AD_____

GRANT NUMBER DAMD17-97-1-7146

TITLE: Strategy Toward the Total Synthesis of Epothilones A and B

PRINCIPAL INVESTIGATOR: Dongfang Meng

CONTRACTING ORGANIZATION: Sloan-Kettering Cancer Center
New York, New York 10021-6007

REPORT DATE: July 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1999	Annual (1 Jul 98 - 30 Jun 99)

4. TITLE AND SUBTITLE

Strategy Toward the Total Synthesis of Epothilones A and B

5. FUNDING NUMBERS

DAMD17-97-1-7146

6. AUTHOR(S)

Meng, Dongfang

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Sloan-Kettering Cancer Center
New York, New York 10021-6007

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Epothilones are a class of promising antibreast cancer candidate. They were synthesized by four strategies. Structure – activity relationships were studied with the synthetic compounds. The first in vivo test of synthetic epothilone D is encouraging.

14. SUBJECT TERMS

Breast Cancer antitumor agents Epothilones. Total synthesis. Structure – activity relationships. In vivo test.

15. NUMBER OF PAGES 108

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

✓ Where copyrighted material is quoted, permission has been obtained to use such material.

✓ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

July 20, 89

PI - Signature Date

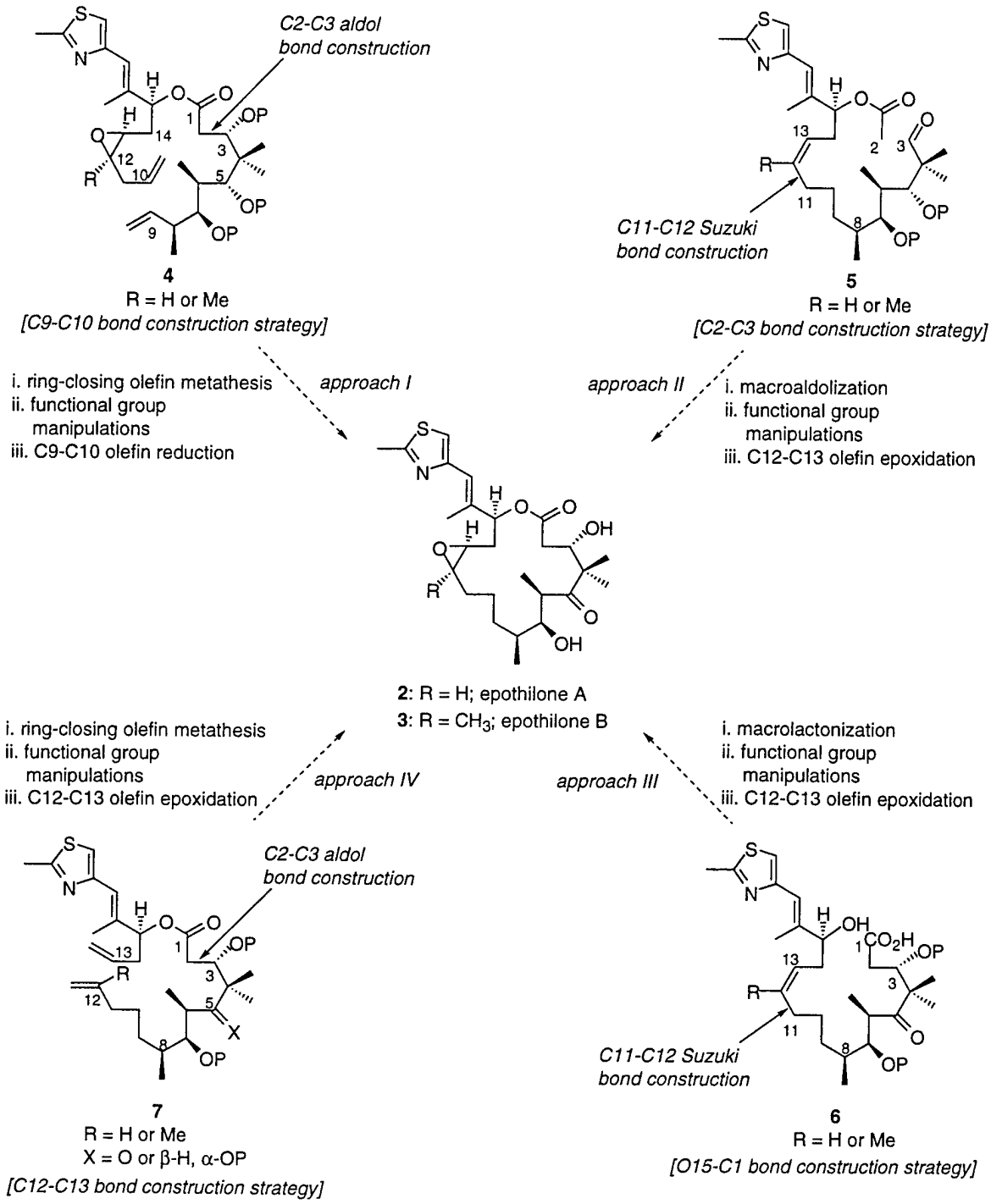
Table of Contents

The epothilones, isolated from the cellulose-degrading myxobacterium *Sorangium cellulosum,* are the first class of microtubule(MT)-stabilizing agents with a taxol-like mechanism of action since the original discovery of taxol. [1,2,3] *In vitro* and in cultured cells, the epothilones mimic all of the biological effects of taxol, one of the most effective drugs for the treatment of breast cancer. Competition binding studies reveal that they share the same MT-binding site and bind with an affinity comparable to that of taxol. It is significant that the epothilones appear to possess several advantages over taxol. First, they exhibit a much lower drop in potency compared to taxol against a multiple drug-resistant cell line. Second, they have much better solubility in water. Third, relative to taxol, the epothilones would appear to be more manageable as targets for total synthesis. It is conceivable that the epothilones could emerge as promising drug candidates for the treatment of breast cancer . With respect to clinical utility, these substances may prove superior to taxol. At the present time, it is difficult to obtain significant quantities of the epothilones through fermentation. It remained to be determined whether or not the epothilones and analogues could be obtained in useful amounts by total synthesis.

In the proposal, a convergent and stereocontrolled path for a total synthesis of epothilones A and B was described. The long term goals included:

1) Total syntheses of epothilones under academic level;
2) Under the guidance of Molecular Modeling studies, design and synthesize simplified epothilone analogues and evaluate their potential as chemotherapeutic agents for the treatment of breast cancer;
3) The development of a practical synthesis of epothilones which would impact on the actual availability of the drug for serious pre-clinical and clinical evaluation. *

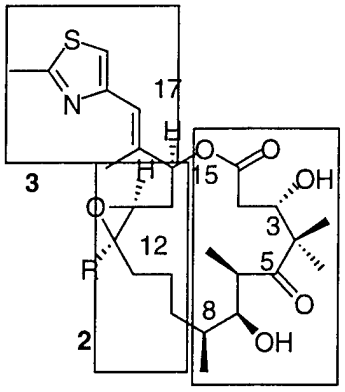
What has been achieved in the past two years in this lab includes:



Scheme 1. Bond construction strategies for total syntheses of epothilone A (**2**) and epothilone B (**3**).

1) Chemically , four synthetic strategies have been attemped (see scheme 1), three of which were successful.[4, 5, 6, 7, 8, 10] Some new methods and new reaction sequences have been used : a) an unprecedented stereocontrolled macroaldolization was used to close the 16 member ring; b) ring-closing olefin metathesis(RCM), as an arising powerful C-C bond formation, was systematically studied on the most complicated substrates. The

theoretically safest strategy to close the ring at C9 – C10 using RCM failed although model compounds worked well in the presence of epoxide and thiozal double bond. The remote functional groups and catalysts both affected the E/Z ratio of the newly formed double bond from metathesis. The trisubstituted olefin required a more reactive molybdenum catalyst without any free hydroxy group on the substrate.

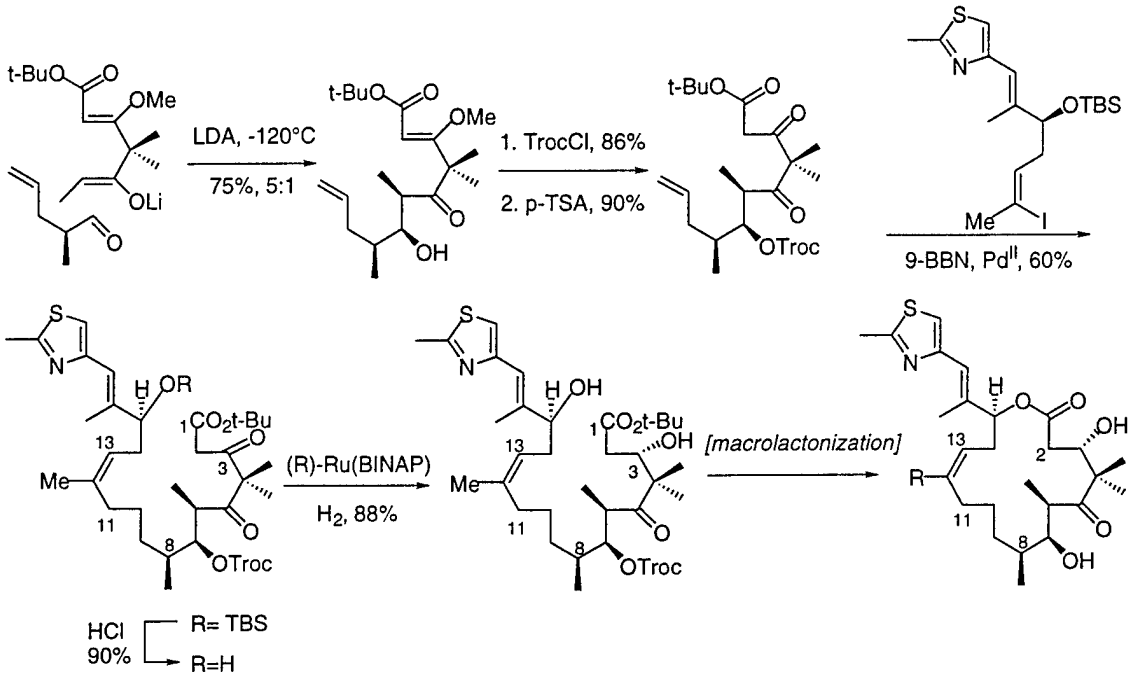


1: epothilone B **1**

scheme 2. The three arbitrarily defined sectores of the epothilones

2) Biologically, the cytotoxicity and tubulin polymerization of epothilones and important synthetic intermediates were studied [4, 5, 6, 8, 10]. Furthermore, a library of epothilone analogues were synthesized for the structure-activity relationship study.[9,11] The first in vivo test was done with synthetic epothelones[11]. The result demonstrate that (scheme 2): a) Epothilones are hundreds to thousands times more effective than Taxol to some drug resistant cell lines and show similar MT-stabilizing efficiency; b) The efficacy is very sensitive to ring size and the modification of the acyl sector (sector 1, C1-C8), sector 2 (C9-C15) and 3 (aryl side chain) are more tolerant although C16-C17 spacer is very important.

*The biologiacal test has been done in some other labs.



Scheme 3

3) A practical synthesis of epothilones has been accompleshed based on the academic level syntheses (see scheme 3). This synthesis provided grams of the epothilones for serious pre-clinical and clinical evaluation (see figure 1)[12,13,14].

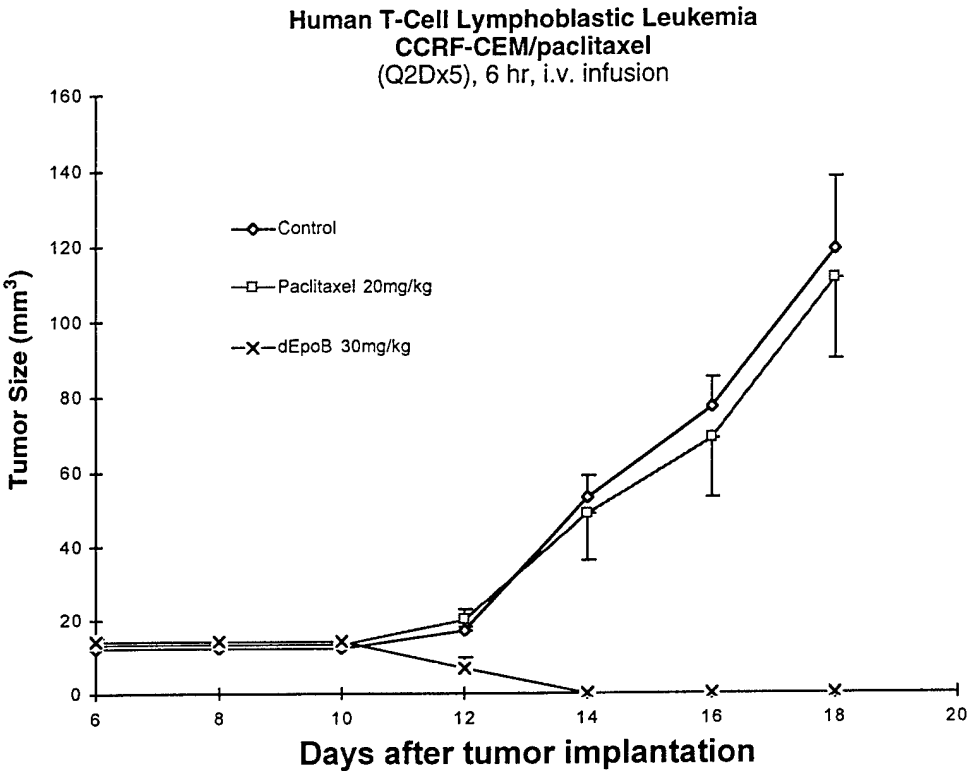


Figure 13. Therapeutic effects of administration of paclitaxel (20 mg/kg) and dEpoB (30 mg/kg) on the human T-cell lymphoblastic leukemia CCRF-CEM/paclitaxel following (Q2Dx5), 6 hr, i.v. infusion. Human T-cell lymphoblastic leukemia (CCRF-CEM/paclitaxe) cells resistant to paclitaxel were inoculated subcutaneously (10^7 cells) into athymic mice on day 0. Every other day, i.v. infusion was given on day 6, 8, 10, 12, 14, 16, and 18. The average tumor volume of the control group on day 12, 14, and 16 was 20±3, 119±22, and 415±62 mm^3, respectively, (mean±SEM, n=3). The vehicle for 6 hr i.v. infusion was 100 mL (Cremophor:ethanol, 1:1) + 3 mL saline

In conclusion, the primary goals of the proposal have been reached, the results showed epothilones are promising to cure breast cancer.

After all of goals in the proposal were accomplished, the total synthesis of another group of potential anti cancer compounds CP-225,917 and CP-263,114 were initiated. The first total synthesis of 7-epi-CP-225,917 and 7-epi-CP-263,114 had been accomplished[15,16,17,18 and 19]. The biological evaluation of the compounds are under going.

References

1) Epothilone, deren Herstellungsverfahren sowie sie enthaltende Mittel". G. Hofle, N. Bedorf, K. Gerth & H. Reichenbach DE 41 38 042 Al, published May 27, **1993** .

2) Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action". D.M. Bollag, P.A. McQuency, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Lazarides & C.m.Woods. *Cancer Res.* **1995,** 55, 2325-2333.

3) Epothilones A and B : Antifungal and Cytotoxic Compounds from *Sorangium cellulosum* (Myxobacteria) Production, Physico- chemical and Biological Properties. K. N.Gerth, G. Bedorf, G. Hofle, H. Irschik & H. Reichenbach: *J. of Antibiotics* **1996,** 49, 560-563.

4) Studies toward a Synthesis of Epothilone A: Use of Hydropyran Template for the Management of Acyclic Stereochemical Relationships, **D. Meng**, E. J. Sorenson, P. Bertinato, **S. J. Danishefsky**, *J. Org. Chem.* **1996**, 61, 7998.

