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Discovery of (*E*)-9,10-Dehydroepothilones through Chemical Synthesis: On the Emergence of 26-Trifluoro-(*E*)-9,10-dehydro-12,13-desoxyepothilone B as a Promising Anticancer Drug Candidate

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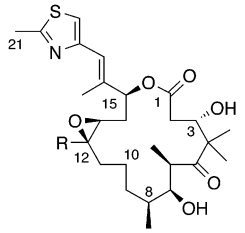
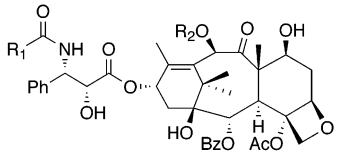
Abstract: We provide a full account of the discovery of the (*E*)-9,10-dehydro derivatives of 12,13-desoxyepothilone B (dEpoB), a new class of antitumor agents with promising in vivo preclinical properties. The compounds, which are to date not available by modification of any of the naturally occurring epothilones, were discovered through total chemical synthesis. We describe how our investigations of ring-closing metathesis reactions in epothilone settings led to the first and second generation syntheses of (*E*)-9,10-dehydro-12,13-desoxyepothilone congener **6**. With further modifications, the synthesis was applied to reach a 26-trifluoro derivative compound (see compound **7**). To conduct such studies and in anticipation of future development needs, the total synthesis which led to the initial discovery of compound **7** was simplified significantly. The total synthesis methodology used to reach compound **7** was then applied to reach more readily formulated compounds, bearing hydroxy and amino functionality on the 21-position (see compounds **45**, **62**, and **63**). Following extensive in vitro evaluations of these new congeners, compound **7** was nominated for in vivo evaluations in xenograft models. The data provided herein demonstrate a promising therapeutic efficacy, activity against large tumors, nonrelapseability, and oral activity. These results have identified compound **7** as a particularly promising compound for clinical development. The excellent, totally synthetic, route to **7** makes such a program quite feasible.

Introduction

Epothilones A (**1**) and B (**2**) are naturally occurring cytotoxic macrolides which were initially isolated by Höfle and co-workers from the mycobacterium *Sorangium cellulosum* (Figure 1).[1] Interest in epothilones originated from the discovery that their mode of antitumor activity mimicked that of the established clinically useful taxoids (**3** and **4**).[2] It has been shown that the taxoids[3] and epothilones interrupt the dynamic mechanism of microtubule assembly/disassembly via microtubulin stabilization.



1: Epothilone A (EpoA), R = H
2: Epothilone B (EpoB), R = CH_3



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

Figure 1. Structures of epothilones and taxoids.

This mode of drug induced intervention initiates cell death through apoptosis. In contrast to paclitaxel, the epothilones seem to exhibit near imperviousness to the defenses of otherwise

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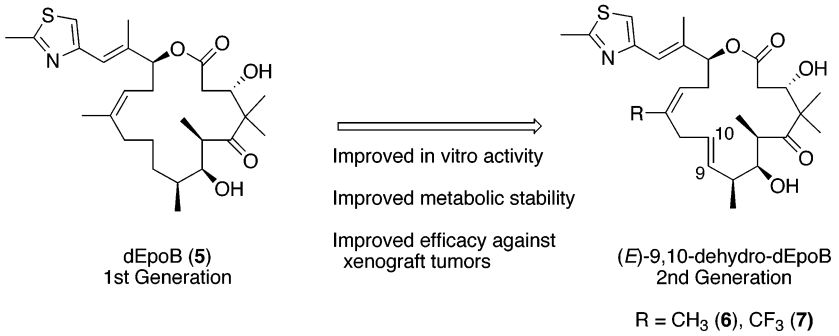

Figure 2. Anticancer drug candidates.

multidrug resistant cells.[4] It was this property, as well as more promising formulatibility, which prompted great interest in epothilones as anticancer drug candidates.[5]

Following extensive multidisciplinary research directed to the biology, chemistry, pharmacology, toxicology, and biosynthesis of the epothilones, three agents, including one from our program (vide infra), have already been advanced to phase I and phase II clinical trials.[6]

Taking advantage of our then pioneering total syntheses of epothilones A and B,[7] preliminary biological studies with selected probe structures suggested that the 12,13-oxido linkage of the macrolactone is a locus of nontumor selective toxicity.[8] This perception led us to an extended evaluation of 12,13-desoxyEpoB (dEpoB, **5**) and related desoxy congeners (Figure 2). The desoxy congeners indeed seemed to exhibit less nontumor directed toxicity[9] relative to epothilone B and a far broader apparent therapeutic index than either EpoB or the taxoids. The advantages of dEpoB relative to paclitaxel, at the level of nude mouse xenograft models, is particularly dramatic with resistant tumors. dEpoB (**5**) is currently in phase II clinical trials.

More recently our laboratory reported that incorporation of *E*-9,10 unsaturation in the macrolide framework of compound **5** (dEpoB) resulted in a marked increase in potency and in metabolic stability.[10] These properties gave rise to favorable outcomes in xenograft tumor studies with nude mice. From this family, 26-trifluoro-(*E*)-9,10-dehydroepothilone (**7**) has emerged as a most promising candidate for drug development.[10b] Herein we provide a full account of the novel synthetic chemistry which led to the discovery of compound **7** and its striking performance with human cancers in murine models.

Our epothilone program has been directed to two intertwined goals. The first was the study of the structure−activity relationship of carefully crafted analogues with enhanced biological properties. The second goal was the exploration of various strategies to develop a practical total synthesis of drug candidates. Our laboratory does not have access to epothilones of natural origin. Only through total synthesis could we enter into a sustainable discovery and development program. Moreover, we hoped to evaluate structures of either enhanced or diminished complexity which could not readily be reached from naturally occurring epothilones. Hence, the total synthesis we practiced should be adaptable to gaining access to chemical "space" not accessible from naturally occurring epothilones.

