Perspective

Complex Target-Oriented Synthesis in the Drug Discovery Process: A Case History in the dEpoB Series

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Introduction

The discovery of new, effective, and safe drugs to treat various diseases is a multifaceted undertaking. Chemistry, particularly synthetic chemistry, tends to enter into such ventures at a relatively late, but still critical, point. Generally, the disease syndrome has been reasonably characterized in diagnostic terms. These days, such a disease characterization would generally have been followed by identification of its underlying biochemical and even genetic basis. From this level of insight there follows identification of fixed molecular targets and, optimally, a screening protocol (hopefully of high throughput) by which the likely value of various agents can be rapidly assessed.

Typical screens are directed toward enzymes, cell surface receptors, or intracellular signaling elements. The structure of these biotargets for screening may be comprehended at varying levels of sophistication ranging from virtually no knowledge to a rather detailed grasp of the conformation in solution or in the crystalline state.

At this stage of the process, there is, at best, a presumptive relationship between the performance of an agent in an assay and the likelihood of its value viz à viz the disease target, which is, of course, the real purpose of the exercise. Given the difficulties and costs associated with assessments that are more amenable to readouts at the level of impact on disease, or even amelioration of its adverse symptoms, the molecular target screening approach is certainly understandable. At the screening level, the key desiderata tend to be potency (suggested by binding affinity) or specificity to a particular target if pertinent isoforms of the protein are available for parallel assessment.

Synthetic chemistry tends to become deeply involved in the discovery process as the screening exercise begins to provide hints of new directions. Of course, given detailed information about the biological target, synthesis, in conjunction with insights from computational chemistry, can generate particularly promising screening candidates. This pathway to progress forms the basis of the much-discussed rational drug design approach. Alternatively, random screenings of industrial sample collections generated from previous drug discovery programs may be used to identify coherent directions for the discovery project. This approach is now being strongly augmented by access to combinatorial libraries generated through diversity chemistry. Ultimately, it becomes the responsibility of synthesis to identify the most promising structures to be advanced for development.

Of greatest pertinence to the study described below is another source of screening candidates en route to lead structures, i.e., the kingdom of natural products. Such substances have contributed immensely to the drug discovery process (including antibiotics, antihypercholestremic agents, hormones, vitamins, and cardiotonic agents, etc.). In this report, we focus on naturally occurring cytotoxic agents of potential clinical value in the chemotherapy of cancer.

Given the impressive track record of natural products based leads (including natural products themselves) in drug discovery, the tendency of certain firms to dilute or even close down this form of screening is puzzling. Indeed, agents obtained from natural sources or congeners of such agents have played a useful role in the clinical level in cancer chemotherapy (cf. inter alia mitomycins, etoposides, and anthracyclines). Certainly, one of the outstanding clinically useful agents, which has come from phytochemical sources, is paclitaxel (Figure 1).[1−3] This drug, developed by the Bristol Myers Squibb Corp., has emerged as a front-line resource in the treatment of various cancers. Given the extensive literature that is already available on the chemistry, pharmacology, and clinical performance of paclitaxel, we will not again review this agent per se. However, paclitaxel will continually reappear in our discussion as a benchmark reference point for the principal focus of this paper, which is a family of compounds known as the epothilones. We shall attempt to show how complex target-oriented synthesis played a critical role in the discovery process pertinent to an agent, 12,13-desoxyepothilone B (vide infra). The near three-year project that we describe suggests that other drug candidate leads, emanating from fairly sophisticated natural product structure types, provide excellent frameworks for integrating advanced target directed synthesis into drug development.

A brief history of the epothilones in the broad context of paclitaxel is in order. Epothilones A (**1**, EpoA) and B (**2**, EpoB) along with other minor related constituents (Figure 1) are cytotoxic macrolide natural products that were isolated from the myxobacterium *Sorangium cel-*

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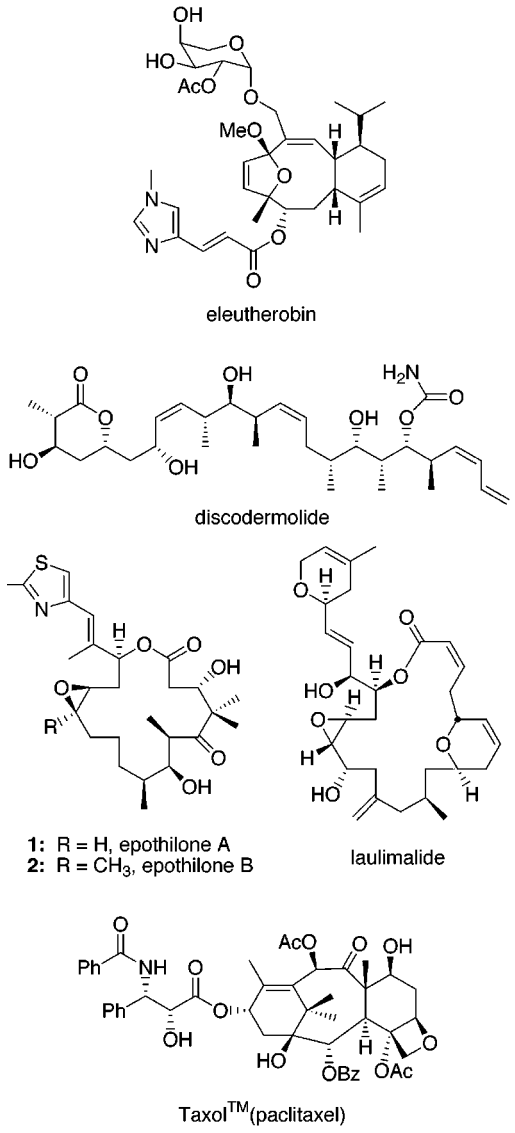


Figure 1. Chemical structure representations of some recently discovered naturally occurring molecules that stabilize microtubule assemblies.

lulosum,[4,5] which were harvested off the shores of the Zambezi River in the Republic of South Africa. These compounds exhibit biological effects that are analogous to those of paclitaxel.[6−9]Although the 16-membered ring structures of the epothilones bear little obvious homology to the structurally complex taxanes, both classes of molecules would appear to share, at least to some extent, a common biological target. These agents apparently function by stabilizing cellular microtubule assemblies, thus inducing the formation of hyperstable tubulin polymers in both cultured cells and microtubule protein.[10] Since microtubules are vital for mitosis, motility, secretion, and proliferation,[11,12] the observed anticancer effects of the epothilones and the taxanes have been attributed to their ability to initiate cell death by stabilization of such assemblies.[13] Apparently, the epothilones and taxanes bind to either the same site or to similar allosteric sites. Thus, when both substrate ([^{3}H]paclitaxel) and inhibitor (EpoA or EpoB) concentrations were varied and analyzed, a classical competitive inhibition pattern was obtained for both epothilone A and B.[13]

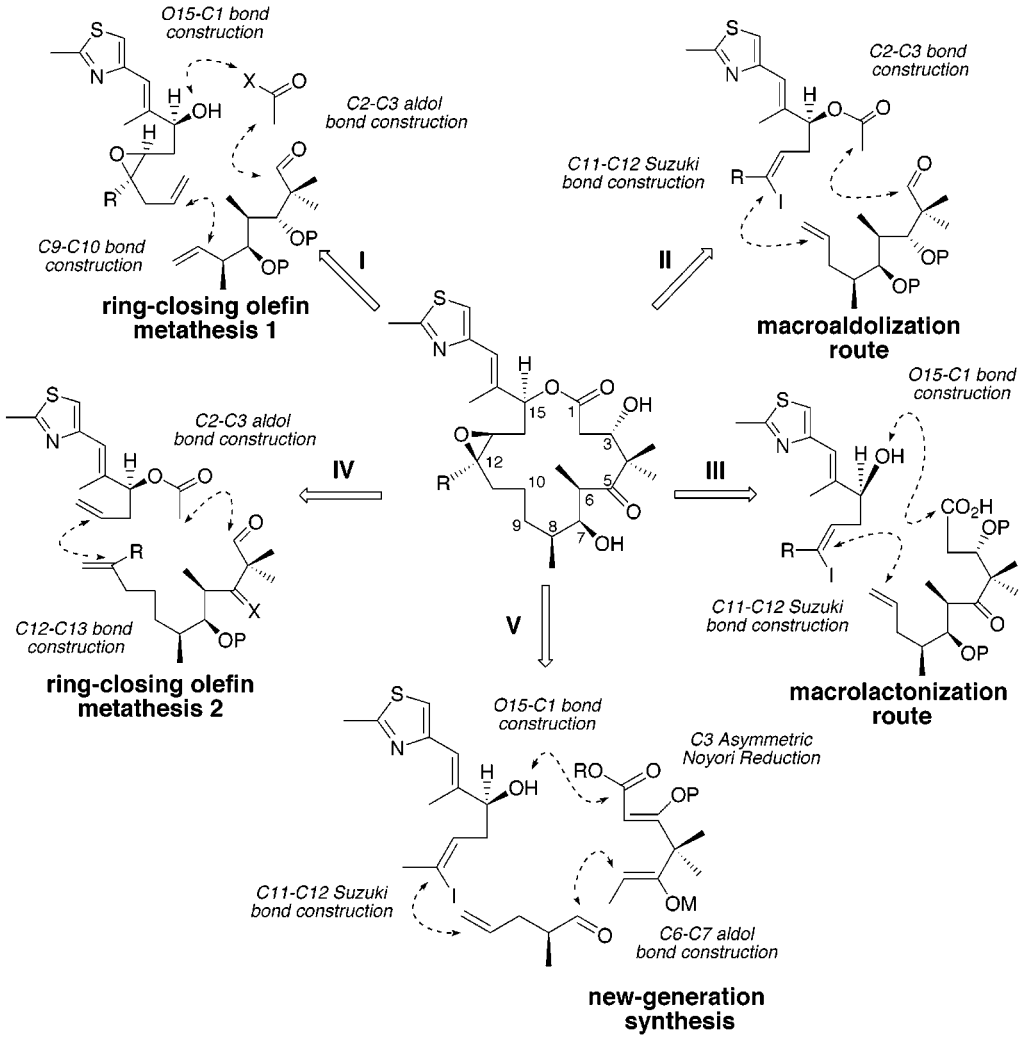
While it is generally believed that the antitumor action of the epothilones and taxanes result from their interaction with microtubules,[14] recent data suggest that a broader view of the cytotoxic action, at least of the latter, may be appropriate. Thus, it has been established that in addition to their stabilizing effect exerted on microtubule arrays, the taxanes also induce several early response and cytokine genes.[15−17] Moreover, several biologically oriented studies have suggested that the taxanes may indeed act by additional mechanisms since the tubulin binding and microtubule stabilizing effects of these agents do not account for their observed cytotoxic efficacy relative to other antimitotic molecules.[18] Interestingly, a recently discovered paclitaxel-induced cytokine gene is *cox-2*, which catalyzes the formation of prostaglandins. Indeed, this induction may be responsible for the immediate hypersensitivity reactions associated with paclitaxel administration.[19] Although limited information on the physiological effects of the epothilones on gene expression has been reported in the literature, a prior study[20] demonstrated that, unlike paclitaxel, the epothilones do not activate macrophages to synthesize proinflammatory cytokines and nitric oxide. Since it is suspected that the paclitaxel-induced proliferation of macrophages may actually be responsible for the endotoxin-like clinical side effects observed upon its administration, there is room to hope that the epothilones may not induce such undesired side-effects.

Other genes that effect gene transcription, including *krox-24*, have also been identified that appear relevant to the observed pharmacology of the taxanes. *Krox-24* is a zinc-finger transcription factor whose expression is deficient in several tumor cell lines, and this defect equates with tumor formation.[21] Moreover, enhanced expression of *krox-24* can prevent oncogenic transformations,[22] and thus, taxane-mediated induction of *krox-24* expression might indeed augment the microtubule-stabilizing effect of the taxanes. These studies reveal that both the beneficial and adverse pharmacological effects encountered during systemic paclitaxel administration may not originate exclusively from stabilization of microtubule assemblies but may arise from effect of the agent on gene expression. Similar experiments performed on one of the weaker epothilones, EpoA, demonstrated no upregulation of *krox-24* mRNA expression. From these results, future studies to document the effects of the epothilones on drug-induced gene expression are certainly warranted.

Other naturally occurring, nontaxoid molecules, the discodermolides[23] and eleutherobins[24] (Figure 1), that stabilize microtubule assemblies and exhibit activities similar to paclitaxel in various assays have been discovered. The commonality of cellular targets for these various drugs would not be anticipated in light of their structural dissimilarity. More recently, however, an extract from the marine sponge *C. mycofijiensis* has identified another potent stabilizer of microtubule assemblies, laulimalides and isolaulimalides[25] (Figure 1), whose structure is remarkably similar to the epothilone class of natural products.

Ojima has recently proposed a common pharmacophore uniting these structurally diverse molecules.[26] The model is based on a correlation of the NMR-defined conformation of a paclitaxel analogue, nonataxel,[27] with several low-energy conformations of epothilone B, eleutherobin, and discodermolide that were obtained from a molecular

Scheme 1. Retrosynthetic Disconnection of the Previous Syntheses (I–IV) and New Synthesis (V) of the Epothilones



dynamics study. This computer-generated model proposes a common pharmacophore that reconciles the extensive structure–activity data that have been reported for these molecules. Snyder et al. have also reported a unified receptor model for the microtubule binding of paclitaxel and epothilone, which accommodates much of the SAR observations for both drugs.[28] Perhaps the most intriguing discovery unearthed during the early investigations of the epothilones is that EpoB is notably more potent (both in vitro and in vivo) than paclitaxel in inhibiting cell growth.[10] Furthermore, while the microtubule assembly target of each agent is apparently the same, the epothilones are far superior in their ability to retain activity against multidrug-resistant (MDR) cell lines and tumors where paclitaxel (Taxol) and other major chemotherapeutic agents fail.[10,14–16] Given the perception that MDR is all too often responsible for the breakdown of efficacy of paclitaxel, this finding served to trigger interest in the epothilone family.

Total Synthesis of Epothilones

Intrigued by the impressive biological profile exhibited by the epothilone class of molecules in early in vitro screens and motivated by their apparent amenability to synthesis relative to paclitaxel, an excursion directed toward this class of molecules seemed to be appropriate. The ultimate goal of our journey was to devise an efficient, highly convergent total synthesis of the epothilones, thereby providing material for preliminary biological testing, both in vitro and in vivo. While the epothilones are available by fermentation,[4,5] the more biologically active epothilone B was particularly scarce and was, in any case, not at all available to our laboratory. Only through chemical synthesis would we have a chance to examine the epothilones.

If a synthesis could be accomplished, strategic analogues might be fashioned to enable a preliminary SAR profile. We anticipated that a small "library" of cleverly designed compounds could generate a suitably modified analogue that might find status as a cancer chemotherapy agent. In addition to considerations of potency, we were particularly interested in the MDR reversing ability of the epothilones in comparison to their MDR susceptible taxoid counterparts.[10] Toward this end, we soon embarked in a multidisciplinary venture directed to these goals.[29] Indeed, we accomplished the first total synthesis of epothilones A and B[30] and were soon joined by others.[31] Although the epothilones promised to be structurally less demanding, from the standpoint of total synthesis, than the taxanes, we foresaw several issues concerning this venture that would require careful attention. The structures of the epothilones invite retrosyn-

thetic disconnection into two regions: the polypropionate domain (C1−C8) and the *O*-alkyl sector (C12−C15). The four discontiguous stereocenters of the acyl domain are housed between two potentially labile β-hydroxy carbonyl moieties and insulated from the *O*-alkyl sector by three contiguous methylene groups, which make up an achiral "hinge" region (C9−C11). The *O*-alkyl center contains an α,β-unsaturated thiazole linkage and a C12−C13 *cis*-epoxide moiety that is insulated by a single methylene group from C15.

Scheme 1 outlines the synthetic strategies pursued in our laboratory toward the goal of the synthesis of the epothilones. Paths I−IV were part of our first-generation approaches, while path V depicts our recently reported route, which is rather more practical. While assembly of the medley of structural elements contained in the epothilones was certainly feasible, several obstacles were encountered during the course of our journey that required active consideration. We supposed that the presence of the achiral "hinge" spacer element would pose a certain difficulty in communicating stereochemical information from one domain with the other. Consequently, in each instance we chose a highly convergent approach wherein the two sectors containing the stereochemical information were prepared independently and then assembled in a late stage merger.

The first part of this account will chronicle our previous efforts and successes in the total synthesis of the epothilones. Subsequently, we will include a description of our new synthesis of EpoB. We then go on to report our findings regarding the significant biological activity of these remarkable compounds. For reasons that will become obvious, we will focus on dEpoB and its potentiality in a clinical setting. We hope that the recollections provided herein will capture the indispensable role of synthesis in the discovery process. In offering this retrospective, we will emphasize that the role of synthesis in the undertaking goes beyond simply rendering dEpoB available, important as that service surely is. Rather, our synthetic experiences influenced how we came to think about this whole family of molecules and provided the bases for a synergistic collaboration.

First-Generation Syntheses of the Epothilones

Initially, we proposed to complete the crucial "merger" of the two chiral regions through a ring-closing olefin metathesis (RCOM), path I, Scheme 1.[29,30] We envisioned construction of the macrocycle through RCOM at the C9−C10 bond.[32] Reducing this line of thought to practice would require assembly of the *O*-alkyl and acyl domains through a merger of fragments **A**, **B**, and **C** (Figure 2).

First Generation Synthesis of the *O*-Alkyl Domain. In practice, the appropriate *O*-alkyl moiety (**A**, Figure 2) was fashioned in two ways, both relying heavily on a line of chemistry developed in our laboratory in the 1980s.[33] Thus, aldehyde **5**[34] or chiral lactaldehyde **8**[35] (Scheme 2) functioned as a heterodienophile in the context of a Lewis acid catalyzed diene−aldehyde cyclocondensation (LACDAC) reaction with butadiene **6**, to afford either a racemic dihydropyran-4-one[36] or a chiral dihydropyran-4-one **9**.[37,38] The *rac*-dihydropyran-2-one could be effectively resolved by a lipase-mediated kinetic resolution[39] after Luche reduction[40] of the C4-ketone to afford the desired chiral dihydropyran-4-ol **7**. Alternatively, reduction of the chiral ketone **9**, protection as the TIPS ether, and dissolving metal reduction of the benzyloxymethyl (BOM) group afforded the chiral alcohol **10**. Dess−Martin oxidation of **10** provided the ketone **11**. The latter could be condensed with the phosphine oxide **12** in a Horner reaction[41] to afford optically pure **7** after removal of the C4-TIPS protecting group.

