J|A|C|S
ARTICLES
Published on Web 06/22/2002

Highly Concise Routes to Epothilones: The Total Synthesis and Evaluation of Epothilone 490

Kaustav Biswas,[†] Hong Lin,[†] Jon T. Njardarson,[†] Mark D. Chappell,[†] Ting-Chao Chou,[‡] Yongbiao Guan,[‡] William P. Tong,[||] Lifeng He,[§] Susan B. Horwitz,[§] and Samuel J. Danishefsky*[,†,⊥]

Contribution from Laboratory for Bioorganic Chemistry, Preclinical Pharmacology Core Facility and Analytical Pharmacology Core Facility, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027, and Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461

Received March 19, 2002

Abstract: A concise modular laboratory construction of the epothilone class of promising antitumor agents has been accomplished. For the first time in the epothilone area, the new synthesis exploits the power of ring-closing olefin metathesis (RCM) in a stereospecific way. Previous attempts at applying RCM to epothilone syntheses have been repeatedly plagued by complete lack of stereocontrol in the generation of the desired 12,13-olefin geometry in the products. The isolation of epothilone 490 (**3**) prompted us to reevaluate the utility of the RCM procedure for fashioning the 10,11-olefin, with the *Z*-12,13-olefin geometry already in place. Olefin metathesis of the triene substrate **12** afforded the product diene macrolide in stereoselective fashion. For purposes of greater synthetic convergency, the C3-(*S*)-alcohol was fashioned late in the synthesis, using chiral titanium-mediated aldol conditions with the entire *O*-alkyl fragment as a C15 acetate as the enolate component. Examination of the effects of protecting groups on the RCM process showed that deprotection of the C7 alcohol has a beneficial effect on the reaction yield. Performing the RCM as the last synthetic step in the sequence afforded a 64% yield of only the desired *E*-olefin. Selective diimide reduction of the new 10,11-olefin yielded 12,13-desoxyepothilone B, our current clinical candidate, demonstrating the utility of this new RCM−reduction protocol in efficiently generating the epothilone framework. Furthermore, the new olefin was selectively funtionalized to demonstrate the advantage conferred by this route for the construction of new analogues for SAR studies, in cytoxicity and microtubule affinity screens. Also described is the surprisingly poor in vivo performance of epothilone 490 in xenografts in the light of very promising in vitro data. This disappointing outcome was traced to unfavorable pharmacokinetic features of the drug in murine plasma. By the pharmacokinetic criteria, the prognosis for the effectiveness of **3** in humans is, in principle, much more promising.

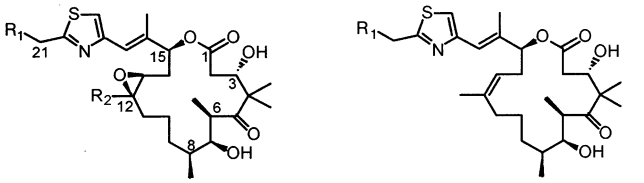
Introduction

Microtubules are polymeric structures that are an integral part of all eukaryotic cells.[1] During mitosis, the dynamics of microtubule polymerization and depolymerization are finely controlled, and any variation in the rate of polymerization can profoundly effect cellular replication. Effected cells are unable to pass through a checkpoint in the cell cycle and enter into programmed cell death. There are two major classes of chemotherapeutic agents that induce mitotic arrest by disrupting microtubule dynamics, those that depolymerize tubulin and those that stabilize tubulin polymers. The most well-known tubulin-stabilizing agent is Taxol (paclitaxel), which is currently a front-line anticancer agent.[2] The current clinical success of Taxol, and the related taxane Taxotere (docetaxel), in combating a variety of human carcinomas has indeed been suggestive of the potential efficacy of other tubulin-stabilizers in cancer therapy.

However, Taxol is less than an ideal drug. Its shortcomings include multidrug resistance (MDR) susceptibility and lack of aqueous solubility. The latter condition compels recourse to formulation vehicles such as cremophores, which have their own associated toxicities.[3] Given the well-established usefulness of

* To whom correspondence should be addressed. E-mail: s-danishefsky@ski.mskcc.org.
[†] Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute.
[‡] Preclinical Pharmacology Core Facility, Sloan-Kettering Institute.
[||] Analytical Pharmacology Core Facility, Sloan-Kettering Institute.
[§] Department of Molecular Pharmacology, Albert Einstein College of Medicine.
[⊥] Department of Chemistry, Columbia University.

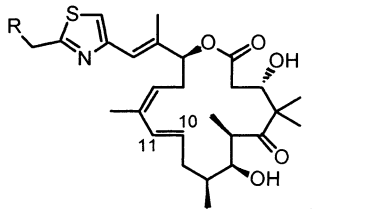
(1) Avila, J.; Nido, J. D. In *Cytoskeleton*; Hesketh, J. E., Pryme, I. F., Eds.; JAI, Greenwich, CT, 1995; Vol. 1, p 47.
(2) Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15.
(3) (a) Rowinsky, E. K.; Eisenhauer, E. A.; Chaudhry, V.; Arbuck, S. G.; Donehawer, R. C. *Semin. Oncol.* **1993**, *20*, 1. (b) Fletcher, B. S.; Kujubadu, D. A.; Perrin, D. M.; Herschman, H. R. *J. Biol. Chem.* **1992**, *267*, 4338. (c) Tsuji, 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. I* **1996**, *97*, 42. (e) Giannakakou, P.; Sackett, D. L.; Kang, Y.-K.; Zhan, Z.; Buters, J. T.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem.* **1997**, *272*, 17118 and references therein.



R_1, R_2 = H, epothilone A
R_1 = H, R_2 = CH_3, epothilone B
R_1 = OH, R_2 = CH_3, epothilone F

1 R_1 = H, 12,13-desoxyepothilone B
2 R_1 = OH, 12,13-desoxyepothilone F

Figure 1. Structures of epothilones.



3, R = H, epothilone 490
4, R = OH, 10,11-dehydrodesoxyepothilone F

Figure 2. Structures of 10,11-dehydroepothilones.

Taxol against a variety of oncological indications, it is not surprising that there has been a continuing interest in agents which share the mechanism of action of Taxol while offering potential therapeutic advantages. One target of opportunity would be the discovery of a drug, operating in the mechanistic framework of Taxol, which exhibits a much greater robustness toward disablement via the onset of multidrug resistance.