Our particular interest in synthesizing and examining epothilones with additional nuclear unsaturation, was first prompted by epothilone 490 (**12**, (*E*)-10,11-dehydro-dEpoB). Epothilone 490, a recently isolated natural product, which

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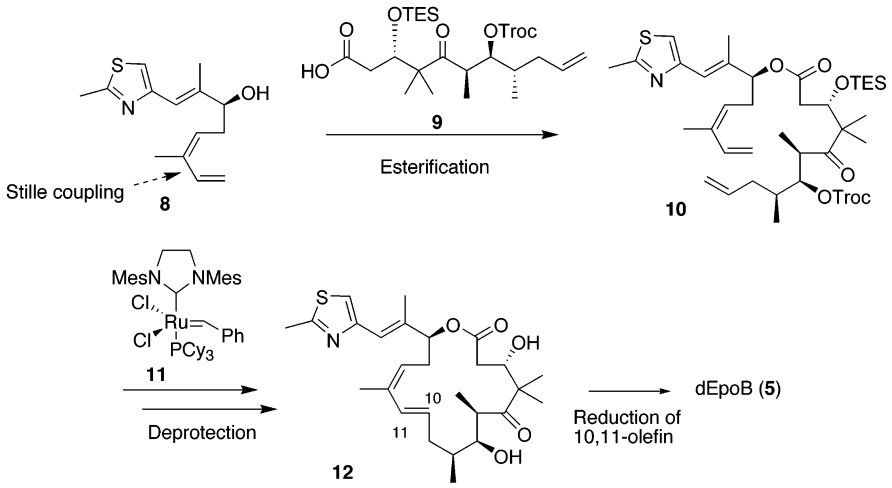
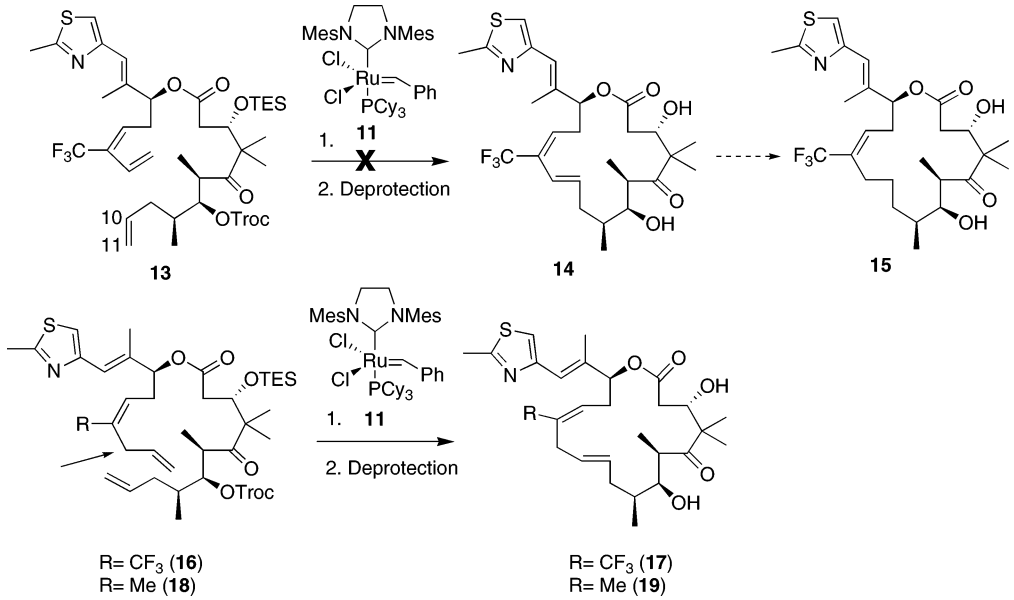
Scheme 1. Synthesis of Epothilone 490 via a Ring-Closing Metathesis



Scheme 2. Synthesis of 17-Membered Epothilones via a Ring-Closing Metathesis



showed highly favorable cell culture cytotoxicity profiles, is available only in small quantities from fermentation.[11] Our previous synthesis of dEpoB could be redirected to yield epothilone 490 by a novel RCM strategy (see progression of **8** → **10** → **12** (Scheme 1)). Remarkably the 10,11-olefin constructed through RCM emerged cleanly in the *E*-configuration. Epothilone 490 (**12**) was then converted to dEpoB (**5**) by regioselective diimide reduction of the *E*-10,11-olefin. Unfortunately, the in vivo performance of epothilone 490, in xenografts, turned out to be surprisingly poor. This difficulty was soon traced to unfavorable pharmacokinetic features of the compound.

Though epothilone 490 (**12**) was no longer a development candidate in our program, we postulated that the synthetic route we had demonstrated for its synthesis could be applied toward the synthesis 26-trifluoro-epo490 (**14**) or, indeed, trifluoro-dEpoB (**15**).[12] Accordingly, we synthesized RCM substrate **13** and tested the proposed ring-closing metathesis reaction. Suprisingly, at the time, a range of conditions which had been used for the ring-closing metathesis reactions of the parent methyl substrate **10** failed to provide the desired **14**.[13] Clearly the nonimplementability of the RCM reaction in the context of compound **13** was due to the presence of the fluorines at the 26-position.

To probe the scope of the deleterious effect of the trifluoromethyl group on the RCM reaction, we inserted a one carbon "spacer" between this group and olefin linkage. Indeed, the ring-closing metathesis of **16** provided the desired 17-membered epothilone macrolide **17** in 57% yield, again exclusively in the *E*-10,11 configuration.[14] This was for us a telling experiment. It suggested that whatever was the precise reason by which the CF_3 group at C12 abrogates the proposed RCM in the case of **13**, it could be countered by inclusion of appropriate spacers.

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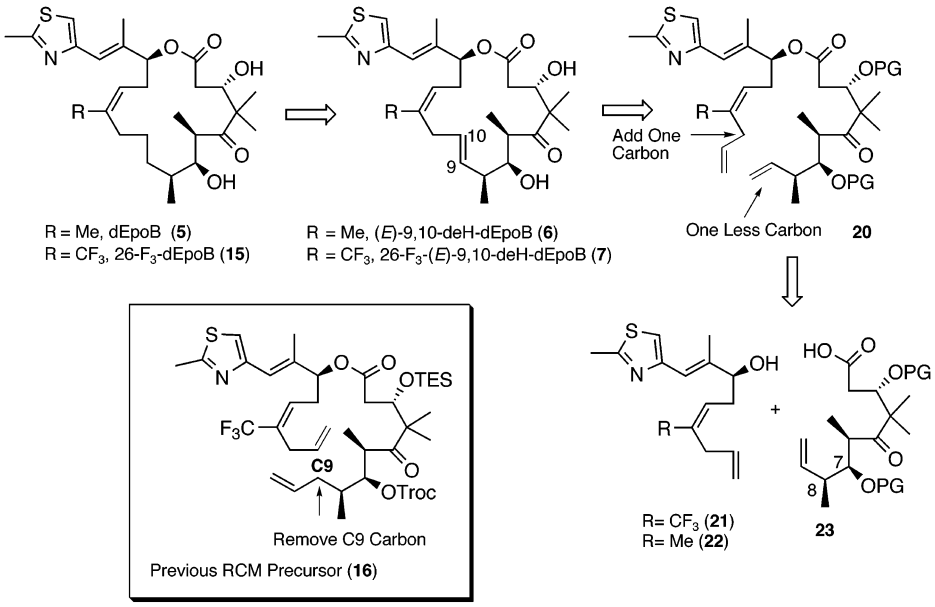

Figure 3. Retrosynthesis of the (*E*)-9,10-dehydroepothilones and 26-trifluoro-dEpoB.