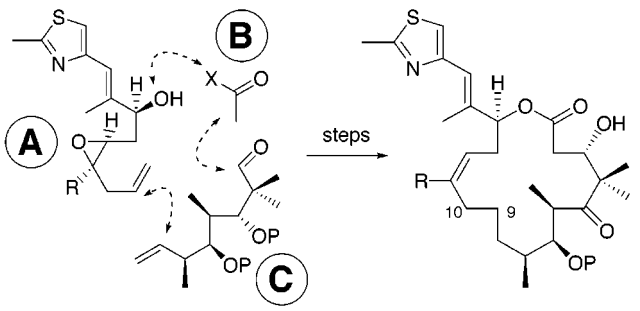
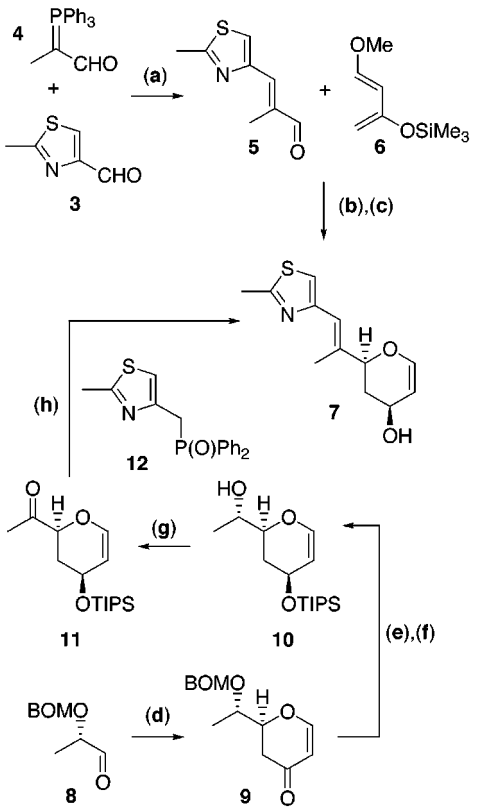


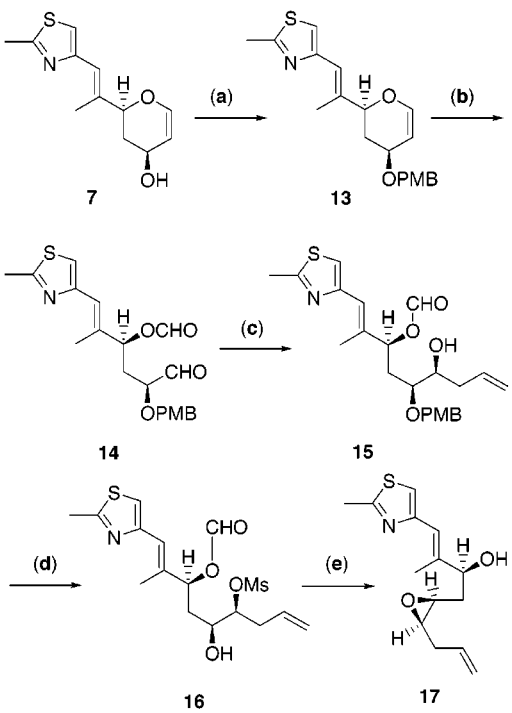
Figure 2. General concept for preparation of epothilones **A** and **B** through ring-closing olefin metathesis coupling to generate the C9−C10 bond by merger of fragments **A**, **B**, and **C**.

Scheme 2. Preparation of the Chiral Dihydropyran-4-ol (7) from a Lewis Acid Catalyzed Diene−Aldehyde Cyclocondensation (LACDAC) Route[a]



[a] Key: (a) C_6H_6, reflux (83%); (b) (**6**), $BF_3{\cdot}OEt_2$, CH_2Cl_2; then CSA (65%); (c) (i) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C to rt (99%); (ii) Lipase-30, vinyl acetate, DME, rt (45%; 93% ee); (iii) K_2CO_3, MeOH, rt; (d) *trans*-1-methoxy-3-trimethylsilyloxy-1,3-butadiene (**6**), $MgBr_2{\cdot}OEt_2$, THF, -10 °C; then AcOH, H_2O (93%); (e) (i) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C; (ii) TIPSCl, imidazole, DMF, 0 °C to rt (87% overall); (f) Na, NH_3 (l), THF, −78 °C; then MeOH, −78 to +25 °C (92%); (g) Dess−Martin periodinane, pyridine, CH_2Cl_2, rt (98%); (h) (i) **12**, *n*-BuLi, THF, −78 °C; then **11**, THF, −78 °C to rt (87%); (ii) *n*-Bu_4NF, THF, rt; BOM = CH_2OCH_2Ph; TIPS = Si(*i*-Pr)$_3$.

Scheme 3. Preparation of the Requisite *O*-Alkyl Moiety (17) for Ring-Closing Olefin Metathesis (RCOM)[a]

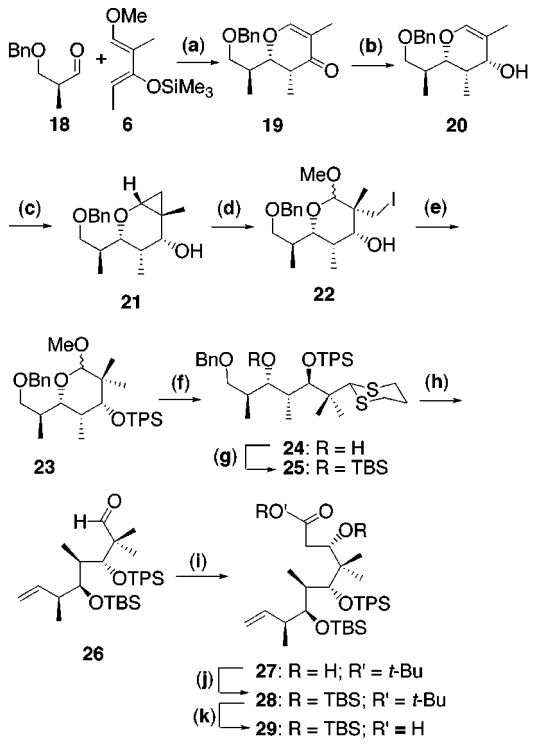


[a] Key: (a) PMBCl, NaH, DMF, 0 °C to rt; (b) 3,3-dimethyldioxirane, K_2CO_3, CH_2Cl_2, 0 °C; then $NaIO_4$, H_2O/THF (92%); (c) allyl triphenylstannane, $SnCl_4$, CH_2Cl_2, −78 °C (98% of **15** + epimer (4:1)); (d) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (ii) DDQ, CH_2Cl_2/H_2O (20:1), 0 °C to rt (93% overall); (e) (i) $LiN(SiMe_3)_2$, THF, −78 to 0 °C; (ii) K_2CO_3, MeOH/H_2O (78% of the *cis*-epoxide); PMB = *p*-$OMeC_6H_4CH_2$; Ms = SO_2CH_3.

Protection of **7** as the *p*-methoxybenzyl (PMB) ether afforded dihydropyran **13** (Scheme 3). At this juncture, glycal **13** was suitably functionalized for cleavage of the C2−C3 bond. Epoxidation and subsequent oxidative solvolysis of the C2−C3 glycal bond generated the acyclic bis(formaldehyde) **14**, which would be part of the future C12−C13 epoxide in **17** (Scheme 3). Subsequent tin-mediated allylation of the C1-aldehyde, in the presence of the formate ester, gave rise to alcohol **15** of the required absolute configuration. Following mesylation, oxidative removal of the PMB protecting group afforded **16**, which underwent treatment with $LiN(SiMe_3)_2$ to provide the desired epoxide **17** (**A**, Figure 2).

First-Generation Synthesis of the Acyl Domain. The C1−C11 domain of the epothilones has been the scene of many variations in strategy and synthetic design.[42] Indeed, synthesis of the polypropionate region at first appeared quite formidable because of the need to gain facile control over the stereocenters at C3, C6, C7, and C8 in a concise manner. In our first-generation synthesis of the C1−C11 acyl domain, we again approached the central goal of stereospecificity by containing the stereocenters placed at C3, C6, C7, and C8 in a rigid cyclic template that could be accessed through cyclocondensation chemistry (vide supra).[43] In the event, Lewis acid mediated cyclocondensation[44] of the chiral aldehyde **18**[45] and butadiene **6**[46] afforded the dihydropyran-4-one **19** (Scheme 4). Reduction of the C4 ketone to the C4 alcohol and subsequent hydroxyl-directed Conia−Simmons−Smith cyclopropanation[47] of the glycal double

Scheme 4. Preparation of the Acyl Fragment via LACDAC Chemistry[a]

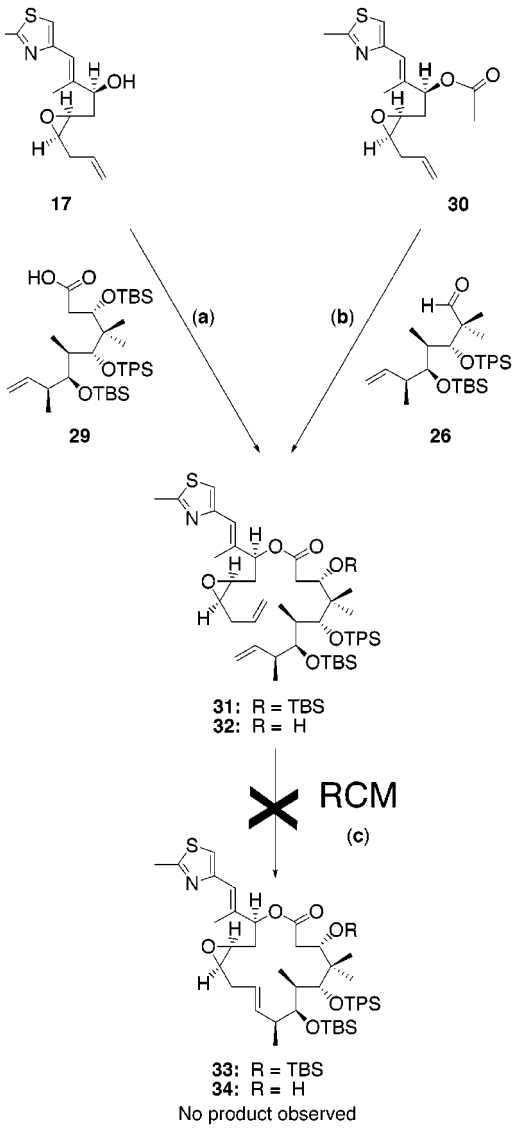


[a] Key: (a) $TiCl_4$, CH_2Cl_2, −78 °C; then CSA, PhH, rt (87%); (b) $LiAlH_4$, Et_2O, −78 °C (91%); (c) Et_2Zn, CH_2Cl_2, Et_2O, rt (93%); (d) NIS (7 equiv), MeOH, rt; (e) (i) *n*-Bu_3SnH, AIBN (cat.), PhH, reflux (80% from **21**); (ii) Ph_3SiCl, imid., DMF, rt (97%); (f) 1,3-propanedithiol, $TiCl_4$, CH_2Cl_2, −78 to −40 °C (78%); (g) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, 0 °C (98%); (h) (i) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2/H_2O (19:1), rt (89%); (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C to rt (90%); (iii) CH_3PPh_3Br, $NaN(SiMe_3)_2$, $PhCH_3$, 0 °C to rt (76%); (iv) $PhI(OCOCF_3)_2$, $CH_2Cl_2/CH_3CN/H_2O$, rt (85%); (i) *t*-$BuOC(O)CH_2Li$, THF, 0 °C (90%; ca. 2.5:1 mixture of C3 epimers in favor of **27**); (j) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, rt; (k) TESOTf, 2,6-lutidine, CH_2Cl_2, rt (90% overall); Bn = CH_2Ph; TMS = $SiMe_3$; TPS = $SiPh_3$; TBS = Si(*t*-Bu)Me_2.

bond afforded the cyclopropane **21**. A regioselective solvolytic fragmentation of the cyclopropane in **21** was accomplished by the agency of *N*-iodosuccinimide (NIS) to afford the methyl glycoside **22**. Following reductive deiodination, subsequent protection afforded the fully functionalized methyl glycoside **23**. Lewis acid-catalyzed dithiane ring opening[48] of the methyl glycoside liberated the future C3−C9 portion of the acyl domain of epothilone after protection of the hydroxyl group as the TBS ether to afford **25**. Finally, olefin formation and exposure of the formyl moiety provided compound **26** (**C**, Figure 2).

First-Generation Ring-Closing Metathesis: Investigation of C9−C10 Bond Construction. Prior to the actual metathesis event, coupling of **17** (**A**, Figure 2) and **26** (**C**, Figure 2) via an ester linkage was required. As suggested in Figure 2, the RCOM substrates could be prepared from the merger of fragments **A**, **B**, and **C**. Two methods were employed in this connection. Realizing that a carboxylic acid coupling to allylic alcohol **17** (**A**, Figure 2) would be ideal in the metathesis approach, it was envisioned that an appropriate acid would arise from the union of acyl fragment **B** and polypropionate fragment **C** [i.e., [**A** + (**B** + **C**)]]. Thus, condensation of the previously prepared **26** (i.e., **C**) with lithiated *tert*-butyl

Scheme 5. Preparation of First-Generation RCOM Substrates and Attempted RCOM[a]



[a] Key: (a) **29**, EDC, CH_2Cl_2, 4-DMAP, rt; then **17** (78%); (b) **26** + **30**, LDA, THF, −78 °C (6:1 mixture of C3 epimers in favor of **32**; 85%); (c) $RuCl_2(PCy_3)_2$=CHPh, PhH, 25 °C, 24 h; Bn = CH_2Ph; TMS = $SiMe_3$; TPS = $SiPh_3$; TBS = Si(*t*-Bu)Me_2.

acetate (i.e., **B**) generated a mixture of diastereomeric alcohols in which the major product was shown to have the requisite 3*S* configuration (Scheme 4). TBS protection of ester **27** afforded **28**, and then subsequent ester hydrolysis generated the desired acid, **29** (Scheme 4). Finally, the resultant acid **29** (i.e., **B** + **C**) could be esterified with alcohol **17** (i.e., **A**) to afford the desired RCOM substrate **31** (Scheme 5).

Our second approach involved assembly of the fragments in Figure 2 in the order [(**A** + **B**) +**C**]. Thus, the (*Z*)-vinyl iodide **17** was acylated to afford **30**, and the resultant lithium enolate of **30** was condensed with aldehyde **26** (Scheme 5). In the event, the aldol reaction afforded an 85% yield of a ca. 5:1 mixture of C3 epimers with the desired diastereomer (**32**) comprising the major product.

Initial efforts in the ring-closing metathesis approach were attempted with substrates **31** and **32** (Scheme 5). However, after employing a variety of catalysts and

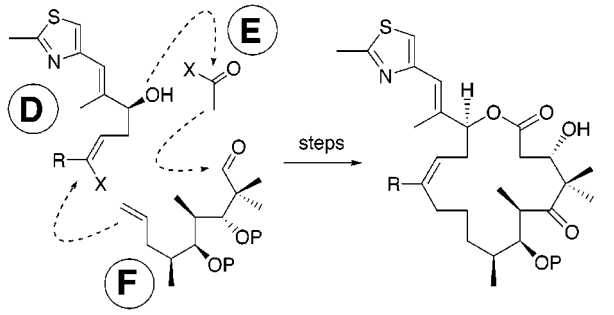


Figure 3. General concept for the Suzuki coupling to generate the C12−C13 double bond of epothilone by merger of fragments **C**, **D**, and **E**. The first successful synthesis of epothilones A and B.

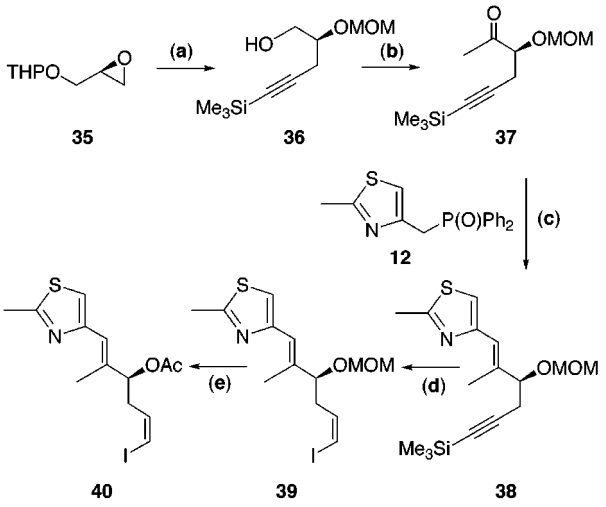
experimental conditions, no cyclized systems (**33** or **34**) were observed. Other substrates were prepared to further probe this unexpected failure; however, no observable reaction was realized. Model systems later suggested that the dense functionality between C3 and C8 had undermined macrocycle formation by RCOM.

***B*-Alkyl Suzuki Strategy.** With the apparent failure of the first ring-closing olefin metathesis approach, we next contemplated the possibility of forming the future C11−C12 bond via a *B*-alkyl Suzuki coupling[49] strategy (paths II and III, Scheme 1). Indeed, the successful synthesis of both epothilones A and B, first accomplished by our laboratories, was achieved through a modified Suzuki strategy. The concept for this route is generalized in Figure 3.

A successful Suzuki coupling pathway would require the merger of the *Z*-haloalkene **D**, the two-carbon extension **E**, and an acyl fragment **F**, as outlined in Figure 3. Using these constructs, we anticipated that the fashioning of the 16-membered macrolide could proceed though two possible routes. We conjectured that the macrolide might be produced by an intramolecular macrolactonization (Scheme 1, path II) wherein the two-carbon acyl fragment, **E**, coupled to fragment **F** would undergo a *B*-alkyl Suzuki merger with the *Z*-vinyl halide, **D** [i.e., [(**D** + (**E** + **F**)]]. Alternatively, we hypothesized an intramolecular aldolization (Scheme 1, path III) wherein the Suzuki merger might occur between the acylated *Z*-vinyl iodide (**D** + **E**) and the polypropionate fragment **F** [i.e, [(**D** + **E**) + **F**]]. In any event, the Suzuki coupling maneuver would be useful only upon introduction of the *β*-epoxide moiety in a stereoselective as well as regioselective manner. All efforts were directed to reaching a substrate, which would allow us toward probe this question.