Such considerations were, doubtless, involved in research which led to the discovery of the family of novel macrolides, the epothilones. These compounds were isolated from the cellulose-degrading myxobacterium, *Sorangium cellulosum*, harvested off the shores of the Zambezi river in South Africa in the late 1980s (Figure 1). Initial disclosure of antifungal activity in 1993[4] was followed by reports of "Taxol-like" microtubule stabilizing-capability induced cytotoxicity in 1995.[5] Our total syntheses of the first discovered epothilones, A and B (Figure 1),[6] provided material for corroborating in vitro studies as well as for the first published in vivo evaluations.[7] Modifications of our early total syntheses also provided congeners for SAR mapping.[8] Following concerns about serious toxicities noted in our in vivo studies of fully synthetic epothilone B, we came to examine 12,13-desoxyepothilone B (dEpoB, **1**, Figure 1) in which the epoxide, a potentially serious source of indiscriminate cytotoxicity, is deleted. On the basis of its extensive and highly encouraging package of preclinical data in mice and dogs, **1** has emerged as a rather promising candidate for more advanced oncological assessments.[9] Indeed, dEpoB is presently being evaluated in human clinical trials. The compound is currently produced either through genetically engineered bacterial fermentation[10] or via chemical total synthesis. The latter method has also produced ample quantities of **1** of suitable purity and toxicology characteristics to be acceptable for extensive human clinical studies. The 21-hydroxy derivative, epothilone F, has also been of interest because of added aqueous solubility, a major problem in paclitaxel administration. We have synthesized and shown that 12,13-desoxyepothilone F (**2**) is equally effective in treatment of murine tumor xenografts as dEpoB, and superior to other clinically advanced derivatives.[9,11]

The progress of a pharmaceutically relevant investigational program in our laboratory setting is critically dependent upon facile access to significant quantities of purified material for preclinical and clinical evaluations. Our initial academic level efforts in the epothilone arena were rewarding and intellectually enriching, especially with regard to the application of LACDAC reactions developed in the 1980s in this laboratory for the stereoselective construction of carbon frameworks with programmable relative and absolute stereochemistry.[12] However, faced with pressing needs of substantial quantities of various epothilones for biological evaluation and with not having access to fermentation-derived material, we have been revisiting the matter of total synthesis on a continuing basis. Our studies are directed in the first instance to new strategy-level solutions. Significant thematic departures have indeed been developed and applied to the epothilones and have provided efficient access to multigram quantities of purely synthetic material to enable drug-discovery and evaluation campaigns.[13]

Several considerations drove the research described below. First, we continue to seek syntheses which could be suitable for eventual large-scale manufacture of desoxyepothilone B. At the same time, we hoped that the new synthesis would provide access to explore novel epothilones, in particular, the recently isolated macrolide, epothilone 490 (**3**, Figure 2).[14] This com-

(4) Höfle, G.; Bedorf, N.; Gerth, K.; Reichenbach, H. (GBF). DE-B 4138042; *Chem. Abstr.* **1993**, *120*, 52841.
(5) (a) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Leisch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325. (b) Kowalski, R. J.; Terhaar, E.; Longley, R. E.; Gunasekera, S. P.; Lin, C. M.; Day, B. V.; Hamel, E. *Mol. Biol. Cell* **1995**, *6*, 2137.
(6) (a) Balog, A.; Meng, D. F.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801. (b) Su, D.-S.; Meng, D. F.; Bertinato, P.; Balog, A., Sorensen; E. J.; Danishefsky, S. J.; Zheng, Y. H.; Chou, T. C.; He, L. F.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757. (c) Meng, D. F.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073. For initial reports of other epothilone syntheses, see: (d) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 166. (e) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 525. (f) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Nincovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268. (g) Schinzer, D.; Limberg, A.; Bauer, A.; Bohm, O. M.; Cordes, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 523. (h) May, S. A.; Greico, P. A. *Chem. Commun.* **1998**, 1597. (i) Sawada, D.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 209. (j) Martin, H. J.; Drescher, M.; Mulzer, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 581. (k) White, J. D.; Carter, R. G.; Sundermann, K. F. *J. Org. Chem.* **1999**, *64*, 684. (l) Zhu, B.; Panek, J. S. *Org. Lett.* **2000**, *2*, 2575, (m) Bode, J. W.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 3611. (n) Fürstner, A.; Mathes, C.; Grela, K. *Chem. Commun.* **2001**, *12*, 1057. (o) Taylor, R. E.; Chen, Y. *Org. Lett.* **2001**, *3*, 2221. (p) Valluri, M.; Hindupur, R. M.; Bijoy, P.; Labadie, G.; Jung, J. C.; Avery, M. A. *Org. Lett.* **2001**, *3*, 3607. (q) Ermolenko, M. S.; Potier, P. *Tetrahedron Lett.* **2002**, *43*, 2895.
(7) (a) Chou, T. C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798. (b) Chou, T. C.; Zhang, X. G.; Balog, A.; Su, D.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642.
(8) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434 and references therein.
(9) Chou, T. C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.
(10) Tang, L.; Shah, S.; Chung, L.; Carney, J.; Katz, L.; Khosla, C.; Julien, B. *Science* **2000**, *287*, 640.
(11) (a) Lee, C. B.; Chou, T.-C.; Zhang, X. G.; Wang, Z. G.; Kuduk, S. D.; Chappell, M. D.; Stachel, S. J.; Danishefsky, S. J. *J. Org. Chem.* **2000**, *65*, 6525. (b) Lee, C. B.; Wu, Z.; Zhang, F.; Chappell, M. D.; Stachel, S. J.; Chou, T. C.; Guan, Y.; Danishefksy, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 5249.
(12) (a) Danishefsky, S. J. *Aldrichimica Acta* **1986**, *19*, 59. (b) Danishefsky, S. J. *Chemtracts*: *Org. Chem.* **1989**, *2*, 273.
(13) Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Glunz, P.; Danishefksy, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 7050.
(14) Kosan Biosciences Inc., Hayward, CA, private communication. The related 12-desmethy-10,11-dehydro compound, in the epo A series, has also been isolated recently; see: Hardt, I. H.; Steinmetz, H.; Gerth, K.; Sasse, F.; Reichenbach, H.; Hofle, G. *J. Nat. Prod.* **2001**, *64*, 847.