To gain a quick assessment of the effect of the C12-trifluoromethyl group on the likely biological profile of the epothilones, we prepared the 17-membered epothilone macrolide **19** in high yield using again the stereoselective RCM reaction to produce, exclusively, the *E*-9,10-olefin (Scheme 2).

In vitro and mouse plasma stability studies of compounds of **17** and **19** revealed some interesting facts. The first was that the introduction of the three fluorine atoms at the 26-position had improved the plasma stability of the drug 2-fold. The second teaching was that both the 17-membered lactones **17** and **19** had retained their cytotoxicity due to the presence of the *E*-9,-10-olefin. This was a significant and suggestive finding, since the 17-membered epothilone without the 9,10-olefin is virtually inactive.[15]

We reasoned that the synthesis of 26-trifluoro-dEpoB (**15**) containing the usual 16-membered ring could be accomplished via a highly convergent strategy, related to that employed in the synthesis of 27-trifluoro-[17]ddEpoB (**17**). This strategy envisioned the formation of a *E*-9,10-olefin via a ring-closing metathesis reaction (Figure 3).[16] We anticipated that chemoselective reduction of the *E*-9,10-olefin of **6** and **7** would furnish dEpoB (**5**) and the desired 26-trifluoro-dEpoB (**15**). The RCM precursor would be prepared by the union of the two fragments (**21** or **22**) and **23** through an esterification reaction. Coupling partner **23** would be constructed by deletion of the methylene spacer group (found in our earlier routes cf. compound **16**) between the secondary methyl groups at C8. As noted above, the in vitro level findings with the 17-membered epothilones containing the skipped diene arrangement (see **17** and **19**) underscored the need for a corresponding investigation of the biological consequences of such a diene in the familiar 16-membered lactone setting. Given the presence of the cis 12,13-olefin in a dEpoB context, such a skipped diene would necessarily contain a 9,10-double bond.

Alkylation of the oxazolidinone **24**[17] with the readily synthesized trifluoro and methyl allyl iodides allowed for the C15 stereocenter to be set in the appropriate absolute configuration[18] with high diastereomeric access (see products **25** and **26** in Scheme 3). The latter were converted to their corresponding Weinreb amides[19] and thence to **27** and **28** en route to **21** and **22** by nucleophilic methylation (MeMgBr) and appropriate Horner−Wittig olefinations.[20] While the use of such oxazolidinones as chiral auxiliaries had been pioneered by Evans and associates,[18] its application to the synthesis of optically defined glycolates by alkylation (rather than by hydroxylation) had not been developed.

The synthesis of the polypropionate fragment **34** was enabled by two critical aldol reactions, which established the relative configuration of the C3, C6, and C7 stereocenters (Scheme 4). The first aldol reaction involved reaction of the Z-enolate of the ethyl ketone **29** with Roche aldehyde **30**[21] to provide the desired **31** with high diastereoselectivity. Recourse to **30**, allowed by this synthesis, is a significant advantage over the use of earlier aldehydes which required resolution for the attainment of enantiomerically pure starting materials.

Protection of the C7-alcohol followed by hydrolysis of the acetal provided the desired aldehyde **32** and set the stage for the second aldol reaction. Reaction of **32** with Duthaler's DAG (diacetone glucose) Ti-enolate[22] afforded the desired hydroxy

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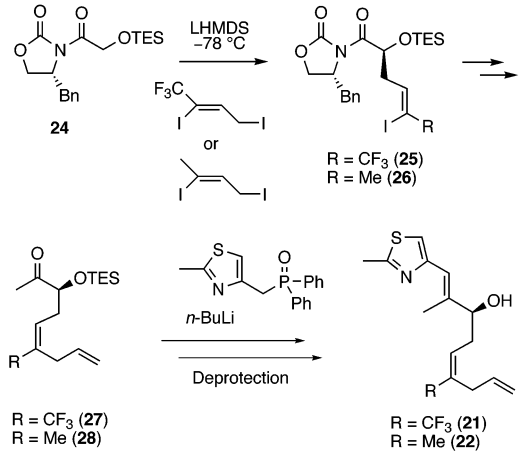
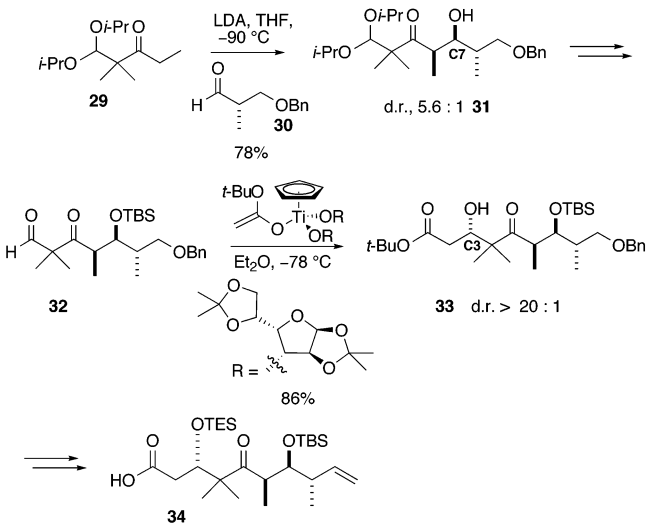
Scheme 3. Synthesis of Fragments **21** and **22**



Scheme 4. Synthesis of Acid **34**



tert butyl ester **33** with very high (>95%) diastereoselectivity. The latter was then converted to the desired acid **34** in straightforward steps.