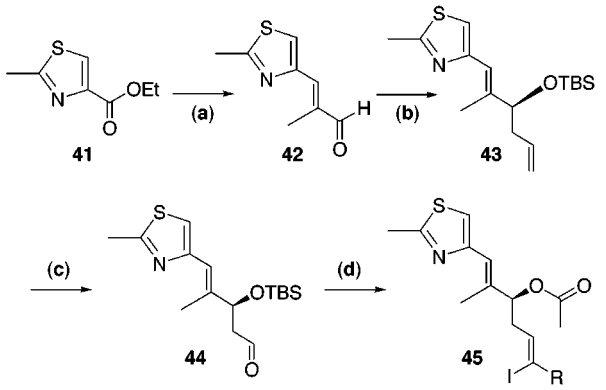
Preparation of the (*Z*)-Haloalkene for *B*-Alkyl Suzuki Coupling. Schemes 6 and 7 outline the syntheses developed for the preparation of the requisite chiral vinyl iodide moiety, **D**. In our first route, we chose to contain the absolute stereochemistry at future C-15 of the epothilones in the context of the commercially available chiral alcohol, (*R*)-(+)-glycidol.[50] Thus, (*R*)-(+)-glycidol was protected as the THP (tetrahydropyran) ether (**35**), and then the electrophilic epoxide was used to alkylate the lithium salt of (trimethylsilyl)acetylene to afford the hydroxy acetylene, **36** (Scheme 6). The primary alcohol was converted to the methyl ketone (**37**) through classical methods. Introduction of the thiazolyl moiety was accomplished by treating ketone **37** with phosphine oxide **12** in an Emmons reaction. To conclude

Scheme 6. First-Generation Synthesis of the Vinyl Iodide Moiety (40) Used in the Total Synthesis of Epothilone A[a]



[a] Key: (a) (i) $Me_3SiCCLi$, $BF_3{\cdot}OEt_2$, THF, −78 °C (76%); (ii) MOMCl, *i*-Pr_2Net, $Cl(CH_2)_2Cl$, 55 °C (85%); (iii) PPTS, MeOH, rt (95%); (b) (i) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C to rt; (ii) MeMgBr, Et_2O, 0 °C to rt (85% for two steps); (iii) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt (93%); (*c*) **12**, *n*-BuLi, THF, −78 °C; then **37**, THF, −78 °C to rt (97%); (d) (i) NIS, $AgNO_3$ $(CH_3)_2O$ (64%); (ii) dicyclohexylborane, Et_2O, AcOH (65%); (e) (i) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt (86%); (ii) Ac_2O, pyridine, 4-DMAP, CH_2Cl_2, rt (99%); PPTS = pyridinium p-toluenesulfonate; MOMCl = methoxymethylchloride; TPAP = *tetra*-*n*-propylammonium perruthenate; NMO = *N*-methyl-morpholine *N*-oxide.

Scheme 7. Second-Generation Synthesis of the Vinyl Iodide Moiety (45) Used in the Total Synthesis of Epothilones A and B[a]

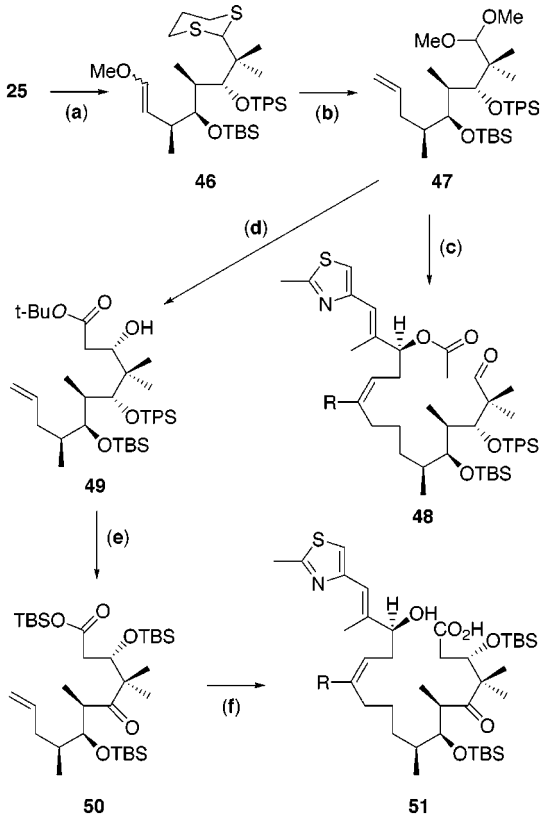


[a] Key: (a) (i) DIBAL, −78 °C (90%); (ii) $Ph_3P{=}C(Me)CHO$ (80%); (b) (i) Keck allylation, 5 days, 50% conversion (85−90% BORSM) or Brown allylboration, $Ipc_2BCH_2CH{=}CH_2$ (85%); (ii) TBSOTf, 2,6-lutidine, −78 °C (90%); (c) (i) OsO_4, NMO (95%); (ii) $Pb(OAc)_4$, Na_2CO_3 (85%); (d) (i) $CH_3CH_2PPh_3I$, BuLi, I_2, NaHMDS (60%); (ii) HF·pyridine, THF (98%); (iii) Ac_2O, DMAP, pyridine (100%).

the synthesis, the TMS acetylene was converted to the corresponding iodoacetylene and then reduced to the (*Z*)-iodoalkene (**39**) through the action of dicyclohexylborane.[51] Finally, deprotection of the MOM protecting group and acetylation afforded the desired (*Z*)-vinyl iodide, **40**.

Our second-generation synthesis of the (*Z*)-vinyl iodide fragment commenced with the readily prepared thiazolyl ester, **41**, and in practice proved to be more concise than our original (*R*)-glycidol based route. Thus, DIBAL reduction of **41** and subsequent Wittig olefination afforded enal **42** that could be allylated with high enantioselectivity

Scheme 8. Preparation of Macoaldolization and Macrolactonization Substrates through *B*-Alkyl Suzuki Merger with *Z*-Vinyl Iodide, 40 or 45[a]

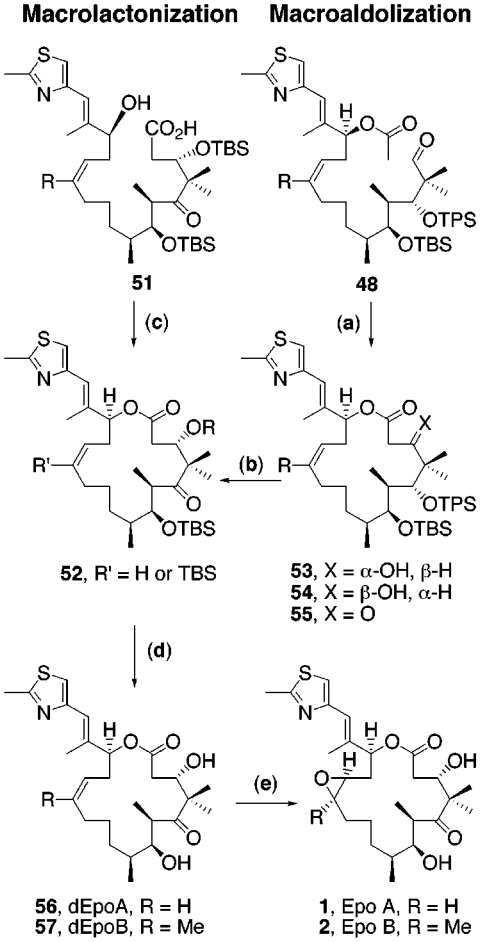


[a] Key: (a) (i) DDQ, CH_2Cl_2/H_2O (89%); (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C to 0 °C (90%); (iii) $MeOCH_2PPh_3Cl$, *t*-BuOK, THF, 0 °C to rt (86%); (b) (i) *p*-TsOH, dioxane/H_2O, 0 °C to rt (99%); (ii) CH_2PPh_3Br, NaHMDS, $PhCH_3$, 0 °C to rt (76%); (iii) $PhI(OCOCF_3)_2$, MeOH/THF, rt, 0.25 h (92%); (c) (i) **45**, 9-BBN, THF, rt; then **40** or **45**, $PdCl_2(dppf)_2$, Cs_2CO_3, Ph_3As, H_2O/DMF, rt (75%); (ii) *p*-TsOH/dioxane/H_2O, 50 °C (85%); (d) (i) *p*-TsOH, dioxane/H_2O (5:1), 50 °C; (ii) *tert*-butyl acetate, LDA, THF, −78 °C; (e) (i) HF·pyridine, pyridine, THF, rt (98%); (ii) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (96%); (iii) Dess−Martin periodinane, CH_2Cl_2, rt (89%); (iv) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (95%); (f) (i) 9-BBN, THF, rt; then **40** or **45**, $PdCl_2(dppf)_2$, Cs_2CO_3, Ph_3As, H_2O/DMF, rt (56%); (ii) K_2CO_3, MeOH/H_2O (84%).

using conditions described by either Brown[52] or Keck[53] (Scheme 7). The resultant alcohol was protected as the TBS ether to afford **43**. Subsequent dihydroxylation and oxidative cleavage afforded aldehyde **44**, which could then be converted to the desired vinyl iodide through a Wittig reaction with the known phosphorane.[54] Protecting group manipulation as shown in Scheme 7 afforded the desired (*Z*)-vinyl iodide, **45**.

With the appropriate (*Z*)-vinyl iodide in hand, we directed our attention to the preparation of the polypropionate moiety suitable for eventual Suzuki merger. We envisioned merger of these two segments to proceed, as described above, through one of two polypropionate segments **47** or **50** (Scheme 8). The polypropionate variations permitted formation of the macrocycle via either macroaldolization or macrolactonization (vide infra). Thus, beginning with thioacetal **46**, a one-carbon homologation sequence afforded the desired dimethyl acetal, **47**. Hydroboration of the *B*-alkyl Suzuki coupling partner **47** and direct subjection of the resultant borane to either vinyl iodide, **40** or **45**, under Suzuki conditions afforded the desired macroaldolization precursor, **48**.

Scheme 9. Macrolactonization and Macroaldolization Approaches to the Total Synthesis of Epothilones A and B[a]



[a] Key: (a) KHMDS, THF, −78 °C, 0.001M (51%; 6:1 α/β); (b) (i) Dess−Martin periodinane, CH_2Cl_2, rt (84%); (ii) $NaBH_4$, MeOH, THF, −78 °C to rt (80% for two steps); (iii) HF·pyridine, pyridine, THF, rt (99%); (iv) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (93%); (c) 2,4,6-trichlorobenzoyl chloride, TEA, 4-DMAP, toluene, rt (88%); (d) HF·pyridine, THF, rt (99%); (e) 3,3-dimethyldioxirane, CH_2Cl_2, −35 °C (49%; ≥16:1 mixture of diastereomers in favor of 1); TPS = $SiPh_3$; TBS = Si(*t*-Bu)Me_2.

The macrolactonization candidate substrate was likewise prepared from dimethyl acetal **47**. Thus, **47** was further elaborated by deprotection of the dimethyl acetal and subsequent treatment of the liberated aldehyde center with lithiated *tert*-butyl acetate (the Rathke anion) as outlined in Scheme 8. Although the aldol reaction in this series afforded a 2:1 mixture of diastereomers, the undesired product could be successfully converted to the desired β-hydroxy ester through an oxidation−reduction sequence (Scheme 8). The C5 protecting group in **49** was removed with buffered HF−pyridine, and the desired C5 ketone in **50** was generated after two additional steps (Scheme 8). Finally, hydroboration of the highly advanced coupling partner, **50**, was successfully accomplished through the action of 9-BBN to afford the desired borane, which was coupled under Suzuki conditions with the appropriate vinyl iodide (**40** or **45**) to afford the desired macrolactonization substrate **51**.

Macrolactonization and Macroaldolization Approaches. Having successfully achieved C11−C12 bond formation through Suzuki coupling, we then sought to close the macrocyclic ring. One of our first attempts would involve a straightforward macrolactonization route (Scheme 9). Compound **51**, prepared as described above, underwent smooth macrolactonization under Yamaguchi conditions[55] to afford the desired macrolactone, **52**, in 88% yield. An alternative and most interesting cyclization approach involved the macroaldolization of compound **48**. This approach was possible because the *gem*-dimethyl substitution at C4 prevents enolization of the aldehyde. In the event, deprotonation of compound **48** (see Scheme 9) with KHMDS in THF (−78 °C) did indeed stereoselectively close the macrocycle, resulting in the preferential formation (6:1) of the desired (*S*)-C3 alcohol **53**. The undesired epimer, **54**, could be recycled through ketone **55** via an oxidation−reduction sequence allowing a very efficient access to the desired β-hydroxy lactone, **53**. Deprotection of **52** to desoxyepothilone A (or B) **56** (or **57**) was achieved with HF·pyridine. Completion of the synthesis was accomplished by introduction of the C12−C13 epoxide with DMDO under carefully controlled conditions to afford a 49% yield (≥16:1 mixture of diastereomers in favor of the desired) of epothilone A, **1** or epothilone B, **2**. Indeed, the introduction of the 12,-13-oxido linkage was both possible and stereoselective in the very late stages of the synthesis.

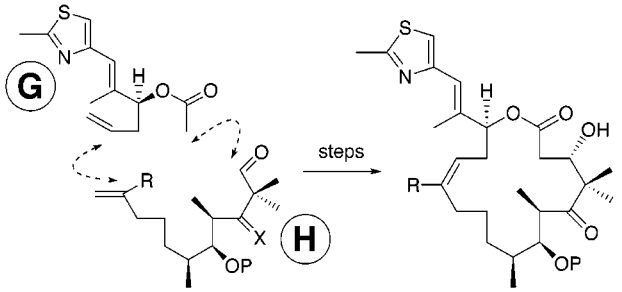
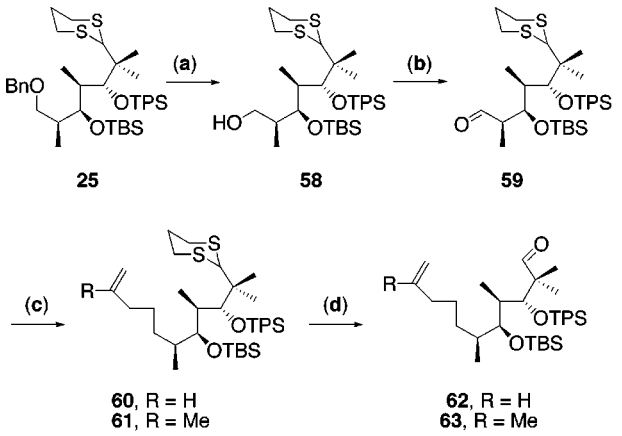


Figure 4. General concept for RCOM to generate the C12−C13 double bond of epothilone by merger of fragments **G** and **H**.

Scheme 10. Synthesis of the C12-C13 RCOM Substrates[a]



[a] Key: (a) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2/H_2O, rt (90%); (b) $(COCl_2)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 to 0 °C (90%); (c) (i) 3-butenylmagnesium bromide, Et_2O, −78 to 0 °C; or (ii) 4-iodo-2-methyl-1-butene, *t*-BuLi, 2.1 equiv, Et_2O, −78 to −50 °C; then **59**, Et_2O, −78 to 0 °C; (iii) thiocarbonyl diimidazole, 4-DMAP, 95 °C; (iv) *n*-Bu_3SnH, AIBN, C_6H_6, 80 °C; (d) $PhI(OCOCF_3)_2$, $CH_2Cl_2/CH_3CN/H_2O$, rt (85%); Bn = CH_2Ph; TMS = $SiMe_3$; TPS = $SiPh_3$; TBS = Si(t-Bu)Me_2.

Second-Generation Ring-Closing Metathesis: Investigation of C12−C13 Bond Construction. Having

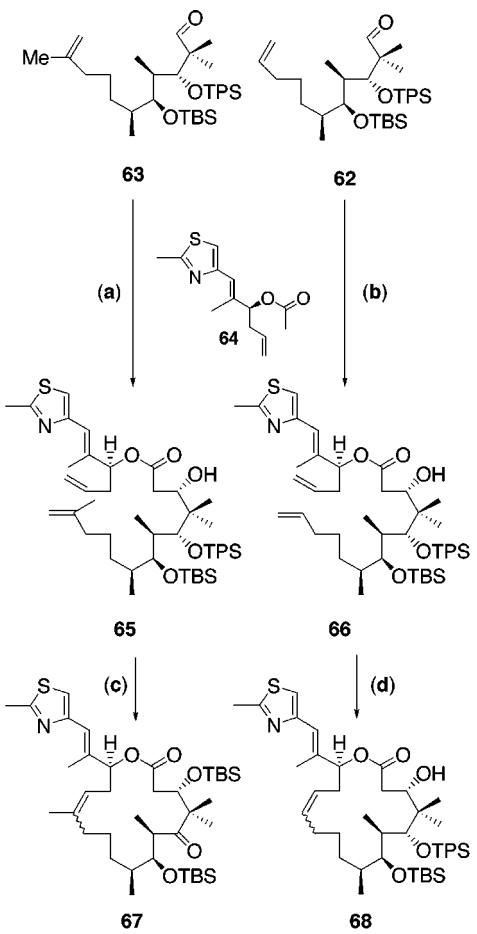
accomplished our central goal in the total synthesis of both EpoA and Epo B, we were in a position to reinvestigate the possibility of an intramolecular olefin methathesis. We envisioned that the two chiral moieties, **G** and **H**, could be effectively coupled via an intramolecular olefin metathesis route (Figure 4). Indeed, this route was attractive because we were now certain of the ability to epoxidize the double bond of the fully assembled macrolide (vide supra) in a stereoselective manner.

To investigate the possibility of a C12−C13 bond merger, we began with the previously prepared dithiane **25**. A two-step deprotection−oxidation sequence afforded the desired aldehyde **59**. Then, Grignard addition of either 3-butenylmagnesium bromide or 4-lithio-2-methyl-1-butene and subsequent deoxygenation at C9 afforded **60** and **61**, respectively (Scheme 10). Finally, the dithiane protecting group was cleaved to liberate aldehydes **62** and **63**.

Scheme 11 outlines the general synthesis of the RCOM substrates. Thus, intermolecular aldolization with the lithium enolate of acetate **64** and either **62** or **63** afforded the requisite substrates (**66** and **65**, respectively) for RCOM attempts. We were hopeful that olefin metathesis would be more productive on these substrates since there would be increased tether length between the C12-olefin and branched positions located in the polypropionate. Indeed, this proved correct, and eventually a second-generation C12−C13 RCOM approach was developed.[56] In these studies, we utilized both the ruthenium-based Grubbs catalyst[57] and the molybdemum-based Schrock catalysts[58] to mediate the metathesis. Although the gross yield of cyclized material (**67** and **68**) was in general quite high, this route was disabled by the formation of mixtures of C12−C13 *Z*/*E* isomers that are separable only with the greatest of difficulty. In the end, attempted RCOM on a collection of substrates revealed that the stereochemical course of the olefin metathesis was a function of the nature of the substituents on the acyl chain. However, no perturbations were discovered that significantly favored formation of the desired natural *Z* compounds. While the mixtures of (*E*)- and (*Z*)-olefin isomers produced in these reactions did indeed prove useful in our early SAR studies, the separation of products was particularly inconsistent with our goal to produce substantial material for in vivo investigations.

As the total synthesis phase of the work was bearing fruit, our primary attentions turned toward the evaluation of epothilones A and B in vitro and in vivo. We would also use the synthesis practiced thus far to secure access to strategic analogues that would chart an SAR profile. Before going into the results of these exercises, and presenting the progression of events somewhat out of sequence, we first conclude the description of our synthesis studies. Though the LACDAC-based syntheses described until now had many interesting features and, if practiced with appropriate persistence, would produce enough compound for early biological assessments (vide infra), the early results of these evaluations underscored the need for larger quantities of the end-game structures. Our choices here were to continue the synthesis as it was being conducted with even greater assiduity, or to devise a new strategy. We strongly preferred the latter track for producing much larger amounts of desired drug candidates (vide infra). In essence, the pressure of the biological findings to be described shortly forced us to raise the bar in our expectations of the effort from an academically pleasing exercise, to one that would produce significant quantities for detailed, in vivo investigation. We emphasize that we were not actually dealing with the question of a practical total synthesis in the sense of future commercialization. The lesser, but still formidable, problem that did engage our attention was that of producing adequate material for extensive animal testing without turning our laboratory into a factory.

