J|A|C|S
COMMUNICATIONS

Published on Web 02/06/2003

Complex Target-Oriented Total Synthesis in the Drug Discovery Process: The Discovery of a Highly Promising Family of Second Generation Epothilones

Alexey Rivkin,[†] Fumihiko Yoshimura,[†] Ana E. Gabarda,[†] Ting-Chao Chou,[‡] Huajin Dong,[‡] William P. Tong,[§] 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

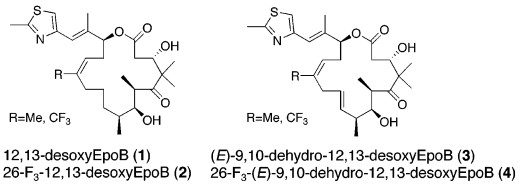
Received December 11, 2002 ; E-mail: s-danishefsky@ski.mskcc.org

The emergence of the epothilones as promising new anticancer agents has led to a worldwide effort to synthesize new analogues, and to establish their SAR with a view for identifying and developing later generation agents for clinical evalution.[1] Human clinical trials seeking to assess issues of toxicity, optimal dosage, and likely efficacy of several epothilones as drugs are well underway.[2] For instance, 12,13-desoxyepothilone B (**1**) (cf. inter alia (dEpoB or EpoD)), initially developed in our laboratory via total synthesis, is now undergoing human clinical trials following highly favorable findings in various in vivo animal settings (Figure 1).[3]

It might have been thought that given the massive effort which has already been expended on the epothilones, there is little to be gained from still another such exploration in search of new congeners. We show below that this need not be the case. In seeking to deepen our grasp of the epothilone SAR estate, we initially undertook the preparation of 12,13-desoxyepothilone analogues containing a 12-trifluoromethyl group (cf. **2**). Our menu of epothilone synthesis strategies, then in existence, failed to encompass this goal. The problem arose from a breakdown in the feasibility of ring-forming olefin metathesis in a context where we were trying to form a $C_{10}-C_{11}$ olefin with a CF_3 group in place at C_{12} (case 1).[4] Earlier, we had demonstrated that viability for RCM is restored when there is a carbon spacer between the CF_3 group and the RCM reaction center (case 2).[5] Of course, this resulted in formation of a 17-membered macrolide. We wondered whether we could reach the 16-membered ring by moving the acyl side vinyl group to C_8 (see projected case 3).

To explore this new ring-forming strategy (vide infra), we also targeted (*E*)-9,10-dehydro-dEpoB, that is, compound **3**. At the time we undertook this effort, it was thought that it was a known compound, indeed with a disappointing early in vitro screening profile.[6] Hence efforts directed to **3** were viewed solely from the perspective of it functioning as a synthetic intermediate to validate the feasibility of RCM in the context of establishing a C_9-C_{10} double bond. Of course, if all went smoothly, selective reduction of the 9,10-double bond of **3** might constitute an even more efficient route to **1** than is currently practiced[7] (vide infra).

The key findings disclosed herein are as follows. (i) A highly concise route to reach the synthesis targets has been accomplished; (ii) contrary to our expectations, *compound **3** had actually not been prepared before*; this family of epothilones is new; (iii) moreover, early indications suggest that several new (*E*)-9,10-dehydro-dEpoB congeners exhibit highly promising in vitro and in vivo potencies as well as highly encouraging pharmacokinetic properties, rendering them exciting prospects for advancement to become the next generation of tubulin directed anticancer drugs.



