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Having accomplished total syntheses of epoithilone A and B, we have been engaged in a new stage of investigation that involves development of efficient strategies for a large scale epothilone preparation and search for more potent analogues. Efficient and processable syntheses of key building blocks of 12,13-desoxyepothilone B (dEpoB) by catalytic asymmetric induction has been achieved. dEpoB is a potent anticancer agent, showing a highly promising therapeutic potential in the currently undergoing phase I study. The syntheses of two epothilone analogues, 15(S)-aza-12,13-desoxyepothilone B and the epimeric 15(R)-aza-12,13-desoxyepothilone B have been accomplished. Tubulin binding and cytotoxicity profiles of these analogues have also been investigated. Another epothilone, 12,13-desoxyepothilone F (dEpoF), was synthesized and evaluated for antitumor potential. The results from an *in vitro* assay reveal that this new analogue is highly active against various tumor cell lines with a potency comparable to that of dEpoB. In particular, the growth of resistant tumor cells is inhibited by dEpoF at concentrations where paclitaxel (Taxol®) is basically ineffective. A preliminary assessment of its *in vivo* activity is also promising. The new analogue, containing an additional hydroxyl group at C21, provides advantages over other epothilones in terms of water solubility and can serve as a readily functionalizable handle to produce other useful compounds for pertinent biological studies.

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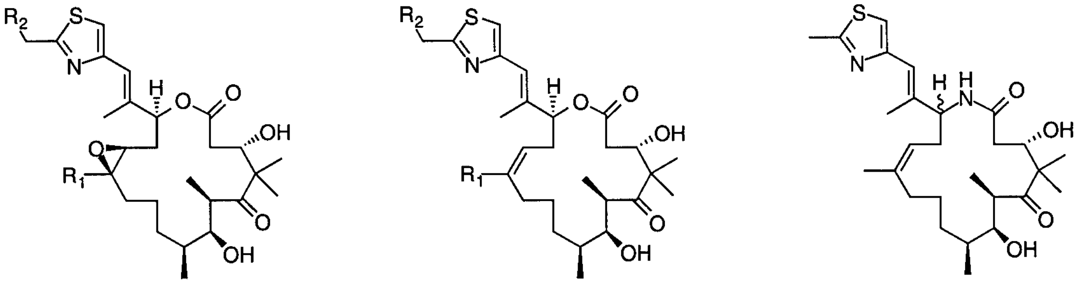
Introduction

The epothilones are a family of naturally occurring cytotoxic macrolides that were isolated from the mycobacterium *Sorangium cellulosum.*[1,2] Despite minimal structural homology with the taxoids, the epothilones manifest biological effects similar to those of paclitaxel (Taxol®) on microtubule and cultured cells.[3] While these two classes of compounds share the same mode of action, the epothilones retain remarkable potency against multidrug resistant tumor cells.[4,5] They may also offer advantages relative to paclitaxel in terms of formulatabililty. Due to the exciting potential of the epothilones for clinical development, they have attracted considerable attention in cancer research as possible agents for cancer chemotherapy.[6]



1

2

3

1a R_1 = H, R_2 = H, Epothilone A
1b R_1 = CH_3, R_2 = H, Epothilone B
1c R_1 = H, R_2 = OH, Epothilone E
1d R_1 = CH_3, R_2 = OH, Epothilone F

2a R_1 = H, R_2 = H, Epothilone C
2b R_1 = CH_3, R_2 = H, Epothilone D
2c R_1 = H, R_2 = OH, Desoxyepothilone E
2d R_1 = CH_3, R_2 = OH, Desoxyepothilone F

3a α-15, epi-Aza-dEpoB
3b β-15, Aza-dEpoB

Figure 1. Structure of Epothilones and Desoxyepothilones

Given the biological ramifications of the epothilones, they have engendered a great deal of attention from the standpoint of total synthesis. Indeed, several total syntheses of naturally occurring epothilones including the present research in these laboratories have been accomplished.[7-21] Subsequently, syntheses of numerous analogues have served to establish a rather detailed map of the structure-activity relationships (SAR) based on *in vitro* and *in vivo*[22] assays.[6] Recently, extensive *in vivo* experiments demonstrated that the less cytotoxic 12,13-desoxyepothilone B (**2b**, dEpoB) manifests a more promising therapeutic profile than does epothilone B (**1b**, EpoB) itself.[23,24] In our studies in mice, the desoxy compound, dEpoB, was well tolerated and is virtually curative against otherwise resistant xenograft tumors. Although the desoxy derivatives are present as minor components from fermentation, the more biologically significant dEpoB is apparently scarce in that it is a secondary constituent within a family of minor B components from fermentation.

While our previous studies relied entirely on the fully synthetic dEpoB,[25,26] our first goal is to develop a practical total synthesis of dEpoB which can furnish material appropriate for a full scale evaluation in humans if findings in higher animals so indicate. Secondly, we hope to continue our search for analogues that may be more potent and readily accessible. In particular, we would like to ascertain the bioprofiles of nitrogen based desoxyepothilone systems (**3a** and **3b**), in macrolactam rather than macrolactone settings. Another interesting candidates are epothilones E and F, each of which possesses a 21-hydroxyl group.[14,27,28] In spite of the putative role of the thiazole as a key pharmacophore,[29,30] the 21-hydroxyl group does not abrogate activity. Another particularly exciting prospect associated with having a primary hydroxyl group is that of enhanced aqueous solubility, thereby providing major simplifications in issues of formulation. Furthermore, the primary hydroxyl group could be utilized as a staging point for further elaboration. All of these goals require successful syntheses of epothilones and rigorous biological evaluations.

Chemical Syntheses of Epothilone Analogues and Biological Evaluation

1. Practical Synthesis of dEpoB. In our previously described routes to dEpoB,[25,26] three subunits were built and combined (Figure 2). The route to building block **C** has already been shown to be amenable to major scale-up. Building block **B** was prepared by auxiliary-mediated allylation of a suitable propionate derivative. The largest impediment to major scale-up was in the synthesis of the **A** subunit, which had previously required eight steps, exclusive of generating the required reagents. Several of these maneuvers required rather sophisticated and expensive chemistry, and prospects for serious scale-up of these protocols were daunting. Thus, we engaged in new synthetic developments which would render the **A** fragment readily available in multigram quantities, utilizing easily processable chemistry. These advances have major favorable consequences for a plant-level synthesis of dEpoB.

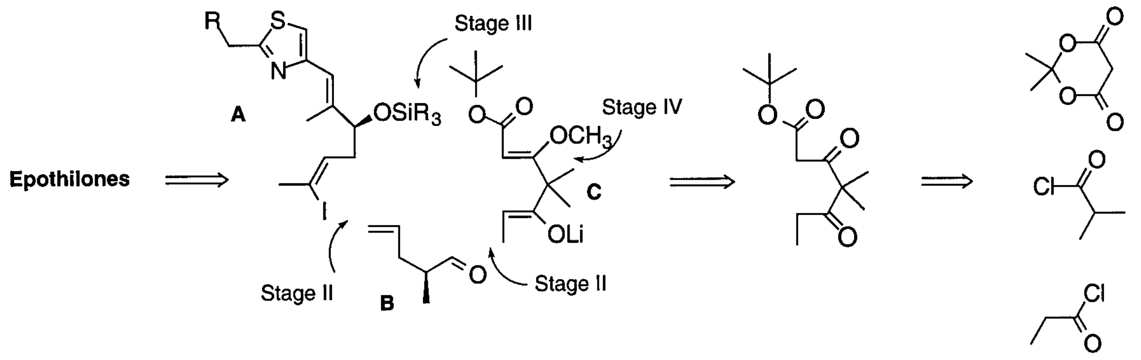
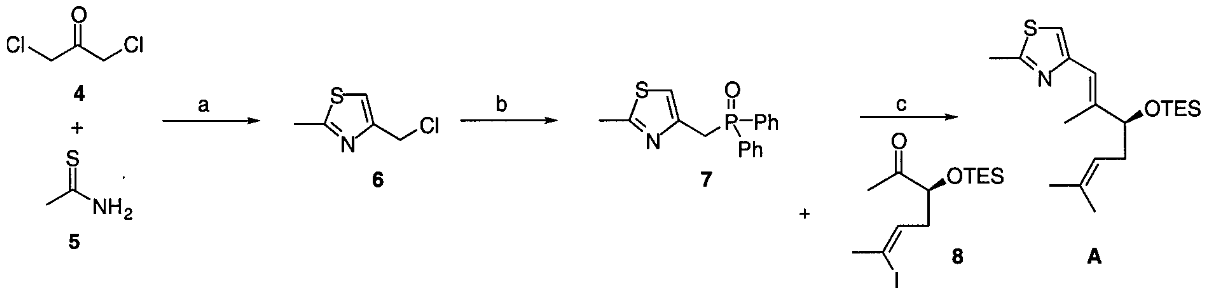


Figure 2. Retrosynthetic Analysis and Epothilone Building Blocks

The new route for the synthesis of the key vinyl iodide fragment required smooth access to large quantities of phosphine oxide **7**. This subunit is easily prepared in two steps on a 100 g scale as shown in Scheme 1. The second subunit required for building the **A** segment is methyl ketone **8**. The condensation between **7** and **8** is conducted in 98% yield on a multigram scale to afford **A**. However, for this chemistry to be valuable, a straightforward synthesis of **8**, amenable to plant-level scale-up, had to be accomplished.

