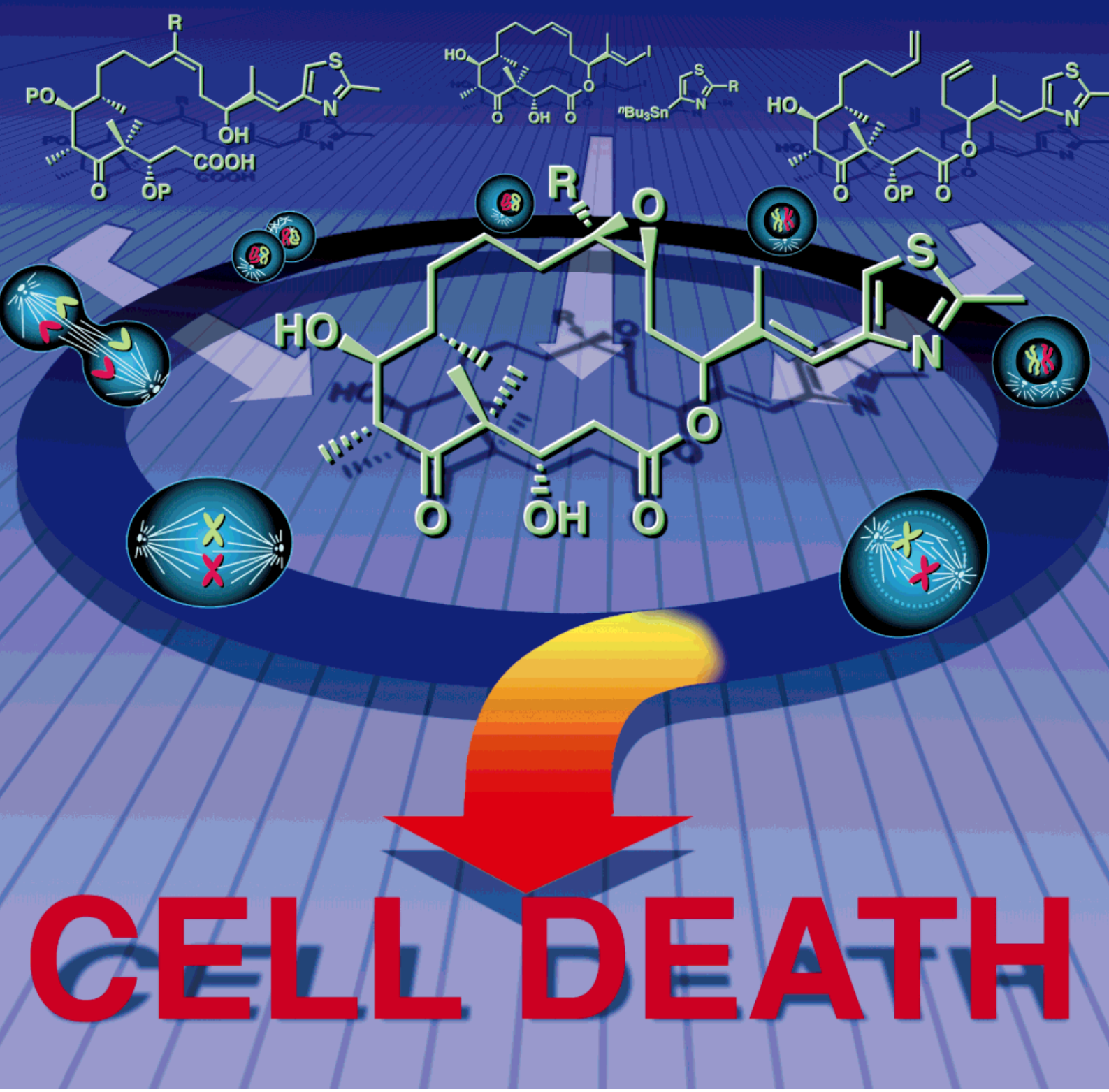
EPOTHILONES



CELL DEATH

Chemical Biology of Epothilones**

K. C. Nicolaou*, Frank Roschangar, and Dionisios Vourloumis

Dedicated to Professor E. J. Corey on the occasion of his 70th birthday

In July of 1996 a paper appeared in *Angewandte Chemie* revealing the structures of a remarkable new class of antitumor agents represented by epothilones A and B. This publication became a milestone in the epothilone story, which began in the late 1980s with their isolation from a species of myxobacteria and gathered momentum in the early 1990s when their taxol-like mechanism of action against tumor cells was discovered. The realization of their unique potential as anticancer agents marked an intense race for their total synthesis, structural modification, and biological investigation. A number of epothilones have now been recognized as superior to taxol in terms of potency and effectiveness against drug-resistant tumor cells, including taxol-resistant cell lines. In this article we describe the fascinating saga of these substances and discuss their total synthesis, chemical biology, and potential in cancer chemotherapy.

Keywords: antitumor agents · epothilones · natural products · structure – activity relationships · total synthesis

1. Introduction

Earlier this century, the word "cancer" was not even mentioned in "polite" company. We have made great strides since then, and today we do not only talk about cancer openly, but can actually find answers to questions about many aspects of cancer prevention, detection, treatment, and recovery. Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells that invade and disrupt other tissues and spread to other areas of the body. If the spread is not controlled, it can result in death. Both external factors (for example, chemicals, radiation, and viruses) and internal factors (for example, hormones, immune conditions, and inherited genes) can be responsible for the development of cancer. Causal factors may act together, or in sequence, to initiate or promote carcinogenesis. Ten or more years often pass between exposures or mutations and detectable cancer.[1]

Cancer is a growing public health problem whose estimated worldwide new incidences are over six million cases per year.[2] In the USA it is estimated that one in two men and one in three women will develop cancer during their lifetime. According to American Cancer Society (ACS) statistics, around 1382400 new cancer cases are expected to be diagnosed in 1997 in the USA alone, and around 560000 Americans are expected to die of cancer—that is more than 1500 people a day, averaging approximately one death per minute. This rate makes it the second leading cause of death in the USA, exceeded only by cardiovascular disease that accounts for one of every four deaths. The financial costs of cancer are immense, both to the individual and to society as a whole. The National Cancer Institute (NCI) estimates overall costs for cancer at $104 billion in 1997: $35 billion for direct medical costs, $12 billion for morbidity costs (cost of lost productivity), and $57 billion for mortality costs. Treatment of breast, lung, and prostate cancers account for over half of the direct medical costs.[2]

Breast cancer is the second most common cancer, after skin cancers, among women. The ACS estimates that in 1997 approximately 180200 new cases of invasive breast cancer will be diagnosed in women, and an estimated 1400 cases will be diagnosed in men in the USA.[2] Breast cancer related deaths will top 44190 (43900 women, 290 men) in 1997, making this type of cancer the second major cause of cancer death in women after lung cancer. Ovarian cancer, termed "the silent disease" due to the lack of obvious signs or symptoms until late in its development, accounts for 4% of all cancers in women, and is predicted to cause about 14200 deaths in the USA in 1997. It ranks fifth as a cause of cancer deaths among women, causing more deaths than any other cancer of the female reproductive system. Prostate cancer, aside from skin

[*] Prof. Dr. K. C. Nicolaou, Dr. F. Roschangar, Dr. D. Vourloumis
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 619-784-2469
E-mail: kcn@scripps.edu
and
Department of Chemistry and Biochemistry
University of California, San Diego
9500 Gilman Drive, La Jolla, California 92093 (USA)

[**] Abbreviations are given in the appendix.

  1433-7851/98/3715-2015 $ 17.50+.50/0

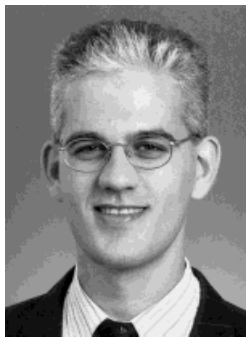
cancer, is the most common cancer in American males, and is developed in approximately one out of every five American men during their lifetime. The ACS estimates that in 1997, around 209 900 new cases of prostate cancer will be diagnosed in the United States and 41 800 men will die of this disease, thus making it the second leading cause of cancer death in men, exceeded only by lung cancer, and accounting for 14 % of male cancer-related deaths. Prostate cancer is most common in North America and northwestern Europe, although it is more rare in Asia, Africa, Central America, and South America.[2]

A major share of the anticancer drug market is commanded by the complex diterpene taxol (paclitaxel), whose discovery from the Pacific Yew Tree in 1971[3] and culmination into a billion dollar drug today represents a remarkable story. Developed and sold by Bristol-Myers Squibb in the 1990s, taxol is currently available in more than 60 countries. It is mainly used for the treatment of a variety of solid tumors commonly encountered with ovarian and breast cancers.[4] Taxotere (docetaxel), developed by Rhone-Poulenc Rorer (France), has more recently been approved for the treatment of similar indications.[5] As seen in Table 1, major drugs used today in the treatment of cancer patients include, in addition to taxol, the hormones Lupron (leuprolide acetate) and Zoladex (goserelin), the nonsteroidal anti-estrogen Nolvadex (tamoxifen), and the cytotoxic agents Paraplatin (carboplatin), as well as the biological-response modifiers Neupogen (filgrastim) and Intron A (interferon alpha-2 b).[6]

The success story of taxol[7] demonstrated once again the wealth of mother nature in terms of biologically active molecules as cures for disease.[8] Aspirin[9] and penicillin[10] are two additional classic examples of such discoveries. These stories will certainly not be the last. In the late 1980s, a new tale of cytotoxic natural products began to unfold. The epothilones A and B (Figure 1) were discovered by Höfle, Reichenbach, and their coworkers at the Gesellschaft für Biotechnologische Forschung (GBF) in Germany.[11] These compounds were isolated from culture extracts of the cellulose-degrading myxobacterium *Sorangium cellulosum* (Myxococcales) strain So ce90, first found in soil collected from the banks of the Zambesi River in South Africa, and were initially found to exhibit a narrow antifungal spectrum against the fungus *Mucor hiemalis* only.[12] Figure 2 shows growing cells of *Sorangium cellulosum* So ce90 (left) and spore capsules (sporangioles, diameter approximately 15-20 μm) of the same organism (right). The latter are formed within the swarm colonies of many yellowish-orange to brown-black fruiting bodies.[13] In this state, the spores survive in dry soil for more than 10 – 20 years. Due to their activity against *Mucor hiemalis*, the epothilones and spirangienes (compounds isolated from the same organism) were first tested as potential antifungal and pesticide agents,[11, 14] but field experiments proved the epothilones to be too toxic (see Section 2). In the meantime, scientists at Merck in the USA had independently isolated epothilones A and B and made the remarkable discovery that these substances kill tumor cells through a mechanism of action similar to that of taxol, namely through induction of tubulin polymerization to microtubules and microtubule stabilization.[15] This observation was later confirmed by the GBF scientists.[12] Furthermore, the Merck scientists found in displacement experiments that epothilones A and B were competitive inhibitors of [^{3}H]taxol binding, with almost identical IC_{50} values to that of taxol, and that these new compounds retained a much greater toxicity against Pgp-expressing multiple drug resistant (MDR) cells (PgP = P-glycoprotein).[15]

K. C. Nicolaou is the Chairman of the Department of Chemistry at The Scripps Research Institute, La Jolla, California where he holds the Darlene Shiley Chair in Chemistry and the Skaggs Professorship of Chemical Biology. He is also Professor of Chemistry at the University of California, San Diego.


K. C. Nicolaou

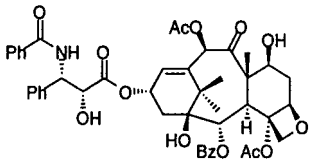
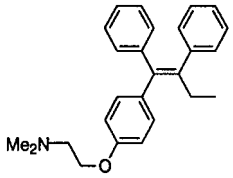
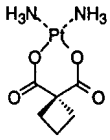

F. Roschangar


D. Vourloumis

Frank Roschangar, born in 1969 in Hannover, Germany, received his B. Sc. degree from the University of Cologne in 1991 and his Ph. D. degree from Rice University, Texas, in 1996 under the supervision of Prof. M. A. Ciufolini where he accomplished an enantiomericly pure total synthesis of camptothecin. He joined the Nicolaou group in 1996 and has been involved in the synthesis of various epothilones. Currently, he holds a position as a research scientist at Glaxo-Wellcome, North Carolina.

Dionisios Vourloumis, born in 1966 in Greece, received his B. Sc. degree from the University of Athens and his Ph. D. from West Virginia University under the direction of Professor P. A. Magriotis, in 1994, working on the synthesis of novel enediyne antibiotics. Since joining Professor Nicolaou's group in 1996, he has been involved in the total syntheses of epothilones A and B, eleutherobin, sarcodictyins A and B, and analogues thereoff.

Table 1. The biggest-selling agents for cancer and cancer-related treatments in 1996.[6]

Active substance group	Trade name (chemical name)	Structure/description	Marketer (worldwide sales [US-Dollar])	Indication
Antineoplastics				
Taxoid	Taxol (Paclitaxel)		Bristol-Meyers Squibb (813×10^6)	treatment of primary ovarian cancer in combination with cisplatin,and for metastatic ovarian cancer where standard platinum- or anthracycline-containing therapy has failed
Hormone	Lupron (Leuprolide acetate)	an agonist of the naturally occurring decapeptide gonadorelin	TAP Pharmaceuticals (810×10^6)	treatment of advanced prostate cancer as an alternative to castration, of endometriosis and central precocious puberty, and for the presurgical management of patients with anaemia caused by benign febroid tumours
	Zoladex (Goserelin)	a synthetic analogue of gonadorelin	Zeneca Pharmaceuticals (563×10^6)	treatment of prostate carcinoma advanced breast cancer, endometriosis, and endometrial thinning
Anti-Oestrogens	Nolvadex (Tamoxifen)		Zeneca Pharmaceuticals (561×10^6)	treatment of hormonally responsive breast cancer; can consistently extend survival rates for up to 5 years[b]
Cytotoxic agent	Paraplatin (carboplatin)		Bristol-Myers Squibb (373×10^6)	treatment of ovarian cancer
Biological Response Modifiers				
	Neupogen (Filgrastim)	a 175 amino acid protein manufactured by recombinant DNA technology and produced by Escherichia coli bacteria	Amgen (1.02×10^9)	regulation and control of bacteria-fighting white blood cells called neutrophils; prevents drop in white blood cells during chemotherapy and radiotherapy
	Intron A (Interferon alpha-2b)	an interferon, which belongs to a group of naturally occurring proteins that were first discovered as a result of their ability to prevent viral replication	Schering-Plough Corporation (524×10^6)	treatment of chronic hepatitis C, basal cell carcinoma, AIDS-related Kaposi's sarcoma, carcinoids, genital warts, and multiple myeloma; adjuvant treatment for malignant melanoma

[a]Also called the luteinizing hormone-releasing hormone. [b] The antiestrogenic effects may be related to its ability to compete with estrogen for binding sites in target tissues such as the breast.

These observations gave rise to a great deal of excitement, anticipating the possible development of these compounds as anticancer agents, particularly in view of their effectiveness against a number of taxol-resistant tumor cell lines. A series of miscalculations, however, prompted the major players in the epothilone business from pursuing patent applications for cancer indications, and the natural substances found themselves as "orphan compounds" as far as patent protection and companies willing to develop them was concerned. This was not to be the end of the story, however, as we will see below.

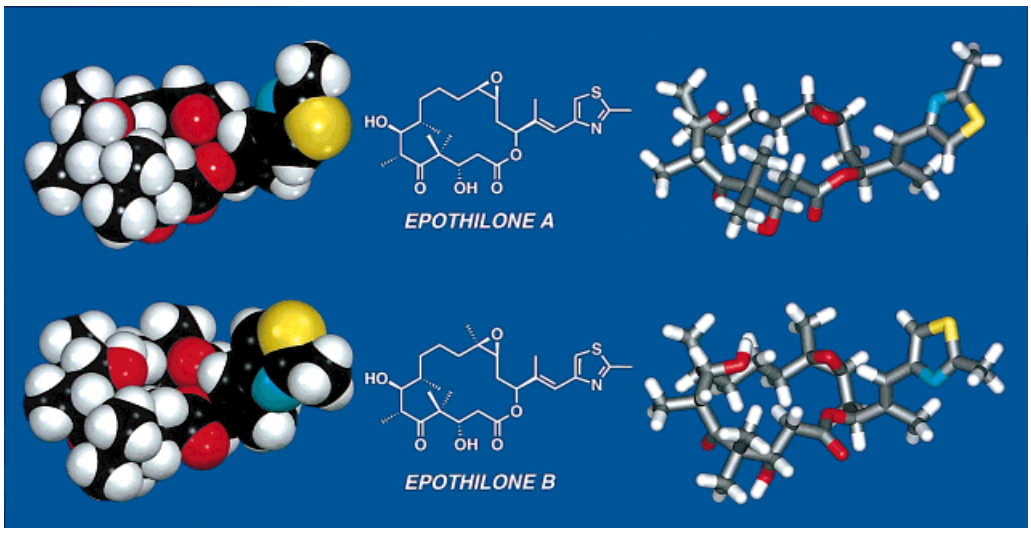


Figure 1. Computer-generated space-filling, ChemDraw, and stick models of epothilones A and B. Carbon: black; oxygen: red; nitrogen: blue; sulfur: yellow; hydrogen: white (we thank C. N. C. Boddy for the computer graphics).

Although the gross structures of the epothilones were revealed in the original German patent by Höfle et al. in the early 1990s[11] and by the Merck group in 1995,[15] it was not until July 1996 that the absolute stereochemistry

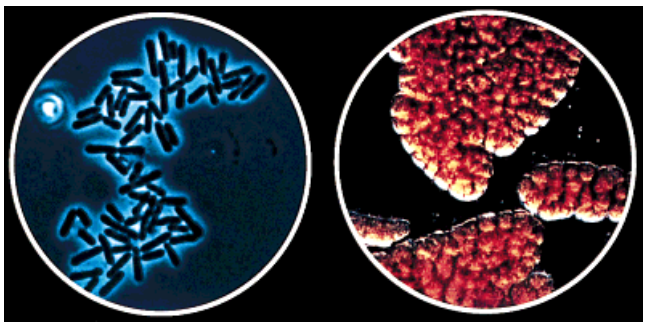
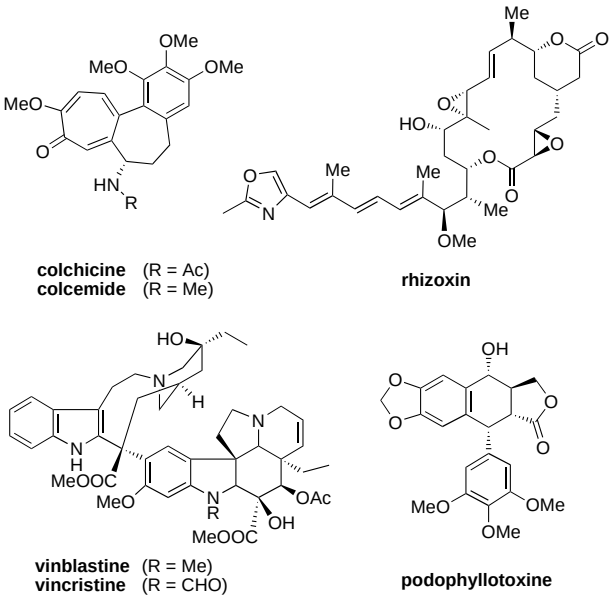


Figure 2. The epothilone-producing myxobacterium *Sorangium cellulosum*: growing cells (left) and spore capsules (right) (courtesy of Dr. H. Reichenbach).

of the epothilones A and B was reported by the German scientists.[12] The structural assignments were made on the basis of spectroscopic[12, 16] and X-ray crystallographic data,[12] and the compounds were named *epothilones* after their structural subunits, *epo*xide, *thi*azol*e* and ket*one*. Seemingly, the carbon backbone of the macrocycle is largely flat, and the side-chain with the thiazole moiety adopts an equatorial position. Apparent structural similarities with taxol are a) a main framework; b) considerable lipophilicity, partly in the form of several methyl groups, including a geminal dimethyl funtionality; and c) a side chain. The release of the absolute stereochemical structures of the epothilones[12] marked the beginning of a new era in their destiny, with efforts to synthesize them assuming top priority in several laboratories around the world. A number of groups had a head start, by learning of their structures prior to publication, and within a short span of time, three research groups had dispatched manuscripts for publication describing their total syntheses. A spate of subsequent reports elaborated on the synthesis and biological evaluation of numerous analogues. In this article, we will review the chemical biology of these fascinating compounds, and discuss their potential in cancer chemotherapy.

2. Biological Properties of Epothilones

Initial investigations by the GBF group focused on the action of the epothilones against fungi, bacteria, and a variety of animal cell lines.[11] These studies revealed only a narrow spectrum of antifungal activity but a rather dramatic effect against oomycetes such as *Phytophotora infestans*, the causative species of the dreaded potato-blight disease.[11] Although greenhouse experiments were encouraging regarding their potential applications in agriculture,[17] the early interest in the epothilones soon subsided due to their failure in field experiments and their apparent phytotoxicity. Soon it was discovered that the compounds also had powerful activity against mouse fibroblast and leukemia cells (2 ng mL^{-1})[13, 18] and strong immunosuppressive action as revealed by their cytotoxicity against human T-cells.[18, 19] The compounds were also tested at the NCI in the USA and proved to be highly active against a panel of cells, including breast and colon cancer cell lines.[20] But it was not until 1995, when a team from Merck in the USA reported their findings on the mode of action of epothilones,[15] that interest in these compounds resurfaced again, this time with much more excitement and momentum. During a high-throughput screening program to discover taxol-like tubulin polymerization agents, the Merck group subjected tens of thousands of compounds to biological assays.[15] Their only hits were epothilones A and B. An investigation of compounds with homology to the epothilones, such as the 16-membered macrocyclic substances erythromycin,[21] chalcomycin,[22] carbomycin,[23] and rosamycin,[24] revealed no active compounds (the 16-membered macrocyclic natural product rhizoxin (Scheme 1),[25] however, is a known



Scheme 1. Selected compounds promoting depolymerization of tubulin.

microtubule-destabilizing agent[26, 27]).[15] The uniqueness of the epothilones immediately placed them in the same class as taxol, whose tubulin-binding mechanism of action was discovered by Horwitz in 1979.[28] The Merck group compared the effects of the epothilones and taxol on tubulin and microtubules and reported higher potencies for both epothilones A and B as tubulin polymerization agents (epothilone B > epothilone A > taxol). Most significantly, all three compounds were shown to compete for the same binding site within their target protein.[15, 29] Furthermore, the epothilones were found to exhibit similar kinetics in their induction of tubulin polymerization, and gave rise to microscopic pictures of stabilized microtubules and damaged cells that were essentially identical to those obtained with taxol.[15] Perhaps the most exciting property of the epothilones is their superiority over taxol as a killer of tumor cells, particularly MDR cell lines, including a number resistant to taxol.[15, 29] In some of the cytotoxicity experiments, epothilone B demonstrated a 2000 – 5000-fold higher potency than taxol, a striking enough observation to awaken and stimulate the interest of many in the academic community and the pharmaceutical industry. Moreover, in vivo experiments, carried out recently at Sloan Kettering in New York involving subcutaneous

implantations of tumor tissues to SCID mice, proved the superiority of epothilone B over taxol.[30] Before we proceed to the next phase of research within the epothilone field, however, it will be instructive to discuss further the molecular and cellular biology of tubulin and tubulin binding agents, and how epothilones relate to them.

2.1. Epothilones, Tubulin, Microtubules, and the Cytoskeleton

Tubulin polymerization – depolymerization[31] plays an important role in the cell cycle (Figure 3), particularly during mitosis. Tubulin is a heterodimeric protein composed of globular α- and β-tubulin subunits (which are amongst the most highly conserved proteins known),[32, 33] and represents the monomeric building block of microtubules. Microtubules, in turn, are one of the fundamental structural components of the cytoskeleton in all eukaryotic cells. They serve both as structural beams and conveyer belts within cells.[27, 34–37] Microtubules rigidify the cell[38] and translocate vesicles, granules, organelles,[39] and chromosomes through special attachment proteins.[38–40] As part of the cytoskeleton, microtubules help develop and maintain the shape and structure of the cell as needed. They may operate alone, or in conjunction with other proteins to form more complex structures, such as cilia, centrioles, or flagella, which are used for cellular movement. The cytoskeleton is the "nanolevel web" made out of filamentous protein networks that dynamically organize the interior of living cells; it is usually transparent and, therefore, invisible under the microscope. Despite its importance to the functioning of the cell, the cytoskeleton is usually left out of cell drawings, so it is crucial to remember its existence and dynamic role.