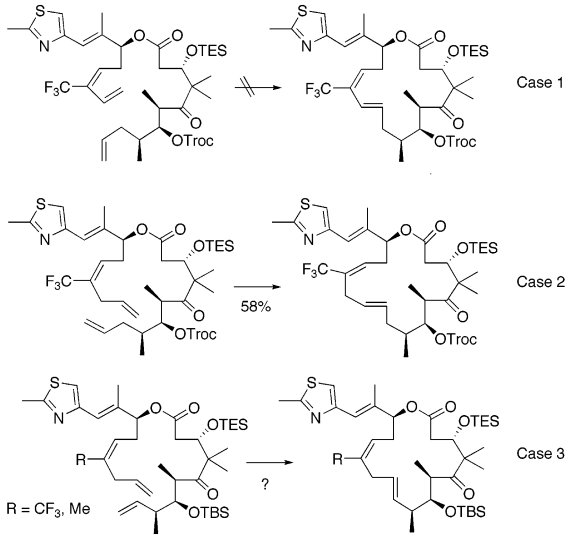
12,13-desoxyEpoB (**1**) (*E*)-9,10-dehydro-12,13-desoxyEpoB (**3**)
26-F_3-12,13-desoxyEpoB (**2**) 26-F_3-(*E*)-9,10-dehydro-12,13-desoxyEpoB (**4**)

Figure 1. Structures of epothilones.

The synthesis of the RCM precursors commenced with the preparation of acyl sector **11** (Scheme 1). Ketone **5**[7c] was subjected to an aldol reaction with the readily available aldehyde **6**.[8] Upon deprotonation and reaction of "lithio" **5** with **6**, smooth condensation gave rise to aldol product **7**, with acceptible diastereoselectivity (dr, 5.7:1). Protection of the C_7 alcohol as a TBS silyl ether followed by hydrolysis of the diisopropyl acetal group afforded keto aldehyde **8**, setting the stage for the second aldol reaction.

Following the previously practiced "titano" *tert*-butyl ester method,[7c] with the new aldehyde **8** as the coupling partner, aldol product **9** was obtained in high diastereoselectivity and high yield (dr > 20:1). Protection of the C_3 alcohol of **9** with a TES silyl group was followed by deprotection of the benzyl ether. The remaining straightforward steps to **11** are shown in Scheme 1.

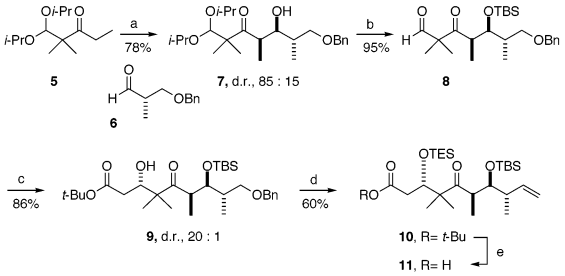
Esterification of the resultant hydroxyketone **12**[9] with the C_1-C_9 acid fragment **11** provided the corresponding RCM cyclization precursor **14** in 81% yield (Scheme 2). The ring-closing metathesis



[†] Laboratory for Bioorganic Chemistry.
[‡] Preclinical Pharmacology Core Facility.
[§] Analytical Pharmacology Core Facility.
[||] Columbia University.

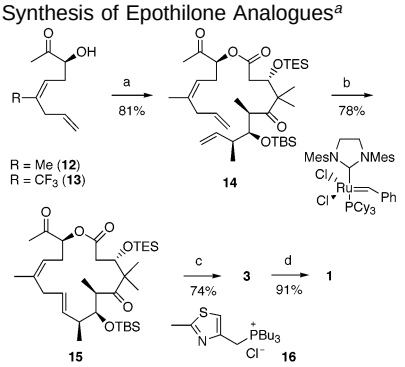
10.1021/ja029695p CCC: $25.00

Scheme 1. Synthesis of Acyl Sector **11**[a]



[a] Reagents and conditions: (a) LDA, THF, −90 °C (78% based on aldehyde); (b) (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, −40 to −20 °C, 97%, (ii) *p*-TsOH·H_2O (cat.), THF−H_2O (4:1), 64 °C, 98% (two steps, 95%); (c) *t*-butyl acetate, LDA, $CpTiCl(OR)_2$ (R = 1,2:5,6-di-*O*-isopropylidene-α-L-glucofuranos-3-*O*-yl), Et_2O, −78 °C, 86%, (dr > 20:1); (d) (i) TESCl, imidazole, DMF, 0 °C to room temperature, 98%, (ii) H_2, Pd/C (10%), EtOH, 83%, (iii) TPAP, NMO, CH_2Cl_2, 95%, (iv) $MePPh_3I$, *n*-BuLi, THF, −78 to −5 °C, 78% (four steps, 60%); (e) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to room temperature.