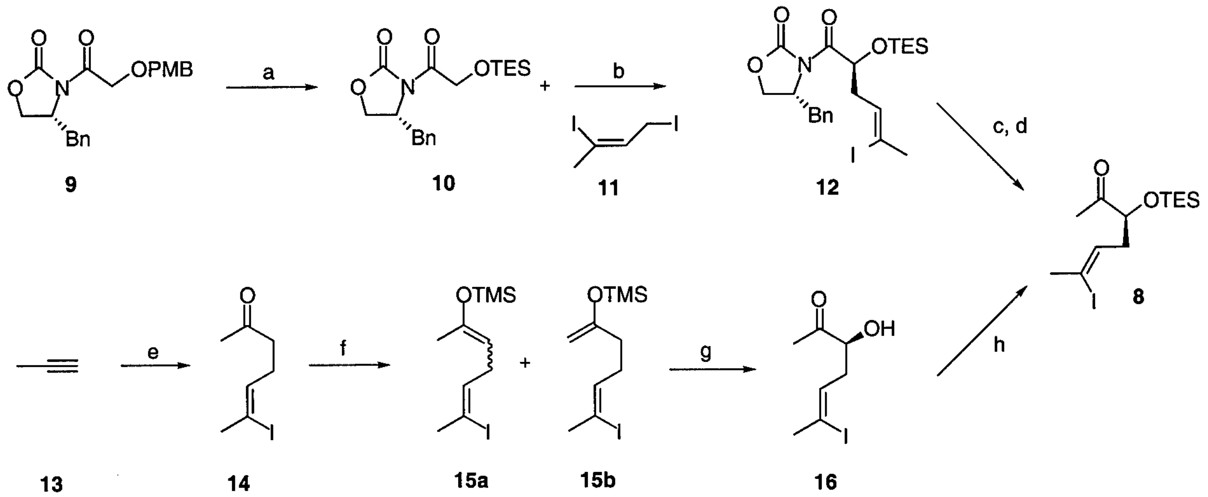
Scheme 1[a]



[a] (a) (i) acetone, (ii) $ZnCl_2$, MeOH, reflux, 60%; (b) $HOPPh_2$, Cs_2CO_3, Bu_4NI, CH_2Cl_2, 97%; (c) (i) n-BuLi. THF, -78 °C, 30 min, (ii) **8**, -78 °C to rt, 98%.

Two such routes have now been developed. In the first approach (Scheme 2) following conversion of **9** to **10,** a highly diastereoselective alkylation of lithio **10** with **11** produces **12.** Diiodide **11** is available from 2-butynol in two steps, and compound **12** was advanced in three steps to **8.** A second route, while somewhat less selective, reaches **8** even more easily, in only four steps via asymmetric dihydroxylation. This synthesis begins with the known reaction of propyne with B-iodo-9-BBN and methyl vinyl ketone to produce **14,** which reacts (in multigram scale) with trimethylsilyl iodide to provide an 88:12 (**15a** : **15b**) mixture of silyl enol ether isomers. Asymmetric dihydroxylation of this material, afforded **16** (87% ee, 55% yield in two steps). Finally, silylation of **16** completes the synthesis of ketone **8** and therefore **A**.

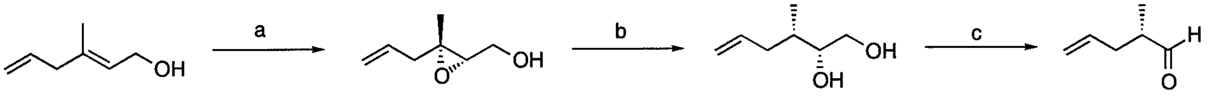
Scheme 2[a]



[a] (a) (i) $TiCl_4$, 0 °C, 87%, (ii) TESCl, imidazole, DMF, 84%; (b) LHMDS, **11**, -78 °C, 81%; (c) (i) HOAc:THF:H_2O (3:1:1), (ii) CH_3ONHCH_3, $AlMe_3$, (iii) TESCl, DMF, 88% overall; (d) MeMgBr, 0 °C, 93%; (e) I-9-BBN, hexanes, (ii) methyl vinyl ketone, hexanes, (iii) 3 N NaOH, PhMe, 100 °C, 65%; (f) TMSI, HMDS, CH_2Cl_2, -20 °C to rt; (g) 1 mol % of OsO_4, AD-mix-, $MeSO_2NH_2$, t-BuOH:H_2O (1:1), 55%-two steps; (h) TESCl, imidazole, DMF, 85%.

With a view to generating fragment **B** by strictly catalytic asymmetric methods, its synthesis was revisited (Scheme 3). For this purpose, we synthesized subunit **17**, which is prepared from isoprene. Asymmetric epoxidation of **17** provides **18**, which undergoes reductive cleavage at the more substituted center to furnish diol **19**. Following periodate cleavage as shown, building block **B** is in hand. While this method bypasses recourse to a chiral auxiliary, its ultimate advantage in terms of scale-up to the multigram levels in a plant-type setting awaits demonstration. As our early synthesis, an aldol condensation joins fragments **B** and **C**. Subsequently, a palladium-mediated B-alkyl Suzuki merger joins **A** with **B-C**. With the carbon skeleton in place, a catalytic Noyori reduction provides the desired stereochemistry at C3 and macrolactonization leads, shortly afterward, to dEpoB.

Scheme 3[a]

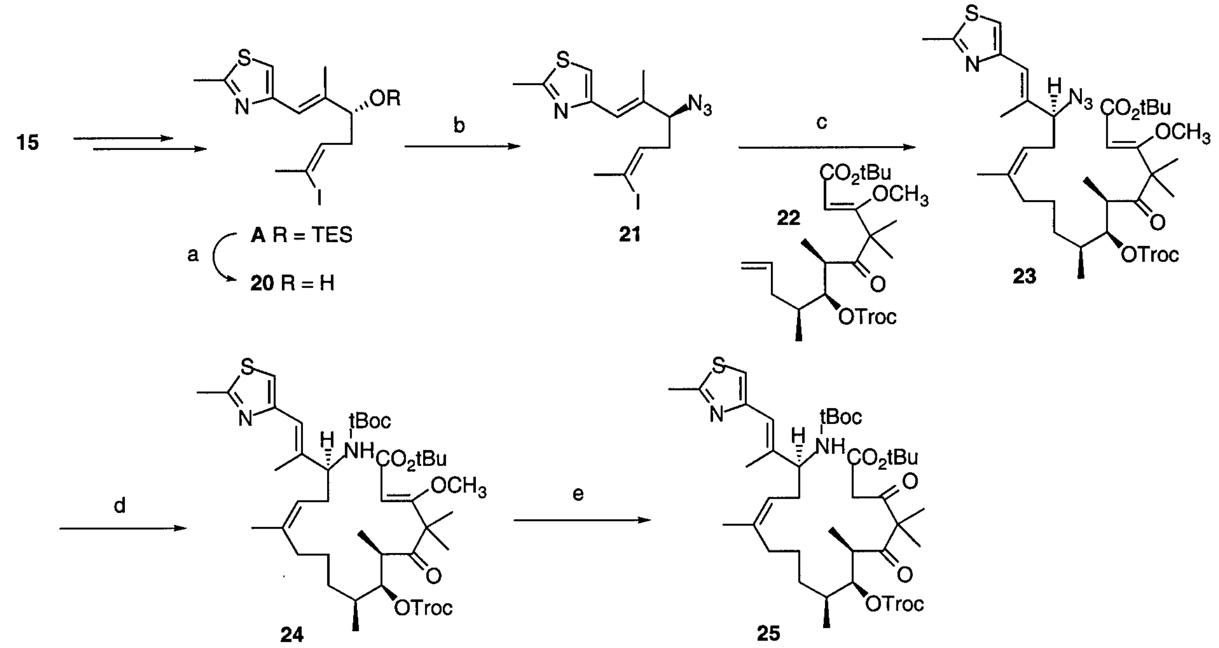


17 **18** **19** **B**

[a] (a) t-BuOH, Ti(Oi-Pr)$_4$, (+)-DET, CH_2Cl_2, 98%, 82% ee; (b) $NaCNBH_3$, BF_3·OEt_2, THF, 52%; (c) $NaIO_4$, THF:H_2O, 81%.

2. Synthesis and Evaluation of Aza Analogue of Epothilones. Next, we examined the feasibility of the new route in the synthesis of the Aza-analogues (**3a** and **3b**). In order to introduce a nitrogen function, we explored the possibility of Mitsunobu substitution at C15 with inversion of stereochemistry. First, asymmetric dihydroxylation of **15** with enantiomeric ligands conveniently afforded both (R)- and (S)-**A**. Deprotection of **A** afforded alcohol **20** (Scheme 2). The latter cleanly underwent Mitsunobu inversion to provide azide **21**. It was found that a palladium-mediated B-alkyl Suzuki cross-coupling could be conducted with the azide in place. In the context of the C2-C3 enol ether, Staudinger reduction of **23** is possible, leading, to **24** after nitrogen protection with a *t*Boc group.