The allylic alcohols **21**, **22**, and the C_1-C_9 acid fragment **34** were united through an EDCI esterification protocol, thus providing the RCM precursors **36** and **37**, respectively (Scheme 5). Ring-closing metathesis reactions of **36** and **37** were carried out using catalyst **11**[23] in toluene. These reactions did indeed provide the trans isomers **39** and **41**. However, the major products were **40** and **42** which had arisen from the obvious alternate RCM pathway. These unwanted RCM isomers predominated over the desired **39** and **41** by ratios of ca. 3:1. Finally, deprotection of the silyl ethers of **39** and **41** with HF-pyridine led to the desired (*E*)-9,10-dehydroepothilones **6**[24] and **7**. As planned, the latter were converted to dEpoB (**5**) and 26-trifluoro-dEpoB (**15**) via diimide reduction of the *E*-9,10-olefin.[25]

By corresponding methodology, we synthesized the (*E*)-9,-10-dehydro-dEpoF (**45**) (see Scheme 5). The selective diimide reductions of the (*E*)-9,10-olefins validated the structures of the various synthetic intermediates described above, thereby prompting re-evaluation of previous assignments in the literature.[24]

Examination of synthetic analogues (**6**, **7**, and **45**), in cell culture settings (see Supporting Information), revealed them to exert increased potency on various sensitive and MDR tumor cell lines that are exhibited by our current clinical candidate, dEpoB (**5**). The impressive cell growth inhibition exhibited by epothilones **6**, **7**, and **45** across a range of various drug-resistant tumors prompted determination of the blood plasma stability of these new (*E*)-9,10 congeners. We recall that epothilone 490 (cf. compound **12** with a methyl group at C-12) exhibits very poor plasma stability. Indeed, it was this plasma instability which had blocked further development of **12**. By contrast, on exposure of **6**, **7**, and **45** to murine plasma, we observed a much slower drug degradation as compared to dEpoB (**5**) (by a factor of ca. 7). This stability constitutes a substantial advance, from a drug availability perspective, relative to dEpoB (**5**), not to speak of epothilone 490 (**12**). Based on the preliminary cell culture and pharmacokinetic data of the (*E*)-9,10-dehydro derivatives **6** and **7**, it would be appropriate to advance them for in vivo investigations. Such studies are, of course, rather more sensitive to drug availability than in vitro measurements.[10]

This requirement, and indeed the possible eventual need to prepare multigram quantities of 9,10-dehdyro derivatives for further development, prompted a significant reassessment of our total synthesis route. Of course, the single most serious problem was that the RCM reaction on **36**−**38** produced **39**, **41**, and **43** only as minor products. The major pathway involved an RCM reaction which was strictly confined to the *O*-alkyl sector of **36**−**38**, leading primarily to the undesired **40**, **42**, and **44**. Accordingly, it was decided to attempt to defer introduction of the thiazole (by olefination) after the RCM (vide infra).

With eventual processiblity in multigram scales as our goal, the syntheses of the alkyl and acyl fragments entering into the RCM reaction were restructured (Scheme 6). Compound **48** was readily synthesized as shown. It was used to alkylate oxazolidinone **24** in high diastereomeric excess. Following deprotection of the OTES group and nucleophilic methylation, compound **50** was in hand. This α-hydroxyketone would serve as the acyl group acceptor in formation of the central ester to prepare all the critical RCM precursors. An obvious concern was the possible vulnerability of such a hydroxyketone as an acyl acceptor to partial racemization or diversion to regioisomeric α-ketols.

A serious problem in our earlier synthesis of the acid fragment **34** was the very expensive and technically demanding Duthaler chemistry[22] to generate the desired *S* stereochemistry at the C3. To circumvent this problem, the aldol reaction was carried out without any chiral auxiliary to provide a 1:1 mixture of the corresponding *β*-hydroxy ketone (Scheme 7). Reagent-controlled

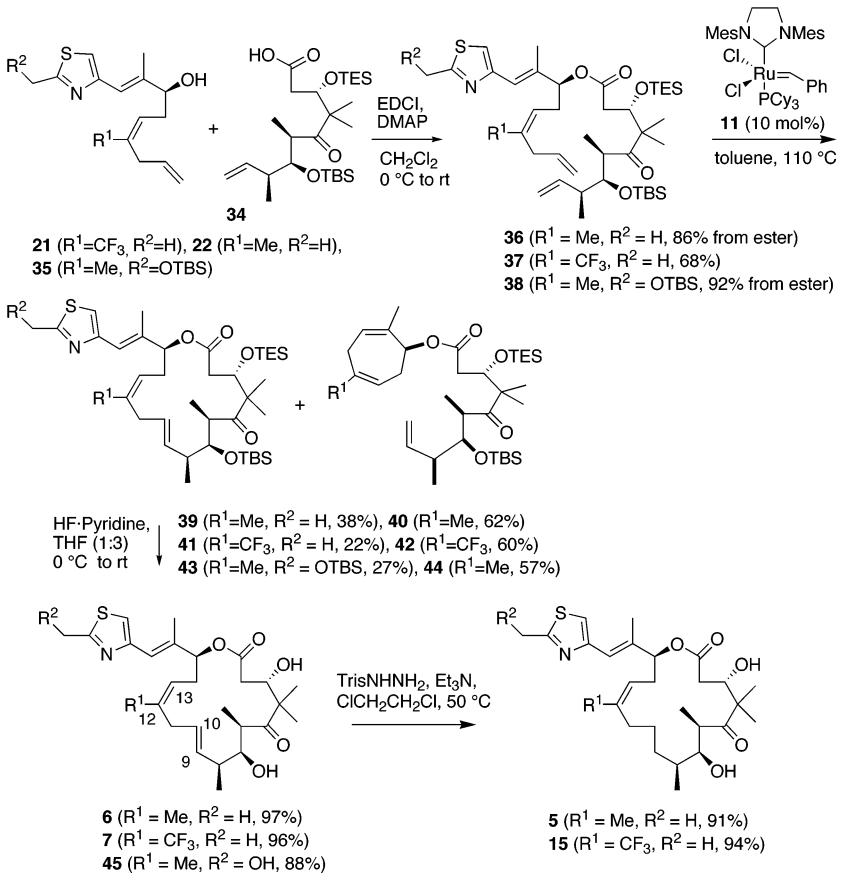
(22) Duthaler, R. O.; Herold, P.; Lottenbach, W.; Oretle, K.; Reidiker, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 495−497.

(23) Initial report: (a) Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247−2250. RCM reviews: (b) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446−452. (c) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18−29. (d) *Alkene Metathesis in Organic Chemistry*; Fürstner, A., Ed.; Springer: Berlin, 1998. (e) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012−3043. (f) Schrock, R. R. *Top. Organomet. Chem.* **1998**, *1*, 1−36.