Scheme 11. Second-Generation RCOM: Synthesis of Substrates and Investigation of C12-C13 Bond Construction[a]



[a] Key: (a) (i) LDA, THF, −78 °C (65%; α/β, 1:1); (ii) Dess−Martin periodinane, CH_2Cl_2, rt; (iii) $NaBH_4$, MeOH, THF, −78 °C to rt (ca. 92% for two steps); (b) (i) LDA, THF, −78 °C (70%; α/β, 1:1); (ii) Dess−Martin periodinane, CH_2Cl_2, rt; (iii) $NaBH_4$, MeOH, THF, −78 °C to rt (ca. 92% for two steps); (c) (i) HF·pyridine, pyridine, THF, rt (93%); (ii) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (85%); (iii) Dess−Martin periodinane, CH_2Cl_2 (94%); (iv) $Mo(CHMe_2Ph)(N(2,6\text{-}(i\text{-}Pr)_2C_6H_3))(OCMe(CF_3)_2)_2$, 20 mol %, C_6H_6, 0.001 M, 55 °C, 2 h, 86% (1:1 mixture of *E*- and *Z*-olefin isomers); (d) $RuBnCl_2(Pcy_3)_2$, 50 mol %, C_6H_6, 0.001 M, rt, 24 h (81%, 1:9 mixture of *Z*- and *E*-olefin isomers); TPS = $SiPh_3$; TBS = Si(t-Bu)Me_2.

Figure 5 provides an overall view of our earliest thoughts on this problem. Our new synthesis embraces three key discoveries that were each crucial to the success of the program.[59] First, we hoped that the difficult C1−C11 polypropionate domain might yet be assembled through a stereoselective aldol condensation of the achiral C5−C6 (*Z*)-metalloenolate system **J** and homochiral aldehyde **K** to generate the C6−C7 bond. Second, a *B*-alkyl Suzuki coupling between the vinyl iodide construct **L** and an alkyl borane would form the C11−

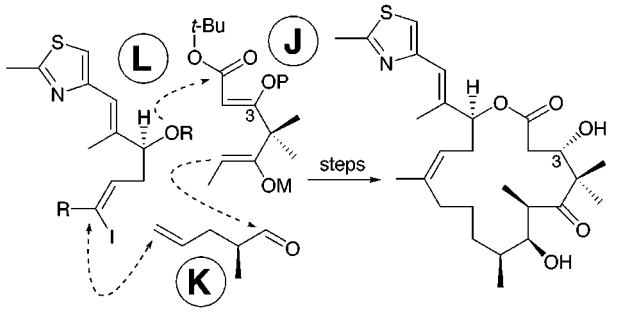


Figure 5. General concept for the new synthesis of dEpoB by merger of fragments **J**, **K**, and **L**. The key steps in the new, practical total synthesis are a stereospecific aldol reaction, *B*-alkyl Suzuki coupling, and stereoselective Noyori reduction of the C3-ketone.

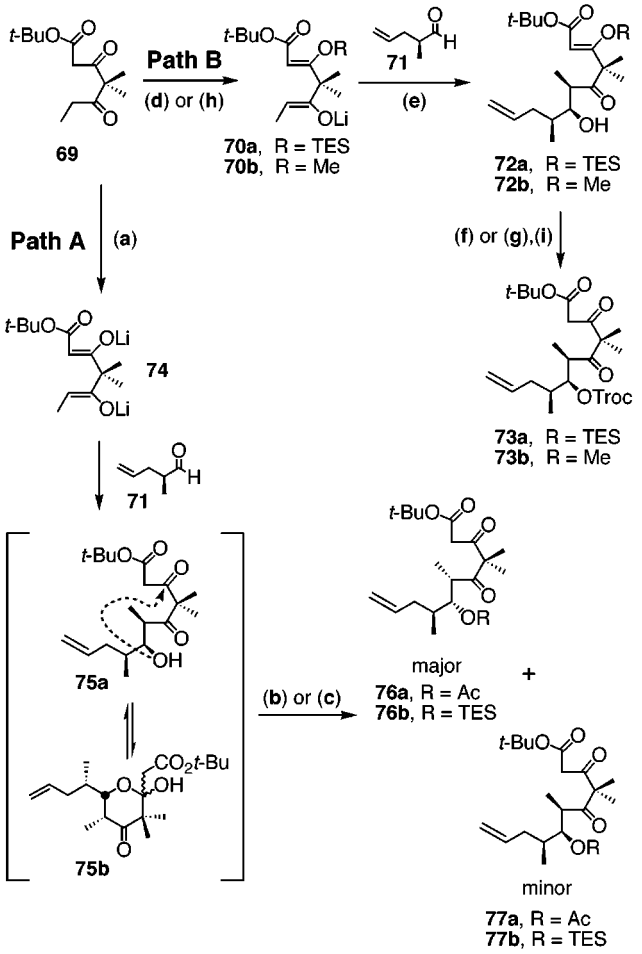
C12 bond. Finally, late-stage and stereoselective reduction of the C3-ketone to the requisite C3-alcohol would be required. If indeed an aldol coupling of constructs **J** and **K** would allow us to introduce the troublesome C1−C6 fragment as a single achiral building block followed by late-stage reduction of the C3-ketone, a major simplification relative to our earlier generation LACDAC based method would have been accomplished. Thus, we would be asking of the aldol coupling a syn connectivity between the emerging stereocenters at C6−C7 concomitant with the requisite anti relationship at C7 relative to the resident chirality at C8. Needless to say, it would be necessary to steer the aldol condensation to C6 in preference to the more readily enolizable center at C2.

In view of this challenge, we could chart two distinct itineraries: First, a *B*-alkyl Suzuki merger of constructs **K** and **L**, followed by a post-Suzuki aldol condensation between the Suzuki coupled product (**K** + **L**) and the enolate **J** [e.g., [**J** + (**K** + **L**)]] (Figure 5). Our second route envisaged a key aldol condensation between constructs **J** and **K**, followed by a B-alkyl Suzuki coupling to effect the merger of the aldolate (**J** + **K**) with the vinyl iodide, **L** [e.g., [(**J** + **K**) + **L**]] (Figure 5). In practice, it soon became apparent that the post-Suzuki aldol condensation route [e.g., [**J** + (**K** + **L**)]] was less desirable. Indeed, the aldol condensation of the constructs (**K** + **L**) with the enolate **J** provided consistently a 2.5:1 mixture of diastereomers. Such ratios undermined our hopes for a practical synthesis. Accordingly, we directed our efforts toward developing the total synthesis of dEpoB utilizing the second route [e.g., [(**J** + **K**) + **L**]].

Dianion Equivalents Corresponding to the Polypropionate Domain of Epothilone B

We turned first to the problem of orchestration of the C1−C7 polypropionate sector to realize positionally specific aldolization at C6 (see arrow in structure J). Three tactical approaches were surveyed toward this end. As outlined in Scheme 12, the aldol reaction (Path A) was initially performed directly between aldehyde **71** and the (*Z*)-lithium enolate **74**. Indeed, this bond construction could be achieved; however, the resultant hydroxyl function at C7 tended to cyclize to the C-3 carbonyl group, thereby affording a rather unmanageable mixture of hydroxy ketone (**75a**) and lactol (**75b**) products. We note that lactol formation could be reversed following treatment of the crude aldol product under the conditions shown (Scheme 12); however, under these conditions an

Scheme 12. Dianion Equivalents Corresponding to the Achiral Polypropionate Fragment J[a]



[a] Key: (a) 2.2 equiv of LDA, THF, −50 °C; (b) TESCl, imidazole, DMF, 12 h (55%, two steps), 2−3:1 ds; (c) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 12 h (58%, two steps), 2−3:1 ds; (d) NaH, TESOTf, −50 °C (86%); (e) LDA, THF, −120 °C (75%, 5.4:1 ds); (**f**) TrocCl, pyridine, CH_2Cl_2; then 0.5 M HCl/MeOH (86%, two steps); (g) TrocCl, pyridine, CH_2Cl_2 (86%); (h) $TMSCHN_2$, Hunig's base, CH_3CN, MeOH (81%); (i) *p*-TSA, acetone, rt (90%).

inseparable 2−3:1 mixture of diastereomeric products, **76** (**a** or **b**)/**77** (**a** or **b**) was obtained. This avenue was further impeded when it became apparent that neither the acetate nor TES moieties introduced to reverse the lactolization in the direction of **76a** or **76b**, respectively, were stable to the conditions of the remainder of the synthesis. Unfortunately, more durable blocking groups could not be introduced onto the C7-hydroxyl center by trapping of the desired hydroxy ketone **75a**.

Although our initial attempts in this direction proved impractical, our efforts were not a complete loss in that we were able to confirm that the critical aldol reaction with (*S*)-aldehyde **71** did indeed provide the desired C6−C7 syn and C7−C8 anti relationship (by anti-Felkin−Anh addition) as the major diastereomer.[60] At this point, we realized that simply engaging the C3 carbonyl group of the nucleophile in another functional arrangement might alleviate the problem of undesired lactolization. Thus we sought to contain the C3 carbonyl group as an enol ether (path B, Scheme 12). Using this strategy, the unwanted cyclization did not occur, and the C7 hydroxyl group could be readily protected with a more robust moiety. As reported in our recent disclosure,[61] the tri-

ethylsilyl (TES) enol ether (**70a**) is prepared from tricarbonyl system **69**. Moreover, the resultant C3-enol, C6-enolate ether successfully underwent an aldol coupling with aldehyde **71**. After a period of trial and error, we discovered that the C7-hydroxyl moiety could be readily protected as the 2,2,2-trichloroethoxycarbonate (Troc) ester. We were also pleased to discover that the aldol reaction between enol ether **70a** and aldehyde **71** gave rise to a 1:5.5 mixture of C7−C8 syn and desired C7−C8 anti isomers, and furthermore, these diastereomers were readily separated by flash column chromatography. Dissappointingly, our initial success in this regard did not translate well in attempted scale-ups (>1 g), and the aldol reaction between **70a** and aldehyde **71** eventually proved to be troublesome. We soon discovered that the resultant C3 TES enol ether in **70a** (R = TES) was prone to decomposition under the very basic conditions of the aldol reaction and was also quite sensitive to silica gel chromatography.

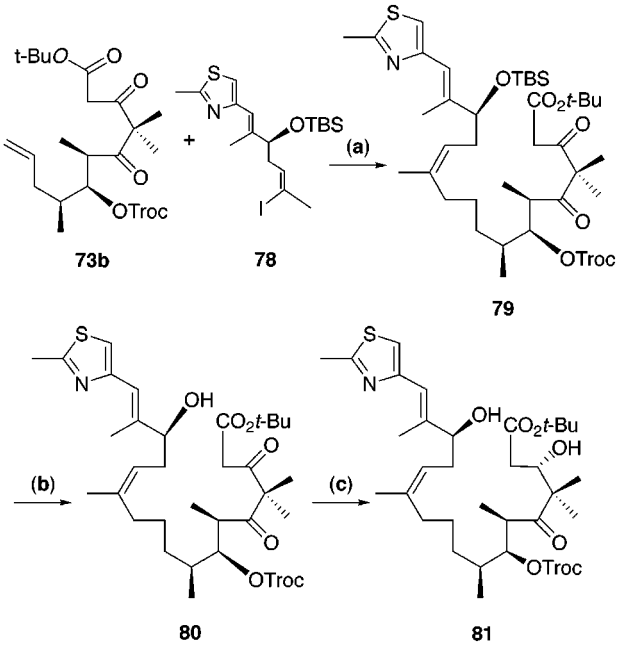
As a result, we sought to modify the C3 protecting group to provide a more stable moiety that would survive both the basic aldol reaction conditions and silica gel chromatography. To this end, we investigated the usefulness of a methyl enol ether linkage.[62] Happily, we discovered that the requisite methyl enol ether, **70b**,[63] could be readily prepared from trimethylsilyl diazomethane and $TMSCHN_2$ with Hunig's base in high yield and as a single olefin isomer (path B, Scheme 12). Fortunately, the aldol reaction performed on the methyl enol ether also afforded the same diastereoselectivity as the TES enol ether. Moreover, the diastereomers (**72b**, R = Me, and its C8 epimer) obtained in the aldol reaction were also readily separated by flash column chromatography on silica gel. Without the difficulties encountered by decomposition, the C7 hydroxyl moiety could be readily protected as the Troc ester to afford **73b**. Fortunately, the reaction could be conducted on larger scales.

***B*-Alkyl Suzuki Merger.** A second key component of our more practical total synthesis of epothilone B includes a *B*-alkyl Suzuki merger,[64] Scheme 13, which can be conducted between the previously described vinyl iodide[65] **78** and, remarkably, the extensively functionalized **73b**. While we were somewhat apprehensive that the tricarbonyl arrangement might be sensitive to the hydroboration conditions necessary for the preparation of the organoborane at C11, our concerns were allayed as the coupling step was accomplished without difficulty. With this milestone accomplished, the resultant TBS-protected product (**79**) was hydrolyzed to afford the requisite C15-hydroxy ester **80**.

Stereoselective Noyori Reduction. With the successful completion of the diastereoselective aldol condensation and *B*-alkyl Suzuki coupling phases, the selective asymmetric reduction of the C3-ketone now emerged as the next key issue. The choice of a suitable reducing agent for this transformation would be determined with the goal of optimizing diastereoselectivity and chemoselectivity. Selective asymmetric reduction of the C3-ketone without concomitant reduction of the ketone at C5 would be crucial. The reader will note that, unlike our earlier generation LACDAC based syntheses, the goal would have to be accomplished without guidance from a C5 hydroxyl derivative.

We chose to focus on the Ru(BINAP) species developed by Noyori[66] and others,[67] which displays excellent chiral recognition and has demonstrated the ability to catalyze

Scheme 13 *B*-Alkyl Suzuki Coupling and Asymmetric Noyori Reduction in the New, Practical Total Synthesis of dEpoB[a]

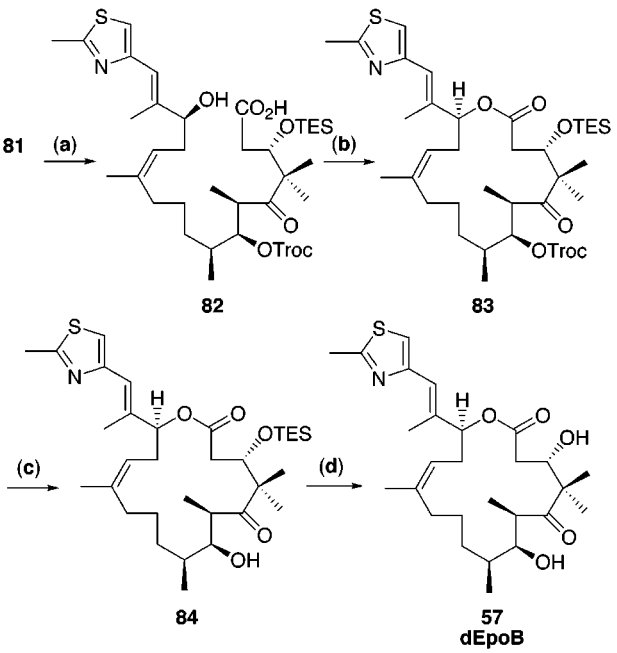


[a] Key: (a) 9-BBN; then **73b**, $Pd(dppf)_2Cl_2$, Ph_3As, Cs_2CO_3, H_2O,DMF, rt, 2 h (60%); (b) 0.4 N HCl/MeOH (96%); (c) (*R*)-Ru(BINAP), H_2, 1200 psi, MeOH, HCl, 25 °C, 7 h (88%).

the asymmetric reduction of various β-keto esters.[68] We eventually settled on the modified Noyori catalyst $[RuCl_2(BINAP)]_2 \cdot NEt_3$]. The possibility of concurrent reduction of the olefinic linkage was an obvious source of concern. We were also concerned that the δ-carbonyl moiety in our substrate might adversely affect the outcome of the stereo- and/or regiocontrol in the asymmetric reduction because of the inherent ability of the ruthenium species to chelate both 1,3- and 3,5-dicarbonyl arrangements. Further investigation of this matter revealed that in the reduction of β,δ-diketo esters, the C3-ketone is at first selectively hydrogenated to give the 3-hydroxy ester and then subsequently the C5 carbonyl group is hydrogenated to afford the dihydroxy ester.[69] We hoped that the presence of the sterically demanding *gem*-dimethyl functionality in **80** would preclude the undesired chelation effect and subsequent reduction of the C5-ketone under the Noyori reduction conditions.

During our early examinations of the asymmetric Noyori reduction, it became apparent that reduction of various diketo esters in this series was critically dependent upon the amount of acid present in the reaction.[70] Previous reports of these types of failures have been attributed to the presence of low-level basic impurities in the substrate. In our hands, the presence of stoichiometric HCl was absolutely required for successful, diastereoselective reduction at C3. In the absence of acid or in the presence of only 0.5 equiv of acid, either no reduction or reduction with poor diastereoselectivity was observed for the C3-ketone. Furthermore, each reaction mixture was contaminated with a considerable amount of product resulting from reduction of the C11−C12 olefinic moiety. We suspected that the presence of the basic thiazolium nitrogen in **80** may coordinate with the ruthenium catalyst resulting in slow reaction rates and poor levels of asymmetric reduction. However, upon the

Scheme 14. Completion of the Synthesis of dEpoB[a]



[a] Key: (a) (i) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C to rt; (ii) then 0.1N HCl/MeOH (77%, two steps); (b) 2,4,6-trichlorobenzoylchloride, TEA, 4-DMAP, $PhCH_3$ (78%); (c) SmI_2, cat. NiI_2, −78 °C, THF (95%); (d) HF·pyridine, THF (98%); (e) DMDO, CH_2Cl_2 (80%).

addition of stoichiometric HCl, the thiazole moiety would perhaps be protonated or partially complexed resulting in reestablishment of the appropriate catalytic cycle.

After several attempts, we chose to perform the asymmetric hydrogenation at 1200 psi and ambient temperature. At higher pressures (>1300 psi), the chemoselectivity of the reduction was compromised and resulted in the reduction of both alkene groups. At lower pressures (<1000 psi), dramatically slower rates of reduction along with poorer diastereoselectivity in the reduction at C3 were observed. While there are reports of asymmetric Noyori reductions at elevated temperatures (ca. 80 °C) proceeding at lower pressures (50 psi), we encountered decomposition of the epothilone substrates at these elevated temperatures.

In the event, the Noyori reduction of ketone **80** with the dimerized Noyori catalyst $[RuCl_2((R)\text{-BINAP})]_2[NEt_3]$ successfully effected the asymmetric hydrogenation of the 3-oxo group of **80** to afford diol **81** with excellent diastereoselectivity (>95:5, no minor diastereomer observed by ^1H NMR) and in high yield. Moreover, the asymmetric Noyori reduction afforded excellent diastereocontrol and displayed superb chemoselectivity without concomitant reduction of the remote di- and trisubstituted olefins[71] or subsequent reduction of the C5-ketone.

