Total Synthesis of Epothilone B, Epothilone D, and *cis*- and *trans*-9,10-Dehydroepothilone D

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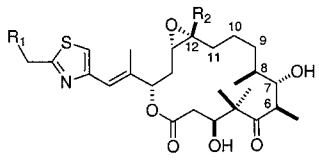
Received February 20, 2001

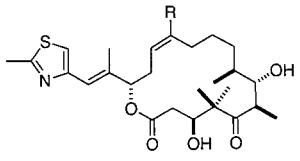
Abstract: The phosphonium salt **35**, representing one of the two principal subunits of the epothilones, was prepared from propargyl alcohol via heptenone **22.** A Wittig reaction of the phosphorane from **35** with aldehyde **33**, obtained from aldol condensation of ketone **27** with aldehyde **28**, afforded **37**. Seco acid **42** derived from **37** underwent lactonization to give *cis*-9,10-dehydroepothilone D (**43**) which was selectively reduced with diimide to yield epothilone D (**4**) and, after epoxidation, epothilone B (**2**). An alternative route to epothilone D employed alkyne **39**, obtained from **33**, in a Castro−Stephens reaction with allylic bromide **34** to furnish enyne **40**. The latter was semi-hydrogenated to provide **37**. Alkyne **46**, prepared from alcohol **45**, was converted to *trans*-vinylstannane **47** which, in a Stille coupling with allylic chloride **50**, gave **51**. Seco acid **52** derived from **51** underwent lactonization to give *trans*-9,10-dehydroepothilone D (**54**). Bioassay data comparing the antiproliferative activity and tubulin polymerization of **43** and **54** with epothilone B (**2**), epothilone D (**4**), and paclitaxel (**7**) showed that the synthetic analogues were less potent than their natural counterparts, although both retain full antiproliferative activity against a paclitaxel-resistant cell line. No significant difference in potency was noted between cis analogue **43** and its trans isomer **54**.

Introduction

The isolation and structural elucidation of epothilones A−F (**1**−**6**) by Höfle and Reichenbach from the soil myxobacterium *Sorangium cellulosum*[1] brought to light a remarkable new family of macrolides which has been the source of a large body of research directed toward the anticancer properties of these substances.

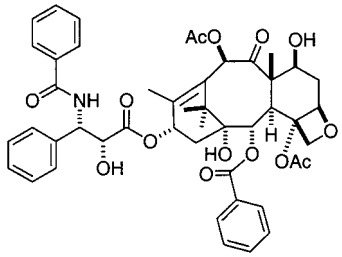


$R_1 = R_2 = H$, Epothilone A (**1**)
$R_1 = H$, $R_2 = Me$, Epothilone B (**2**)
$R_1 = OH$, $R_2 = H$, Epothilone E (**5**)
$R_1 = OH$, $R_2 = Me$, Epothilone F (**6**)



R = H, Epothilone C (**3**)
R = Me, Epothilone D (**4**)

As microtubule-stabilizing agents with a mechanism of action similar to that of paclitaxel (Taxol, **7**),[2] the epothilones exhibit exceptional promise as a new lead in cancer chemotherapy, especially where conventional treatments are ineffective.[3] The advantages shown by the epothilones over paclitaxel include their increased water solubility, their more rapid action in vitro,



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and their effectiveness against tumor cells which exhibit multiple drug resistance.[4] Epothilone B (**2**), which is approximately 50 times more active than paclitaxel and which is available in kilogram quantities from fermentation of *S. cellulosum*, along with epothilone D (**4**), now appears to be the optimal candidates for drug development from among the naturally occurring epothilones and a large ensemble of synthetic analogues.[5] It was these substances which initially provided the focus for our synthetic efforts directed toward the epothilones.[6]

At the outset, our primary goal was the development of a synthetic route to **2** which would embody the highest possible degree of stereocontrol. We considered it especially important to establish clean *Z* geometry of the 12,13-trisubstituted double bond and to set in place the stereocenters at C6, C7, and C15 with complete stereochemical accuracy. As our plan for a convergent synthesis unfolded, it became evident that an attractive means for connecting two major subunits such as A and B would be via C−C bond formation at C9−C10 (Scheme 1). This plan would not only allow the incorporation of

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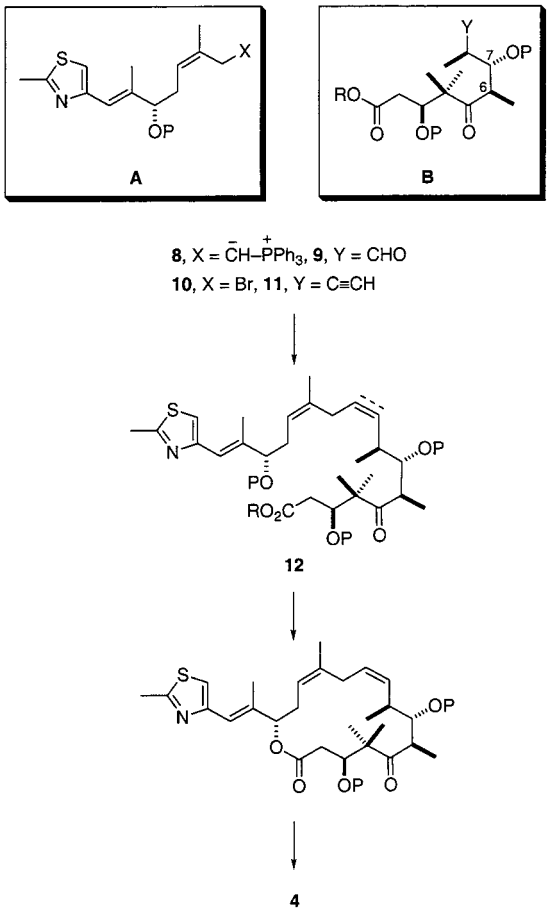
10.1021/ja010454b CCC: $20.00
Published on Web 05/16/2001