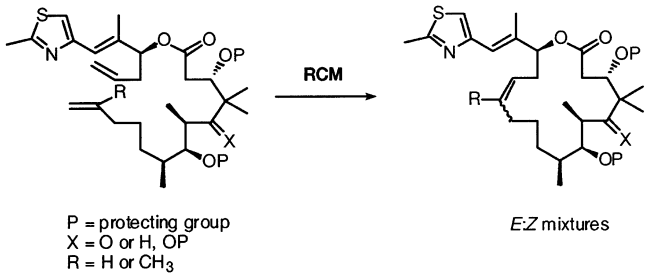


Figure 3. Previous attempts at synthesis of epothilones employing RCM for macrolide contruction, affording *E*:*Z* mixtures of the C12−C13 olefin.

pound, which contains a 10,11-*E*-olefin conjugated to the usual 12,13-unsaturation of dEpoB, showed highly favorable in vitro cytotoxicity in preliminary screens. The prospects for a quality synthesis of epothilone 490 from dEpoB were not inviting.

From a purely synthetic perspective, the presence of the C10−C13 diene unit was suggestive of potential forays into new ways of macrocyclization toward the synthesis of epothilones. Olefin metathesis certainly stands out as a premier methodological advance in carbon−carbon bond formation reactions of recent vintage.[15] Along with the dramatic advances in the field of olefin metathesis, the demonstration of the applicability of ring-closing olefin metathesis (RCM) in the assembly of a complex macrolide by Hoveyda and co-workers in the context of Sch 38516 has opened up new avenues for large-ring construction.[16] Following these elegant studies, significant efforts had been directed to using olefin metathesis for establishing the 12,13-double bond of the epothilones from the earliest days of the problem. Unfortunately, RCM directed to the 12,13-linkage has invariably led to a stereorandom mixture of olefin isomers, separable only with the greatest of difficulty (Figure 3).[6c,f−h]

It was from this background, we considered the possibility of using RCM to construct a 10,11-double bond *in a substrate which already contains the putative 12,13-unsaturation*. In proposing this solution to the problem, we were not unmindful of a recent success enjoyed in our laboratory in the total synthesis of radicicol using RCM to fashion a lactonic diene,[17] along with other recent examples of macrocyclization approaches involving diene−ene RCM.[18] Successful olefin metathesis would produce a 10,11;12,13-diene of the type found in epothilone 490. We also hoped that position-selective hydrogenation of the disubstituted 10,11-olefin would provide access to the 12,13-desoxyepothilones themselves. As seen below, this new strategy for synthesizing epothilones has been realized. When combined with a new and highly convergent way to join key pre-epothilone fragments, a highly modular synthesis from readily available modules has been realized. The prospects for a synthesis of dEpoB which would be practical at the plant level is much enhanced.

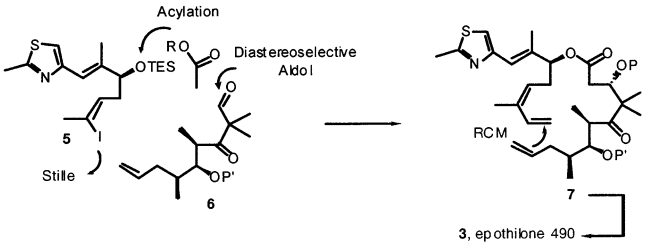


Figure 4. Synthetic plan for epothilone 490.

Specifically, at the level of chemical synthesis, we report (i) the first total synthesis of the potentially important epothilone 490 (**3**) and the related 21-hydroxy derivative (**4**) via the nonprecedented application of highly stereoselective RCM to this series of drug prospects, (ii) application to a particularly straightforward total synthesis of the highly promising 12,13-desoxyepothilone B, and (iii) exploitation of the new olefinic functionality of epothilone 490 to reach novel epothilones. Moreover, we report in vitro as well as the first in vivo assessment of **3**. Pharmacokinetic studies of fully synthetic epothilone 490 help place these findings in xenografts in perspective.

Results and Discussion

The synthetic plan envisaged a construction of a "seco" acyclic triene (**7**, Figure 4) positioned for diene−ene RCM for macrolide formation. Fortunately, we could draw upon previously disclosed and highly accessible building blocks to pursue a new vision of the epothilone synthesis problem. These are vinyl iodide **5**,[19] and aldehyde **6**.[11b,20] Inspection of the relationship of these two building blocks to goal structure **3** obliges one to deal with two issues. In terms of gross carbon count, there is a need to insert carbons 1 and 2 of the eventual epothilone into the regime, formally as a carboxymethyl spacer function. With C1 and C2 inserted, the carbon network of the two-component array **5** and **6**, while of the appropriate length, is not well-structured to deliver the 10,11-unsaturation of epothilone 490. An intriguing possibility was that of introducing two more carbon based centers to the ensemble. We envisioned that RCM would deliver the conjugated diene linkage of 490 while disposing of the extraneous carbons (vide infra).

The "seco" compound **7** could be accessed from a reassembly of advanced synthetic intermediates (Figure 4). The C11−C15 domain can be acylated with an appropriate C1 acid moiety to construct the C1−C15 ester linkage. The stereoselective formation of the C3 alcohol (in its native *S*-configuration) developed into a major challenge in our earlier efforts, especially in the epothilone F series.[11a] Extensive investigations revealed that the best yields were obtained from a chiral titanium-mediated *tert*-butyl acetate aldol reaction with aldehyde **6**, affording the correct C3 alcohol, *after* construction of the C6, C7, and C8 stereocenters.[20] For the synthesis of our cyclization precursor, acylation with acetic anhydride to generate the C15 acetate (vide infra), followed by an diastereoselective aldol reaction with aldehyde **6** would generate the target compound, with concomitant formation of the C3 stereocenter. Successful formation of

(15) Reviews: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18. (c) *Alkene Metathesis in Organic Chemistry*; Fürstner, A., Ed.; Springer: Berlin, 1998. (d) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012. (e) Schrock, R. R. *Top. Organomet. Chem.* **1998**, *1*, 1.

(16) Xu, Z.; Johannes, C. W.; Salman, S. S.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1996**, *118*, 10926.