Scheme 2. Synthesis of Epothilone Analogues[a]



[a] Reagents and conditions: (a) EDCI, DMAP, CH_2Cl_2, **11**, 0 °C to room temperature (81%, starting from **10**, two steps); (b) toluene, 110 °C, 20 min, 78%; (c) (i) KHMDS, **16**, THF, −78 to −20 °C, 76%, (ii) HF-pyridine, THF, 97% (two steps, 74%); (d) $TrisNHNH_2$, Et_3N, $ClCH_2CH_2Cl$, 50 °C, 91%.

reaction[10] of **14** was then carried out in toluene using the recently described Grubbs catalyst.[11] Happily, the reaction afforded exclusively the *trans* isomer **15** in 78% yield. Installation of the thiazole moiety via the protocol[12] shown in Scheme 2 was followed by deprotection of the two silyl ethers with HF-pyridine, thereby leading to **3**. The structure of **3** was rigorously corroborated by its high yielding conversion to **1**.[9] We note in passing that the total synthesis of **1** has been very substantially simplified relative to previously practiced routes. Thus, the use of the readily available **6**, obtained from the chiral pool, is certainly a large improvement relative to reliance on (*S*)-2-methyl-4-pentenal, whose synthesis requires intervening chiral auxiliaries. The new strategy was then successfully applied to the total synthesis of the corresponding trifluoro analogues **2** and **4**.

With compound **3** of rigorously proven structure in hand, we were surprised to find that its spectral properties were not congruent with those previously reported for a compound presumed to be the same entity. The actual structure of the compound previously assigned as **3** has now been reevaluated and will be disclosed in short order.[13] However, it is clear in retrospect that **3** had not been previously prepared and, in fact, the whole family of (*E*)-9,10-dehydroepothilones reported here is a new genus.

Examination of synthetic analogues (**2**−**4**), in cell culture settings, revealed stronger inhibitory effects on various sensitive and MDR tumor cell lines than are exhibited by our clinical entry dEpoB (**1**) (Table 1). We note that **3** is the first 12,13-desoxyepothilone

Table 1. In Vitro Cytotoxicities (IC_{50}) with Tumor Cell Lines[a]

compound	CCRF-CEM(C) (μM)	C/VBL_{100} (μM)	C/Taxol (μM)
1 (dEpoB)	0.0036	0.016	0.0046
2	0.0041	0.080	0.018
3	0.0009	0.0042	0.0012
4	0.0035	0.0210	0.0057

[a] XTT assay following 72 h inhibition. CCRF-CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF-CEM/VBL_{100} cell line is resistant to vinblastine, and CCRF-CEM/Taxol is resistant to taxol.

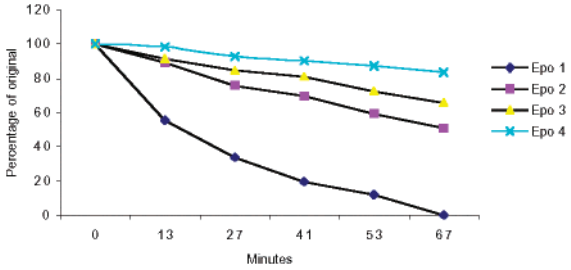


Figure 2. Plasma stability of epothilones **1**−**4** murine plasma.

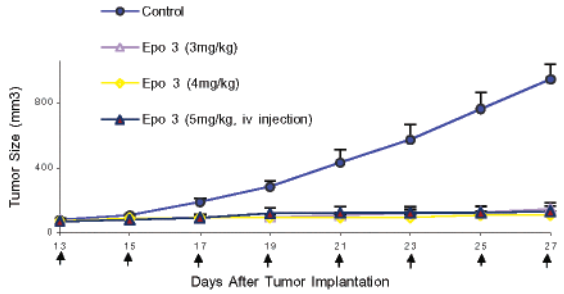


Figure 3. Therapeutic effect of (*E*)-9,10-dehydro-dEpoB (**3**) in nude mice bearing HCT-116 xenograft (iv infusion, Q2Dx7, $n = 3$). Arrows indicate drug administrations.

compound that possesses substantially improved cytotoxicity relative to that of dEpoB (**1**).