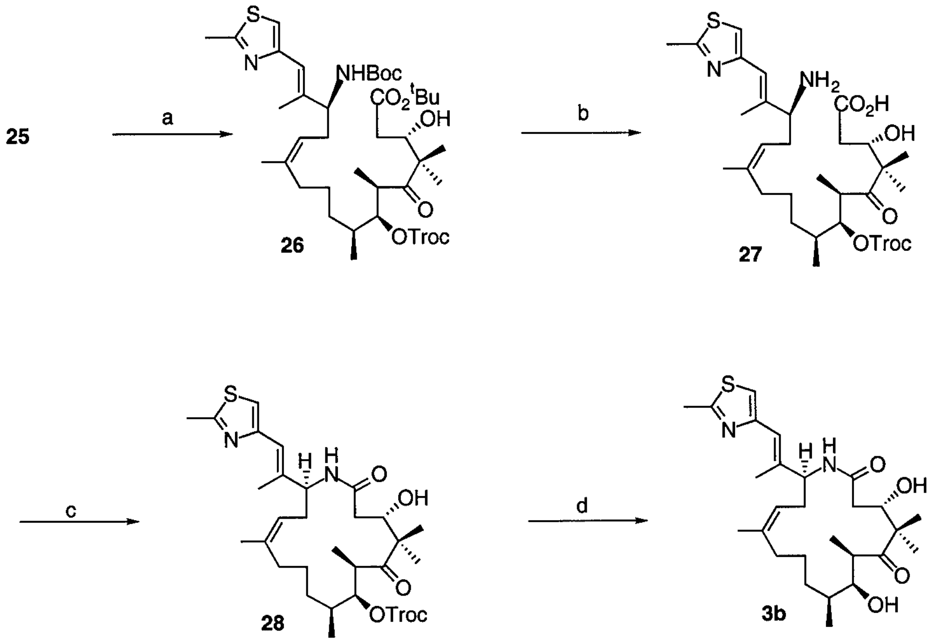
Scheme 4[a]



[a] (a) AcOH:THF:H_2O, 98%; (b) PPh_3, DIAD, HN_3 THF, 66%; (c) 9-BBN, THF then Pd(dppf)Cl_2, $AsPh_3$, Cs_2CO_3, DMF:THF:H_2O, 70%; (d) i) PPh_3, THF, H_2O, 92%; ii) Boc_2O, ACN, Et_3N, 65%; (e) p-TsOH, acetone, 82%.

With the N-tBoc protective function in place at C15, we could conduct the ruthenium-mediated asymmetric Noyori-type reduction at C3 (Scheme 5). The tBoc and tert-butyl ester groups were cleaved concurrently to afford amino acid **27**. A free C3 hydroxyl group also did not interfere with HATU-mediated macrolactamization to afford **28**, albeit in moderate yield. Finally, the C7 Troc group was discharged through the action of zinc dust under the influence of sonication, affording the desired **3b**. In much the same way, the 15(S) precursor (**20**) was converted to 15(R)-epi-aza-dEpoB (**3a**).

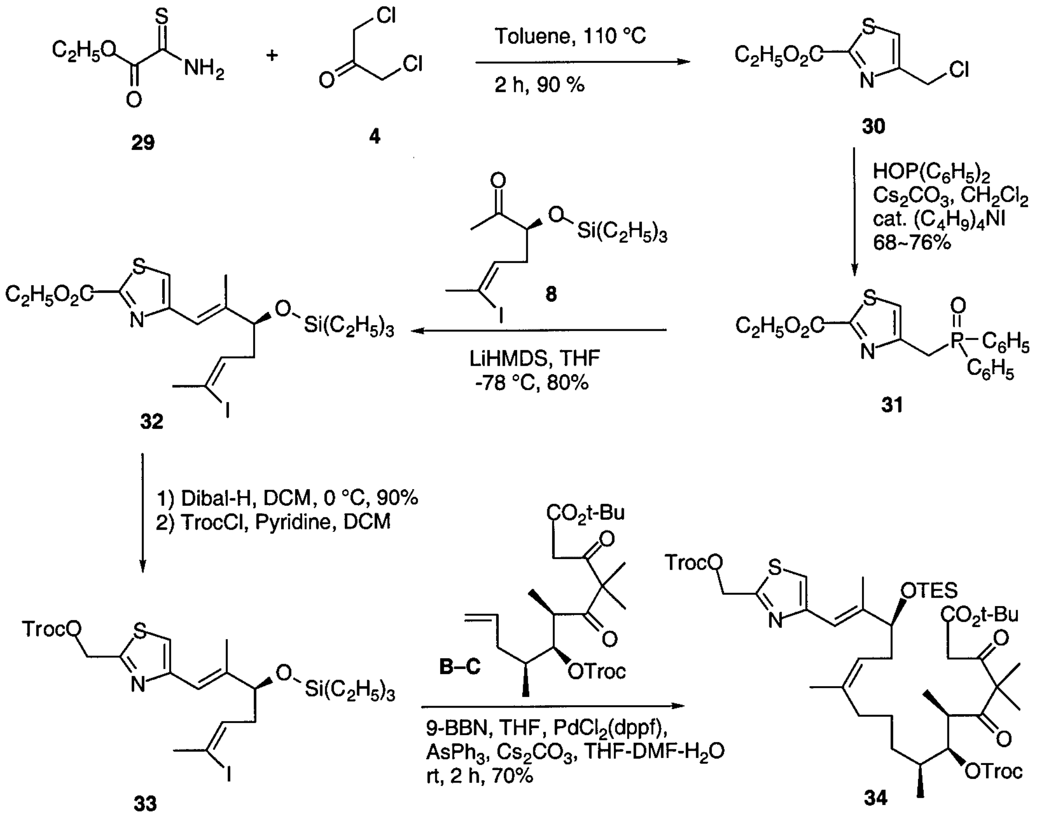
Scheme 5[a]



[a] (a) $[Ru(BINAP)Cl_2]_2$•TEA, HCl/MeOH, H_2 (1200 psi), 78%; (b) THA, CH_2Cl_2; (c) HATU, Hoat, DIPEA, CH_2Cl_2, 50%; (d) Zn, HOAc, >>>.

The fully synthetic 15-aza-12,13-desoxyepothilone B (**3b**) and the epimeric analogue (**3a**) have been tested against a variety of tumor cells in order to evaluate their antitumor activity. Initially, the aza analogues were assayed to determine their relative ability to bind tubulin in comparison to dEpoB. The results indicated that for **3b** microtubulin stabilizing activity was maintained, retaining 75% of the activity of dEpoB. However, the epimeric 15-aza-dEpoB analogue (**3a**) did not appreciably stabilize microtubules within the detection limits of the assay. Hence, a major effect of C15 stereochemistry on the microtubule stabilization properties of the aza agents has been uncovered. Similarly, in cytotoxicity studies, a direct comparison of 15-aza-dEpoB (**3b**) with dEpoB (**2b**), using the CCRF-CEM cell line, showed that it was only slightly less potent than dEpoB (4.8 x less active). By contrast, the corresponding 15-epi-aza-dEpoB (**3a**) analogue displayed severely reduced antitumor activity (21 x less active). Both aza-dEpoB and its corresponding epimer were not active against several benchmark paclitaxel or vinblastine resistant tumor cells (CCRF-CEM/taxol and CCRF-CEM/VBL, respectively) used in earlier studies. By contrast, dEpoB itself is highly active against these resistant lines. In addition, we have successfully epoxidized **3b** to produce the corresponding aza-EpoB analogue. This compound has recently been advanced to phase I clinical trials by the Bristol-Myers Squibb company.