Structurally, microtubules are regular internetworked linear polymers (protofilaments) of highly dynamic assemblies of heterodimers of α- and β-tubulin. When thirteen of these protofilaments are arranged parallel to a cylindrical axis they self-assemble to form microtubes. These polymers form tubes of approximately 24 nm in diameter and up to several μm in length.[41] Figures 4 and 5 display such microtubules and

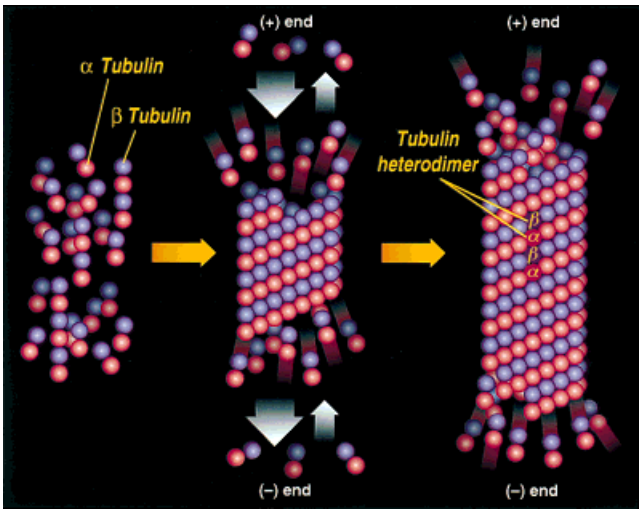


Figure 4. Polymerization of tubulin to microtubules.

demonstrate their assembly from tubulin. Formation of microtubules proceeds by a nucleation-elongation mechanism.[42, 43] Nucleation is the initial phase of the process in which preformed heterodimers of α- and β-tubulin assemble in the presence of Mg^{2+}, guanosine triphosphate (GTP), and microtubule-associated proteins (MAPs). This process is relatively slow until a short microtubule is formed, triggering the much faster elongation phase. The elongation phase involves extension of the microtubule nucleus at both ends by a reversible, noncovalent addition of tubulin heterodimers to form growing oligomers which become linear rows of tubulin beads. The tubulin units within the protofilaments are held together by stronger bonds within the α/β tubulin dimers, and weaker and reversible bonds formed during microtubule assembly between the α/β-dimers. The microtubules are formed within the cell in an area called the "aster". Their dipolar structures consist of a (+)-end, which is kinetically more dynamic, and a (−)-end, which is less dynamic (Figures 4 and 5). Although both ends can either grow or dissociate, it is the (+)-end that usually grows faster than the (−)-end, and net growth occurs at the (+)-end, while net shortening takes place at the (−)-end.[44] When both of these dynamic processes occur at once, the microtubule is said to be treadmilling.[45]

The growth and dissolution of microtubules is regulated by bound GTP molecules. Each tubulin dimer carries two

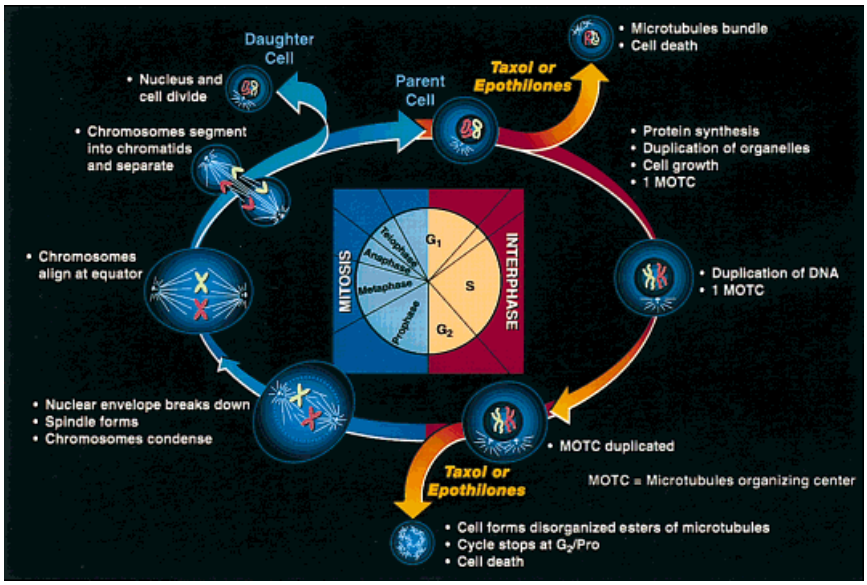


Figure 3. Schematic diagram of the cell cycle showing the inhibition of mitosis by taxol and the epothilones. MOTC = Microtubular organism center.

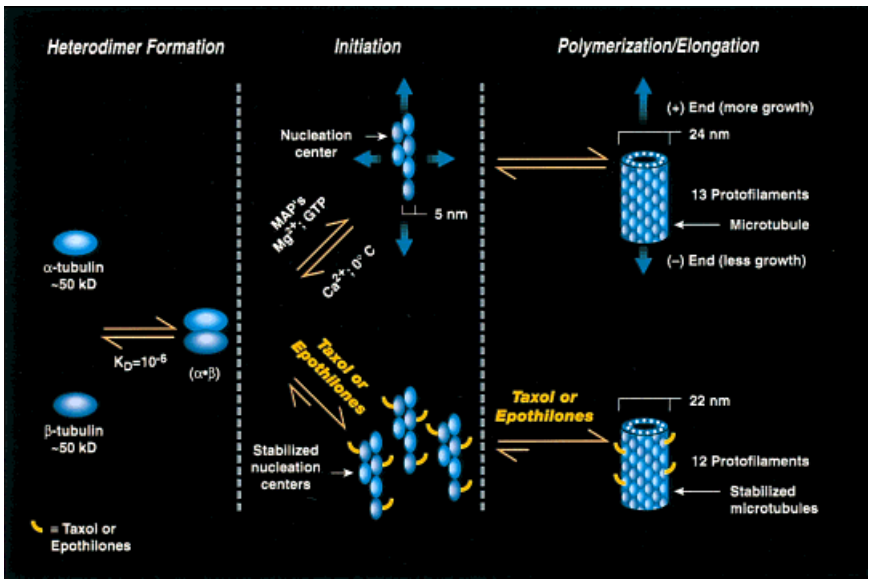


Figure 5. Dynamic equilibria of tubulin-microtubules.

GTP molecules, but only the one on the β subunit of the tubulin dimers appears to function.[46] During polymerization, the GTP molecule hydrolyzes to guanosine diphosphate (GDP) and orthophosphate (P_i). The presence of the GTP, GDP, and P_i forming a "cap" on the ends of the microtubules facilitates further growth due to their higher affinity for additional tubulin subunits.[47] While the half-life of tubulin at 37 °C is nearly a full day, that of a given microtubule may be only about 10 min. Consequently, microtubules are in a constant state of flux responding to the needs of the cell. This state is called "dynamic instability"[42, 48] and is controlled by regulatory processes within the cell. Thus, microtubule growth is promoted in a dividing or moving cell, but more controlled in a stable polarized cell. The regulatory control is exerted by adding (for growth) or hydrolyzing (for shrinkage) GTP on the ends of the microtubule.

As major components of the cellular apparatus known as the mitotic spindle, the microtubules also play a crucial role in mitosis, which is the process during cell replication in which the duplicated genetic material in the form of chromosomes is partitioned equally between the two daughter cells.[49] When cells enter mitosis, the cytoskeletal microtubule network (mitotic spindle) is dismantled by melting at the center, and two dipolar spindle-shaped arrays of microtubules are formed outwards from the centrosome. In vertebrate cells, the centrosome acts as the major site of microtubule nucleation (microtubule-organizing center or MTOC) by lowering the critical concentration of tubulin required for polymerization and anchoring the (−)-ends of the resulting microtubules.[50] At metaphase, the chromosomes are assembled to an equatorial position on the mitotic spindle by the dynamic action of microtubules.[51] At anaphase the microtubule dynamics change and the chromosomes partition and move to the new spindle poles on the dynamic microtubules, where the new cells are being formed.[52] In this process, the parent cell duplicates its chromosomes to provide each of the two daughter cells with a complete set of genes. When it is time for a eukaryotic cell to divide, the microtubules literally pull its chromosomes apart pushing them into the compartments of the two emerging daughter cells. The rate by which microtubules change their length increases by 20- to 100-fold during mitosis relative to the rate during interphase. These rapid dynamics are extremely sensitive to tubulin-interactive agents which exert their antimitotic action at the metaphase to anaphase transition (Figure 6).[48, 53] Compounds displaying considerable structural diversity, such as vinblastine,[42, 54, 55] colchicine,[55, 56] taxol, and the epothilones, block mitosis by intervening at this important juncture in the cell cycle (see Section 2.2 for more on tubulin binding agents). These and other similar drugs may exert their antiproliferative and cytotoxic effects at this cell cycle checkpoint by supressing spindle microtubule dynamics.[57]

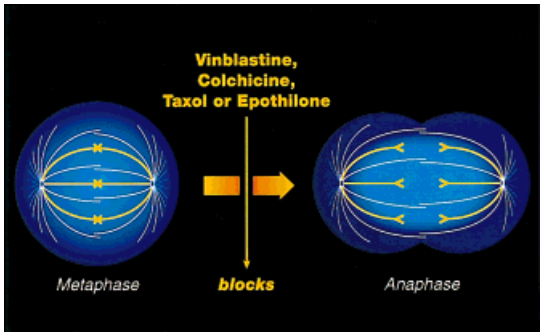


Figure 6. Blocking of mitotic spindle division by antimitotic agents.

Microtubule dynamics can also be suppressed both in vitro and in vivo by MAPs,[58] the cell- and tissue-type specific cellular proteins.[27, 34] MAPs are high molecular weight proteins (200–300 kDa) or tau proteins (20–60 kDa).[27, 59] One end of the MAP binds monomeric or polymerized tubulin, thus speeding up polymerization and facilitating assembly and stabilization of the microtubules. The other end of the MAP binds to vesicles or granules. It is believed that some of these MAPs may bind to special sites on α-tubulin after it is incorporated into the microtubule. Such sites include points of acetylation or removal of tyrosine residues from the carboxyl terminus of tubulin and are important markers for stabilized microtubules because they disappear upon depolymerization. Interestingly, a tau protein suppresses steady-state dynamic instability of microtubules in vitro in a manner which is qualitatively indistinguishable from that of taxol.[60] This observation is consistent with the notion that antimitotic drugs may mimic the actions of naturally occurring regulatory ligands. The MAPs include kinensin and dynein which "walk" along the microtubules in opposite directions. A number of MAPs have head domains that bind to microtubules and ATP,

thus acting as ATPase motors. Their tail domains may bind to the organelle to be moved, but the mechanism by which energy, generated by the ATP breakdown, is converted into vectorial transport is not known. In summary, MAPs are auxiliary "machines" that accelerate tubulin polymerization, serve as motors for vesicles and granules, and essentially control cell compartmentalization.

2.2. Epothilones, Tubulin Binding Agents, and Cell Death

A number of anticancer drugs possessing diverse molecular structures exert their cytotoxicity by disrupting microtubule dynamics.[27, 34, 35, 61] Most of these compounds, including the well-established chemotherapeutic agents colchicine,[55, 56] colcemid,[62] podophyllotoxin,[63, 64] vinblastine,[42, 54, 55] and vincristine[42, 54, 55] (see Scheme 1), operate by interfering with the formation and growth of microtubules and preventing polymerization of microtubules by diversion of tubulin into other types of aggregates,[56, 65] thereby promoting net depolymerization and inhibition of cell proliferation at mitosis. This class of antimitotic compounds further includes combretastatin,[64] maytansine,[66, 67] rhizoxin (Scheme 1),[25] phomopsin,[67] the dolastatins,[66-68] cryptophycins,[69] benzimidazoles (such as nocadazol),[42, 48, 70] and the curacins.[71] At appropriate concentrations, these drugs inhibit the formation of spindle microtubules or depolymerize existing ones.[42, 48]

Figure 7 depicts graphically the different complex formations of vinblastine, colchicine, and taxol (see also Figure 5) with microtubules. Vinblastine binds to the ends of microtubules with high affinity, and its potent cytotoxicity appears

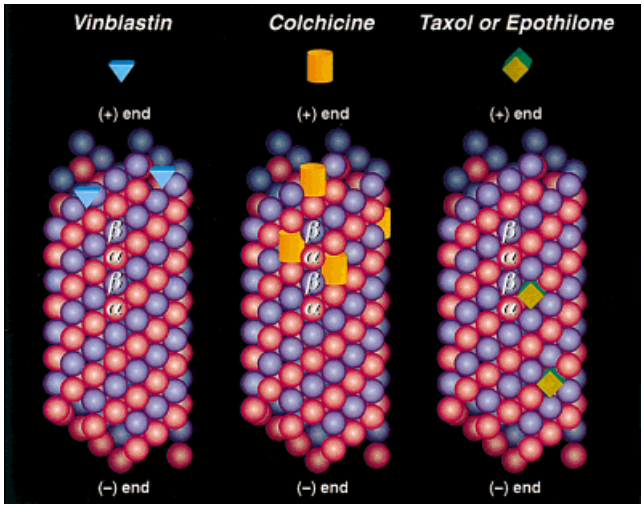
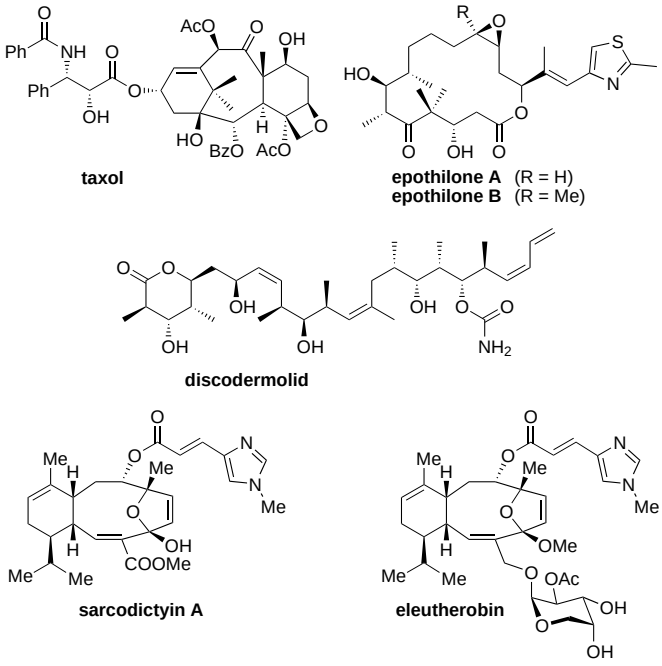


Figure 7. Microtubule – ligand complexes.

to be due to a relatively small number of end-binding molecules.[42] Colchicine, on the other hand, first binds to free tubulin, and the formed complexes are incorporated into the microtubules at the growing ends in relatively low concentrations, but show profound effects on the microtubule dynamics.[56] In contrast to the antimitotic drugs described above, taxol disturbs the polymerization – depolymerization dynamics of microtubules in vitro, with cell death as the net result, by binding to the polymeric microtubules and stabilizing them against depolymerization (see Scheme 2 for examples of tubulin-polymerizing natural products).[72] Despite



Scheme 2. Selected natural products with tubulin polymerization and microtubule stabilization properties.

their seemingly little structural similarities to taxol, the epothilones appear to act by the same mechanism and bind to the same general regions as taxol does.[15, 29] Although the epothilones displace taxol from its receptor, they must bind in a slightly different manner to microtubules as suggested by their action against taxol-resistant tumor cells, which contain mutated tubulin. Each tubulin molecule (α/β-tubulin heterodimer) on the microtubules contains a taxol binding site. High-resolution electron microscopy revealed that the taxol binding site is located between the protofilaments formed from α- and β-tubulin units.[29] Taxol and epothilone binding markedly reduces the rate of α/β-tubulin dissociation. Therefore, taxoids and epothilones act as a bracket to augment and stabilize the pool of tubulin polymers.[73]

Taxol has also been shown to nucleate tubulin polymerization in vitro without the presence of the GTP that is required for normal polymerization.[28, 74] Figure 8 demonstrates the cellular effects of epothilone B with cultures of PtK_2 cells of *Potorous tridactylis*. The top picture (control) shows the PtK_2 cells with their nuclei (blue) surrounded by tubulin (red). One of the cells (center) is in the anaphase showing the chromosomes (bright color) being pulled apart towards the poles. On treatment with epothilone B (bottom), however, the cells appear to be in disarray with their nuclei (blue) fragmented in irregular shapes and the tubulin (red) aggregated in distinct wedge-shaped bundles. Thus, by interacting with tubulin, the epothilones block nuclear division and kill the cell by initiating apoptosis ("gene-

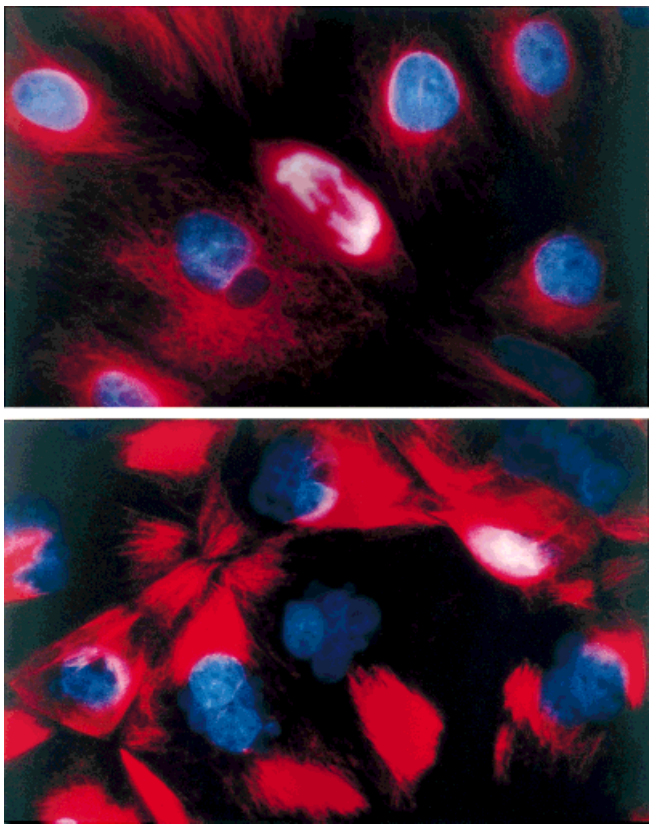


Figure 8. Effect of epothilones on PtK2 cells. See text for explanation (picture courtesy of Drs. F. Sasse and H. Reichenbach).

directed cellular self-destruction" or programmed cell death). Recently, Hamel and co-workers examined the actions of epothilones A and B with additional colon and ovarian carcinoma cell lines and compared them with the action of taxol.[29] Thus, the Pgp-overexpressing MDR colon carcinoma line SW620 and the taxol-resistant ovarian tumor cell line KBV-1 retained susceptibility to the epothilones, whereas taxol revealed its weakness. With *Potorous tridactylis* kidney epithelial (PtK_2) cells, examined by indirect immunofluorescence, epothilone B proved to be the most active, inducing extensive formation of microtubule bundles. Furthermore, it was confirmed that the epothilones are not substrates for Pgp (as originally reported by Bollag et al.[15]) and that subtle differences between taxol and the epothilones exist in terms of their mechanism of action on microtubules. This observation was revealed by differences in microtubule morphology. Researchers at the University of Freiburg, Germany, have recently demonstrated that epothilone A initiates apoptosis in neuroblastoma cells just as taxol does.[75] However, unlike taxol, epothilone A was active against a constitutively Pgp-expressing MDR neuroblastoma cell line (SK-N-SH),[76] and moreover, its efficacy was not diminished despite the increase of the Pgp level during administration of the drug.[15] While taxol's ability to polymerize tubulin is associated with nearly stoichiometric binding to its target,[77] its stabilization effect on microtubules depends on a relatively small number of molecules binding to the microtubules. When taxol molecules bind to microtubules, they render them extremely stable and static,[73] making cell division impossible, and killing the cells as they begin to divide. Since cancer cells divide more frequently than healthy cells, taxol damages tumors where runaway cell division occurs most profoundly. But other rapidly dividing cells such as white blood cells and hair cells can also be attacked, and consequently side effects are experienced by patients taking the drug. Chemotherapy with taxol, therefore, is frequently accompanied by suppression of the immune system, deadening of sensory nerves, nausea, and hair loss (neutropenia, peripheral neuropathy, and alopecia).[78]

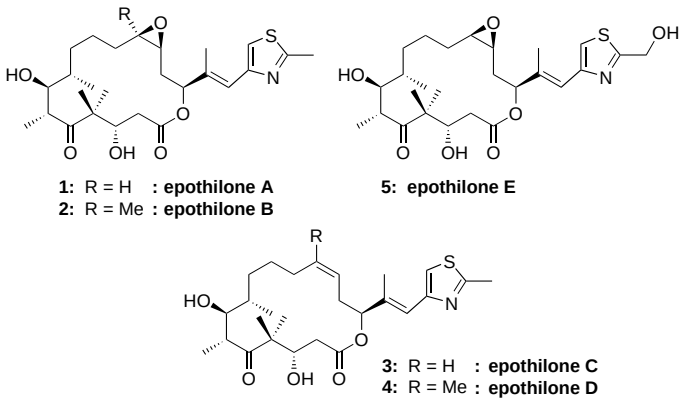
Taxol exhibits endotoxin-like properties by activating macrophages,[79–81] which in turn synthesize proinflammatory cytokines[82] and nitric oxide.[83] It was recently found that epothilone B, despite its similarities to taxol in its effects on microtubules, lacked any IFN-γ-treated murine macrophage stimulatory activity as measured by nitric oxide release (nor did it inhibit nitric oxide production).[84] It was concluded that epothilone-mediated microtubule stabilization does not trigger endotoxin-signalling pathways, which may translate in clinical advantages for the epothilones over taxol in terms of side effects.[85] Like taxol and the epothilones, the polyketide marine natural product discodermolide,[86] and the recently discovered eleutherobin[87] and sarcodictyin A[88] (see Scheme 2) also exhibited cytotoxic properties against tumor cells by interfering with tubulin-microtubule dynamics.

3. Chemistry of the Epothilones

Soon after the recognition of the importance of the epothilones, a number of groups around the world began to pursue strategies for their total synthesis. Several groups rushed into the synthesis and it was a very close finish among three groups.[19] The structures of epothilones are considerably less complex than that of taxol. Nevertheless, the epothilones posed a considerable challenge to synthetic chemists and, most importantly, offered opportunities for the discovery and development of new synthetic technologies and strategies. Of particular interest were the 16-membered macrolide ring, the seven stereocenters, and the thiazole side-chain, whose nitrogen and sulfur atoms held potential complications. While the splendor of the molecular architecture and the highly congested nature of epothilones A and B can be seen in Figure 1, Scheme 3 presents the five naturally occurring epothilones (A – E) identified to date. The topical nature of the field has already elicited highlight reviews from Wessjohann,[89] Kalesse,[90] and Finlay.[91] In the sections below we will first describe the various total syntheses of epothilones A (**1**) and B (**2**) and then discuss the design, synthesis and biological evaluation of the many analogues reported. It should be noted at this point that the Danishefsky group was the first to accomplish total syntheses of epothilones A and B.

3.1. The Nicolaou Strategies to Epothilones

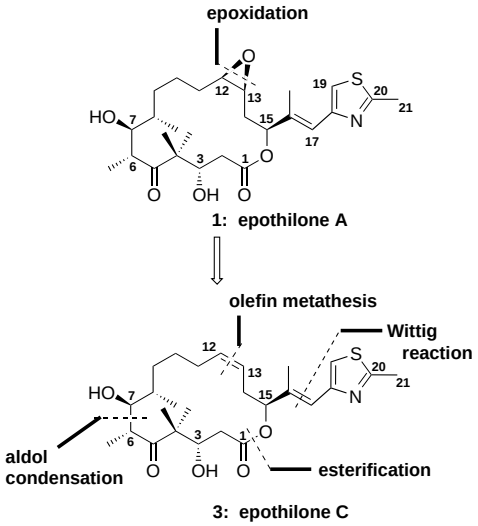
It was on a visit to the Gesellschaft für Biotechnologische Forschung (GBF) in Germany on April 18, 1996, that one of us (KCN) met Professor G. Höfle. Listening to his epothilone



Scheme 3. Structures of the naturally occurring epothilones A (**1**) – E (**5**).

story was both fascinating and stimulating, and quite sufficient to convince one interested in chemistry, biology, and medicine that a research program in the field was warranted. But there was one problem: no stereochemical assignments had been revealed up to that time, and Höfle was not ready to release them yet. Nevertheless, as he had promised, on May 15, 1996, Höfle dispatched a manuscript to us (later to appear in *Angewandte Chemie*[12]), fully assigning the proper stereochemistries of epothilones A (**1**) and B (**2**). The relocation of our group into the newly constructed Arnold and Mabel Beckmann Center for Chemical Sciences on May 28, 1996, marked the official embarkation date of our epothilone project at Scripps.

