Application of Ring-Closing Metathesis Reactions in the Synthesis of Epothilones[⊥]

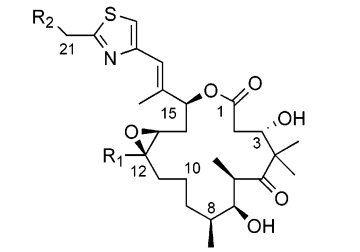
Alexey Rivkin, Young Shin Cho, Ana E. Gabarda, Fumihiko Yoshimura, and Samuel J. Danishefsky*

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, and Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027

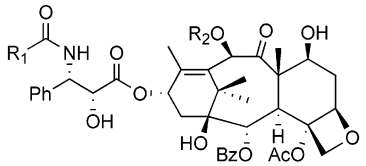
Received December 18, 2003

There is wide interest in the epothilones, which like the taxoids initiate cytotoxicity through microtubule stabilization. Briefly described is an application of a ring-closing metathesis reaction toward the synthesis of epothilones as carried out in our laboratory. This has led to the discovery of the (*E*)-9,10-dehydroepothilones as second-generation anticancer drug candidates.

The epothilone macrolides (**1**−**4**), initially discovered by Höfle and co-workers from fermentation sources, have elicited a great deal of interest as drug candidates in cancer chemotherapy (Figure 1).[1] The large interest in epothilones initially originated from the fact that their mode of antitumor activity mimicked that of the established clinically useful taxoids (**5**, **6**), which initiate cytotoxicity through microtubule stabilization.[2] The taxoids are currently the frontline drugs being used to fight a variety of different cancers.[3] One of the main liabilities of the taxoid drugs is their susceptibility to disablement by multiple drug resistance (MDR). This vulnerability of taxol prompted interest in the epothilones, which were shown to exhibit virtual imperviousness to the defenses of otherwise multidrug-resistant cells.[2,4] Following extensive multidisciplinary research directed to the biology, chemistry, pharmacology, toxicology, and biosynthesis of the new structural epothilones, three agents, including compound **7** (vide infra), have already been advanced to phase I and phase II clinical trials, with additional variants being readied for investigational review.[5]



1: R_1 = H, R_2 = H, Epothilone A (EpoA)
2: R_1 = CH_3, R_2 = H, Epothilone B (EpoB)
3: R_1 = H, R_2 = OH, Epothilone E (EpoE)
4: R_1 = CH3, R_2 = OH, Epothilone F (EpoF)



5: R_1 = Ph, R_2 = Ac Paclitaxel (Taxol)
6: R_1 = *t*-BuO, R_2 = H Docetaxel (Taxotere)

Figure 1. Structures of epothilones and taxoids.

In 1996, our laboratory reported the first total synthesis of epothilones A (**1**) and B (**2**) and were soon joined by others.[6] During our preliminary synthetic studies with the epothilones, we concluded that the 12,13-oxido linkage of the epothilones is a locus of non-tumor selective toxicity. Accordingly, this linkage was "edited", initially through chemical synthesis, to provide 12,13-desoxyEpoB (dEpoB, **7**) and related desoxy congeners (Figure 2).[7] While significantly less cytotoxic than EpoB (**2**) itself, dEpoB is roughly equipotent, in xenograft models, with paclitaxel and doxetaxel, the currently leading tubulin-directed anticancer drugs. dEpoB benefits from a much broader therapeutic index in xenograft models than are exhibited by taxoids or by epothilones that contain epoxides.[8] The major advantages of dEpoB in these nude mouse xenograft models is particularly dramatic with resistant tumors. dEpoB, our first-generation drug candidate, has recently been entered into phase II human clinical trials.

More recently, our laboratory reported the discovery of a new family of second-generation epothilone drug candidates, the (*E*)-9,10-dehydroepothilones **8** and **9** (Figure 2), which possessed remarkable curative properties.[9] The incorporation of *E*-9,10 unsaturation in the context of the usual *Z*-12,13 olefin (see compound **7**) resulted in a marked increase in in vitro potency and metabolic stability of the drug. The beneficial effects of the incremental C9−C10 unsaturation have been extended to in vivo experiments in the settings of xenograft mice. From this family, 26-trifluoro-(*E*)-9,10-dehydroepothilone (**9**) has emerged as another drug candidate suitable for development.[10] Agent **9** achieves tumor shrinkage and disappearance in human xenografts in nude mice at well-tolerated doses and complete remission for over two months with early rapid recovery of body weight to the pretreatment control levels. The therapeutic safety margin of **9** is perhaps the widest reported to date for a prospective cancer therapeutic agent.

