likewise, the replacement of the 2-methyl group on the thiazole ring by relatively small substituents such as CH_2OH, CH_2NH_2, CH_2F, SCH_3, or CH_2CH_3 is well tolerated. More bulky substituents result in a substantial loss in potency.[21,61]

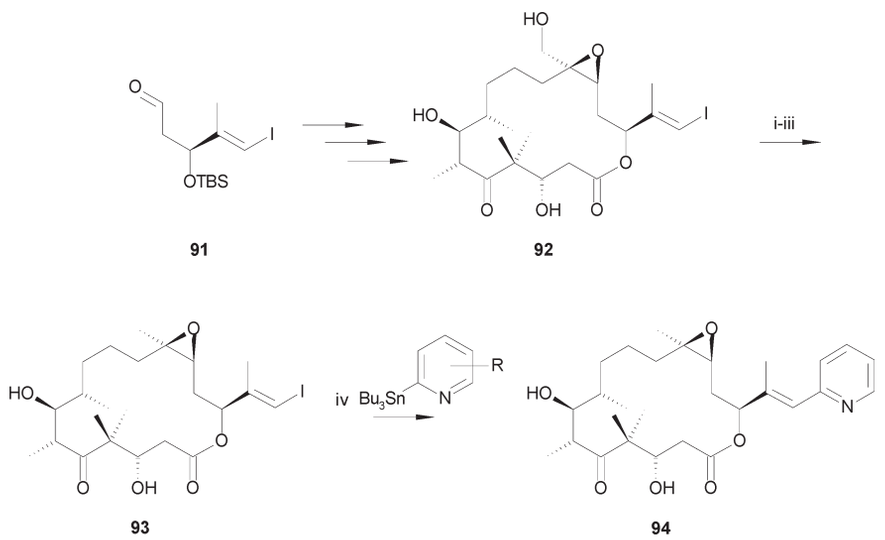
The most significant contributions to the area of heterocycle modifications in epothilones have come from a collaborative effort between the Nicolaou group at the TSRI and the group at Novartis. Thus, Nicolaou and coworkers have developed a highly efficient approach to the synthesis of epothilone analogs with variations in heterocycle structure, which is based on vinyl iodide **93** as a central intermediate[54,82] (Scheme 18).

As exemplified in Scheme 18 for the pyridine-based epothilone B analog **94**, intermediate **93** undergoes cross coupling reactions with a variety of (hetero)aromatic stannanes to form the corresponding epothilone B analogs. Vinyl iodide **93** itself is obtained from aldehyde **91** *via* hydroxy epoxide **92** (Scheme 18), which is prepared through the sequence of reactions depicted in Scheme 9 for the synthesis of **39** from **31**. Quite remarkably, the vinyl iodide moiety in **91** (and subsequent intermediates) is sufficiently stable to be compatible with all sets of reaction conditions encountered on the way to **93**.

Pyridine-based epothilone B analog **94** is almost equipotent with **2** in cancer cell proliferation assays, thus indicating that the presence of a 5-membered heterocycle attached to C17 is not a prerequisite for highly potent biological activity. (The IC_{50}-value for growth inhibition of the human epidermoid cancer cell line KB-31 is 0.30 nM for **94** *vs*. 0.19 nM for **2**).[82] On the other hand, Nicolaou's work has clearly uncovered that epothilone B-like cellular activity in pyridine-based analogs of **2** (*i.e.* sub-nM IC_{50}s for growth inhibition) requires the *N*-atom in the heterocycle to be positioned *ortho* to the attachment point of the linker between the heterocycle and the macrocyclic skeleton. Shifting the *N*-atom to an alternative



Scheme 17 Ref. 73: i: $Pd(Ph_3)_4$, NaN_3, 45 °C, 60–70%. ii: Me_3P, 71%. iii: EDCI, HOBt, 65%.



Scheme 18 Ref. 54*b*: i. Tos-Cl, Et_3N, DMAP, CH_2Cl_2, 0 °C→25 °C,. ii. NaI, acetone, 25 °C, 78% (2 steps). iii. $NaBH_3CN$, DMPU, 45 °C, 70%. iv. $PdCl_2(MeCN)_2$, CuI, $AsPh_3$,DMF, 25 °C, 66%.

position such as in analogs **95** or **96** (Fig. 5) results in a significant (> one order of magnitude) decrease in cellular potency[82]). In fact, **95** and **96** are even less potent than a compound whose side-chain incorporates a plain phenyl group rather than a heterocyclic ring, *i.e.* **97** (Fig. 5).[82] (Note, however, that **97** is still one order of magnitude less active than **2**[82]). On the basis of the available data it is difficult to judge, whether the differences in cellular potency between epothilone B analogs **94–97** is related to differences in ligand–target interactions alone or whether they may also reflect changes in other parameters such as *e.g.* cellular uptake.