Scheme 1



functionality in a domain of the macrolide, which has not been extensively explored from the viewpoint of analogue synthesis, but would also offer an opportunity to constrain a region of the perimeter thought to be somewhat flexible.[7] The latter point impinges upon an important issue relevant to the three-dimensional structure of the epothilones, and to the relationship of this structure to that of paclitaxel. The solid-state structure of **2** revealed by X-ray crystallographic[1b] analysis shows that the carbon atoms attached to C9 and C10 are antiperiplanar, resulting in an almost perfect zigzag alignment of the C7−C12 segment. Structural studies of epothilone A (**1**) using NMR techniques suggest that the major conformer in solution is similar to that of **2** in the crystal, although the C6−C11 segment of the perimeter shows flexibility, and ca. 20% of a minor conformer can be seen.[8] One current view of the pharmacophoric relationship between paclitaxel (**7**) and the epothilones overlays C6−C9 of **7**, where a trans antiperiplanar orientation is enforced by the fusion of the B and C rings, with C11−C8 of **2**.[9] Incorporation of a cis double bond at C9−C10 of **4** would rigidify this segment of the macrolide perimeter and would remove its conformational homology with **7** by enforcing a syn coplanar arrangement of the four carbons C8−C11. It might therefore be expected that the conformational change induced by a cis 9,10 double bond would impact the biological activity of **4**. By contrast, a trans double bond at C9−C10 of **4** would constrain the C8−C11 domain of the macrolide perimeter to an antiperiplanar orientation, thereby restoring conformational homology with paclitaxel.[10]

Two approaches were projected for installing a *cis*-olefin at C9−C10 of **4** and, hence, **2**. The first envisioned a Wittig olefination which would connect phosphorane **8** with aldehyde **9**, while the second option postulated alkylation of the anion from terminal alkyne **11** with allylic bromide **10**. The latter route would generate a seco acid **12** which could be semi-hydrogenated to obtain the substance produced from **8** and **9**. Alternatively, the alkyne **11** could be used to produce a *trans*-vinylstannane, and Stille coupling with **10** would then lead to a *trans*-9,10-dehydroepothilone in which the antiperiplanar orientation of atoms around C9−10 was fixed and immutable. The versatility offered by these approaches to epothilone synthesis appeared inviting, especially since **8** and **11** could in principle be acquired from **10** and **9**, respectively, through straightforward transformations. Efforts therefore began toward synthesis of the coupling partners in the set **8**−**11**.

Results and Discussion

Synthesis of Subunits A and B. Since early installation of the *Z* trisubstituted C12−C13 double bond of **4** was judged to be of pivotal importance, our route began with construction of this moiety in a form which would permit extension from each terminus in succession toward subunit A. Carbocupration of propargyl alcohol has been shown to proceed with clean regio- and stereoselectivity to give iodo alcohol **13**,[11] and although the yield of this conversion is low, the minimal cost of reagents makes this an acceptable process for constructing a functionalized trisubstituted alkene suitable for our purpose. After protection of **13** as its THP ether **14**, the latter was subjected to halogen−metal exchange with *tert*-butyllithium. Transmetalation with cuprous cyanide then gave an alkenylcopper species which underwent conjugate addition to (*S*)-3-acryloyl-4-benzyloxazo-

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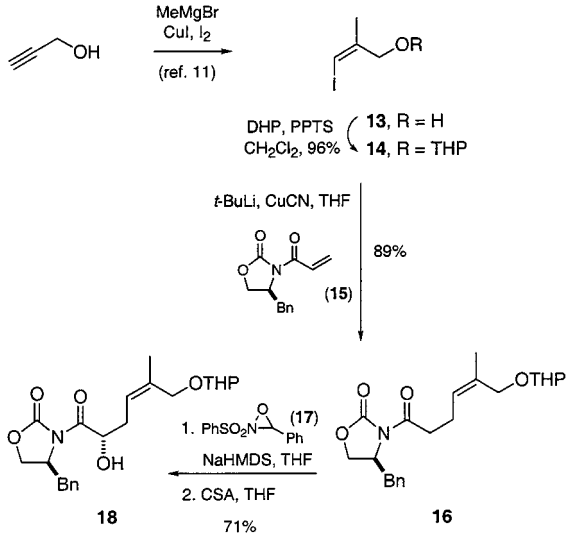
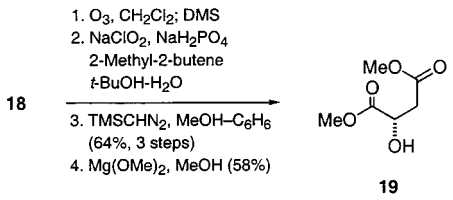
Scheme 2