The momentum of our epothilone program over the past several years has closely intertwined goals.[11] The first was the preparation of carefully crafted and hypothesis-driven epothilone analogues, through paths made accessible by "molecular editing" enabled by total synthesis. The second goal was the exploration of various strategies to develop a practical total synthesis of epothilone drug candidates. To serve our purpose, a synthetic path must furnish ample material for a full-scale evaluation. Over the past seven

[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Instititute for their pioneering work on bioactive natural products.

* To whom correspondence should be addressed. E-mail: s-danishefsky@ski.mskcc.org.

10.1021/np030540k CCC: $27.50
Published on Web 01/24/2004

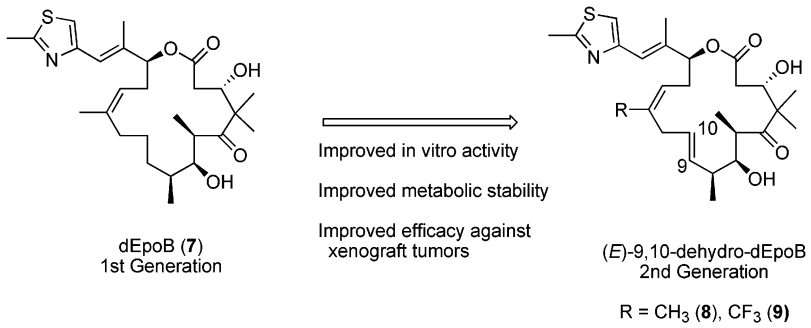


Figure 2. Structures of epothilone drug candidates.

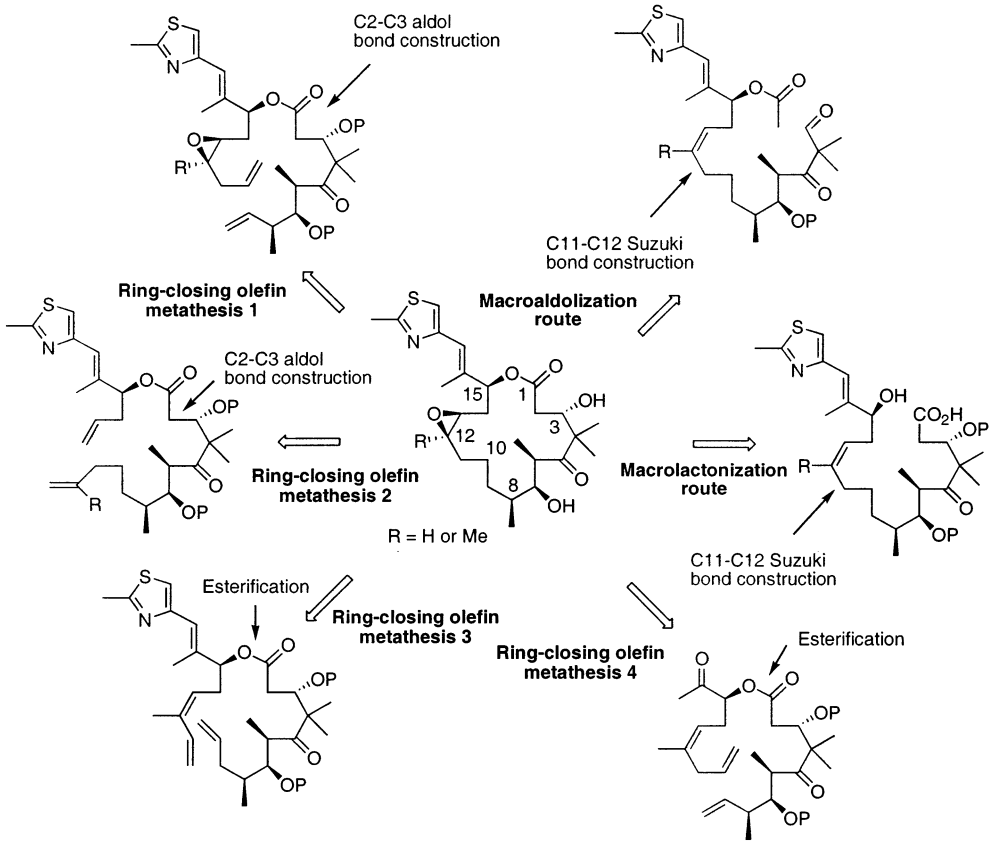


Figure 3. Our past strategies for the total synthesis of epothilones.