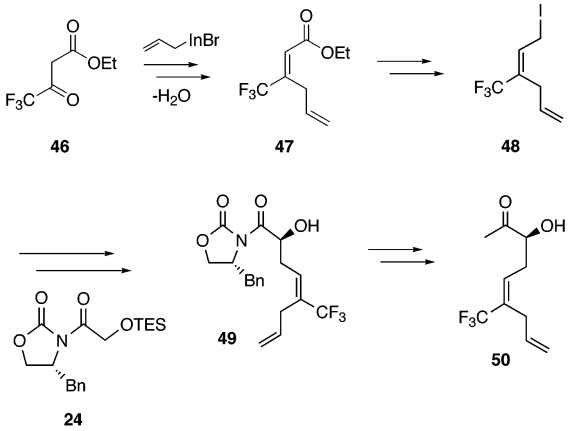
(24) (a) White, J. D.; Carter, R. G.; Sundermann, K. F.; Wartmann, M. *J. Am. Chem. Soc.* **2001**, *123*, 5407−5413. (b) White, J. D.; Carter, R. G.; Sundermann, K. F.; Wartmann, M. *J. Am. Chem. Soc.* (Addition/Correction) **2003**, *125*, 3190−3190. With compound **6** of rigorously proven structure in hand, we were surprised to find that its spectral properties were not congruent with those previously reported (see ref 24a) for a compound presumed to be the same entity. The actual structure of the compound previously assigned as **6** has now been re-evaluated (see ref 24b). In retrospect, it is clear that **6** had not been previously prepared and, in fact, the whole family of (*E*)-9,10-dehydroepothilones reported here is a new genus.

(25) (a) Corey, E. J.; Mock, W. L.; Pasto, D. J. *Tetrahedron Lett.* **1961**, *2*, 347−352. (b) Pasto, D. J.; Taylor, R. T. *Org. React.* **1991**, *40*, 91−155.

Scheme 5. Synthesis of (*E*)-9,10-Dehydroepothilones



Scheme 6. Processible Synthesis of Fragment **50**



asymmetric reduction of the derived keto function (see compound **52**) using Noyori conditions[26] generated the desired S stereochemistry at the C3 in high diastereomeric excess. The now available β-hydroxy ester **53** was transformed to acid **34** in several steps following earlier protocols.[10a]

Remarkably, esterification of the resultant hydroxyketones **50** and **54** with the C_1-C_9 acid fragment **34** provided the corresponding RCM cyclization precursors **55** and **56** (Scheme 8) without noticeable racemization at C15, or loss of integrity of the initial α-ketol linkage. The ring-closing metathesis reaction of **55** and **56** was carried out using catalyst **11**[23] in toluene. This reaction, now uncomplicated by alternate metathesis pathways, provided exclusively the trans isomers **57** and **58** in high yields. Fortunately, installation of the thiazole moiety via a Wittig reaction proceeded with high *E*/*Z* selectivity and yield to provide **6** and **7** following deprotection of the two silyl ethers.[27]

We considered whether the incorporation of C9−C10 olefin in epothilone B (**2**, EpoB) would alter its biological profile in the same direction as was the case with its 12,13-desoxy counterparts. Toward this end, we studied the epoxidation of **6** with 2,2′-dimethydioxirane (DMDO). The reaction indeed proceeded with high chemoselectivity at the more substituted C12−C13 olefin. There was obtained an 87% yield of a 1:2.6 ratio of the (*E*)-9,10-dehydroepothilone B (**59**) and its diastereomer bearing the α-12,13-oxirane (structure not shown).[28] In vitro studies with **59** (whose configurations at C12 and C13 was established by its reduction to afford Epo B) revealed it to be roughly 2−4-fold more potent than the parent EpoB (**2**) in various cell lines. While compound **59** proved to be the most potent epothilone we encountered in our program, its narrow therapeutic index in xenografts, as well as its difficult accessibility (vide supra), served to reduce its priority for the preclinical development. Interestingly, the unnatural α-oxirane possessed a substantially lower in vitro activity.

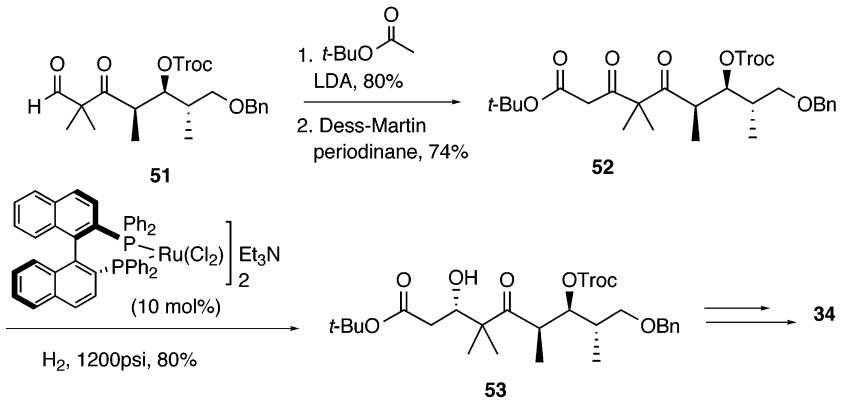
Another advantage of the restructured (second generation) synthesis described above is that a variety of heterocycles can

(26) Noyori, R.; Ohkuma, T.; Kitamura, M.; Takaya, H.; Sayo, N.; Kumobayashi, H.; Akutagawa, S. *J. Am. Chem. Soc.* **1987**, *109*, 5856−5858.

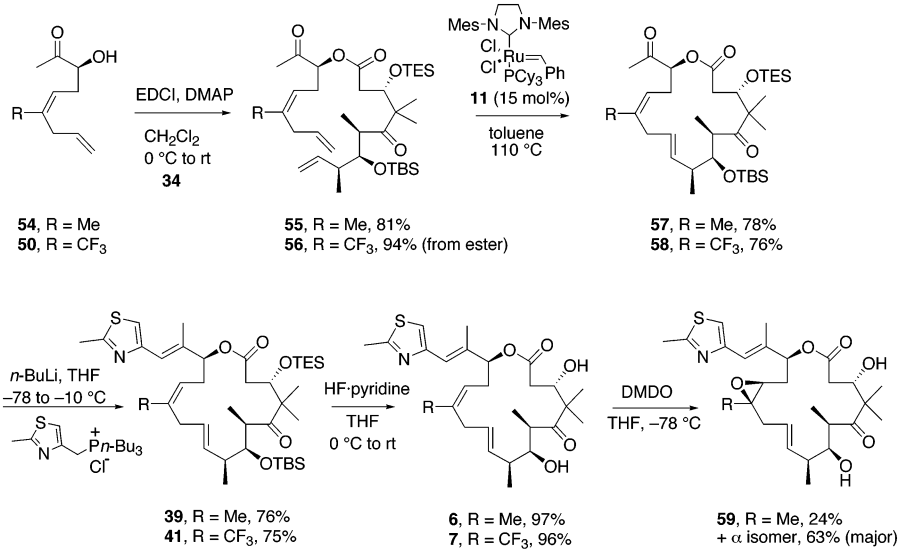
(27) Hindupur, R. M.; Panicker, B.; Valluri, M.; Avery, M. A. *Tetrahedron Lett.* **2000**, *42*, 7341−7344. Attempts to apply Avery's protocol for the installation of the thiazole gave the desired product in low yield and with poor *E*/*Z* selectivity.