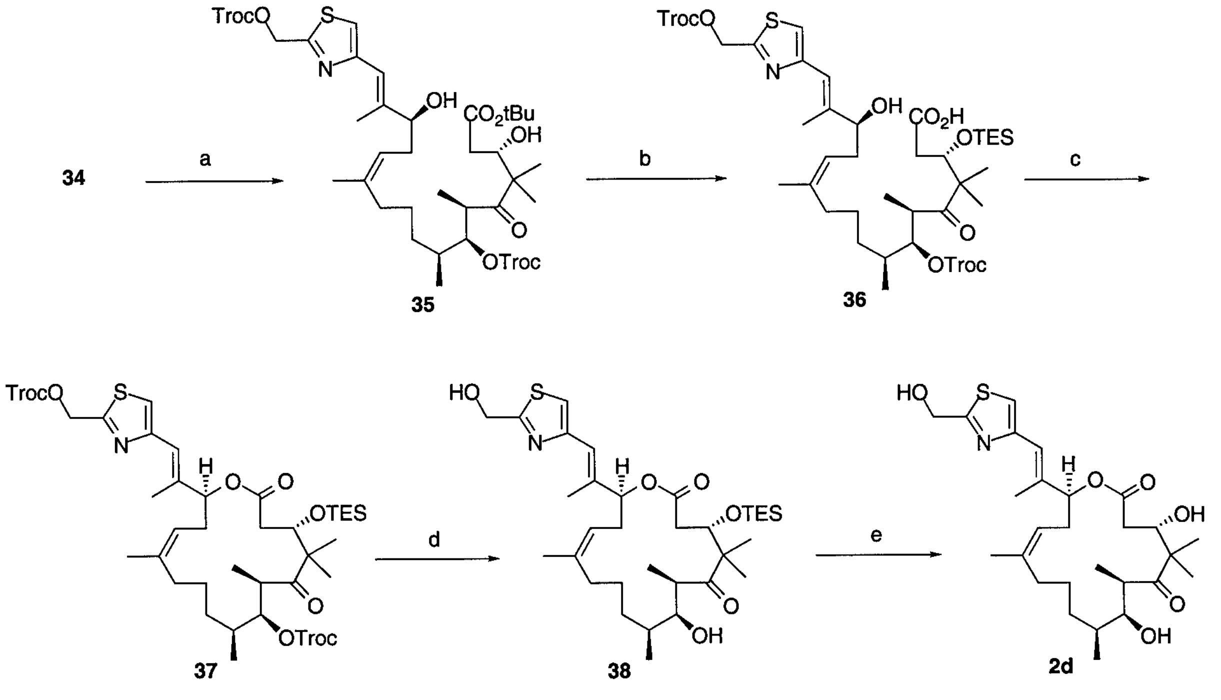
3. Synthesis of dEpoF. The preparation of a Left-Wing fragment for the synthesis of dEpoF (**2b**) has also been explored using the same Wittig type reaction as the key transformation. First, the commercially available ethyl oxamate (**29**) was condensed with 1,3-dichloroacetone to give 2,4-disubstituted thiazole **30**. Subsequent P-alkylation with diphenylphosphine oxide afforded the requisite reagent **31**. The olefination reaction of the anion of the phosphine oxide **31** with methyl ketone **8** proceeded smoothly to furnish **32** as a single geometric isomer in good yield. Reduction of the ethyl ester of **6** by Dibal-H followed by protection with TrocCl generated primary alcohol **33** which could then be connected with the Right-Wing fragment **BC** to give **34** via palladium catalyzed *B*-alkyl Suzuki coupling reaction. This new route is much shorter and more convergent than the corresponding previous sequence involving 10 linear transformations. In particular, the usage of methyl ketone **8** as a universal hinge conveniently allows for the introduction of diversity to the structure of the thiazole moiety.



Scheme 6. New Route to the Left-Wing Fragment for the Synthesis of dEpoF

With the successful union of **33** and **B-C**, stereoselective reduction at C3 was attempted using diketone **34** and C15 free hydroxy derivative of **34** (Scheme 7). . Although the Noyori reduction afforded the desired alcohol **35**, C15 methyl ether was produced in an almost equal quantity presumably due to the solvolysis by the solvent methanol. While this very surprising susceptibility to solvolysis of **34** and related congeners at C15 was certainly detrimental to the efficiency of our total synthesis, we moved on to our goal. Having established all of the necessary stereocenters, the *t*-butyl ester was unmasked with simultaneous protection of the C3 and C15 alcohols by TESOTf. Selective desilylation of the C15 TES group with methanolic HCl provided **36** setting the stage for cyclization. Macrolactonization of this seco-acid, according to the Yamaguchi protocol,[46,47] afforded fully protected lactone **37** in 60~70 % yield. The removal of the two Troc protecting groups was performed through the agency of samarium (II) iodide[48] or zinc, both in good yields. Finally, standard fluoride induced removal of the C3 silyl group yielded the desired 12,13-desoxyepothilone F (**2d**).

Scheme 7[a]



[a] a) 5% $[Ru(BINAP)Cl_2]_2$•TEA, HCl/MeOH, H_2 (1200 psi), 78%; b) (i) TESOTf, 2,6-lutidine, CH_2Cl_2, -78 °C to rt, 8 h, (ii) HCl-CH_3OH, 0 °C, 70%; c) 2,4,6-trichlorobenzoyl chloride, $(C_2H_5)_3N$ then 4-DMAP, toluene, slow addition 3 h, 60-70%; d) Zn, AcOH-THF, rt, 1 h, 86% or cat. NiI_2/SmI_2, THF, -78 to -40 °C, 87%; e) HF•Pyridine, THF 0 °C to rt, 91%.

4. Evaluation of Antitumor Potential of dEpoF. The fully synthetic dEpoF has been evaluated against a variety of cell types in order to evaluate its antitumor potential. As shown in Table 1, dEpoF exhibited high cytotoxicity activity against a range of sensitive and resistant tumor cell lines tested. In particular, high potency and relatively low cross-resistances were observed for dEpoF against sensitive and MDR cell lines, respectively. Direct comparison of dEpoF with dEpoB indicates that the new compound possesses a comparable potency. It is noteworthy that dEpoF consistently outperforms other anticancer agents such as taxol, vinblastine, etoposide and actinomycin in inhibiting the growth of MDR tumor cells.

Table 1. Potency of dEpoF, dEpoB and Taxol against various tumor cell growth *in vitro*

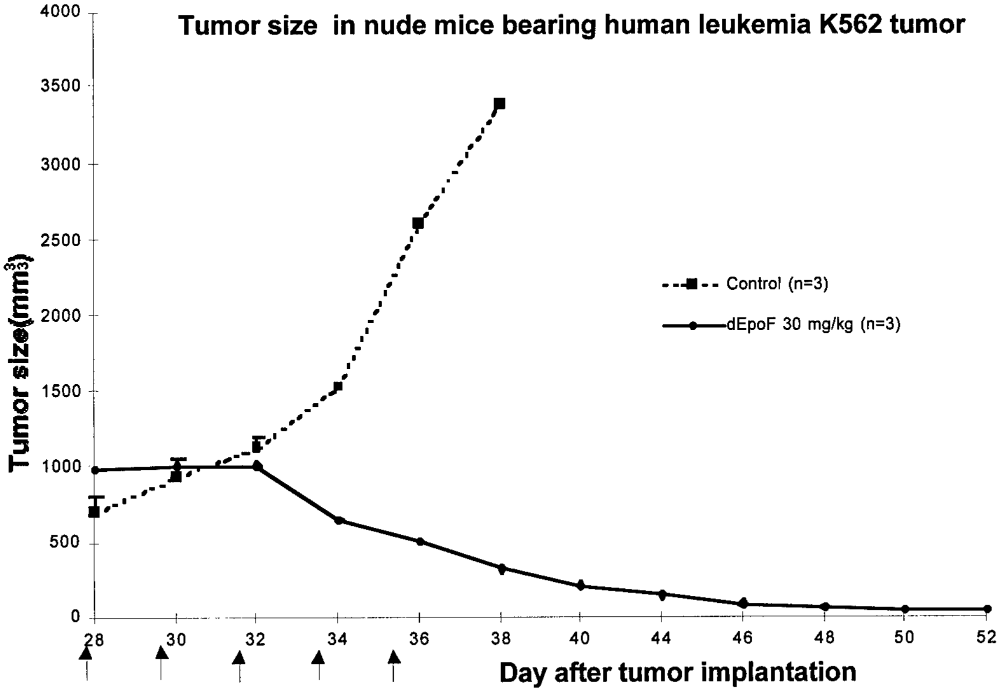
Tumor Cell Lines	IC$_{50}$ (mM)[a]			
	dEpoF	dEpoB	Taxol	Others
Human T-cell AL Leukemia				
CCRF-CEM	0.0027	0.0095	0.0021	0.00063[b], 0.290[c]
CCRF-CEM/VBL$_{100}$	0.047 (17.4 x)	0.017 (1.8 x)	4.140 (1971 x)	0.332[b] (527 x)
CCRF-CEM/VM$_1$	0.0049 (1.8 x)	0.014 (1.5 x)	0.0066 (3.18 x)	3.44[c] (117 x)
CCRF-CEM/Taxol	0.0053 (2.0 x)	0.0162 (1.7 x)	0.120 (57 x)	
Hamster Lung Fibroblasts				
DC-3F	0.0017	0.0019	0.0135	0.00025[d]
DC-3F/ADII	0.0136 (8.0 x)	0.0073 (3.8 x)	0.583 (43.2 x)	0.00153[d] (61.2 x)
DC-3F/ADX	0.0223 (13.1 x)	0.0288 (15.2 x)	20.19 (1496 x)	0.4092[d] (1637 x)
Human CM Leukemia				
K562	0.0021	0.0036	0.0029	
Human Mammary Carcinoma				
MX-1	0.0042	0.0221	0.0394	0.00184[e]

a) Cell growth inhibition was measured by XTT tetrazonium assay after 72 h incubation for cell growth as described previously in ref. 23. The values were determined with six to seven concentrations of each drug using a computer program. The cross-resistance are shown in parentheses. b) Vinblastin (VBL). c) Etoposide (VP-16). d) Actinomycin D (AD). e) Epothilone B (EpoB).

We then turned our attention to the *in vivo* efficacy of dEpoF. Thus, the therapeutic effect of dEpoF was evaluated in athymic mice bearing a human leukemia K562 xenograft. The animal experiments were performed according to the slow IV infusion protocol developed in our previous studies.[23,24] As depicted in Figure 2, treatment of the mice with dEpoF (30 mg/kg) readily induced reduction in the size of tumor to the point of remission. While the preliminary *in vivo* results with this sensitive tumor clearly look promising, more revealing experiments are necessary to assess its full promise.

With the encouraging biological results, we next examined the aqueous solubility of dEpoF using an HPLC-based method.[49] Indeed, dEpoF was found to be 2.5 times more water soluble than dEpoB. Although literature estimates of paclitaxel aqueous solubility vary considerably,[50] it has been noted that epothilones are approximately 30 times more water soluble than paclitaxel.[1] Considering these observations and the problems associated paclitaxel administration, the present analogue appears to be a promising candidate that may bring significant improvement in the formulation of the active drug.