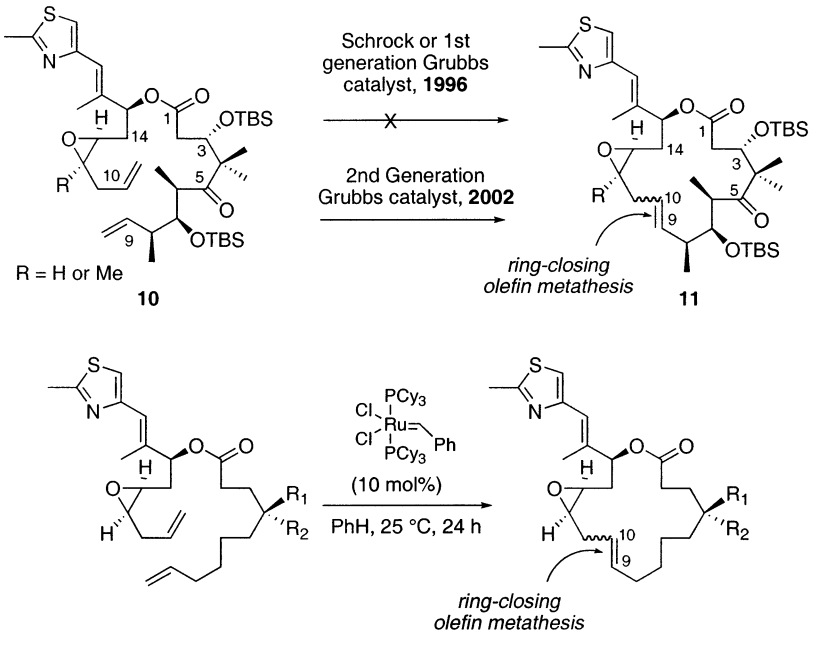
years, we have reported several different approaches for the synthesis of the epothilones, which allowed for extensive SAR studies by identifying the regions of the epothilones that are amenable to chemical modification. The synthesis approaches relied on macrolactonization, macroaldolization, and ring-closing metathesis for the closure of the 16-membered macrolide. The focus of this review will be on the application of the ring-closing metathesis reaction toward the syntheses of epothilones as practiced in our laboratory. We show how this chemistry led to the eventual discovery of our second-generation drug candidates, the (*E*)-9,10-dehydroepothilones (Figure 3).

Our initial approaches for the total synthesis of epothilones A (**1**) and B (**2**) were based on a ring-closing metathesis reaction to construct the 9,10-olefin with the 12,13-epoxide functionality already in place (Scheme 1).[7] Regrettably, all attempts to effect the RCM reaction of **10** (R = H) using the Schrock (molybdenum based) or the early version of Grubbs (ruthenium based) catalyst failed to provide the desired product **11**. In contrast, RCM reactions of models **12** and **13** provided the desired compounds **14** and **15** in high yield. It appeared that the steric congestion between C3 and C8 was detrimental to the success of the RCM reaction. Interestingly, six years later Sinha and coworkers did synthesize **10** and demonstrated that the RCM reaction does indeed achieve cyclization.[12] This success required recourse to a second-generation catalyst which was not available at the time we were carrying out our studies.

Our inability to carry out the RCM to form the 9,10-olefin prompted us to pursue a strategy based on the *B*-alkyl Suzuki method. This chemistry enabled conclusion of the first syntheses of epothilones A and B.

Our interest in improving upon the overall synthesis of epothilones A and B prompted us to investigate the possibility of an intramolecular ring-closing olefin metathesis to form the C12−C13 olefin. This double bond could be converted into the desired epoxide. With increased spacing between the C12 olefin and the branched positions of the polypropionate region, the ring-closing metathesis approach proved to be quite successful (Scheme 2).[7] In these studies, cyclizations were conducted using earlier variants of the Grubbs catalyst and the Schrock catalyst. Although the yields of the cyclization products were gener-