One of the most recent additions to the series of side-chain-modified analogs of **2** prepared by the Nicolaou group through

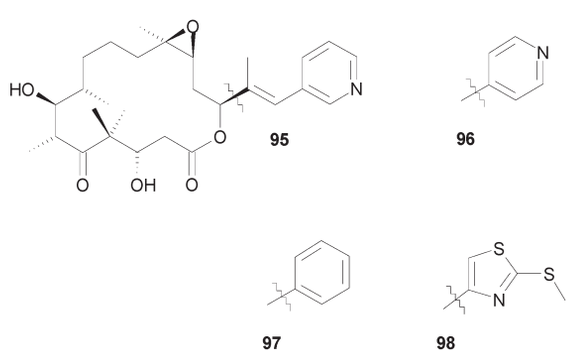


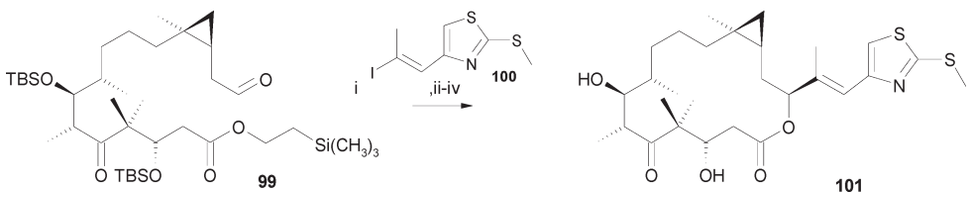
Fig. 5 Side-chain-modified analogs of **2** prepared by Nicolaou *et al*. *via* intermediate **93** (Scheme 18).[82,61]

intermediate **93** is 20-desmethyl-20-methylsulfanyl-epothilone B **98** (Fig. 5).[61] This compound has also been prepared by the Novartis group through semi-synthesis and it has recently entered phase I clinical trials sponsored by Novartis.[83] Compound **98** is a more potent antiproliferative agent than either **2** or paclitaxel, 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 **2** and 4.7 nM for paclitaxel.[83] Like **2**, **98** retains full activity against cancer cells overexpressing the Pgp drug efflux pump and it has demonstrated potent antitumor activity in experimental human tumor models.[83]

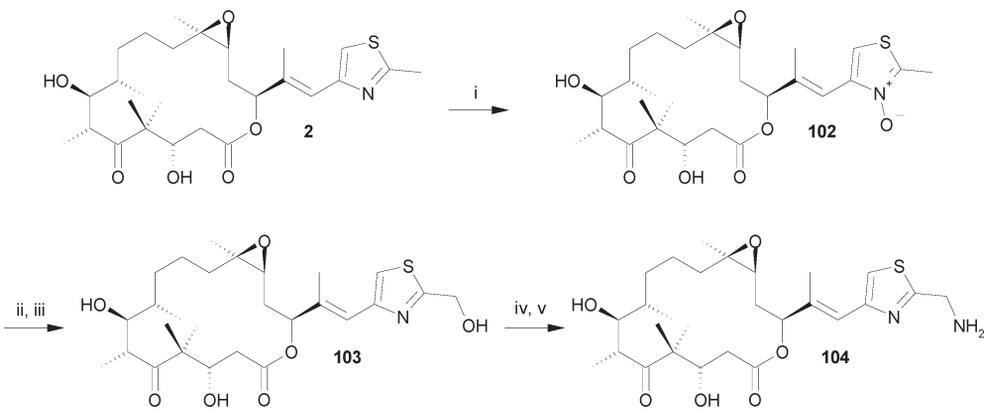
Employing aldehyde **99**, obtained in 18 linear steps from geraniol, and vinyl iodide **100** as key building blocks, Nicolaou *et al*. have also prepared the C12–C13-cyclopropane analog of **98**, *i.e.* compound **101** (Scheme 19).[84] **101** binds to stabilized microtubules with 27.4-fold enhanced affinity over **2** ($\Delta\Delta G^{35\,°C} = -8.2$ kJ mol^{-1})[85] and in some cases has been found to be a more potent antiproliferative agent *in vitro* than either **2** or **98**.[84]

Modifications of the thiazole moiety in epothilones have also been performed through semi synthesis.[79,80] This approach has led to the discovery of C21-amino epothilone B **104** as a potent and more water-soluble analog of **2** (Scheme 20), a compound which is currently undergoing phase I clinical trials sponsored by BMS.[23,86] The synthesis of **104** proceeds *via* epothilone F **103** as a key intermediate (Scheme 20),[23] which can be efficiently accessed from **2** through a process developed by Höfle *et al*.[79] Epothilone F **103** may be also obtained from **2** by biotransformation.[87,88]