Figure 3. Therapeutic effect of dEpoF in nude mice bearing human leukemia K562 xenograft



Key Research Accomplishments

- Development of a practical route for the plant scale preparation of desoxyepothilone B (dEpoB).
- Synthesis of dEpoB to provide sufficient amounts for higher animal studies.
- Successful total syntheses of new 21-hydroxy, 15-aza- and epi-aza desoxy epothilone analogues.
- In vitro antitumor evaluation of the new analogues.
- In vivo evaluation of dEpoF.
- Discovery of desoxyepothilone F (dEpoF) as a potent anticancer agent.

Reportable Outcomes

1. "The Total Synthesis and Antitumor Activity of 12,13-Desoxyepothilone F: An Unexpected Solvolysis Problem at C15, Mediated by Remote Substitution at C21," Chul Bom Lee, Ting-Chao Chou, Xiu-Guo Zhang, Zhi-Guang Wang, Scott D. Kuduk, Mark D. Chappell, Shawn J. Stachel and Samuel J. Danishefsky *J. Org. Chem.* **2000**, *in press.*
2. "On the Total Synthesis and Preliminary Biological Evaluation of 15(*R*) and 15(*S*) Aza-dEpoB: A Mitsunobu Inversion at C15 in Pre-Epothilone Fragments," Shawn J. Stachel, Mark D. Chappell, Chul Bom Lee, Samuel J. Danishefsky, Ting-Chao Chou, Lifeng He, and Susan B. Horwitz *Org. Lett.* **2000**, *2*, 1637.
3. "En Route to a Plant Scale Synthesis of the Promising Antitumor Agent 12,13-Desoxyepothilone B," Mark D. Chappell, Shawn J. Stachel, Chul Bom Lee and Samuel J. Danishefsky, *Org. Lett.* **2000**, *2*, 1633.
4. "A Synthesis of Desoxyepothilone F (21-Hydroxy-12,13-desoxyepothilone B): A Compound Possessing Potent in vitro and in vivo Antitumor Activity, and Uses thereof," Samuel J. Danishefsky, Chul Bom Lee and Ting-Chao Chou, *U.S. Patent "pending."*
5. "Chemical Synthesis and Antitumor Activity of 12,13-Desoxyepothione F" Chul Bom Lee, Ting-Chao Chou and Samuel J. Dnishefsky, *Era of Hope (poster)*, US Army Breast Cancer Research Program, Atlanta, GA, June, 2000.

Conclusions

In summary, a highly efficient and practical route for dEpoB systems has been developed. Using this synthetic route, various epothilone analogues have been produced through total syntheses to provide sufficient amounts for biological studies that will support a full and searching evaluation of dEpoB and other promising epothilones at the clinical level. The feasibility of converting an oxygen at C15 to a nitrogen by Mitsunobu inversion reaction in seco dEpoB systems gives access to various novel structural variants of the system. Aza systems, however, do not appear to be as clinically promising as dEpoB in the preliminary screening. Another epothilone analogue that possesses an additional hydroxy group at C21 has also been synthesized. The strategy based on convergent merger of the two key fragments in the current synthesis of dEpoF, compared to the dEpoB synthesis, proved less efficient due to the unexpected acid induced susceptibility. Nevertheless, the *in vitro* and *in vivo* tumor growth inhibition experiments demonstrated the hydroxy analogue possesses high antitumor activity. Given the promising *in vivo* profile of the closely related dEpoB, the need for further investigation with dEpoF is readily apparent. Overall, our successful chemical sythesis of these epothilone derivatives has made it possible to study all too important clinical aspects of epothilones, which otherwise would have been difficult.

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Appendices

- Reprints of published papers.
- Curriculum vitae

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Education and Experience:

1999-Present	Postdoctoral Research Fellow Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, New York Director: Professor Samuel J. Danishefsky
1992-98	Ph.D. in Organic Chemistry Department of Chemistry, Stanford University, Stanford, California. Advisor: Professor Barry M. Trost
1991-92	Research and Head Teaching Assistant Institute for Basic Sciences, Seoul National University, Seoul, Korea. Chairman: Professor Kyungtae Kim
1990-91	Second Lieutenant (Mandatory Military Service) Korea the Third Military Academy, Yeong-Cheon, Korea.
1988-90	M.S. in Chemistry Department of Chemistry, Seoul National University, Seoul, Korea. Advisor: Professor Eun Lee
1984-88	B.S. in Chemistry College of Natural Sciences, Seoul National University, Seoul, Korea. General Advisor: Professor Hasuck Kim

Research Experience:

1999-Present
- Postdoctoral Research Fellow, Sloan-Kettering Institute for Cancer Research.
- Professor Samuel J. Danishefsky
- *Synthesis of Potent Anticancer Agents, Epothilone Analogs.*

  Studies directed toward development and semi-practical total synthesis of potent anticancer agents, epothilone analogues.

1992-98
- Graduate Research Assistant, Department of Chemistry, Stanford University.
- Professor Barry M. Trost
- *Asymmetric Alkylation of Allylic gem-Dicarboxylates and Total Syntheses of Sphingofungins E and F.*

  Development of the Pd-catalyzed asymmetric catalytic allylic alkylation of *gem*-dicarboxylates. Enantioselective total syntheses of novel antifungal agents, sphingofungins E and F.

1991-92
- Research Associate, Institute for Basic Sciences, Seoul National University.
- Professor Eun Lee
- *Free Radical Reactions of Aryl Ethers and β-Alkoxyacrylates.*

  Synthetic and mechanistic studies on the translocation of 3-arylpropyl radicals. Stereoselective synthesis of 5- or 6-membered oxacycles using β-alkoxyacrylate derivatives.

1988-90
- Graduate Research Assistant, Department of Chemistry, Seoul National University
- Professor Eun Lee
- *Total Syntheses of Novel Aza-δ-lactam Antibiotics.*

  Synthesis of nitrogen analogues of carbapenem from aspartic acid. Efficient synthesis of pyridazine derivatives from α-amino acids.

1987-88
- Undergraduate Research Assistant, Seoul National University.
- Professor Eun Lee
- *A Study on the Chirality Transfer via the Claisen Rearrangements.*

  Investigation on various Claisen rearrangement reactions for the the stereoselective construction of quaternary angular methyl groups in the total synthesis of sesquiterpenoids.

Teaching Experience:

1996	Teaching Assistant: Frontiers in Organic Chemistry (ACS continuing education program: Professor Barry M. Trost); Stanford University.
1993	Teaching Assistant for Chem 36 (Chemical separations: Professor Keith O. Hodgson); Stanford University.
1991	Head Teaching Assistant for Experimental General Chemistry and Organic Chemistry (Professor Kukjo Shin and Professor Eun Lee); Seoul National University.
1989	Teaching Assistant for Organic Chemistry (Organic synthesis lab course: Professor Eun Lee); Seoul National University.
1988	Teaching Assistant for General Chemistry (Lab course for freshman students: Professor Sangyeop Lee); Seoul National University.

Honors and Awards:

1999-	Postdoctoral Fellowship, US Army Breast Cancer Research Program.
1997-98	Pharmacia & Upjohn Graduate Fellowship.
1997	The Roche Award for Excellence in Organic Chemistry, Hoffmann La Roche.
1996-97	Veatch Fellowship: The Franklin Veatch Memorial Foundation, Stanford University.
1992-94	Korean Government Fellowship: The Ministry of Education, Republic of Korea.
1988-90	Graduate Scholarship: Seoul National University.
1985-88	University Merit Scholarship: Seoul National University.

Publications:

1. "Transition Metal-Catalyzed Asymmetric Allylic Alkylation (AAA) Reactions", Barry M. Trost and Chul Bom Lee In *"Catalytic Asymmetric Synthesis (2nd Ed.)"*, I. Ojima (Ed), VCH Publishers Inc., New York, NY, **2000**, Chapter 8.
2. "The Total Synthesis and Antitumor Activity of 12,13-Desoxyepothilone F: An Unexpected Solvolysis Problem at C15, Mediated by Remote Substitution at C21," Chul Bom Lee, Ting-Chao Chou, Xiu-Guo Zhang, Zhi-Guang Wang, Scott D. Kuduk, Mark D. Chappell, Shawn J. Stachel and Samuel J. Danishefsky *J. Org. Chem.* **2000**, *in press.*
3. "α-Acetoxysulfones as "Chiral Aldehyde" Equivalents," Barry M. Trost, Matthew L. Crawly and Chul Bom Lee, *J. Am. Chem. Soc.* **2000**, *122*, 6120.