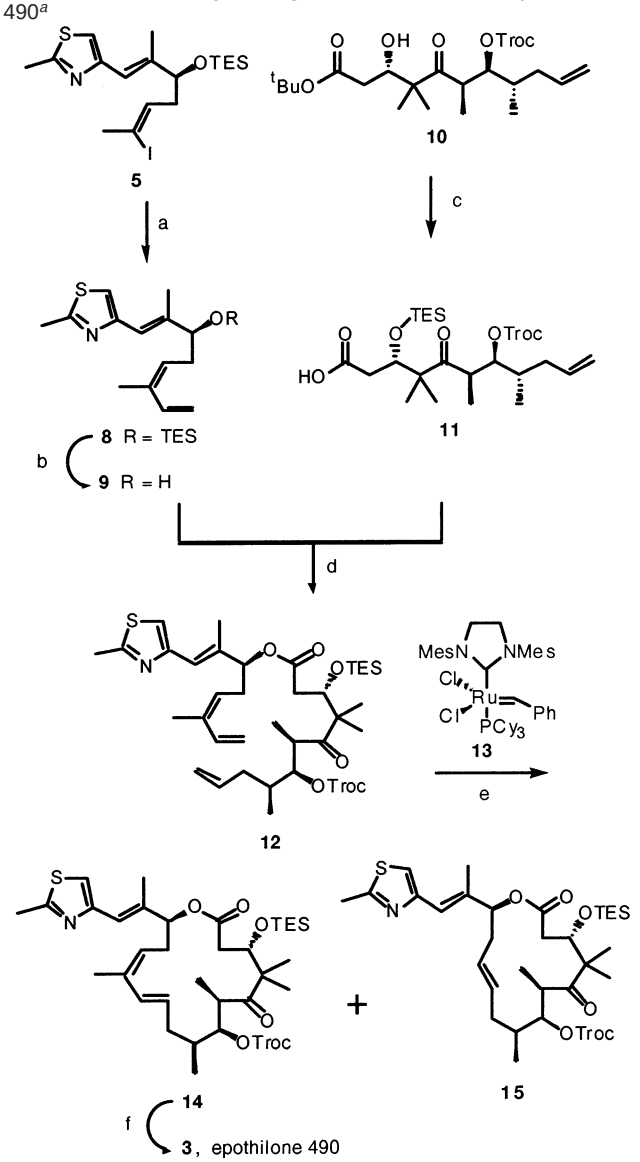
(17) (a) Garbaccio, R. M.; Danishefsky, S. J *Org. Lett.* **2000**, *2*, 3127. (b) Garbaccio, R. M.; Stachel, S. J.; Baeschlin, D. K.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10903.

(18) (a) Cabrejas, L. M. M.; Rohrbach, S.; Wagner, D.; Kallen, J.; Zenke, G.; Wagner, J. *Angew Chem., Int. Ed.* **1999**, *38*, 2443. (b) Dvorak, C. A.; Schmitz, W. D.; Poon, D. J.; Pryde D. C.; Lawson, J. P.; Amos, R. A.; Meyers, A. I. *Angew. Chem., Int. Ed.* **2000**, *39*, 1664.

(19) Chappell, M. D.; Stachel, S. J.; Lee, C. B.; Danishefsky, S. J. *Org. Lett.* **2000**, *2*, 1633.

(20) Wu, Z.; Zhang, F.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 4505.

Scheme 1. Initial Ring-Closing Metathesis Route to Epothilone 490[a]



[a] Reagents and conditions: (a) $Pd_2(dba)_3$, CH_2=$CHSnBu_3$, PPh_3, DMF, 50 °C, 96%; (b) TBAF, THF, 0 °C, 92%; (c) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 92%; (d) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 76%; (e) **13** (10 mol %), CH_2Cl_2 (0.002 M), 35 °C, 50% (**14**:**15** 3:1); (f) Zn, THF, AcOH, 86%; HF·pyr, THF, 0 °C, 90%.

the C3 (*S*)-alcohol late in the synthesis would obviate potential pitfalls in the construction of the C6−C8 stereotriad. With these design elements in mind, we embarked first upon the total synthesis of epothilone 490.

Given our quest for practicality, we insisted on convergent solutions to accomplish the C1−C2 interpolation and the creation of the diene functionality. In the event, Stille coupling[21] of **5** with vinyl *n*-tributyltin afforded **8** (Scheme 1). Cleavage of the silyl-protecting group afforded **9**. Our initial approach commenced with EDCI/DMAP-mediated esterification of the resulting allylic alcohol **9** with the C1 acid fragment **11**, obtained by deprotection of known *tert*-butyl ester **12**.[11b] This reaction yielded the cyclization precursor, triene **12**. Exposure of **12** to the RCM reaction with the second-generation ruthenium metathesis catalyst **13**[22] in methylene chloride gave a mixture of two compounds in a 3:1 ratio, with a total yield of 50%.[23] The major component of the product mixture was identified as the desired *trans*-substituted diene product **14**, along with the 14-membered macrolide **15** as a minor product, seemingly arising from a metathesis reaction involving the internal 12,13-olefin. Deprotection of the Troc and silyl groups *led to fully synthetic epothilone 490 (**3**), identical in all respects to an authentic sample*. The formation of the *E*-10,11-double bond was highly stereoselective and helped to confirm the stereochemistry of epothilone 490 to be as shown.

Following a similar series of reactions, we proceeded to synthesize the 21-hydroxyl variant of the new compound, the 10,11-dehydro version of desoxyepothilone F, compound **4**. Starting with the known Troc-protected 21-hydroxy vinyl iodide **16**,[11a] Stille coupling gave diene **17** (Scheme 2). Deprotection of the silyl group followed by esterification and RCM afforded **20**. Deprotection of the Troc and triethylsilyl groups afforded 21-hydroxy diene **4**.

A Surprising Substrate Effect on RCM Yield. Although, our initial foray into a new RCM manifold afforded a moderate yield of the macrolide, we were pleased to observe only the desired *E*-olefin in the reaction mixture. Examination of the sequence of steps that led to the construction of the cyclization precursor suggested a different order of conjoining the fragments in fewer total steps. Since the C3 (*S*)-stereocenter is constructed by a chiral titanium-mediated acetate aldol reaction,[20] we decided to attempt this reaction at a late stage, *with the entire O-alkyl fragment serving as part of the chiral nucleophile as its C15 acetate*. In this context, the allylic alcohol **9** was acylated to obtain the desired acetate **21** (Scheme 3). Following the protocol of Duthaler,[24] the lithium enolate of **21** was treated with the chiral titanium reagent to generate the chiral titanium enolate. Addition of aldehyde **6** afforded the desired aldol product, **22**, *as a single diastereomer*.[25]

Mindful of the fact that the newer ruthenium metathesis catalysts are tolerant of a wide variety of functional groups, we decided to attempt an RCM reaction on **22**, without protection of the C3 alcohol moiety. Treatment of **22** with catalyst **13** afforded the desired product in 41% yield, *with none of the 14-membered macrolide being observed*. Deprotection of the C7 Troc-protecting group in the usual way afforded epothilone 490.