Amongst the many strategies we considered, the olefin metathesis approach[92] shown in Scheme 4 was, perhaps, the



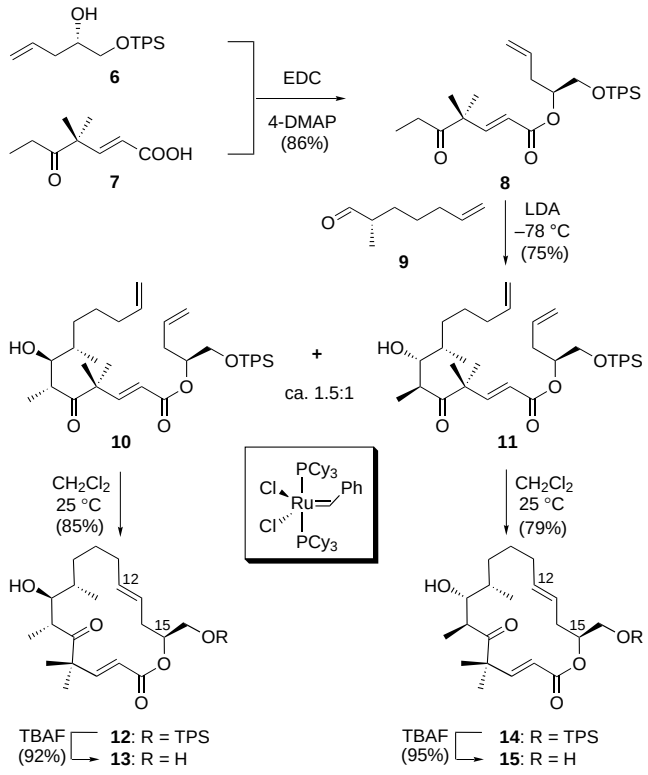
Scheme 4. Nicolaou's olefin metathesis strategy to epothilones A (**1**) and C (**3**): retrosynthetic analysis and strategic bond disconnections (Nicolaou et al.).[96, 97]

most intriguing. Despite the elegant precedents for such reactions from the groups of Schrock,[93] Grubbs,[94] and Hoveyda,[95] we were faced with a number of dilemmas and questions:

- Will the macrocycle be formed under the metathesis conditions?
- Will the stereochemical outcome of this reaction favor the *Z*-olefin geometry as required, or the *E* geometry?
- Will the thiazole moiety interfere with the catalyst?
- Will an epoxidation be possible in the presence of the α,β-unsaturated thiazole side-chain, and if so, which of the two diastereomeric epoxides will be formed?

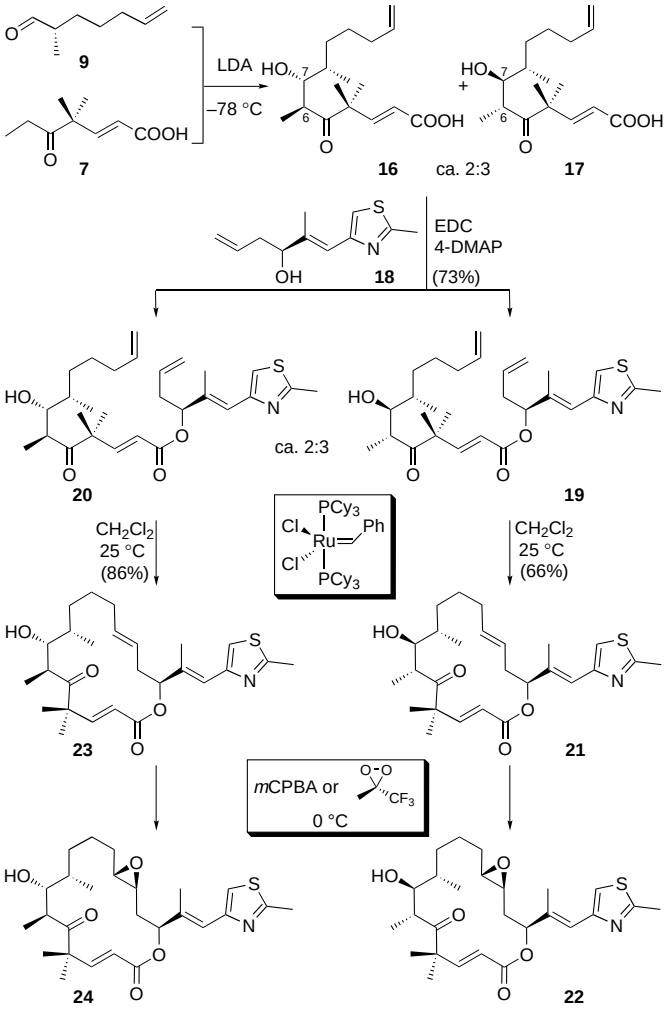
While there were no guaranteed answers to these questions, we immediately recognized that those were the ingredients for an exciting and challenging synthetic adventure, and hence we hesitated no more. In addition to the olefin metathesis, this strategy would require a Wittig olefination, an esterification, and an aldol reaction (Scheme 4). Furthermore, the strategy appeared flexible and convergent enough to deliver both epothilones A and B and, most significantly, a series of analogues for biological screening.

The first generation studies[96, 97] summarized in Scheme 5 were designed and carried out in order to test the olefin metathesis concept with substrates of minimal functionality. A "fortunate" misassignment of the newly generated double bond geometry in products **13** and **15** as *Z* (later we proved by X-ray crystallographic analysis that the stereochemistry of this olefin was in fact *E*,[97] as shown in Scheme 5) encouraged us to proceed to the next stage.



Scheme 5. Nicolaou's olefin metathesis strategy to epothilone A: testing the concept with first generation model studies (Nicolaou et al.).[96, 97]

The second generation model studies (Scheme 6)[97] were designed to test the feasibility of the olefin metathesis strategy in the presence of the thiazole side-chain, and whether a selective epoxidation was viable. As seen in Scheme 6, the experiments were encouraging and both concerns could be dismissed, even though the stereochemistry of the C12 – C13 double bond was incorrect.

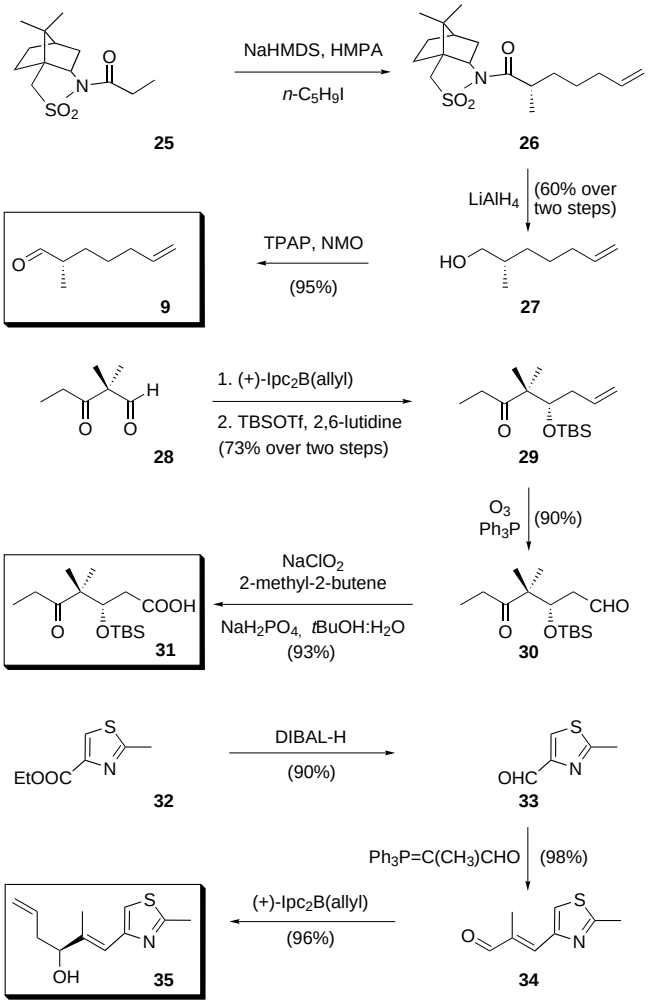


Scheme 6. Nicolaou's olefin metathesis strategy to epothilone A: second generation model studies for testing the feasibility of the concept in the presence of the thiazole moiety (Nicolaou et al.).[97]

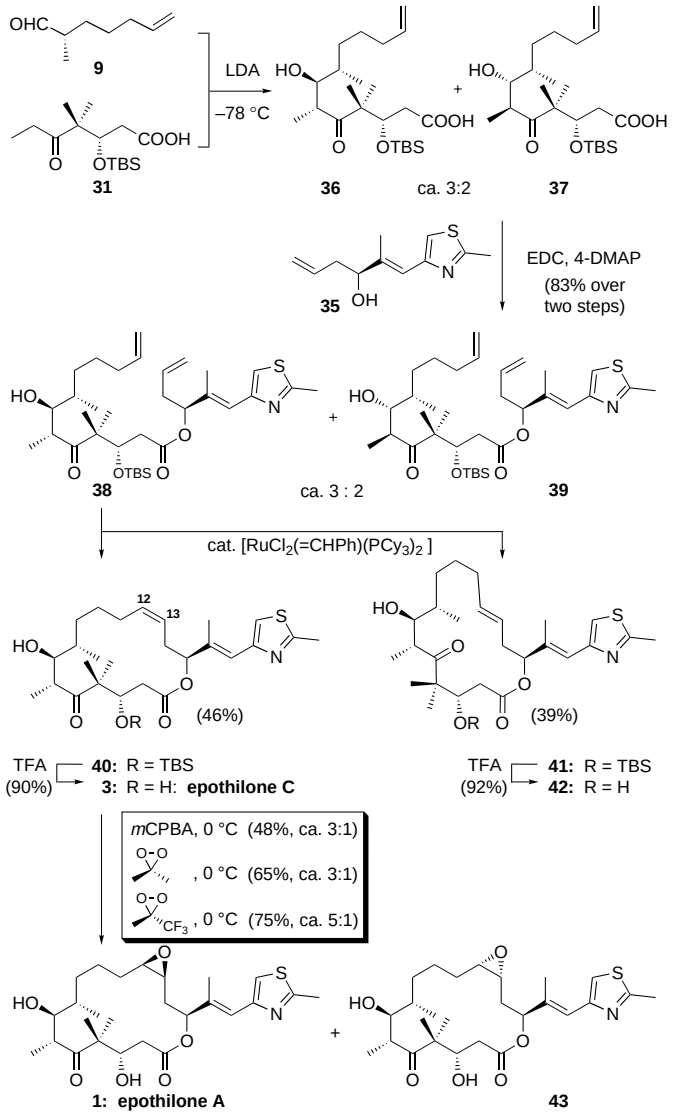
Comforted by the information obtained thus far regarding a possible total synthesis of epothilones by the designed olefin metathesis approach, we embarked on the construction of the requisite building blocks. Scheme 7 summarizes these constructions leading to enantiomerically enriched fragments **9**, **31**, and **35**.[97–99] These fragments were assembled sequentially, as shown in Scheme 8,[97, 98] affording the olefin metathesis precursors **38** and **39**. To our delight, the desired precursor **38** entered smoothly into the olefin metathesis reaction forming a mixture of *Z* and *E* macrocyclic olefins **40** (46%) and **41** (39%). Furthermore, the correct geometrical isomer, epothilone C (**3**), underwent selective epoxidation at the C12–C13 double bond to afford epothilone A (**1**) and its diastereomer **43**. The degree of diastereoselectivity in the final step depended upon the epoxidizing agent used [**1**:**43** ≈ 3:1[97, 98] (*m*CPBA, 0 °C); 3:1[98] (dimethyldioxirane, 0 °C); 5:1[98] (methyl(trifluoromethyl)dioxirane, 0 °C); 13:1[100] (methyl peroxycarboximidic acid, 25 °C)]. Our first total synthesis of epothilone A (**1**) was accomplished in November 1996.

Our explorations of the olefin metathesis reaction within the epothilone field would not end here. Prompted by our desire to produce a large epothilone library for chemical biology studies, we sought to develop a solid phase synthesis of epothilone A, which—we projected—would be amenable to powerful split-pool combinatorial methods for constructing compound libraries.[101–103] Thus, the solid phase synthesis of epothilones A (**1**) and C (**3**) was pursued and accomplished as outlined in Scheme 9. The highlight of this assembly is the simultaneous formation and release of the macrocycle from the resin. A further noteworthy feature of this synthesis is the delivery of four isomers of epothilone C (two C6–C7 aldol and two C12–C13 olefinic isomers), which could be chromatographically separated and further reacted to afford the corresponding epoxides. Application of this technology to the construction of combinatorial epothilone libraries will be presented in Section 4.2.

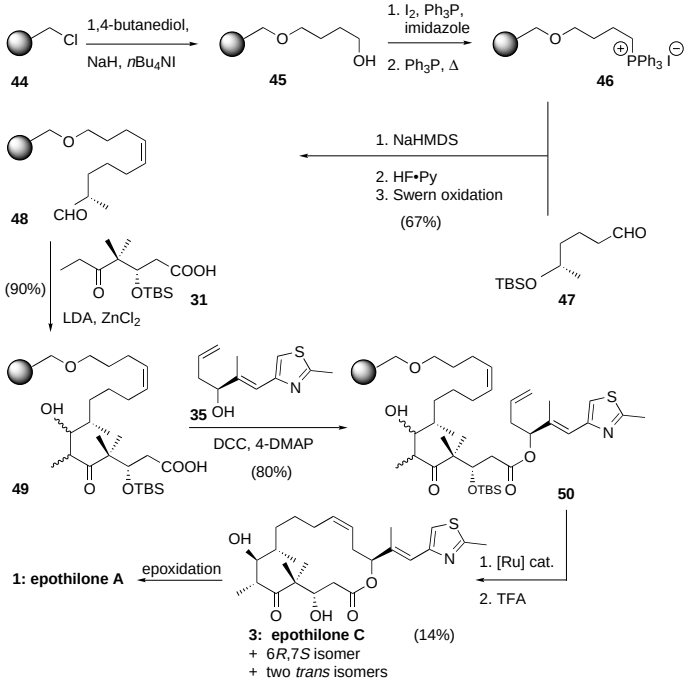
Simultaneously, as the olefin metathesis campaign was proceeding, a second strategy based on a macrolactonization approach (Scheme 10) had been initiated.[99, 101, 104] The objective of this strategy was to avoid the geometric mixtures of macrocyclic olefins of the metathesis-based synthesis, which, wonderful as it was for the purpose of library construction, suffered from low efficiency when applied to a specific target. Another reason for the macrolactonization alternative was



Scheme 7. Nicolaou's synthesis of key intermediates **9**, **31**, and **35** (Nicolaou et al.).[97, 99]



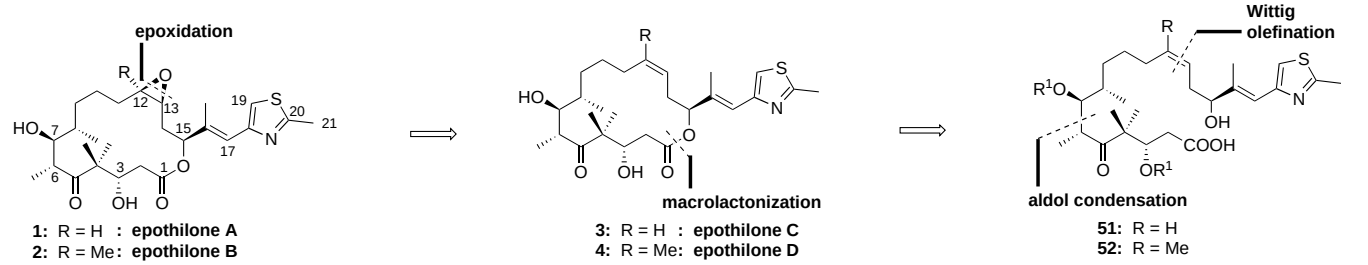
Scheme 8. Nicolaou's olefin metathesis strategy to epothilones A (**1**) and C (**3**): total synthesis in solution (Nicolaou et al. and Yang et al.).[97, 98]



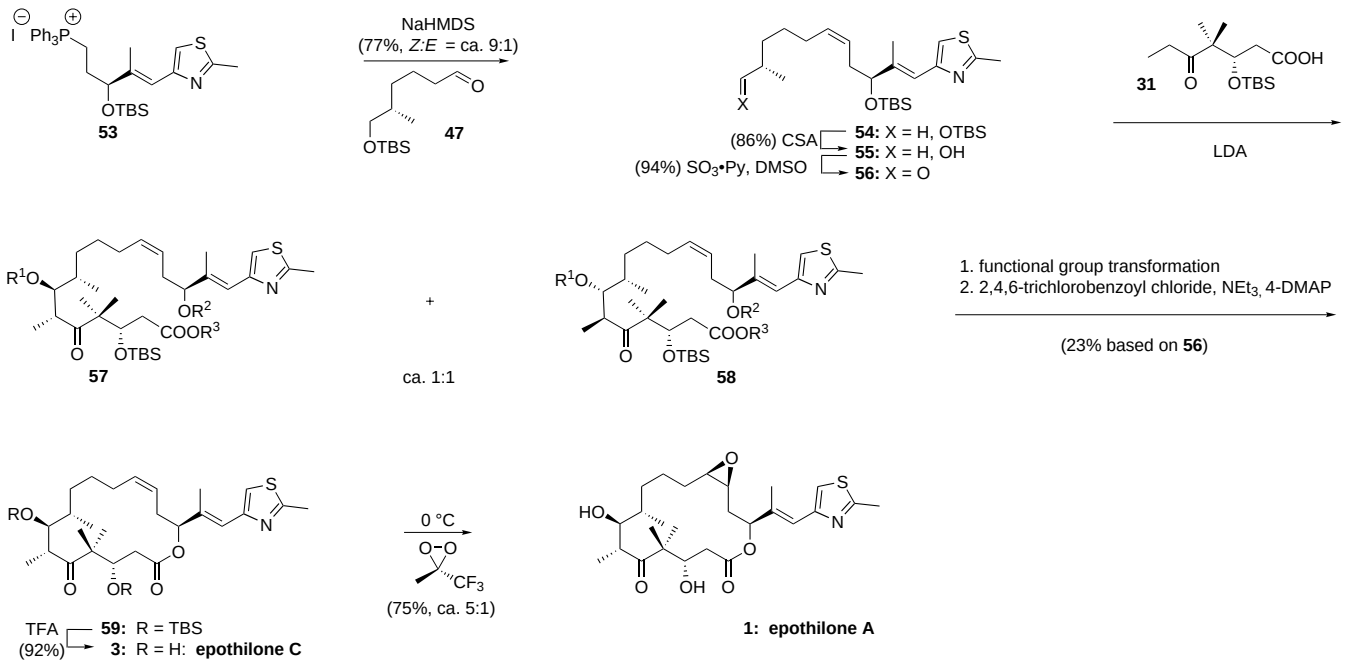
Scheme 9. Nicolaou's olefin metathesis strategy to epothilones A (**1**) and C (**3**): solid phase synthesis. [Ru] = [$RuCl_2$(=CHPh)(PCy_3)$_2$] (Nicolaou et al.).[101]

the perception of a more convenient and efficient synthesis of epothilones B (**2**) and D (**4**), for which the metathesis approach would surely encounter a more severe challenge due to the trisubstituted C12 – C13 double bond. As delineated in Schemes 11 and 12, the macrolactonization strategy to epothilones A (**1**) and C (**3**),[99, 104] and B (**2**) and D (**4**)[99, 101] proceeded smoothly and with a high degree of stereoselectivity at C12 – C13 (**53** + **47** → **54**; Z:$E \approx 9$:1). A further improvement in the aldol diastereoselectivity was also achieved (6*S*,7*R*:6*R*,7*S* ≈ 3.5:1) by the use of the reduced fragment **60** in the aldol step (**60** + **61** → **62** + **63**, Scheme 12).[99] The epoxidation of epothilone D (**4**) to epothilone B (**2**) with methyl(trifluoromethyl)dioxirane proceeded in 85 % yield and approximately 5:1 diastereoselectivity. The stereocontrolled construction of the requisite fragments **60** and **61** for this approach was carried out as summarized in Scheme 13.

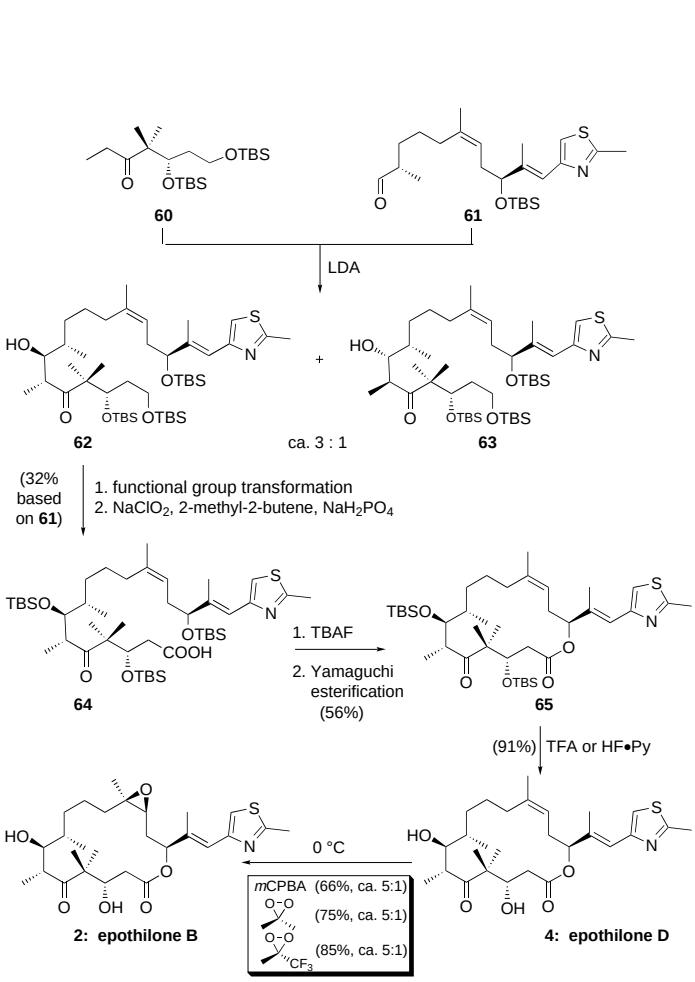
The emergence of epothilone E (**5**)[105] as a naturally occurring substance with important biological activity, coupled with our desire to develop yet another approach to epothilones, prompted our next excursion within the field. The olefin metathesis/Stille coupling strategy shown in Scheme 14 would serve particularly well in a projected synthesis of side-chain analogues of epothilone A,[106] and, potentially, of analogues of epothilone B. A demonstration of the power of this strategy is summarized in Scheme 15, with the first total synthesis of epothilone E (**5**). The methods described above were utilized to construct not only the natural epothilones (A – E), but most importantly, allowed the generation of a series of interesting analogues for biological screening, as shown in Section 4.