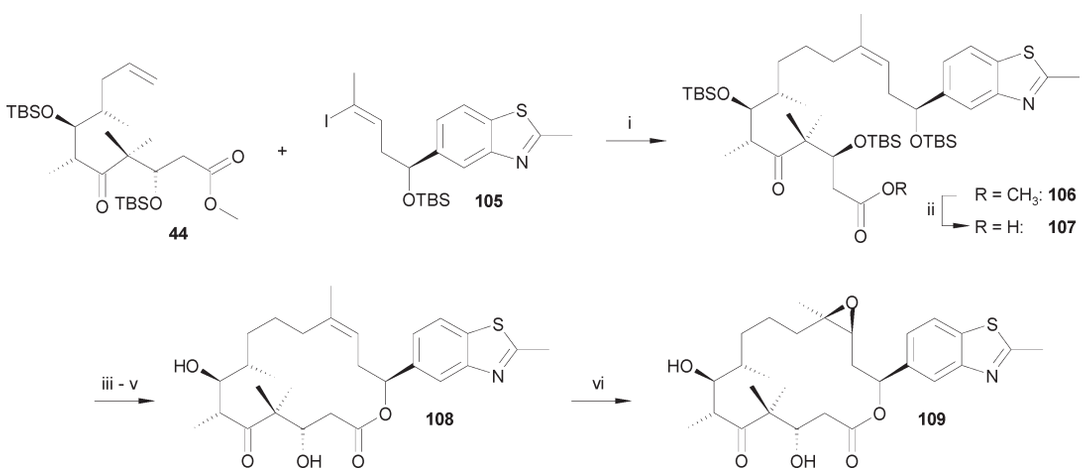
In addition to the simple replacement of the pendant heterocycle, our own group has also investigated modifications, which eliminate rotation about the C17–C18 bond and thus lead to complete rigidization of the epothilone side-chain[89,90] (*cf*. also[91]). The synthesis of



Scheme 19 Ref. 84: $CrCl_2$, $NiCl_2$ (cat.), 4-*tert*.-butyl pyridine, DMSO, 25 °C. ii. TBAF, THF, 25 °C, 42% (2 steps). iii. 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, 0 °C, then 4-DMAP, toluene, 75 °C, 32% (15(*S*)-isomer). iv. CF_3COOH–CH_2Cl_2 (20% v/v), 25 °C, 71%.



Scheme 20 Refs. 23,79: i. MCPBA, CH_2Cl_2, 48%. ii. $(CF_3CO)_2O$, 2,6-lutidine, CH_2Cl_2, 75 °C. iii. aq. NH_3, MeOH, 81% (2 steps). iv. DPPA, THF, 94%. v. $P(CH_3)_3$, THF–H_2O, 91%.



Scheme 21 Ref. 90: i. a. Olefin **44** (1.25 equiv.), 9-BBN, THF, RT, 4 h; b. add to mixture of Cs_2CO_3 (1.5 equiv.), $PdCl_2(dppf)_2$ (0.1 equiv.), Ph_3As (0.2 equiv.), vinyl iodide **105** (1 equiv.), DMF, −10° C→ RT, 16 h, 60%. ii. LiOH, *i*-PrOH–H_2O 4 : 1, 50 °C, 7 h, 88%. iii. TBAF, THF, RT, 86%. iv. a. 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0° C, 15 min; b. dilute with toluene, add to solution of DMAP in toluene, 75 °C, 63%. v. HFxpyridine, THF, 73%. vi. Dimethyldioxirane (DMDO), acetone, −50° C, 46%.

structures of this type is exemplified in Scheme 21 for benzothiazole-based analog **109** and relies on the same strategic principles that had been successfully applied to the synthesis of *trans*-epothilone A **51** (Scheme 11).[90]

Thus, construction of the C11–C12 bond was achieved through *B*-alkyl Suzuki coupling between intermediate **44** and vinyl iodide **105** and the resulting coupling product **106** was elaborated into epothilone D analog **108** in 4 steps including ester saponification, selective removal of the C15-OTBS-protecting group, and Yamaguchi-type macrolactonization and protecting group removal. Epoxidation of **108** with DMDO at −50 °C proceeded with *ca*. 6 : 1 selectivity and gave pure **109** in 46% yield after chromatographic purification. Except for the epoxidation step, which was based on the $MeReO_3$–H_2O_2 system[92] in all other cases, the same strategy was successfully employed in the synthesis of related analogs incorporating quinoline-, benzoxazole-, and benzimidazole-type sidechains.[90] Analogs of this type are generally more potent inhibitors of human cancer cell proliferation than the respective parent compounds **2** or **4**, with the activity increase being more pronounced for the deoxy-type structures (*e.g.* **108**, which is 6-fold more potent than **4** against both the drug-senstive as well as the multidrug-resistant human epidermoid cancer cell lines KB-31 and KB-8511, respectively[90]). Interestingly, the observed increase in antiproliferative activity does not seem to be a consequence of more effective interactions with tubulin/microtubules,[90] although the 180° torsion angle about the C16–C17–C18–N bond, as enforced in **108**/**109** and related analogs, accurately matches the torsion about this bond in the bioactive (tubulin-bound) conformation of **1**.[72] In contrast, a conformational equilibrium between a 0° and the 180° torsion angle about the C16–C17–C18–N bond is observed for **1** free in solution.[72] Given the fact that **4** is currently undergoing phase I/II clinical trials, the improved antiproliferative activity of **108** and related analogs could make these compounds interesting candidates for further development.