4. "On the Total Synthesis and Preliminary Biological Evaluation of 15(*R*) and 15(*S*) Aza-dEpoB: A Mitsunobu Inversion at C15 in Pre-Epothilone Fragments," Shawn J. Stachel, Mark D. Chappell, Chul Bom Lee, Samuel J. Danishefsky, Ting-Chao Chou, Lifeng He, and Susan B. Horwitz *Org. Lett.* **2000**, *2*, 1637.
5. "En Route to a Plant Scale Synthesis of the Promising Antitumor Agent 12,13-Desoxyepothilone B," Mark D. Chappell, Shawn J. Stachel, Chul Bom Lee and Samuel J. Danishefsky, *Org. Lett.* **2000**, *2*, 1633.
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7. "Asymmetric Alkylation of Allylic *Gem*-Dicarboxylates," Barry M. Trost, Chul Bom Lee and Jochen M. Weiss, *J. Am. Chem. Soc.* **1995**, *117*, 7247.
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Manuscripts Submitted or in Preparation:

1. "Asymmetric Alkylation of Allylic geminal-Dicarboxylates I: Initial Studies Using Malonate Esters as Nucleophile," Barry M. Trost and Chul Bom Lee, *J. Am. Chem. Soc. submitted.*
2. "Asymmetric Alkylation of Allylic geminal-Dicarboxylates II: Enantioselective Synthesis of Allylic Esters," Barry M. Trost and Chul Bom Lee, *J. Am. Chem. Soc. submitted.*
3. "Total Syntheses of Sphingofungins E and F: Enantioselective Alkylation of a *gem*-Diacetate with Azlactones," Barry M. Trost and Chul Bom Lee, *J. Am. Chem. Soc. in preparation.*
4. "Chemistry of geminal Dicarboxylates," Chul Bom Lee, *Tetrahedron. in preparation.*

Patents:

"A Synthesis of Desoxyepothilone F (21-Hydroxy-12,13-desoxyepothilone B): A Compound Possessing Potent in vitro and in vivo Antitumor Activity, and Uses thereof" Samuel J. Danishefsky, Chul Bom Lee and Ting-Chao Chou. *U.S. Patent "pending."*

Presentations:

1. "Chemical Synthesis and Antitumor Activity of 12,13-Desoxyepothilone F" Chul Bom Lee, Ting-Chao Chou and Samuel J. Danishefsky; *Era of Hope*, Breast Cancer Research Program, US Army Medical Research and Material Command, Atlanta, GA (June 2000).
2. "Palladium Catalyzed Asymmetric Alkylation and Total Syntheses of Sphingofungins E and F" Chul Bom Lee; *Department Colloquium*, Department of Chemistry, Columbia University, New York, NY (September 1999).
3. "Asymmetric Alkylation of Allylic *gem*-Dicarboxylates and Total Syntheses of Sphingofungins E and F" Chul Bom Lee; *Presentation of Dissertation*, Department of Chemistry, Stanford University, Stanford, CA (December 1998).
4. "Asymmetric Alkylation of Allylic *gem*-Dicarboxylates," Chul Bom Lee; *The Roche Symposium*, Hoffmann-La Roche Inc., Nutely, NJ (June 1997).
5. "Asymmetric Alkylation of Allylic *gem*-Dicarboxylates: Methodology and Synthetic Applications," Barry M. Trost and Chul Bom Lee; *Division of Organic Chemistry, 213th ACS National Meeting* (Poster), San Francisco, CA (April 1997).
6. "Asymmetric Alkylation of Allylic *gem*-Dicarboxylates," Barry M. Trost, Chul Bom Lee and Jochen M. Weiss; *10th Annual William S. Johnson Symposium in Organic Chemistry* (Poster) Stanford University, Stanford, CA (October 1995).
7. "Stereoselective Synthesis of Tetrahydrofurans and Tetrahydropyrans Via Free Radical Mediated Cyclizations of β-Alkoxyacrylates," Eun Lee, Jin Sung Tae, Chulbom Lee; '92 *Annual Meeting of The Korean Chemical Society,* Seoul, Korea (April 1992).

References:

Professor Samuel J. Danishefsky, Sloan-Kettering Institute for Cancer Research, MSKCC.
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Professor Eun Lee, Department of Chemistry, Seoul National University.

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On the Total Synthesis and Preliminary Biological Evaluations of 15(*R*) and 15(*S*) Aza-dEpoB: A Mitsunobu Inversion at C15 in Pre-Epothilone Fragments

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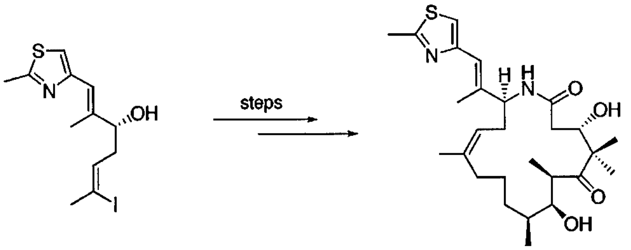
ABSTRACT



The syntheses of two epothilone analogues, 15(*S*)-aza-12,13-desoxyepothilone B and the epimeric 15(*R*)-aza-12,13-desoxyepothilone B, are described. A Mitsunobu inversion was utilized for elaboration of pre-epothilone fragments to the corresponding macrolactam. Tubulin binding and cytotoxicity profiles of these analogues are presented.

The previous Letter set forth the background of the epothilone project from our perspective. In particular, it described a key asymmetric dihydroxylation reaction. We took recourse to AD mix-α[1] (see **3** → **4**) to reach the 15(*S*) enantiomer (**5a**), and shortly thereafter dEpoB (**1**). Not surprisingly, recourse to AD mix-*β* leads to the antipodal 15(*R*) stereoseries (see compound **6**) en route to **7** and **7a** (Scheme 1).

With compounds **5a** and **7a** in hand, we wondered about the possibility of displacement of the C-15 hydroxyl centers, thereby enabling the introduction of nitrogen-based nucleophiles.[2] Assuming strict inversion of configuration could be realized, it was hoped that the 15(*R*) alcohol (**7a**) would lead eventually to a 15(*S*) aza analogue of dEpoB (**2**) while the 15(*S*) alcohol (**5a**) would pave the way for reaching the epimeric 15(*R*) aza series (vide infra **17**). In this way, we could ascertain the bioprofiles of such new dEpoB systems, in macrolactam rather than macrolactone settings.[3] We further hoped to evaluate the consequences of permuting the C-15 stereochemistry on the tubulin binding and cytotoxicity profiles.[4] Finally, we hoped to evaluate the capacity of these

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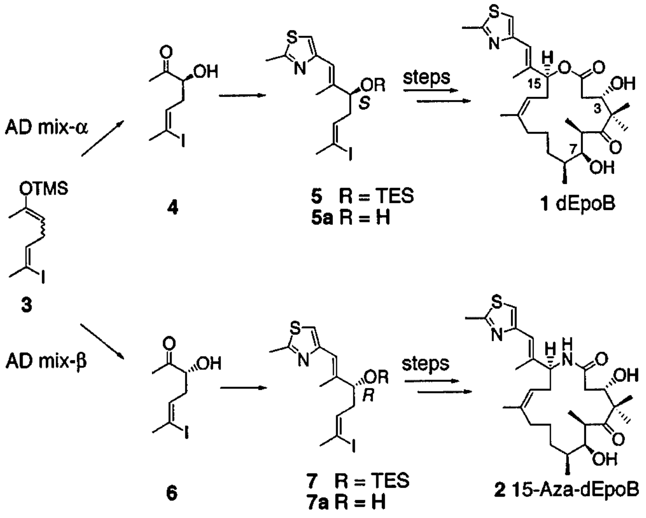
(1) (a) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L, Morikawa, K.; Wang, Z.-M.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. (b) Aldrichimica Acta **1994**, *27*, 70.

(2) (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Luo, Y.; Blaskovich, M. A.; Lajoie, G. A. *J. Org. Chem.* **1999**, *64*, 6106.

10.1021/ol005932m CCC: $19.00

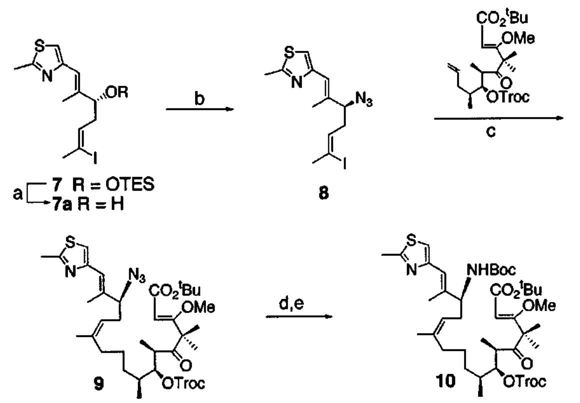
Scheme 1. Asymmetric Dihydroxylation



aza compounds to function in the context of otherwise resistant tumors. An account of the chemical synthesis of our targets and their preliminary evaluation is provided herein.

Deprotection of **7** as shown afforded alcohol **7a** (Scheme 2). The latter underwent Mitsunobu inversion,[2] as indicated, to provide azide **8**. While the reaction worked smoothly, a great deal of effort was necessary to enable the successful integration of this azido function into our synthetic schemes.[5]

Scheme 2. Mitsunobu Inversion[a]

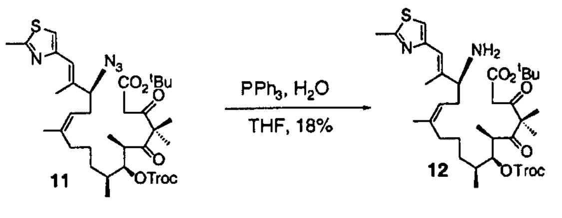


Conditions: a) HOAc:THF:H$_2$O, 98%; b) PPh$_3$, DIAD, HN$_3$, THF, 66%; 9-BBN, THF then Pd(dppf)Cl$_2$, AsPh$_3$, CsCO$_3$, DMF 70%; d) PPh$_3$, THF, H$_2$O, 92%; e) Boc$_2$, ACN, Et$_3$N, 65%.