Scheme 3

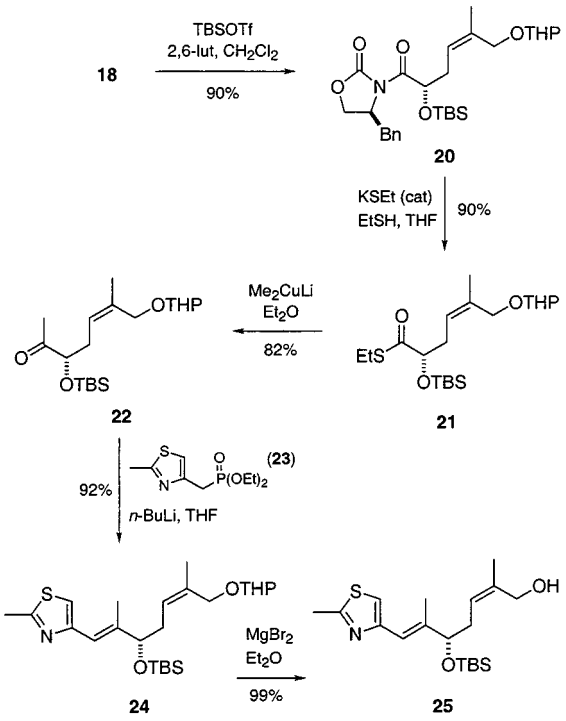


lidin-2-one (**15**).[12] The sodium enolate of the resulting oxazolidinone **16** was hydroxylated[13] using Davis' oxaziridine[14] **17** to afford alcohol **18** with diastereoselectivity >98:2 (Scheme 2). The (*S*) configuration of the hydroxyl substituent in **18** was confirmed by its ozonolytic degradation to dimethyl (*S*)-maleate (**19**) (Scheme 3).

After protection of **18** as its silyl ether **20**, the chiral auxiliary was removed with ethanethiol containing a catalytic quantity of potassium ethanethiolate.[15] The resulting thioester **21**, obtained in nearly quantitative yield, proved to be an excellent substrate for introduction of the requisite methyl ketone **22** through reaction with lithium dimethylcuprate. A Wadsworth–Emmons olefination of this ketone with the lithium anion of the known phosphonate **23**[16] furnished the *E,Z* diene **24**, accompanied by a trace (<5%) of the *Z,Z* isomer. The latter was easily removed from the mixture by rapid chromatography, leading to a fully stereocontrolled preparation of **24**. Since we next intended to elaborate this substance at the primary ether terminus, a means had to be found for removing the THP block without unmasking the silyl ether. Magnesium bromide in ether[17] served well for this purpose and afforded primary alcohol **25** in an overall yield of 36% for the seven steps from **14** (Scheme 4). The stable alcohol **25**, representing the subunit A required for assembly of **12** and thus **4**, provided a convenient staging point for access to **10** and **8** and was readily acquired in quantities as large as 10 g by the foregoing route.

The second subunit B needed for the convergent plan outlined in Scheme 1 presents a more demanding exercise, particularly

Scheme 4



with regard to installation of the stereocenters at C6 and C7. Although an aldol construction is an attractive option for creating the C6–C7 linkage of B and has been employed in many approaches to this segment of the epothilones,[5] the double stereodifferentiating anti-Felkin pathway which is required to generate the correct stereochemical triad at C6, 7, 8 has only recently been achieved in a stereocontrolled fashion. Thus, Nicolaou, after obtaining an initially disappointing 3:1 stereoselectivity,[6d] was eventually able to accomplish this aldol connection in a process which favored the anti-Felkin mode by 10:1.[6k] Mulzer improved this ratio to 95:5,[6o] and in his synthesis of **2** Schinzer obtained a stereoselectivity of 9:1.[6l] Our own studies with double stereodifferentiating aldol reactions have demonstrated that seemingly minor structural variation in the substrates or in the reaction conditions can exert a profound influence on the stereochemical outcome. In particular, the presence of a strongly ligating substituent such as a *p*-methoxybenzyl ether adjacent to the aldehyde component can play a pivotal role in steering an enolate toward one face of the carbonyl through the operation of a chelation effect.[18] This effect, which was noted during our synthesis of the polypropionate segment of rutamycin B,[19] has also been recognized by Mulzer in the context of a chelation-controlled aldol reaction that furnished the same anti,syn stereotriad seen in **29**.[20] Application of this principle to our plan for the synthesis of subunit B led to the proposal that the enolate of ketone **27**, previously synthesized by Nicolaou from keto aldehyde **26**,[6d] should be reacted with (*S*)-aldehyde **28** bearing a *p*-methoxybenzyloxy (PMBO) substituent for secondary chelation with the aldehyde carbonyl. Our previous experience suggested that an ether that ligates less strongly to an alkali metal cation than PMBO would result in diminished stereoselectivity,[19] and this indeed proved to be the case (vide infra).