5) Studies toward a Synthesis of Epothilone A: Stereocontrolled Assembly of the Acyl Region and Models for Macrocyclization, P. Bertinato, E. J. Sorenson, **D. Meng**, **S. J. Danishefsky**, *J. Org. Chem.* **1996**, 61, 8000.

6) Total Synthesis of (-)-Epothilone A, A. Balog, **D. Meng**, T.Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorenson, **S. J. Danishefsky**, *Angew. Chem. Int. ED. Engl.* **1996**, 35, 2801.

7) Remote Effects in Macrolide Formation Through Ring Forming Olefin Metathesis: An Application to the Synthesis of Fully Active Epothilone Congeners, **D. Meng**, D.-S. Su, P. A. Balog, Bertinato, E. J. Sorenson, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *J. Am. Chem. Soc.*, **1997** ,119, 2773.

8) The Total Synthesis of (-)-Epothilone B: An Extension of the Suzuki Coupling Method and Insights Into Structure-Activity Relation of the Epothilones, D.-S. Su, **D. Meng**, P. Bertinato, A. Balog, E. J. Sorenson, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, *Angew. Chem. Int. ED. Engl.* **1997**, 36, 757.

9) Stereoselective Syntheses and Evaluation of Compounds in the 8-Desmethylepothilone A Series: Some Surprising Observations Regarding Their Chemical and Biological Properties, A. Balog, P. Bertinato, D.-S. Su, **D. Meng**, E. J. Sorenson, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *Tet. lett.* **1997**, 38, 4529

10) The Total synthesis of Epothilones A and B. **D. Meng**, P. Bertinato, A. Balog, D.-S. Su, T.Kamenecka, E. J. Sorenson, **S. J. Danishefsky**, *J. Am. Chem. Soc.* **1997** ,119, 10073.

11) Structure-Activity Relationships of the Epothilones and the First In Vivo Comparison with Paclitaxel. D.-S. Su, A. Balog, **D. Meng**, P. Bertinato, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *Angew. Chem. Int. ED. Engl.* **1997**, 36, 2093.

12) Farnesyl Transferase Inhibitors Cause Enhanced Mitotic Sensitivity to Taxol and Epothilones. M. M. Moasser, L. Sepp – Lorenzino, N. E. Kohl, **D. Meng**, A. Balog, D.-S. Su, **S. J. Danishefsky**, N. Rosen , *Proc. Nat. Acad. Sci.* **1998**, 95, 1369.

13) Desoxyepothilone B: an Efficacious Microtubule – targeted Antitumor Agent with a Promising *in vivo* Profile Relative to Epothilone B. T.-C. Chou, X. G. Zhang, A. Balog, D.-S. Su, **D. Meng**, K. Savin, J. R. Bertino, **S. J. Danishefsky**, *Proc. Nat. Acad. Sci.* **1998**, 95, 9642

14) The Microtubule – stabilizing Agents Epothilones A and B and Their Desoxy – derivatives Induce Mitotic Arrest and Apoptosis in Human Prostate Cancer Cells. L. Sepp – Lorenzino, A. Balog, D. –S. Su, **D. Meng**, N. Tmaul – Holmes, H. I. Scher, **S. J. Danishefsky** and N. Rosen. *Prostate Cancer and Prostatic Diseases*, **1999**, 2, 41

15) A Useful α, α– Annulation Reaction of Enamine. A. Frontier, **D. Meng, S. J. Danishefsky**. *Tetrahedron*, **1998**, 54, 12721.

16) An Approach to the Total Syntheses of CP – 225, 917 and CP – 263, 114: Creation of a Matrix Structure by Sequential Aldol Condensation and Intramolecular Heck Ring Closure. O. Kwon, D.-S. Su, **D. Meng**, W. Deng, D. C. D'Amico, **S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* **1998**, 37, 1877.

17) A Stereospecific Geminal Alkylation Scheme en route to CP – 225, 917 and CP – 263, 114. O. Kwon, D.-S. Su, **D. Meng**, W. Deng, D. C. D'Amico, **S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* **1998**, 37, 1880.

18) A Stereospecific Thio-Mediated Cleavage of a Spirocyclobutanone: Synthesis of a fully Functional Precursor to the CP Compounds **D. Meng, S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* **1998**, 38, 1485.

19) Discovery Through Total Synthesis of the Remarkable Chemistry at C7 in the CP Compounds: Is CP263,114 A Fermentation Product? **D. Meng**, Qiang Tan and **S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* accepted

Key Research Accomplishments:

- the first total syntheses of epothilones A, B, C and D;

- the first structure-activity relationship study of epothilones A, B, C and D;

- the first in vivo test of epothilones A, B, C and D;

- the first practical synthesis of epothilones A, B, C and D;

- the first total syntheses of 7-epi-CP-225,917 and 7-epi-CP-263,114.

Reportable Outcome:

-Publications:

1) Studies toward a Synthesis of Epothilone A: Use of Hydropyran Template for the Management of Acyclic Stereochemical Relationships, **D. Meng**, E. J. Sorenson, P. Bertinato, **S. J. Danishefsky**, *J. Org. Chem.* **1996**, 61, 7998.

2) Studies toward a Synthesis of Epothilone A: Stereocontrolled Assembly of the Acyl Region and Models for Macrocyclization, P. Bertinato, E. J. Sorenson, **D. Meng**, **S. J. Danishefsky**, *J. Org. Chem.* **1996**, 61, 8000.

3) Total Synthesis of (-)-Epothilone A, A. Balog, **D. Meng**, T.Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorenson, **S. J. Danishefsky**, *Angew. Chem. Int. ED. Engl.* **1996**, 35, 2801.

4) Remote Effects in Macrolide Formation Through Ring Forming Olefin Metathesis: An Application to the Synthesis of Fully Active Epothilone Congeners, **D. Meng**, D.-S. Su, P. A. Balog, Bertinato, E. J. Sorenson, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *J. Am. Chem. Soc.*, **1997** ,119, 2773.

5) The Total Synthesis of (-)-Epothilone B: An Extension of the Suzuki Coupling Method and Insights Into Structure-Activity Relation of the Epothilones, D.-S. Su, **D. Meng**, P. Bertinato, A. Balog, E. J. Sorenson, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, *Angew. Chem. Int. ED. Engl.* **1997**, 36, 757.

6) Stereoselective Syntheses and Evaluation of Compounds in the 8-Desmethylepothilone A Series: Some Surprising Observations Regarding Their Chemical and Biological Properties, A. Balog, P. Bertinato, D.-S. Su, **D. Meng**, E. J. Sorenson, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *Tet. lett.* **1997**, 38, 4529

7) The Total synthesis of Epothilones A and B. **D. Meng**, P. Bertinato, A. Balog, D.-S. Su, T.Kamenecka, E. J. Sorenson, **S. J. Danishefsky**, *J. Am. Chem. Soc.* **1997** ,119, 10073.

8) Structure-Activity Relationships of the Epothilones and the First In Vivo Comparison with Paclitaxel. D.-S. Su, A. Balog, **D. Meng**, P. Bertinato, **S. J. Danishefsky**, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *Angew. Chem. Int. ED. Engl.* **1997**, 36, 2093.

9) Farnesyl Transferase Inhibitors Cause Enhanced Mitotic Sensitivity to Taxol and Epothilones. M. M. Moasser, L. Sepp – Lorenzino, N. E. Kohl, **D. Meng**, A. Balog, D.-S. Su, **S. J. Danishefsky**, N. Rosen , *Proc. Nat. Acad. Sci.* **1998**, 95, 1369.

10) Desoxyepothilone B: an Efficacious Microtubule – targeted Antitumor Agent with a Promising *in vivo* Profile Relative to Epothilone B. T.-C. Chou, X. G. Zhang, A. Balog, D.-S. Su, **D. Meng**, K. Savin, J. R. Bertino, **S. J. Danishefsky**, *Proc. Nat. Acad. Sci.* **1998**, 95, 9642

11) The Microtubule – stabilizing Agents Epothilones A and B and Their Desoxy – derivatives Induce Mitotic Arrest and Apoptosis in Human Prostate Cancer Cells. L. Sepp – Lorenzino, A. Balog, D. –S. Su, **D. Meng**, N. Tmaul – Holmes, H. I. Scher, **S. J. Danishefsky** and N. Rosen. *Prostate Cancer and Prostatic Diseases*, **1999**, 2, 41

12) A Useful α, α– Annulation Reaction of Enamine. A. Frontier, **D. Meng**, **S. J. Danishefsky**. *Tetrahedron*, **1998**, 54, 12721.

13) An Approach to the Total Syntheses of CP – 225, 917 and CP – 263, 114: Creation of a Matrix Structure by Sequential Aldol Condensation and Intramolecular Heck Ring Closure. O. Kwon, D.-S. Su, **D. Meng**, W. Deng, D. C. D'Amico, **S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* **1998**, 37, 1877.

14) A Stereospecific Geminal Alkylation Scheme en route to CP – 225, 917 and CP – 263, 114. O. Kwon, D.-S. Su, **D. Meng**, W. Deng, D. C. D'Amico, **S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* **1998**, 37, 1880.

15) A Stereospecific Thio-Mediated Cleavage of a Spirocyclobutanone: Synthesis of a fully Functional Precursor to the CP Compounds **D. Meng, S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* **1998**, 38, 1485.

16) Discovery Through Total Synthesis of the Remarkable Chemistry at C7 in the CP Compounds: Is CP-263,114 A Fermentation Product? **D. Meng**, Qiang Tan and **S. J. Danishefsky**, *Angew. Chem. Int. Ed. Engl.* accepted

Patent:

- A process for a stereocontrolled total synthesis of the micritubule stabilizing agents Epothilones A and B and related analogs, **D. Meng**, S. J. Danishefsky, et al, .in pending.

Degrees Obtained:

Master of Science, Columbia University, 1998, June.
(Ph.D. defense will be held in this summer.

Employment;

Offers from Several Pharmaceutical Companies. The offer from Merck is taken.

Studies toward a Synthesis of Epothilone A: Use of Hydropyran Templates for the Management of Acyclic Stereochemical Relationships

Dongfang Meng,[†,‡] Erik J. Sorensen,[†]
Peter Bertinato,[†] and Samuel J. Danishefsky*[,†,‡]

Laboratory for Bioorganic Chemistry, 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 August 30, 1996

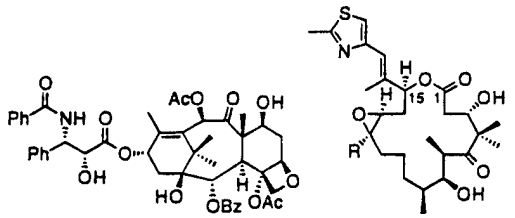
Taxol has been approved for chemotherapeutic clinical application against ovarian carcinomas. It is also undergoing extensive evaluation for other indications. While taxol is not a curative agent, it is already a useful chemotherapeutic resource.[1]

The best indications arising from tissue culture and *in vitro* experiments are that taxol functions by inhibition of cellular mitosis through binding to and stabilization of microtubule assemblies.[2] Presumably, this property is pertinent to the human patient.

Unfortunately, taxol is far from an ideal drug. Thus, difficulties with respect to formulation and susceptibility to multiple drug resistance (MDR) complicate its applicability.[3] At the present writing, no major improvements in drug performance have been realized from any substantially modified analogs of taxol or its close relative, taxotere.[4]

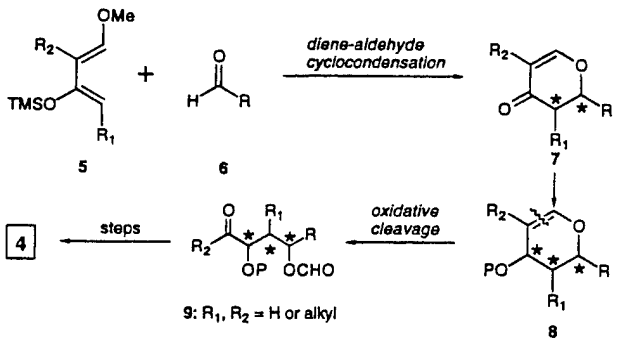
New agents that function by microtubule stabilization are clearly of great interest.[2] In this connection, there has already been considerable attention directed toward the bacterial-derived metabolites epothilone A (**2**) and B (**3**), which were first identified as antifungal cytotoxic agents by Höefle *et al.*[5a,b] and subsequently encountered by a group based at the Merck corporation.[6] The report of the Merck scientists on the epothilones indicated that they are powerful cytotoxic agents that seem to function through stabilization of microtubules by binding to taxol-binding domains. Given the possibilities that these agents themselves, or appropriately modified derivatives, might function as alternatives to taxol, attention from the standpoint of organic synthesis is warranted.

Augmenting the biological rationale for such a venture are the chemical incentives associated with several novel structural features of the epothilones. Thus, the presence of a thiazole moiety, as well as a *cis* epoxide and geminal dimethyl groups are among the issues to be addressed. Not the least intriguing feature is the array of three contiguous methylene groups that serves to insulate the two functional domains of the molecules. This achiral "spacer element" actually complicates prospects for continuous chirality transfer and seems to call for a strategy of merging two stereochemically committed substructures. Herein, we direct our attention to a synthesis of compound **4**, confident that, in principle, such a structure could be converted to the epothilones themselves, and to related screening candidates.



1: taxol™ **2**: R = H; epothilone A **3**: R = CH_3; epothilone B

The identification of compound **4** as a synthetic intermediate provided an opportunity to illustrate the power of hydropyran matrices in addressing problems associated with the control of stereochemistry in acyclic intermediates. Some years ago, we described the synthesis of dihydropyrones through what amounts to overall cyclocondensation of suitably active dienes and aldehydic heterodienophiles.[7]



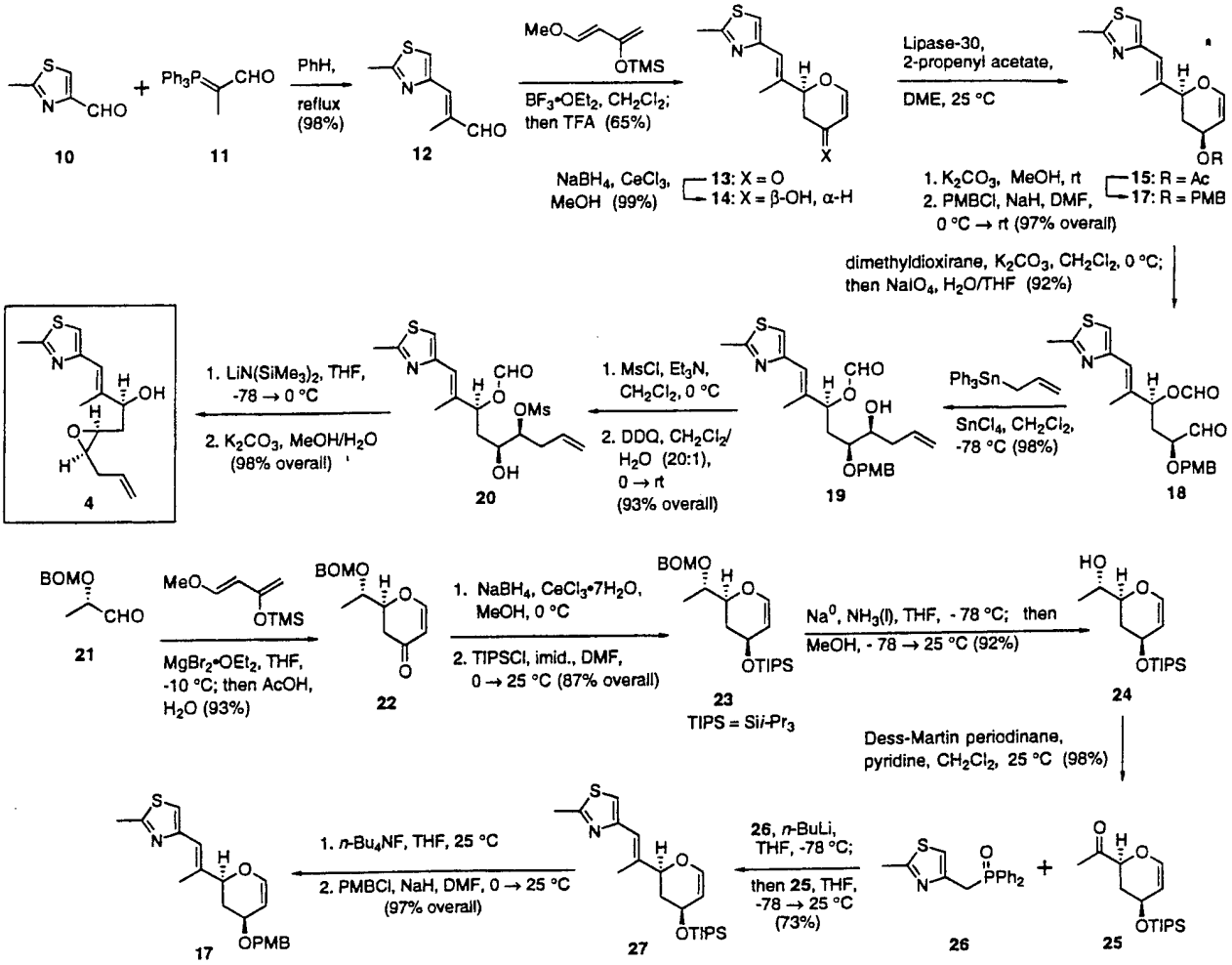
High margins of stereoselectivity can be realized in assembling such matrices (*cf.* **5** + **6** → **7**). Moreover, the hydropyran platforms service various stereoselective reactions (see formalism **7** → **8**). Furthermore, the products of these reactions are amenable to ring-opening schemes, resulting in the expression of acyclic fragments with defined stereochemical relationships (*cf.* **8** → **9**).[8]

We describe the application of two such routes for the synthesis of compound **4**. Route 1, which does not *per se* involve control over the issue of absolute configuration, commences with the known aldehyde **10**.[9] Homologation, as shown, provided enal **12**. Cyclocondensation of **12** with the known diene,[10] under BF_3 catalysis, led to racemic dihydropyrone **13**. Luche reduction[11] of **13** provided compound **14**. At this point we were well positioned to take advantage of our previously introduced lipase methodology for resolution of glycal derivatives through enzymatically mediated kinetic resolution.[12] Thus, carbinol **14** was treated with lipase 30 and isopropenyl acetate (following the prescriptions of Wong),[13] and

† Sloan-Kettering Institute for Cancer Research.
‡ Columbia University.