Eventually, a passage was navigated. It was found that a palladium-mediated *B*-alkyl Suzuki cross-coupling[6] could be conducted with the azide in place (see **8** → **9**). In the context of the C2−C3 enol ether, Staudinger reduction of **9** is possible, leading, after nitrogen protection, to **10**.[7]

By contrast, in a related azido substrate **11** containing the C3 ketone, Staudinger reduction en route to **12** could be conducted in our hands in only 18% yield (Scheme 3).

Scheme 3. Staudinger Reduction of Azido-*β*-keto Ester



Reduction of the azide to the amine is apparently complicated by Schiff base formation (inter- or intramolecular) between the in situ generated 15-amino functionality and the 3-keto group.[8]

With the N-^{t}Boc protective function in place at C15, we could conduct the ruthenium-mediated asymmetric Noyori-type reduction[9] at C3 (see transformation **10** → **14**, Scheme 4). By contrast, the corresponding reduction at the stage of **11** was not successful. Apparently the azide function is not compatible with the strongly acidic Noyori reduction protocol, though it does not seem to be converted to the amino group under these conditions.

With **14** in hand, the ^{t}Boc and *tert*-butyl ester groups were cleaved concurrently to afford amino acid **15**. This reaction could be conducted in the presence of a free C3 hydroxyl group. This group also did not interfere with HATU-mediated macrolactamization[10] to afford **16**, albeit in only 50% yield. Finally, the C7 Troc group was discharged through the action of zinc dust under the influence of sonication, affording the desired **2**.[11] In much the same way, the 15(*S*) precursor (**5**) was converted to 15(*R*)-epi-aza-dEpoB (**17**).

The fully synthetic 15-aza-12,13-desoxyepothilone B (**2**) and the epimeric 15-aza-12,13-desoxyepothilone B (**17**) analogues have been tested against a variety of tumor cells in order to evaluate their antitumor activity. Initially, the aza

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(3) (a) Compound **2** was first reported in the patent literature: Vite, G. D.; Borzilleri, R. M.; Kim, S.-H.; Johnson, J. A. Patent WO 99/102514, 1999. It was then reported at (b) the 219th National Meeting of the American Chemical Society, San Francisco, March 2000, by one of us (S.J.D.; ORGN 43) and then by (c) Vite, G.; Borzilleri, R.; Fooks, C.; Johnson, J.; Kim, S.-H.; Leavitt, K.; Mitt, T.; Regueiro-Ren, A.; Schmidt, R.; Zheng, P.; Lee, F. (ORGN 286).

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(6) (a) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishikawa, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314. (b) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457. (c) Johnson, C. R.; Braun, M. P. *J. Am. Chem. Soc.* **1993**, *115*, 11014.

(7) (a) Vaultier, M,; Knouzi, N.; Carrie, R. *Tetrahedron Lett.* **1983**, *24*, 763. (b) Taylor, E. C.; Pont, J. L. *Tetrahedron Lett.* **1987**, *28*, 379. (c) Knouzi, N.; Vaultier, M.; Toupet, L.; Carrie, R. *Tetrahedron Lett.* **1987**, *28*, 1757. (d) Nagarajan, S.; Ganem, S. *J. Org. Chem.* **1987**, *52*, 5044.

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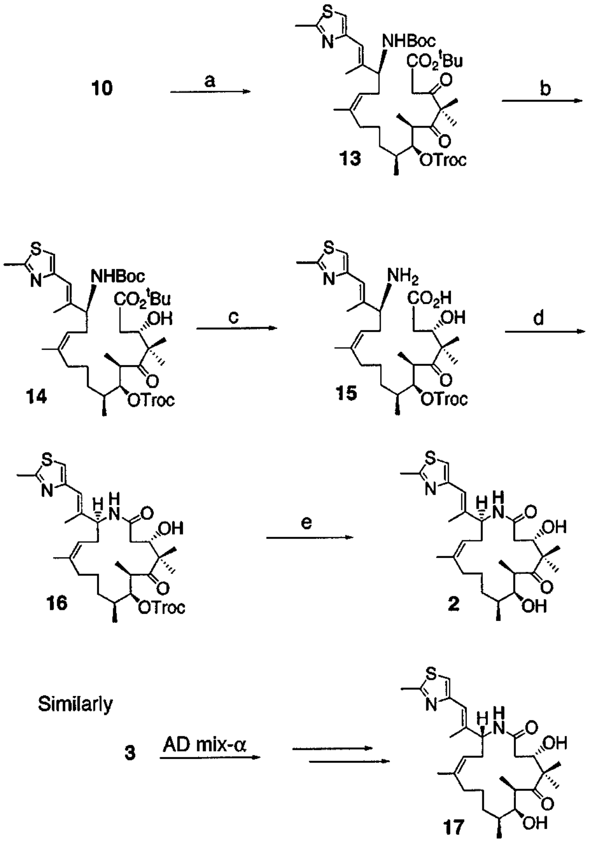
Scheme 4. Synthesis of Aza-dEpoB (2)[a]



Conditions: a) p-TsOH, acetone, 82%; b) $[Ru(BINAP)Cl_2]_2$- Et_3N, 0.12M HCl/MeOH, H_2, 1200psi, 78%; c) TFA, CH_2Cl_2; d) HATU, Hoat, DIPEA, CH_2Cl_2, 50%; e) zinc dust, HOAc, sonication.

analogues (**2** and **17**) were assayed to determine their relative ability to bind tubulin in comparison to dEpoB. The results indicated that for 15-aza-dEpoB (**2**) microtubulin stabilizing activity was maintained, retaining 75% of the activity of dEpoB. However, the epimeric 15-aza-dEpoB analogue (**17**) did not appreciably stabilize microtubules within the detection limits of the assay. Hence, a major effect of C15 stereochemistry on the microtubule stabilization properties of the aza agents has been uncovered. Similarly, in cytotoxicity studies, a direct comparison of 15-aza-dEpoB (**2**) with dEpoB (**1**), using the CCRF-CEM cell line, showed that it was only slightly less potent than dEpoB (4.8× less active). By contrast, the corresponding 15-epi-aza-dEpoB (**17**) analogue displayed severely reduced antitumor activity (21× less active). We also noted that, both aza-dEpoB (**2**) and its corresponding epimer (**17**) were not active against several benchmark paclitaxel or vinblastine resistant tumor cells (CCRF-CEM/taxol and CCRF-CEM/VBL, respectively) used in earlier studies. By contrast, dEpoB itself is highly active against these resistant lines.[12] In addition, we have successfully epoxidized **2** to produce the corresponding aza-EpoB analogue. This compound has recently been advanced to phase 1 clinical trials by the Bristol-Myers Squibb company.[3c]

In summary, the feasibility of carrying out an oxygen to nitrogen Mitsunobu inversion in seco dEpoB systems portends access to various novel structural variants of the system. For the moment, however, aza systems **2** and **17** do not appear to be as clinically promising as dEpoB.

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En Route to a Plant Scale Synthesis of the Promising Antitumor Agent 12,13-Desoxyepothilone B

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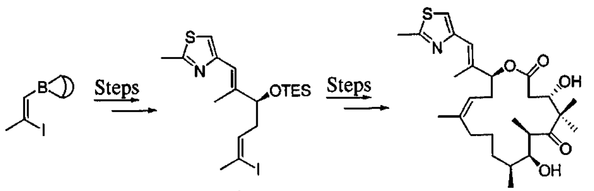
ABSTRACT



A

12,13-desoxyepothilone B
dEpoB

Efficient and processable syntheses of key building blocks of the antitumor agent 12,13-desoxyepothilone B (dEpoB) by catalytic asymmetric induction are herein described.

Interest in the epothilone family of natural products on the part organic chemists was incubated, no doubt, by the promising biological profiles of the A and B isomers, particularly the latter.[1,2] These compounds seem to share a common tubulin-centered mechanistic framework with paclitaxel, yet offer potential advantages in terms of formulability and performance in vitro toward Taxol insensitive tumor cells.[3] Likewise, the interesting and insightful structural features of the epothilones challenge the collective ingenuity of the science of organic synthesis. The field has benefited from a continuing series of disclosures,[2,4] seeking to improve upon the three initial feasibility demonstrations.[5–7]

As early as 1997, using our then intricate (though stereospecific) academic type synthesis,[5b] enough epothilone B was garnered to show for the first time that the initial Merck reports[3] on the favorable in vitro biological profiles of epothilones were extendable to human tumors transplanted in murine hosts in a xenograft setting.[8] However, during these

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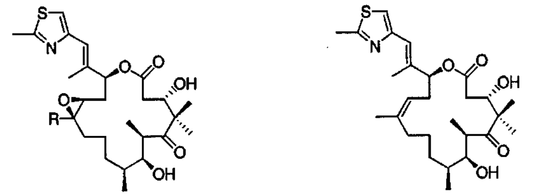
10.1021/ol0059302 CCC: $19.00

investigations, some potentially serious toxicity problems were uncovered using agent **2** (Figure 1). These findings cast some doubt as to whether exploitable therapeutic indices could be found with epothilone B (EpoB). During this era, we demonstrated that much of the toxicity of EpoB could be abrogated through the use of 12,13-desoxyepothilone B (dEpoB, **3**).[9,10] Vast superiority of dEpoB relative to EpoB and to paclitaxel has been demonstrated in a variety of competitive in vivo settings, and the results have been published elsewhere.[2b,11] Presently, dEpoB has advanced to toxicology and efficacy studies in dogs, en route to a full-scale clinical evaluation.[12]



1 epothilone A (R = H)
2 epothilone B (R = CH_3)

3 12,13-desoxyepothilone B (dEpoB)

Figure 1. Epothilones.