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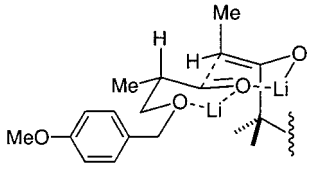
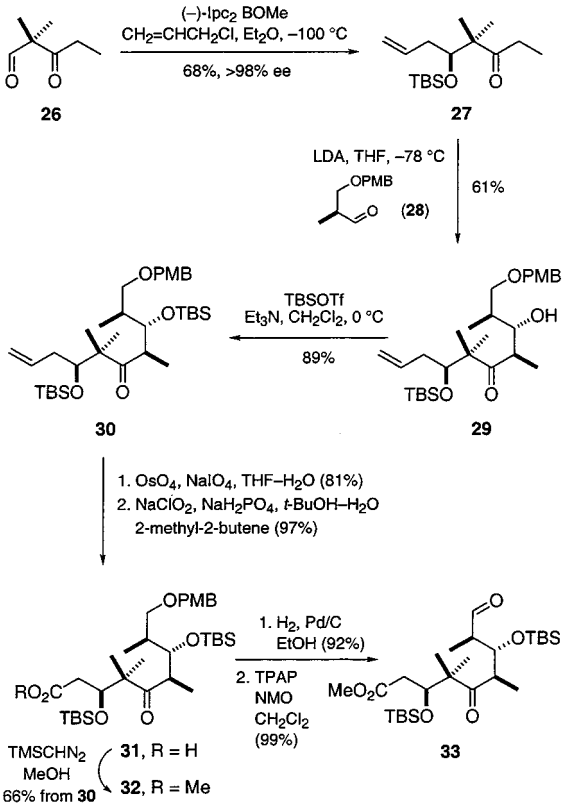


Figure 1. Possible transition state for the anti-Felkin aldol reaction of **27** with **28** invoking double chelation.

Scheme 5



Aldol condensation of **27** with **28**, the latter prepared from methyl (*S*)-3-hydroxy-2-methylpropionate, yielded anti-Felkin product **29** as the sole stereoisomer (Scheme 5).[21] The importance of the PMB ether in **28** is without question here since its replacement by a TBS ether resulted in much lower stereoselectivity in this aldol reaction. Results published by Schinzer[6l] imply that there are structural component(s) of the enolate which participate in chelation to the metal cation and thereby reinforce the anti-Felkin course of this aldol reaction, but our observations are not in accord with this view since structural variation in the ketone had little effect on the stereoselectivity of this reaction. The explanation proposed by Mulzer for the stereochemical outcome of this aldol reaction envisions a boatlike transition state in which a single lithium cation is coordinated to the benzyl ether, the aldehyde carbonyl, and the enolate oxygen.[20] An alternative model for the transition state of the reaction of **27** with **28** is presented in Figure 1, where *double metal chelation* involving *both* lone pairs of the aldehyde oxygen is invoked in a chairlike transition state. There is ample precedent in the literature,[22] including crystal structures of bis chelated carbonyl compounds, to support the concept of double chelation as proposed in Figure 1. In the present instance, it would be secondary chelation between the benzyl ether and aldehyde carbonyl which directs the addition of the *Z* enolate of **27** toward the *re* face of **28**. Whatever the true explanation for the stereoselectivity observed in the coupling of **27** and **28**, our result was most welcome since it avoided the need to remove stereoisomers of **29** from the reaction.

After protection of alcohol **29** as its silyl ether **30**, a degradative sequence was implemented which transformed the terminal vinyl group of **30** by oxidative cleavage to carboxylic acid **31** and then to methyl ester **32**. Hydrogenolysis of the *p*-methoxybenzyl ether of **32** gave a primary alcohol which was oxidized directly to aldehyde **33** with a catalytic quantity of Ley's reagent in the presence of *N*-methylmorpholine-*N*-oxide.[23] Aldehyde **33** represents subunit B in our strategic plan (Scheme 1) and can be viewed either as a masked version of **9** or the direct precursor of terminal alkyne **11**.

Assembly of Subunits. Our initial tactic for connecting subunits A and B envisioned the Wittig olefination shown in Scheme 1 which would set in place a C9−C10 cis double bond. It was anticipated that a triene such as **12** would provide access to a seco acid precursor of epothilone D (**4**) by selective hydrogenation of the disubstituted alkene, but it was also our intention to advance **12** toward a *cis*-9,10-dehydroepothilone by leaving the double bond in place so that this analogue could be evaluated against its congeners for antimitotic activity.

In preparation for the Wittig coupling leading to **12**, alcohol **25** was first converted to bromide **34** by reaction with methanesulfonic anhydride followed by displacement of the allylic mesylate with lithium bromide. The allylic bromide **34** was then homologated to phosphonium bromide **35** by displacement with the phosphorane **36** obtained from methyltriphenylphosphonium bromide and *n*-butyllithium. The Wittig reaction of **33** with the phosphorane prepared from **35** with lithium hexamethyldisilylazide, when carried out under carefully controlled conditions, afforded the pure *E,Z,Z*-triene **37** in excellent yield (Scheme 6).