Conclusions

The discovery by Bollag *et al*. that epothilones A and B are microtubule stabilizers and thus inhibit human cancer cell growth by a paclitaxel-(Taxol®-)like mechanism of action has triggered a remarkable research effort in natural product chemistry and cancer biology alike. Being only the second family of structures with the ability to inhibit microtubule depolymerization,[93] epothilones offered the first opportunity for the development of a true second gen-

eration of "paclitaxel-like" anticancer agents and thus rapidly turned into important lead structures for anticancer drug discovery. Once the potential of these compounds had been recognized, the power of modern organic chemistry, applied to the problems of natural product total synthesis as well as the specific derivatization of complex structures obtained from biological sources, then enabled the preparation of a wealth of structural analogs for structure–activity studies in a remarkably short period of time. This article has highlighted some of the structural variables that have been investigated as part of this research and the synthetic chemistry that needed to be developed as a prerequisite for such studies. Progress in chemistry was strongly linked to the rapid pharmacological evaluation of new compounds synthesized, with the data generated by pharmacologists and biochemists providing the basis (and the motivation) for the design and synthesis of new generations of structural analogs by organic chemists. One of the limitations still encountered in this process is the current lack of high-resolution structural information for tubulin/microtubules and although not discussed in this Perspective, it should be noted that different pharmacophore models have been developed for epothilones to provide a structural basis for the rational design of new analogs.[94] These models have been recently complemented (or perhaps superseded) by the determination of the tubulin-bound, bioactive conformation of **1** by NMR spectroscopy.[72] Collectively, this information should aid further progress in analog design, including structures which might deviate from the natural product scaffold more significantly than those investigated to date (and therefore could offer the potential for additional pharmacological differentiation).

So far, five clinical development compounds have been reported in the literature as a result of epothilone-based research efforts (*vide supra*). Of these, one compound (epothilone B, **2**) is produced directly by fermentation, three are obtained through semi-synthesis from **2** and in one case (epothilone D, **4**) the material employed in the early clinical trials may have been prepared by total synthesis (although this is not documented in the literature and **2** may also be available by alternative approaches (*vide supra*)). However, given the state of research in the area of epothilone synthesis, fully synthetic epothilone analogs clearly represent technically feasible clinical development candidates. In fact, it is the author's conviction that such compounds will soon appear on the clinical trial landscape or may already be investigated in humans even today.

With all of this said and even in light of the encouraging results that have emerged from ongoing clincial trials, it still remains to be seen whether any of the current development compounds will ever become a clinically useful anticancer drug. However, a number of alternative structures are already available which might warrant clinical testing and ongoing research efforts will no doubt produce additional such alternatives. It is thus the author's belief that epothilone-derived anticancer agents will eventually find their way into clinical practice.

Acknowledgements

The author would like to thank all former collaborators at Novartis for their help and inspiration over the last several years. In particular I want to acknowledge the support by Dr A. Flörsheimer, Dr T. O'Teilly, and Dr M. Wartmann, who worked with me on the epothilone program at Novartis since its very beginning. My sincere thanks also goes to Professor K. C. Nicolaou and his group at the The Scripps Research Institute for a very productive collaboration.