In retrospect, the ability to control the desired C3 stereochemistry of the late stage intermediate **80** had permitted us to introduce the C1−C6 fragment into the synthesis as an achiral building block.

Completion of the Synthesis of dEpoB. With these critical issues favorably resolved, no significant obstacles remained in the total synthesis. The conversion of **81** to dEpoB (**57**), Scheme 14, was effected using previously developed methods.[29,30] Thus, the diol **81** was successfully hydrolyzed to the requisite hydroxy acid **82** by treatment with triethylsilyl trifluromethanesulfonate (TESOTf). Subsequent macrolactonization according to Yamaguchi conditions afforded the fully protected macrolactone **83**. Samarium(II) iodide proved to be singularly effective in accomplishing the reduction of the 2,2,2-trichloroethoxy carbonate (Troc) moiety in **83** to afford the C7 alcohol **84**.[72] Finally, standard HF·pyridine deprotection of the C3 TES group afforded the desired dEpoB **57**. As previously shown, the latter can be epoxidized to afford the natural product epothilone B (**2**) with a high degree of chemo- and stereoselectivity.

Discovery of Surprising Long-Range Effects on the Double Diastereoface Selectivity in an Aldol Condensation. During our investigations directed toward a practical synthesis of dEpoB, we were surprised as well as pleased to discover an unanticipated bias in the relative diastereoface selectivity observed in the aldol condensation between the *Z*-lithium enolate **J** and aldehyde **K**, Figure 5. Indeed, the aldol reaction proceeds with the expected simple diastereoselectivity to afford as the major product the C6−C7 syn relationship shown in Scheme 12; however, the C7−C8 relationship of the principal product was anti.[73] The observed syn/anti relationship between C6−C7/C7−C8 in the aldol reaction between the (*Z*)-lithium enolate of **69** and chiral aldehyde **71** was not anticipated on the basis of the traditional models for predicting the stereochemical outcome of these reactions.[74] These fortuitous and intriguing discoveries prompted us to investigate the cause for this favorable result.

Superficially, the relative face selectivity exhibited in the aldol condensation is contrary to the predicted models for double stereodifferentiation encompassed in the Felkin rules.[75] Our unexpected results were reconcilable when viewed in the context of intramolecular interactions present during the transition state that might induce a facial bias in the approach of the enolate, thus leading to the observed C7/C8 anti relationship. Indeed, Evans,[76] Hoffman,[77] Heathcock,[71,72] and Roush[78] have examined similar systems in the recent past. Their studies examined reactions involving *Z*(O)-enolates (*Z*-crotylboronates)[77a,b] and allylboron reagents[77,78] with α-branched chiral aldehydes for which the Felkin−Anh rules for diastereoselection fail to adequately rationalize the observed stereochemistry. After examination of the Felkin−Anh transition state, Evans concluded that the presence of a destabilizing gauche-pentane interaction causes the anti-Felkin−Anh product to be formed preferentially.[75] However, recently, Roush has proposed a more refined perception of this model to account for attrition in anti selectivity with α-methyl chiral aldehydes.[79] The Roush model proposes that in the reacting Curtin-Hammett conformer, the larger protected ether group (OR) of the aldehyde is distanced from the R′ group of the enolate (**I**) to avoid an unfavorable *syn*-pentane interaction (Figure 6, **I**). Thus, the crux of the model focuses on the minimization of unnecessary steric interactions between the largest functionalities of the enolate and the α-branched aldehyde in the reacting ensemble. Consequently, the observed anti-Felkin−Anh selectivity displayed in these reactions is in general typical for "α-methyl" aldehydes.

We note, however, that the model implied in Figure 6 does not precisely correspond to our situation since our discoveries using aldehyde **71** (Figure 6, **II**) and related congeners are devoid of the sterically demanding ether moiety (Figure 6, I) that is usually involved in fashioning

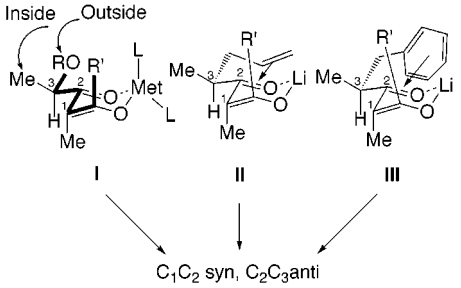
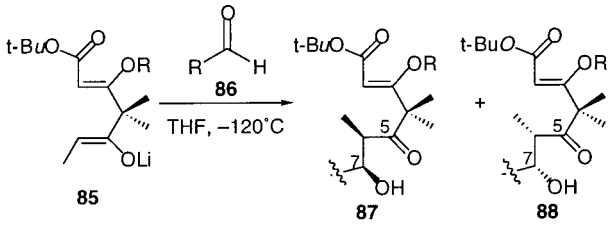


Figure 6. Proposed transition-state models for the aldol condensation of aldehydes, **86**, and enolate, **85** in Table 1 and Table 2.

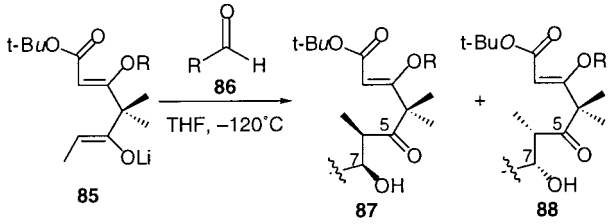
Table 1. Results of Aldol Condensations of Various Acyclic Aldehydes 86 with Enolate 85



Entry	Aldehyde R =	Ratio (**87**:**88**) (C7:C8) (syn:anti)	
a		1	5.5
b		1	1.3
c		1	2.0
d		11	1.0
e		1	2.0
f		1	4.0

anti diastereoface selectivity.[80] Indeed, the branching moieties present in our aldehyde **71** (methyl vs propenyl) are not significantly different in their steric constraints. Rather, it is our finding that the conformational bias in our substrates is dependent on a very particular relationship between the formyl moiety and unsaturation in the pendant side chain. As a result of our studies, we proposed an early model wherein the presence of unsaturation at C4−C5 in the aldehyde moiety provides a subtle stabilizing nonbonded interaction between the unsaturation in the aldehyde and the carbonyl of the enolate (Figure 6, **II**).[81] Indeed other examinations of aldol reactions of chiral α-methyl aldehydes has suggested that nonbonded interactions play an important role in determining aldehyde diastereofacial selectivities in the reaction of Z-enolates.[82] Thus we propose that the olefin−aldehyde interaction somehow stabilizes the transition state leading to the observed major anti-Felkin diastereomer **75a**.[83]Table 1 outlines the results of aldol reactions between the enolate **85** and various other aldehydes **86**. In light of our findings, we probed further into the source of this unpredicted stereochemical outcome. During the course of our investigations, we identified a particular significance in the intervening tether

Table 2. Results of Aldol Condensations of Various Benzenoid Aldehydes 86 with Enolate 85



Entry	Aldehyde (**86**)	Ratio (**87**:**88**) (C7:C8)	
a		1	5.0
b		1	2.1
c		1	1.2
d		1	2.8
e		1	4.2
f		1	5.0
g		1	5.4
h		1	1.2

length between the site of unsaturation and the formyl group. Thus, reduction of the double bond of the side chain led to a sharply diminished selectivity affording a 1.3:1 mixture of diastereomeric products (entry b, Table 1). Likewise, lengthening of the tether beyond that found in entry a led to a 2:1 ratio of diastereomers (entry c, Table 1). By contrast, shortening the tether (entry d, Table 1) gave strong syn diastereoface selectivity consistent with previous findings with this particular aldehyde.[80] The results of entry e, Table 1, wherein the steric factors are virtually equivalent (propyl versus 2-propenyl at the branching site) demonstrate a small, but clear preference for the C7−C8 anti product, presumably reflecting the special effect of the olefin-aldehyde interaction. The Roche aldehyde[84] (entry f, Table 1), a substrate well-known for its tendency to favor the anti-Felkin−Anh adduct,[85] performs as expected to afford a 4:1 mixture of anti/syn diasteromeric products.

We next contemplated whether the unsaturation site could be encompassed in the context of a properly positioned benzenoid linkage (Figure 6, **III** and Table 2). We were intrigued to discover that excellent diastereoface selectivity was obtained in the aldol condensation of the (*Z*)-lithium enolate **85** with the benzyl-substituted aldehyde, entry a, Table 2.

On the basis of our proposed model, functional group substitution about the aromatic ring could significantly effect the donating ability of the ring. Consequently, we examined the effects of para-positioned functional groups on the resultant C7−C8 relationship (entries e−h, Table 2). Some minor slippage in the anti/syn ratio is seen in the electron-deficient *p*-bromo substrate (entry e, Table 2). The benchmark ratio seen in entry a is restored with

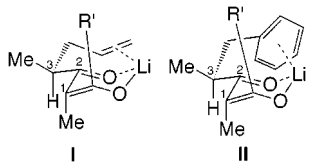


Figure 7. Potential transition state for diastereoselective aldol reaction involving a potential lithium cation bridging the olefin (I) or aryl moiety (II).

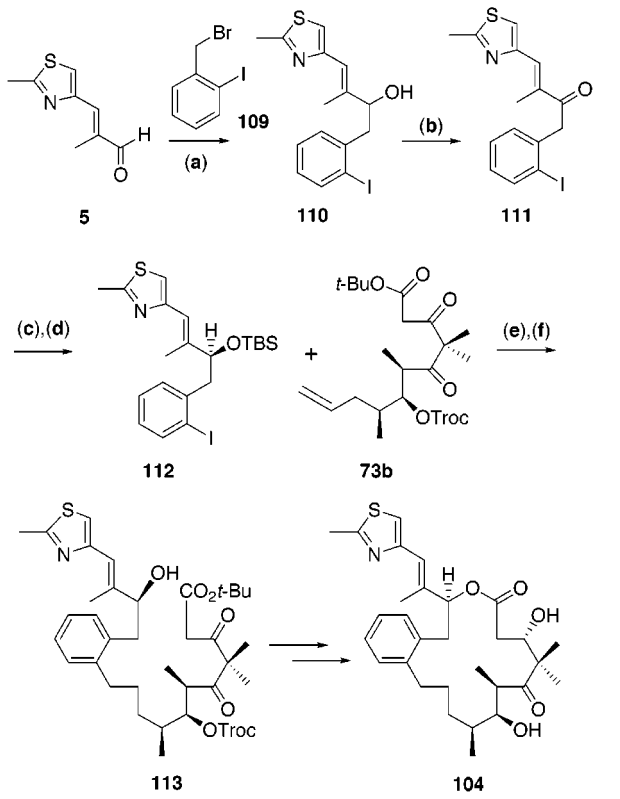
the electron-rich *p*-methoxy substrate (entry f, Table 2), while a small improvement was realized with the *p*-dimethylamino derivative (entry g, Table 2). By contrast, in the strongly electron-deficient *p*-nitrophenyl substrate (entry h, Table 2), the C7−C8 anti selectivity was severely abrogated. Clearly, the "aryl effect" is closely coupled to the electron-donating ability of the ring.

In contrast to the reconcilable data observed with para-substituted substrates, a range of ortho substituents (entries b−d, Table 2) all resulted in significant weakening of the C7−C8 anti selectivity. We take these data to suggest that ortho substitution results in some steric inhibition of the rotamer in which the faces of the aromatic ring and formyl group are parallel (see structure **III**, Figure 6).

We interpret our data to support a stabilizing through-space interaction of a donor olefinic linkage with the aldehyde function as the likely source of preference of conformers **II** and **III** leading to the sense of attack anticipated by the Roush model that leads to the anti-Felkin products. However, an alternative, yet attractive hypothesis that rationalizes the observed anti-Felkin−Anh diastereoselectivity has been suggested wherein a Li cation bridges the olefin (or π system of the aryl moiety) and the enolate (Figure 7).[86] Thus, intramolecular bridging in the transition state could indeed enhance the facial approach of the enolate that leads to the C7−C8 anti diastereomer.

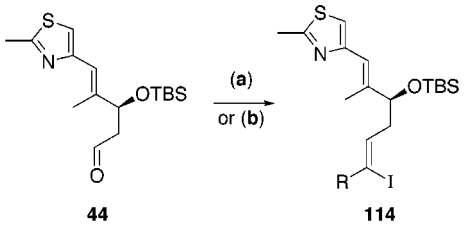
More recently, modification of our new synthesis provided relatively facile access to analogue **104** (Scheme 15). Analogue **104** bears a fused phenyl ring at C12−C13 and was prepared as outlined in Scheme 15. In general, the synthesis of this compound followed an analogous route to that traversed in our new synthesis (vide supra) and centered on the preparation of the aryl iodide *B*-alkyl Suzuki coupling partner **112**. Thus, zinc-mediated nucleophilic addition[87] of 2-iodobenzylbromide **109**[88] with aldehyde **5** afforded racemic **110** in 67% yield. Oxidation of *rac*-**110** followed by asymmetric reduction to **(*S*)-110** afforded only modest enantioselectivities. The highest enantiomeric excess observed for the reduction of ketone **111**, by methods that are generally effective using (*R*)-2-methyl-CBS-oxazaborolidine,[89] was 60%. While far from ideal, this enantioselectivity margin was sufficient for our purposes. Subsequent protection of the resultant alcohol as the TBS ether provided the desired aryl iodide, **112**, suitable for Suzuki coupling. Palladium-mediated Suzuki coupling of **112** and tricarbonyl **73b** afforded, after C15 deprotection, the hydroxy ester **113**. Then hydrolysis of the *tert*-butyl ester in **113** afforded the desired C15 hydroxy acid (not shown) for Yamaguchi macrolactonization. The diastereomeric products (3−4:1 mixture of ds; epimers at C15 resulting from incomplete enantioselective reduction by the CBS catalyst) could be separated by flash column chromatography after macrolactonization to afford, as the major product, the analogue

Scheme 15. Preparation of the Novel Analog 104 for Both in Vitro and in Vivo Biological Studies[a]



[a] Key: (a) Zn, CuCN, LiCl, THF, then BF_3, **52** (67%); (b) Swern oxidation (90%); (c) CBS reduction, 60% ee (89%); (d) TBSOTf, 2,6-lutidine, CH_2Cl_2 (90%); (e) 9-BBN, THF, 2 h; then **56**, $Pd(dppf)_2Cl_2$, Cs_2CO_3, Ph_3As, H_2O, DMF, rt, 6 h (70%); (f) 0.5 M HCl/MeOH, THF, rt, 6 h (85%).

Scheme 16. General Procedure for the Synthesis of C-12-Modified Analogs for in Vivo Biological Studies[a]



[a] Key: (a) $XPh_3P(CH_2)_nOTBS$, BuLi, rt; then I_2, −78 °C; then NaHMDS, −78 to −30 °C and then **44**; (b) $XPh_3P(CH_2)_nCH_3$, BuLi, rt; then I_2, −78 °C; then NaHMDS, −78 to −30 °C and then **44**.

containing the desired "natural" stereochemistry at C15.[90] The remainder of the synthesis was carried out according to our previous protocols (vide supra) to obtain analogue **104**.

Access to other C12-modified epothilone analogues[91] was secured by modifying the alkyl group geminal to the vinyl iodide as depicted in Scheme 16. Thus, Wittig olefination of the appropriate phosponium salt with aldehyde **44** afforded good yields of the requisite vinyl iodides **114** that were used in the synthesis of analogues **95**−**99**. Although *Z*/*E* mixtures were obtained, these diastereomers were separable with the desired *Z* isomer being predominant. Similar modifications of the Wittig reaction in Scheme 16 also afforded the alkyl substituted (*Z*)- and (*E*)-vinyl iodides that were used in the synthesis

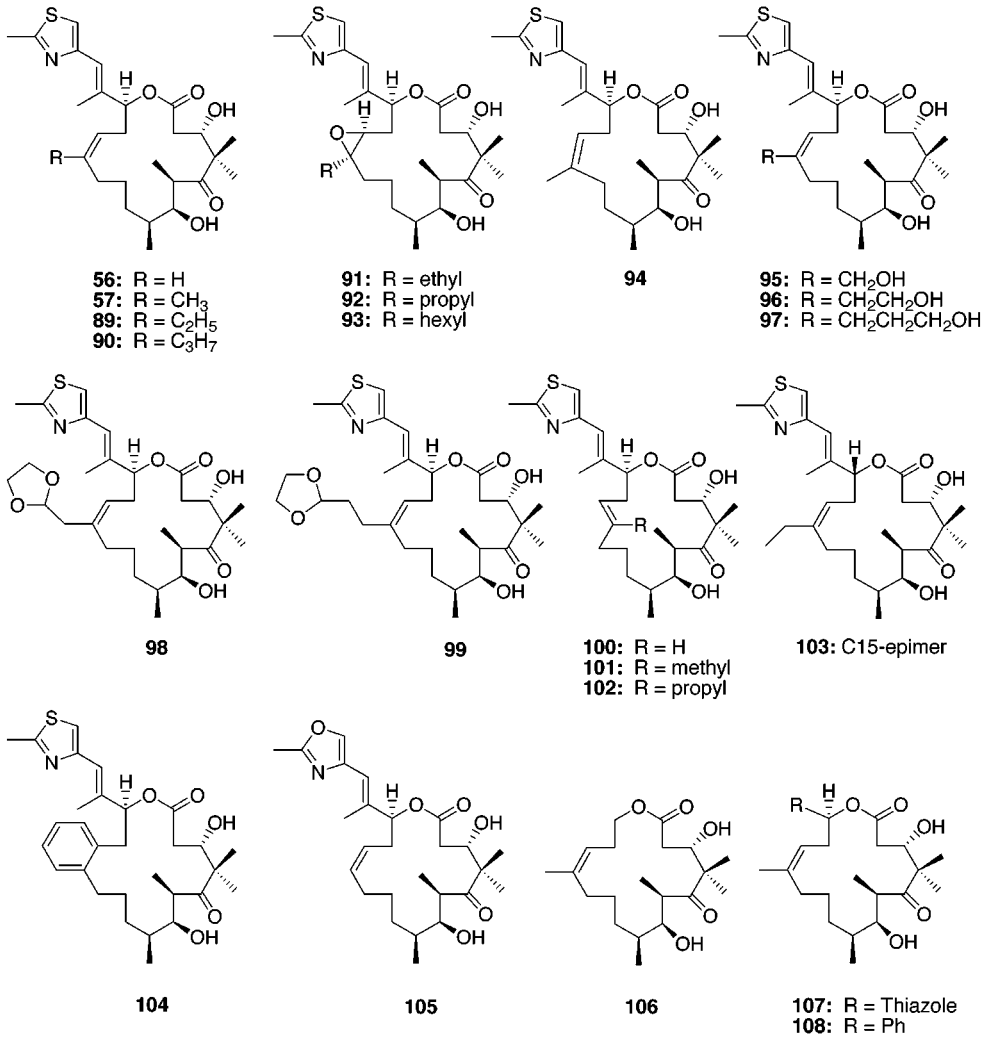


Figure 8. Fully synthetic epothilone analogs that were prepared for SAR studies.