(1) Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. *Taxane Anticancer Agents*; American Cancer Society: San Diego, 1995.
(2) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature (London)* **1979**, *277*, 665.
(3) Landino, L. M.; Macdonald, T. L. In *The Chemistry and Pharmacology of Taxol® and its Derivatives*; Farin, V., Ed.; Elsevier: New York, 1995; Chapter 7, p 301.
(4) Gueritte-Voegelein, F.; Guenard, D.; Dubois, J.; Wahl, A.; Marder, R.; Muller, R.; Lund, M.; Bricard, L.; Potier, P. In *Taxane Anticancer Agents*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; American Cancer Society: San Diego, 1995; Chapter 14, p 189.
(5) (a) Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560. (b) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567.
(6) Bollag, D. M.; McQueney, P. A. Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.
(7) Danishefsky, S. J. *Aldrichim. Acta* **1986**, *19*, 59.
(8) Danishefsky, S. J. *Chemtracts* **1989**, *2*, 273.
(9) (a) Shafiee, A.; Mazloumi, A.; Cohen, V. I. *J. Heterocycl. Chem.* **1979**, *16*, 1563. (b) Schafiee, A.; Shahocini, S. *J. Heterocycl. Chem.* **1989**, *26*, 1627.
(10) Danishefsky, S. J.; Kitahara, T. *J. Am. Chem. Soc.* **1974**, *96*, 7807.
(11) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

S0022-3263(96)01673-8 CCC: $12.00



the reaction was stopped after *ca.* 48% conversion, providing acetate **15** in addition to the enantiomerically related free glycal **16**. Compound **15** was further advanced to the *p*-methoxybenzyl (PMB)-protected system **17**. At this juncture, reaction of **17** with dimethyldioxirane[14] generated an intermediate (presumably the corresponding glycal epoxide) that, upon treatment with sodium metaperiodate, gave rise to aldehyde formate **18**. Lewis acid-promoted allylation of **18** afforded carbinol **19** in which the formate ester had nicely survived. Unfortunately, **19** was accompanied by its *anti* stereoisomer (not shown here) (4:1). Although this 4:1 mixture of diastereomers was obtained in an excellent yield of 98%, it was not possible to obtain the desired stereoisomer **19** in pure form at this stage. Mesylation of the secondary alcohol, followed by deprotection (see **19** → **20**) and cyclization, as indicated, gave compound **4**, a substance that could be separated from the stereoisomeric *trans* epoxide.

Needless to say, in this synthesis, only *ca.* half of the dihydropyrone was secured through the process of kinetic resolution. While, in theory, several of our synthetic strategems contemplate the possible use of each enantiomer of **15** to reach epothilone itself, we sought to implement another route to allow for full enantiomeric convergence. The logic of this route is that the chirality of a "dummy" stereogenic center is communicated to the emerging pyran following previously established principles of tunable diastereoselection in the cyclocondensation reaction.[7,8] We proceeded as follows. Cyclocondensation of lactaldehyde derivative **21**[15] with the indicated diene, under ostensible chelation control, afforded **22**. The side chain ether could then be converted to the methyl ketone **25** as shown (see **22** → **23** → **24** → **25**). Finally, an Emmons condensation of **25** with the phosphine oxide **26**[16] as shown in Scheme 4 afforded compound **27** as a single geometrical isomer. A straightforward protecting group adjustment then afforded the previously encountered **17**. This route cogently illustrates the concept of stereochemical imprinting through a carbon center that eventually emerges in planar form after conferring enantioselection to subsequently derived stereocenters. The use of the dihydropyrone-based logic for securing the stereochemical elements of the epothilones, as well as the identification of a possible strategy for macrocyclization will be described in the paper that follows.

Acknowledgment. This work was supported by NIH Grant No. CA 28824. Postdoctoral Fellowships are gratefully acknowledged by E.J.S. (NSF, CHE-9504805) and P.B. (NIH, CA 62948).

Supporting Information Available: Experimental procedures and spectroscopic data for the compounds illustrated in the reactions (compounds **12**–**15**, **17**–**20**, **4**, and **22**–**27**) (9 pages).

JO961673W

(12) (a) Berkowitz, D. B.; Danishefsky, S. J. *Tetrahedron Lett.* **1991**, *32*, 5497. (b) Berkowitz, D. B.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1992**, *114*, 4518.

(13) Hsu, S.-H.; Wu, S.-S.; Wang, Y.-F.; Wong., C.-H. *Tetrahedron Lett.* **1990**, *31*, 6403.

(14) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, 35, 1381.

(15) Heathcock, C. H.; Young, S. D.; Hagen J. P.; Pirrung, M. C. White, C. T.; VanDerveer, D. *J. Org. Chem.* **1980**, *45*, 3846.

(16) The known 2-methyl-4-chloromethylthiazole (see: Marzoni, G. *J. Heterocycl. Chem.* **1986**, *23*, 577) was transformed to **26** (*cf.* Toh, H. T.; Okamura, W. H. *J. Org. Chem.* **1983**, *48*, 1414.

Studies toward a Synthesis of Epothilone A: Stereocontrolled Assembly of the Acyl Region and Models for Macrocyclization

Peter Bertinato,† Erik J. Sorensen,†
Dongfang Meng,†,‡ and Samuel J. Danishefsky*,†,‡

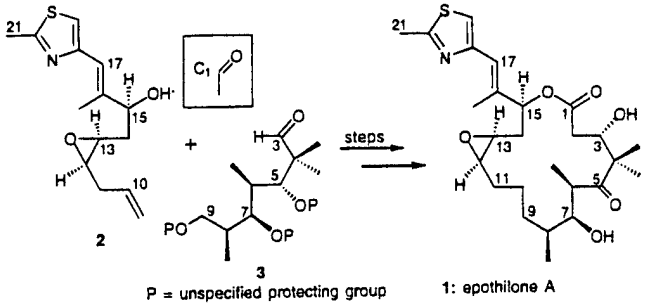
Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027

Received August 30, 1996

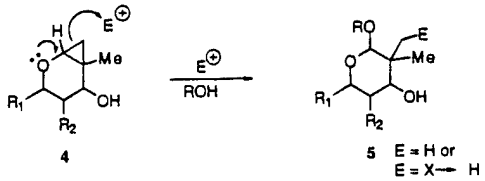
In our previous paper, we described a synthesis of the "alkoxy" segment of epothilone A **1** (see compound **2**, Scheme 1) encompassing carbons 10–21.[1] In this paper, we address the synthesis of another fragment encoding the stereochemical information of acyl section carbons 3–9. It was envisioned that the aldehydo center (C_3) of the formal target **3** would serve as an attachment site to a nucleophilic construct derived from compound **2** (requiring placement of a two-carbon insert, as suggested in Scheme 1), through either inter- or intramolecular means. In such a context, it would be necessary to deal independently with the stereochemistry of the secondary alcohol center eventually required at C_3. One of the interesting features of system **3** is the presence of geminal methyl groups at carbon 4 (epothilone numbering). It was our hope to again use a dihydropyran strategy to assemble a cyclic matrix corresponding, after appropriate disassembly, to a viable equivalent of system **3**. *We hoped to expand upon our dihydropyran paradigm to include the synthesis of gem dimethyl containing cyclic and acyclic fragments.* The particular reaction type we had in mind for this purpose is generalized under the heading of transformation of **4** → **5** (see Scheme 2). At this juncture, we deliberately avoid commitment as to the nature of the electrophile, E. Accordingly, we leave for the moment unaddressed the question as to whether a reduction would or would not be necessary in going from structure type **5** to reach the intended generalized target **3**.

Once again, our opening step consisted of a stereochemically tunable version of the diene–aldehyde cyclocondensation reaction[2] (Scheme 3)—in this instance drawing upon chelation control in the merger of the readily available enantiomerically homogeneous aldehyde **6** with the known diene **7**.[3] Indeed, as precedent would have it, under the influence of titanium tetrachloride there was produced substantially a single isomer shown as compound **8**.[4] In the usual and stereochemically reliable way,[5] the dihydropyrone was reduced to the corresponding glycal **9**. At this point, we utilized a directed Simmons–Smith reaction for the conversion of glycal **9** to cyclopropane **10**.[6] This compound is indeed an interesting structure in that it corresponds in one sense to a cyclopropano version of a *C*-glycoside. At the same time, the cyclopropane is part of a cyclopropylcarbinyl alcohol system with attendant possibilities for rearrangement.[7] It was our intention to cleave the *C*-glycosidic bond of the cyclopropane in a fashion that would elaborate the geminal methyl groups, leaving in its wake a solvent-derived glycoside with the desired aldehyde oxidation state at C-3 (see hypothesized transformation **4** → **5**, Scheme 2). In early efforts, the nonoxidative version of the projected reaction (*i.e.*, $E^+ = H^+$) could not be reduced to practice. Instead, products clearly attributable to the ring-expanded system **11**[8] were identified.

Scheme 1. Convergent Strategy for a Total Synthesis of Epothilone A (1)



Scheme 2. Glycal Cyclopropane Solvolysis Strategy for the Introduction of Geminal Methyl Groups



Fortunately, however, the desired sense of cyclopropane opening, under the influence of the ring oxygen, was achieved by subjecting compound **10** to oxidative opening with *N*-iodosuccinimide.[9] The intermediate iodomethyl compound, obtained as a methyl glycoside **12**, when exposed to the action of tri-*n*-butyltin hydride, gave rise to pyran **13** containing the geminal methyl groups. Protection of this alcohol (see **13** → **14**), followed by cleavage of the glycosidic bond, revealed the acyclic dithiane derivative **15** which can serve as a functional version of the hypothetical aldehyde **3**.

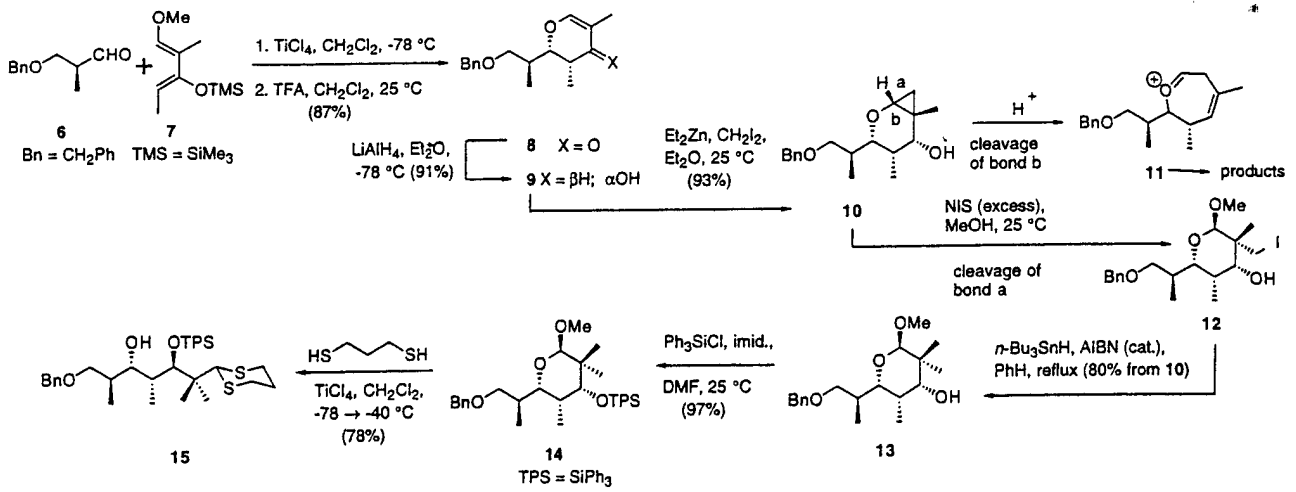
We have also begun to explore possible ways of combining fragments relating to **2** and **3** in a fashion to reach epothilone and congeners thereof. Mindful of the pio-

† Sloan-Kettering Institute for Cancer Research.
‡ Columbia University.

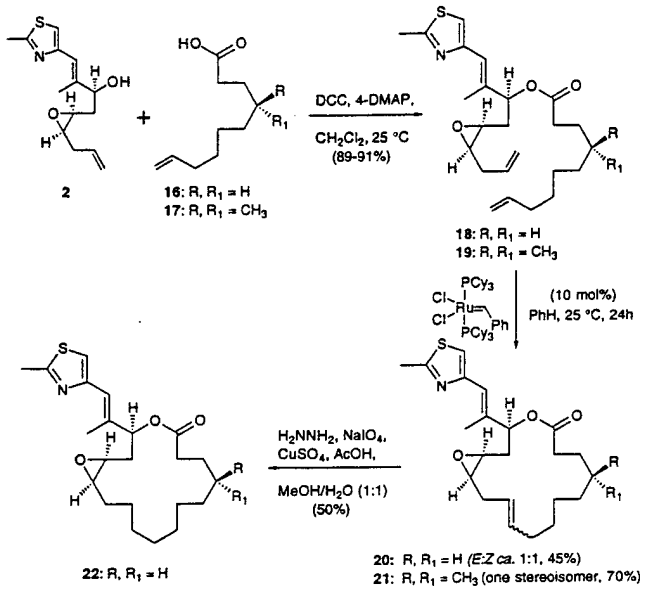
(1) Meng, D.; Sorensen, E. J.; Bertinato, P.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 7998–7999.
(2) Danishefsky, S. J. *Aldrichim. Acta* **1986**, *19*, 59.
(3) Danishefsky, S. J.; Yan, C.-F.; Singh, R. K. Gammill, R. B.; McCurry, P. M., Jr.; Fritsch, N.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101*, 7001.
(4) (a) Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F.; Maring, C. J.; Springer, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1256. (b) Danishefsky, S. J.; Myles, D. C.; Harvey, D. F. *J. Am. Chem. Soc.* **1987**, *109*, 862.
(5) Danishefsky, S. J. *Chemtracts Org. Chem.* **1989**, *2*, 273.
(6) (a) Winstein, S.; Sonnenberg. J. *J. Am. Chem. Soc*, **1961**, *83*, 3235. (b) Dauben, W. G.; Berezin, G. H. *J. Am. Chem. Soc.* **1963**, *85*, 468. (c) Furukawa, J.; Kawabata, N.; Nishimura, J. *Tetrahedron* **1968**, *24*, 53. For selected examples, see: (d) Boeckman, R. K., Jr.; Charette, A. B.; Asberom, T.; Johnston, B. H. *J. Am. Chem. Soc.* **1991**, *113*, 5337. (e) Hoberg, J. O.; Bozell, J. J. *Tetrahedron Lett.* **1995**, *36*, 6831.
(7) Wenkert, E.; Mueller, R. A.; Reardon, E. J., Jr.; Sathe, S. S.; Scharf, D. J.; Tosi, G. *J. Am. Chem. Soc.* **1970**, *92*, 7428.
(8) For example, exposure of **10** to acidic methanol gave rise to an epimeric mixture of seven- membered mixed acetals, presumably through the addition of methanol to oxocarbenium ion **11**. This most interesting transformation is under active study in our laboratory.
(9) For interesting Hg(II)-induced solvolyses of cyclopropanes that are conceptually similar to the conversion of **10** to **12**, see: (a) Collum, D. B.; Still, W. C.; Mohamadi, F. *J. Am. Chem. Soc.* **1986**, *108*, 2094. (b) Collum, D. B.; Mohamadi, F.; Hallock, J. S. *J. Am. Chem. Soc.* **1983**, *105*, 6882. Following this precedent, we did, in fact, accomplish a Hg(II)-induced solvolysis of cyclopropane **10**, although this transformation proved to be less efficient than the reaction shown in Scheme 3.