References and notes

1 See, *e.g.*,F. McLaughlin, P. Finn and N. B. La Thangue, *Drug Discovery Today*, 2003, **8**, 793–802.
2 P. B. Schiff, J. Fant and S. B. Horwitz, *Nature*, 1979, **277**, 665–667.
3 M. A. Jordan, *Curr. Med. Chem.: Anti-Cancer Agents*, 2002, **2**, 1–14.
4 (*a*) G. Höfle, N. Bedorf, K. Gerth, and H. Reichenbach, German Patent Disclosure, DE 4138042, 1993; *Chem. Abstr.*, 1993, **120**, p. 52841; (*b*) K. Gerth, N. Bedorf, G. Höfle, H. Irschik and H. Reichenbach, *J. Antibiot.*, 1996, **49**, 560–564.
5 D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides and C. M. Woods, *Cancer Res.*, 1995, **55**, 2325–2333.
6 K.-H. Altmann, G. Bold, G. Caravatti, N. End, A. Flörsheimer, V. Guagnano, T. O'Reilly and M. Wartmann, *Chimia*, 2000, **54**, 612–621.
7 R. J. Kowalski, P. Giannakakou and E. Hamel, *J. Biol. Chem.*, 1997, **272**, 2534–2541.
8 K.-H. Altmann, M. Wartmann and T. O'Reilly, *Biochim. Biophys. Acta*, 2000, **1470**, M79–M91.
9 A. Wolff, A. Technau and G. Brandner, *Int. J. Oncol.*, 1997, **11**, 123–126.
10 P. Giannakakou, D. L. Sackett, Y. K. Kang, Z. Zhan, J. T. Buters, T. Fojo and M. S. Poruchynsky, *J. Biol. Chem.*, 1997, **272**, 17118–17125.
11 G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth and H. Reichenbach, *Angew. Chem., Int. Ed.*, 1996, **35**, 1567–1569.
12 K. C. Nicolaou, F. Roschangar and D. Vourloumis, *Angew. Chem., Int. Ed.*, 1998, **37**, 2014–2045.
13 C. R. Harris and S. J. Danishefsky, *J. Org. Chem.*, 1999, **64**, 8434–8456.
14 J. Mulzer, H. J. Martin and M. Berger, *J. Heterocycl. Chem.*, 1999, **36**, 1421–1436.
15 K. C. Nicolaou, A. Ritzén and K. Namoto, *Chem. Commun.*, 2001, 1523–1535.
16 For more recent syntheses of epothilones not included in refs. 12–15 *cf.*, *e.g.*: (*a*) R. I. Storer, T. Takemoto, P. S. Jackson and S. V. Ley, *Angew. Chem., Int. Ed.*, 2003, **42**, 2521–2525; (*b*) Z.-Y. Liu, Z.-C Chen, C.-Z. Yu, R.-F. Wang, R. Z. Zhang, C.-S. Huang, Z. Yan, D.-R. Cao, J.-B. Sun and G. Li, *Chem. Eur. J.*, 2002, **8**, 3747–3756; (*c*) J. Liu and C.-H. Wong, *Angew. Chem., Int. Ed.*, 2002, **41**, 1404–1407; (*d*) J. Sun and S. C. Sinha, *Angew. Chem., Int. Ed.*, 2002, **41**, 1381–1383; (*e*) M. S. Ermolenko and P. Potier, *Tetrahedron Lett.*, 2002, **43**, 2895–2898; (*f*) N. Martin and E. J. Thomas, *Tetrahedron Lett.*, 2001, **42**, 8373–8377; (*g*) H. J. Martin, P. Pojarliev, H. Kahlig and J. Mulzer, *Chem. Eur. J.*, 2001, **7**, 2261–2271; (*h*) M. Valluri, R. M. Hindupur, P. Bijoy, G. Labadie, J.-C. Jung and M. A. Avery, *Org. Lett.*, 2001, **3**, 3607–3609; (*i*) R. M. Hindupur, B. Panicker, M. Valluri and M. A. Avery, *Tetrahedron Lett.*, 2001, **42**, 7341–7344; (*j*) B. Zhu and J. S. Panek, *Eur J. Org. Chem.*, 2001, **9**, 1701–1714.
17 Strictly speaking, only the absolute stereochemistry of epothilone B had been determined with certainty at this point.[11].
18 (*a*) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg and Z. Zang, *Angew. Chem., Int. Ed.*, 1996, **35**, 2399–2401; (*b*) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg and Z. Zang, *Angew. Chem., Int. Ed.*, 1997, **36**, 166–168.
19 A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D. S. Su, E. J. Sorensen and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 1996, **35**, 2801–2803.
20 D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm and M. Cordes, *Angew. Chem., Int. Ed.*, 1997, **36**, 523–524.
21 M. Wartmann and K.-H. Altmann, *Curr. Med. Chem.: Anti-Cancer Agents.*, 2002, **2**, 123–148.
22 K.-H. Altmann, *Mini Rev. Med. Chem.*, 2003, **3**, 149–158.
23 R. M. Borzilleri and G. D. Vite, *Drugs of the Future*, 2003, **27**, 1149–1163.
24 M. D. Chappell, S. J. Stachel, C. B. Lee and S. J. Danishefsky, *Org. Lett.*, 2002, **2**, 1633–1636.
25 The most precise structural information currently available for tubulin at the protein level stems from a 3.7 A electron crystallography structure of a two-dimensional tubulin polymer sheet with bound docetaxel: E. Nogales, S. G. Wolf and K. H. Downing, *Nature*, 1998, **391**, 199–203.
26 I. Molnar, T. Schupp, M. Ono, R. Zirkle, M. Milnamow, B. Nowak-Thompson, N. Engel, C. Toupet, A. Stratmann, D. D. Cyr, J. Gorlach, J. M. Mayo, A. Hu, S. Goff, J. Schmid and J. M. Ligon, *Chem. Biol.*, 2000, **7**, 97–109.
27 B. Julien, S. Shah, R. Ziermann, R. Goldman, L. Katz and C. Khosla, *Gene*, 2000, **249**, 153–160.
28 (*a*) K. Gerth, H. Steinmetz, G. Höfle and H. Reichenbach, *J. Antibiot.*, 2001, **54**, 144–148; (*b*) K. Gerth, H. Steinmetz, G. Höfle and H. Reichenbach, *J. Antibiot.*, 2001, **53**, 1373–1377.
29 L. Tang, L. S. Shah, L. Chung, J. Carney, L. Katz, C. Khosla and B. Julien, *Science*, 2000, **287**, 640–642.
30 R. L. Arslanian, C. D. Parker, P. K. Wang, J. R. McIntire, J. Lau, C. Starks and P. J. J. Licari, *J. Nat. Prod.*, 2002, **65**, 570–572.
31 H. J. Martin, M. Drechser and J. Mulzer, *Angew. Chem., Int. Ed.*, 2000, **39**, 581–583.
32 A. Fürstner, C. Mathes and C. W. Lehmann, *Chem. Eur. J.*, 2001, **7**, 5299–5317.
33 D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1997, **119**, 10073–10092.
34 (*a*) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, F. Roschangar, F. Sarabia, S. Ninkovic, Z. Yang and J. I. Trujillo, *J. Am. Chem. Soc.*, 1997, **119**, 7960–7973; (*b*) K. C. Nicolaou, S. Ninkovic, F. Sarabia,