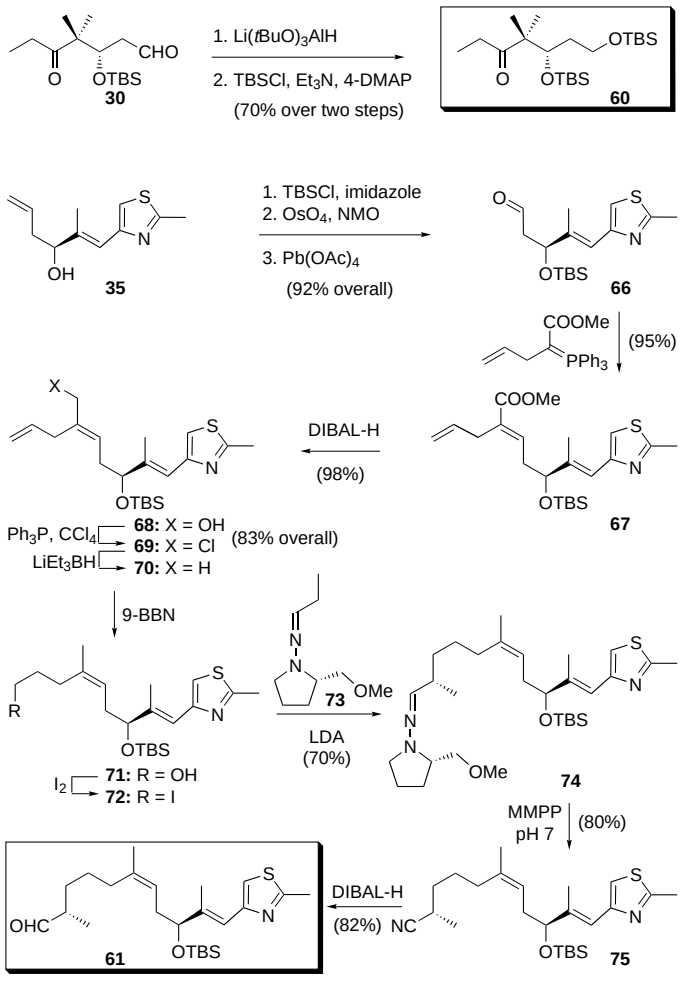
Scheme 10. Nicolaou's macrolactonization strategy to epothilones A – D: retrosynthetic analysis and strategic bond disconnections (Nicolaou et al.).[99, 104]



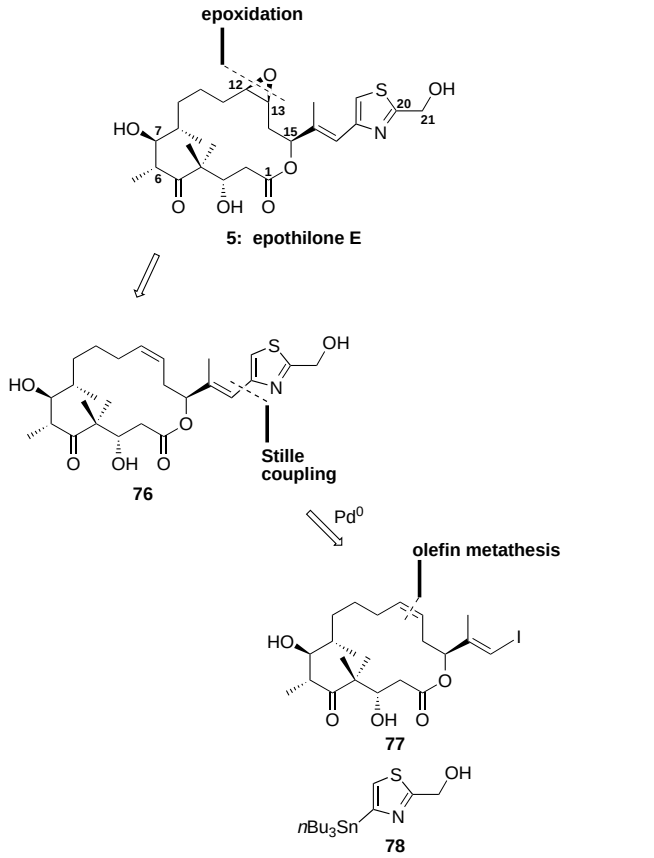
Scheme 11. Nicolaou's total synthesis of epothilones A (**1**) and C (**3**) based on the macrolactonization approach (Nicolaou et al.).[99, 104]



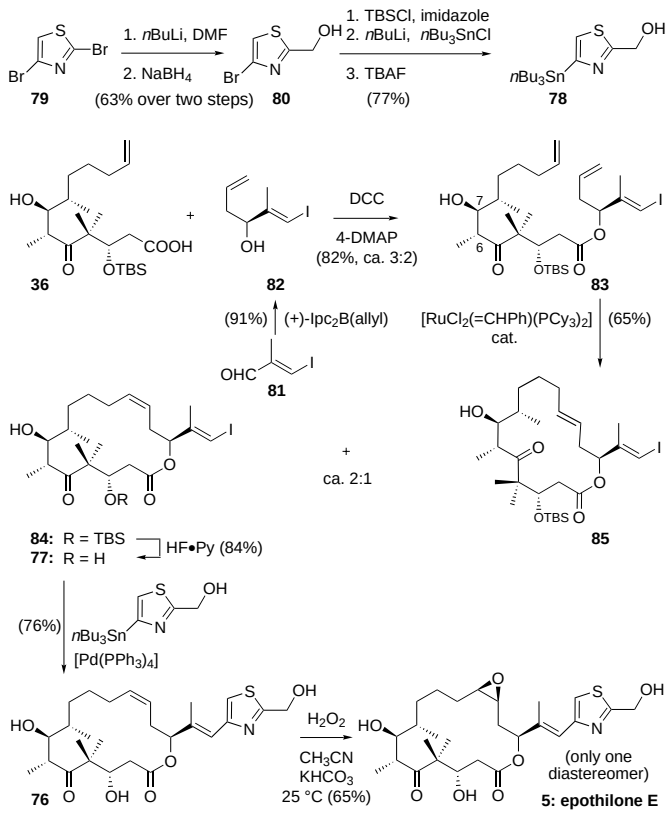
Scheme 12. Nicolaou's macrolactonization approach: stereoselective total syntheses of epothilones B (**2**) and D (**4**) (Nicolaou et al.).[99, 101]



Scheme 13. Nicolaou's synthesis of key intermediates **60** and **61** (Nicolaou et al.).[99]



Scheme 14. Nicolaou's olefin metathesis/Stille coupling strategy to epothilone E (**5**): retrosynthetic analysis and strategic bond disconnections (Nicolaou et al.).[106]

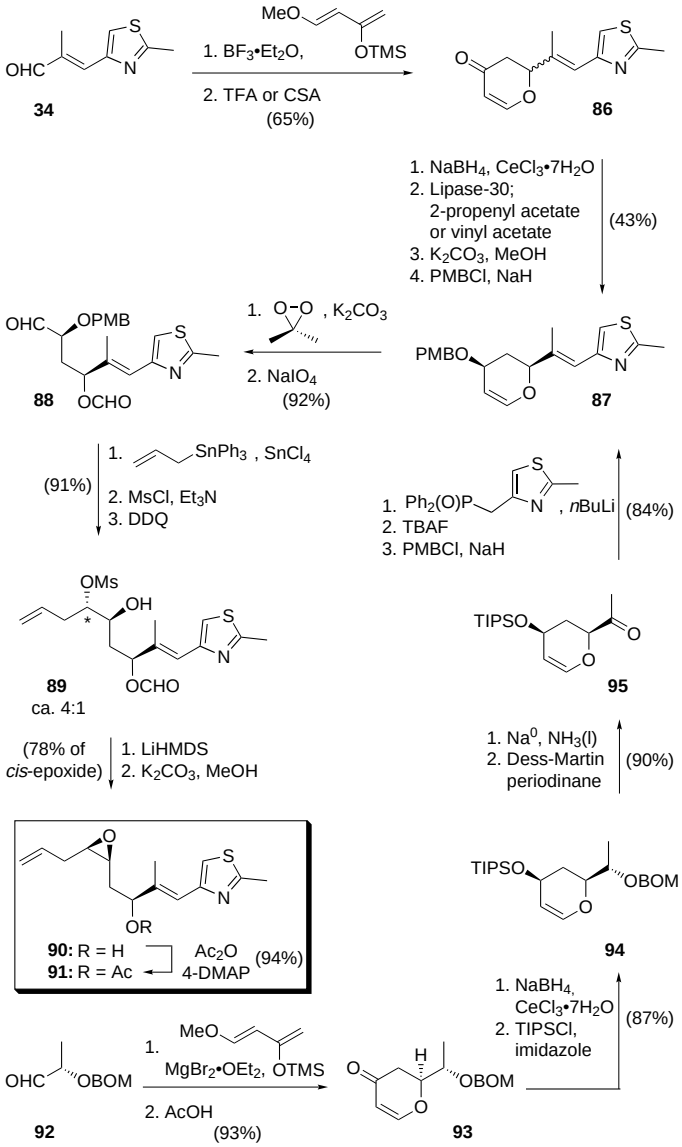


Scheme 15. Nicolaou's olefin metathesis/Stille coupling strategy to epothilone E (**5**): total synthesis (Nicolaou et al.).[106]

3.2. The Danishefsky Strategies to the Synthesis of Epothilones

The Danishefsky group made major contributions to the epothilone field, being the first to accomplish a total synthesis of both epothilones A (**1**)[107] and B (**2**)[108] and their desoxy precursors (epothilones C (**3**) and D (**4**), respectively).[107, 108] Their strategies included an elegant macroaldolization reaction,[107–109] an olefin metathesis approach,[109, 110] and a macrolactonization-based process[109] for the construction of the macrocycle. In addition, a number of interesting reactions and sequences were applied, including Suzuki-type couplings and dihydropyran formation and rupture, as means to install functionality and control stereochemistry.

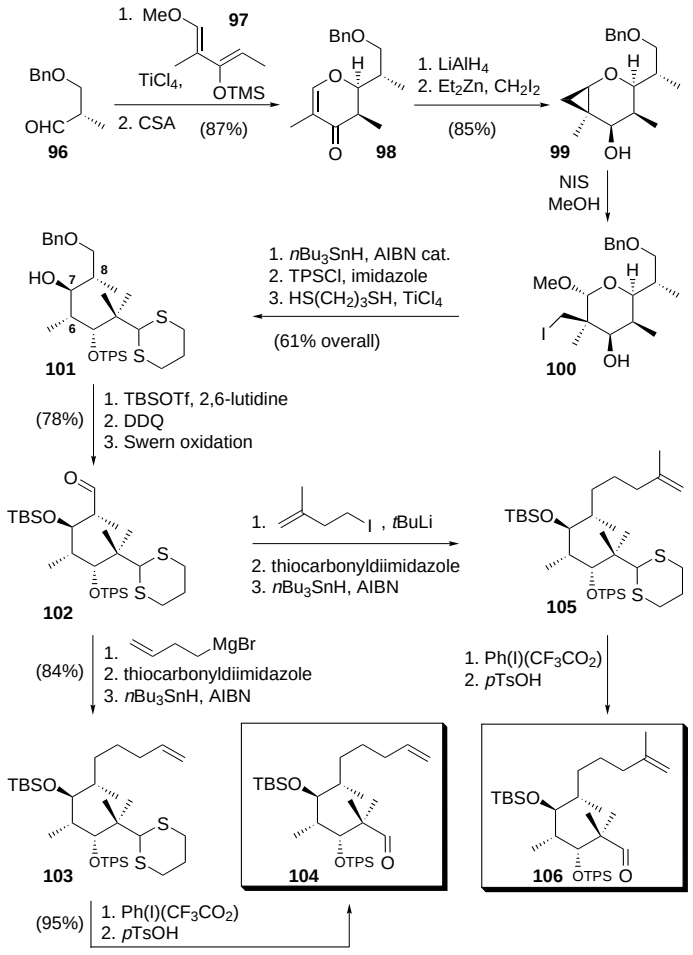
Thus, the group was able to demonstrate the use of dihydropyran templates synthesized from the Danishefsky diene,[111] for the stereocontrolled constructions of their required fragments **90** and **91** as shown in Scheme 16.[109, 112]



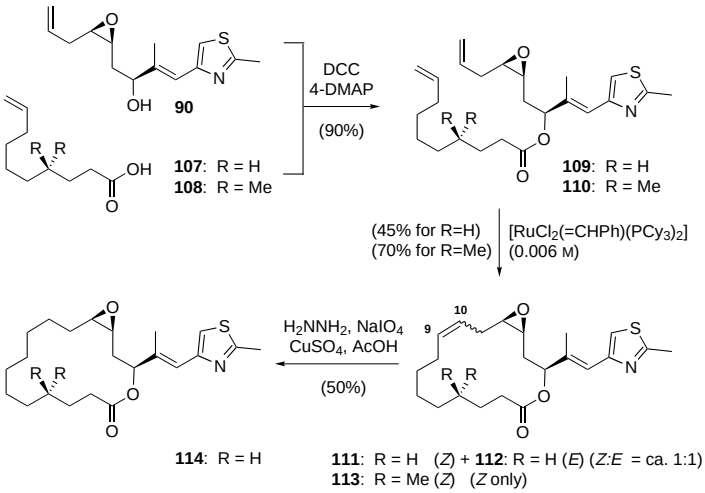
Scheme 16. Danishefsky's utilization of dihydropyran templates as controlling elements in the diasterosynthesis of epothilone fragments **90** and **91** (Meng et al.).[109, 112]

Further elaboration along similar lines allowed the synthesis of the C3 – C12 segments **104** and **106** (Scheme 17) necessary for the synthesis of epothilones A (**1**) and B (**2**), respectively.[109, 110] In both schemes, sequential formation and opening of the dihydro- and tetrahydropyran systems was the key tactic for introduction of the stereochemistry onto the final open-chain intermediates. An early model study by the Danishefsky group for the elaboration of the epothilone macrocycle through olefin metathesis, targeting the C9 – C10 bond, was encouraging (Scheme 18), but unfortunately proved less fruitful in the real case.[109, 113]

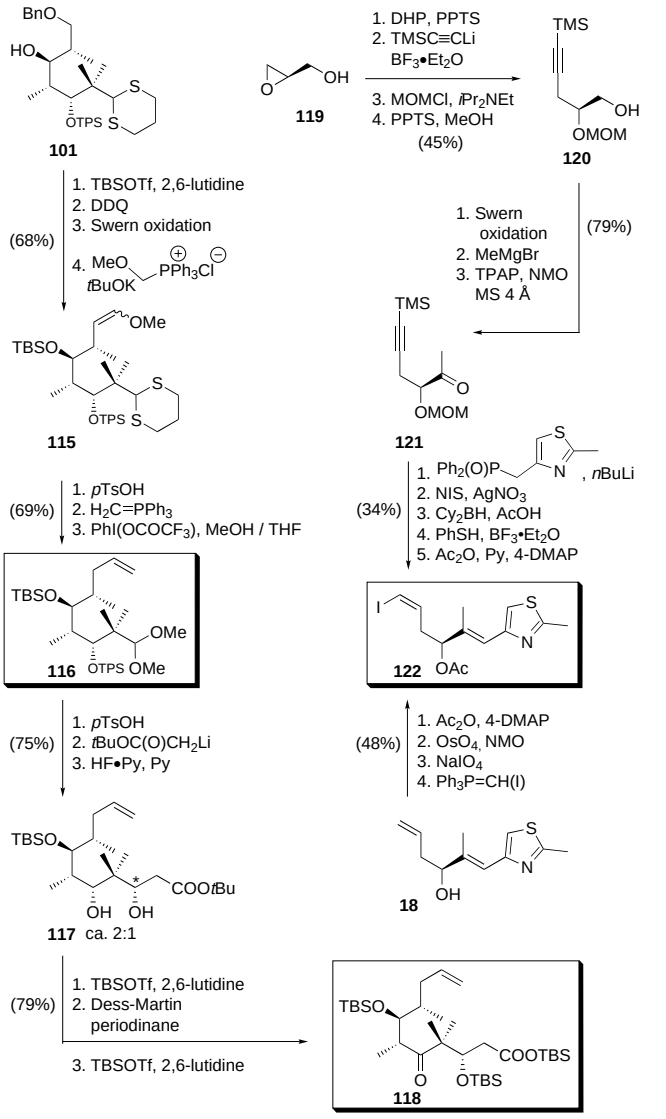
The first breakthrough in the Danishefsky group came with the employment of the macroaldolization strategy for the construction of epothilones A (**1**) and C (**3**) (Schemes 19 and 20).[108, 109] First, the advanced intermediates **116** and **122** were constructed in a stereocontrolled fashion as outlined in Scheme 19. In assembling the fragments (Scheme 20), a stereospecific Suzuki coupling allowed the union of intermediates **116** and **122** to form compound **123**, which underwent a stereoselective ring closure under basic conditions, leading to the desired macrocycle **59**. Subsequent functional group manipulations and epoxidation led to epothilones C (**3**) and A (**1**) in good overall yield and stereoselectivity (Scheme 20). Danishefsky's second approach to epothilone A (**1**) proceeded through intermediates **118** and **122**



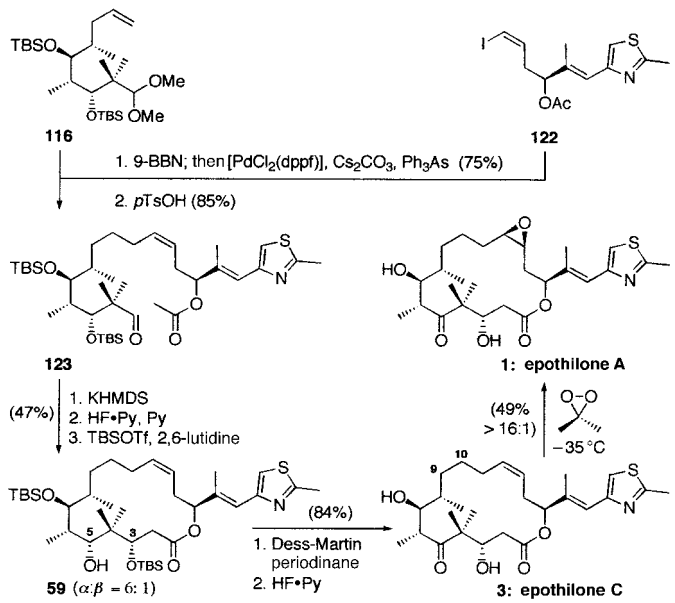
Scheme 17. Danishefsky's stereoselective synthesis of the epothilone C3 – C12 fragments **104** and **106** (Meng et al. and Bertinato et al.).[109,110]



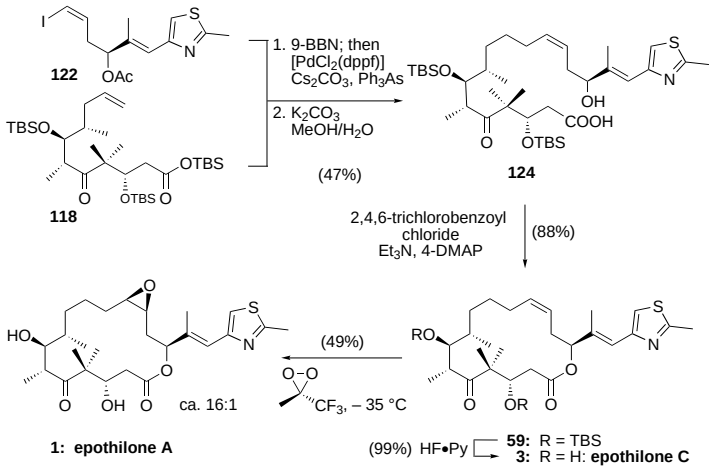
Scheme 18. Danishefsky's model study targeting the C9 – C10 double bond through olefin metathesis (Meng et al. and Bertinato et al.).[109, 113]



Scheme 19. Danishefsky's synthesis of advanced intermediates **116**, **118**, and **122** for the macroaldolization and macrolactonization strategies towards epothilone A (Balog et al. and Su et al.).[107, 108]



Scheme 20. Danishefsky's total syntheses of epothilones A (**1**) and C (**3**) through the macroaldolization strategy (Su et al. and Balog et al.).[108, 109]



Scheme 21. Danishefsky's synthesis of epothilones A (**1**) and C (**3**) by the macrolactonization approach (Meng et al.).[109]

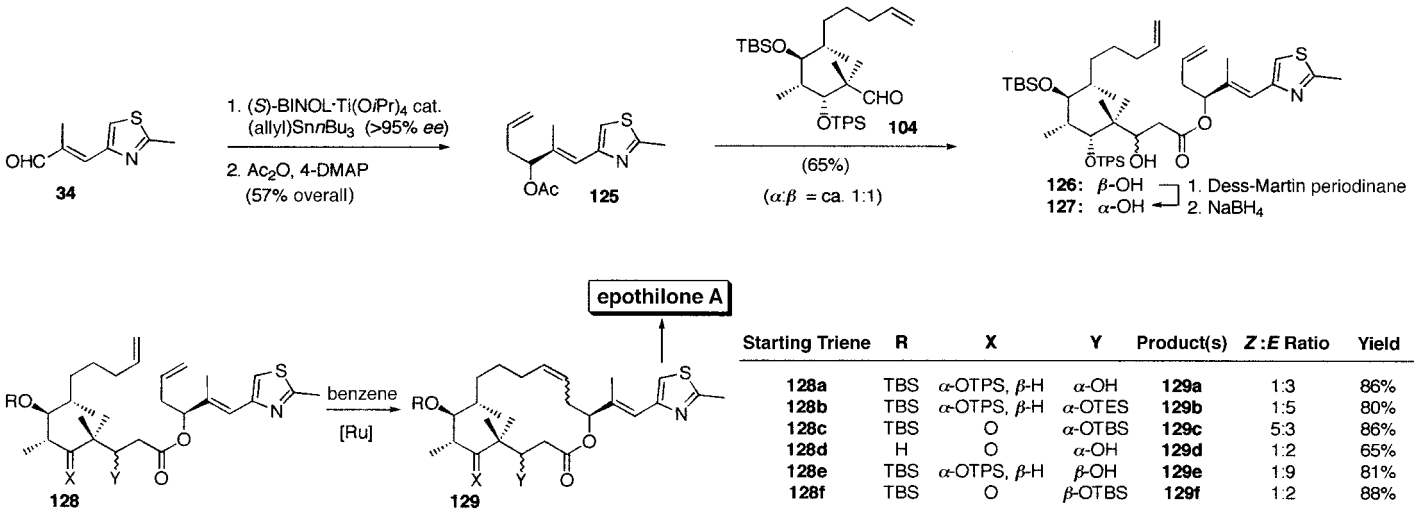
(Scheme 21), and involved a stereospecific Suzuki coupling to join them, as well as a macrolactonization reaction to form the macrocyclic framework.[109]

Employing the olefin metathesis approach, but now targeting the C12 – C13 double bond, the Danishefsky group carried out an interesting and revealing study (Scheme 22).[110] By varying the substituents on the open-chain precursor **128**, they observed different ratios for the *Z*:*E* cyclic olefins ranging from around 5:3 to 1:9. Using their macroaldolization strategy, the group has also synthesized epothilones B (**2**) and D (**4**) as shown in Scheme 23.[108] Again, a stereospecific Suzuki coupling (**116** + **130** → **131**), followed by base treatment and functional group manipulation resulted in ring closure (**131** → **65**), to afford the desired framework, which served admirably as a precursor to both epothilones D (**4**) and B (**2**).

Finally, a remarkable olefin metathesis reaction (Scheme 24) involving a trisubstituted olefin and the molybdenum-based Schrock catalyst[93] served as the basis for a conceptually different total synthesis of epothilones D (**4**) and B (**2**).[109] In this case, however, the C12 – C13 double bond was formed as a mixture of *Z*:*E* isomers in an approximately 1:1 ratio.

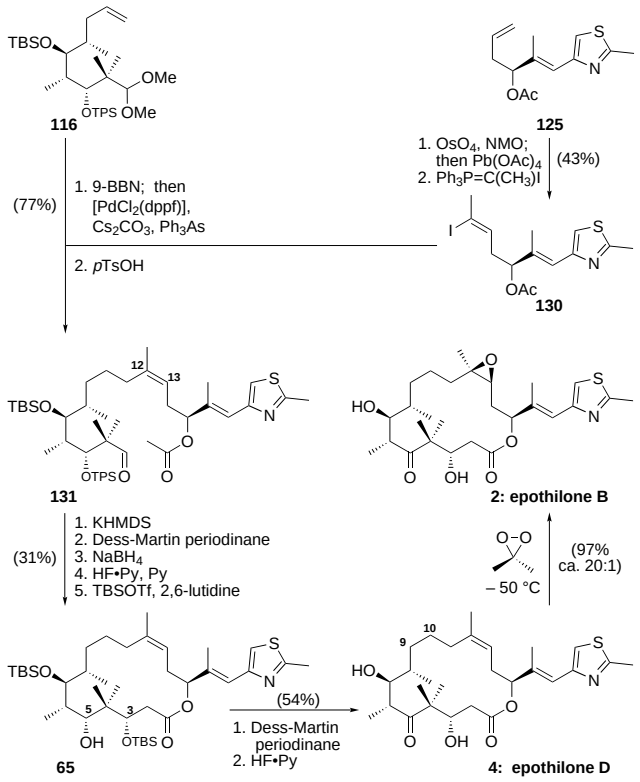
3.3. The Schinzer Strategy to the Synthesis of Epothilones A and C

The Schinzer group, working in Braunschweig (not far from the GBF where the epothilones were first discovered) independently developed an olefin metathesis approach to epothilones A (**1**) and C (**3**) as shown in Schemes 25 and 26.[114, 115] Their design required the key intermediates **137**, **141**, and **146**, which were obtained by asymmetric synthesis according to the sequences depicted in Scheme 25.[114] The formation of a single (6*R*,7*S*) diastereoisomer in the aldol condensation of ethyl ketone **137** with aldehyde **9** under the influence of LDA was most impressive, and was attributed to the influence of the acetonide moiety (Scheme 26).[115] Attachment of the side-chain by esterification, ring closure through olefin metathesis, and epoxidation with dimethyldioxirane, then led to epothilones (**3**) and A (**1**).