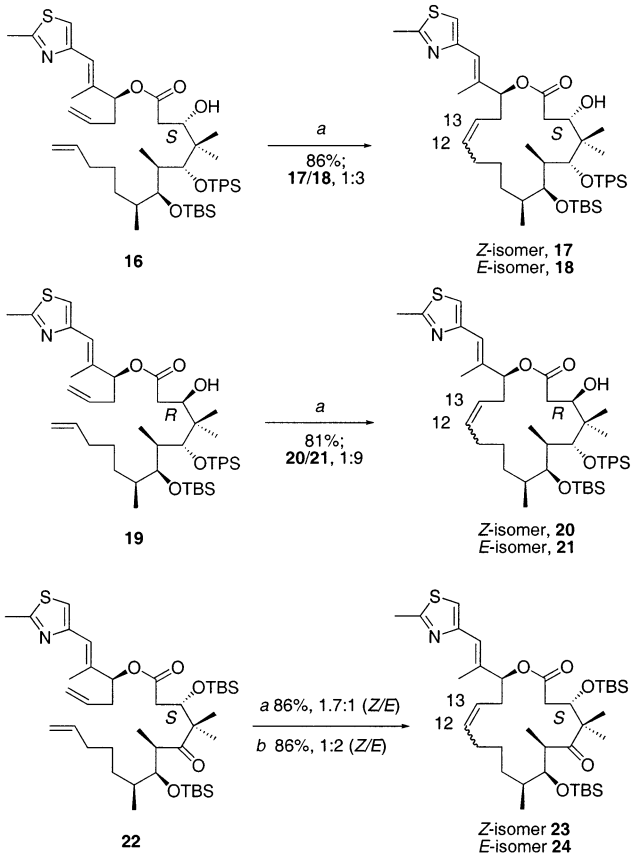
Scheme 1. Ring-Closing Metathesis Strategy 1



12: R_1, R_2 = H
13: R_1, R_2 = CH_3

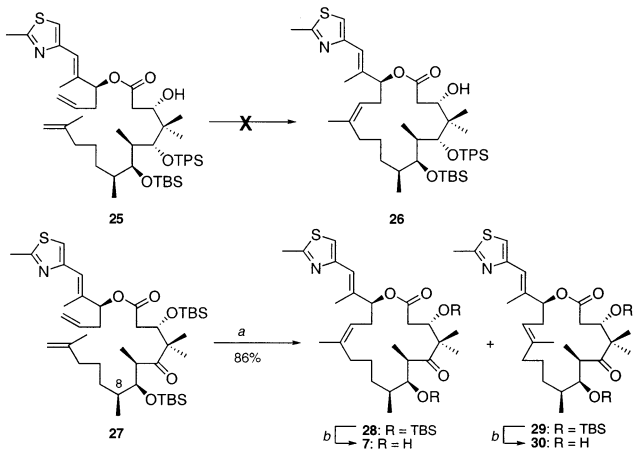
14: R_1, R_2 = H (*E*:*Z* *ca.* 1:1; 45%)
15: R_1, R_2 = CH_3 (one isomer; 70%)

Scheme 2. Ring-Closing Metathesis Strategy 2[a]



[a] (a) $RuBnCl_2(PCy_3)_2$, 50 mol %, C_6H_6, 0.001 M, rt, 24 h; (b) $Mo(CHMe_2Ph)$-$(N(2,6\text{-}(i\text{-}Pr)_2C_6H_3))(OCMe(CF_3)_2)_2$ 20 mol %, C_6H_6, 0.001 M, rt, 1 h.

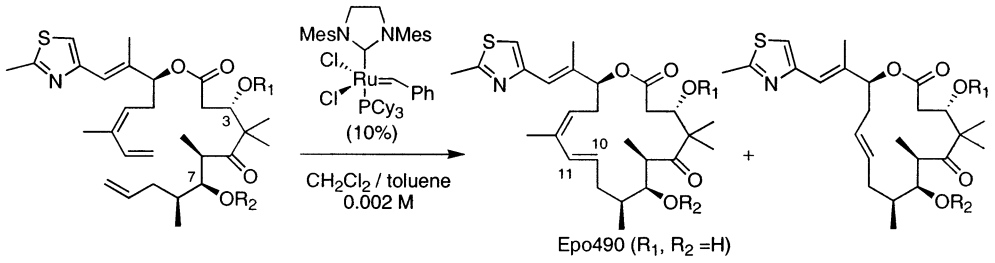
Scheme 3. Ring-Closing Olefin Metathesis Strategy 2[a]