The change in ratios of the 16- and 14-membered macrolide rings upon deprotection of the C3 alcohol suggested a surprising substrate effect on the macrocyclization step. We began to wonder about the potential effect of deblocking the C7 alcohol on the metathesis reaction as well. Therefore, we decided to perform a series of RCM reactions in which we varied the protection status of the C3 and the C7 alcohols in all of the possible combinations (Table 1). The results were indeed quite dependent on the presence of the protecting groups. The 14-membered macrolide was observed only when the substrate was fully protected. More importantly, *the yield of the reaction*

(21) Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1.

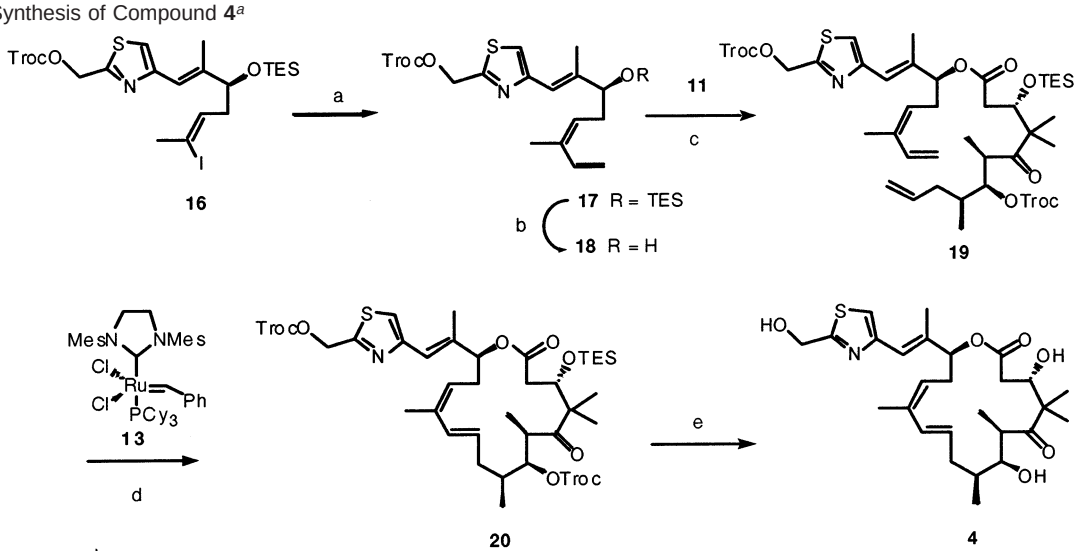
(22) Initial report: Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247.

(23) No reaction was observed with the first-generation bis(cyclohexyl)ruthenium Grubbs catalyst, while treatment with the Schrock molybdenum catalyst led to decomposition of the starting material.

(24) Duthaler, R. O.; Herold, P.; Lottenbach, W.; Oretle, K.; Reidiker, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 495.

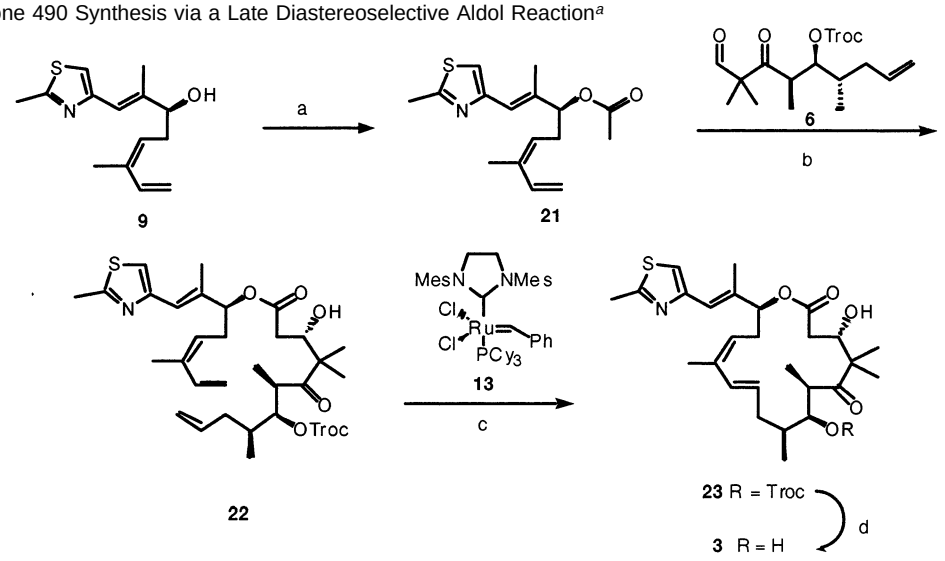
(25) The identity of the product was verified by treatment with TESCl to generate the C3 TES ether, which was identical to **12**, as determined by ^1H and ^{13}C NMR and optical rotation. Furthermore, **22** was converted to epothilone 490, verifying the (*S*)-stereochemistry at C3.

Scheme 2. Synthesis of Compound **4**[a]



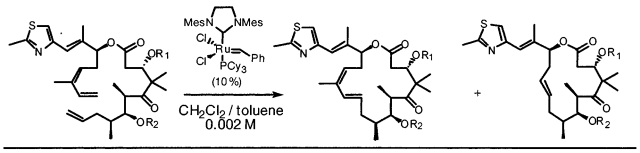
[a] Reagents and conditions: (a) $Pd_2(dba)_3$, $CH_2{=}CHSnBu_3$, PPh_3, DMF, 78%; (b) AcOH, THF, H_2O, 89%; (c) EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 88%; (d) **13** (10 mol %), CH_2Cl_2 (0.002 M), 35 °C, 40%; (e) Zn, THF, AcOH, 70%; HF·pyr, THF, 0 °C, 80%.