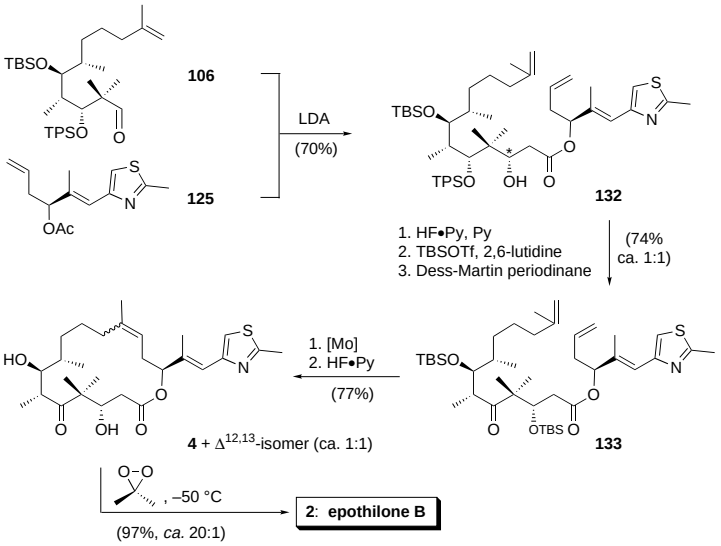


Starting Triene	R	X	Y	Product(s)	*Z*:*E* Ratio	Yield
128a	TBS	α-OTPS, β-H	α-OH	**129a**	1:3	86%
128b	TBS	α-OTPS, β-H	α-OTES	**129b**	1:5	80%
128c	TBS	O	α-OTBS	**129c**	5:3	86%
128d	H	O	α-OH	**129d**	1:2	65%
128e	TBS	α-OTPS, β-H	β-OH	**129e**	1:9	81%
128f	TBS	O	β-OTBS	**129f**	1:2	88%

Scheme 22. Danishefsky's olefin metathesis studies towards the total synthesis of epothilones A and C [Ru] = $RuCl_2(=CHPh)(PCy_3)_2$] (Meng et al.).[109, 110]



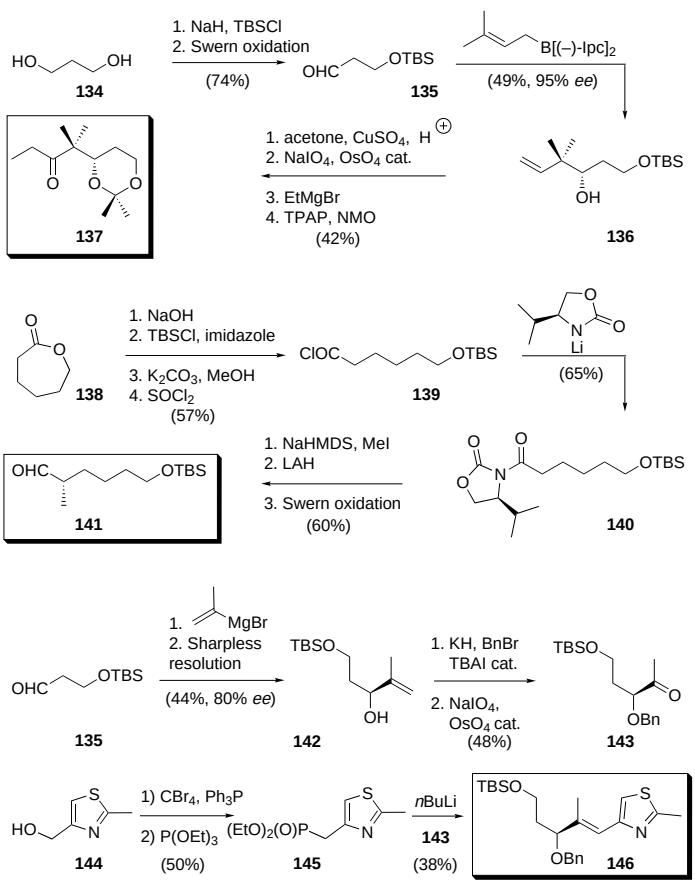
Scheme 23. Danishefsky's total synthesis of epothilones B (**2**) and D (**4**) by macroaldolization (Su et al. and Meng et al.).[108, 109]



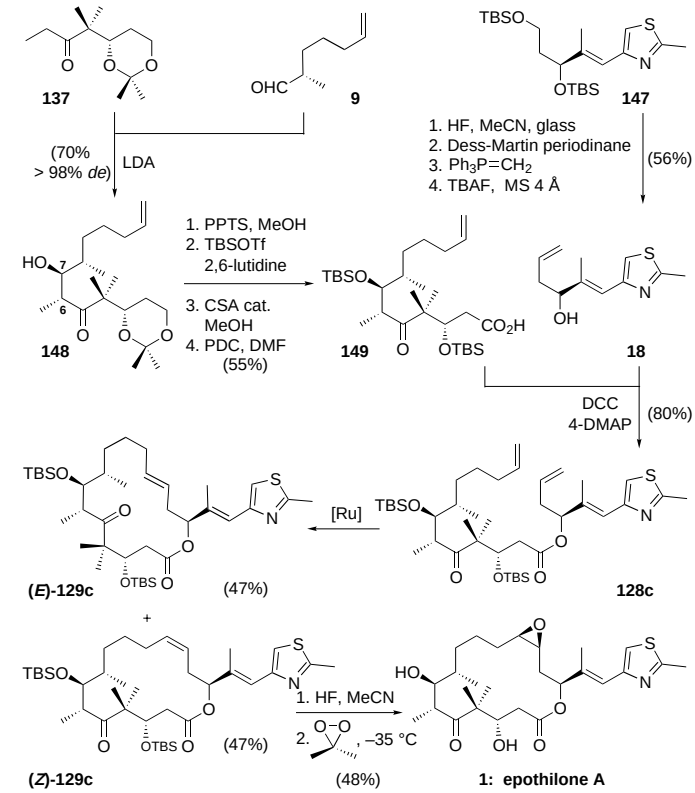
Scheme 24. Danishefsky's total synthesis of epothilones B (**2**) and D (**4**) by ring-closing olefin metathesis. [Mo] = [Mo(=CHMe$_2$Ph){N(2,6-(*i*Pr)$_2$C$_6$H$_3$)}(OCMe(CF$_3$)$_2$)$_2$] (Meng et al.).[109]

3.4. Miscellaneous Other Approaches to Epothilones

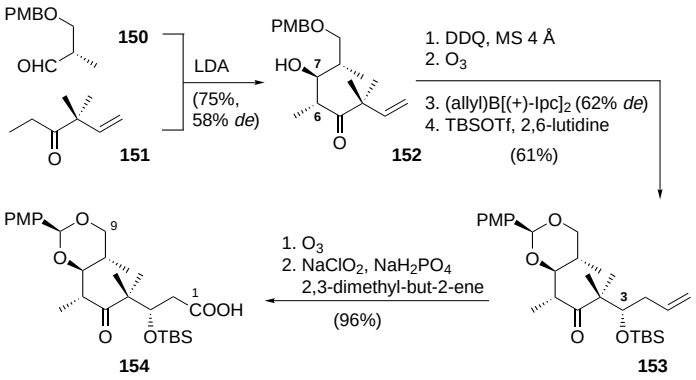
A number of other groups also made important contributions to the epothilone field, in terms of model studies and synthesis of key intermediates. Amongst them are those of Mulzer,[116, 117] Kalesse – Meyer,[118] Wessjohann,[119] Taylor,[120] and De Brabander.[121] Thus, by using elegant sequences, Mulzer et al.[116, 117] synthesized the C1 – C9 and C11 – C21 epothilone fragments **154** and **161** as shown in Schemes 27 and



Scheme 25. Schinzer's asymmetric synthesis of intermediates **137**, **141**, and **146** for the total synthesis of epothilone A (**1**) (Schinzer et al.).[112]

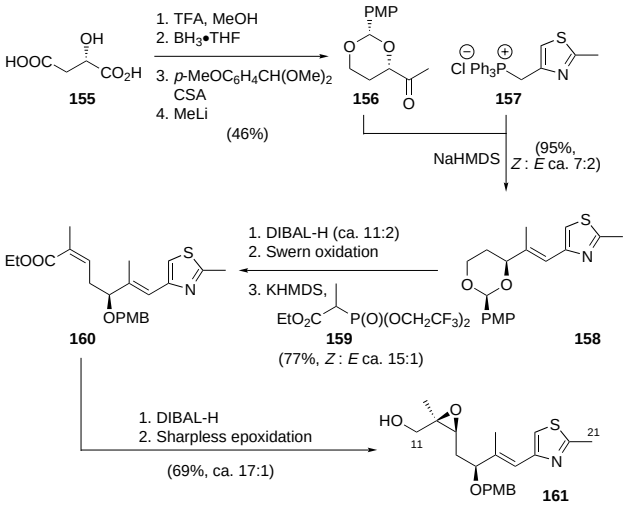


Scheme 26. Schinzer's total synthesis of epothilones A (**1**) and C (**3**) by olefin metathesis. [Ru] = [RuCl$_2$(=CHPh)(PCy$_3$)$_2$] (Schinzer et al.).[115]



Scheme 27. Mulzer's synthesis of the C1 – C9 epothilone fragment **154** (Mulzer et al.).[116]

28, respectively. The Kalesse – Meyer contribution[118] resulting in the asymmetric synthesis of building block **166** is shown

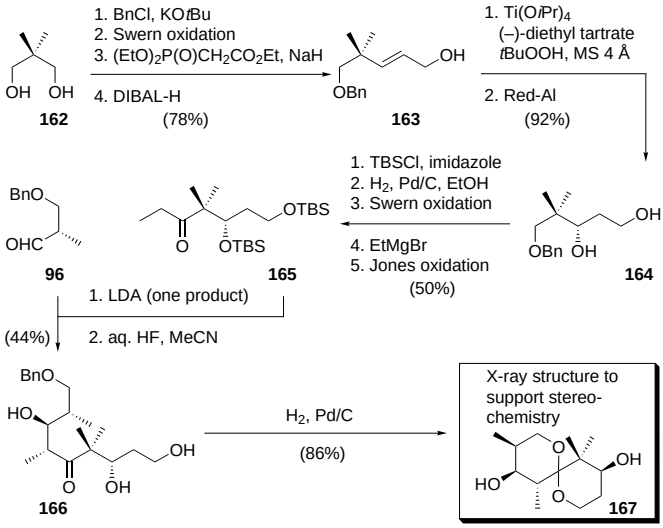


Scheme 28. Mulzer's synthesis of the C11 – C21 epothilone fragment **161** (Mulzer et al.).[117]

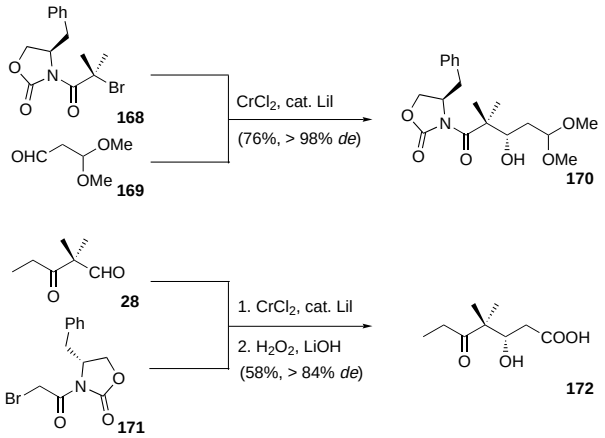
in Scheme 29. Based on their recently developed chromium(II)-mediated Reformatsky reaction, Gabriel and Wessjohann[119] devised routes to two different C1 – C5 fragments (**170** and **172**) of epothilones as outlined in Scheme 30. The work of Taylor and Haley[120] involved the asymmetric construction of the key thiazole-containing intermediate **35** and an olefin metathesis model study as summarized in Scheme 31. Asymmetric routes to key intermediates **9** (C7 – C11) and **31** (C1 – C6) were developed by De Brabander et al.[121] as depicted in Scheme 32.

4. Chemical Synthesis and Biological Properties of the Designed Epothilones

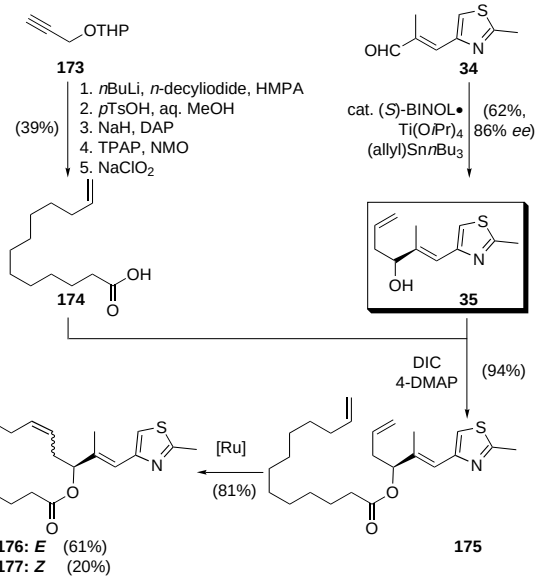
The successful crusade for the total synthesis of the natural epothilones paved the way for the chemical synthesis of a large number of designed epothilones for chemical biology studies. Thus, a new campaign began for the design, synthesis, and biological evaluation of epothilone libraries. Tables 2 – 6 list the analogues synthesized by the two principal groups



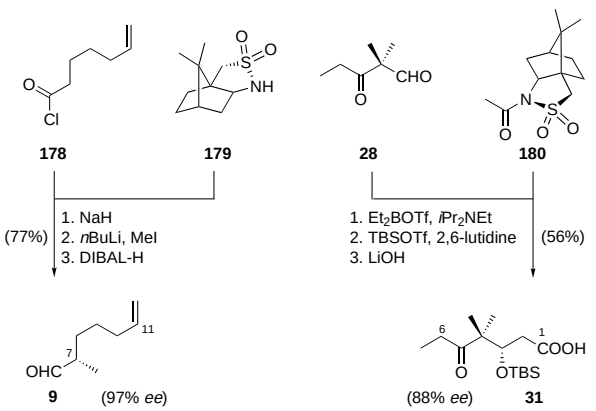
Scheme 29. The Kalesse – Meyer synthesis of the C1 – C9 epothilone fragment **166** (Claus et al.).[118]



Scheme 30. Wessjohann's application of the chromium-Reformatsky reaction for the asymmetric synthesis of C1 – C6 epothilone fragments **170** and **172** (Gabriel et al.).[119]

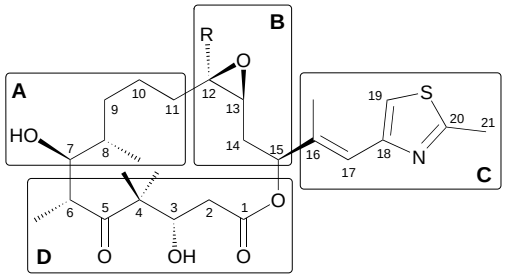


Scheme 31. Taylor's enantioselective synthesis of thiazole side chain **35** and olefin metathesis model studies. [Ru] = [$RuCl_2$(=CHPh)(PCy_3)$_2$] (Taylor et al.).[120]



Scheme 32. De Brabander's enantioselective synthesis of Nicolaou's key intermediates **9** and **31**(De Brabander et al.).[121]

involved in this campaign, namely ours and the Danishefsky group. The Höfle and Schinzer teams also made contributions to this area, even though their results remain, for the most part, unreported. For discussion purposes, the epothilone structure is divided into four regions, A – D, as shown in Scheme 33. The design of analogues was directed primarily at simplifying the molecular structure for purposes of gaining more-facile access to the target molecules and at answering

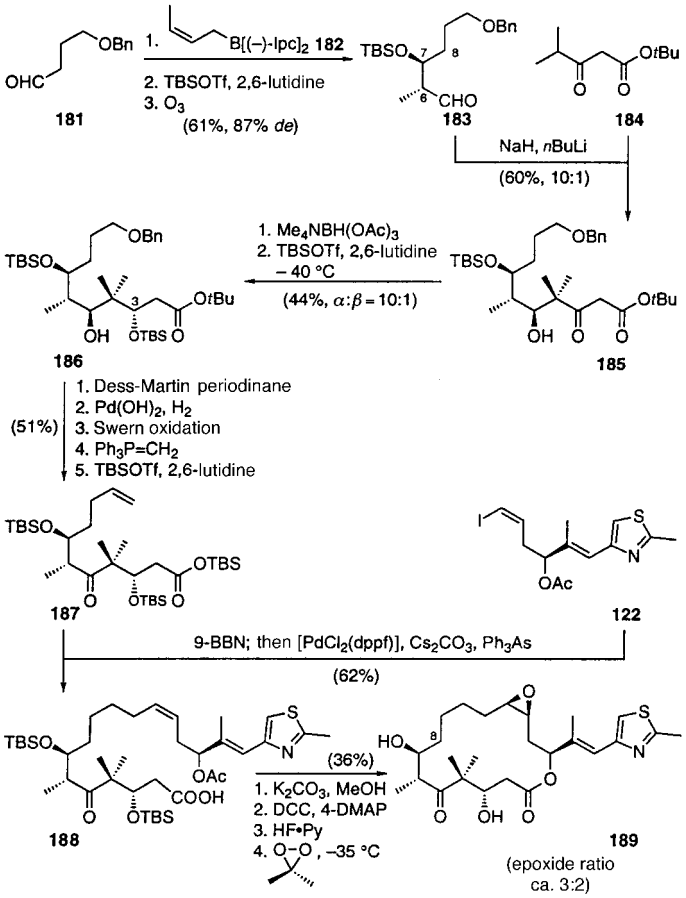


Scheme 33. Regions A – D of the epothilone molecule where structural variations were performed.

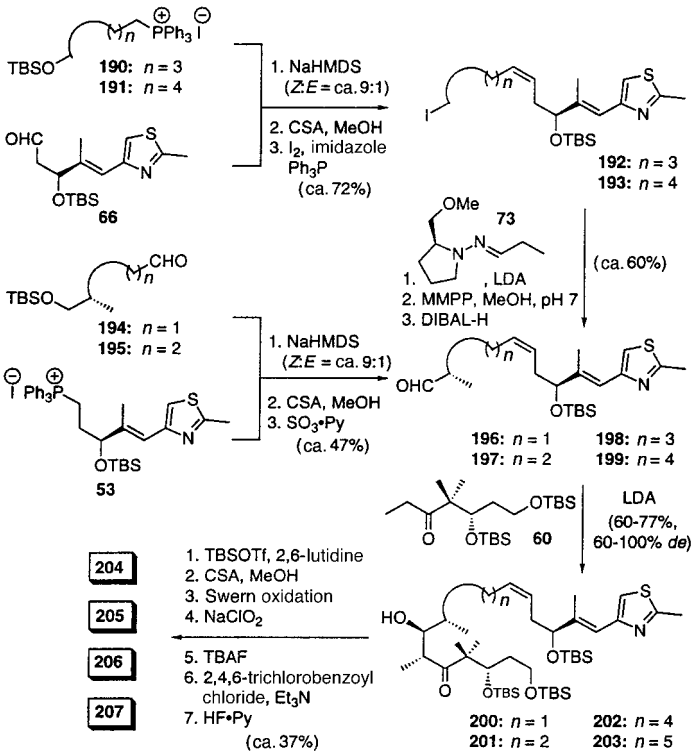
questions regarding the requirement of certain functionalities and stereochemical elements for activity, as well as the introduction of novel structural elements. To achieve the synthesis of these analogues, a number of novel methods and strategies were developed, as we will briefly discuss. The compounds shown in Tables 2 – 6 were constructed by applying the previously discussed methods, or by implementing the sequences demonstrated in Schemes 34 – 38.

4.1. Solution-Phase Synthesis of Epothilone Analogues

The solution-phase synthesis of 8-desmethylepothilone A (**189**); (region **A** modification) was accomplished by both Danishefsky et al. (macrolactonization approach)[122] and Nicolaou et al. (olefin metathesis approach).[102] The Danishefsky synthesis is summarized in Scheme 34. The 14-, 15-, 16-, and 17-membered ring desoxyepothilones (**204** – **207**) were synthesized by the Nicolaou group using the macro-

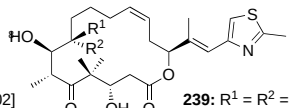
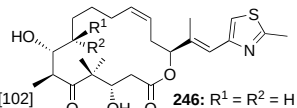
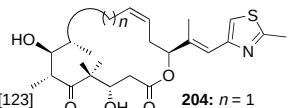
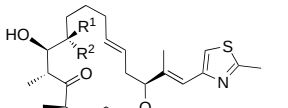
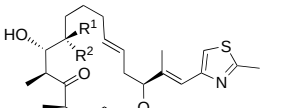
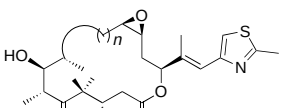
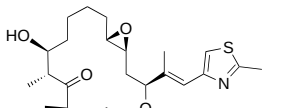
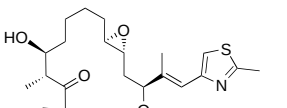
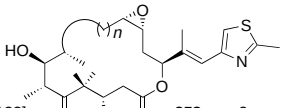
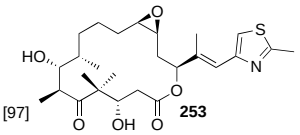
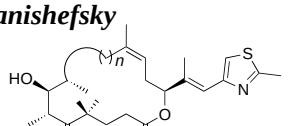
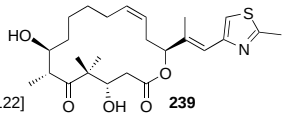
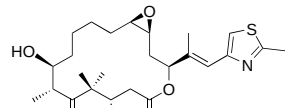


Scheme 34. Danishefsky's synthesis of 8-desmethylepothilone A (region A modification; see Table 2; Ballog et al.).[122]

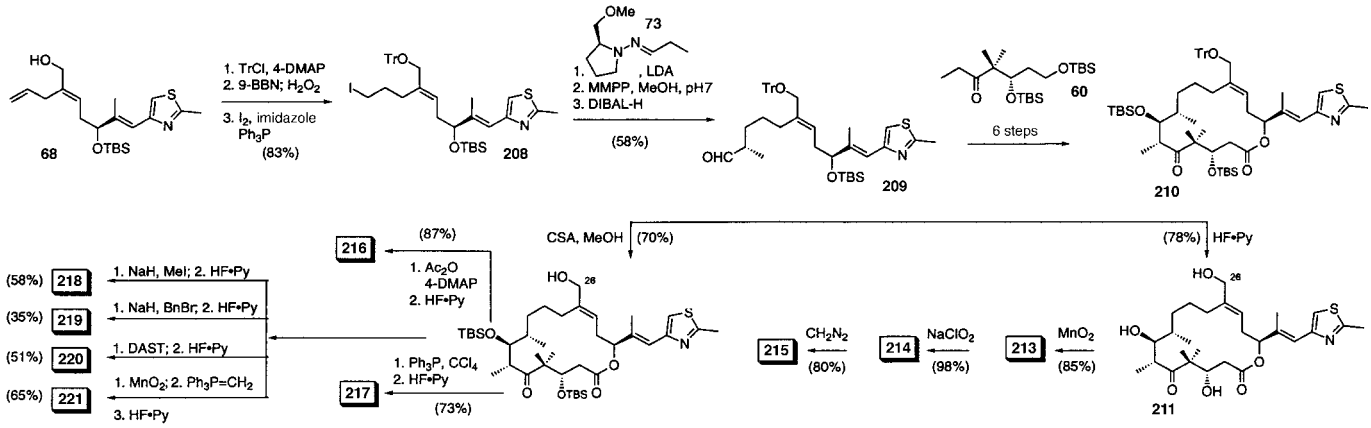


Scheme 35. Synthesis of 14-, 15-, 17-, and 18-membered epothilones A by the macrolactonization strategy (Region A modifications; see Table 2; Nicolaou et al.).[123]

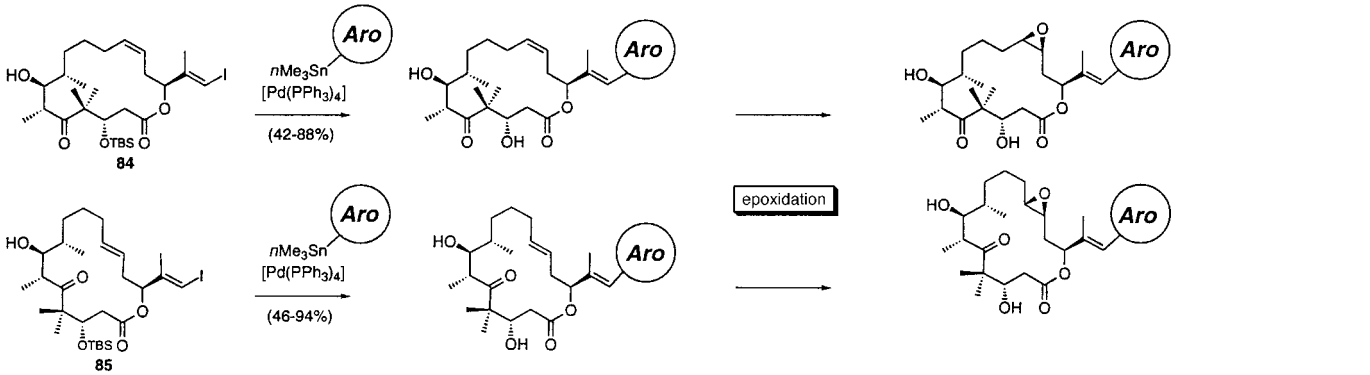
Table 2. Structures and tubulin polymerization properties of region A-modified epothilone analogues.

Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]
Nicolaou								
[102] [102] [102]		**239:** $R^1 = R^2 = H$ 23 **240:** R^1 = Me, $R^2 = H$ 11 **241:** $R^1 = R^2$ = Me 2	[102] [102] [102]		**246:** $R^1 = R^2 = H$ 23 **247:** R^1 = Me, $R^2 = H$ 8 **248:** $R^1 = R^2$ = Me 5	[123] [123] [123] [123]		**204:** $n = 1$ 6 **205:** $n = 2$ 9 **206:** $n = 4$ 12 **207:** $n = 5$ 41
[102] [102] [102]		**242:** $R^1 = R^2 = H$ 24 **243:** R^1 = Me, $R^2 = H$ 11 **244:** $R^1 = R^2$ = Me 3	[102] [102] [102]		**249:** $R^1 = R^2 = H$ 21 **250:** R^1 = Me, $R^2 = H$ 11 **251:** $R^1 = R^2$ = Me 4	[123] [123] [123] [123]		**254:** $n = 1$ 4 **255:** $n = 2$ 5 **256:** $n = 4$ 7 **257:** $n = 5$ 21
[102]		**245** 21	[102]		**252** 27	[123] [123] [123]		**258:** $n = 2$ 3 **259:** $n = 4$ 5 **260:** $n = 5$ 29
			[97]		**253** 25			
Danishefsky								
[30]		**205':** $n = 2$ –	[122]		**239** –	[30]		**189** –

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[15] and Nicolaou et al.[102]

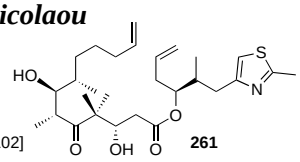
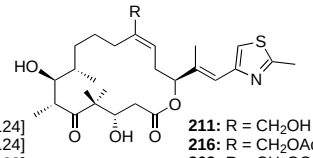
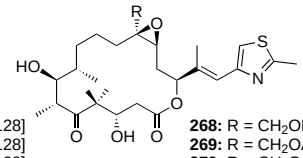
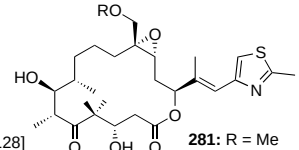
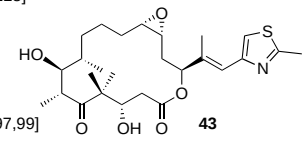
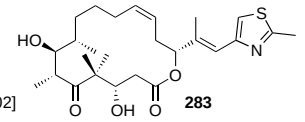
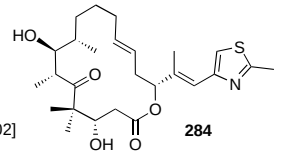
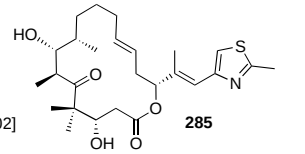
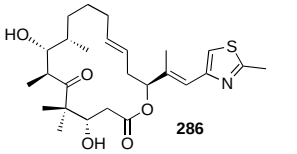
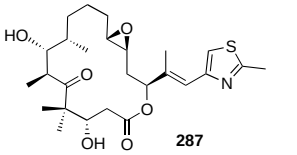
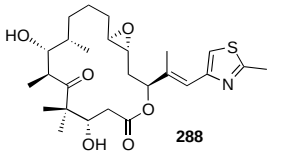
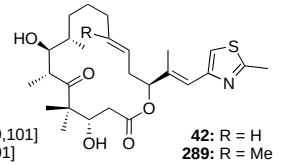
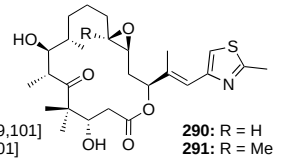
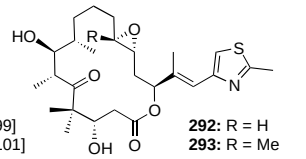
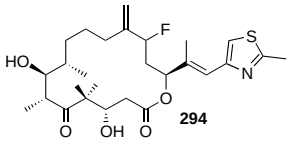
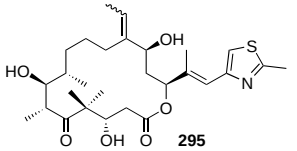
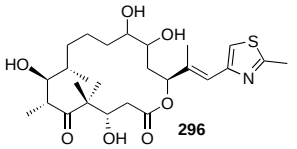
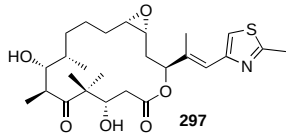
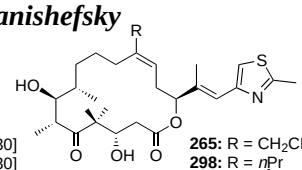
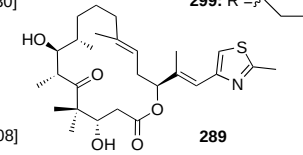
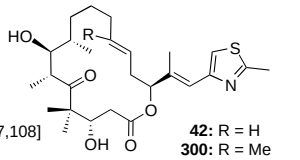
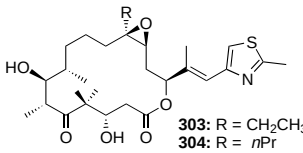
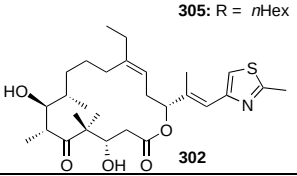


Scheme 36. Synthesis of C26-modified epothilones (region B modifications; see Table 3; Nicolaou et al.).[124]



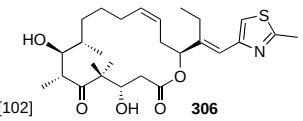
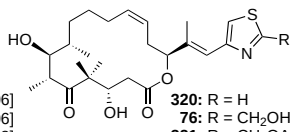
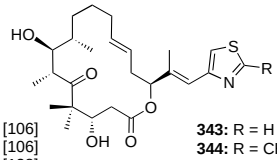
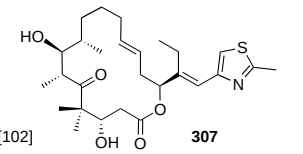
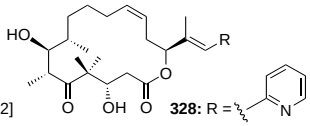
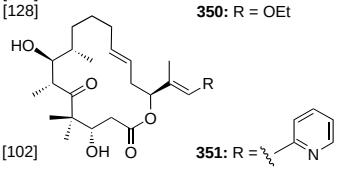
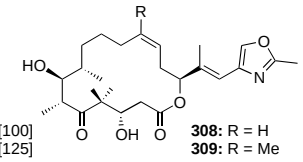
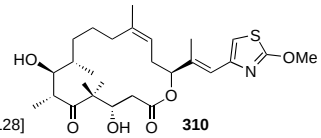
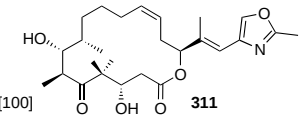
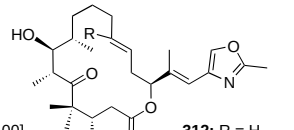
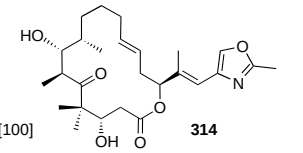
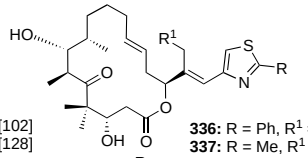
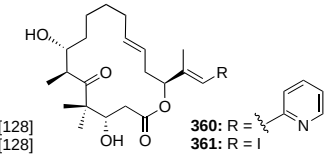
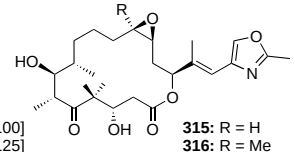
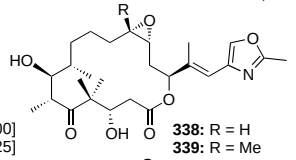
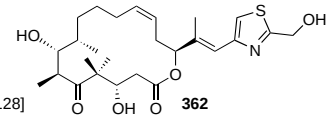
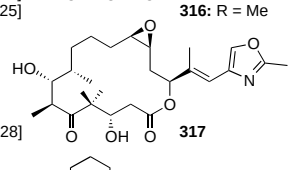
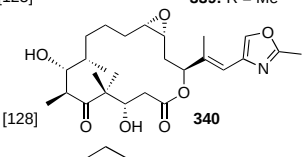
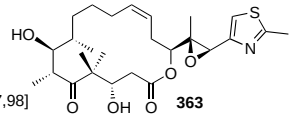
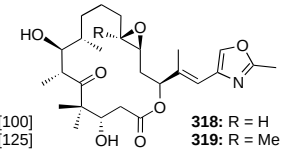
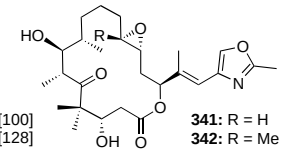
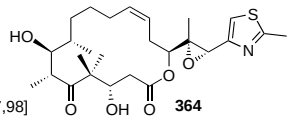
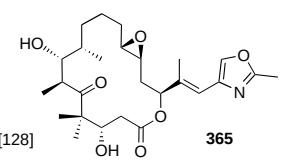
Scheme 37. Synthesis of side-chain modified epothilones by Stille coupling (region C modifications; see Table 4; Nicolaou et al).[106] Aro = aromatic group.

Table 3. Structures and tubulin polymerization properties of region B-modified epothilone analogues.

Ref.	Structure	Tubulin polymerization [%][a]
Nicolaou		
[102]	 **261**	18
[124]	 **211:** R = CH_2OH	52
[124]	**216:** R = CH_2OAc	66
[128]	**262:** R = $CH_2OC(O)tBu$	43
[128]	**263:** R = $CH_2OC(O)Ph$	69
[124]	**218:** R = CH_2OMe	47
[124]	**219:** R = CH_2OBn	62
[124]	**220:** R = CH_2F	83
[124]	**217:** R = CH_2Cl	88
[128]	**264:** R = CH_2I	11
[128]	**265:** R = CH_2CH_3	66
[128]	**266:** R = CH_2NHAc	10
[128]	**221:** R = $CH{=}CH_2$	95
[128]	**267:** R = $C{\equiv}CH$	81
[124]	**213:** R = CHO	64
[124]	**214:** R = CO_2H	12
[124]	**215:** R = CO_2Me	12
[128]	 **268:** R = CH_2OH	29
[128]	**269:** R = CH_2OAc	51
[128]	**270:** R = $CH_2OC(O)tBu$	6
[128]	**271:** R = $CH_2OC(O)Ph$	5
[128]	**272:** R = CH_2OMe	6
[128]	**273:** R = CH_2OBn	12
[128]	**274:** R = CH_2F	93
[128]	**275:** R = CH_2Cl	69
[128]	**276:** R = CH_2I	41
[128]	**277:** R = CH_2CH_3	79
[128]	**278:** R = $CH{=}CH_2$	94
[128]	**279:** R = CHO	87
[128]	**280:** R = CO_2Me	19
[128]	 **281:** R = Me	2
[128]	**282:** R = Ac	25
[97,99]	 **43**	17
[102]	 **283**	10
[102]	 **284**	10
[102]	 **285**	15
[97]	 **286**	13
[97]	 **287**	19
[97]	 **288**	16
[97,99,101] [99,101]	 **42:** R = H **289:** R = Me	76 72
[97,99,101] [99,101]	 **290:** R = H **291:** R = Me	92 84
[97,99] [99,101]	 **292:** R = H **293:** R = Me	17 63
[128]	 **294**	29
[128]	 **295**	31
[102]	 **296**	12
[97]	 **297**	20
Danishefsky		
[30]	 **265:** R = CH_2CH_3	–
[30]	**298:** R = *n*Pr	–
[30]	**299:** R = 	–
[108]	 **289**	–
[107,108]	 **42:** R = H	–
[30]	**300:** R = Me	–
[30]	**301:** R = *n*Pr	–
[30]	 **303:** R = CH_2CH_3	–
[30]	**304:** R = *n*Pr	–
[30]	**305:** R = *n*Hex	–
[30]	 **302**	–

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[15] and Nicolaou et al.[102]

Table 4. Structures and tubulin polymerization properties of region C-modified epothilone analogues.

Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]
Nicolaou								
[102]	 **306**	34	[106]	 **320:** R = H	31	[106]	 **343:** R = H	41
			[106]	**76:** R = CH_2OH	34	[106]	**344:** R = CH_2OH	40
			[128]	**321:** R = CH_2OAc	2	[128]	**345:** R = CH_2OAc	1
			[128]	**322:** R = CH_2F	57	[106]	**346:** R = $(CH_2)_5OAc$	2
			[106]	**323:** R = $(CH_2)_5OAc$	3	[106]	**347:** R = Piperidyl	5
			[106]	**324:** R = Piperidyl	18	[106]	**348:** R = SMe	71
			[106]	**325:** R = SMe	92	[102]	**349:** R = Ph	16
			[102]	**326:** R = Ph	25	[128]	**350:** R = OEt	2
			[128]	**327:** R = OEt	3			
[102]	 **307**	48	[102]	 **328:** R =	51	[102]	 **351:** R =	61
[100]	 **308:** R = H	75	[106]	**329:** R = 	13	[106]	**352:** R =	2
[125]	**309:** R = Me	93	[106]	**330:** R = 	16	[106]	**353:** R =	26
[128]	 **310**	62	[106]	**331:** R = 	34	[106]	**354:** R =	2
			[106]	**332:** R = 	63	[106]	**355:** R =	57
[100]	 **311**	18	[106]	**333:** R = 	4	[106]	**356:** R =	1
			[106]	**334:** R = 	6	[106]	**357:** R =	2
[100]	 **312:** R = H	43	[128]	**335:** R = 	1	[128]	**358:** R =	1
[125]	**313:** R = Me	54	[106]	**77:** R = I	26	[106]	**359:** R = I	2
[100]	 **314**	20	[102]	 **336:** R = Ph, R^1 = H	12	[128]	 **360:** R =	8
			[128]	**337:** R = Me, R^1 = Me	39	[128]	**361:** R = I	21
[100]	 **315:** R = H	58	[100]	 **338:** R = H	24	[128]	 **362**	8
[125]	**316:** R = Me	93	[125]	**339:** R = Me	71			
[128]	 **317**	21	[128]	 **340**	14	[97,98]	 **363**	20
[100]	 **318:** R = H	64	[100]	 **341:** R = H	46	[97,98]	 **364**	9
[125]	**319:** R = Me	95	[128]	**342:** R = Me	71			
[128]	 **365**	6	[128]	**369**	8	[97]	**371**	22

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[15] and Nicolaou et al.[102]

Table 4. (Continued).

Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]
[97]	**366**	12	[97]	**370**	7	[97]	**372**	16
[97]	**367**	23				[97]	**373**	14
[97]	**368**	22				[97]	**374**	26
Danishefsky								
[30]	**308**	–	[30]	**376**	–	[30]	**377**	–
[30]	**375**	–				[30]	**378**	–

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[15] and Nicolaou et al.[102]

lactonization strategy as outlined in Scheme 35,[123] while the Danishefsky group reported the 15-membered desoxyepothilone B (**205′**).[30] These compounds and their epoxidized counterparts are represented in Table 2.

A stereoselective entry into a series of desoxyepothilones substituted in position 26 (region **B**) were synthesized in our group utilizing the macrolactonization strategy as demonstrated in Scheme 36 for compounds **213**–**221**.[124] Most of

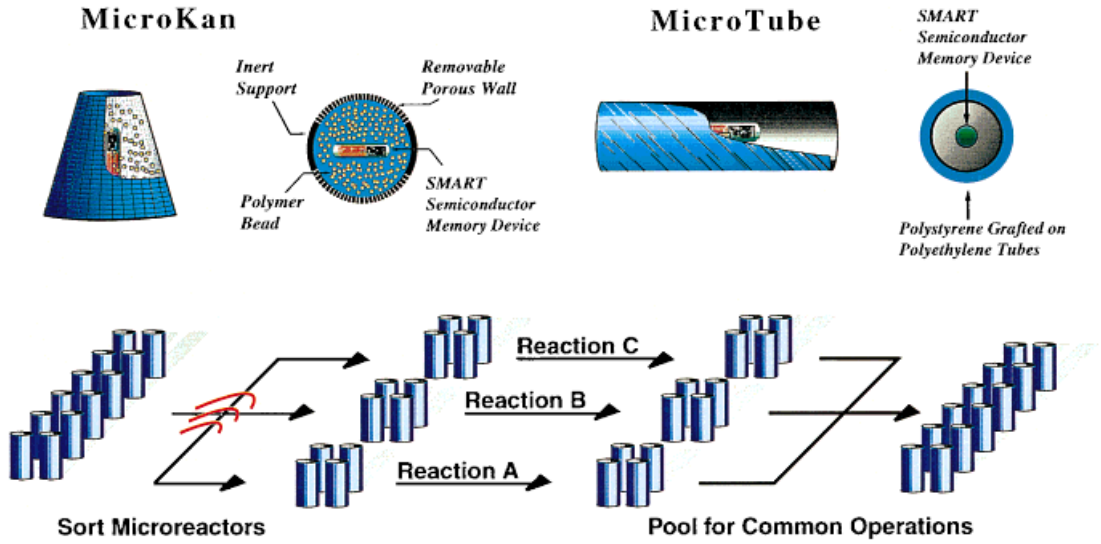
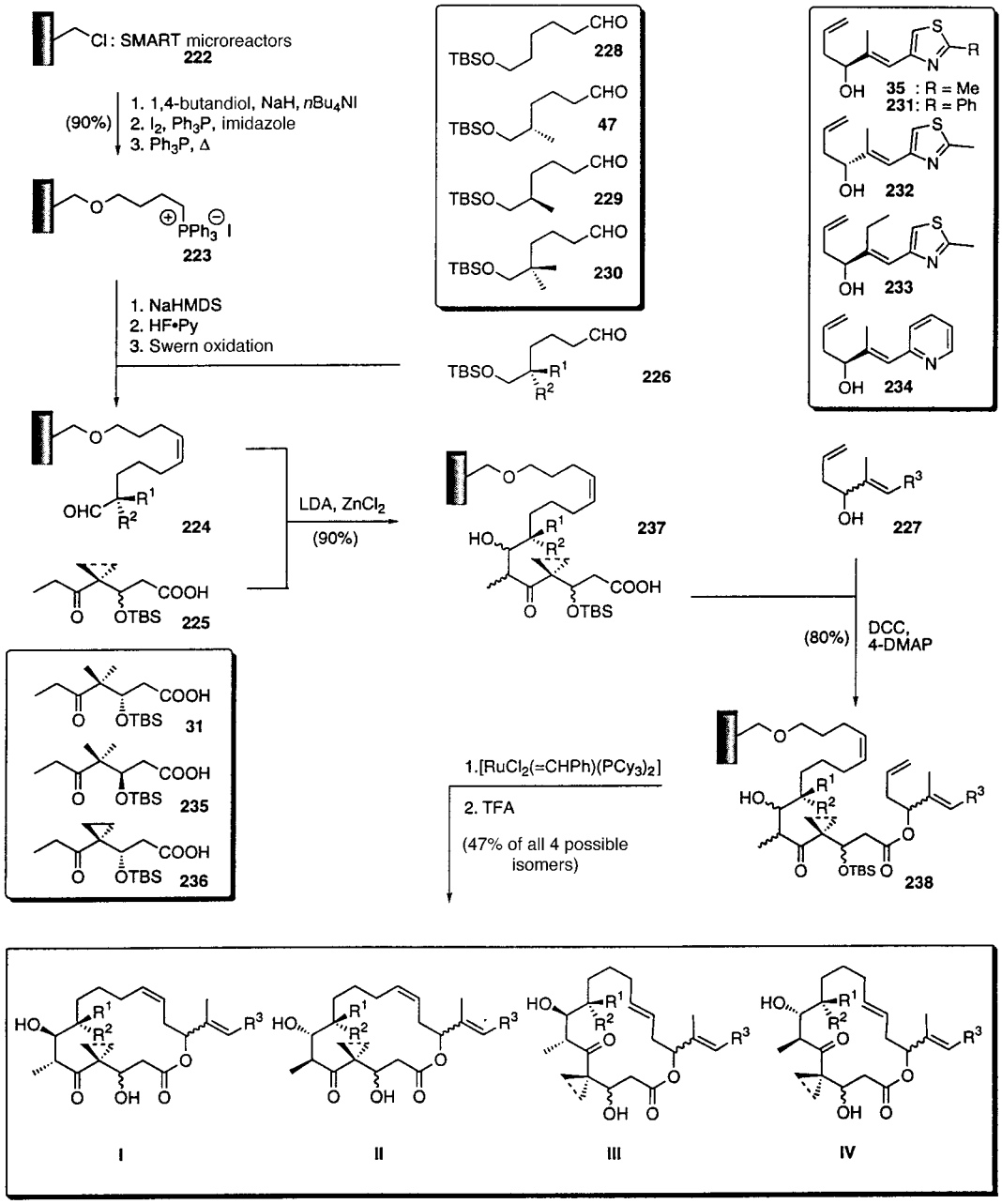


Figure 9. Top: SMART microreactores. Bottom: general strategy for the radiofrequency encoded pool-split combinatorial synthesis of epothilones (Nicolaou et al.).[102, 103]



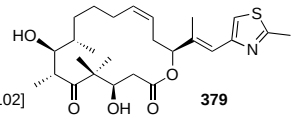
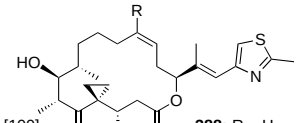
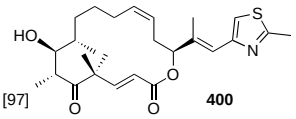
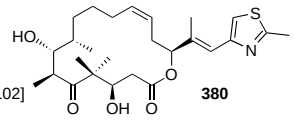
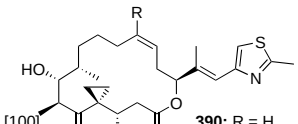
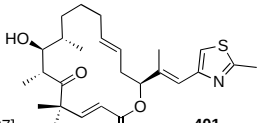
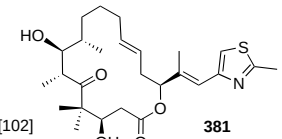
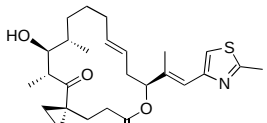
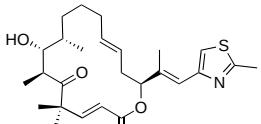
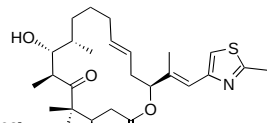
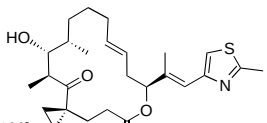
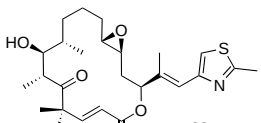
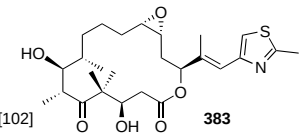
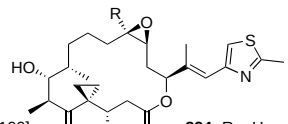
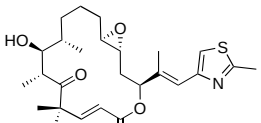
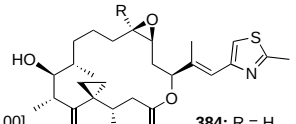
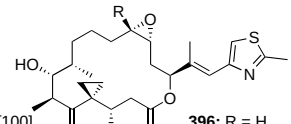
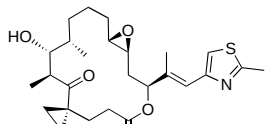
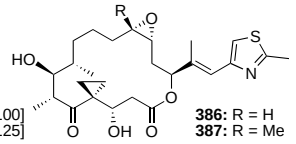
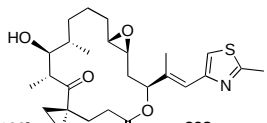
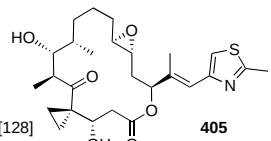
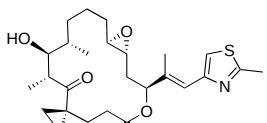
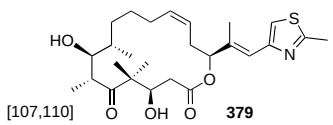
Scheme 38. Synthesis of epothilone libraries by split-pool-REC (modifications in regions **A**–**D** (seen Tables 2–6; Nicolaou et al.).[102]

these materials were further elaborated to the corresponding epoxides and are listed in Table 3. The olefin metathesis/Stille coupling strategy developed for epothilone E (see Scheme 15 above) was applied to the synthesis of a number of side-chain epothilone analogues (region **C**) as generalized in Scheme 37.[106] The use of a late-stage Stille coupling in this strategy allowed convergent and rapid access to several interesting compounds of this family (Table 4).