Although the Wittig coupling of **33** with **35** was in most respects satisfactory, the strongly basic conditions of the reaction along with the fact that stoichiometry was difficult to control on a small scale, prompted investigation of an alternative method for linking subunits A and B. A protocol which effected direct coupling of A with B rather than preliminary homologation of the bromide **34** to **35** would confer obvious advantage, but this implied that a one-carbon homologation of aldehyde **33** would be needed. In practice, homologation of **33** was readily achieved through its condensation with dimethyl diazomethylphosphonate[24] (**38**), leading to terminal alkyne **39**. The latter was now poised for displacement of bromide from **34**, which was accomplished via a modified Castro−Stephens[25] coupling using cuprous iodide in the presence of triethylamine.[26] This reaction yielded dienyne **40** along with a small amount of the conjugated diyne resulting from oxidative self-coupling of **39**. Semihydrogenation of **40** over Lindlar's catalyst in hexane[27] gave **37** (Scheme 7), identical with the product obtained from Wittig coupling of **33** and **35**.

The three secondary silyl ethers of **37** are in sufficiently similar stereochemical environments to make selective unmasking of the ether at C15 seem implausible. Indeed, a variety of

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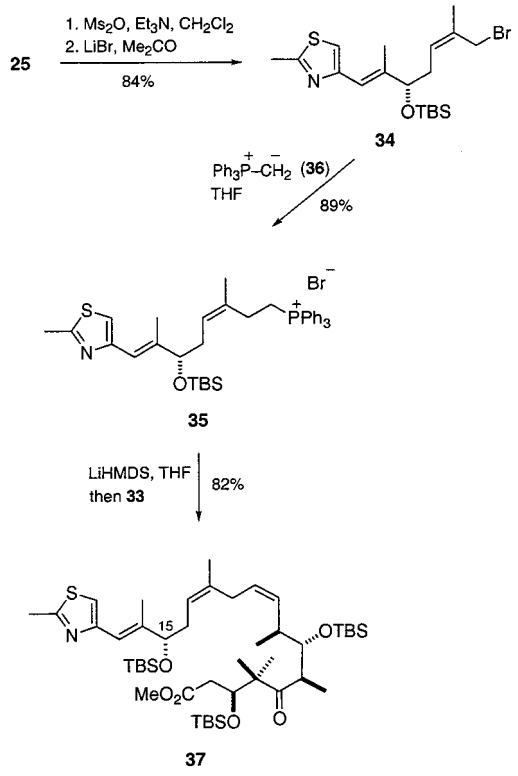
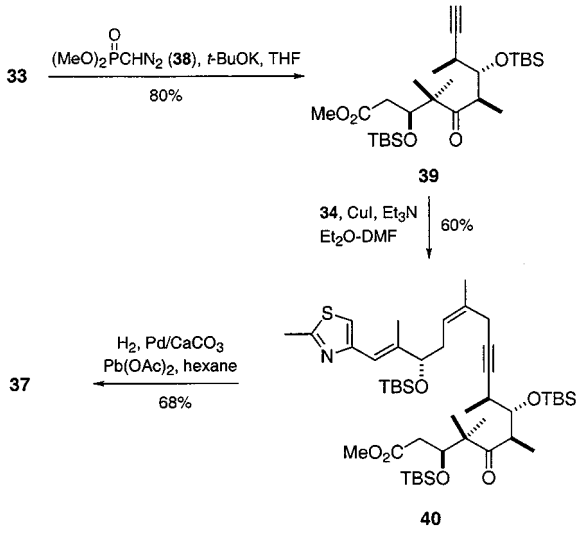
Scheme 6

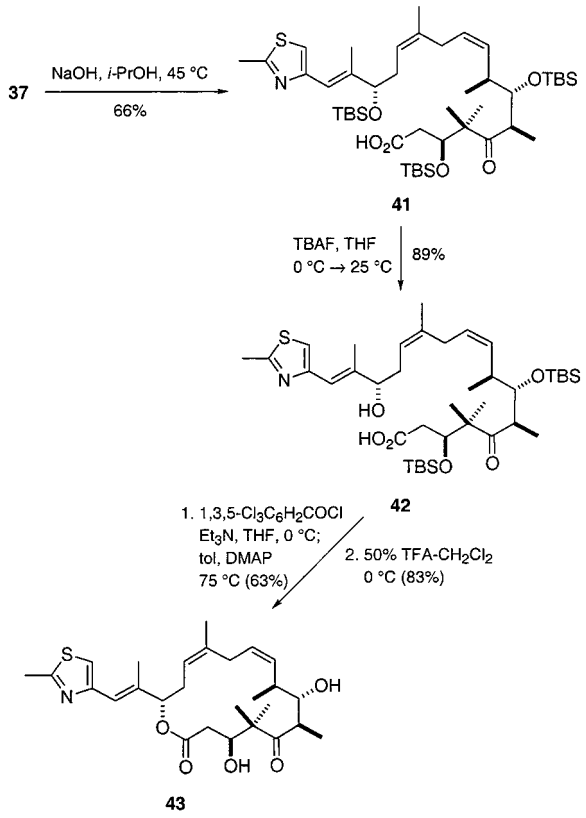


Scheme 7

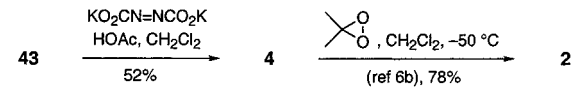


reagents known to cleave silyl ethers displayed little or no selectivity with **37**. However, after saponification of **37**, the resultant carboxylic acid **41** underwent selective desilylation at C15 to afford hydroxy acid **42** in excellent yield (Scheme 8). Similarly selective desilylation was noted by Nicolaou in the case of the 9,10 dihydro version of carboxylic acid **41**.[6d] A possible explanation for the markedly different results obtained in the deprotection of ester **37** and acid **41** may involve intramolecular silyl transfer from the C15 oxygen to an intermediate carboxylate anion; however, no evidence for a transient silyl ester was seen in the reaction with **41**. Nevertheless, this result suggested that the hydroxyl and carboxyl centers can achieve proximity and augured well for the ensuing macrolactonization. It is possible based on conformational analysis of **41** that the *cis*-9,10-olefin encourages this proximity, but a "folding" effect was not manifest in the lactonization of