Scheme 3. Epothilone 490 Synthesis via a Late Diastereoselective Aldol Reaction[a]



[a] Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 98%; (b) LDA, Et_2O, −78 °C, then $CpTiCl(OR)_2$ (R = 1,2:5,6-di-*O*-isopropylidine-α-L-glucofuranos-3-*O*-yl), −78 °C to −30 °C, then **6**, −78 °C, 85%; (c) **13** (10 mol %), CH_2Cl_2 (0.002 M), 35 °C, 41%; (d) Zn, THF, AcOH, 86%.

Table 1. Effect of Alcohol Protection and Different Solvents on RCM Yield[a]



12, R_1 = TES, R_2 = Troc	35% / 58%[b]	15% / 6%[b]
22, R_1 = H, R_2 = Troc	41% / 57%	0% / 0%
24, R_1 = TES, R_2 = H	57% / n.d.[c]	0% / n.d.[c]
25, R_1 = H, R_2 = H	64% / 55%	0% / 0%

[a] Reactions in CH_2Cl_2 were run for 5.5 h at 35 °C, reactions in toluene for 25 min at 110 °C. [b] Done with 20 mol % catalyst at 0.0005 M dilution. [c] Not determined.

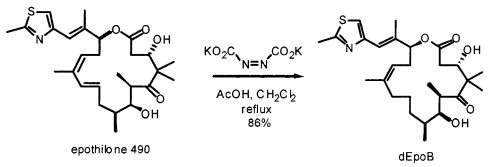
almost doubled upon use of a substrate where C7 is free. In fact, RCM of the fully deprotected substrate afforded the product epothilone 490 in 64% yield, with no observed *Z*-isomer of the C10−C11 olefin. This reaction represents the best yield obtained to date in construction of the epothilone scaffold in the B series with RCM, with no laborious separation of undesired olefin isomers involved in the purification process. Interestingly, when we carried out this same series of reactions in refluxing toluene, this substrate effect was diminished, with 55−58% yields observed across the various substrates.[26]

The origin of this substrate effect has not yet been determined. Intriguingly, we note that both the C3 and the C7 alcohols are β- to carbonyl groups, suggesting a possible contribution of intramolecular hydrogen bonding in imparting a degree of rigidity to the cyclization precursor.[27] Clearly, this effect does not seem to affect the reaction yield at higher temperature, in a less polar solvent.

(26) Toluene is a preferred solvent for scale-up processes; indeed, compound **22**, derived from the acetate aldol as shown in Scheme 3, was successfully subjected to metathesis conditions at 1 mmol scale in toluene at 110 °C as a proof of principle experiment. See Supporting Information for details. We thank Dr. Kana Yamamoto for suggesting the use of refluxing toluene conditions in these reactions

(27) For examples and discussion of similar protecting-group effects on RCM reactions in the synthesis of salicylihalamides, see: (a) Fürstner, A.; Thiel, O.; Blanda, G. *Org. Lett.* **2000**, *2*, 3731. (b) Fürstner, A.; Dierkes, T.; Thiel, O.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286 and references therein.

Scheme 4. Diimide Reduction of 10,11-Olefin: New Synthesis of dEpoB



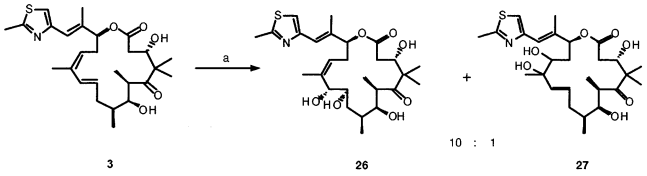
Selective Diimide Reduction of 10,11-Olefin: A New Route to dEpoB. The successful application of RCM to the synthesis of the diene epothilones of the 490 series led us to examine whether we could access our clinical candidate dEpoB by this newly described endgame. Attainment of this goal would involve a selective hydrogenation of the disubstituted C10−C11 *E*-olefin, in the presence of the trisubstituted C12−C13 *Z*-olefin and the "benzylic" trisubstituted C16−C17 olefin. A variety of metal-catalyzed and homogeneous hydrogenation conditions were examined, but they suffered from either over- or under-reduction.[28] Diimide-based reductions are known to be extremely sensitive to steric effects in distinguishing differentially substituted olefins.[29] Therefore, we turned our attention to diimide as a reducing agent to convert epothilone 490 to dEpoB. This goal was successfully accomplished by treatment of fully synthetic **3** with in situ generated diimide (86% yield, Scheme 4).

By focusing on a new section of the carbon skeleton for generation of an olefin, we have been able to successfully access the epothilone framework using an RCM−reduction protocol. Needless to say, the inspiration for this strategy was the isolation and identification of epothilone 490. During this process, we utilized the semipractical syntheses of advanced intermediates **5** and **6**, and fashioned the epothilone scaffold by a novel sequence of highly efficient reactions. The total synthesis reported herein is far more convergent and far less dependent on technically demanding reactions than the route which had already produced multigram quantities of clinical grade dEpoB. However, evaluation as to the feasibility of scale-up of the new route, in a plant context, has not been conducted at this writing.

Selective Functionalization of the 10,11-Olefin. The successful reduction reaction also indicated that selective functionalization of the newly generated C10−C11 olefin was feasible to enable a SAR profile of that sector of epothilones. Therefore, we report on the synthesis and preliminary evaluation of some novel epothilones available via epothilone 490. We subjected dienes in this series to dihydroxylation, epoxidation, and cyclopropanation conditions. Treatment of **3** with catalytic osmium tetroxide in the presence of NMO resulted in the formation of a 10:1 mixture, where the major product was identified as **26** (Scheme 5). The minor product arises from the dihydroxylation of the 12,13-olefin.