4.2. Solid-Phase Synthesis of Combinatorial Libraries of Epothilones

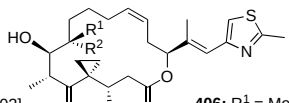
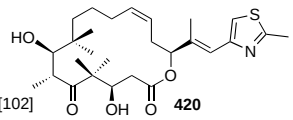
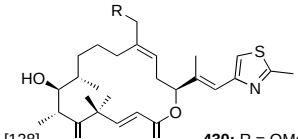
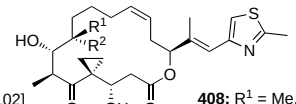
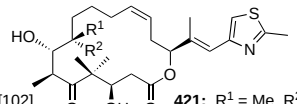
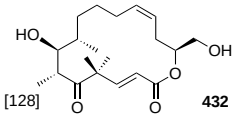
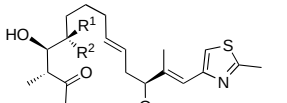
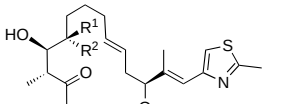
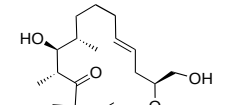
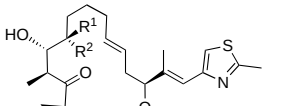
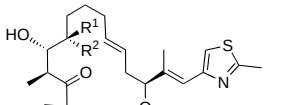
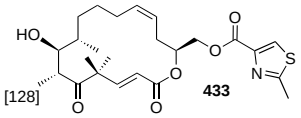
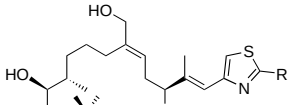
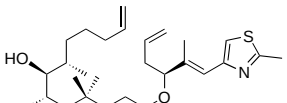
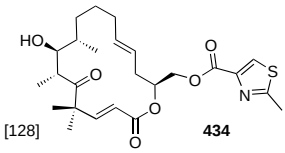
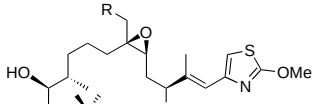
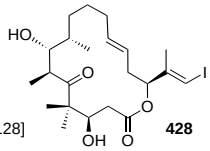
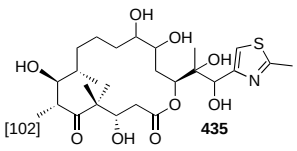
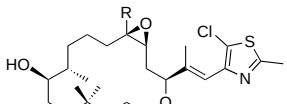
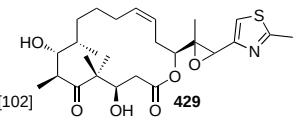
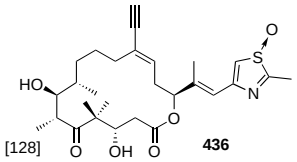
Our group applied the solid-phase radiofrequency-encoded chemistry (REC)[103] pool-split strategy to combinatorial synthesis for the construction of an epothilone library using both Microkans and Microtubes (Figure 9).[102] This approach resulted in the rapid assembly of several epothilone analogues with modifications in regions **A**–**D** (Scheme 38). Furthermore, this chemistry demonstrated a new concept for natural product synthesis and library generation. Particularly appealing was the ability to employ the olefin metathesis reaction to form and simultaneously cleave the macrocycle (desoxyepothilones **I**–**IV**) from the solid support. The epothilones so generated were chromatographically separated and epoxidized to yield a total of eight final epothilones (two diastereomeric *syn* aldol products times the two olefin diastereoisomers (*Z* and *E*) times two diastereomeric epoxides) from each microreactor, as discrete compounds and in milligram quantities.

Table 5. Structures and tubulin polymerization properties of region D-modified epothilone analogues.

Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]
Nicolaou								
[102]	 **379**	16	[100] [125]	 **388:** R = H **389:** R = Me	17 22	[97]	 **400**	34
[102]	 **380**	23	[100] [125]	 **390:** R = H **391:** R = Me	13 5	[97]	 **401**	58
[102]	 **381**	21	[100]	 **392**	25	[97]	 **402**	20
[102]	 **382**	24	[100]	 **393**	18	[97]	 **22**	46
[102]	 **383**	25	[100] [125]	 **394:** R = H **395:** R = Me	10 27	[97]	 **403**	28
[100] [125]	 **384:** R = H **385:** R = Me	20 19	[100] [125]	 **396:** R = H **397:** R = Me	9 18	[100]	 **404**	3
[100] [125]	 **386:** R = H **387:** R = Me	6 18	[100]	 **398**	31	[128]	 **405**	1
			[100]	 **399**	18			
Danishefsky								
[107,110]	 **379**							

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[15] and Nicolaou et al.[102]

Table 6. Structures and tubulin polymerization properties of multiple regions-modified epothilone analogues.

Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]	Ref.	Structure	Tubulin polymerization [%][a]
Nicolaou								
[102]	 **406:** R^1 = Me, R^2 = H	5	[102]	 **420**	3	[128]	 **430:** R = OMe	20
[102]	**407:** R^1 = R^2 = Me	4				[128]	**431:** R = OBn	8
[102]	 **408:** R^1 = Me, R^2 = H	1	[102]	 **421:** R^1 = Me, R^2 = H	5	[128]	 **432**	4
[102]	**409:** R^1 = R^2 = Me	5	[102]	**422:** R^1 = R^2 = Me	1			
[102]	 **410:** R^1 = Me, R^2 = H	9	[102]	 **423:** R^1 = Me, R^2 = H	3	[128]	 **13**	17
[102]	**411:** R^1 = R^2 = Me	5	[102]	**424:** R^1 = R^2 = Me	4			
[100]	 **412:** R^1 = Me, R^2 = H	5	[102]	 **425:** R^1 = Me, R^2 = H	7	[128]	 **433**	1
[102]	**413:** R^1 = R^2 = Me	4	[102]	**426:** R^1 = R^2 = Me	5			
[128]	 **414:** R = CH_2F	11	[128]	 **427**	18	[128]	 **434**	1
[128]	**415:** R = OMe	25						
[128]	 **416:** R = OH	9	[128]	 **428**	17	[102]	 **435**	13
[128]	**417:** R = F	91						
[128]	 **418:** R = CH_2OH	16	[102]	 **429**	7	[128]	 **436**	17
[128]	**419:** R = CHO	5						

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[15] and Nicolaou et al.[102]

4.3. Structure – Activity Relationships of Epothilones

As we have seen, chemical synthesis allowed the preparation of a large number of designed epothilones. Selected libraries of these compounds are given in Tables 2 – 6, together with tubulin polymerization data. Tables 7 and 8 list cytotoxicity results for a number of highly active compounds identified from screening of these libraries. Figure 10 summarizes the conclusions of these investigations regarding the structural requirements for biological activity within the epothilone structure.

Inspection of Tables 2 – 6 reveals the effect on biological activity of structural changes by region (**A** – **D**, Scheme 33). Thus, as seen from Table 2, modifications in region **A** (C8 – C11), such as ring size (with the exception of the 18-membered epothilone A (**206**) that exhibited significant tubulin polymerization activity), change of the C8 stereochemistry, and addition or removal of a methyl group at C8 resulted in considerable loss of biological action.

In contrast, changes in region **B** (C12 – C15) are well tolerated (see Table 3). With regards to the C12 – C13 functionality, it was of interest to find that:

- Both the olefin and the epoxide were active.
- Both epoxide stereoisomers exhibited high activity.
- Changing the geometry of the double bond had little effect on the activity of the desoxyepothilones or their epoxide counterparts.

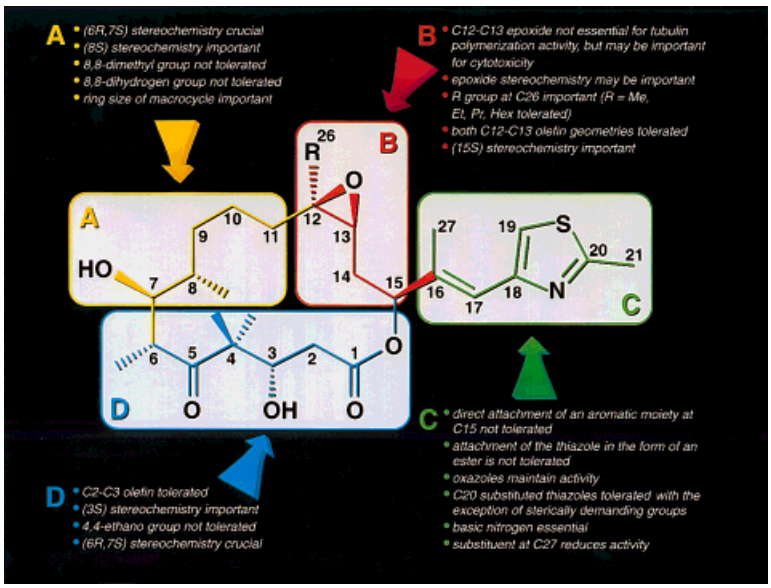


Figure 10. Structure – activity relationship of the epothilones.

Since epothilone B was more active than A, the role of the C12 substituent was studied extensively. The majority of C12 analogues synthesized were very active—some rivaling epothilone B—demonstrating the potential of such modifications in finding a suitable drug candidate. Finally, inversion of the C15 configuration led to a significant decrease of tubulin polymerization activity.

Region **C** modified compounds are exhibited in Table 4 and reveal less tolerance than region **B** analogues. Specifically, depletion or direct attachment of the aromatic moiety at C15, as well as replacement of the C20 methyl group with bulkier alkyl or aryl substituents, resulted in a loss of tubulin binding and cytotoxic properties. Hydrogen atom or thiomethyl substitution at C19 was, however, well tolerated. Furthermore, replacement of the C16 methyl with an ethyl group, and of the thiazole by a number of structurally diverse aromatic moieties turned out to be, in general, detrimental to biological activity. Notable exceptions were the corresponding oxazoles,[100, 125] 2-pyridyl-[102] and 2-thiazyl-containing[106] compounds, which exhibited properties comparable to the natural epothilones.

Modification in region **D** led to interesting findings as presented in Table 5. For example, inversion of the C3 stereochemistry resulted in significantly reduced potency, as did substitution of the C4 *gem* dimethyl group with a 4,4-ethano moiety. Similar loss of activity was observed, when the C5 ketone was reduced, or when the C5, C6 and C7 substituents were removed. Interestingly, however, when an *E* olefin was introduced at C2 – C3, the resulting compound retained considerable potency. Table 6 lists a series of compounds in which modifications were attempted simultaneously in more than one region (**A** – **D**) of the epothilone structure. Thus far, results from such concurrent changes have not yielded any breakthroughs in term of bioactive compounds.

The cytotoxicity data shown in Tables 7 and 8 (next page) for a number of potent epothilone analogues compare well with those of the natural epothilones and hold considerable promise as lead compounds and drug candidates. As in the case of epothilones A and B, the exciting aspect of these designed analogues resides in their ability to act against drug-resistant tumor cells, including taxol-, vinblastine- and etoposide-resistant cell lines. The idea of in vivo epoxidation of desoxyepothilones is an intriguing hypothesis, which may explain the observation that some of these compounds exhibited strong cytotoxic properties, while others were less active, despite their potent tubulin-polymerizing properties.

Since it was suggested that epothilones and taxol share a common pharmacophore,[12, 16] Winkler and Axelsen proposed a model for the active sites common to these structurally dissimilar substances in 1996.[126] They based their molecular mechanics calculations on structure-activity relationship data of taxol, indicating which parts of this material appear to be essential for biological activity. More recently, Ojima reported similar molecular modeling studies attempting to correlate conformations of taxol analogues, epothilones, and discodermolide.[127]

Using the naturally derived epothilones A – D, the Höfle group in Germany constructed approximately 100 derivatives and studied their biological action.[134] Figure 10 summarizes a number of conclusions on the structure – activity relationships (SARs) drawn on the basis on the chemical biology studies, carried out so far on the epothilones. New designs can now be directed towards those modifications that look most promising in the quest for suitable candidates for drug development.

5. Summary and Future Perspectives

Coming at the heels of the taxol era, whose development as a billion dollar drug was accompanied by a wealth of new scientific knowledge, the epothilones burst onto the scene as the most promising new candidates for cancer chemotherapy of the 1990s. Particularly attractive is their taxol-like mechanism of action and their superiority over taxol in causing death to taxol-resistant tumor cells. Their novel, but in comparison to taxol, less complex molecular structure

Table 7. Biological properties[a] of taxol, the natural epothilones, and selected synthetic epothilone analogues. (Nicolaou et al.).[102,128]

Screening	Induction of tubulin polymerization		Inhibition of carcinoma cell growth[b]			
	Quant. assay[a]		Ovarian[d]		Breast[e]	
	polymer	Glutamate assay[c]	Parental 1A9	β-tubulin mutations 1A9PTX10	1A9PTX22	MCF7
Compound	formed [%][f]	EC_{50} [μM]	IC_{50} [nM]	IC_{50} [nM]	IC_{50} [nM]	
taxol	50	4.7	1.4	32	38	4.2
epothilone A (**1**)	76	4.6	2.2	20	5.9	5.1
epothilone B (**2**)	98	3.4	0.13	1.0	0.31	1.0
epothilone C (**3**)	72	8.3	32	> 100	100	38
epothilone D (**4**)	94	3.9	6.5	23	9.0	9.3
epothilone E (**5**)	95	–	> 100	50	20	–
22	46	–	32	> 100	> 100	–
42	76	9.8	60	> 100	100	> 100
211	52	–	40	80	> 100	–
217	88	–	80	> 100	> 100	–
218	47	–	30	> 100	65	–
219	62	–	18	65	> 100	–
220	83	–	0.5	7.5	6	–
221	95	–	5	30	20	–
263	69	–	40	> 100	> 100	–
268	29	–	50	30	90	–
269	51	–	20	7	40	–
270	6	–	80	20	95	–
274	93	–	0.2	0.4	0.2	–
275	69	–	0.4	0.6	0.25	–
276	41	–	10	50	10	–
278	94	–	0.05	1.0	0.05	–
279	87	–	5	30	5	–
289	84	7.5	61	> 100	85	75
290	92	6.2	2.0	18	3.0	5.4
291	84	5.6	1.0	8.5	1.0	1.8
293	63	13	6.0	30	6.5	14
295	31	–	50	50	> 100	–
308	75	6.1	68	> 100	90	74
309	93	3.3	8.0	30	12	> 100
313	54	6.0	32	> 100	> 100	68
315	58	5.3	3.0	25	8.0	6.1
316	93	–	0.12	1.1	–	–
318	64	7.8	3.5	32	9.5	> 100
325	92	–	9	22	28	–
328	51	7.6	32	> 100	70	57
332	63	–	10	28	25	–
339	71	6.1	1.5	11	3.0	6.2
341	46	8.1	4.8	34	9.0	5.7
342	71	–	8	65	17	–
343	41	–	20	> 100	45	–
348	71	–	15	> 100	20	–
351	61	11	82	> 100	> 100	78

[a] See Table 1. [b] Cell growth was evaluated by measurement of the increase in cellular protein.[129] [c] Assay performed according to literature procedures.[102, 130] The EC_{50} value is defined as the drug concentration resulting from a 50% reduction in supernatant protein relative to control values. [d] The parental ovarian line, derived as a clone of line A2780,[131] was used to generate taxol-resistant cell lines by incubating the cells with increasing concentrations of taxol with verapamil;[132] the cells were grown in the presence of drug for 96 h. [e] The MCF7 cells were obtained from the National Cancer Institute drug screening program;[133] cells were grown in the presence of the drug for 48 h. [f] Polymer formed relative to that formed with GTP.

prompted intense research activities with the intention of developing practical synthetic routes for their production, and of synthesizing analogues for biological evaluation. Both objectives were met to a large extent by a number of laboratories, even though much remains to be done. Amongst the challenges remaining are:

- Synthetic routes even more efficient than the ones so far developed, which can be adapted for large scale production.
- Determination of the pharmacological and toxicological profiles of the natural epothilones and selected synthetic analogues in a variety of animal models for choosing suitable drug candidates.
- Clinical evaluation of such candidate compounds for the treatment of a variety of cancers.

In addition to their prospects as new weapons against cancer, these naturally occurring substances have already stimulated new inventions and discoveries in the areas of chemistry and biology. If epothilone B elicited our admiration by virtue of its novel structure and biological action, our failure thus far to surpass its potency against tumor cells left us

Table 8. Biological properties[a] of taxol, the natural epothilones, and selected synthetic epothilone analogues (Danishefsky et al.).[30]

Compound	parental CCRF-CEM IC_{50} [nM]	CCRF-CEM/VBL IC_{50} [nM]	CCRF-CEM/VM_1 IC_{50} [nM]
taxol	2.0	4140	2.0
epothilone A (**1**)	3.0	20	3.0
epothilone B (**2**)	0.2	1.0	2.0
epothilone C (**3**)	22	12	13
epothilone D (**4**)	9.0	17	14
42	52	35	111
265	21	77	–
298	39	67	–
299	3.0	9.0	–
300	90	262	94
301	90	254	–
302	55	197	–
303	1.0	7.0	–
304	4.0	6.0	–
305	27	49	–
308	30	49	–
375	98	146	–

[a] The cytotoxicities of the tested compounds were determined by the growth of human lymphoblastic leukemic cells CCRF-CEM, or their sublines resistant to vinblastine and taxol (CCRF-CEM/VBL) or resistant to etoposide (CCRF-CEM/VM-1).

in awe of nature's exquisite molecular engineering abilities. While natural evolution through combinatorial chemistry took nature millions of years to reach this lethal weapon, synthetic chemists should be able to accelerate this process by imagination and logical design and arrive at even more appropriate candidates for selective medical intervention. It is expected that by summarizing the results in the field, this review article will encourage and facilitate further research and development. Our hope is that the chapter on the chemistry and biology of epothilones will soon expand to include medicine as well. Since the submission of this review the following relevant articles appeared in the literature (up to February 28, 1998).[135–137]

Appendix: List of Abbreviations

Ac	acetyl
AIBN	2,2′-azobisisobutyronitrile
9-BBN	9-borabicyclo[3.3.1]nonane
BINOL	1,1-bi-2-naphthol
Bn	benzyl
BOM	benzyloxymethyl
CSA	10-camphorsulfonic acid
Cy	cyclohexyl
DAP	1,3-diaminopropane
DAST	(diethylamino)sulfur trifluoride
DCC	1,3-dicyclohexyl carbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHP	3,4-dihydro-2*H*-pyran
DIBAL-H	diisobutylaluminum hydride
4-DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
dppf	1,1′-bis(diphenylphosphanyl)ferrocene
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
HMDS	bis(trimethylsilyl)amide
HMPA	hexamethylphosphoramide
Ipc	isopinocampheyl
LAH	lithium aluminum hydride
LDA	lithium diisopropyl amide
*m*CPBA	*m*-chloroperoxybenzoic acid
MMPP	monoperoxyphthalic acid, magnesium salt
MOM	methoxymethyl
Ms	methanesulfonyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMO	4-methylmorpholine *N*-oxide
OTf	trifluoromethanesulfonate
PDC	pyridinium dichromate
PMB	*p*-methoxybenzyl
PMB-Cl	*p*-methoxybenzylchloride
PPTS	pyridinium *p*-toluenesulfonate
Py	pyridine
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBS	*tert*-butyldimethylsilyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TMS	trimethylsilyl
TPAP	tetra-*n*-propylammonium perruthenate
Tr	triphenylmethyl
TsOH	*p*-toluenesulfonic acid

We are pleased to acknowledge the important contribution of our collaborators on this project, whose names appear in the original publications. We are grateful to Prof. Höfle for bringing the epothilone story to our attention and for his encouragement, and to Professor S. J. Danishefsky for helpful discussions. This work was supported by The Skaggs Institute for Chemical Biology, the CaPCURE Foundation, Novartis, the National Institutes of Health, USA, and the Deutsche Forschungsgemeinschaft (fellowship to F.R.). We also wish to thank our friends from industry (Merck Sharp & Dohme, Dupont-Merck, Pfizer, Schering-Plough, Amgen, and Glaxo Wellcome) for their support of our programs.

Received: January 8, 1998 [ZA269IE]
German version: *Angew. Chem.* **1998**, *110*, 2120 – 2153

[1] a) C. S. Muir, *Cancer Res.* **1990**, *50*, 6441 – 6448; b) B. N. Ames, L. S. Gold, *Angew. Chem.* **1990**, *102*, 1233 – 1246; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1197 – 1208; c) *Intermediate Biomarkers of Precancer and their Application in Chemoprevention* (Eds.: M. Lipkin, B. Levin, Y. S. Kim, G. J. Kelloff), Wiley-Liss, New York, NY, **1992**, p 196 (*J. Cell Biochem. (Suppl. 16G)*); d) J. M. Bishop, *Science* **1987**, *235*, 305 – 311; e) H. M. Schüller, *Biochem. Pharmacol.* **1991**, *42*, 1511 – 1523; f) I. R. Hart, A. Saini, *Lancet* **1992**, *339*, 1453 – 1457; g) D. G. Brodland, J. A. Zitelli, *J. Am. Acad. Dermatol.* **1992**, *27*, 1 – 8.

[2] Information retrieved from the Internet Homepages of the American Cancer Society (ACS; http://www.cancer.org) and of the National Cancer Institute (NCI; http://www.nci.nih.gov).

[3] a) M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, A. T. McPhail, *J. Am. Chem. Soc.* **1971**, *93*, 2325 – 2327; b) T. Junod, *Life* **1992**, *15*, 71 – 76; c) "*Taxane Anticancer Agents: Basic Science and Current Status*": G. I. Georg, T. T. Chen, I. Ojima, D. M. Wyas, ACS Symposium Series **1995**, *583*, 35.

[4] a) E. K. Rowinsky, R. C. Donehower, *New Engl. J. Med.* **1995**, *332*, 1004 – 1014; b) J. E. Cortes, R. Pazdur, *J. Clin. Oncol.* **1995**, *13*, 2643 – 2655.

[5] a) J. N. Denis, A. E. Greene, D. Guénard, F, Gueritte-Voegelein, L. Mangatal, P. Potier, *J. Am. Chem. Soc.* **1988**, *110*, 5917 – 5919; b) L. Mangatal, M. T. Adeline, D. Guénard, F. Gueritte-Voegelein, P. Potier, *Tetrahedron* **1989**, *45*, 4177 – 4190; c) I. Barasoain, C. De Ines, F. Duz, J. M. Andreia, V. Peyrot, D. Leynadier, P. Garcia, C. Briand, G. De Suusa, R. Rahmani, *Proc. Am. Assoc. Cancer Res.* **1991**, *32*, 329; d) I. Ringel, S. B. Horwitz, *J. Natl. Cancer Inst.* **1991**, *83*, 288 – 291; e) J. F. Diaz, J. M. Andreu, *Biochemistry* **1993**, *32*, 2747 – 2755; f) V. Fromes, P. Guonon, M. C. Bissery, A. Fellous, *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 511.

[6] "Annual Report: Top 100 drugs" anonymous, *MedAdNews* **1997**, *16*, 11 – 91.

[7] K. C. Nicolaou, W.-M. Dai, R. K. Guy *Angew. Chem.* **1994**, *33*, 15 – 44.

[8] K. C. Nicolaou, R. K. Guy, J. L. Gunzer, *MedChem News* **1997**, *7*, 12 – 18.

[9] a) K. D. Rainsford, *Aspirin and the Salicylates*, Thetford Press, Thetford, England, **1984**; b) G. Weissmann, *Sci. Amer.* **1991**, *268*(1), 84 – 90.

[10] a) *The beta-Lactam Antibiotics: Penicillins and Cephalosporins in Perspective* (Ed.: S. Selwyn), Hodder and Stoughten, London, **1980**; b) *Murder, Magic, and Medicine* (Ed.: J. Mann), Oxford University Press, London, **1994**, pp. 124 – 140.

[11] G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach (GBF), DE-B 4138042, **1993** [*Chem. Abstr.* **1993**, *120*, 52841].

[12] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671 – 1673; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567 – 1569.

[13] b) K. Gerth, N. Bedorf, G. Höfle, H. Irschik, H. Reichenbach, *J. Antibiot.* **1996**, *49*, 560 – 563.

[14] G. Höfle, N. Berdorf, K. Gerth, H. Reichenbach (GBF), DE-B 4211055, **1993** [*Chem Abstr.* **1993**, *119*, 180598].

[15] a) D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325 – 2333; for a recent review on the biology of epothilones, see b) D. M. Bollag, *Exp. Opin. Invest. Drugs* **1997**, *6*, 867 – 873.

[16] S. F. Victory, D. G. Vander Velde, R. K. Jalluri, G. L. Grunewald, G. I. Georg, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 893 – 898.

[17] J. P. Pachlatko, Novartis, Personal communication.

[18] D. Schinzer, *Eur. Chem. Cron.* **1997**, 7 – 10.

[19] S. Borman, *Chem. Eng. News* **1996**, *74*(52), 24 – 26.

[20] a) Developmental Therapeutics Program of the National Cancer Institute (NCI), USA: M. R. Grever, S. A. Schepartz, B. A. Chabner, *Semin. Oncol.* **1992**, *19*, 622 – 638.

[21] P. B. Fernandes, *Antimicrob. Newsl.* **1987**, *4*, 25 – 34.

[22] D. C. Jordan, *Antibiotics (USSR)* **1967**, *1*, 446 – 450.

[23] a) A. Abou-Zeid, A. I. El-Dewany, *Indian Chem. Manuf.* **1978**, *16*, 17 – 25; b) E. E. Schmid, *Antibiot. Chemother. (Basel)* **1971**, *17*, 52 – 66.

[24] A. Baumüller, U. Hoyme, P. O. Madsen, *Antimicrob. Agents Chemother.* **1977**, *12*, 240 – 242.

[25] a) S. B. Kaye, P. Workman, M. A. Graham, J. Cassidy, D. Jodrell, *Cancer Surv.* **1993**, *17*, 371-396; b) E. Hamel, *Pharmacol. Ther.* **1992**, *51*, 31 – 51; c) D. R. Budman, *Semin. Oncol.* **1992**, *19*, 639 – 645.

[26] M. Takahashi, S. Iwasaki, H. Kobayashi, S. Okuda, T. Murai, Y. Sato, *Biochim. Biophys. Acta* **1987**, *926*, 215 – 223.

[27] *Microtubules* (Eds.: J. S. Hyams, C. W. Lloyd), Wiley-Liss, New York, **1994**.

[28] P. B. Schiff, J. Fant, S. B. Horwitz, *Nature* **1979**, *277*, 665 – 667.

[29] R. J. Kowalski, P. Giannakakou, E. Hamel, *J. Biol. Chem.* **1997**, *272*, 2534 – 2541.