(28) Stachel, S. J.; Danishefsky, S. J. *Tetrahedron Lett.* **2001**, *42*, 6785−6787.

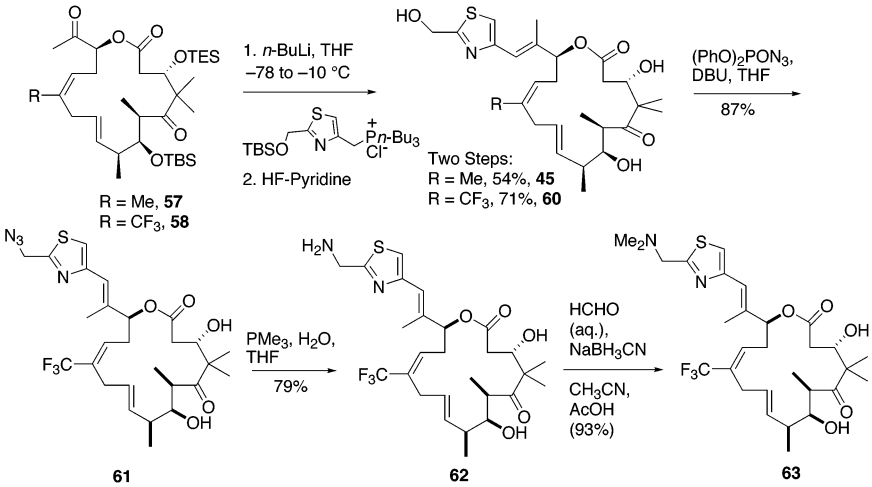
Scheme 7. Processible Synthesis of Acid **34**



Scheme 8. Processible Synthesis of (*E*)-9,10-Dehydroepothilones



Scheme 9. Diversification of C21 of (*E*)-9,10-Dehydroepothilones



be installed via ketone intermediates **57** and **58**. This point is well highlighted by the synthesis of (*E*)-9,10-dehydro-dEpoF (Scheme 9). Wittig reaction of the ketone with the appropriate phosphonium ylides afforded the desired (*E*)-9,10-dehydro-dEpoF compounds **45** and **60** in high yield and with high *E*/*Z* selectivity. Furthermore, we were able to efficiently convert the 21-hydroxy **60** to derivatives of the type **62** and **63**, containing amino functionality at C21 in several steps as shown in Scheme 9.

The fully synthetic epothilone analogues have been evaluated against a variety of cell types to determine their antitumor potential. As shown in tabular form in the Supporting Information, all of the compounds exhibited high cytotoxic activity against a variety of sensitive and resistant tumor cell lines.[29]

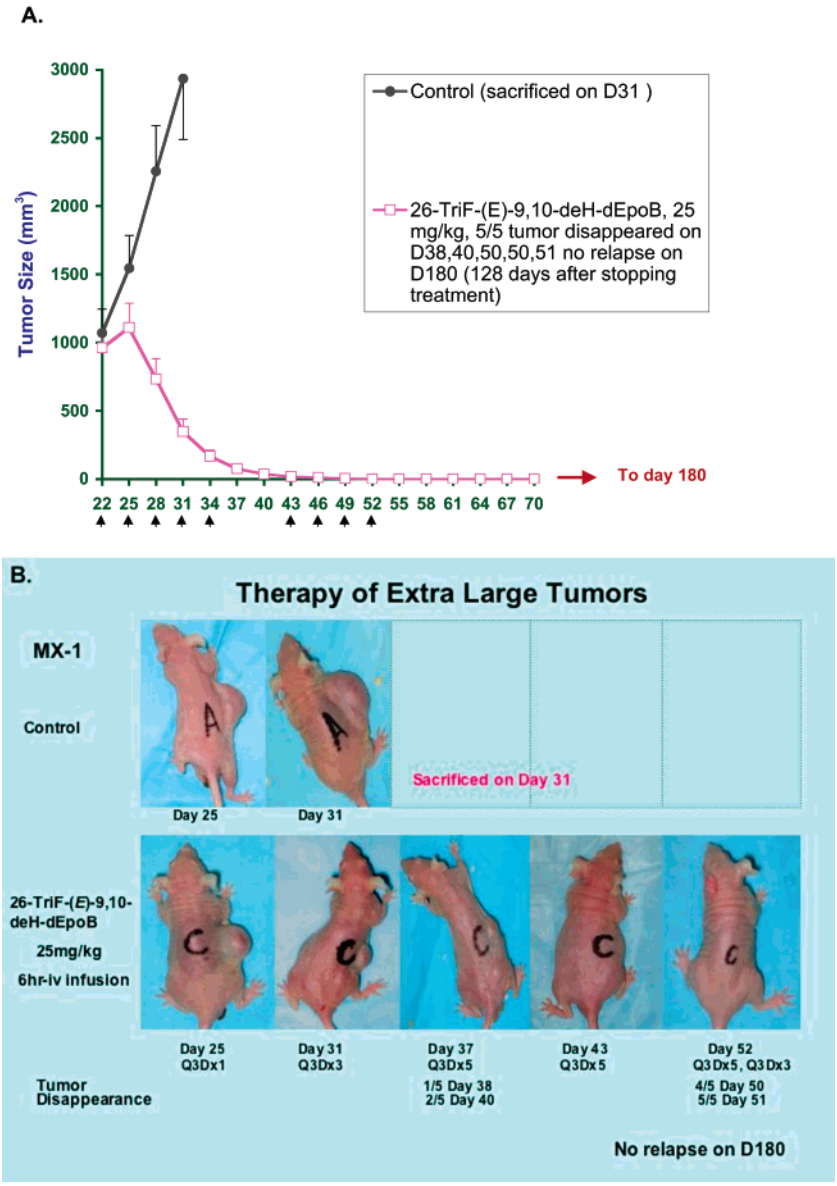


Figure 4. Therapy of extra large MX-1 tumor xenograft. MX-1 tumor tissue (50 mg) was implanted sc on day 0. On day 22 (D22) when tumor size reached 960 ± 132 mg (about 3.4% of body weight), Fludelone 25 mg/kg, 6 hr iv infusion, Q3Dx5 was given on D22, D25, D28, D31, and D34 as indicated by arrows. The second cycle of treatment, following 9 day rest, was given on D43, D46, D49, and D52. (a) Tumor size changes in the vehicle treated control (●) and Fludelone treated group (□) ($n = 5$ each). Observation was continued Q3D up to D180 (128 days following cessation of treatment on D52). b. Photographs for the nude mice (one mouse each selected from the control group and the treated group) taken on D25, D31, D37, D43, and D52. No relapse was observed on D180.