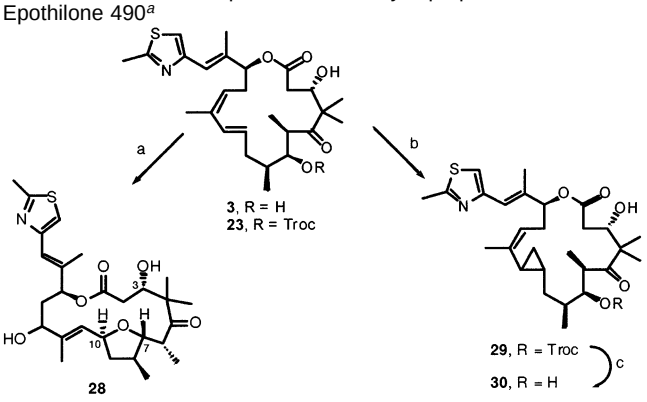
The stereochemistry at C10 and C11 of **26** was determined by X-ray crystallography, as depicted in Figure 5a. Inspection of a Macromodel-derived minimized (**MM2**) structure of epothilone 490, Figure 5b, shows that the "external" face of the 10−11 olefin is more available to reagents. This model

Scheme 5. Selective Dihydroxylation of 10,11-Olefin with OsO_4[a]



[a] Reagents and conditions: (a) OsO_4 (0.2 equiv), NMO (1.0 equiv), acetone:H_2O (9:1), −25 °C, 68%.

Scheme 6. Selective Epoxidation and Cyclopropanation of Epothilone 490[a]



[a] Reagents and conditions: (a) **3**, DMDO, CH_2Cl_2, −78 °C − rt, silica gel, 47%; (d) **23**, CH_2N_2, $Pd(OAc)_2$, Et_2O, 0 °C, 35%; (c) Zn, THF, AcOH, sonication, 85%.

suggests a rationalization of the product stereochemistry we observe in the dihydroxylation reaction.

Interestingly, exposure of **3** to the action of 2,2′-dimethyldioxirane, with the intent of generating an epoxide, gave rise to tetrahydrofuran-containing macrocycle **28** upon silica gel purification.[30] Compound **28** arises from epoxidation of the 12,13-olefin and S_N2'-type participation of the C-7 hydroxyl group (Scheme 6). Finally, treatment of **23** with diazomethane in the presence of $Pd(OAc)_2$,[31] followed by deprotection, afforded the vinyl cyclopropane **30**.[32]

The new analogues obtained from epothilone 490 exhibited a range of in vitro cytotoxities[9] and microtubule stabilizing ability,[33] as shown in Table 2. Indeed, the microtubule stabilizing ability closely parallels the observed cytotoxicity data.

The impressive cell growth inhibition exhibited by epothilone 490 across a range of various drug-resistant tumors led us to determine its efficacy in an in vivo setting, in nude mice bearing human tumor xenografts. Fortunately, our straightforward synthesis of **3** allowed us to indulge these interests. To our surprise, epothilone 490 did not demonstrate any meaningful inhibitory effect on the growth of the implanted tumors, as compared to control mice (data not shown). These data were surprising given the favorable prognosis based on in vitro protocols.

In addressing this problem, we recalled that dEpoB itself evidenced a worrisome bioinstability in murine plasma. However, it had much longer half-lives in higher organisms,

(28) Attempted conditions: H_2, Pd/C; H_2, Rh/Al_2O_3; H_2, Wilkinson's catalyst; H_2, PtO_2 (Adam's catalyst); H_2, Crabtree's catalyst.

(29) (a) Corey, E. J.; Mock, W. L.; Pasto, D. J. *Tetrahedron Lett.* **1961**, 347. (b) Pasto, D. J.; Taylor, R. T. *Org. React.* **1991**, *40*, 91. Importantly, J. D. White and co-workers have previously reported the reduction of a C9−C10 olefin during their synthesis of the epothilones with diimide, see ref 6k.

(30) The stereochemistry of macrocycle **28** was assigned on the basis of the analysis of 2D COSY and NOESY spectra, assuming that all the existing stereochemistry remained untouched under the mild reaction conditions.

(31) Denmark, S. E.; Stavenger, R. A.; Faucher, A.-M.; Edwards, J. P. *J. Org. Chem.* **1997**, *62*, 3375 and references therein.

(32) Stereochemistry of the new cyclopropane ring is undetermined at this writing. The 12-desmethyl version of this cyclopropyl analogue has recently been reported at the ACS National Meeting in Orlando, April 2002: Pabba, P. K.; Taylor, R. E. *Abstr. Pap. Am. Chem. Soc.* **2002**, *223*, 436-Orgn.

(33) Gaskin, F.; Cantor, C. R.; Shelanski, M. L. *J. Mol. Biol.* **1974**, *89*, 737.

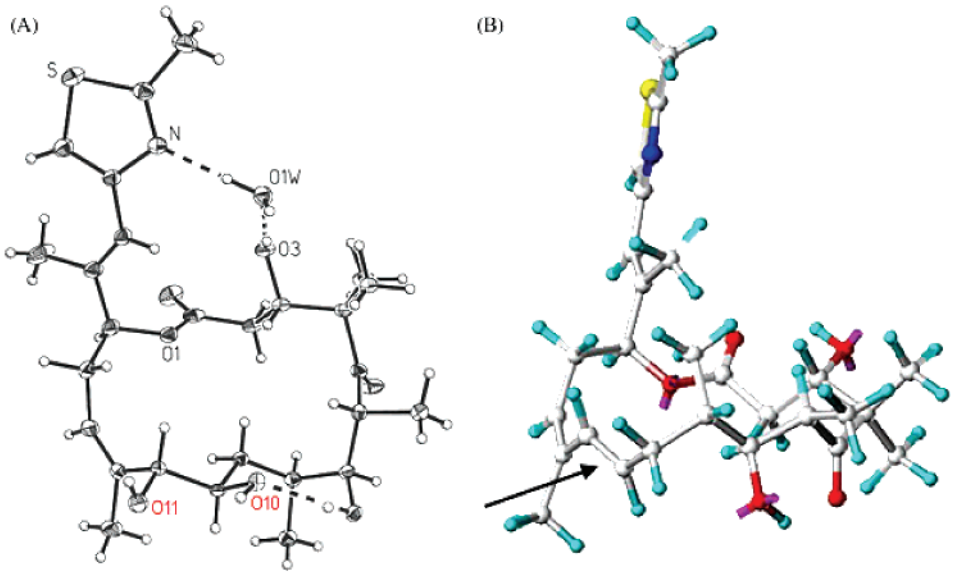


Figure 5. (a) X-ray structure of **26**, showing the stereochemistry of the dihydroxylation product to be the "external" diol. The new oxygens at C10 and C11 are in red. (b) Macromodel-derived minimized conformation of epothilone 490, demonstrating the easier access to the "external" face of the 10,11-olefin (marked by arrow) to reagents.