[a] (a) $Mo(CHMe_2Ph)(N(2,6\text{-}(i\text{-}Pr)_2C_6H_3))(OCMe(CF_3)_2)_2$ 20 mol %, C_6H_6, 0.001 M, 55 °C, 2 h, 86%, **28**/**29** 1:1; (b) HF·pyr, THF, rt, 2 h 90%.

ally quite good, the ratios of *E*/*Z* isomers were not favorable toward the desired *Z* isomer. A considerable effort to improve the stereoselectivity of the olefin metathesis reaction with the goal of reaching the natural *Z* series was undertaken. A handful of RCM precursors, including **16**, **19**, and **22**, differing in the substitution pattern of the polypropionate region were tested. The results from these studies showed that the stereochemical course of the olefin metathesis was in some instances sensitive to the nature of the substituents along the acyl chain. For example, changing the stereochemistry at C3 of **16** from *S* to *R* led to further bias toward the formation of the undesireable *E* isomer (see **19** → **20** and **21**). Conversion of the C5-secondary alcohol to a carbonyl led to **22**, which gave a *Z*/*E* ratio of 1.7/1 at least in one case. Interestingly, the olefin geometry in this reaction was also sensitive to the catalyst employed. As seen from these data, structural permutations of the polyproponiate region led to conformational shifts which had subtle effects on the precomplexation of the vinyl group of one sector to the terminal metal-carbene complex derived from the other sector. However, the main goal, i.e., a stereospecific route to the *Z*-12,13-olefin of epothilones by metathesis, was not realized.

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



31, R_1 = TES, R_2 = Troc	35% / 58%[b]	15% / 6%[b]
32, R_1 = H, R_2 = Troc	41% / 57%	0% / 0%
33, R_1 = TES, R_2 = H	57% / n.d.[c]	0% / n.d.[c]
34, 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.

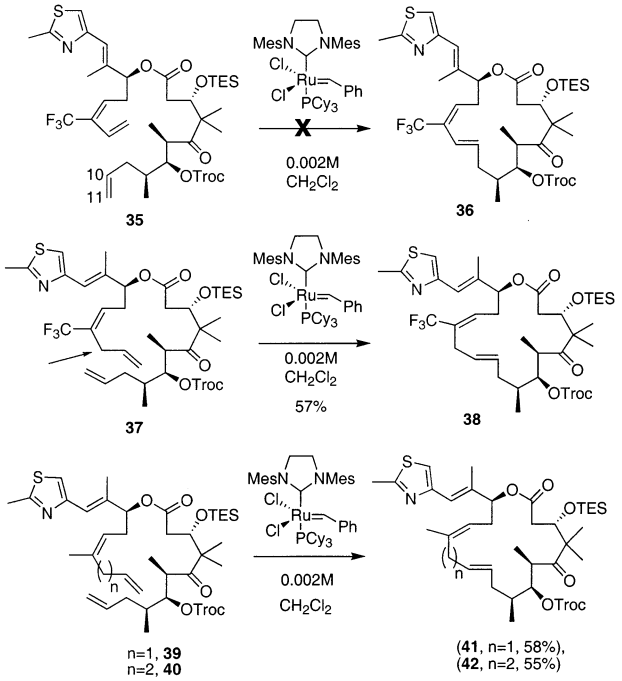
The dramatic effect of the substitution pattern of the polypropionate region of the ring-closing metathesis reaction was again demonstrated during the synthetic studies of epothilone B (Scheme 3).[7] When we tried to carry out the RCM reaction on **25** using Schrock catalyst, the reaction completely failed to provide any of the desired product **26**. However, once the C3 alcohol was protected as the TBS silyl ether and the C5 silyl ether was converted to the ketone group, the RCM reaction of **27** with the Schrock catalyst gave a 1:1 mixture of the *Z* isomer **28** and *E* isomer **29** in 86% combined yield. Due to difficulties in obtaining the desired *Z* olefin stereoselectively through RCM reactions, we instigated the optimization of the synthesis of dEpoB and epothilone B using the *B*-alkyl Suzuki reaction as the key coupling step to fashion the C11−C12 bond.

Eventually we returned to investigating RCM reactions in epothilone settings in the context of a proposed synthesis of the recently isolated epothilone 490. The latter showed highly favorable cytotoxicity in preliminary in vitro screens. The strategy for the synthesis of this epo490 was based on a stereoselective RCM reaction to form the *E*-10,11-olefin (Table 1).[13] Initially, the RCM reaction of **31** with second-generation Grubbs catalyst provided the desired substituted diene product along with the 14-membered macrolide in a 3:1 ratio and 50% combined yield. The formation of the *E*-10,11-olefin proceeded with high stereoselectivity.