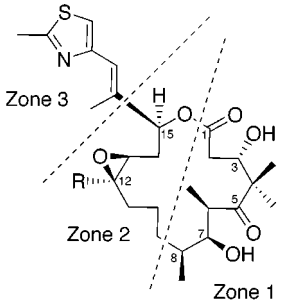


Figure 9. Results of our SAR studies demonstrated by dividing the regions of the epothilones into zones that are tolerable to chemical modification.

of analogues **89**−**93** and **100**−**103** in Figure 9. Finally, the desired (*Z*)- or (*E*)-vinyl iodide could be incorporated into our new synthesis affording sufficient quantities of these C-12 modified analogues for both in vitro and in vivo studies.

Biological Evaluation of Epothilones

Although paclitaxel is currently used in the treatment regimen in a variety of solid forms of cancer, it can be rendered ineffective by a number of mechanisms including overexpression of the energy-dependent drug transport protein P-glycoprotein (PgP).[92,93] Overexpression of PgP generally results in broad-spectrum resistance to many structurally and mechanistically diverse anticancer agents. This phenomenon constitutes a potentially critical element of the multidrug-resistance (MDR) phenotype. Indeed, MDR may be reversed by certain reversing agents that appear promising when co-administered with the anticancer agent.[94] While it may perhaps be possible to overcome P-glycoprotein resistance by changes in the schedule of administration,[95] a search for paclitaxel substitutes with improved performance in vitro and in vivo has met with limited success.[96,97] Interestingly, we recently reported that farnesyl transferase inhibitors coadministered with paclitaxel, and the epothilones, have shown promising results by enhancing the mitotic sensitivity of tumor cells in a synergistic manner.[98] However, the clinical acceptability of such drug−drug combinations is open to serious question.

While our long-term goals encompassed the discovery, evaluation, and development of new epothilone-like compounds with improved clinical efficacy, we started out at a more modest level. Our first subgoal was to chart the general SAR characteristics of epothilones A and B and well-selected congeners with respect to three questions: (i) tubulin assembly and affinity, (ii) in vitro cytotoxicity against various cell lines, and (iii) efficiency in the context of MDR cell lines.

Table 3. Relative Efficacy of Various Analogs against the Sensitive CCRF-CEM and Resistant CCRF-CEM/VBL Cell Lines

analog	CCRF-CEM IC_{50} (nM)	CCRF-CEM/VBL IC_{50} (nM)
paclitaxel	2.1	>4000
Epothilone A (**1**)	3.0	200
Epothilone B (**2**)	0.2	1.0
56 (depoA)	22	12
57 (depoB)	9.0	17
89	21	77
90	39	67
91	1.0	7.0
92	4.0	6.0
93	27	49
94	>10 000	>10 000
95	49	>2000
96	2	1033
97	9.5	167
98	4.3	20
99	80	409
100	52	35
101	90	262
102	90	254
103	55	197
104	2050	4300
105	30	49
106	>10 000	>10 000
107	>3000	1200
108	1800	>5000

Figure 8 and Table 3 depict the range of structures prepared in this laboratory for this program and the in vitro cytotoxicity of each analogue when evaluated against both sensitive CCRF-CEM and resistant CCRF-CEM/VBL cell lines. Table 3 shows the relative toxicity of each of the analogues to both the sensitive CCRF-CEM and resistant CCRF-CEM/VBL cell lines.[99] While paclitaxel showed strong cross-resistance to the CCRF-CEM/VBL, most epothilones demonstrate little or no cross-resistance. Thus, EpoB and dEpoB were 6.1-fold and 1.8-fold resistant, respectively, whereas clinically used vinblastine and paclitaxel (Taxol) were 527-fold resistant and 1,971-fold resistant, respectively, against the multidrug resistant CCRF-CEM/VBL cell line. Indeed, EpoB is ca. 3400 times more active than paclitaxel against CCRF-CEM/VBL in cell-culture cytotoxicity studies. In another experiment, the actinomycin D resistant hamster lung tumor cell line, DC-3F, demonstrated a 13 000-fold resistance to actinomycin D and a 388-fold resistance to paclitaxel. By contrast, the resistant DC-3F cell line was only 28-fold resistant to EpoB and exhibited no observable resistance to dEpoB. In general, among the various compounds and cell lines tested in our laboratory, dEpoB exhibited the least cross-resistance to several drug-resistant cell lines.

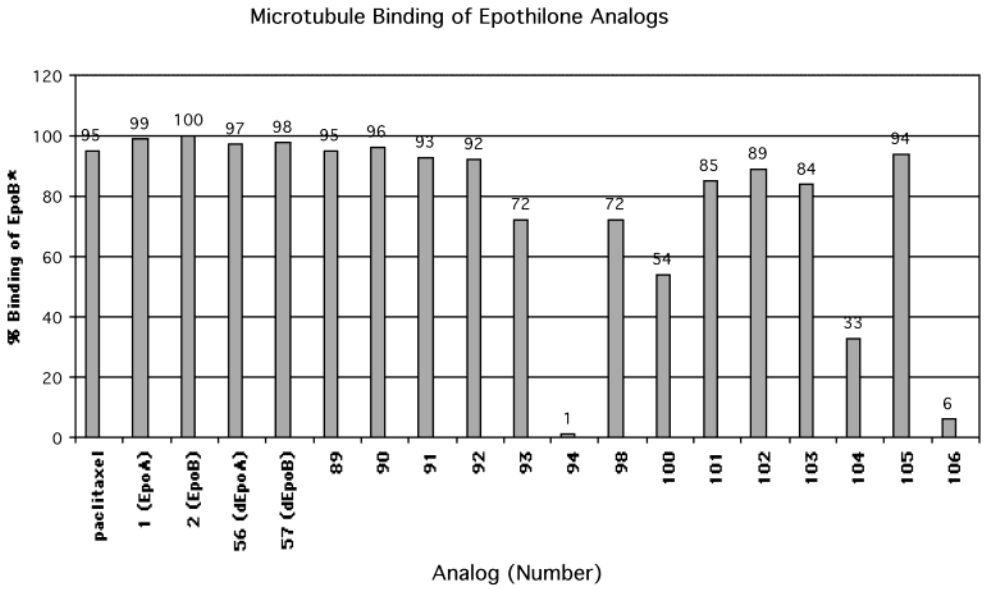
Table 4 depicts graphically the relative ability of selected key analogues to bind tubulin relative to EpoB. Indeed, the microtubule stabilizing ability closely parallels the observed cytotoxicity data available against the sensitive CCRF-CEM cell lines (compare Table 3 and Table 4). In this graph, the degree of microtubule formation was assayed in the presence of 10 μM of each of the tested analogues. The degree of microtubule assembly in the presence of 10 μM epothilone B is defined as 100%.

The results of the SAR study obtained from our collection of analogues prompted us to divide the structure of epothilone into an acyl sector (C1−C8, zone 1), an *O*-alkyl sector (C9−C15, zone 2), and a pendant aryl sector that projects from C15 (zone 3) as depicted graphically in Figure 9.

The acyl sector (zone 1) of the epothilones proved to be the most intolerant of modification. For example, inversion of stereochemistry at C3 (*S* to *R*) or reduction at C5 resulted in serious arrest of activity. Analogues with functionality at C3, C5, C6, C7, and C8 removed or significantly modified demonstrated both diminished tubulin binding activity and diminished cytotoxicity (structures not shown). Truncation of the achiral hinge region generated a 15-membered macrolide, **106**, which demonstrated a major loss of in vitro tubulin polymerization/depolymerization ability (Table 4).

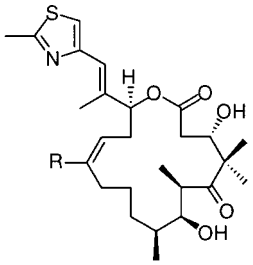
Next, we consider the *O*-alkyl sector (zone 2). This proved to be remarkably tolerant to modification with basic maintenance of in vitro function. The (*Z*)-desoxy compounds **56** (dEpoA) and **57** (dEpoB) retain most, if

Table 4. Relative Ability of Selected Epothilone Analogs To Bind Microtubules Relative to the Parent Compound EpoB



*Formation of microtubules in the presence of 10 μM of the tested compounds. Microtubules formed in the presence of 10 μM epothilone B is defined as 100%.

Table 5. Susceptibility of Epothilone Analog To Succumb to the MDR Resistance Phenotype Is a Function of the Tether Length of the C12 Alkyl Chain



Entry (No.)	Compound R =	CCRF-CEM IC_{50} (nM)	CRCF-CEM/VBL IC_{50} (nM)	Resistance
1 (**57**)	CH_3 (dEpoB)	9.5	17	1.8
2 (**98**)		4.3	20	4.7
3 (**99**)		80	409	5.1
4 (**95**)	CH_2OH	49	>2000	>50
5 (**96**)	CH_2CH_2OH	32	1033	33
6 (**97**)	$CH_2(CH_2)_2OH$	9.5	167	18
7	Paclitaxel	2.1	4140	1971

not all, of the cytotoxicity and tubulin binding ability of the parent counterparts, **1** and **2**, respectively. Since the C12-methyl-substituted compounds **2** (EpoB) and **57** (dEpoB) are more active than the desmethyl compounds **1** (EpoA) and **56** (dEpoA), we chose to investigate the limits of substitution at C12 in both the parent and desoxy systems. Substitution at C12 with ethyl, propyl, or hexyl in the parent system analogues **91**−**93** was well tolerated as was substitution of ethyl (**89**) and propyl (**90**) in the desoxy series. Surprisingly, even the (*E*)-desoxy compounds **100**−**102** maintain significant biological activity, although reduced relative to the parent *Z* series (compare **57** and **101**, for example). Finally we investigated the effect of inversion of stereochemistry at C15 (**103**) which revealed that the cyctotoxic and microtubule stabilizing activity is still maintained, albeit in attenuated form.

We further examined substitution at C12 while being particularly attentive to the effects of polarity. We were intrigued to discover that the more polar functional groups had a profound effect on the susceptibility of the compound to succumb to multidrug-resistance (Table 5). Thus, the highly polar alcohol functionality provided analogues (**95**−**97**) that were MDR substrates and were presumably exported out of the cell more efficiently by the drug efflux transport protein, PgP. Interestingly, the tether length of the alkyl chain in the alcohol series significantly effected the MDR susceptibility with the longer tether length being associated with less susceptibility to the MDR phenotype and stronger potency against both the sensitive and resistant cell lines. Likewise, the effect of tether length on potency is clearly demonstrated in the analogues **98** and **99** wherein analogue **98** is roughly 20-fold more potent than **99**, Table 5. However, the ethylidene acetals, **98** and **99**, which are in the aldehyde oxidation state, but protected as the ethylene glycol acetals, are not MDR substrates and both retain activity against the resistant CCRF-CEM/VBL cell lines (Table 5).

We also examined the permissiveness of the "aryl" zone to structural modification. Analogues prepared for this purpose demonstrated that the replacement of thiazole by oxazole is well tolerated. Thus, **105** is equipotent with **57** (dEpoB) in both tubulin polymerization and cytotoxicity assays. The more drastic substitution of phenyl in place of the thiazolyl moiety led to a structure, which retained 60% of the activity of **2** in the tubulin polymerization assay, although there was a slight loss of cytotoxicity. Deletion of the entire unsaturated thiazole moiety as in **106** exhibited very low cytotoxicity (IC_{50} > 10.0 mM) and demonstrated almost no activity in tubulin binding assays. The olefinic spacer element connecting the aromatic rings with C15 was examined via compounds **107** and **108** and demonstrated a major loss of cytotoxicity. These analogues illustrate that "zone 3" requires the olefinic spacer linking the macrolide at C15 to an aromatic subsection which is then substantially amenable to modification. Thus, while zone 3 is tolerant of modification, the aryl functionality in some form may not be deleted.

Finally, the novel analogue **104**, described above, in which C12−C13 is confined within an aromatic ring was examined. Intriguingly, preliminary evaluation of this compound demonstrated considerable tubulin binding. However, poor cyctotoxicity was observed in vitro. Conceivably, this result could be attributed to an issue of transport rather than mechanistic value.

Although we did not prepare a massive collection of compounds, the SAR data obtained from our pool of analogues was more than adequate. It also allowed for the selection of candidates for more advanced in vitro and in vivo studies. Nicolaou et al. have reported an exhaustive SAR study and substantially, which prompted the same general conclusions.[100] In summary, it is clear that the polypropionate sector constitutes a "hot-spot" region with great sensitivity to structural change. In comparison, the *O*-alkyl and aryl sectors exhibit regions of significant tolerance, both in the cytotoxicity assays and in tubulin binding assays.

In Vivo Analysis: Discovery of a Potential EpoB Problem

Attention was first directed to the in vivo efficacy of EpoB in athymic mice with various xenograft tumors. Our focus on EpoB was motivated by the interplay of several considerations. As pointed out at the outset, we had no access to any fermentation products. Hence, our choice was first dictated by considerations of in vitro data described above, focusing on highest tubulin binding affinity, potency, and efficacy in the MDR context. Given the more extensive resource commitment associated with animal work, and given the fact that we were making our compounds by total synthesis, we could not help but consider the issue of ease of access in rendering our selections. While we did evaluate some side-chain analogues projecting from C12, these were more difficultly available from a synthetic standpoint and correspondingly received less attention than fully synthetic EpoB.

Indeed, our preliminary in vitro studies had indicated that the naturally occurring parent compound, EpoB (**2**), was the most potent of the epothilone compounds (vide supra). Thus, EpoB and paclitaxel were administered

Table 6. Therapeutic Effect of EpoB, dEpoB, and Paclitaxel in SCID Mice Bearing Human MX-1 Xenograft*

drug	dose (mg/kg)	avg body wt change (g) day 7	day 11	day 13	day 15	day 17	avg tumor size (tumor/control) day 11	day 13	day 15	day 17	deaths
control		27.2	+0.8	+1.1	+1.9	+0.6	1.00	1.00	1.00	1.00	0/8
57	15	27.1	+0.8	+1.1	+1.6	+1.5	0.65	0.46[a]	0.49[a]	0.41[b]	0/6
	25[e]	**27.0**	**+0.4**	**+0.7**	**+1.0**	**+0.7**	**0.38**[a]	**0.11**[b]	**0.05**[c]	**0.04**	**0/6**
2	0.3	26.9	+0.5	+0.4	−0.3	−1.2	1.00	.071	0.71	0.84	0/7
	0.6[f]	27.4	−0.3	−1.3	−2.1	−2.1	1.08	0.73	0.81	0.74	3/7[h]
paclitaxel	5	26.9	−0.1	+0.4	+1.1	+1.2	0.54	0.46	0.40[a]	0.45[b]	0/7
	10[g]	27.6	−2.7	−1.1	−0.3	+2.2	0.43	0.37	0.12	0.11	4/7[h]

*MX-1 tissue, 50 μL/mouse, was implanted into SCID mice on day 0. Every other day i.p. (intraperitoneal) treatments were given on days 7, 9, 11, 13, 15. The average tumor volumes of the control group on day 11, 13, 15, and 17 were 386 ± 120, 915 ± 245, 1390 ± 324, and 1903 ± 319 mm^3 (mean ± SEM), respectively. [a] $P < 0.05$. [b] $P < 0.01$. [c] $P < 0.005$. [d] $P < 0.001$. [e] One out of six mice with no detectable tumor on day 35. [f] Three mice died of drug toxicity on day 17. [g] Four mice died of drug toxicity on day 13, 13, 13, 15. [h] P values were not shown due to toxic lethality.

Table 7. Toxicity of Epothilone B and Desoxyepothilone B in Normal Nude Mice

group	dose, schedule, and route of administration	deaths
control		0/4
EpoB (2)	**0.6 mg/kg**, QDx4, i.p.	**8/8**[a]
dEpoB (4)	**25 mg/kg**, QDx4, i.p.	**0/6**

[a] Mice died of toxicity on day 5, 6, 6, 7, 7, 7, 7, 7.

weekly to SCID mice bearing the paclitaxel-sensitive CCRF-CEM xenograft. In this experiment, EpoB and paclitaxel demonstrated similar reduction of tumor size, although no cures ensued. To evaluate the susceptibility of EpoB to MDR-resistant xenografts, the chemotherapeutic potential of EpoB was assayed using human lymphoblastic leukemia, CCRF-CEM/VBL, tumor xenografts implanted into SCID mice. The tumor bearing SCID mice were treated with either EpoB (0.7 mg/kg, intra peritoneal, i.p.) or paclitaxel (2.0 mg/kg, i.p.) on days 7, 8, 9, 10, 14, and 15.[29] Indeed, the mice treated with EpoB demonstrated more significant reduction in the size of the tumor when compared to the control mice than did those treated with paclitaxel (data not shown), although a full cure was not obtained.

Subsequently, the therapeutic effects of EpoB and paclitaxel were compared and evaluated in athymic mice bearing an MX-1 xenograft.[101] Nude mice bearing the MX-1 xenograft were treated with either EpoB (0.3 or 0.6 mg/kg, Q2Dx5, i.p.) or paclitaxel (5 mg/kg, Q2Dx5, i.p.), Table 6. This early in vivo probe pointed to a potentially serious toxicity issue for EpoB. Its tolerated doses were not therapeutically adequate when compared to the clinically effective paclitaxel.

At this stage, it seemed that the object of our total synthesis, EpoB, did not manifest therapeutic ratios in mice, such as to suggest that it would emerge as a drug. *It had soon become apparent that, at the biological level, we had reached the kind of crisis point which was all too familiar to us as synthetic chemists.* Often in synthesis when a favored pathway turns out to be unworkable, there is launched a process of rationalization followed by improvisation. Ideas which, a priori, seemed less attractive than the route which was being followed, begin to garner more notice and adherence in the light of a breakdown of the once favored course.

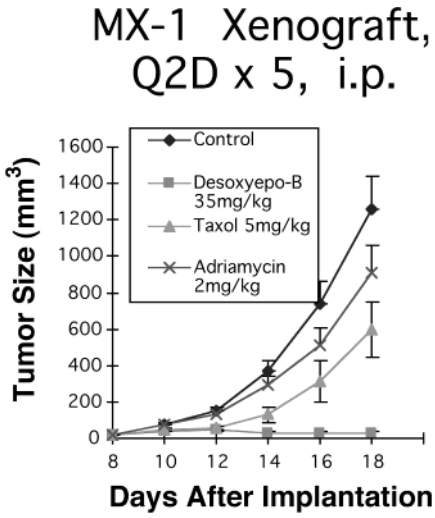


Figure 10. Therapeutic effect of dEpoB, paclitaxel, and adriamycin in nude mice bearing the human mammary carcinoma MX-1 xenograft. MX-1 tissue preparation 100 μL/mouse was implanted s.c. on day 0. Every other day i.p. treatments were given on day 8, 10, 12, 14, and 16 with dEpoB 35 mg/kg (■), paclitaxel 5 mg/kg (▲), adriamycin 2 mg/kg (×), and vehicle (DMSO, 30 μL) treated control (◆). For paclitaxel, 2/10 mice died of toxicity on day 18. For adriamycin, 1/10 mice died of toxicity on day 22. For dEpoB, 10/10 mice survived and were subjected to the second cycle of treatment at 40 mg/kg on day 18, 20, 22, 24, and 26. This led to 3/10 mice tumor-free up to day 80, whereas 7/10 mice were with markedly suppressed tumors and were sacrificed on day 50.