S0022-3263(96)01674-X CCC: $12.00

Scheme 3. Enantioselective Synthesis of Compound 15



Scheme 4. Construction of Epothilone Model Systems 20–22 by Ring-Closing Olefin Metathesis



neering studies of Schrock[10a] and Grubbs[10b] and the recent ground-breaking disclosure of Hoveyda,[11] we wondered about the possibility of realizing such an approach *en route* to our goal.[12] The matter was first examined with two model ω-unsaturated acids **16** and **17** that were used to acylate alcohol **2** to provide esters **18** and **19**, respectively (see Scheme 4). These compounds did indeed undergo olefin metathesis macrocyclization in the desired manner under the conditions shown. In the case of substrate **18**, **20** was obtained as a mixture of *E*- and *Z*- stereoisomers (*ca.* 1:1). Diimide reduction of **20** was then conducted to provide homogeneous **22** in 50% yield. The olefin metathesis reaction was also extended to compound **19** bearing geminal methyl groups corresponding to their placement at C4 of epothilone A. Once again, olefin metathesis occurred, this time curiously producing olefin **21** as a single entity in 70% yield (stereochemisty tentatively assigned as *Z*). Substantially identical results were obtained through the use of Schrock's molybdenum alkylidene metathesis catalyst.

Having shown that olefin metathesis is equal, in principle, to the challenge of constructing the 16-membered ring containing both the required epoxy and thiazolyl functions of our target system, we have started to project a synthesis of epothilone A itself. Clearly, with these fragments in hand, a variety of strategies for their combination, culminating in either carbon–carbon bond formation or macrolactonization, can be entertained, and these are being evaluated. At the present writing, however, it is appropriate to point out that no successful olefin metathesis reaction has yet been realized from *seco*-systems bearing a full compliment of functionality required to reach epothilone. These negative outcomes may merely reflect a failure to identify, as yet, a suitable functional group constraint pattern appropriate for macrocylization.[13] Many possibilities remain to be screened. Accordingly, intramolecular olefin metathesis is still included in a variety of ring-forming options currently being evaluated for reaching epothilone A.

Acknowledgment. This work was supported by NIH Grant No. CA 28824. Postdoctoral Fellowships are gratefully acknowledged by E.J.S. (NSF, CHE-9504805) and P.B. (NIH, CA 62948). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses.

Supporting Information Available: Experimental procedures and spectroscopic data for the compounds illustrated in the schemes (compounds **8**–**10**, **12**–**16**, and **18**–**22**) (6 pages).

JO961674O

(10) (a) Schrock, R. R.; Murdzek, J. S.; Bazan, G. C.; Robbins, J.; DiMare, M.; O'Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875. (b) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039. For reviews of ring-closing metathesis, see: (c) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (d) Schmalz, H.-G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1833.

(11) Houri, A. F.; Xu, Z.; Cogan, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1995**, *117*, 2943.

(12) For recent examples of ring-closing metathesis, see: (a) Martin, S. F.; Chen, H.-J.; Courtney, A. K.; Liao, Y.; Pätzel, M.; Ramser, M N.; Wagman, A. S. *Tetrahedron* **1996**, *52*, 7251. (b) Fürstner, A.; Langemann, K. *J. Org. Chem.* **1996**, *61*, 3942.

(13) Substrates containing the full complement of oxygenated functionality, including the trisubstituted olefin and thiazolyl moiety, were screened for ring-closing olefin metathesis. In an effort to favor ring closure through the rigidification of the carbon backbone, a *seco* structure possessing a cyclic isopropylidene ketal bridging a C3–C5 diol relationship was prepared and subjected to ring-closing metathesis. In one instance, we also screened a substrate containing functionality that would lead to the C12–C13 epoxide, but lacking this function, *per se*. In spite of these setbacks, efforts to fashion the macrolide of epothilone A through ring-closing metathesis are continuing A full account of these studies will be disclosed in due course.

ANGEWANDTE CHEMIE

International Edition in English

Reprint

Total Synthesis of (−)-Epothilone A**

Aaron Balog, Dongfang Meng, Ted Kamenecka, Peter Bertinato, Dai-Shi Su, Erik J. Sorensen, and Samuel J. Danishefsky*

Epothilones A and B were isolated from the myxobacteria of the genus *Sorangium*. The full structures of these compounds, determined from an X-ray crystallographic analysis, were recently communicated by Höfle.[1] Interest in the total synthesis of the epothilones arises from several considerations. First, they contain substructural motifs that pose intrinsic problems for a total synthesis. Not uncommonly, solutions to novel structural challenges carry with them important lessons for organic chemistry, which transcend the particular goal under investigation. Moreover, the biological profiles of the epothilones warrant multidisciplinary scientific attention. As is well known by now, taxol is already a useful resource in chemotherapy against ovarian and breast cancers.[2] Furthermore, its range of applicability seems to be expanding under continuing clinical scrutiny. The mechanism of the cytotoxic action of taxol, at least at the in vitro level, involves stabilization of microtubule assemblies.[3] A series of complementary in vitro investigations with the epothilones indicated that they function by the same mechanism as the taxoids, apparently down to the binding sites to their protein target.[4] Moreover, the epothilones surpass taxol in terms of cytotoxicity and far surpass it as regards in vitro efficacy against drug-resistant cells. Since multiple drug resistance (MDR) is one of the serious limitations of taxol,[5] any agent that promises relief from this problem merits serious attention. Furthermore, formulatability of the epothilones is claimed to be more straightforward than is the case with taxol. Accordingly, we have undertaken the total synthesis of epothilones, focusing first on epothilone A (**1**). Herein we report for the first time the total synthesis of this target.

Carbons 9 through 11 insulate the chiral domains embracing carbons 3 through 8 on the acyl side of the macrolactone, and carbons 12 through 15 on the alkyl side. We reasoned from the outset that the prospects of transmitting stereochemical information from one of the segments to the other were bleak. Accordingly, it seemed more prudent to deal with the stereochemistry of each segment individually. In the acyl segment this required solution to both the relative and absolute configurations of the "polypropionate-like" network. In the alkyl segment, two possibilities presented themselves. In one instance, the C12–C13 epoxide would be included in the unit to be merged with the acyl-related substructure. In that case it would be necessary to secure the relative as well as absolute stereochemical relationships of carbons 15, 13, and 12. As matters transpired, we came to consider omitting the epoxide from the alkyl-side moiety undergoing coupling. This strategy would be feasible only if the epoxide could be introduced with acceptable stereocontrol after closure of the macrocycle.

In an earlier disclosure[6] we described the synthesis of compound **4**, which contains most of the requisite stereochemical information required for the acyl fragment. This intermediate was reached by a novel, oxidatively induced, solvolytic cleavage of the cyclopropanopyran **3**. We also described a construct containing the alkyl-side coupling partner embodying the absolute and relative stereochemistry at carbons 15, 13, and 12, which was not used in the studies described herein.[7]

Several potential connection sites attracted attention for the union of the alkyl and acyl domains. At some point an acylation would be required to establish an ester (or lactone) bond (see bold arrow *2*, Scheme 1). Furthermore, an aldol condensation seemed to be called for in fashioning a C2–C3 connection. Less obvious was the timing of this aldol step. It could be considered for the elongation of the C3–C9 construct to prepare it for acylation of the C-15 hydroxyl group. As matters developed, however, we employed a bolder possibility: the closure of the macrolide by a virtually unprecedented macroaldolization.[8] This risky but otherwise attractive option is implied by bold arrow *3*.

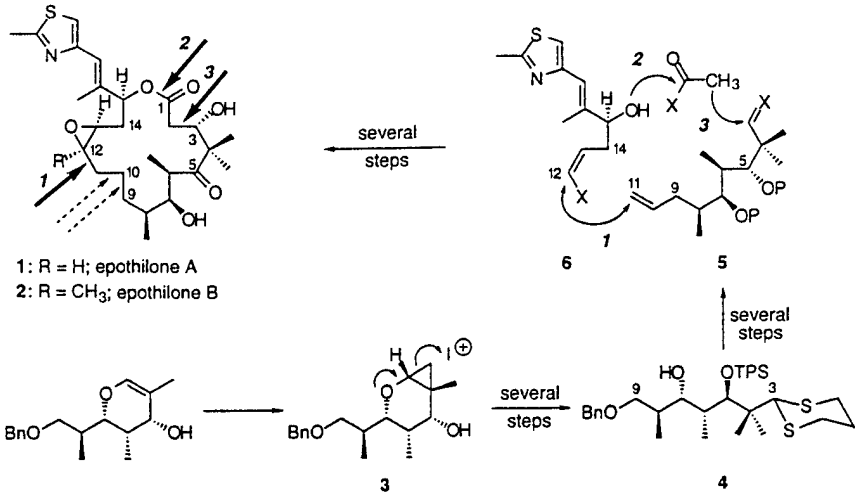
Considerable debate and experimentation attended the decision on the first union between the acyl and alkyl fragments (see bold arrow *1*). As alluded to in earlier publications,[6, 7] and as will be expanded upon in subsequent disclosures, significant resistance was encountered against proposed bond formation between carbons 9 and 10 or between carbons 10 and 11, for which the epoxide would be included in the alkyl coupling partner. Complications had arisen from unanticipated difficulties in fashioning acyl and alkyl reactants with the appropriate complementarity for merger across either of these bonds. We thus turned to the possibility of establishing the initial merger between carbons 11 and 12. This approach dictated deletion of the oxirane linkage from the O-alkyl coupling partner. After examination of several permutations, we settled upon generalized systems **5** and **6** to enter the first-stage coupling reaction. A de novo synthesis of a usable substrate corresponding to generalized system **5**, starting from **4**, would be necessary.

The steps leading from **4** to **11** are shown in Scheme 2. Protection of the future C-7 alcohol (see compound **7**) was followed by cleavage of the benzyl ether and oxidation to form aldehyde **8**. Elongation of the aldehyde to the terminal allyl-containing fragment **10** proceeded through enol ether **9** (mixture of *E* and *Z* geometrical isomers). Finally, the dithiane linkage was oxidatively cleaved under solvolytic trapping conditions[9] to give rise to specific coupling component **11**.

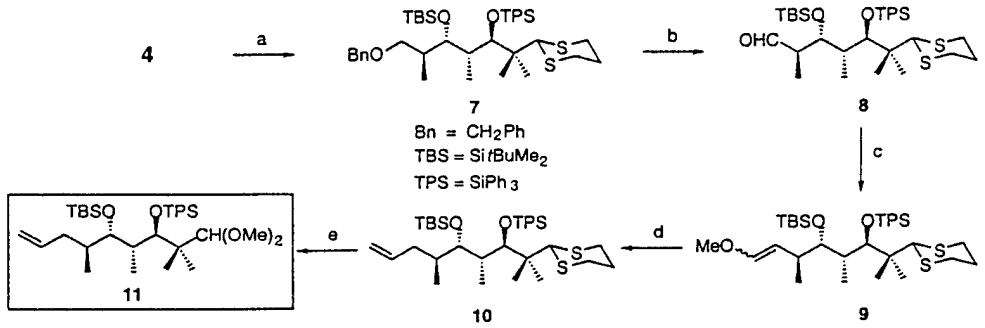
[*] Prof. S. J. Danishefsky,[+] Dr. A. Balog, D. Meng,[+] Dr. T. Kamenecka, Dr. P. Bertinato, Dr. D.-S. Su, Dr. E. J. Sorensen
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: Int. code +(212)772-8691

[+] Other address:
Department of Chemistry, Columbia University
Havemeyer Hall, New York, NY 10027 (USA)

[**] This research was supported by the National Institutes of Health (grant number CA-28824). Postdoctoral fellowship support is gratefully acknowledged by E. J. S. (N. S. F., CHE-9504805), A. B. (N. I. H., CA-GM 72231), P. B. (N. I. H., CA 62948), T. K. (N. I. H., A10-9355). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses. Professor Dr. G. Höfle of the Gesellschaft für Biotechnologische Forschung is gratefully acknowledged for providing natural epothilone A for comparative analysis. We also thank Professor Gunda Georg of the University of Kansas for bringing this problem to our attention.

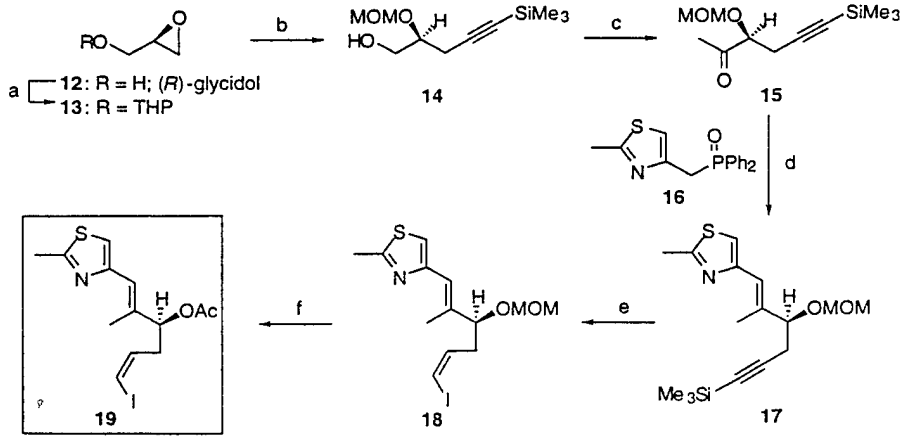


Scheme 1.



Scheme 2. a) *t*-$BuMe_2OTf$ (Tf = trifluoromethanesulfonate), 2,6-lutidine, CH_2Cl_2, 98%; b) 1. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2/H_2O, 89%; 2. $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C → RT, 90%; c) $MeOCH_2PPh_3Cl$, *t*-BuOK, THF, 0 °C → RT, 86%; d) 1. *p*-TsOH, dioxane/H_2O, 50 °C, 99%; 2. CH_3PPh_3Br, sodium hexamethyldisilazide (NaHMDS), $PhCH_3$, 0 °C → RT, 76%; e) $PhI(OCOCF_3)_2$, MeOH/THF, RT, 0.25 h, 92%.