Scheme 8



Scheme 9



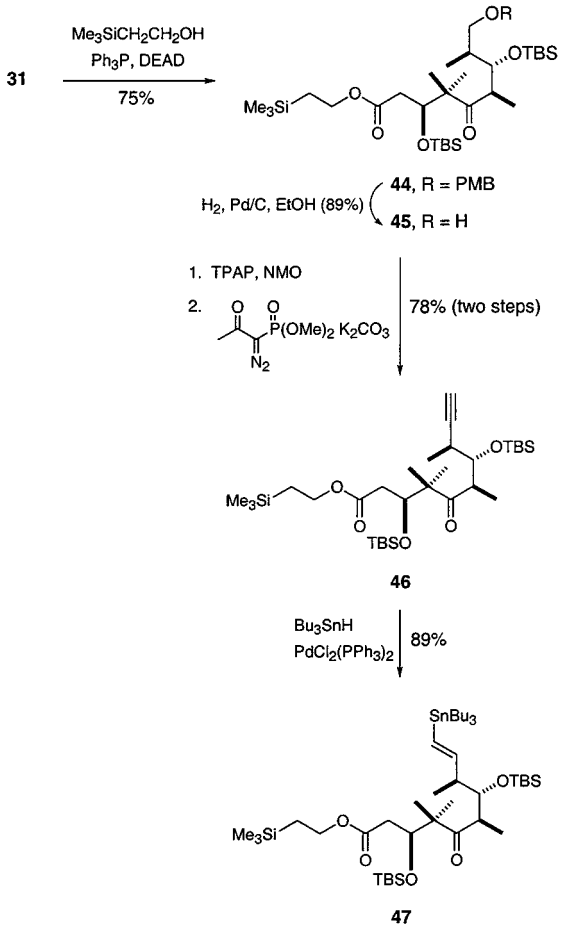
seco acid **42**. Under Yamaguchi conditions,[28] **42** gave the bis *tert*-butyldimethylsilyl ether of 9,10-dehydroepothilone D in a yield virtually identical with that obtained by others[6] for macrolactonization of the dihydro analogue of the seco acid. Removal of the two remaining silyl ethers from the macrolactone with trifluoroacetic acid gave 9,10-dehydroepothilone D (**43**).

Attempts to selectively hydrogenate the disubstituted olefin of **43** using Wilkinson's catalyst were unsuccessful. However, reduction of **43** with diimide,[29] generated from dipotassium azodicarboxylate, was moderately selective and resulted in epothilone D (**4**) in acceptable yield (Scheme 9). Final epoxidation of **4** with dimethyldioxirane under conditions described by Danishefsky[6a] afforded epothilone B (**2**), whose ^{1}H and ^{13}C NMR spectra exactly matched those of an authentic sample.

Stille Coupling Route. The synthesis of *cis*-9,10-dehydroepothilone D (**43**) via Castro−Stephens coupling of alkyne **39** with bromide **34** provides only modest advantage over the Wittig pathway employing **33** and **35**, but it does offer an opportunity for replacing the cis double bond at the C9−C10 position in the macrolide perimeter by a *trans*-olefin. Stille coupling[30] of a *trans*-vinylstannane derived from **39** with an allylic halide corresponding to **34** was initially envisioned as the means of access to *trans*-9,10-dehydroepothilone D. However, the modest (66%) yield of **41** from the saponification of **37** indicated that a further modification to this plan would be desirable, namely

(28) Inanaga, J.; Kirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
(29) Pasto, D. J.; Taylor, R. T. *Org. React.* **1991**, *40*, 91.
(30) Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1.

Scheme 10

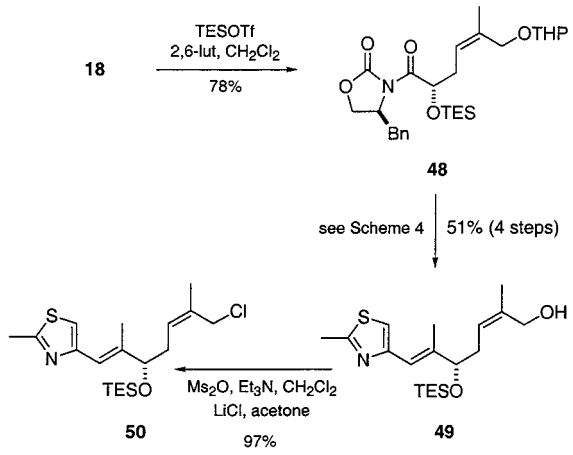


installation of an ester which could be removed efficiently and simultaneously with the C15 silyl-protecting group. An obvious choice for this purpose was a (trimethylsilyl)ethyl ester, and **31** was therefore esterified with 2-(trimethylsilyl)ethanol using Mitsunobu conditions to provide **44**. Hydrogenolysis removed the *p*-methoxybenzyl ether from **44**, and oxidation of alcohol **45** afforded an aldehyde which was reacted with Bestmann's reagent[31] to give terminal alkyne **46**. Hydrostannylation of the latter in the presence of a palladium dichloride catalyst furnished vinylstannane **47** (Scheme 10).