During studies of this RCM reaction, it was discovered that altering the substitution of the C3 and C7 alcohols had a significant impact on the outcome of the reaction. Interestingly, removal of one or both of the protecting groups prevented the formation of the undesired 14-membered products. The best yield of the RCM reaction was obtained from the fully deprotected substrate **34**, which afforded epothilone 490 in 64% yield. In contrast to earlier 12,13-olefin RCM studies, the substitution of the polypropionate region did not have any effect on *E*/*Z* selectivity, but did affect the ring size. The origin of this substrate effect was postulated to be the consequence of hydrogen bonding between the C3 and C7 alcohols and their corresponding *B*-carbonyl groups, thus contributing to the rigidity of the cyclization precursor.

In the context of our studies with epothilone 490, we decided to synthesize a 26-trifluoro analogue of the compound (Scheme 4).[14] Suprisingly, the ring-closing metathesis reactions of **35** under the same conditions used for the parent methyl substrate **31** failed to provide the desired **36**. The incapacitation of the RCM reaction was obviously due to the presence of the fluorines at the 26 position, because in their absence the RCM reaction worked very well.

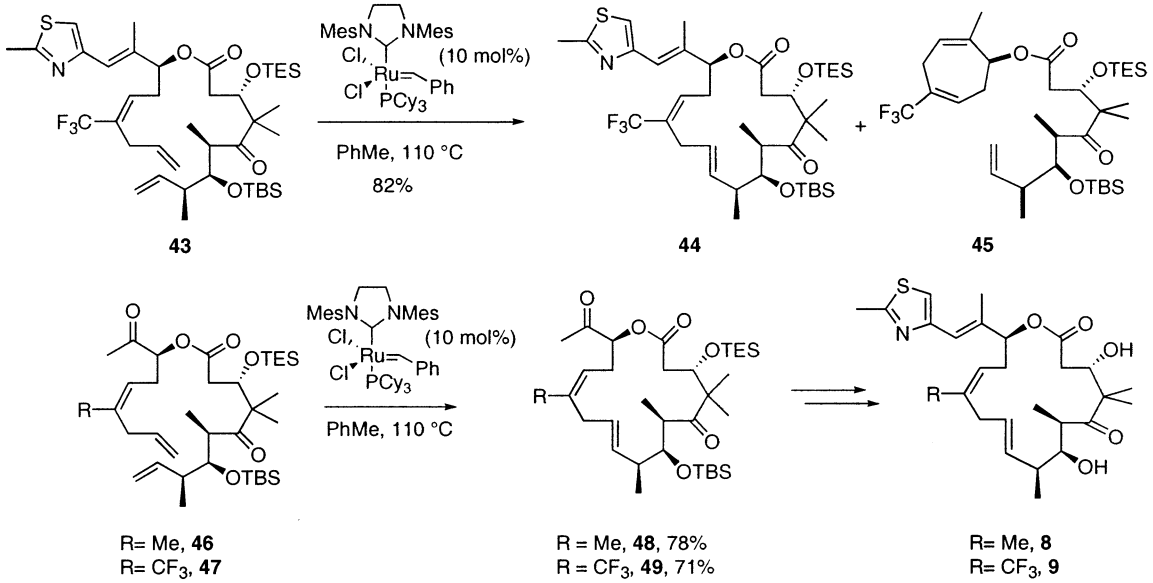
Scheme 4. Synthesis of 17- and 18-Membered Epothilones via Ring-Closing Olefin Metathesis Strategy



To explore the deleterious effect of the trifluoromethyl group on the RCM, we undertook to add one carbon "spacer" between this group and the reacting olefin. Indeed, the ring-closing metathesis of **37** using the second-generation Grubbs catalyst provided the desired 17-membered epothilone macrolide in 57% yield and exclusively the *E*-10,11-olefin. We also showed that the 17- and 18-membered epothilone macrolides **41** and **42** could be prepared in high yield using stereoselective RCM reaction to form exclusively the *E*-10,11-olefin (Scheme 4).[15] Even though the 18-membered epothilone **42** proved to possess low in vitro activity, the 17-membered epothilone **41** suprisingly retained a substantial in vitro activity.