Since our laboratory lacks access to fermentation-derived epothilones, total synthesis constituted our only recourse to produce material for biological investigations. Indeed, all of the in vivo evaluations have been conducted on fully synthetic dEpoB. The present goal is to develop a practical total synthesis of dEpoB which can furnish material appropriate for a full scale evaluation in humans if, as expected, findings in higher and larger animals so indicate.

In our previously described improved routes to dEpoB,[13,14] three subunits were built and combined. The route to building block **C** (see Figure 2) has already been shown to be amenable to major scale-up. As previously described by Overman,[15] building block **B** was prepared by auxiliary-mediated allylation of a suitable propionate derivative, using methodology first promulgated by Evans and associates.[16] The largest impediment to major scale-up was in the synthesis of the **A** subunit, which had previously required eight steps, exclusive of generating the required reagents. Several of these maneuvers required rather sophisticated and expensive chemistry, and prospects for serious scale-up of these protocols were daunting. The disclosure herein now describes new synthetic developments which render the **A** fragment readily available in multigram quantities, utilizing easily processable chemistry. These advances have major favorable consequences for a plant-level synthesis of dEpoB.

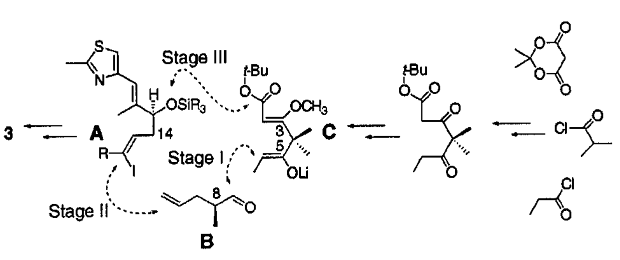
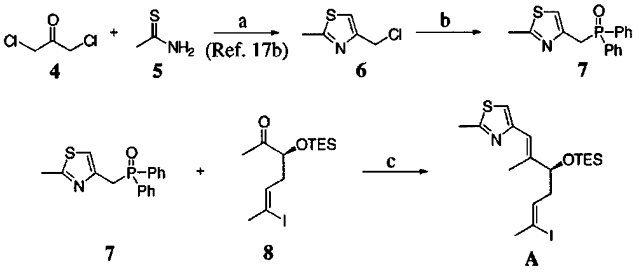


Figure 2. Epothilone building blocks.

The new route for the synthesis of the key vinyl iodide fragment required smooth access to large quantities of phosphine oxide **7**. This subunit is in fact easily prepared *in two steps* on a 100 g scale as shown in Scheme 1.[17] The second subunit required for building the **A** segment is methyl ketone **8** (vide infra). The Horner-like condensation[18] between **7** and **8** is conducted in 98% yield on a multigram scale to afford **A**. However, for this chemistry to be valuable, a straightforward synthesis of **8**, amenable to plant-level scale-up, had to be accomplished.

Scheme 1[a]



[a] (a) (i) acetone, (ii) $ZnCl_2$, MeOH, reflux, 60%; (b) $HOPPh_2$, Cs_2CO_3, Bu_4NI, CH_2Cl_2, 97%; (c) (i) **7**, *n*-BuLi. THF, −78 °C, 30 min, (ii) **8**, −78 °C to rt, 98%.

Fortunately, two such routes have now been developed. In the first approach (Scheme 2) following conversion of **9**

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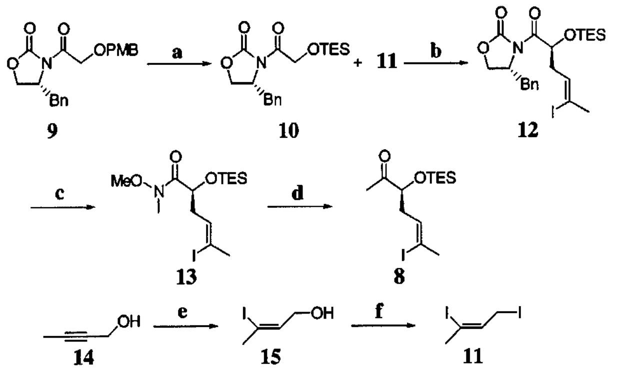
Scheme 2^a

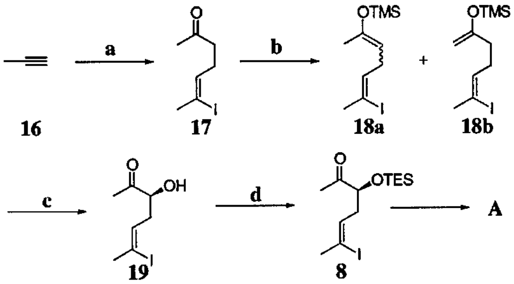


^{a} (a) (i) $TiCl_4$, 0 °C, 87%, (ii) TESCl, imidazole, DMF, 84%; (b) LHMDS, 11, −78 °C, 81%; (c) (i) HOAc:THF:H_2O (3:1:1), (ii) CH_3ONHCH_3, $AlMe_3$, (iii) TESCl, DMF, 88% overall; (d) MeMgB, 0 °C, 93%; (e) (i) RedAl, 0 °C to rt, (ii) I_2, −78 °C to rt, Et_2O; (f) TMSI, CH_2Cl_2, 0 °C, 81%−two steps.

to **10** (84%),[19,20] a highly diastereoselective alkylation of lithio **10** with **11** produces **12** (>25:1 de) in 81% yield.[19,20] As was previously reported,[21] diiodide **11** is available from 2-butynol in two steps as shown. Finally, compound **12** was advanced in three steps to **8** by recourse to the Weinreb amide **13**.[22]

A second route, while somewhat less selective, reaches **8** even more easily, in only four steps via asymmetric dihydroxylation[23] (Scheme 3). This synthesis begins with the known reaction of propyne with *B*-iodo-9-BBN and methyl vinyl ketone to produce **17**,[24] which reacts (in multigram scale) with trimethylsilyl iodide to provide an 88:12 (**18a**:**18b**) mixture of silyl enol ether isomers **18**.[25] Asymmetric dihydroxylation[23] of this material, under the conditions shown, afforded **19** (87% ee, 55% yield−two steps). Finally, triethylsilylation of **19** completes the synthesis of ketone **8** and therefore **A**.

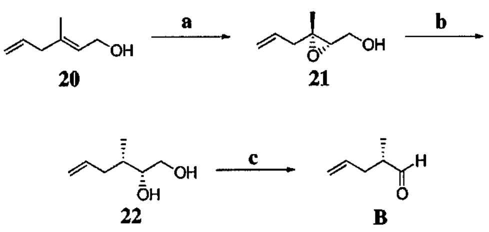
Scheme 3^a



^{a} (a) (i) I-9-BBN, hexanes, (ii) methyl vinyl ketone, hexanes, (iii) 3 N NaOH, PhMe, 100 °C, 65%; (b) TMSI, HMDS, CH_2Cl_2, −20 °C to rt; (c) 1 mol % of OsO_4, AD-mix-α, $MeSO_2NH_2$, *t*-BuOH:H_2O (1:1), 55%−two steps; (d) TESCl, imidazole, DMF, 85%.

With a view to generating fragment **B** by strictly catalytic asymmetric methods, its synthesis was revisited (Scheme 4).

Scheme 4^a



^{a} (a) *t*-BuOH, Ti(O*i*-Pr)$_4$, (+)-DET, CH_2Cl_2, 98%, 82% ee; (b) $NaCNBH_3$, BF_3·OEt_2, THF, 52%; (c) $NaIO_4$, THF:H_2O, 81%.

For this purpose, we synthesized subunit **20**, which is prepared from isoprene by known chemistry.[26] Asymmetric epoxidation[27] of **20** provides **21**, which undergoes reductive cleavage[28] at the more substituted center to furnish diol **22**. Following periodate cleavage as shown, building block **B** is in hand. While this method bypasses recourse to a chiral auxiliary, its ultimate advantage in terms of scale-up to the multigram levels in a plant-type setting awaits demonstration.

As previously described,[13,14] a novel aldol condensation joins fragments **B** and **C**. Subsequently, a palladium-mediated *B*-alkyl Suzuki[29] merger joins **A** with **B**−**C**. With the carbon skeleton in place, a catalytic Noyori reduction[30] provides the desired stereochemistry at C3 and macrolactonization leads, shortly afterward, to dEpoB.[14] While we always remain open to possibilities for still greater practicality, we are now already confident that compound availability through total synthesis will support a full and searching evaluation of dEpoB and other promising epothilones at the clinical level.

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