The most salient features of our findings are as follow: One can expect a loss of ca. an order of magnitude in replacement of the C12−C13 β-epoxide by an *E*-12,13 double bond (compare EpoB and dEpoB in the sensitive CCRF-CEM cell line). Another expectation, is that inclusion of an *E*-9,10 double bond in addition to the *Z*-12,13-olefin leads to a significant increase in cytotoxicity across several cell lines.

Still another instructive trend was seen in comparing 26-trifluoro-(*E*)-9,10-dehydro-dEpoB (**7**) (Fludelone) with the corresponding (*E*)-9,10-dehydro compound **6**. Inclusion of the three fluorine atoms at C26 attenuates cytotoxicity of **7** by up to a factor of 5 relative to **6** in CCRF-CEM leukemic cells. This attenuation effect of the 12-trifluoromethyl function is also seen in compounds lacking the 9,10-unsaturation (compare dEpoB (**5**) and 26-trifluoro-dEpoB (**15**)).

Given these data, and given the accessibility of these 9,10-dehydro compounds (including 12-trifluoromethyl congeners) through chemical synthesis, we were in a position to initiate in vivo experiments on our most promising compounds. We describe here some particularly striking and promising results with compound **7** (Fludelone), which has emerged as a most exciting possibility for advancement to clinical evaluation. In vivo experiments were carried out using human tumor xenografts in immunodeficient nude mice. For all their imperfections, such models in oncology are widely used in evaluating[30] the potential of antitumor lead compounds en route to clinical development.

(29) See ref 9a−c for the preliminary therapeutic evaluations of (*E*-9,10-dehydroepothilones. A comprehensive account of the therapeutic evaluations of the (*E*)-9,10-dehydroepothilones will be reported in due course.

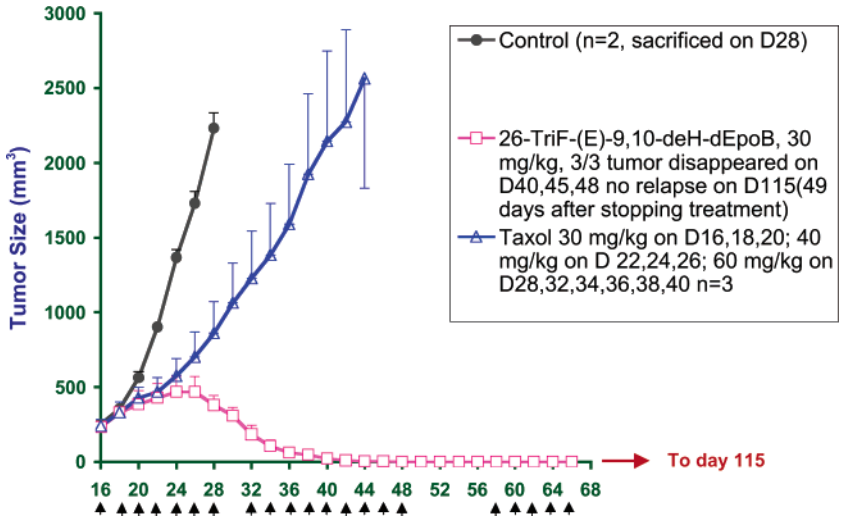


Figure 5. Therapeutic effects against human mammary carcinoma MX-1 xenograft by Fludelone or paclitaxel (Taxol) via oral administration. Female nude mice were used. Fludelone 30 mg/kg (□) ($n = 3$) was given orally Q2Dx7 beginning D16 after tumor implantation and then Q2Dx9 on D32−48, as indicated by arrows. All three mice's tumor disappeared on D40, 45, and 48. For consolidation therapy, the third cycle of treatment was given Q2Dx5 from D58−66 when all mice were tumor free on D48. There was no relapse on D115 (49 days after stopping treatment). Control (●) ($n = 2$) received vehicle only. Parallel comparative experiment was carried out with Paclitaxel 30 mg/kg (△) ($n = 3$), orally beginning D16, Q2Dx3 and then the dose was increased to 40 mg/kg, Q2Dx3 (D22−26) and then to 60 mg/kg, Q2Dx3 (D28−40).

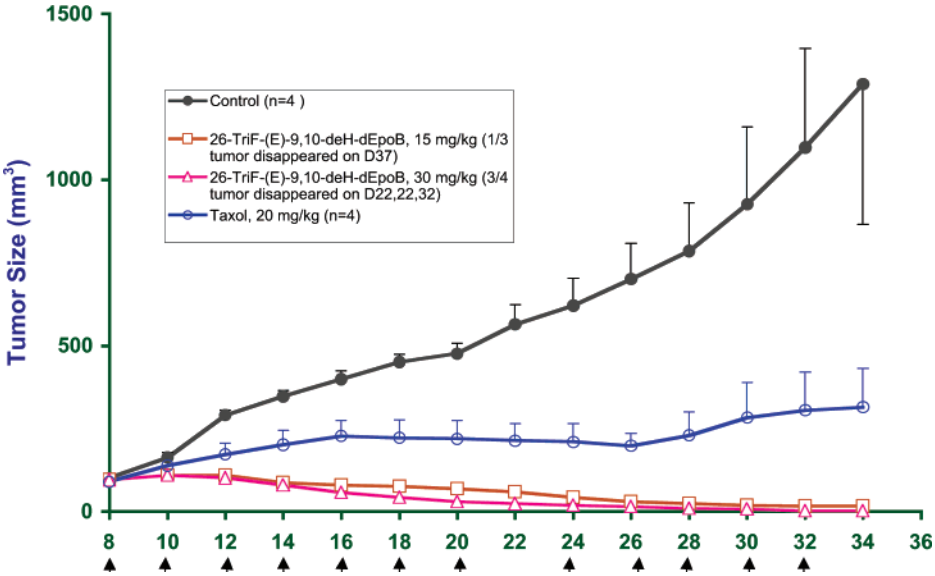


Figure 6. Therapeutic effects against the drug resistant human T-cell lymphoblastic leukemia CCRF-CEM/Paclitaxel xenograft by Fludelone and Paclitaxel. Tumor tissue of CCRF-CEM/Paclitaxel (44-fold resistant in vitro), 50 mg/mouse was implanted sc into nude mice on day 0. Treatment of 6 hr iv infusion started on D8 with Fludelone 15 mg/kg (○) ($n = 3$) and 30 mg/kg (△) ($n = 4$) or Paclitaxel 20 mg/kg (○) ($n = 4$). Q2Dx7 (D8-D20) skipped D22 dose and then resumed treatment Q2Dx5 on D24, 26, 28, 30, and 32, as indicated by arrows. The control group (○) ($n = 4$) received vehicle only. For Fludelone at 15 mg/kg, $^1/_3$ of mice's tumor disappeared on D37, and at 30 mg/kg, $^3/_4$ of mice's tumor disappeared on D22 and 32).