D. Vourloumis, Y. He, H. Vallberg, M. R. V. Finlay and Z. Yang, *J. Am. Chem. Soc.*, 1997, **119**, 7960–7973.
35 D. Schinzer, A. Bauer, O. M. Böhm, A. Limberg and M. Cordes, *Chem. Eur. J.*, 1999, **5**, 2483–2491.
36 C. R. Harris, S. D. Kuduk, A. Balog, K. Savin, P. Glunz and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1999, **121**, 7050–7062.
37 D. Meng, D. S. Su, A. Balog, P. Bertinato, E. J. Sorensen, S. J. Danishefsky, Y. H. Zheng, T. C. Chou, L. He and S. B. Horwitz, *J. Am. Chem. Soc.*, 1997, **119**, 2733–2734.
38 D. S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y. H. Zheng, T. C. Chou, L. He and S. B. Horwitz, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 757–759.
39 D. S. Su, A. Balog, D. Meng, P. Bertinato, S. J. Danishefsky, Y. H. Zheng, T. C. Chou, L. He and S. B. Horwitz, *Angew. Chem., Int. Ed.*, 1997, **36**, 2093–2096.
40 K. C. Nicolaou, N. Winssinger, J. Pastor, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou and E. Hamel, *Nature*, 1997, **387**, 268–272.
41 K. C. Nicolaou, D. Vourloumis, T. Li, J. Pastor, N. Winssinger, Y. He, S. Ninkovic, F. Sarabia, H. Vallberg, F. Roschangar, N. P. King, M. R. Finlay, P. Giannakakou, P. Verdier-Pinard and E. Hamel, *Angew. Chem., Int. Ed.*, 1997, **36**, 2097–2103.
42 I. Hardt, H. Steinmetz, K. Gerth, F. Sasse, H. Reichenbach and G. Höfle, *J. Nat. Prod.*, 2000, **64**, 847–856.
43 T. C. Chou, X. G. Zhang, A. Balog, D. S. Su, D. Meng, K. Savin, J. R. Bertino and S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 9642–9647.
44 T. C. Chou, X. G. Zhang, C. R. Harris, S. D. Kuduk, A. Balog, K. A. Savin, J. R. Bertino and S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 15798–15802.
45 J. Rothermel, M. Wartmann, T. Chen and T. Hohneker, *Semin. Oncol.*, 2003, **30**(Suppl 6), 51–55.
46 D. Spriggs, J. Dupont, S. Pezzulli, J. Larkin, J. Cropp, R. Johnson and A. L. Hannah, AACRNCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics, Nov 17–21, 2003, Boston, MA, USA. Abstract-A248. Published as a Supplement to *Clin. Cancer Res.*, vol. 9, issue 16.
47 K. Holen, A. Hannah, Y. Zhou, G. Cropp, R. Johnson, J. Volkman, K. Binger, D. Alberti and G. Wilding, AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics, Nov 17–21, 2003, Boston, MA, USA. Abstract-A261. Published as a Supplement to *Clin. Cancer Res.*, vol. 9, issue 16.
48 A. Balog, C. R. Harris, K. Savin, X.-G. Zhang, T.-C. Chou and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 1998, **37**, 2675–2678.
49 C. B. Lee, T.-C. Chou, X.-G. Zhang, Z.-G. Wang, S. D. Kuduk, M. D. Chappell, S. J. Stachel and S. J. Danishefsky, *J. Org. Chem.*, 2000, **65**, 6525–6533.
50 C. B. Lee, Z. Wu, F. Zhang, M. D. Chappell, S. J. Stachel, T.-C. Chou, Y. Guan and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2001, **123**, 5249–5259.
51 R. O. Duthaler, P. Herold, W. Lottenbach, K. Oertle and M. Riediker, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 495–496.
52 J. Johnson, S. H. Kim, M. Bifano, J. DiMarco, C. Fairchild, J. Gougoutas, F. Lee, B. Long, J. Tokarski and G. D. Vite, *Org. Lett.*, 2000, **2**, 1537–1540.
53 K. C. Nicolaou, M. R. V. Finlay, S. Ninkovic and F. Sarabia, *Tetrahedron*, 1998, **54**, 7127–7166.
54 (*a*) K. C. Nicolaou, D. Hepworth, M. R. Finlay, N. P. King, B. Werschkun and A. Bigot, *Chem. Commun.*, 1999, 519–520; (*b*) K. C. Nicolaou, D. Hepworth, N. P. King, M. R. Finlay, R. Scarpelli, M. M. Pereira, B. Bollbuck, A. Bigot and B. Werschkun, *Chem. Eur. J.*, 2000, **6**, 2783–2800.
55 K. C. Nicolaou, S. Ninkovic, M. R. Finlay, F. Sarabia and T. Li, *Chem. Commun.*, 1997, 2343–2344.
56 R. A. Newman, J. Yang, M. R. V. Finlay, F. Cabral, D. Vourloumis, L. C. Stevens, L. P. Troncoso, X. Wu, C. J. Logothetis, K. C. Nicolaou and M. N. Navone, *Cancer Chemother. Pharmacol.*, 2001, **48**, 319–326.
57 K. H. Altmann, K. C. Nicolaou, M. Wartmann and T. O'Reilly, *Proc. Am. Assoc. Cancer Res.*, 2001, **42**, Abstract-1979.
58 K. H. Altmann, G. Bold, G. Caravatti, D. Denni, A. Flörsheimer, A. Schmidt, G. Rihs and M. Wartmann, *Helv. Chim. Acta*, 2002, **85**, 4086–4110.
59 (*a*) Y. Tu, Z.-X. Wang and Y. Shi, *J. Am. Chem. Soc.*, 1996, **118**, 9806–9807; (*b*) Z.-X. Wang, Y. Tu, M. Frohn, J.-R. Zhang and Y. Shi, *J. Am. Chem. Soc.*, 1996, **118**, 9806–9807.
60 K. C. Nicolaou, K. Namoto, A. Ritzén, T. Ulven, M. Shoji, J. Li, G. D'Amico, D. Liotta, C. T. French, M. Wartmann, K.-H. Altmann and P. Giannakakou, *J. Am. Chem. Soc.*, 2001, **123**, 9313–9323.
61 K. C. Nicolaou, A. Ritzén, K. Namoto, M. B. Ruben, F. Diaz, J. M. Andreu, M. Wartmann, K.-H. Altmann, A. O'Brate and P. Giannakakou, *Tetrahedron*, 2002, **58**, 6413–6432.