Table 8. Therapeutic Effects of Desoxyepothilone B, Paclitaxel, Adriamycin, and Camptothecin in SCID Mice Bearing MDR Human MCF-7/Adr Tumor*

drug	dose (mg/kg)	avg body wt change (g) day 8	day 11	day 13	day 15	day 17	avg tumor size (tumor/control) day 11	day 13	day 15	day 17	deaths
control	0	25.0	+2.0	+2.3	+3.1	+3.7	1.00	1.00	1.00	1.00	0/8
57	**35**	**25.0**	**0.3**	**+0.7**	**0.6**	**+0.8**	**0.31**[b]	**0.27**[c]	**0.30**[c]	**0.34**[a]	**0/8**
paclitaxel	6	25.3	+1.7	+1.8	+0.8	+0.9	0.57	0.66	0.85	0.90	0/7
	12	24.5	+0.7	−1.3	−2.4	0	0.50	0.51	0.32	0.40	3/7[d]
adriamycin	2	25.6	+0.2	−0.4	−0.6	−0.4	0.70	0.68	0.84	0.78	0/8
	3	24.6	+0.5	−1.3	−3.2	−1.6	0.66	0.83	0.57	0.53	3/6[d]

*MCF-7/Adr cells, 3 × 10^6/mouse, were implanted into SCID mice on day 0. Every other day, i.p. treatments were given on days 8, 10, 12, 14, and 16. The average tumor size of control group on day 11, 13, 15, and 17 was 392 ± 84, 916 ± 210, 1499 ± 346, and 2373 ± 537mm^3, respectively (mean ± SEM). [a] $P < 0.05$. [b] $P < 0.01$. [c] $P < 0.005$. [d] P values were not shown due to toxic lethality.

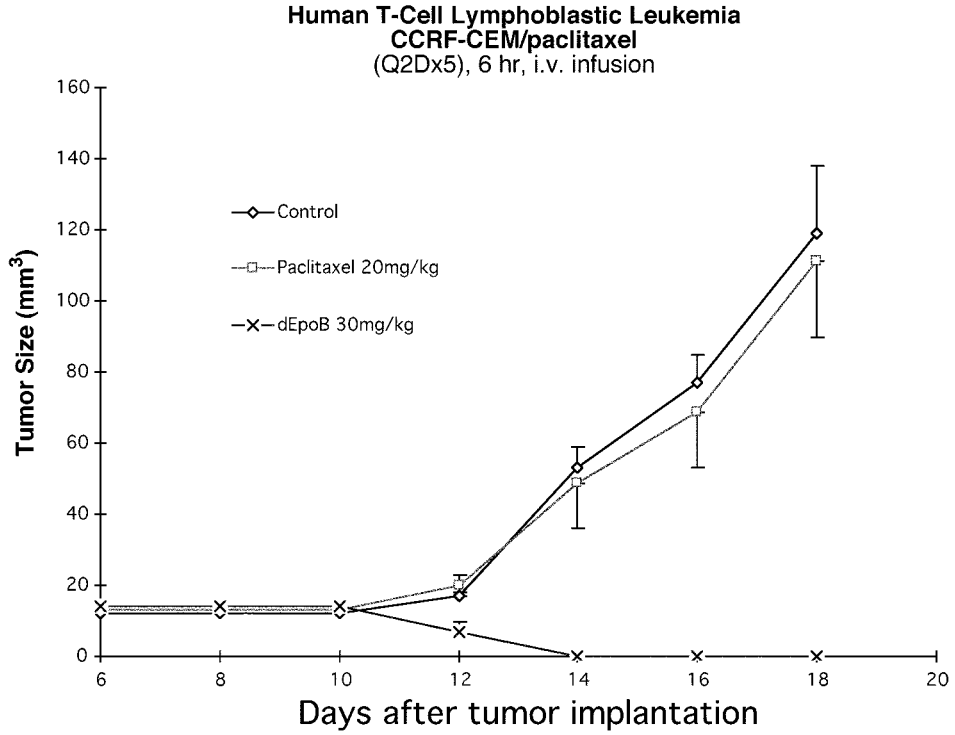


Figure 11. Therapeutic effects of administration of paclitaxel (20 mg/kg) and dEpoB (30 mg/kg) on the human T-cell lymphoblastic leukemia CCRF-CEM/paclitaxel following (Q2Dx5), 6 h, i.v. infusion. Human T-cell lymphoblastic leukemia (CCRF-CEM/paclitaxe) cells resistant to paclitaxel were inoculated subcutaneously (10^7 cells) into athymic mice on day 0. Every other day, i.v. infusion was given on day 6, 8, 10, 12, 14, 16, and 18. The average tumor volume of the control group on day 12, 14, and 16 was 20 ± 3, 119 ± 22, and 415 ± 62 mm^3, respectively (mean $\pm$ SEM, $n = 3$). The vehicle for 6 h i.v. infusion was 100 mL (Cremophor/ethanol, 1:1) + 3 mL of saline.

The analogy in our scenario here was that the maximally potent compound, EpoB, was failing to provide sufficiently workable therapeutic indices in mice. We then began to theorize that perhaps EpoB could be dissected into two pharmacological components. In this view, born in an atmosphere of crisis, the overall structure had a useful cytotoxic capacity presumably related to its tubulin assembly affinity. However, perhaps there was another parallel source of bioreaction, not particularly directed to relief of tumor burden, manifesting a parallel baseline toxicity which inevitably compromised the clinical advancement of EpoB.

While indulging this line of thinking, we began to wonder whether the 12,13-oxido linkage might be a site of vulnerability providing the undesired baseline toxicity. Quite naturally, this took us back to the 12,13-desoxy derivative, dEpoB (**57**). While this compound was less potent in the in vitro cytotoxicity screens (Table 3) relative to EpoB (**2**), in the tubulin affinity assay (Table 4) the performances of the two compounds were actually comparable. We wondered whether the cytotoxicity differential between the two compounds might not correspond to a gross toxicity factor of the 12,13-oxido linkage that undermined the therapeutic index of **2** with no useful antitumor advantage.

Of course, from the standpoint of our synthesis, dEpoB (**57**) enters the stream one step earlier than EpoB itself. Furthermore, dEpoB is itself a very minor fermentation product (EpoD) in the mixture that provides EpoB. Hence, if the compound prepared by chemical synthesis were shown to be useful, perhaps it could in the long run, arise from optimization of the fermentation process. Thus, as is often the case in chemical synthesis, necessity rather than a priori analysis was the forerunner of invention. Elsewhere, we have told the story of the emergence of dEpoB (EpoD) at the biological level.[102] Our first line of inquiry involved head-to-head comparisons of dEpoB versus EpoB. Early on, we compared the tumor agents as to toxicity in normal athymic mice on a daily i.p. schedule shown in Table 7. Remarkably, it was found that dEpoB is much less toxic at 25 mg/kg than is EpoB at 0.6 mg/kg. The comparison with athymic mice bearing human MX-1 xenograft was no less striking. For example, when nude mice bearing MX-1 xenografts were challenged with dEpoB (30 mg/kg, Q2Dx5, i.p.) marked tumor regression and cures ensued (Table 6).[101] However, at the very low doses needed to allow for survival of mice treated with EpoB (i.p.) there was little benefit (Table 6). On the basis of the data in Tables 6 and 7 dEpoB appeared to us to be much more promising than EpoB itself with respect to useful therapeutic index.

From this point, we rapidly shifted our interest toward the anticancer effects of dEpoB. We next turned to a comparison of dEpoB with two major drugs, adriamycin and paclitaxel (Table 8 and Figure 10). Figure 10 demonstrates graphically an experiment performed wherein MX-1 bearing xenograft mice were treated with dEpoB (35−40 mg/kg, Q2D, i.p.) beginning on day 8 after tumor implantation. At the end of this treatment period, five of 10 mice had no detectable tumor and three remained tumor free on day 60. In parallel experiments, mice were treated with either paclitaxel or adriamycin. The paclitaxel group (5 mg/kg, Q2D) tumor sizes were reduced but the tumors continued to grow during treatment. The therapeutic effects of adriamycin (2 mg/kg,

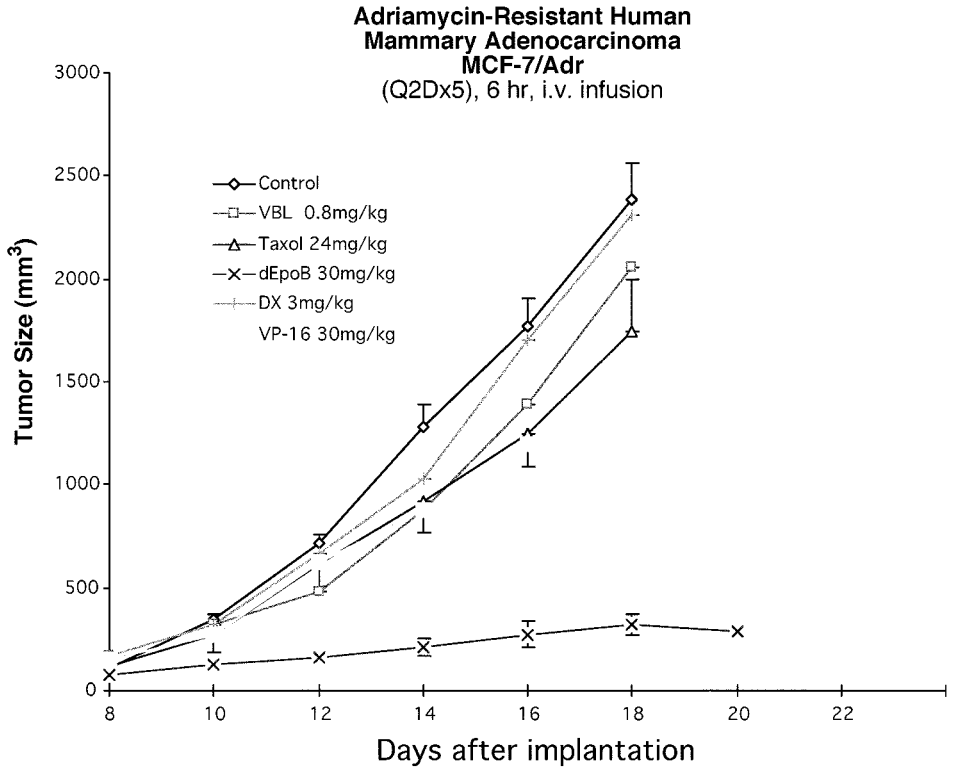


Figure 12. Therapeutic effects of administration of vinblastine (0.8 mg/kg), paclitaxel (24 mg/kg), dEpoB (30 mg/kg), and doxorubicin (3 mg/kg) on the adriamycin-resistant human mammary adenocarcinoma MCF-7/Adr following (Q2Dx5), 6 h, i.v. infusion. Adriamycin-resistant human mammary adenocarcinoma (MCF-7/Adr) tissue 50 mg was implanted subcutaneously into nude mice on day 0. Every other day, 6 h i.v. infusion (control, dEpoB, and paclitaxel) and i.v. injection (VBL, DX, and VP-16) were given on day 8, 10, 12, 14, and 16. The average tumor volume of the control group on day 14, 16, and 18 was 1281 ± 145, 1767 ± 161, and 3181 ± 203 mm^3 (mean $\pm$ SEM, $n = 5$), respectively. The vehicle for the 6 h i.v. infusion was 100 mL (Cremophor/ethanol, 1:1) + 3.5 mL of saline. The vertical bars are the standard errors of means for the control, dEpoB, and paclitaxel. VBL, vinblastine; DX, doxorubicin (adriamycin); VP-16 (etoposide).

Q2D) on the MX-1 xenografts were much weaker when compared with dEpoB or paclitaxel.

Similarly, the therapeutic effects of dEpoB were also evaluated in athymic mice bearing xenografts of human mammary adenocarcinoma, MCF-7/Adr, resistant to adriamycin. The MCF-7/Adr cells selected were 3.9-fold resistant to adriamycin, 46-fold resistant to paclitaxel, and only 2.4-fold resistant to dEpoB. As depicted in Table 8, both paclitaxel (12 mg/kg) and adriamycin (3 kg/mg) at clinically effective doses proved too toxic to be therapeutically beneficial. By contrast, dEpoB (35 mg/kg) demonstrated negligible toxicity as demonstrated by minimal body weight changes. Furthermore, at 35 mg/kg, dEpoB significantly reduced tumor size by 66−73% when compared to the control group.

These early studies were somewhat unfair to paclitaxel in that they were conducted i.p. whereas paclitaxel is more effective when administered by i.v. infusion. Furthermore these experiments were conducted with DMSO as the vehicle whereas paclitaxel functions best in a Cremophore/EtOH solution. Nonetheless, the data shown in Table 8 and Figure 10 constituted to us an impressive showing on the part of dEpoB and a strong reason to persist in the synthetic endeavor whose results have been related above.

Subsequently, we expanded our efforts by examining the effects of various formulations, routes, and schedules of i.v. administration.[91] We discovered that slow IV infusion (6 h, Q2D, 30 mg/kg × 5 doses) of dEpoB in nude mice bearing human xenograft tumors was most effective. We now "leaned over backward" to be more than fair to paclitaxel and adopted a Cremophore vehicle that had been optimized for this agent. In the event, desoxyepothilone B, **57**, performed similarly to paclitaxel in sensitive tumor xenografts such as the non-MDR MX-1 wherein each demonstrated a complete cure (data not shown). Similar results were also obtained for both paclitaxel and dEpoB when challenged with the non-MDR HT-29 colon tumor (data not shown) and non-MDR SK-OV-3 ovarian tumor. Since the superior effects observed by slow infusion administration of dEpoB were more clearly observed against MDR tumors, we directed our efforts toward these models. For instance, dEpoB (6 h, Q2D, 30 mg/kg × 5 doses, i.v.) demonstrated a full curative effect when administered to nude mice bearing the resistant human lymphoblastic T-cell leukemia, CCRF-CEM/paclitaxel, that was 57-fold resistant to paclitaxel (Figure 11).[103]Likewise, athymic mice implanted with the paclitaxel-resistant human mammary adenocarcinoma, MCF-7/Adr, xenograft markedly benefited from a reduction in the size of established tumors (average tumor size reduction, 89% versus 27%, dEpoB versus paclitaxel) when treated with dEpoB (6 h, Q2D, 30 mg/kg × 5 doses).[91] However, a full cure was not achieved in this experiment.

Finally, the beneficial effects of dEpoB relative to other commonly used anti-cancer agents were clearly demonstrated when nude mice bearing refractory MCF-7/Adr tumor xenografts were challenged with frontline chemotherapeutic agents such as paclitaxel (24 mg/kg), adria-

mycin (3 mg/kg), vinblastine (0.8 mg/kg), and etoposide (VP-16).[91] In these experiments, adriamycin demonstrated a lack of therapeutic effect even at the nearly lethal dose, and vinblastine, etoposide, and paclitaxel showed little therapeutic effect (Figure 12).

Based upon the promising profile of dEpoB, we also performed in vivo studies with other desoxy analogues such as **90** and **98** that had demonstrated favorable in vitro efficacy. We anticipated that modification at C12 (e.g., propyl or ethylidene acetal) would lead to improvement of the pharmacological effects similar to that observed in dEpoB (C12 methyl) relative to dEpoA (C12 proton), where placement of a methyl group at C12 improves the activity. However, our supposition proved incorrect and, in fact, proved to be deleterious in practice. Thus, analogues **90** and **98** showed markedly higher toxicity and less impressive efficacy than the lead compound dEpoB when administered to nude mice bearing human xenografts (data not shown).

Conclusions and Future Directions

Although dEpoB is naturally occurring (epothilone D), it is isolated as a minor fermentation product compared to the principal constituents, epothilones A and B. We were obliged to make recourse to chemical synthesis as a means for the discovery of the biological attributes of this promising compound. Through these rigorous biological examinations and structure activity profiles, we have designated dEpoB as our leading candidate for clinical application. Further development of dEpoB and other more recently developed analogues as potential clinical candidates continues to remain the main focus of our research.

In this paper, we have demonstrated, through example, the interactivity between chemical synthesis and drug discovery in the case of dEpoB. It was chemical synthesis that provided us with this compound in significant amounts required for the extensive biological experiments described herein. Moreover, it was chemical synthesis that contributed to our thinking on the chemical personality of dEpoB.

We suspect that there are many more opportunities for bringing synthesis to bear in the drug discovery process. We speak here of syntheses that will produce substantial amounts of materials rather than the minute symbolic sums that are often the endpoint of many contemporary academic marathons. It is this type of challenge that constitutes a major new frontier and incitement to the science of chemical synthesis. Obviously, to succeed in such a context will require very careful selection of target structures as to appropriate complexity and careful attention to the prognosis for interesting biology. Ideally, those who conduct the synthesis will also be positioned to follow and even to instruct the course of biological evaluation but this may not always be possible.

As to the findings themselves, we observe that in the course of these synthetic endeavors we have demonstrated the reach of the *B*-alkyl Suzuki coupling (**73b** + **78** → **79**) to embrace new levels of structural complexity. Similarly, the critical Noyori reduction (**80** → **81**) has also been conducted in a setting that is far more elaborate than has previously been thought possible for this reaction. Finally, in the assembly of the polypropionate domain, we have uncovered the *remarkable effect of a properly positioned, but still remote locus of unsaturation, on the stereochemical course of the carbon−carbon bond-forming event*.

In regard to dEpoB itself, we are continuing in the exploration and development of this and related 12,13-desoxy agents. At the level of rodent xenograft studies, the most remarkable properties of dEpoB have been its remarkably low toxicity, its high antitumor activity, and its seeming invulnerability to the MDR phenotype, at least in athymic mice. Our next steps in the development of dEpoB involve toxicological evaluation in larger animals and assessment of antitumor effects in such animals. These studies will start very soon. Chemical synthesis is proving to be equal to this new challenge. Pending favorable prognoses, which we fully expect, from these continuing investigations, it could be appropriate to take dEpoB, or a related compound, forward for clinical evaluation.