The synthesis of the alkyl fragment started with commercially available (*R*)-glycidol **12**, which was converted, via its THP derivative **13**, into alcohol **14**. After cleavage of the tetrahydropyran blocking group, the resultant alcohol was smoothly converted into the methyl ketone **15**, as shown. The latter underwent an Emmons-type homologation with phosphane oxide **16**.[7, 10] The resultant alkyne **17** was then converted, via compound **18**, into *Z*-iodoalkene **19**[11] (Scheme 3).



Scheme 3. a) Dihydropyran (DHP), pyridinium *p*-toluenesulfonate (PPTS), CH_2Cl_2, RT; b) 1. $Me_3SiCCLi$, $BF_3 \cdot OEt_2$, THF, −78 °C; 2. methoxymethyl chloride (MOMCl), iPr_2NEt, $Cl(CH_2)_2Cl$, 55 °C; 3. PPTS, MeOH, RT; c) 1. $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78 °C → RT; 2. MeMgBr, Et_2O, 0 °C → RT; 3. tetra-*n*-propylammonium perruthenate (TPAP), *N*-methylmorpholine *N*-oxide (NMO), 4 Å mol. sieves, CH_2Cl_2, 0 °C → RT; d) **16**, *n*BuLi, THF, −78 °C, then **15**, THF, −78 °C → RT; e) 1. *N*-iodosuccinimide, $AgNO_3$, $(CH_3)_2CO$; 2. Cy_2BH, Et_2O, AcOH; f) PhSH, $BF_3 \cdot OEt_2$, CH_2Cl_2, RT; 2. Ac_2O, Py, 4-dimethylaminopyridine (4-DMAP), CH_2Cl_2, RT.

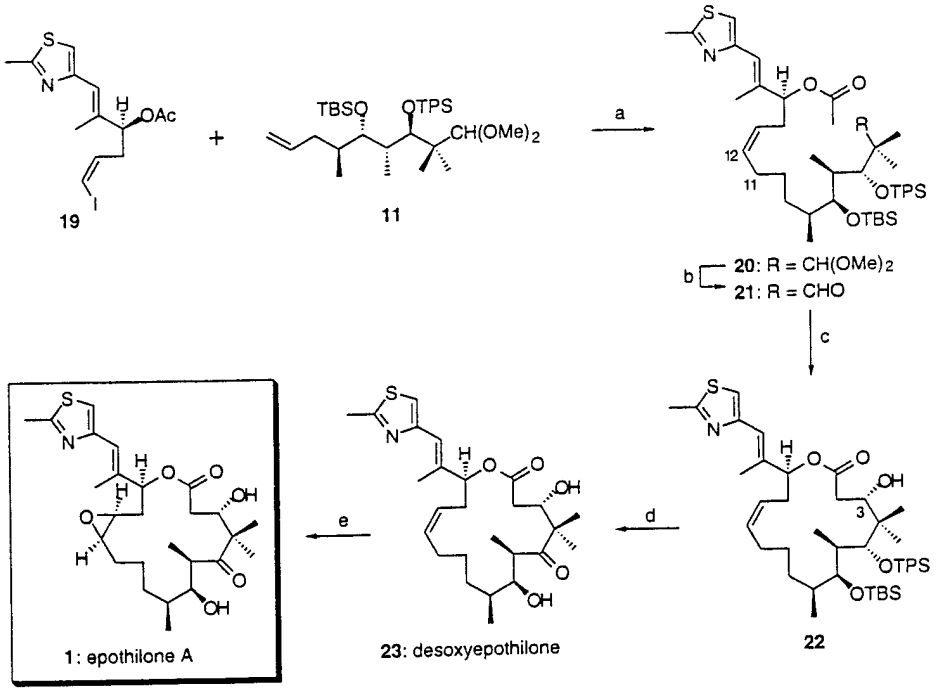
The coupling of the two fragments, the all-critical first coupling, was achieved by a *B*-alkyl Suzuki carbon–carbon bond construction.[12] Hydroboration of the pre-acyl fragment **11** with 9-BBN furnished the mixed borane, which under the conditions indicated cross-coupled to iodoolefin **19** to give **20** in 71% yield. Upon cleavage of the acetal, aldehyde **21** was in hand.

With **21** we could explore the aggressive strategy of employing the methyl group of the C-1 bound acetoxy function as the nucleophilic component in a macroaldolization.[8] In the event, deprotonation was accomplished with potassium hexamethyldisilazide in THF at −78 °C. Remarkably, these conditions gave rise to a highly stereoselective macroaldolization, resulting in the formation of the C-3 (*S*)-alcohol **22** (Scheme 4). The heavy preponderance of **22** was favored when its precursor potassium aldolate was quenched at about 0 °C. When the aldolate was protonated at lower temperature, higher amounts of the C-3 (*R*) compound were detected. In fact, under some treatments, the C-3 (*R*) epimer predominates. This matter remains to be fully sorted out. At present, we are able to generate highly favorable C-3 (*R*):C-3 (*S*) ratios if the quenching is performed on an analytical scale. In preparative-scale experiments, we can reliably obtain **22** and its C-3 epimer in a 6:1 ratio.

Having fashioned compound **22**, we could converge on our subgoal, desoxyepothilone (**23**). This objective was accomplished by selective removal of the triphenylsilyl (TPS) group in **22**, followed, sequentially, by selective silylation of the C-3 alcohol, oxidation of the C-5 alcohol, and, finally, fluoride-induced cleavage of the two silyl ethers.

Examination of a model based on the crystal structure of epothilone[1] suggested that the oxirane is disposed on the exterior face of the macrolide. In the event, oxidation of **23** was carried out with dimethyl dioxirane under the conditions shown. The major product of this reaction was (−)-epothilone A (**1**), whose identity was established by NMR and infrared spectroscopy, mass spectrometry, optical rotation, and chromatographic comparisons with authentic material kindly provided by Professor Höfle.[13]

While subject to further improvements, the synthesis in its present form, already provides us with workable amounts of epothilone A. More importantly, it provides routes to congeners not available from the natural product itself. The biological properties of some of these now accessible probe structures, as well as several of the chemical issues raised during the course of this synthesis, are receiving continuing attention.[14]



Scheme 4. a) **11**, 9-borabicyclo[3.3.1]nonane (9-BBN), THF, RT, then $PdCl_2(dppf)_2$, (dppf = 1,1'-bis(diphenylphosphino)ferrocene) $CsCO_3$, Ph_3As, H_2O, DMF, **19**, RT, 71%; b) TsOH, dioxane, H_2O, 50 °C; c) KHMDS, THF, −78 °C, 51%; d) 1. HF·Py, Py, THF, RT, 97%; 2. *t*BuMe$_2$SiOTf, 2,6-lutidine, CH_2Cl_2, −25 °C, 93%; 3. Dess–Martin periodinane, CH_2Cl_2, 87%; 4. HF·Py, THF, RT, 99%; e) dimethyl dioxirane, CH_2Cl_2, 0.5 h, −50 °C, 45% (≧20:1).

Received: October 17, 1996 [Z 9663 IE]
German version: *Angew. Chem.* **1996**, *108*, 2976–2978

Keywords: C–C coupling · cyclization · epothilone · natural products · synthesis methods

[1] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomberg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567.
[2] G. I. Georg, T. T. Chen, I. Ojima, D. M. Vyas, *Taxane Anticancer Agents*, American Cancer Society, San Diego, **1995**.
[3] P. B. Schiff, J. Fant, S. B. Horwitz, *Nature* **1979**, *277*, 665.
[4] D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325.
[5] L. M. Landino, T. L. MacDonald in *The Chemistry and Pharmacology of Taxol and its Derivatives* (Ed.: V. Farin), Elsevier, New York, **1995**, p. 301.
[6] P. Bertinato, E. J. Sorensen, D. Meng, S. J. Danishefsky, *J. Org. Chem.*, **1996**, *61*, 8000–8001.
[7] D. Meng, E. J. Sorensen, P. Bertinato, S. J. Danishefsky, *J. Org. Chem.*, **1996**, *61*, 7998–7999.
[8] For a previous instance of a keto aldehyde macroaldolization, see C. M. Hayward, D. Yohannes, S. J. Danishefsky, *J. Am. Chem. Soc.* **1993**, *115*, 9345.
[9] G. Stork, K. Zhao, *Tetrahedron Lett.* **1989**, 30, 287.
[10] This Emmons coupling provided about an 8:1 mixture of olefin stereoisomers in favor of *trans*-**17**.
[11] E. J. Corey, J. R. Cashman, T. M. Eckrich, D. R. Corey, *J. Am. Chem. Soc.* **1985**, *107*, 713.
[12] a) N. Miyaura, T. Ishiyama, H. Sasaki, M. Ishi-Kawa, M. Satoh, A. Suzuki, *J. Am. Chem. Soc.* **1989**, *111*, 314; b) N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457.
[13] In addition to epothilone A (**1**), small amounts of a diepoxide mixture, as well as traces of the diastereomeric *cis* C12–C13 monoepoxide (≧20:1) were detected.
[14] For alternative strategies toward the epothilones, see a) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* **1996**, *108*, 2554; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2399; b) D. Schinzer, A. Limberg, O. M. Böhm, *Chem. Eur. J.* **1996**, *2*, 4007.

  0570-0833/96/3523-2803 $ 15.00+.25/0

ANGEWANDTE CHEMIE

International Edition in English

Reprint

Total Synthesis of (−)-Epothilone B: An Extension of the Suzuki Coupling Method and Insights into Structure–Activity Relationships of the Epothilones**

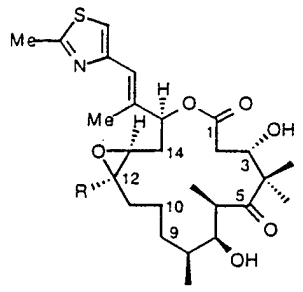
Dai-Shi Su, Dongfang Meng, Peter Bertinato, Aaron Balog, Erik J. Sorensen, Samuel J. Danishefsky,* Yu-Huang Zheng, Ting-Chao Chou, Lifeng He, and Susan B. Horwitz

Recently, synthetic studies directed to epothilone A (**3**)[1,2] culminated in its first total synthesis.[3–5] Our synthesis passed through the *Z*-desoxy compound (**4**), which underwent highly stereoselective epoxidation with 2,2-dimethyldioxirane, under carefully defined conditions, to yield the desired β-epoxide. The same myxobacterium of the genus *Sorangium* that produces **3** also produces epothilone B (**1**). The latter is significantly more potent than **3** both in antifungal screens and in cytotoxicity assays in some cell lines.[6,7] Clearly then, there was a strong rationale for preparing epothilone B (**1**).

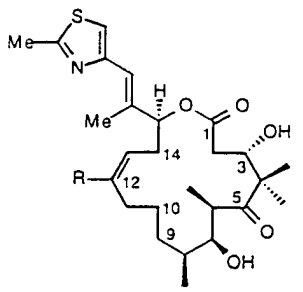
Our interim goal structure was desoxyepothilone B (**2**) or a suitable derivative thereof. With access to such a compound, we could investigate the regio- and stereoselectivity of the epoxidation of the C12–C13 double bond. Not the least interesting issue in the project was the synthesis of *Z*-trisubstituted olefinic precursors of **2** with high margins of stereoselection. In our synthetic route to epothilone A[3] we had employed a palladium-mediated B-alkyl Suzuki coupling[8,9] of the *Z*-vinyl iodide **5** with borane **7** derived from hydroboration of compound **6** with 9-BBN (Scheme 1).

[*] Prof. S. J. Danishefsky,[+] Dr. D.-S. Su, D. Meng,[+] Dr. P. Bertinato, Dr. A. Balog, Dr. E. J. Sorensen
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: Int. code +(212)772-8691
Dr. Y.-H. Zheng, Dr. T.-C. Chou
Laboratory for Biochemical Pharmacology, Sloan-Kettering Institute
L. He, Prof. B. Horwitz
The Department of Molecular Pharmacology
The Albert Einstein College of Medicine
Bronx, NY 10461 (USA)
[+] Additional address:
Columbia University, Department of Chemistry, Havemeyer Hall
New York, NY 10027 (USA)

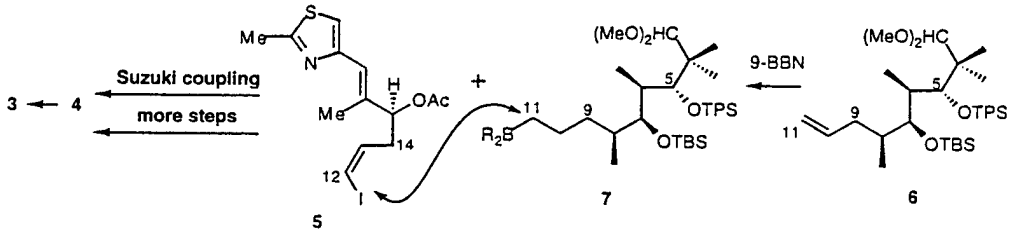
[**] This research was supported by the U. S. National Institutes of Health [grant no. CA-28824 (S.J.D) and CA-39821 (S.B.H.)]. Postdoctoral fellowships are gratefully acknowledged by E. J. S. (NSF, CHE-9504805), A. B. (NIH, CA-GM 72231), and P. B. (NIH, CA-62948). We thank Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectrometric analyses, Prof. Dr. G. Höfle of the Gesellschaft für Biotechnologische Forschung, Braunschweig (Germany), for providing natural epothilone B for comparative analysis, and Prof. Gunda Georg of the University of Kansas for bringing the epothilone problem to our attention.



1: R = Me, epothilone B
3: R = H, epothilone A



2: R = Me, X = desoxyepothilone B
4: R = H, X = desoxyepothilone A



Scheme 1.

Naturally, our first instinct was to apply the same line of thinking to reach a *Z*-trisubstituted olefin en route to **2**. Here, two serious issues had to be addressed. First, it would be necessary to devise a method to prepare vinyl iodide **8**, the trisubstituted analog of **5**. If this goal could be accomplished, we would still face the challenge of conducting the required B-alkyl Suzuki coupling reaction to reach a *Z*-trisubstituted olefin. Such an intermolecular transformation, with a B-alkyl as opposed to a B-alkenyl group, and where the vinyl iodide is not partof a β-iodoenoate (or β-iodoenone), was not precedented.[10]

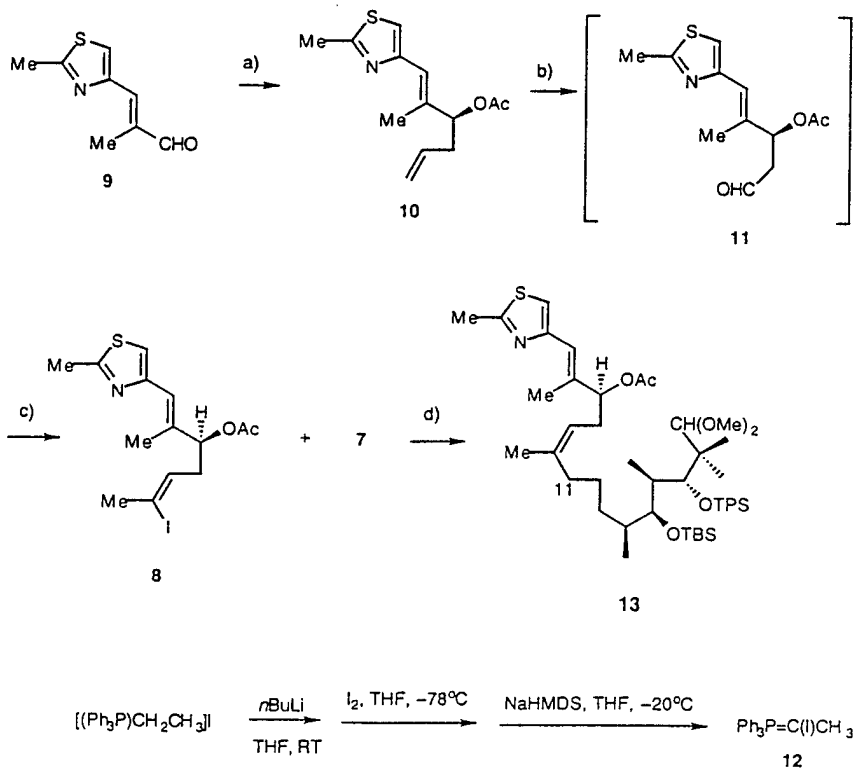
We first dealt with the synthesis of compound **8** (Scheme 2). Our route started with olefin **10**, which was prepared[4] by catalytic asymmetric allylation of **9**[11] followed by acetylation. Site-selective dihydroxylation of **10** followed by oxidative cleavage of the glycol generated the unstable aldehyde **11**. Remarkably, **11** reacted with phosphorane **12**[12] (preparation shown at the bottom of Scheme 2) to afford the *Z*-iodide **8** albeit in modest overall yield. Borane **7** was generated from **6** as previously described.[3] Gratifyingly, the coupling of compound **7** and iodide **8** could be conducted to produce the pure *Z*-olefin **13**.