Remarkably, treatment of MX-1 xenografts with 25 mg/kg dosages of Fludelone resulted in complete tumor disappearance and the absence of any relapse for over 4 months after suspension of treatment (See Figure 4). Most importantly, these therapeutic successes can be achieved either by 6 h iv infusion or by *oral* administration (See Figure 5). On the other hand, treatment of the MX-1 xenografts by oral administration of paclitaxel did not shrink the tumor (see Figure 5). It goes without saying that if this was translatable to the human clinical setting, achievement of oral activity could be of significant advantage.

Paclitaxel-resistant tumor xenografts (Figure 6) as well as human colon carcinoma (HCT-116, Figure 7) can also be cured with Fludelone by iv infusion. The experiments using human mammary carcinoma (MX-1) and human colon carcinoma (HCT-116) xenografts in nude mice lasted 6.0 and 6.6 months, respectively. There was no tumor relapse in either experiment during 4.3 and 5.3 months, respectively, following the cessation of treatment. For the HCT-116 experiment, paclitaxel and Fludelone were compared at 20 mg/kg and both achieved tumor disappearance. The paclitaxel-treated group relapsed 1.1 months

(30) For reports that suggest correlations between xenograft data and clinical activity are good, see ref 30a. For an opposite view, see ref 30b. (a) Fiebig, H. H.; Berger, D. P. Preclinical Phase II trials. In *The Nude Mouse in Oncology Research*; Boven, E., Winograd, B., Eds.; CRC Press: Boca Raton, FL, 1995; p 318. (b) Johnson, J. I.; Decker, S.; Zaharevitz, D. *Br. J. Cancer* **2001**, *84*, 1424−1431.

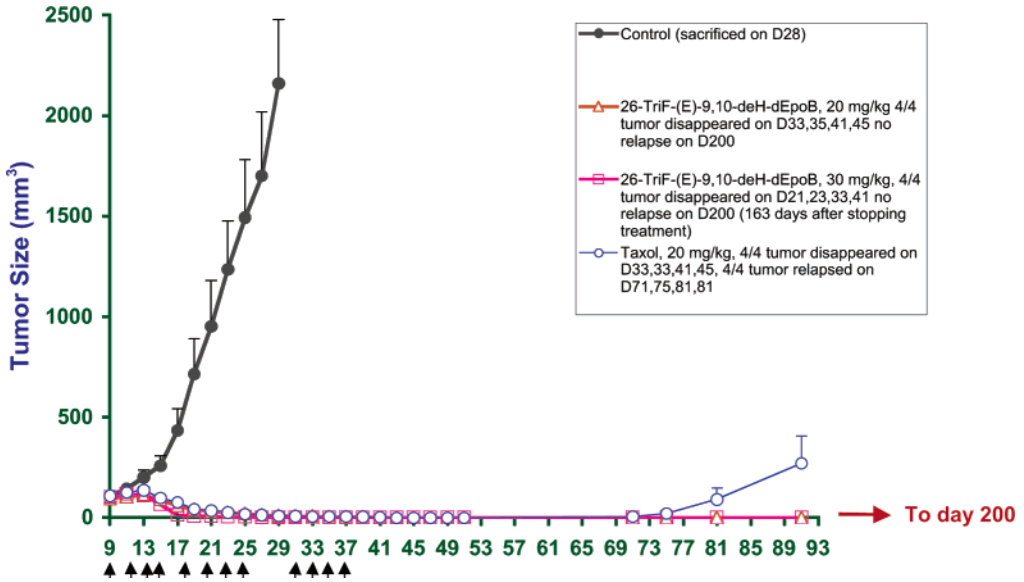


Figure 7. Therapeutic effects against human colon carcinoma HCT-116 xenograft by Fludelone and Paclitaxel. HCT-116 tumor tissue 50 mg/mouse was implanted sc into nude mice on day 0. Treatment of Q2Dx4, 6 hr iv fusion for three cycles was carried out on (D9, 11, 13, 15), (D19, 21, 23, 25), and (D31, 34, 35, 37) with Fludelone 20 mg/kg (△), 30 mg/kg (□), and Paclitaxel 20 mg/kg (○) ($n = 4$ each). Complete tumor disappearance in all mice occurred on (D33, 35, 41, 45), (D21, 23, 33, 41), and (D33, 33, 41, 45) for Fludelone 20 mg/kg, 30 mg/kg and Paclitaxel 20 mg/kg, respectively. There was no tumor relapse for both of the Fludelone treated groups on D200. However, the Paclitaxel treated group (○) suffered relapses on D71, 75, and 81 which represent the relapses on the 34th, 38th, and 41st day after stopping treatment.

after treatment was discontinued, whereas Fludelone-treated animals were tumor free for over 5.3 months.

These results have involved a particularly long and thorough therapeutic study using xenografts and report remarkably long periods of complete remission with parenteral or oral administration of a single antitumor agent. Achievement of complete tumor disappearance and long term remission by oral treatment could well be of particularly great clinical significance if translatable to the human setting.

Conclusion

Only advancement to a human clinical setting will establish whether Fludelone will become a valuable resource for oncologists in the treatment of patients. That this compound was discovered is a consequence of a close linkage between the disciplines of organic chemistry, pharmacokinetics, and cancer pharmacology. That a development program on Fludelone can now be undertaken realistically speaks particularly well as to the power of chemical synthesis. Parenthetically, we note that the manner in which the (*E*)-9,10-dehydroepothilones were discovered underscores the value of natural product leads in drug discovery and the potential applicability of diverted total synthesis,[31] even in multistep settings. Extensive research into the (*E*)-9,10-dehydroepothilone B family, in the context of chemistry, biology and oncology, continues.

Acknowledgment. This research was supported by the National Institutes of Health (Grant Numbers: CA-28824 (S.J.D.), CA-08748 (T.C.C.). Postdoctoral fellowship support is gratefully acknowledged be A.R. (NIH Cancer Pharmacology Training Grant, T32-CA62948), F.Y. (Uehara Memorial Foundation), and A.E.G. (Goodwin Fellow, Therapeutics Center of Memorial Sloan-Kettering Cancer Center). The authors wish to thank Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute, and CA-08748) for mass spectral and NMR analysis.

Supporting Information Available: Experimental details for preparation and spectral characteristics ofselected compounds and in vitro cytotoxicity data in tabular form. This material is available free of charge via the Internet at http://pubs.acs.org.

JA046992G

(31) cf. Njardarson, J. T.; Gaul, C.; Shan, D.; Huang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 1038−1040.