We speculated that perhaps we could obtain the desired 16-membered epothilone analogues by moving the acyl side vinyl group to C8 (see Scheme 5, **43**).[9] This proposed strategy required the formation the 9,10-olefin via a ring-closing metathesis reaction. Although this type of RCM

Scheme 5. Synthesis of (*E*)-9,10-Dehydroepothilones via Ring-Closing Olefin Metathesis Strategy



reaction had failed previously with the Schrock and first-generation Grubbs catalyst, we hoped that the more active second-generation Grubbs catalyst would afford more favorable results. Happily, the ring-closing metathesis reactions of **43** using the second-generation Grubbs catalyst in toluene provided exclusively the *E*-isomer **44** along with the corresponding seven-membered side product **45** in a 1:3 ratio, respectively, in 82% combined yield.[16] Interestingly, in contrast to the results we obtained during the 10,11-olefin RCM formation (Table 1), we were not able to bias the formation of the desired **44** by carrying out the reaction with a fully deprotected substrate.

Subsequent to our discoveries that the (*E*)-9,10-dehydroepothilones possessed remarkable curative proterties against xenograft tumors, we decided to synthesize gram quantities of the compounds for extensive in vivo studies. To accomplish this, we had to improve the yield of the RCM reaction by avoiding the formation of the seven-membered side product **45**. Ultimately, we carried out the RCM reaction of **46** and **47** in the absense of the thiazole-substituted olefin, obtaining exclusively the *E*-isomers **48** and **49** in 78% and 81% yields, respectively (Scheme 5).[9] Later, we installed the thiazole moiety via a Wittig reaction in high yield and with high *E*/*Z* selectivity to provide the desired (*E*)-9,10-dehydroepothilones **8** and **9** following deprotection.

We note, parenthetically, that this study serves to underscore the potential applicability of directed total synthesis, even in a multistep setting, in the quest for new substances of material clinical benefit. The point is worth making in the current research environment, which favors recourse to massive numbers of compounds for screening, in preference to smaller numbers of more carefully crafted, hypothesis-driven candidate structures. In particular, the suggestive powers of teachings derived from natural products warrant close and continuing study.

References and Notes

(1) (a) Höfle, G.; Bedorf, N.; Gerth, K.; Reichenbach, H. German Patent DE-B 4,138,042, 1993. (b) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schumburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int., Ed. Engl.* **1996**, *35*, 1567−1569. (c) Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560−563.

(2) 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*, 2325−2333.

(3) (a) 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*, 1797−1805. (b) 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*, 273−279. (c) Rowinsky, E. K.; Donehower, R. C. *N. Engl. J. Med.* **1995**, *332*, 1004−1014.

(4) (a) Horwitz, S. B. *Ann. Oncol.* **1994**, *5* (Suppl. 6), S3−6. (b) Moos, P. J.; Fitzpatrick, F. A. *Proc. Natl. Acad. Sci.* **1998**, *95*, 3896−3901. (c) Kirikae, F.; Ma, Z.; Kuduk, S. D.; Slater, J. C.; Takeuchi, C. S.; Bounaud, P.-Y.; Nakano, M. *Biochem. Biophys. Res. Commun.* **1996**, *227*, 227−235.

(5) For more information about clinical trials of dEpoB, visit: www.kosan.com.

(6) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801−2803.

(7) Meng, D.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073−10092.

(8) Chou, T.-C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798−15802.

(9) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Chou, T.-C.; Dong, H.; Tong, W. P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 2899−2901.

(10) Chou, T. C.; Dong, H.; Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Tong, W. P.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 4761−4767.

(11) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434−8456.

(12) Sun, J.; Sinha, S. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 1381−1383.

(13) Biswas, K.; Lin, H.; Njardarson, J. T.; Chappell, M. D.; Chou, T. C.; Guan, Y.; Tong, W. P.; He, L.; Horwitz, S. B.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 9825−9832.

(14) Rivkin, A.; Biswas, K.; Chou, T. C.; Danishefsky, S. J. *Org. Lett.* **2002**, *4*, 4081−4084.

(15) Rivkin, A.; Njardarson, J. T.; Biswas, K.; Chou, T. C.; Danishefsky, S. J. *J. Org. Chem.* **2002**, *124*, 7737−7740.

(16) Unpublished results. Full details will be provided in due course.

NP030540K