62 K. C. Nicolaou, F. Sarabia, S. Ninkovic, M. R. Finlay and C. N. C. Boddy, *Angew. Chem., Int. Ed.*, 1998, **37**, 81–84.
63 R. L. Arslanian, L. Tang, S. Blough, W. Ma, R.-G. Qiu, L. Katz and J. R. Carney, *J. Nat. Prod.*, 2002, **65**, 1061–1064.
64 K. Biswas, H. Lin, J. T. Njardson, M. D. Chappell, T.-C. Chou, Y. Guan, W. P. Tong, L. He, S. B. Horwitz and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2002, **124**, 9825–9832.
65 A. Rivkin, J. T. Njardson, K. Biswas, T.-C. Chou and S. J. Danishefsky, *J. Org. Chem.*, 2002, **67**, 7737–7740.
66 A. Rivkin, K. Biswas, T.-C. Chou and S. J. Danishefsky, *Org. Lett.*, 2002, **4**, 4081–4084.
67 A. Rivkin, F. Yoshimura, A. E. Gabarda, T.-C. Chou, H. Dong, W. P. Tong and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2003, **125**, 2899–2901.
68 F. Yoshimura, A. Rivkin, A. E. Gabarda, T.-C. Chou, H. Dong, H. Sukenick, F. F. Morel, R. E. Taylor and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2003, **42**, 2518–2512.
69 T.-C. Chou, H. Dong, A. Rivkin, F. Yoshimura, A. E. Gabarda, Y. S. Cho, W. P. Tong and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2003, **42**, 4762–4767.
70 L. Tang, R.-G. Li, L. Yong and L. Katz, *J. Antibiot.*, 2003, **56**, 16–23.
71 (*a*) J. D. White, R. G. Carter, K. F. Sundermann and M. Wartmann, *J. Am. Chem. Soc.*, 2001, **123**, 5407–5413; (*b*) J. D. White, R. G. Carter, K. F. Sundermann and M. Wartmann, *J. Am. Chem. Soc.*, 2003, **125**, 3190.
72 T. Carlomagno, M. J. J. Blommers, J. Meiler, W. Jahnke, T. Schupp, F. Petersen, D. Schinzer, K.-H. Altmann and C. Griesinger, *Angew. Chem., Int. Ed.*, 2003, **42**, 2511–2515.
73 R. M. Borzilleri, X. Zheng, R. J. Schmidt, J. A. Johnson, S. H. Kim, J. D. DiMarco, C. R. Fairchild, J. Z. Gougoutas, F. Y. F. Lee, B. H. Long and G. D. Vite, *J. Am. Chem. Soc.*, 2000, **122**, 8890–8897.
74 F. Y. Lee, R. M. Borzilleri, C. R. Fairchild, S. H. Kim, B. H. Long, C. Reventos-Suarez, G. D. Vite, W. C. Rose and R. A. Kramer, *Clin. Cancer Res.*, 2001, **7**, 1429–1437.
75 T. C. Chou, O. A. O'Connor, W. P. Tong, Y. Guan, Z. G. Zhang, S. J. Stachel, C. Lee and S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 8113–8118.
76 S. J. Stachel, C. B. Lee, M. Spassova, M. D. Chappell, W. G. Bornmann, S. J. Danishefsky, T.-C. Chou and Y. Guan, *J. Org. Chem.*, 2001, **66**, 4369–4378.
77 D. Schinzer, K.-H. Altmann, F. Stuhlmann, A. Bauer and M. Wartmann, *ChemBioChem*, 2000, **1**, 67–70.
78 N. Lin, K. Brakora and M. Seiden, *Curr. Opin. Invest. Drugs*, 2003, **4**, 746–756.
79 G. Höfle, N. Glaser, M. Kiffe, H.-J. Hecht, F. Sasse and H. Reichenbach, *Angew. Chem., Int. Ed.*, 1999, **38**, 1971–1974.
80 M. Sefkow and G. Höfle, *Heterocycles*, 1998, **48**, 2485–2488.
81 K. C. Nicolaou, N. P. King, M. R. V. Finlay, Y. He, F. Roschangar, D. Vourloumis, H. Vallberg, F. Sarabia, S. Ninkovich and D. Hepworth, *Bioorg. Med. Chem.*, 1999, **7**, 665–697.
82 K. C. Nicolaou, R. Scarpelli, B. Bollbuck, B. Werschkun, M. M. Pereira, M. Wartmann, K.- H. Altmann, D. Zaharevitz, R. Gussio and P. Giannakakou, *Chem. Biol.*, 2000, **7**, 593–599.
83 M. Wartmann, J. Loretan, R. Reuter, M. Hattenberger, M. Muller, J. Vaxelaire, S.-M. Maira, A. Flörsheimer, T. O'Reilly, K. C. Nicolaou and K.-H. Altmann, *Proc. Am. Assoc. Cancer Res.*, 2004, **45**, Abstract-5440.
84 K. C. Nicolaou, P. K. Sasmal, G. Rassias, M. V. Reddy, K.-H. Altmann and M. Wartmann, *Angew. Chem., Int. Ed.*, 2003, **42**, 3515–3520.
85 R. M. Buey, J. F. Diaz, J. M. Andreu, A. O'Brate, P. Giannakakou, K. C. Nicolaou, P. K. Sasmal, A. Ritzén and K. Namoto, *Chem. Biol.*, 2004, **11**, 225–236.
86 G. Vite, G. Höfle, M. Bifano, C. Fairchild, N. Glaser, K. Johnston, A. Kamath, S.-H. Kim, K. Leavitt, F. F.-Y. Lee, T. Leibold, B. Long, R. Peterson, K. Raghavan, A. Reguerio-Ren, Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7–11, 2002, MEDI-018.
87 G. Höfle, H. Reichenbach, K. Gerth, I. Hardt, F. Sasse and H. Steinmetz, PCT Int. Appl., 1999, WO 9965913 A2.
88 W. Li, J. A. Matson, X. Huang, K. S. Lam and G. A. Mcclure, PCT Int. Appl., 2000, WO 2000039276 A2.
89 N. End, G. Bold, G. Caravatti, M. Wartmann and K.-H. Altmann, *Proceedings of ECSOC-4: The 4th International Electronic Conference on Synthetic Organic Chemistry"* 2000, 14311442. Accessible at: http://www.mdpi.org/ecsoc-4.htm.
90 K.-H. Altmann, G. Bold, G. Caravatti, A. Flörsheimer, V. Guagnano and M. Wartmann, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2765–2768.
91 U. Klar, W. Skuballa, B. Buchmann, W. Schwede, T. Bunte, J. Hoffmann and R. Lichtner, in: Anticancer Agents, Frontiers in Cancer Chemotherapy, ACS Symposium Series 796, eds I. Ojima, G.D. Vite and K.-H. Altmann, American Chemical Society, Washington DC, 2001, pp. 131–147.