The impressive cell growth inhibition exhibited by epothilones **2**−**4** across a range of various drug-resistant tumors prompted determination of the blood plasma stability of these new (*E*)-9,10 congeners. For instance, the recently described (*E*)-10,11-dehydro-dEpoB (of case 1 with a CH_3 group at C_{12}) exhibits very poor plasma stability with respect to lactone opening. It is this plasma instability which has stifled advancement of (*E*)-10,11-dehydro-dEpoB. By contrast, on exposure of **2**−**4** to murine plasma, we observed a much slower drug degradation as compared to dEpoB (**1**) by a factor of 7. This stability constitutes a substantial advance from a drug availability perspective relative to dEpoB (Figures 2 and 3).

The combination of the cytotoxicity and plasma stability data encouraged us to synthesize substantial amounts of **3** to determine its in vivo efficacy, in nude mice bearing human tumor xenografts. *The direct and high quality total synthesis described above allowed us to indulge these interests.* Happily, epothilone **3** demonstrated a markedly improved potency in inhibiting the growth of implanted tumors, relative to dEpoB. The improved potency and plasma stability allows very substantial reduction of drug dosing (*an order of magnitude*) in the context of xenografts of **3**.

In summary, delineated above is a powerful stereoselective total synthesis of **3** and, following site-selective diimide reduction, dEpoB (**1**) itself. The described herein strategy was then straightforwardly applied to the preparation of the corresponding trifluoro analogues **2** and **4**. The data reported above in conjunction with the findings

to be reported in due course point to the emergence of a most promising new family of anticancer drugs appropriate for further evaluation en route to the possible advancement to a human clinical setting. Furthermore, the new synthesis strategy comprises a significant practical improvement in the total synthesis of dEpoB. Parenthetically, the study serves to underscore the potential applicability of target directed total synthesis, even in a multistep setting, in the quest for new substances of material clinical benefit.

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

Note Added after ASAP: In the version of this manuscript published on the Web 2/06/2003, the phrase "Thus, the use of the readily available **11**..." on the second page was incorrect and should read "Thus, the use of the readily available **6**...". The final version published 2/10/2003 and the print version are correct.

Supporting Information Available: Experimental procedures and characterization data for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For extensive reviews in this field, see: (a) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434. (b) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2014. Additional references: (c) Florsheimer, A.; Altmann, K. H. *Expert Opin. Ther. Pat.* **2001**, *11*, 951. (d) Chou, T. C.; Zhang, X. G.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798. (e) Chou, T. C.; Zhang, X. G.; Balog, A.; Su, D.; Meng, D. F.; Savin, K.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642.

(2) Chou, T. C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.

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

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

(5) Rivkin, A.; Njardarson, J. T.; Biswas, K.; Chou, T. C.; Danishefsky, S. J. *J. Org. Chem.* **2002**, *67*, 7737.

(6) White, J. D.; Carter, R. G.; Sundermann, K. F.; Wartmann, M. *J. Am. Chem. Soc.* **2001**, *123*, 5407.

(7) (a) Balog, A.; Harris, C.; Savin, K.; Zhang, X. G.; Chou, T. C. *Angew. Chem., Int. Ed.* **1998**, *37*, 2675. (b) Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Glunz, P. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 7050. (c) Lee, C. B.; Wu, Z.; Zhang, F.; Chappell, M. D.; Stachel, S. J.; Chou, T. C.; Guan, Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 5249. (d) 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.

(8) (a) Cohen, N.; Eichel, W. F.; Lopresti, R. J.; Neukom, C.; Saucy, G. *J. Org. Chem.* **1976**, *41*, 3505. (b) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873. (c) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348.

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

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

(11) For an early instance of macrocyclization in a complex setting via a RCM, see: Sinha, S. C.; Sun, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1381.

(12) Hindupur, R. M.; Panicker, B.; Valluri, M.; Avery, M. A. *Tetrahedron Lett.* **2001**, *42*, 7341. 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.

(13) Private communication from Professor J. D. White.

JA029695P