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References

(1) Holmes, F. A.; Walters, R. S.; Theriault, R. L.; Forman, A. D.; Newton, L. K.; Raber, M. N.; Buzdar, A. U.; Frye, D. K.; Hortobagyi, G. N. *J. Natl. Cancer Inst.* **1991**, *83*, 1797.
(2) McGuire, W. P.; Rowinsky, E. K.; Rosenshein, N. B.; Grumbine, F. C.; Ettinger, D. S.; Armstrong, D. K.; Donehower, R. C. *Ann. Intern. Med.* **1989**, *111*, 273.
(3) Rowinsky, E. K.; Donehower, R. C. *N. Engl. J. Med.* **1995**, *332*, 1004.
(4) Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560.
(5) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567.
(6) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck, S. G.; Donehower, R. C. *Seminars Oncol.* **1993**, *20*, 1−15.
(7) Cortez, J. E.; Pazdur, R. *J. Clin. Oncol.* **1995**, *13*, 2643−2655.
(8) Rose, W. C. *Anti-Cancer Drugs* **1992**, *3*, 311−321.
(9) Cass, C. E.; Janowska-Wieczorek, A.; Lynch, M. A.; Sheinin, H.; Hindenburg, A. A.; Beck, W. T. *Cancer Res.* **1989**, *49*, 5798−5804.
(10) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.
(11) Horwitz, S. B.; Fant, J.; Schiff, P. B. *Nature* **1979**, *277*, 665.
(12) Hyams, J. F.; Loyd, C. W. *Microtubules*; Wiley-Liss: New York, 1993.
(13) Kowalski, R. J.; Giannakakou, P.; Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534.
(14) Horwitz, S. B. *Ann. Oncol.* **1994**, 5 Suppl. 6, S3−6.
(15) Moos, P. J.; Fitzpatrick, F. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3896.
(16) Kirikae, T.; Ojima, I.; Kirikae, F.; Ma, Z.; Kuduk, S. D.; Slater, J. C. Takeuchi, C. S.; Bounaud, P.-Y.; Nakano, M. *Biochem. Biophys. Res. Commun.* **1996**, *227*, 227.
(17) Lee, L. F.; Haskill, J. S.; Mukaida, N.; Natsushima, K.; Ting, J. P. *Mol. Cell. Biol.* **1997**, *17*, 5097.

(18) (a) Woods, C. M.; Zhu, J.; McQueney, P. A.; Bollag, D.; Lazarides, E. *Mol. Med.* **1995**, *1*, 506−526. (b) Wahl, A. F.; Donaldson, K. L.; Fairchild, C.; Lee, F. Y. F.; Foster, S. A.; Demers, G. W.; Galloway, D. A. *Nat. Med.* **1996**, *2*, 72−79. (c) Debernadrdis, D.; Sire, E. G.; De Feudis, P.; Vikhanskaya, F.; Valentin, M.; Russo, P.; Parodi, S.; D'Incalci, M.; Broggini, M. *Cancer Res.* **1997**, *57*, 870−874.

(19) (a) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck, S. G.; Donehower, R. C. *Semin. Oncol.* **1993**, *20*, 1−15. (b) Fletcher, B. S.; Kujubu, D. A.; Perrin, D. M.; Herschman, H. R. *J. Biol. Chem.* **1992**, *267*, 4338. (c) Tsujii, M.; Dubois, R. N. *Cell* **1995**, *3*, 493. (d) Essayan, D. M.; Kagey-Sobotka, A.; Colarusso, P. J.; Lichtenstein, L. M.; Ozols, R. F.; King, E. D. *J. Allergy Clin. Immunol.* **1996**, *97*, 42−46.

(20) Muhlradt, P. F.; Sasse, F. *Cancer Res.* **1997**, *57*, 3344.

(21) (a) Huang, R. P.; Liu, C.; Fan, Y.; Mercola, D.; Adamson, E. D. *Cancer Res.* **1995**, *55*, 5054−5062. (b) Huang, R. P.; Fan, Y.; de Bell, I.; Niemeyer, C.; Gottardis, M. M.; Mercola, D.; Adamson, E. D. *Int. J. Cancer* **1997**, *72*, 102−109.

(22) Huang, R. P.; Darland, T.; Okamura, D.; Mercola, D.; Adamson, E. D. *Oncogene* **1994**, *9*, 1366−1377.

(23) Gunasekera, S. P.; Gunasakera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *5*, 4912−4915.

(24) Lindel, T.; Jensen, P. R.; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744−8745.

(25) Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653.

(26) Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Kuduk, S. D.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256−4261.

(27) Ojima, I.; Kuduk, S. D.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Med. Chem.* **1997**, *40*, 279−285.

(28) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P. *Org. Lett.* **1999**, *1*, 43.

(29) Su, D.-S.; Balog, A.; Meng, D.; Bertinato, P.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2093−2096.

(30) For an in-depth description of our first generation synthetic effort, see: (a) Meng, D.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073. (b) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, E. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801.

(31) (a) Nicolaou, K. C.; He, Y.; Vourloumis, D.; Vallberg, H.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2014−2045 and references therein. For recent chemical syntheses of epothilone B, see: (b) Mulzer, J.; Mantoulaidis, A.; Ohler, E. *Tetrahedron Lett.* **1998**, *39*, 8633. (c) Taylor, R. E.; Galvin, G. M.; Hilfiker, K. A.; Chen, Y. *J. Org. Chem.* **1998**, *63*, 9580. (d) May, S. A.; Grieco, P. A. *J. Chem. Soc., Chem. Commun.* **1998**, 1597. (e) Schinzer, D.; Bauer, A.; Schieber, J. *Synlett* **1998**, 861. (f) White, J. D.; Carter, R. G.; Sundermann, K. F. *J. Org.* Chem. **1999**, *64*, 684. For recent syntheses of the epothilones involving antibody catalysis, see: (g) Schulz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14590−14591. (h) Sinha, S. C.; Barbas, C. F., III; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14603−14608. (c) Sinha, S. C.; Keinan, E. *J. Am. Chem. Soc.* **1995**, *117*, 3653−3654.

(32) For a review of ring-closing olefin metathesis, see: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (b) Schmalz, H.-G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1833.

(33) (a) Danishefsky, S. J. *Aldrichim. Acta* **1986**, *19*, 59. (b) Danishefsky, S. J. *Chemtracts* **1989**, *2*, 273.

(34) (a) Shafiee, A.; Mazloumi, A.; Cohen, V. I. *J. Heterocycl. Chem.* **1979**, *16*, 1563. (b) Schafiee, A.; Shahocini, S. *J. Heterocycl. Chem.* **1989**, *26*, 1627.

(35) Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pirrung, M. C.; White, C. T.; VanDerveer, D. *J. Org. Chem.* **1980**, *45*, 3846.

(36) Danishefsky, S. J.; Kitahara, T. *J. Am. Chem. Soc.* **1974**, *96*, 7807.

(37) Meng, D.; Sorensen, E. J.; Bertinato, P.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 7998.

(38) Bertinato, P.; Sorensen, E. J.; Meng, D.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 8000.

(39) (a) Berkowitz, D. B.; Danishefsky, S. J. *Tetrahedron Lett.* **1991**, *32*, 5497. (b) Berkowitz, D. B.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1992**, *114*, 4518. (c) Hsu, S.-H.; Wu, S.-S.; Wang, Y.-F.; Wong, C.-H. *Tetrahedron Lett.* **1990**, *31*, 6403.

(40) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

(41) For examples, see: (a) Lythgoe, B.; Nambudiry, M. E. N.; Ruston, S.; Tideswell, J.; Wright, P. W. *Tetrahedron Lett.* **1975**, 3863. (b) Lythgoe, B. *Chem. Soc. Rev.* **1981**, 449. (c) Toh, H. T.; Okamura, W. H. *J. Org. Chem.* **1983**, *48*, 1414. (d) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098.

(42) Harris, C. R.; Balog, A.; Savin, K.; Danishefsky, S. J.; Chou, T.-C.; Zhang, X.-G. *Actual. Chim. Ther.* **1999**, *25*, 187−206.

(43) (a) Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 8000.

(44) (a) Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F.; Maring, C. J.; Springer, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1256. (b) Danishefsky, S. J.; Myles, D. C.; Harvey, D. F. *J. Am. Chem. Soc.* **1987**, *109*, 862.

(45) (a) Cohen, N.; Eichel, W. F.; Lopresti, R. J.; Neukom, C.; Saucy, G. *J. Org. Chem.* **1976**, *41*, 3505. (b) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873. (c) Meyers, A. I.; Babiak, K. A.; Campbell, H. L.; Comins, D. L.; Fleming, M. P.; Henning, R.; Heuschmann, M.; Hudspeth, J. P.; Kane, J. M.; Reider, P. J.; Roland, D. M.; Shimizu, K.; Tomioka, K.; Walkup, R. D. *J. Am. Chem. Soc.* **1983**, *105*, 5015. (d) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348.

(46) Danishefsky, S. J.; Yan, C.-F.; Singh, R. K.; Gammill, R. B.; McCurry, P. M., Jr.; Fritsch, N.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101*, 7001.

(47) Boeckman, R. K., Jr.; Charette, A. B.; Asberom, T.; Johnston, B. H. *J. Am. Chem. Soc.* **1991**, *113*, 5337.

(48) For an example, see: Egbertson, M.; Danishefsky, S. J. *J. Org Chem.* **1989**, *54*, 11.

(49) (a) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishi-Kawa, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314. (b) For an excellent review see: Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457. (c) Johnson, C. R.; Braun, M. P. *J. Am. Chem. Soc.* **1993**, *11*, 11014. (d) Ohba, M.; Kawase, N.; Fujii, T.; Aoe, K.; Okamura, K.; Fathi-Afshar, R.; Allen, T. M. *Tetrahedron Lett.* **1995**, *36*, 6101.

(50) For an excellent review of the chemistry of glycidol, see: Hanson, R. M. *Chem. Rev.* **1991**, *91*, 437.

(51) Corey, E. J.; Cashman, J. R.; Eckrich, T. M.; Corey, D. R. *J. Am. Chem. Soc.* **1985**, *107*, 713.

(52) Racherla, U. S.; Brown, H. C. *J. Org. Chem.* **1991**, *56*, 401.

(53) Keck, G. E.; Tarbet, K. H.; Geraci, L. S. *J. Am. Chem. Soc.* **1993**, *115*, 8467.

(54) (a) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 2173. (b) Stork, G.; Zhao, K. *J. Am. Chem. Soc.* **1990**, *112*, 5875. (c) Chen, J.; Wang, T.; Zhao, K. *Tetrahedron Lett.* **1994**, *35*, 2827.

(55) (a) Yamaguchi, M.; Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Mulzer, J.; Mareski, P. A.; Buschmann, J.; Luger, P. *Synthesis*, **1992**, 215.

(56) Meng, D.; Su, D.-S.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733.

(57) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039.

(58) Schrock, R. R.; Murdzek, J. S.; Bazan, G. C.; Robbins, J.; DiMare, M.; O'Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875.

(59) For an indepth study of our new total synthesis of epothilone B see: Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Glunz, P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 7050−7062.

(60) The stereochemistry of the diastereomeric products isolated in this reaction were confirmed to be those arising from incomplete relative face selectivity of the aldol reaction (Felkin/anti-Felkin). The stereochemistry of the aldolates was proven by comparison of the ^{1}H NMR spectrum of the major diastereomer with a compound of known stereochemistry that had been independently synthesized.

(61) Balog, A.; Harris, C.; Savin, K.; Zhang, X. G.; Chou, T.-C.; Danishefsky, S. J. *Angew. Chem., Int., Ed. Engl.* **1998**, 2675−2678.

(62) Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Danishefsky, S. J. *Tetrahedron Lett.* **1999**, 2263.

(63) Aoyama, T.; Terasawa, S.; Sudo, K.; Shiori, T. *Chem. Pharm. Bull.* **1984**, *32*, 3759.

(64) (a) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishi-Kawa, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314. (b) For an excellent review, see: Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457. (c) Johnson, C. R.; Braun, M. P. *J. Am. Chem. Soc.* **1993**, *115*, 11014.

(65) (a) Su, D.-S.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757. (b) Balog, A.; Bertinato, P.; Su, D.-S.; Meng, D.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Tetrahedron Lett.* **1997**, *38*, 4529.

(66) Noyori, R. *Tetrahedron* **1994**, *50*, 4259 and references therein.

(67) Ager, D. J.; Laneman, S. A. *Tetrahedron: Asymmetry* **1997**, *8*, 3327 and references therein.

(68) (a) Taber, D. F.; Deker, P. B.; Silverberg, L. J. *J. Org. Chem.* **1992**, *57*, 5990. (b) Taber, D. F.; Silverberg, L. J.; Robinson, E. D. *J. Am. Chem. Soc.* **1991**, *113*, 6639. (c) Schreiber, S. L.; Kelly, S. E.; Porco, J. A.; Sammakia, T.; Suh, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 6210. (d) Taber, D. F.; Wang, Y. *J. Am. Chem. Soc.* **1997**, *119*, 22.

(69) (a) Shao, L.; Seki, T.; Kawano, H.; Saburi, M. *Tetrahedron Lett.* **1991**, *32*, 7699. (b) Shao, L.; Kawano, H.; Saburi, M.; Uchida, Y. *Tetrahedron* **1993**, *49*, 1997.

(70) King, S. A.; Thompson, A. S.; King, A. O.; Verhoeven, T. R. *J. Org. Chem*. **1992**, *57*, 6689.
(71) (a) Taber, D. F.; Silverberg, L. J. *Tetrahedron Lett*. **1991**, *32*, 4227. (b) Taber, D. F.; Silverberg, L. J.; Robinson, E. D. *J. Am. Chem. Soc*. **1991**, *113*, 6639.
(72) (a) Molander, G. A. In *Organic Reactions*; Paquette, L. A., Ed.; John Wiley and Sons: New York, 1994; Vol. 46, p 211. (b) Molander, G. A.; Harris, C. R. *J. Org. Chem*. **1997**, *62*, 7418. (c) Machrouhi, F.; Namy, J.-L.; Kagan, H. B. *Synlett* **1996**, 633.
(73) Heathcock, C. H. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1984; Vol. 3, pp 111−213.
(74) Lodge, E. P.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 2819 and references therein.
(75) (a) Mukaiyama, T. *Org. React*. **1982**, *28*, 203. (b) Masamune, S.; Choy, W.; Petersen, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1.
(76) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem*. **1982**, *13*, 1.
(77) (a) Hoffmann, R. W.; Zeiss, H.-J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 218. (b) Hoffmann, R. W.; Weidmann, U. *Chem. Ber.* **1985**, *118*, 3966. (c) Hoffmann, R. W.; Brinkmann, H.; Frenking, G. *Chem. Ber.* **1990**, *123*, 2387. (d) Brinkmann, H.; Hoffmann, R. W. *Chem. Ber.* **1990**, *123*, 2395. (e) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 555.
(78) (a) Roush, W. R.; Adam, M. A.; Walts, A. E.; Harris, D. J. *J. Am. Chem. Soc.* **1986**, *108*, 3422. (b) Roush, W. R.; Adam, M. A.; Harris, D. J. *J. Org. Chem*. **1985**, *50*, 2000. Roush, W. R.; Palkoitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348. (c) Roush, W. R. In *Comprehensive Organic Synthesis*; Heathcock, C. H., Ed.; Pergamon Press: Oxford, 1991; Vol. 2, p 1.
(79) Roush, W. R. *J. Org. Chem*. **1991**, *56*, 4151−4157.
(80) Heathcock, C. H.; Buse, C. T.; Kleschick, W. A.; Pirrung, M. C.; Sohn, J. E.; Lampe, J. *J. Org. Chem*. **1980**, *45*, 1066.
(81) Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Danishefsky, S. J. *Tetrahedron Lett*. **1999**, 2267.
(82) Gennari, C.; Vieth, S.; Comotti, A.; Vulpetti, A.; Goodman, J. M.; Patterson, I. *Tetrahedron* **1992**, *48*, 4439.
(83) Ghera, E.; Kleinman, V.; Hassner, A. *J. Org. Chem*. **1999**, *64*, 8.
(84) Roush, W. R.; Hoong, L. K.; Palmer, M. A. J.; Straub, J. A.; Palkowitz, A. D. *J. Org. Chem*. **1990**, *55*, 4117.
(85) Reetz, M. T. *Angew. Chem., Int., Ed. Engl.* **1984**, *23*, 556.
(86) (a) Posner, G. H.; Lentz, C. M. *J. Am. Chem. Soc.* **1979**, *101*, 934. (b) Ghera, E.; Kleinman, V.; Hassner, A. *J. Org. Chem*. **1999**, *64*, 8.
(87) (a) Berk, S.; Knochel, P.; Yeh, M. C. P. *J. Org. Chem*. **1988**, *53*, 5791. (b) Yeh, M. C. P.; Knochel, P.; Santa, L. E. *Tetrahedron Lett*. **1988**, *29*, 3887.
(88) L'abbe, G.; Leurs, S.; Sannen, I.; Wim, D. *Tetrahedron* **1993**, *49*, 4439.
(89) For an excellent review of this catalyst, see: Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed. Engl*. **1998**, *37*, 1986.
(90) The stereochemistry of the C-15 stereocenter resulting from the (*R*)-2-methyl-CBS-oxazaborolidine reduction of ketone **55** was not proven rigorously but is predicted based on enantioselective reductions of these systems: Smith, D. B.; Waltos, A. M.; Loughhead, D. G.; Weikert, R. J.; Morgans, D. J.; Rohloft, J. C.; Link, J. O.; Zhu, R. *J. Org. Chem*. **1996**, *61*, 2236.
(91) Chou, T.-C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798.
(92) Giannakakou, P.; Sackett, D. L.; Kang, Y.-K.; Zhan, Z.; Buters, J. T.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem*. **1997**, *272*, 17118−17125 and references therein.
(93) Landino, L. M.; MacDonald, T. L. In *The Chemistry and Pharmacology of Taxol and Its Derivatives*; Favin, V., Ed.; Elsevier: New York, 1995; Chapter 7, p 301.
(94) Ojima, I.; Bounaud, P.-Y.; Bernacki, R. J. *Chemtech* **1998**, *28*, 31−36.
(95) Ahan, Z.; Scala, S.; Monks, A.; Hose, C.; Bates, S.; Fojo, T. *Cancer Chemother. Pharmacol*. **1997**, *40*, 245−50.
(96) Rose, W. C. *Anti-Cancer Drugs* **1992**, *3*, 311−321.
(97) (a) Klar, U.; Graf, H.; Schenk, O.; Rohr, B.; Schulz, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1397. (b) Li, C.; Yu, D. F.; Newman, R. A.; Cabral, F.; Stephens, L. C.; Hunter, N.; Milas, L.; Wallace, S. *Cancer Res*. **1998**, *58*, 2404. (c) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck. S. G.; Donehower, R. C. *Seminars Oncol*. **1993**, *20*, 1−15.
(98) Moasser, M. M.; Lorenzino-Sepp, L.; Kohl, N. E.; Oliff, A.; Balog, A.; Su, D.-S.; Danishefsky, S. J.; Rosen, N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1369−1374.
(99) CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. CCRF-CEM/VBL cell line demonstrates cross-resistance to paclitaxel, adriamycin, and etoposide and was developed after continuous exposure of CCRF-CEM cells to increasing and sublethal (IC_{50}) concentrations of vinblastine.
(100) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2015.
(101) MX-1 is a well-characterized human mammary adenocarcinoma that has been used for many years as a part of the National Cancer Institute's screening panel of experimental tumors.
(102) Chou, T.-C.; Zhang, X.-G.; Balog, A.; Su, D.-S.; Meng, D.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci.* **1998**, *95*, 9642−9647.
(103) The CCRF-CEM/paclitaxel cell line was developed at this institute (T.-C. Chou) after continuous exposure of CCRF−CEM cells with increasing and sublethal (IC_{50}−IC_{90}) concentrations of paclitaxel for 10 months.

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