92 J. Rudolph, K. L. Reddy, J. P. Chang and K. B. Sharpless, *J. Am. Chem. Soc.*, 1997, **119**, 6189–6190.

93 Subsequent to the discovery of the microtubule-stabilizing properties of epothilones, a number of other natural products were recognized to be microtubule-stabilizers. These include, *e.g.*: (*a*) Discodermolide: E. ter Haar, R. J. Kowalski, E. Hamel, C. M. Lin, R. E. Longley, S. P. Gunasekara, H. S. Rosenkranz and B. W. Day, *Biochemistry*, 1996, **35**, 243–250; (*b*) Eleutherobin: B. H. Long, J. M. Carboni, A. J. Wasserman, L. A. Cornell, A. M. Cassaza, P. R. Jensen, T. Lindel, W. Fenical and C. R. Fairchild, *Cancer Res.*, 1998, **58**, 1111–1115; (*c*) Laulimalide: S. L. Mooberry, G. Tien, A. H. Hernandez, A. Plubrurkan and B. S. Davidson, *Cancer Res.*, 1999, **59**, 653–660; (*d*) Peloruside: K. A. Hood, L. M. West, B. Rouwe, P. T. Northcote, M. V. Berridge, St. J. Wakefield and J. H. Miller, *Cancer Res.*, 2002, **62**, 3356–3360; (*e*) Dictyostatin: A. Isbrucker, J. Cummins, S. Pomponi, R. E. Longley and A. E. Wright, *Biochem. Pharmacol.*, 2003, **66**, 75–82.

94 (*a*) I. Ojima, S. Chakravarty, T. Inoue, S. Lin, L. He, S. B. Horwitz, S. D. Kuduk and S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 4256–4261; (*b*) M. Wang, X. Xia, Y. Kim, D. Hwang, J. M. Jansen, M. Botta, D. C. Liotta and J. P. Snyder, *Org. Lett.*, 1999, **1**, 43–46; (*c*) P. Giannakakou, R. Gussio, E. Nogales, K. H. Downing, D. Zaharevitz, B. Bollbuck, G. Poy, D. Sackett, K. C. Nicolaou and T. Fojo, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2904–2909; (*d*) F. Manetti, S. Forli, L. Maccari, F. Corelli and M. Botta, *Il Farmaco*, 2003, **58**, 357–361; (*e*) F. Manetti, L. Maccari, F. Corelli and M. Botta, *Curr. Topics Med. Chem.*, 2004, **4**, 203–217.