With compound **13** in hand, we apply protocols similar to those employed in the synthesis of **4**.[3] Thus, hydrolysis of the acetal linkage led to aldehyde **14**, which was now subjected to macroaldolization (Scheme 3). The highest yield of aldol product was obtained by carrying out the reaction under conditions that produced a 1.5:1 mixture of α/β epimers at C3. Fortunately, we could convert the 3*R* isomer to the required 3*S* epimer by reduction of its derived C3 ketone ($\rightarrow$**15**).[13] Cleavage of the C5 triphenylsilyl ether was followed sequentially by monoprotection (*tert*-butyldimethylsilyl) of the C3 hydroxyl group, oxidation at C5 ($\rightarrow$**16**) and, finally, cleavage of the silyl protecting groups to expose the C3 and C7 hydroxyl groups ($\rightarrow$ **2**).

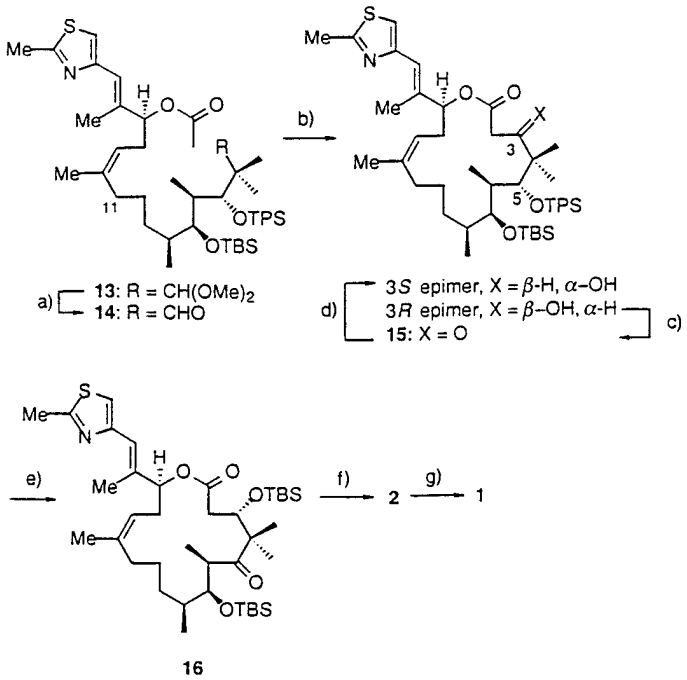
Z-Desoxyepothilone B (**2**) does indeed undergo very rapid and substantially regio- and stereoselective epoxidation[14] to afford epothilone B (**1**), which is identical with an authentic sample (^{1}H NMR, MS, IR , $[\alpha]_D$). Thus, the first total synthesis of (−)-epothilone B has been accomplished.

In retrospect, the total synthesis carries with it several important teachings. Certainly, the stereospecific preparation of the vinyl iodide **8** by use of the rarely employed phosphorane **12**, even with the unstable β-acetoxyaldehyde **11** could not have been predicted.[12] Moreover, the successful Suzuki coupling to construct the trisubstituted *Z*-double bond constitutes an important extension of the prior art.[10, 15] Finally, the highly regio- and stereoselective epoxidation of *Z*-desoxyepothilone B (**2**) with dimethyldioxirane was highly gratifying.

With epothilone B (**1**) and its *Z*-12,13-desoxy precursor (**2**) in hand through total synthesis, we were in a sound position to explore their biological activities. Also available to us from our previous studies[3, 4] were epothilone A (**3**), its *Z*-12,13-desoxy precursor **4**, and the *E* variant (**17**) of the latter. Through an alternative

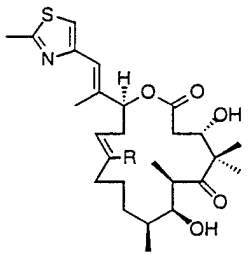


Scheme 2. a) 1. Allyl(tributyl)tin, (*S*)-(−)-2,2′-dihydroxy-1,1′-biphenyl, $Ti(OiPr)_4$, CH_2Cl_2, −20 °C, 60%, >95% *ee*; 2. Ac_2O, Et_3N, 4-dimethylaminopyridine (DMAP), CH_2Cl_2, room temperature (rt) 95%; b) 1. OsO_4, *N*-methylmorpholine *N*-oxide, acetone/H_2O, 0 °C; 2. $Pb(OAc)_4$, C_6H_6, 0 °C; c) **12**, THF, −20 °C, *Z* isomer only, 43% from **10**; d) $[Pd(dppf)_2]$ (dppf = 1,1′bis(diphenylphosphano)-ferrocene), Cs_2CO_3, Ph_3As, H_2O, DMF, rt 77%. THP = tetrahydropyran; TPS = triphenylsilyl.



Scheme 3. a) *p*-TsOH, dioxane/H_2O, 55 °C, 71%; b) potassium bis(trimethylsilyl)-amide (KHMDS), THF, −78 °C, 67%, α/β: 1.5:1; c) Dess–Martin periodinane, CH_2Cl_2, rt; d) $NaBH_4$, MeOH, rt, 80% for two steps; e) 1. HF·pyridine, pyridine, THF, rt, 93%; 2. TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C, 89%; 3. Dess–Martin periodinane, CH_2Cl_2, rt, 67%; f) HF·pyridine, THF, rt, 80% g) dimethyldioxirane, CH_2Cl_2, −50 °C, 70%, 14:1 ratio of *cis* epoxides. TBS = *tert*-butyldimethylsilyl; OTf = trifluoromethanesulfonate.

route we also gained access to the *E* compound in the B series (**18**).[16] All of these compounds manifested the ability to bind to microtubules in the absence of guanosine triphosphate (GTP), biological activity reminiscent of that of Taxol™.[17] They also manifest high levels of cell killing as seen in Table 1. By the cytotoxicity standard, the trisubstituted compounds **1** and **2** outperform their disubstituted counterparts **3** and **4**. The desoxy compounds **2** and **4** are comparable and, in some instances, superior to the natural products (**1** and **3**). In this regard it is interesting that though **18** is still quite active, it is significantly less potent than the corresponding *Z* systems **2** and **4**, and the disubstituted *E* system **17**.



R = H, *trans*- desoxyepothilone A **17**
R = Me, *trans*- desoxyepothilone B **18**

Table 1. Relative efficacy of epothilone compounds against drug-sensitive and resistant CCRF-CEM cell lines [a].

Compound	CCRF-CEM IC_{50} [μM] [b]	CCRF-CEM·VBL IC_{50} [μM] [b]	CCRF-CEM/VM_1 IC_{50} [μM] [b]
epothilone A (**3**)	0.003	0.020	0.003
desoxyepothilone A (**4**)	0.022	0.012	0.013
trans-desoxy A **17**	0.052	0.035	0.111
epothilone B (**1**)	0.0004	0.003	0.002
desoxyepothilone B (**2**)	0.009	0.017	0.014
trans-desoxy B **18**	0.090	0.262	0.094
Taxol™	0.002	3.390	0.002

[a] The cytotoxicities of test compounds were determined by the growth of human lymphoblastic leukemic cells CCRF-CEM or their sublines resistant to vinblastine and taxol (CCRF-CEM/VBL) or resistant to etoposide (CCRF-CEM/VM-1). XTT-microculture tetrazolium/formazan assays [18] were used. [b] The IC_{50} values were calculated based on five or six assays at various concentrations; a median-effect plot [19] was generated with computer software [20].

All proposals addressed to the pharmacological modeling of the epothilones must now take notice of these interesting structural findings. Certainly they are of significant consequence in our own analog strategy program, which is well underway.

Received: December 27, 1997 [Z9934IE]
German version: *Angew. Chem.* **1997**, *109*, 775–777

Keywords: antitumor agents · epothilone · natural products · total synthesis

[1] D. Meng, E. J. Sorensen, P. Bertinato, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 7998.
[2] P. Bertinato, E. J. Sorensen, D. Meng, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 8000.
[3] A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108*, 2976; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801.
[4] D. Meng, D.-S. Su, A. Balog, P. Bertinato, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *J. Am. Chem. Soc.*, in press.
[5] For a subsequent total synthesis of epothilone A, see Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.*, **1997**, *109*, 170; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166; K. C. Nicolaou, F. Sarabia, S. Ninkovic, Z. Yang, *ibid.* **1997**, *109*, 593 and **1997**, *36*, 525; D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *ibid.* **1997**, *109*, 543 and **1997**, *36*, 523. For other synthetic studies directed towards epothilone A, see a) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vollberg, Z. Yang, *ibid.* **1996**, *108*, 2554 and **1996**, *35*, 2399; b) D. Schinzer, A. Limberg, O. M. Böhm, *Chem. Eur. J.* **1996**, *2*, 1477; c) J. Mulzer, A. Mantoulidis, *Tetrahedron Lett.* **1996**, *37*, 9179.
[6] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomberg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567.
[7] D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325.
[8] N. Miyaura, T. Ishiyama, H. Sasaki, M. Ishi-Kawa, M. Satoh, A. Suzuki, *J. Am. Chem. Soc.* **1989**, *111*, 314.
[9] For an excellent review, see N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457.
[10] For some close analogies that differ in important details from the work shown here, see a) N. Miyaura, H. Suginome, A. Suzuki, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2221; b) M. Ohba, N. Kawase, T. Fujii, K. Aoe, K. Okamura, R. Fathi-Afshar, T. M. Allen, *Tetrahedron Lett.* **1995**, *36*, 6101; c) C. R. Johnson, M. P. Braun, *J. Am. Chem. Soc.* **1993**, *115*, 11014.
[11] G. E. Keck, K. H. Tarbet, L. S. Geraci, *J. Am. Chem. Soc.* **1993**, *115*, 8467.
[12] J. Chen, T. Wang, K. Zhao, *Tetrahedron Lett.* **1994**, *35*, 2827.
[13] The stereochemically controlled aldol condensation giving a product with the natural 3*S* configuration as previously described in the epothilone A series [3] was accomplished. However, the overall yield for reaching the 3*S* epimer is better with the protocol described here.
[14] Although precise comparisons are not available, the epoxidation of **2** appears to be more rapid and regioselective than that of **4** [3].
[15] To the best of our knowledge, this is the first successful Suzuki coupling between a B-alkyl system and an unconjugated, trisubstituted alkene.
[16] Compound **18** was prepared by a stereorandom olefin metathesis route which also led to **2**. These geometric isomers could be separated only with great difficulty. Details of the nonstereoselective route will be provided in a subsequent fuller disclosure.
[17] Experiments conducted by Dr. Susan Horwitz at the Albert Einstein College of Medicine, Bronx, NY (USA): Microtubule protein (MTP) was purified from calf brains by two cycles of temperature-dependent assembly and disassembly [21]. In control assembly experiments, MTP (1 mg mL^{-1}) was diluted in assembly buffer containing 0.1 M MES (2-(*N*-morpholino)ethanesulfonic acid), 1 mM EGTA (1,2-di(2-aminoethoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid), 0.5 mM $MgCl_2$, 1 mM GTP, and 3 M glycerol, pH 6.6. The concentration of tubulin in MTP was estimated to be about 85%. Assembly was followed spectrophotometrically at 350 nm, 35 °C for 40 min, by monitoring changes in turbidity as a measure of polymer mass [22]. Drugs were tested at a concentration of 10 μM in the absence of GTP. Microtubule formation was verified by electron microscopy. To determine the stability of microtubules assembled in the presence of GTP or drug, turbidity was monitored for 40 min after the reaction temperature was shifted to 4 °C.
[18] D. A. Scudiero, R. H. Shoemaker, D. K. Paul, A. Monks, S. Teiney, T. H. Hafziget, J. J. Currens, D. Sheriff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827.
[19] T.-C. Chou, D. Rideout, *Synergism and Antagonism in Chemotherapy*, Academic Press, New York, **1991**, pp. 61–102.
[20] J. Chou, T-C. Chou, IBM-PC software and manual, Biosoft, Cambridge, UK, **1987**.
[21] R. C. Weisenberg, *Science* **1972**, *177*, 1104.
[22] F. Gaskin, C. R. Cantor, M. L. Shelanski, *J. Mol. Biol.* **1974**, *89*, 737.

Communications to the Editor

Remote Effects in Macrolide Formation through Ring-Forming Olefin Metathesis: An Application to the Synthesis of Fully Active Epothilone Congeners

Dongfang Meng,[†,‡] Dai-Shi Su,[†] Aaron Balog,[†]
Peter Bertinato,[†] Erik J. Sorensen,[†]
Samuel J. Danishefsky,*[,†,‡] Yu-Huang Zheng,[§]
Ting-Chao Chou,[§] Lifeng He,[∥] and Susan B. Horwitz[∥]

Laboratories for Bioorganic Chemistry and Biochemical Pharmacology
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, New York 10021
Department of Molecular Pharmacology
The Albert Einstein College of Medicine
Bronx, New York 10461

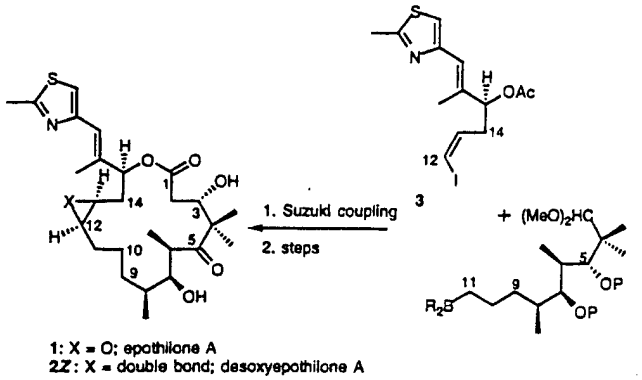
Received December 12, 1996

Recently, we achieved the first synthesis of epothilone A (structure **1**).[1] Aside from numerous chemical issues which must be addressed in accomplishing such a synthesis, interest in the epothilone class of compounds is further heightened by claims (thus far based solely on *in vitro* measurements) that the epothilones may constitute a useful group of anticancer agents, operating through the same mechanism of action as paclitaxel.[2] It has further been suggested, again on the basis of *in vitro* data, that the epothilones offer advantages relative to paclitaxel in terms of ease of formulation and potency toward drug resistant cell lines.

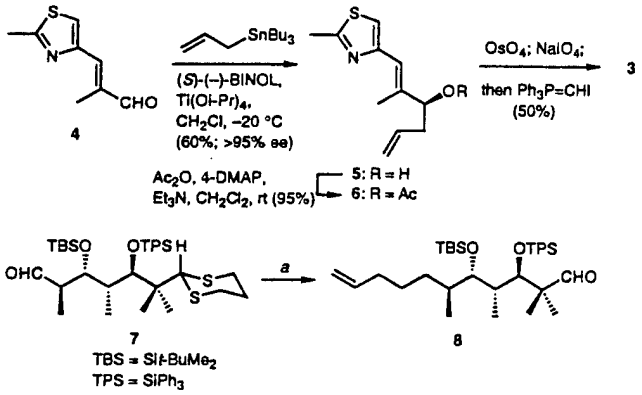
In our synthesis of epothilone A (**1**), we passed through the desoxycompound **2Z**. We showed, for the first time, that the action of dimethyldioxirane on compound **2Z** results in a highly diastereoselective epoxidation, providing compound **1**. The strategy we employed to construct compound **2Z** provided strict control over the geometry of the C12−C13 double bond through a *B*-alkyl Suzuki coupling reaction of *cis* vinyl iodide **3** with an appropriate borane (Scheme 1).