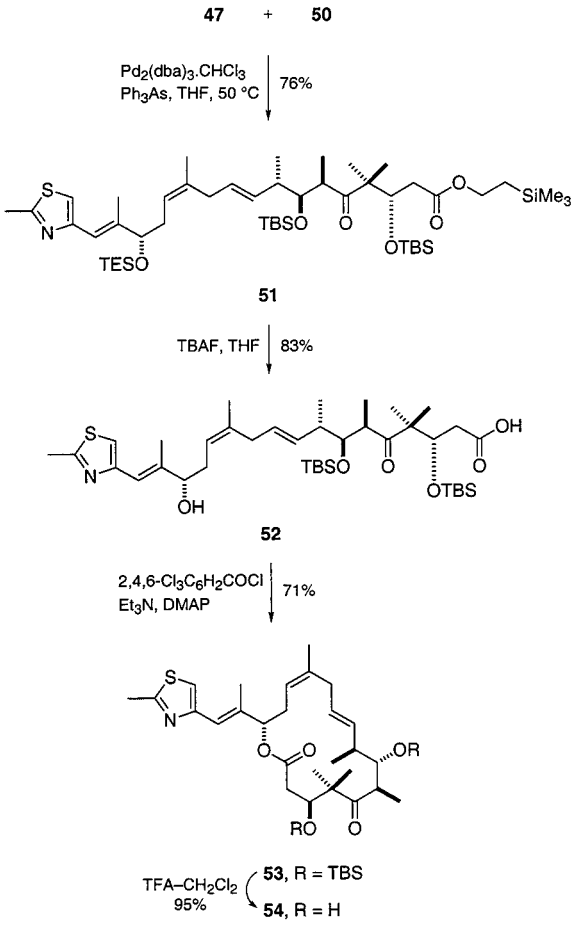
Although removal of the TBS ether at C15 of carboxylic acid **41** had led in high yield to seco acid **42** in which silyl ethers at C3 and C7 were retained, it was not clear that similar selectivity would pertain to deprotection at C15 of a tris(silyl) ether bearing both a trans double bond and a (trimethylsilyl)ethyl ester. For this reason, it was decided to replace the TBS protection of **25** with the corresponding triethylsilyl group (TES) so that unmasking of a seco ester carrying different silyl ethers would now be unambiguous. This modification necessitated a return to **18**, which was protected as TES ether **48** with triethylsilyl triflate. The latter was advanced to alcohol **49** by a four-step sequence exactly analogous to that used for the conversion of **20** to **25** (see Scheme 4). For Stille coupling purposes, the allylic chloride **50** derived from **49** was found to be more effective than the corresponding bromide (Scheme 11).

Coupling of **47** with **50** in the presence of catalytic dipalladium tris(dibenzylideneacetone)chloroform complex and triphenylarsine[32] proceeded in high yield and gave the 9*E*,12*Z*,16*E*-

Scheme 11



Scheme 12



heptadecanoate **51** (Scheme 12). As expected, exposure of **51** to tetra-*n*-butylammonium fluoride cleaved both the (trimethylsilyl)ethyl ester and the triethylsilyl ether but left *tert*-butyldimethylsilyl ethers at C3 and C7 intact. The resulting seco acid **52** underwent facile macrolactonization to **53**, and subsequent removal of the remaining pair of TBS ethers with trifluoroacetic acid furnished *trans*-9,10-dehydroepothilone D (**54**).

Biological Data

The antiproliferative activity of **43** and **54** was assessed in vitro using a panel of human cancer cell lines. As illustrated in Table 1, **43** was 20- to 30-fold less potent than natural epothilone

(31) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521.

(32) Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991**, *113*, 9585.

Table 1. Comparison of Antiproliferative Activity of **43** and **53** with Natural Epothilones and Paclitaxel

compound	$IC_{50\ values}$[a]					
	KB-31[b]	KB-8511[c]	A549[d]	HCT-116[e]	PC-3M[f]	MCF-7[g]
epothilone B (**2**)	0.17	0.16	0.16	0.34	0.32	0.29
epothilone D (**4**)	1.94	1.00	4.62	4.48	7.40	2.31
paclitaxel (**7**)	2.67	841.80	5.19	4.88	6.62	3.26
cis-9,10-dehydroepothilone D (**43**)	59.39	28.54	109.03	101.83	146.47	72.00
trans-9,10-dehydroepothilone D (**54**)	103.70	70.37	108.27	109.97	146.80	95.03

[a] Values are expressed in nM and represent the mean of three independent experiments. [b] Epidermoid cancer cell line. [c] Multidrug-resistant subline of KB-31 overexpressing P-glycoprotein. [d] Lung cancer cell line. [e] Colon cancer cell line. [f] Prostate cancer cell line. [g] Breast cancer cell line

Table 2. Tubulin Polymerization by Epothilones and Paclitaxel.[a]

compound[b]	polymerization (%)[c]
epothilone A (**1**)	71
epothilone B (**2**)	95
epothilone D (**4**)	88
paclitaxel (**7**)	53
cis-9,10-dehydroepothilone D (**43**)	56
trans-9,10-dehydroepothilone D (**54**)	36

[a] Porcine microtubule protein was used in this assay. [b] Concentration is 5 μM. [c] Extent of tubulin polymerization is expressed relative to that induced by 25 μM **2**, which was defined as 100%.