[30] D.-S. Su, A. Ballog, D. Meng, P. Bertinato, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 2178 – 2181; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2093 – 2096.

[31] For an excellent review in this field, see J. Avila, J. D. Nido, in *Cytoskeleton Vol. 1* (Eds.: J. E. Hesketh, I. F. Pryme), JAI, Greenwich, CT, **1995**, pp. 47 – 85.

[32] K. F. Sullivan, *Annu. Rev. Cell Biol.* **1988**, 687 – 716.

[33] R. G. Burns, *Cell Motil. Cytoskeleton* **1991**, *20*, 181 – 189.

[34] *Microtubules* (Ed.: P. Dustin), Springer, Berlin, **1984**, pp 482 ff.

[35] M. A. Jordan, R. J. Toso, D. Thrower, L. Wilson, *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9552 – 9556.

[36] For a recent review on microtubule structures and dynamics, see R. H. Wade, A. A. Hyman, *Curr. Opin. Cell Biol.* **1997**, *9*, 12 – 17.

[37] L. Wilson, M. A. Jordan, *Chem. Biol.* **1995**, *2*, 569 – 573.

[38] B. R. Brinkley, *Cold Spring Harbor Symp. Quant. Biol.* **1982**, *46*, 1029 – 1040.

[39] a) E. H. Ball, J. J. Singer, *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 123 – 126; b) M. S. Cooper, A. H. Cornell-Bell, A. Chernjawsky, J. W. Dani, S. J. Smith, *Cell* **1990**, *61*, 135 – 145.

[40] a) M. M. Rasenick, N. Wang, K. Yan, *Adv. Second Messenger Phosphoprotein Res.* **1990**, *24*, 381 – 386; b) J. M. Scholey, *Nature* **1990**, *343*, 118 – 120.

[41] L. A. Amos in *Microtubules* (Eds.: K. Roberts, J. Hyams), Academic Press, New York, **1979**, *129*, pp 135 – 148.

[42] a) T. Mitchison, M. W. Kirschner, *Nature* **1984**, *312*, 237-242; b) M. W. Kirschner, T. Mitchison, *Cell* **1986**, *45*, 329-342; c) M. W. Kirschner, T. Mitchison, *Nature* **1986**, *324*, 621-622; d) J. Avila, *FASEB J.* **1990**, *4*, 3284-3290.

[43] a) E. Schulze, M. Kirschner, *J. Cell Biol.* **1986**, *102*, 1020 – 1031; b) L. Cassimeris, N. K. Pryer, E. D. Salmon, *J. Cell Biol.* **1988**, *107*, 2223 – 2231.

[44] a) L. T. Haimo, *Methods Cell Biol.* **1982**, *24*, 189 – 206; b) P. R. Burton, R. H. Himes, *J. Cell Biol.* **1978**, *77*, 120 – 133; c) E.-M. Mandelkow, E. Mandelkow, *J. Mol. Biol.* **1979**, *129*, 135 – 148.

[45] R. L. Margolis, L. Wilson, *Cell* **1978**, *13*, 1 – 8.

[46] a) L. U. Cassimeris, R. A. Walker, N. K. Pryer, E. D. Salmon, *Bioessays* **1987**, *7*, 149 – 154; b) H. P. Erickson, E. T. O'Brien, *Annu. Rev. Biophys. Biomol. Struct.* **1992**, *21*, 145 – 146; c) V. I. Gelfand, A. D. Bershadsky, *Annu. Rev. Cell Biol.* **1991**, *7*, 93 – 116; d) E. D. Salman in *Mitosis* (Eds.: J. S. Hyams, B. R. Brinkley), Academic Press, New York, **1989**, pp. 119 – 181.

[47] L. Wilson, M. A. Jordan, *Chem. Biol.* **1995**, *2*, 569 – 573.

[48] M. Kirschner, T. J. Mitchison, *Cell* **1986**, *45*, 329 – 342.

[49] a) J. R. McIntosh, *Trends Biochem. Sci.* **1984**, *9*, 195 – 198; b) C. Mazia in *The Cell, Vol.* III (Eds.: J. Brachat, A. E. Mirsky), Academic Press, London, **1961**, pp. 77 – 412.

[50] a) B. R. Brinkley, *Annu. Rev. Cell Biol.* **1985**, *1*, 145 – 172; b) M. DeBrabander, *Cell Biol. Int. Rep.* **1982**, *6*, 901 – 915.

[51] J. R. McIntosh, C. M. Pfarr, *J. Cell Biol.* **1991**, *115*, 577 – 585.

[52] G. Gorbsky, *Bioassays* **1992**, *14*, 73 – 80.

[53] a) R. Li, A. W. Murray, *Cell*, **1991**, *66*, 519 – 531; b) M. A. Hoyt, L. Totis, B. T. Roberts, *Cell* **1991**, *66*, 507 – 517.

[54] B. A. Boggs, M. L. Gonzales-Garey, W. E. O'Brien, S. B. Barlow, F. Cabral, *Cell. Pharmacol.* **1993**, *1*, S95 – S101.

[55] S. Timashiff, J. Andreu, M. Gorbunoff, F. Medranot, V. Prakash, *Cell. Pharmacol.* **1993**, *1*, S27 – S33.

[56] a) R. J. Toso, M. A. Jordan, K. W. Farrell, B. Matsumoto, L. Wilson, *Biochemistry* **1993**, *32*, 1285-1293; b) S. B. Hastie, *Pharmacol. Ther.* **1991**, *51*, 377 – 401.

[57] M. A. Jordan, L. Wilson, in *Taxane Anticancer Agents*, (Eds.: G. I. Georg, T. T. Chen, I. Ojima, D. M. Vyas), American Chemical Society, Washington, DC, **1995**, pp. 138 – 153.

[58] J. B. Olmsted, C. F. Asnes, L. M. Parysek, H. D. Lyon, G. M. Kidder, *Ann. NY Acad. Sci.* **1986**, *466*, 292-305.

[59] a) M. Goedert, R. Jakes, M. G. Spillantini, R. A. Crowther, in *Microtubules* (Eds.: J. Hyams, C. Lloyd), Wiley-Liss, New York, **1994**, pp. 183 – 200; b) G. Wiche, C. Oberkanins, A. Himmler, *Int. Rev. Cytol.* **1991**, *124*, 217 – 273.

[60] D. Panda, B. L. Goode, S. C. Feinstein, L. Wilson, *Biochemistry* **1995**, *34*, 11117 – 11127.

[61] F. I. Raynaud, I. R. Judson, *Drugs Future* **1996**,*21*, 65 – 70.

[62] C. L. Rieder, R. E. Palazzo, *J. Cell Sci.* **1992**, *102*, 387 – 392.

[63] R. B. Natale, *Semin. Oncol.* **1997**, *24*, 29 – 37.

[64] D. L. Sackett, *Pharmacol. Ther.* **1993**, *59*, 163 – 228.
[65] a) E. Hamel, *Med. Res. Rev.* **1996**, *16*, 207 – 231; b) W. B. Derry, L. Wilson, M. A. Jordan, *Biochemistry* **1995**, *34*, 2203 – 2211; for a review see c) L. Wilson, M. A. Jordan, in *Microtubules* (Eds.: L. Hyams, C. Lloyd), Wiley-Liss, New York, **1994**, pp. 59 – 84.
[66] E. Hamel, *Cell. Pharmacol.* **1993**, *1*, S47 – S52
[67] E. Hamel, *Pharmacol. Ther.* **1992**, *55*, 31 – 51.
[68] a) K. Yamada, H. Kigoshi, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1479 – 1489; b) G. R. Pettit, *Prog. Chem. Org. Nat. Prod.* **1997**, *70*, 1 – 79; c) M. Namikoshi, K. L. Rinehart, *J. Ind. Microbiol. Biotechnol.* **1996**, *17*, 373 – 384; d) G. R. Pettit, *Pure Appl. Chem.* **1994**, *66*, 2271 – 2281.
[69] a) C. D. Smith, X. Zhang, S. L. Mooberry, G. M. L. Patterson, R. E. Moore, *Cancer Res.* **1994**, *54*, 3779 – 3784; b) R. E. Moore, T. H. Corbett, G. M. L. Patterson, F. A. Valeriote, *Curr. Pharm. Des.* **1996**, *2*, 317 – 330; c) R. E. Moore, *J. Ind. Microbiol.* **1996**, *16*, 134 – 143; d) T. Corbett, F. Valeriote, P. LoRusso, L. Polin, C. Panchapor, S. Pugh, K. White, J. Knight, L. Juiwanna, *Int. J. Pharmacogn.* **1995**, *33* (Suppl.), 102 – 122.
[70] M. Fighetti, S. Rubino, P. Cappuccinelli, G. Monaco, *Microbiologica (Bologna)* **1988**, *11*, 269 – 277.
[71] a) W. H. Gerwick, P. J. Proteau, D. G. Nagle, E. Hamel, A. Blokin, D. L. Slate, *J. Org. Chem.* **1994**, *59*, 1243 – 1245; b) B. S. Davidson, *Curr. Opin. Biotechnol.* **1995**, *6*, 284 – 291; c) M. C. Pirrung, S. K. Nauhaus, *Chemtracts: Org. Chem.* **1994**, *7*, 128 – 129.
[72] a) M. A. Jordan, R. J. Torso, D. Thrower, L. Wilson, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9552 – 9556; b) J. Liebmann, J. A. Cook, C. Lipschultz, D. Teague, J. Fisher, J. B. Mitchell, *Cancer Chemother. Pharmacol.* **1994**, *33*, 331 – 339.
[73] P. B. Schiff, S. B. Horwitz, *Biochemistry* **1981**, *20*, 3247 – 3252.
[74] a) P. B. Schiff, J. Fant, S. B. Horwitz, *Nature* **1979**, *277*, 665-667; b) E. Hamel, A. A. del Campo, M. C. Lowe, C. M. Lin, *J. Biol. Chem.* **1981**, *256*, 11 887 – 11 894.
[75] A. Wolff, A. Technau, G. Brandner, *Int. J. Oncol.* **1997**, *11*, 123 – 126.
[76] a) U. Moll, A. G. Ostermeyer, R. Haladay, B. Winkfield, M. Frazier, G. Zambetti, *Mol. Cell. Biol.* **1995**, *16*, 1126 – 1137; b) S. C. Goldman, C.-Y. Chen, T. J. Lansing, T. M. Gilmer, M. B. Kastan, *Am. J. Pathol.* **1996**, *148*, 1381 – 1385.
[77] J. Parness, S. B. Horwitz, *Biochemistry* **1981**, *91*, 479-487.
[78] E. K. Rowinsky, E. A. Eisenhauer, V. Chaudhry, S. G. Arbuck, R. C. Donehower, *Semin. Oncol.* **1993**, *20*, 1 – 15.
[79] J. B. Hibbs, R. R. Taintor, Z. Vavrin, *Science* **1987**, *235*, 473 – 476.
[80] A. Ding, B. Chen, N. Fuortes, E. Blum, *J. Exp. Med.* **1996**, *183*, 1899 – 1904.
[81] C. Bogdan, A. Ding, *J. Leukocyte Biol.* **1992**, *52*, 119 – 121.
[82] E. F. Ding, F. Porteu, E. Sanchez, C. F. Nathan, *Science* **1990**, *248*, 370-372.
[83] C. L. Manthey, P.-Y. Perera, C. A. Salkowski, S. N. Vogel, *J. Immunol.* **1994**, *152*, 825 – 831.
[84] P. F. Mühlradt, F. Sasse, *Cancer Res.* **1997**, *57*, 3344 – 3346.
[85] a) J. H. Schiller, B. Storer, K. Tutsch, R. Arzoomanian, D. Alberti, C. Feierabend, D. Spriggs, *J. Clin. Oncol.* **1994**, *12*, 241 – 248; b) D. H. Johnson, D. M. Paul, K. R. Hande, Y. Shyr, C. Blanke, B. Murphy, M. Lewis, R. F. de Vore, *J. Clin. Oncol.* **1996**, *14*, 2054 – 2060.
[86] Total synthesis: a) J. B. Nerenberg, D. T. Hung, P. K. Somers, S. L. Schreiber, *J. Am. Chem. Soc.* **1993**, *115*, 12 621 – 12 622; b) A. B. Smith III, Y. Qiu, D. R. Jones, K. Kobayashi, *J. Am. Chem. Soc.* **1995**, *117*, 12 011 – 12 012; c) S. S. Harried, G. Yang, M. A. Strawn, D. C. Myles, *J. Org. Chem.* **1997**, *62*, 6098 – 6099; biological activity: d) E. ter Haar, R. J. Kowalski, E. Hamel, M. C. Lin, R. E. Longley, S. P. Gunasekera, H. S. Rosenkranz, B. W. Day, *Biochem.* **1996**, *35*, 243 – 250; e) D. T. Hung, J. Chen, S. L. Schreiber, *Chem. Biol.* **1996**, *3*, 287 – 293; f) R. J. Kowalski, P. Giannakakou, S. P. Gunasekera, R. E. Longley, B. W. Day, E. Hamel, *Mol. Pharmacol.* **1997**, *52*, 613 – 622; isolation: g) S. P. Gunasekera, M. Gunasekera, R. E. Longley, G. K. Schulte, *J. Org. Chem.* **1990**, *55*, 4912 – 4915.
[87] Total synthesis: a) K. C. Nicolaou, F. van Delft, T. Ohshima, D. Vourloumis, J. Xu, S. Hosokawa, J. Pfefferkorn, S. Kim, T. Li, *Angew. Chem.* **1997**, *109*, 2631 – 2634; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2520 – 2524; isolation and biological activity: b) W. Fenical, P. R. Jensen, T. Lindel, US-A 5 473 057, **1995** [*Chem. Abstr.* **1996**, *124*, 194297z; c) T. Lindel, P. R. Jensen, W. Fenical, B. H. Long, A. M. Casazza, J. Carboni, C. R. Fairchild, *J. Am. Chem. Soc.* **1997**, *119*, 8744 – 8745.
[88] Total synthesis: a) K. C. Nicolaou, J. Y. Xu, S. Kim, T. Ohshima, S. Hosokawa, J. Pfefferkorn, *J. Am. Chem. Soc.* **1997**, *119*, 11 353 – 11 354; isolation: b) M. D'Ambrosio, A. Guerriero, F. Pietra, *Helv. Chim. Acta* **1987**, *70*, 2019 – 2027; c) M. D'Ambrosio, A. Guerriero, F. Pietra, *Helv. Chim. Acta* **1988**, *71*, 964 – 976; biological activity: d) M. Ciomei, C. Albanese, W. Pastori, M. Grandi, F. Pietra, M. D'Ammbrosio, A. Guerriero, C. Battistini, *Proc. Am. Assoc. Canc. Res.* **1997**, *38*, 5.
[89] L. Wessjohann, *Angew. Chem.* **1997**, *109*, 739 – 742; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 715 – 718.
[90] M. Kalesse, *Eur. Chem. Cron.* **1997**, 7 – 11.
[91] M. R. V. Finlay, *Chem. Ind.* **1997**, *24*, 991 – 996.
[92] For the development of the olefin metathesis as a ring forming reaction, see a) R. H. Grubbs, S. J. Miller, G. C. Fu, *Acc. Chem. Res.* **1995**, *28*, 446-452; b) W. J. Zuercher, M. Hashimoto, R. H. Grubbs, *J. Am. Chem. Soc.* **1996**, *118*, 6634 – 6640; c) P. R. Schwab, H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100 – 110; d) M. Schuster, S. Blechert, *Angew. Chem.* **1997**, *109*, 2124 – 2144; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2036 – 2055; e) J. Tsuji, S. Hashiguchi, *Tetrahedron Lett.* **1980**, *21*, 2955 – 2959; for some earlier pioneering studies on this reaction, see f) T. J. Katz, S. J. Lee, N. Acton, *Tetrahedron Lett.* **1976**, 4247 – 4250; g) T. J. Katz, N. Acton, *Tetrahedron Lett.* **1976**, 4241-4254; h) T. J. Katz, J. McGinnis, C. Altus, *J. Am. Chem. Soc.* **1976**, *98*, 606 – 608; i) T. J. Katz, *Adv. Organomet. Chem.* **1977**, *16*, 283 – 317; for a number of applications of the olefin metathesis reaction in medium and large ring synthesis, see j) A. Fürstner, K. Langemann, *Synthesis* **1997**, 792 – 803; (k) B. C. Borer, S. Deerenberg, H. Bieräugel, U. K. Pandit, *Tetrahedron Lett.* **1994**, *35*, 3191 – 3194; (l) T. D. Clark, M. R. Ghadiri, *J. Am. Chem. Soc.* **1995**, *117*, 12 364 – 12 365; (m) A. F. Houri, Z. Xu, D. A. Cogan, A. H. Hoveyda, *J. Am. Chem. Soc.* **1995**, *117*, 2943 – 2944; (n) A. Fürstner, K. Langemann, *J. Org. Chem.* **1996**, *61*, 3942 – 3943; (o) S. F. Martin, H.-J. Chen, A. K. Courtney, Y. Liao, M. Pätzel, M. N. Ramser, A. S. Wagman, *Tetrahedron* **1996**, *52*, 7251 – 7264; (p) Z. Xu, C. W. Johannes, S. S. Salman, A. H. Hoveyda, *J. Am. Chem. Soc.* **1996**, *118*, 10 926 – 10 927.
[93] R. R. Schrock, J. S. Murdzek, G. C. Bazan, J. Robbins, M. DiMare, M. O'Regan, *J. Am. Chem. Soc.* **1990**, *112*, 3875 – 3886.
[94] P. Schwab, M. B. France, J. W. Ziller, R. H. Grubbs, *Angew. Chem.* **1995**, *107*, 2179 – 2181; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2039 – 2041.
[95] A. F. Houri, Z. Xu, D. A. Cogan, A. Hoveyda, *J. Am. Chem. Soc.* **1995**, *117*, 2943 – 2944.
[96] K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* **1996**, *108*, 2554 – 2556; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2399 – 2401.
[97] K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, F. Roschangar, F. Sarabia, S. Ninkovic, Z. Yang, J. I. Trujillo, *J. Am. Chem. Soc.* **1997**, *119*, 7960 – 7973.
[98] Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.* **1997**, *109*, 170 – 172; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166 – 168.
[99] K. C. Nicolaou, S. Ninkovic, F. Sarabia, D. Vourloumis, Y. He, H. Vallberg, M. R. V. Finlay, Z. Yang, *J. Am. Chem. Soc.* **1997**, *119*, 7974 – 7991.
[100] K. C. Nicolaou, H. Vallberg, N. P. King, F. Roschangar, Y. He, D. Vourloumis, C. G. Nicolaou, *Chem. Eur. J.* **1997**, *3*, 1957 – 1970.
[101] K. C. Nicolaou, N. Winssinger, J. Pastor, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, E. Hamel, *Nature* **1997**, *36*, 757-759.
[102] 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. V. Finlay, P. Giannakakou, P. Verdier-Pinard, E. Hamel, *Angew. Chem.* **1997**, *109*, 2181 – 2187; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097 – 2103.
[103] a) K. C. Nicolaou, X.-Y. Xiao, Z. Parandoosh, A. Senyei, M. P. Nova, *Angew. Chem.* **1995**, *107*, 2476 – 2479; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2289 – 2291; b) E. J. Moran, S. Sarshar, J. F. Cargill, M. M. Shahbaz, A. Lio, A. M. M. Mjalli, R. W. Armstrong, *J. Am. Chem. Soc.* **1995**, *117*, 10 787 – 10 788.

[104] K. C. Nicolaou, F. Sarabia, S. Ninkovic, Z. Yang, *Angew. Chem.* **1997**, *109*, 539 – 540, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 525 – 527.
[105] G. Höfle, GBF Braunschweig, Personal communication.
[106] K. C. Nicolaou, Y. He, F. Roschangar, N. P. King, D. Vourloumis, T. Li, *Angew. Chem.* **1998**, *110*, 89 – 92; *Angew. Chem. Int. Ed.* **1998**, *37*, 84 – 87.
[107] A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108,* 2976 – 2978; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801 – 2803.
[108] D.-S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775 – 777; *Angew. Chem. Int. Ed. Engl*. **1997**, *36*, 757 – 759.
[109] D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, *119*, 10073 – 10092.
[110] D. Meng, D.-S. Su, A. Balog, P. Bertinato, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *J. Am. Chem. Soc.* **1997**, *119*, 2733 – 2734.
[111] S. J. Danishefsky, *Aldrichimica Acta* **1986**, *19*, 59 – 69; b) S. J. Danishefsky, *Chemtracts: Org. Chem.* **1989**, *2*, 273 – 297.
[112] D. Meng, E. J. Sorensen, P. Bertinato, S. J. Danishefsky, *J. Org. Chem*. **1996**, *61*, 7998 – 7999.
[113] P. Bertinato, E. J. Sorensen, D. Meng, S. J. Danishefsky, *J. Org. Chem*. **1996**, *61*, 8000 – 8001.
[114] D. Schinzer, A. Limberg, O. M. Böhm, *Chem. Eur. J.* **1996**, *2*, 1477 – 1482.
[115] D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1997**, *109,* 543 – 544; *Angew. Chem. Int. Ed. Engl*. **1997**, *36*, 523 – 524.
[116] J. Mulzer, A. Mantoulidis, *Tetrahedron Lett.* **1996**, *37*, 9179-9182.
[117] J. Mulzer, A. Mantoulidis, E. Öhler, *Tetrahedron Lett.* **1997**, *38*, 7725 – 7728.
[118] E. Claus, A. Pahl, P. G. Jones, H. M. Meyer, M. Kalesse, *Tetrahedron Lett.* **1997**, *38*, 1359 – 1362.
[119] T. Gabriel, L. Wessjohann, *Tetrahedron Lett.* **1997**, *38*, 1363 – 1366.
[120] R. E. Taylor, J. D. Haley, *Tetrahedron Lett.* **1997**, *38*, 2061 – 2064.
[121] J. De Brabander, S. Rosset, G. Bernardinelli, *Synlett* **1997**, 824 – 826.
[122] A. Balog, P. Bertinato, D.-S. Su, D. Meng, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Tetrahedron Lett.* **1997**, *38*, 4529 – 4532.
[123] K. C. Nicolaou, F. Sarabia , S. Ninkovic, M. R. V. Finlay, C. N. C. Boddy, *Angew. Chem.* **1998**, *110*, 85 – 89; *Angew. Chem. Int.* **1998**, *37*, 81 – 84.
[124] K. C. Nicolaou, S. Ninkovic, M. R. V. Finlay, F. Sarabia, T. Li, *Chem. Commun.* **1997**, 2343 – 2344.
[125] K. C. Nicolaou, F. Sarabia, M. R. V. Finlay, S. Ninkovic, N. P. King, D. Vourloumis, Y. He, *Chem. Eur. J.* **1997**, *3*, 1971 – 1986.
[126] J. D. Winkler, P. H. Axelsen, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2963 – 2966.
[127] S. Chakravarty, I. Ojima, *Abstr. pap. 214th ACS National Meeting*, Las Vegas, NV (USA), **1997**, MEDI-075.
[128] K. C. Nicolaou, M. R. V. Finlay, S. Ninkovic, N. P. King, Y. He, T. Li, F. Sarabia, D. Vourloumis, *Chem. Biol.*, in press; K. C. Nicolaou, N. P. King, M. R. V. Finlay, Y. He, F. Roschangar, D. Vourloumis, H. Vallberg, F. Sarabia, S. Ninkovic, D. Hepworth, *Biorg. Med. Chem.*, in press.
[129] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesh, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107 – 1112.
[130] C. M. Lin, Y. Q. Jiang, A. G. Chaudhary, J. M. Rimoldi, D. G. I. Kingston, E. Hamel, *Cancer Chemother. Pharmocol.* **1996**, *38*, 136 – 140.
[131] B. C. Behrens, T. C. Hamilton, H. Matsuda, K. R. Grotzinger, J. Wang-Peng, K. G. Louie, T. Knutsen, W. M. McKoy, R. C. Young, R. F. Ozols, *Cancer Res.* **1987**, *47*, 414 – 418.
[132] P. Giannakakou, D. L. Sackett, Y.-K. Kang, Z. Zhan, J. T. M. Buters, T. Fojo, M. S. Poruchynsky, *J. Biol. Chem.* **1995**, *272*, 17118 – 17125.
[133] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757 – 766.
[134] Wisschenschaftlicher Jahresbericht, Gesellschaft für Biotechnologische Forschung, Braunschweig, 1996, p 112.
[135] Application of radical-induced opening of trisubstituted epoxides in the synthesis of C1 – C12 segment of epothilones; T. K. Chakraborty, S. Dutta, *Tetrahedron Lett.* **1998**, *39*, 101 – 104.
[136] Chiral synthesis of the C3 – C13 segment of epothilone A; Z. Y. Liu, C. Z. Yu, J. D. Yang, *Synlett,* **1998**, *12*, 1383 – 1384.
[137] Enantioselective synthesis of a C7 – C15 carboxaldehyde segment of epothilone A: P. Bijoy, M. A. Avery, *Tetrahedron Lett.* **1998**, *39*, 209 – 212.
[138] K. C. Nicolaou, *Angew. Chem.* **1996**, *108*, 644 – 664; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 588 – 607.