The studies described herein focused on a different method for the construction of desoxyepothilone A (**2Z**). In particular, we investigated the possibility of a ring-forming olefin metathesis reaction to construct the C12−C13 bond.[3] We were particularly mindful of a precedent furnished by Hoveyda et al.[3b] It was hoped that such an assembly strategy involving components of the type **6** and **8** might lead to an even more direct route to the natural series and analogs thereof. *These studies became of particular interest when it was found, surprisingly, that desoxyepothilone A (2Z) has the full biological activity of epothilone A as manifested through independent investigations at the level of cytotoxicity and polymerization of stable microtubules in the absence of GTP.* Herein we describe a straightforward route to reach substrates needed for olefin metathesis. We also disclose the results of these cyclizations which indicate a remarkable sensitivity to permutations of functionality and stereochemistry at centers far removed from the site of olefin metathesis. Finally, we describe some early but exciting SAR results which indicate that significant structural variances can be introduced in this series with maintenance of full biological function.

Scheme 1



Scheme 2[a]



[a] Key: (a) (i) 3-butenylmagnesium bromide, Et_2O, −78 to 0 °C (92%); (ii) thiocarbonyldiimidazole, DMAP, 95 °C; (iii) Bu_3SnH, AIBN, C_6H_6, 80 °C (83% for two steps); (iv) $(CF_3CO_2)_2IC_6H_5$, MeOH, THF; (v) *p*TSA, dioxane, H_2O, 50 °C (85% for two steps).

Our new strategy commences with aldehyde **4**, a substance available in multigram quantities.[1b,c] An important technological advance in the area was registered when it was found that subjection of aldehyde **4** to the catalytic asymmetric allylation protocol previously described by Keck leads to **5** in >95% enantiomeric excess (Scheme 2).[4] As an aside, we note that **5** was converted in two steps to the previously mentioned vinyl iodide **3**, thereby effecting a major economy in the earlier synthesis. For purposes to be described, compound **5** was simply converted to the ester **6**. The pre-acyl construct **8** was assembled from the dithiane aldehyde **7**[1a,b] in the manner indicated in Scheme 2. We thus had in hand the two subunits required to study ring forming olefin metathesis *en route* to the C12−C13 bond.

The compounds **6** and **8** were joined through a simple intermolecular aldol addition. That this reaction produced an approximately 1:1 mixture of the epimers **9** and **10** was *per se*

[†] Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research.
[‡] Department of Chemistry, Columbia University, Havemeyer Hall, New York, NY 10027.
[§] Laboratory for Biochemical Pharmacology, Sloan-Kettering Institute for Cancer Research.
[∥] The Albert Einstein College of Medicine.

(1) (a) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801. (b) Meng, D.; Sorensen, E. J.; Bertinato, P.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 7998. (c) Bertinato, P.; Sorensen, E. J.; Meng, D.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 8000.

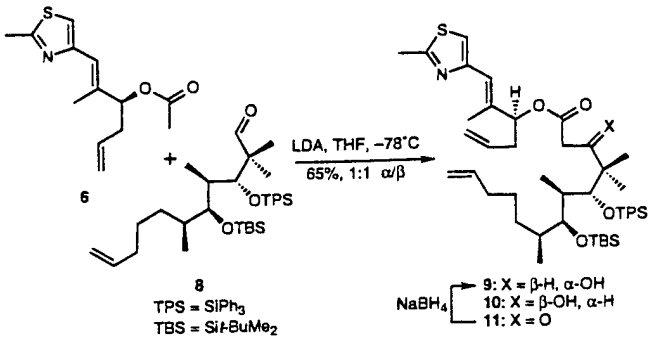
(2) See: Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomberg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567 and references therein.

(3) (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* 1995, *28*, 446. (b) Houri, A. F.; Xu, Z.; Cogan, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1995**, *117*, 2943.

(4) Keck, G. E.; Tarbet, K. H.; Geraci, L. S. *J. Am. Chem. Soc.* **1993**, *115*, 8467.

S0002-7863(96)04275-8 CCC: $14.00

Scheme 3



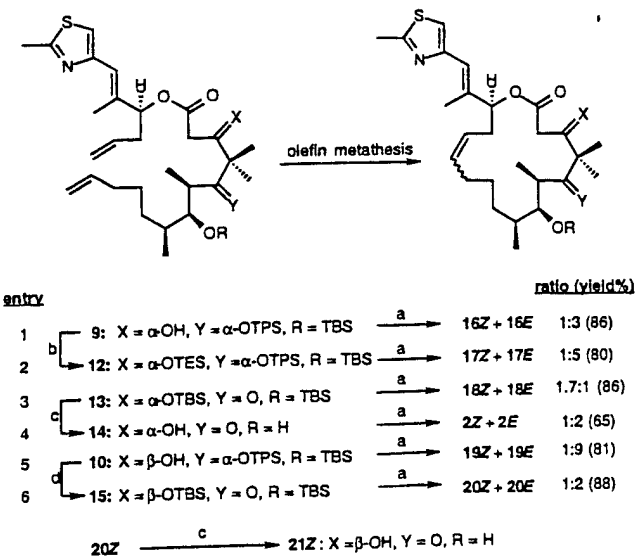
of no consequence, since the latter could be converted to the former through an oxidation/reduction sequence (i.e., **10** → **11** → **9**) (Scheme 3). Indeed, much was learned chemically and biologically from having both the 3*S* (cf. **9**) and 3*R* (cf. **10**) series available to us. From the core compounds **9** and **10**, we could easily fashion substrates **12**−**14**. We were then in a position to study the ring-forming olefin methathesis (ROM) reaction (Scheme 4).

Cyclization reactions were conducted under the conditions shown in Scheme 4 for compounds **9**, **10**, and **12**−**14**. As seen, we could readily obtain products containing the *E* C12−C13 double bond (see formation of **19***E*,*Z*). However, at this writing the highest ratio for the *Z* product is only 1.7:1 (see formation of **18***E*,*Z*). We note that, with all protecting groups identical, the proportion of *E* product increases upon changing from the 3*S* to 3*R* series (see formation of **19***E*,*Z* and **20***E*,*Z*). Similarly, keeping C-3 and C-7 constant but permutating C-5 (see ROM substrates **13** and **14**) affords more of the *Z* olefin product (see formation of **18***E*,*Z* and **2***E*,*Z*).

Using this chemistry, we could easily access the fully deprotected *cis*-desoxyepothilone A (**2***Z*). Of course, this work constitutes a second synthesis of **2***Z* and a formal total synthesis of **1**.[5] It is noteworthy that the concise route to enantiomerically pure vinyl iodide **3** (see Scheme 2) renders our initial approach to **2***Z* more practical. Nevertheless, the ROM chemistry described herein provides an eminently more workable route to *trans*-desoxyepothilone A (**2***E*). *Remarkably, compound (2E) is fully active as measured by cytotoxicity and microtubule assays. Perhaps equally surprising, biological activity is abrogated in the 3R compounds 21Z and 3-epi-epothilone A (3-epi-1).*[6]

(5) It has been brought to our attention through the popular media that another total synthesis of epothilone A has been subsequently completed utilizing ring-closing olefin metathesis. Nicolaou, K. C.; et al. *Angew. Chem., Int. Ed. Engl.* **1997**, *37*, 166.

Scheme 4[a]



[a] Key: (a) $RuBnCl_2(PCy_3)_2$ (50 mol %), C_6H_6, 0.001 M, rt, 24 h; (b) TESCl, imidazole, DMF, (80%); (c) pyridine hydrofluoride, THF, rt; (d) (i) pyridine hydrofluoride, pyridine, THF, rt, (93%); (ii) TBSOTf, 2,6-lutidine, −35 °C, (95%); (iii) Dess−Martin periodinane, (87%) (TBS = *tert*-butyldimethylsilyl; TPS = triphenylsilyl; TES = triethylsilyl).

In summary, a route to (*E*)- and (*Z*)-desoxyepothilones using ROM technology has been accomplished. Through this and related methodology to be described soon, highly biologically active congeners have been obtained, and a total synthesis driven mapping of the SAR of epothilones is well underway.

Acknowledgment. This research was supported by the National Institutes of Health (grant numbers CA-28824 (S.J.D.) CA-39821 (S.B.H.)). Postdoctoral fellowship support is gratefully acknowledged by E.J.S. (NSF, CHE-9504805), A.B. (NIH, CA-GM 72231), P.B. (NIH, CA 62948). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses.

Supporting Information Available: Preparation of substrates for olefin methathesis (**9**, **10** and **12**−**15**) and compounds **21***Z* and 3-*epi*-epothilone A and relevant biological data (IC_{50} values) as well as all relevant spectral data for compounds **2**−**21** (43 pages). See any current masthead page for ordering and Internet access instructions.

JA964275J

(6) The compound 3-*epi*-epothilone A was produced by treatment of 3-*epi*-desoxyepothlone A (**21***Z*) with dimethyldioxirane at −35 °C.

Pergamon

Tetrahedron Letters, Vol. 38, No. 26, pp. 4529-4532, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00931-3

Stereoselective Syntheses and Evaluation of Compounds in the 8-Desmethylepothilone A Series: Some Surprising Observations Regarding Their Chemical and Biological Properties

[a]Aaron Balog, [a]Peter Bertinato, [a]Dai-Shi Su, [a,c]Dongfang Meng, [a]Erik Sorensen, [a,c]Samuel J. Danishefsky,*

[b]Yu-Huang Zheng, [b]Ting-Chao Chou, [d]Lifeng He and [d]Susan B. Horwitz

Contribution from [a]The Laboratory for Bioorganic Chemistry, [b]The Laboratory for Biochemical Pharmacology, The Sloan-Kettering Institute for Cancer Research, 1275 York Ave., New York, N.Y. 10021,

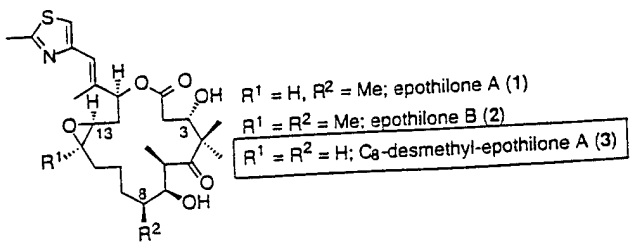
[c]The Department of Chemistry, Columbia University, Havemeyer Hall, New York, N.Y. 10027 and

[d] The Department of Molecular Pharmacology, The Albert Einstein College of Medicine, Bronx, N.Y. 10461

Abstract: The title compounds have been synthesized in a convergent way by recourse to a Weiler type dianion construction. © 1997 Elsevier Science Ltd.

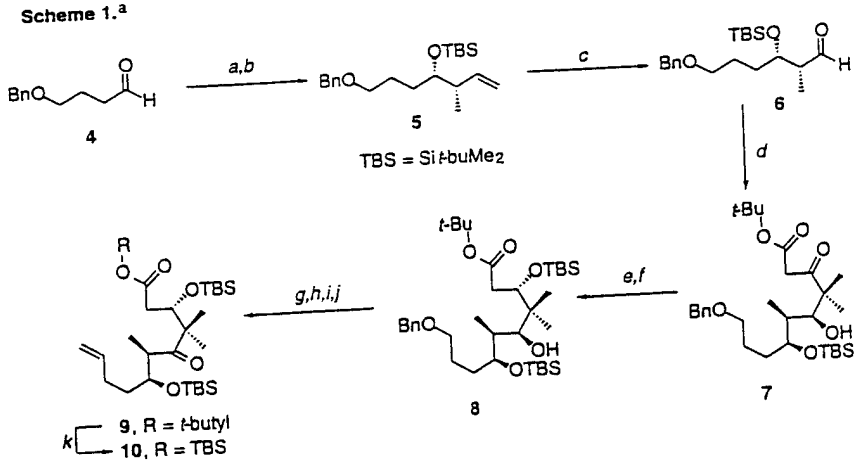
Recently, several groups have described total syntheses of epothilones A (1) and B (2)[1,2] whose mode of antitumor action closely mimics that of taxol ™.[3] Although taxol™ (Paclitaxel) is a clinically proven drug, its formulation continues to be difficult. In addition, taxol induces the multidrug resistance (MDR) phenotype. Hence, any novel agent that has the same mechanism of action as taxol and has the prospect of having superior therapeutic activity warrants serious study.[4]

A central challenge now is that of generating epothilone analogs that are more effective and more readily synthesized than is the case for 1 and 2. Though the syntheses of the natural products can provide ample material for preliminary biological evaluation, the prospect of producing adequate amounts of these compounds for full development would be daunting. One particular area where a structural change could bring significant relief from the complexities of the synthesis would be in the deletion of the C_3 methyl group from the polypropionate domain (see goal system 3). The need to deal with this C_3 chiral center complicates all of the syntheses of epothilone thus far reported. In the context of our own program, deletion of the C_3 methyl group would prompt a major change in synthetic strategy relative to our earlier diene-aldehyde cyclocondensation route.[5]



Asymmetric crotylation[6] (87% ee) of **4** followed by protection led to the TBS ether **5**. The double bond was readily cleaved to give rise to aldehyde **6**. The aldehyde was coupled to the dianion derived from *t*-butyl isobutyrylacetate to provide **7**. The ratio of the C_{5S} (**7** shown here): C_{5R} compound (not shown) is *ca* 10:1. That the Weiler-type β-ketoester dianion chemistry[7] can be conducted in the context of the isobutyryl group prompts several alternate perceptions for still more concise syntheses. Directed reduction of the C_3 ketone of **7** following literature precedents,[8] followed by selective silylation of the C_3 hydroxyl gave a 50% yield of a 10:1 ratio of the required C_{3S} (see compound **8**): to C_{3R} isomer (not shown).[9] The carbinol, produced upon debenzylation, was oxidized to an aldehyde which, following methylenation through a simple Wittig reaction, afforded olefin **9**. Treatment of this compound with TBSOTf provided ester **10** which was used directly in the Suzuki coupling with the vinyl iodide **12** (*vide infra*).

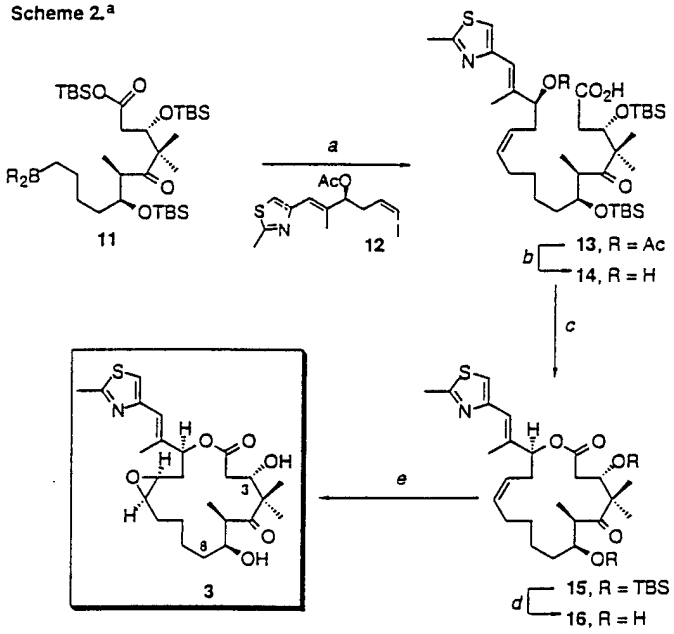
Scheme 1.[a]



[a] a) (Z)-Crotyl-B[(−)-Ipc]$_2$, −78°C, Et_2O, then 3N NaOH, 30% H_2O_2; b) TBSOTf, 2,6-lutidine, CH_2Cl_2 (74% for two steps, 87% ee); c) O_3, CH_2Cl_2/MeOH, −78°C, then DMS, (82%); d) *t*-butyl isobutyrylacetate, NaH, BuLi, 0°C, then **6** (60%, 10:1); e) $Me_4NBH(OAc)_3$, −10°C (50%, 10:1 α/β) or $NaBH_4$, MeOH, THF, 0°C, (88%, 1:1 α/β); f) TBSOTf, 2,6-lutidine, −40°C, (88%), g) Dess-Martin periodinane, (90%); h) $Pd(OH)_2$, H_2, EtOH, (96%); i) DMSO, oxalyl chloride, CH_2Cl_2, −78°C (78%); j) Methyl triphenylphosphonium bromide, NaHMDS, THF, 0°C (85%); k) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (87%).