D (**4**), and 330- to 670-fold less potent than epothilone B (**2**). Interestingly, *trans*-9,10-dehydroepothilone D (**54**) showed biological activity very similar to that of its cis isomer **43** despite an apparent difference in the conformation of these two macrolactones.[33] Thus, the average IC_{50} of **54** for growth inhibition in the cell line panel used in this study was only 1.36-fold higher than that observed for **43**. As noted for epothilones B and D, **43** and **54** retain anti-proliferative activity against KB-8511 cells, a paclitaxel-resistant cell line overexpressing P-glycoprotein (Table 1). While the tubulin polymerization activity of **43** and **54** was lower than of **4** (56, 36, and 88%, respectively) (Table 2), it is conceivable that decreased cellular penetration may contribute to the marked reduction in antiproliferative potency observed for **43** and **54**. The absence of a clear difference in the biological profiles of *cis*-**43** and *trans*-**54** analogues of 9,-10-dehydroepothilone D observed here has a parallel in results previously reported for other epothilone analogues. Thus, epothilones incorporating a *trans*-epoxide or *trans*-olefin at C12−C13 have been shown to possess biological activity comparable to their cis isomer.[34,35] Taken together, these data support the proposition that the C8−C13 region of the epothilone perimeter is relative tolerant of structural modification and suggest that the interaction of this segment of the molecule with tubulin is less stringently defined. Further evaluation of analogues with more extreme deformation in this portion of the structure is planned to define the limit of this tolerance.

Materials and Methods

The following human cancer cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD): non-small cell lung adenocarcinoma A549 (CCL 185), human colon carcinoma HCT116 (ATCC CCL 247), and estrogen-dependent breast carcinoma cell line MCF-7 (ATCC HTB 22). The human metastatic prostate carcinoma PC-3M was obtained from Dr. I. J. Fidler (MD Anderson Cancer Center, Houston, TX). The human KB-31 (drug-sensitive) and KB-8511 (P-gp overexpressing, multidrug-resistant) epidermoid carcinoma cell lines were obtained from Dr. R. M. Baker, Roswell Park Memorial Institute (Buffalo, NY) and have been previously described.[36]

In Vitro Tubulin Polymerization Assay. Induction of tubulin polymerization was determined using a modified version of a previously described microtubule protein centrifugation assay.[37] Briefly, MAP-associated porcine brain tubulin was incubated with 5 μM compound for 20 min at room temperature. The samples were then centrifuged for 15 min at 14 000 rpm to separate polymerized from nonpolymerized microtubule protein. The protein concentration of the supernatant containing the remainder of nonpolymerized, soluble microtubule protein was determined by the Lowry method (DC Assay Kit, Bio-Rad Laboratories, Hercules, CA) using a SpectraMaxPlus photometer (Molecular Devices, Sunnyvale, CA). The reduction in optical density at 750 nm induced by the test compound was compared to that for 25 μM epothilone B (**2**), which served as a positive control.

Determination of Antiproliferative Activity. Antiproliferative assays were performed as previously described.[38] Cells were seeded at 1.5×10^3 cells/well into 96-well microtiter plates and incubated overnight. Compounds were added in serial dilutions on day 1. Subsequently, the cells were incubated for 3 or 4 days (allowing for at least 2 population doublings), and then were fixed with 3.3% v/v glutaraldehyde, washed with water, and stained with 0.05% w/v methylene blue. After the cells were washed, the dye was eluted with 3% HCl, and the optical density was measured at 665 nm with a SpectraMax 340 photometer (Molecular Devices, Sunnyvale, CA). IC_{50} values were determined by mathematical curve-fitting using SoftPro3.0 software (Molecular Devices, Sunnyvale, CA) employing the formula $(OD_{treated} - OD_{start})/(OD_{control} - OD_{start}) \times 100$. The IC_{50} was defined as the drug concentration which resulted in 50% net cell growth compared to control cultures at the end of the incubation period.

Acknowledgment. We are grateful to Professor K. C. Nicolaou, Scripps Research Institute, and Professor Paul A. Grieco, Montana State University, for the NMR spectra of epothilone B, and to Dr. Karl-Heinz Altmann, Jacqueline Loretan, and Robert Reuter, Novartis Pharma AG, for assistance with the biological studies. One of us (R.G.C.) is indebted to the National Institutes of Health for a Postdoctoral Fellowship (F32 CA 76743). This research was assisted by financial support from the National Institutes of Health (GM50574), Pfizer Global Research and Development, and DuPont Pharmaceutical Company.

Supporting Information Available: Experimental procedures, characterization data, and copies of ^1H and ^{13}C NMR spectra for new compounds, epothilone B (**2**), and epothilone D (**4**) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA010454B

(33) A conformational study of **43** and **53** is currently in progress and will be reported in due course.

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