Table 2. In Vitro Cytotoxicities (IC_{50}) with Tumor Cell Lines[a] and Microtubule Binding

cmpd	CCRF-CEM (μM)	CCRF-CEM/$_{VBL100}$ (μM)	CCRF-CEF/$_{VM1}$ (μM)	CCRF-CEM/$_{Taxol}$ (μM)	% tubulin binding[b]
1 (dEpoB)	0.011	0.015	0.016	0.007	100
3	0.025	0.091	0.035	0.032	89
4	0.030	0.202	0.061	0.051	77
26	1.001	99.0	2.35	16.76	31
28	0.761	8.76	n.d.[c]	4.24	inactive
30	0.077	0.141	n.d.[c]	n.d.[c]	84
Taxol	0.0021	0.827	0.003	0.081	n.d.[c]
vinblastine	0.0008	0.122	0.0014	0.018	n.d.[c]

[a] XTT assay following 72-h inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF-CEM/$_{VBL100}$, CCRF-CEM/$_{VM1}$, and CCRF-CEM/$_{Taxol}$ cell lines all overexpress P-glycoprotein and display a multidrug resistance phenotype to MDR-associated oncolytics.[9] [b] Formation of microtubules in the presence of the compounds. Microtubules formed in the presence of dEpoB is defined as 100%.[34] [c] Not determined.

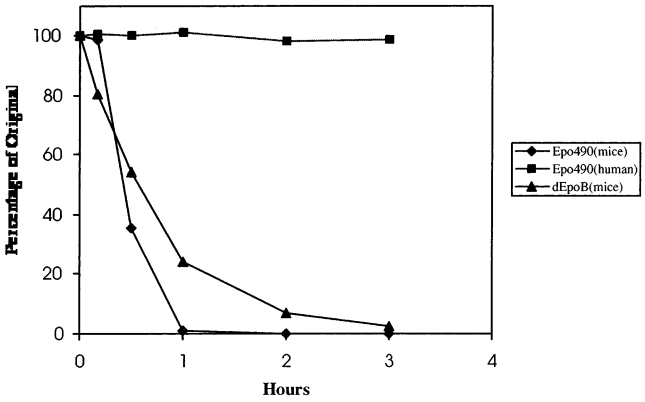


Figure 6. Plasma stability of epothilone 490 and dEpoB in nude mouse and human plasma (see ref 9 for details).

including humans.[9] This trend has been ascribed to higher esterase levels in rodents. The failure of epothilone 490 in our murine xenograft assay, in contrast to its excellent cell-culture inhibitory data, suggested that the pharmacokinetic properties of **3** be evaluated.

Indeed, on exposure of **1** and **3** to murine plasma, the biodegradation of epothilone 490 was even faster than that of dEpoB (Figure 6). Thus, while the murine pharmacokinetics of **1** are far from optimal, drug levels are adequate for major reduction and elimination of murine tumor burden. By contrast, the murine stability of **3** is so poor as to vitiate the potential benefits of the drug. *Encouragingly, the same drug remained essentially unchanged over 3 h in human plasma.*

Conclusions

The development of a clinically useful complex natural product demands easy access to vast quantities of purified material. Traditionally, isolation or fermentation-based methods have been the sole supplier of either the final product or of advanced intermediates which could be easily transformed to the desired drug by semisynthetic means. For example, the success of Taxol as a clinical candidate against solid tumors has been totally contingent upon the ability to procure the final product via semisynthesis from a readily accessible baccatin precursor.[35]

In this regard, *total* chemical synthesis of clinically useful complex natural products has been a much investigated but less productive tool. However, with the rapid development of efficient reaction processes, it could well be possible to gain

(34) See ref 6b for experimental details.
(35) (a) Denis, J. N.; Greene, A. E.; Guenard, D.; Guerittevoegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917. (b) Holton, R. A.; Liu, J. W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2475 and references therein.

rapid access to complex natural products by total synthesis. Our odyssey in the study and development of the epothilone-based family of anticancer agents is testimony to the power of chemical synthesis in supplying multigram quantities of these natural polyketides for preclinical and clinical evaluations of efficacy. In this report, we returned to our early attempts at fashioning the macrolide ring of the epothilones by ring-closing metathesis-based processes, which had been plagued by poor stereocontrol in the past. The identification of a series of natural epothilones with a new olefin at the C10−C11 position, and the development of more reactive metathesis catalysts prompted us to reexamine the utility of this reaction for generation of the epothilone macrocycle.

Herein, we described a construction of the epothilones with ring-closing metathesis. For purposes of greater synthetic convergency, we fashioned the C3 (*S*)-alcohol late in the synthesis, using a chiral titanium-mediated aldol reaction with the entire *O*-alkyl fragment as its C15 acetate as the enolate component. Examination of the effects of protecting groups on the RCM process demonstrated an intriguing substrate effect on reaction yield. Selective diimide reduction of the new olefin yielded desoxyepothilone B, our current clinical candidate, *validating the utility of this new RCM−reduction protocol in generating the epothilone framework.* The beginnings of charting the chemistry-based possibilities for analogue synthesis with **3** well in hand are described. Also described is the surprisingly poor in vivo performance of epothilone 490 in xenografts. This outcome was traceable to unfavorable pharmacokinetic features of the drug in this particular species. *To the extent that plasma stability is predictive of pharmacokinetic performance, the prognosis for the effectiveness of **3** in humans is much more promising*. Parenthetically, this research points to exciting possibilities in drug discovery and refinement centered around organic synthesis in close liaison with in vivo pharmacology and pharmacokinetics.

Acknowledgment. This work was supported by the National Institutes of Health (CA-28824). Postdoctoral support is acknowledged by K.B. (U.S. Army Breast Cancer Research Program, DAMD-17-98-1-8155), H.L. (NIH Cancer Pharmacology Training Grant, T32-CA62948), J.T.N. (General Motors Cancer Research Scholar Program) and M.D.C. (NIH, 1 F32 GM19972-01). We thank Dr. George Sukenick and Ms. Sylvi Rusli (NMR Core Facility, CA-02848) for mass spectral analyses and Joseph Tanski (Columbia University) for X-ray diffraction analysis.

Supporting Information Available: Experimental details for preparation and spectral characteristics of **8**, **9**, **11**−**12**, **14**−**15**, **3**, **17**−**20**, **4**, and **21**−**23** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0262333