The hydroboration of **10** with 9-BBN produced intermediate **11** which, on coupling with the vinyl iodide **12** and *in situ* cleavage of the TBS ester led to **13**. After de-acetylation, the hydroxy acid **14** was in hand. Marcolactonization[10] of this compound produced **15** which, after desilylation, afforded C_3-desmethyldesoxyepothilone (**16**). Finally, epoxidation of this compound with dimethyldioxirane produced the goal structure **3**. The stereoselectivity of epoxidation was surprisingly poor (1.5:1) given that epoxidation of desoxyepothilone A occurred with >20:1 stereoselectivity. *Apparently, the deletion of the C_3 methyl group tilts the conformational distribution of 16 to forms in which the epoxidation by dimethyl dioxirane is less β-selective.*[11]

Scheme 2.[a]



[a] a) $Pd(dppf)_2Cl_2$, Ph_3As, Cs_2CO_3, H_2O, DMF, rt (62%); b) K_2CO_3, MeOH, H_2O (78%); c) DCC, 4-DMAP, 4-DMAP·HCl, $CHCl_3$ (78%); d) HF·pyr, THF, rt (82%), e) 3,3-dimethyl dioxirane, CH_2Cl_2, -35°C (72%, 1.5:1).

Compounds **3** and **16** were tested for cytotoxicity in cell cultures and assembly of tubulin in the absence of GTP. Surprisingly, neither macrolide displayed significant tubulin polymerization.[12] Cytotoxicity studies showed drastically reduced activity in the 8-desmethyl series. Compounds **3** and **16** were approximately 200 times less active than their corresponding epothilone A counterparts (see Table). Recalling earlier SAR findings at both C_3 and C_5, in conjunction with the findings reported here, the polypropionate sector of the epothilones emerges as a particularly sensitive locus of biological function.[2,13] Further studies on the SAR of epothilones congeners, enabled by improved access through synthesis, are ongoing and will be disclosed in due course.

Table 1. *Relative efficacy of epothilone compounds against drug-sensitive and resistant human leukemic CCRF-CEM cell lines.*[a]

Compound	CCRF-CEM IC_{50} (μM)[b]	CCRF-CEM/VBL IC_{50} (μM)[b]	CCRF-CEM/VM_1 IC_{50} (μM)[b]
16	5.00	5.75	6.29
3	0.439	2.47	0.764
epothilone A (1)	0.003	0.020	0.003
desoxyepothilone A	0.022	0.012	0.013
epothilone B (2)	0.0004	0.003	0.002
desoxyepothilone B	0.009	0.017	0.014
taxol®	0.002	3.390	0.002

[a]The cytotoxicities of test compounds were determined by the growth of human lymphoblastic leukemic cells CCRF-CEM, or their sublines resistant to vinblastine and taxol (CCRF-CEM/VBL) or resistant to etoposide (CCRF-CEM/VM-1). XTT-microculture tetrazolium/formazan assays were used.

[b]The IC_{50} values were calculated from 5-6 concentrations based on the median-effect plot using computer software.

Acknowledgments. This research was supported by the National Institutes of Health (Grant numbers CA 28824 (S.J.D.) and CA 39821 (S.B.H.)). Postdoctoral fellowship support is gratefully acknowledged to E.J.S. (NSF CHE-9504805), A.B. (NIH, CA-GM 72231), P.B. (NIH CA 62948)). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses.

REFERENCES

1. (a) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801. (b) Nicolaou, K. C.; He, Y.; Vourloumis, D.; Vallberg, H.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 166. (c) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 525; (d) Schinzer, D.; Limberg, A; Bauer, A.; Bohm, O. M.; Cordes, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 523.
2. Su, D. -S.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Chou, T. -C.; He, L. and Horwitz, S. B. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757.
3. Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomberg, D.; Gerth, K., Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567 and references therein.
4. Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.
5. (a) Danishefsky, S. J. *Chemtracts* **1989**, *2*, 273; (b) Meng, D; Sorensen, E. J.; Bertinato, P. and Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 7998; (c) Bertinato, P.; Sorensen, E. J.; Meng, D. and Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 8000.
6. Brown, H. C.; Bhat, K. S. *J. Amer. Chem. Soc.* **1986**, *108*, 5919.
7. (a) Weiler, L. *J. Am. Chem. Soc.* **1970**, *92*, 6702.; (b) Weiler, L.; Huckin, S. N. *J. Am. Chem. Soc.* **1974**, *96*, 1082.
8. Evans, D. A.; Gauchet-Prunet, J. A.; Carreira, E. M.; Charette, A. B. *J. Org. Chem.* **1991**, *56*, 741.
9. Reduction with sodium borohydride afforded a ca. 1:1 mixture of C_3 epimers.
10. Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.
11. It is interesting to speculate as to whether the effect of the C_8 methyl on the stereoselectivity of epoxidation by dimethydioxirane and the dramatic reduction of biological activity are related. Molecular modeling studies designed to explore this theme are under way.
12. Microtubule protein (MTP) was purified from calf brains by two cycles of temperature dependent assembly and disassembly.[14] In control assembly experiments, MTP (1 mg/mL) was diluted in assembly buffer containing 0.1 M MES (2-(N-morpholino) ethanesulfonic acid), 1 mM EGTA, 0.5 nM $MgCl_2$, 1mM GTP and 3M glycerol, pH 6.6. The concentration of tubulin in MTP was estimated to be about 85%. Assembly was monitored spectrophotometrically at 350 nm, 35°C for 40 min by following changes in turbidity as a measure of polymer mass.[15] Drugs were tested at a concentration of 10 μM, in the absence of GTP. Microtubule formation was verified by electron microscopy. To determine the stability of microtubules assembled in the presence of GTP or drug, turbidity was followed for 40 min after the reaction temperature was shifted to 4°C.
13. Meng, D.; Su, D.-S.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733.
14. Weisenberg, R. C. *Science* **1972**, *177*, 1104.
15. Gaskin, F.; Cantor, C. R.; Shelanksi, M. L. *J. Mol. Biol.* **1974**, *89*, 737.

(*Received in USA* 7 *April* 1997; *accepted* 6 *May* 1997)

J. Am. Chem. Soc. **1997**, *119*, 10073−10092

Total Syntheses of Epothilones A and B

Dongfang Meng,[†,‡] Peter Bertinato,[†] Aaron Balog,[†] Dai-Shi Su,[†] Ted Kamenecka,[†] Erik J. Sorensen,[†] and Samuel J. Danishefsky*[,†,‡]

Contribution from The Laboratory for Bioorganic Chemistry, The Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and The Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027

Received June 12, 1997[®]

Abstract: Convergent, stereocontrolled total syntheses of the microtubule-stabilizing macrolides epothilones A (**2**) and B (**3**) have been achieved. Four distinct ring-forming strategies were pursued (see Scheme 1). Of these four, three were reduced to practice. In one approach, the action of a base on a substance possessing an acetate ester and a nonenolizable aldehyde brought about a remarkably effective macroaldolization see (**89** → **90** + **91**; **99** → **100** + **101**), simultaneously creating the C2−C3 bond and the hydroxyl-bearing stereocenter at C-3. Alternatively, the 16-membered macrolide of the epothilones could be fashioned through a C12−C13 ring-closing olefin metathesis (e.g. see **111** → **90** + **117**; **122** → **105** + **123**) and through macrolactonization of the appropriate hydroxy acid (e.g. see **88** → **93**). The application of a stereospecific *B*-alkyl Suzuki coupling strategy permitted the establishment of a *cis* C12−C13 olefin, thus setting the stage for an eventual site- and diastereoselective epoxidation reaction (see **96** → **2**; **106** → **3**). The development of a novel cyclopropane solvolysis strategy for incorporating the geminal methyl groups of the epothilones (see **39** → **40** → **41**), and the use of Lewis acid catalyzed diene−aldehyde cyclocondensation (LACDAC) (see **35** + **36** → **37**) and asymmetric allylation (see **10** → **76**) methodology are also noteworthy.

The introduction of taxol (paclitaxel) (**1**) into cancer chemotherapy is testimony to the synergism of broadly based contributions from many areas of scientific expertise *en route* to the clinic. The original isolation and structure work, which also served to identify the cytotoxicity of the drug, was accomplished by Wall and co-workers.[1] In a seminal paper, Horwitz identified the *in vitro* mode of action of taxol, demonstrating its ability to stabilize microtubule assemblies.[2] This finding gave impetus to a wider range of pharmacological investigations of critical importance. The development of improved methods from phytochemical sources for obtaining baccatin III, and improved chemical methods for converting baccatin III to paclitaxel, provided the drug in ample quantities for human trials.

On the basis of favorable findings that issued from these evaluations, paclitaxel, developed by the Bristol Myers-Squibb Co., was approved for chemotherapeutic application against ovarian carcinomas. Since then, this drug has been undergoing extensive evaluations for other indications and is being incorporated in a variety of clinical contexts. Though it is often not a curative agent, paclitaxel is emerging as a useful main line chemotherapeutic resource. There being no evidence to the contrary, it is assumed that the *in vivo* mode of action and antitumor properties of paclitaxel arise from inhibition of cellular mitosis through the Horwitz mechanism. The question of whether or not this mode of action is actually operative in the human patient is not easily addressed.

Although having many advantages, paclitaxel is not an ideal drug.[3] One major problem with this agent, useful as it is, has to do with difficulties in its formulation. Paclitaxel is a rather insoluble substance in water, thereby necessitating awkward forms of clinical administration. Perhaps even more serious is the fact that paclitaxel is subject to a significant attenuation of therapeutic value through the onset of multiple drug resistance (MDR). Although there has been extensive structure−activity work in the paclitaxel area, to our knowledge the only modified compound presently being evaluated in human clinical trials is the close relative, taxotere.[4]

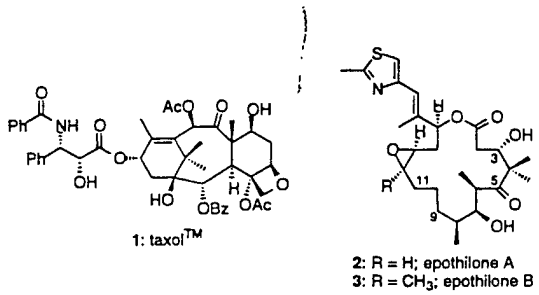


Figure 1. Structures of taxol (**1**) and epothilones A (**2**) and B (**3**).

Given the high interest engendered by taxoids as a consequence of their clinical usefulness, the search for new agents that function by a comparable mechanism is of great interest. It is in this connection that the recently discovered bacterial metabolites epothilone A (**2**) and B (**3**) have attracted considerable attention. These compounds were first identified as antifungal cytotoxic agents by Höfle and co-workers.[5] During the course of a screening program aimed at the identification of substances with a paclitaxel-like mode of action, a group based at Merck found that the epothilones are powerful cytotoxic agents which function through stabilization of cellular microtubules.[6] The strong implication of the Merck research effort was that the epothilones share in the paclitaxel mode of action.

† Sloan-Kettering.

‡ Columbia University.

[®] Abstract published in *Advance ACS Abstracts,* October 1, 1997.

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.

(2) Horwitz, S. B.; Fant, J.; Schiff, P. B. *Nature* **1979**, *277*, 665.

(3) Landino, L. M.; MacDonald, T. L. In *The Chemistry and Pharmacology of Taxol® and its Derivatives*; Farin, V., Ed.; Elsevier: New York, 1995; Chapter 7, p 301.

(4) Gueritte-Voegelein, F.; Guenard, D.; Dubois, J.; Wahl, A.; Marder, R.; Muller, R.; Lund. M.; Bricard, L.; Potier, P. In *Taxane Anticancer Agents*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; American Cancer Society: San Diego, 1995; Chapter 14, p 189.

(5) (a) Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560. (b) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567.

S0002-7863(97)01946-X CCC: $14.00

Indeed, these compounds seem to adhere to the "taxol binding domains" of the microtubule assemblies. In light of the possibility that the epothilones, or suitably modified derivatives, might find a role in cancer chemotherapy, this series also merits multidisciplinary attention. Among the scientific enterprises which we felt to be warranted in the case of the epothilones would be research directed to their total synthesis.

Unlike the situation with paclitaxel, where it was clear from the outset that total synthesis would be unlikely to impact upon the actual availability of the drug itself,[7] the simpler structures of the epothilones invited the hope that chemical synthesis could improve accessibility to the desired agents. Thus, organic chemistry could contribute to the development of an epothilone-based drug effort through a highly efficient total synthesis or possibly by delivering much simpler structures that still manifest chemotherapeutically useful biological activity.

Moreover, several intrinsically challenging chemical issues require attention in any total synthesis venture aimed at the epothilones. Quickly recognized in the case of epothilone A is the presence of a thiazole moiety, a *cis*-epoxide (C12−C13) and, somewhat unusual for a macrolide, the presence of geminal methyl groups at C4. Not the least noteworthy feature of the synthesis problem is inherent in the array of three contiguous methylene groups which serves to insulate the two domains of the epothilones that bear stereochemical imprints. The acyl section, numbered from carbons 1−8, presents a constellation of four chiral centers whose proper emplacement would require careful management. An agenda dealing with the synthesis of this domain must also include programs for elaborating and maintaining a potentially unstable β-hydroxy ester linkage at C3. The oxidation state at C3 must be cleanly differentiated from that at C5 where a ketonic group is to emerge. This entire polypropionate sector is insulated by carbons 9, 10, and 11 from the chiral *O*-alkyl domain comprising carbons 12−15. The already mentioned *cis*-epoxide, connecting carbons 12 and 13 (disubstituted in the case of epothilone A and trisubstituted in the case of epothilone B), is insulated by a single methylene group from carbon 15, which bears an allylic alcohol and a thiazole-based version of an α-methyl styryl linkage.

None of these issues in isolation pose an insurmountable obstacle to the capabilities of contemporary organic chemistry. However, taken together, they constitute a significant challenge to the goal of a stereocontrolled total synthesis of the epothilones. Below, we provide a summary of our activities that eventually accomplished this goal[8] for the first time for both epothilone A[8c] and epothilone B.[8e] In a concurrent time frame, studies from other laboratories accomplished these goals.[9,10] Moreover, the field of epothilone synthesis has spawned a host of interesting disclosures of potential impact on the long term total synthesis goal.[11]

Overall Synthetic Strategies

In pursuit of this program, a variety of initiatives were considered for assembling epothilone systems, including the natural products themselves. It is well to identify the main themes that were followed, often in parallel. In the earliest phase, our thinking was much influenced by the presence of the achiral domain encompassing carbons 9, 10, and 11. As noted above, this achiral region insulates the chiral "*O*-alkyl and acyl" sectors of the molecule from one another. We felt that the presence of this spacer element would pose a considerable difficulty in communicating stereochemical information from one chiral locus to the other. *Rather, it seemed appropriate to build the two chiral domains independently and to join them through carbon−carbon bond formation somewhere in the C9−C11 sector.* In this way, the integrity of chiral centers at C8 and C12, that are terminal to their respective chiral enclaves, would not be placed at risk in the crucial "merger" phase. One obvious possibility which presented itself in this regard was that of ring-forming olefin metathesis[12−14] (see approach I, Scheme 1). In this connection we were mindful of a seminal precedent disclosed by Hoveyda et al. in the context of synthesizing a macrolactam. Indeed, other laboratories, active in the epothilone field recognized, as did we, the potential pertinence of olefin metathesis to the epothilone area.[13]

(6) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.

(7) For total syntheses of paclitaxel (taxol™), see: (a) Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvannan, K.; Sorensen, E. J. *Nature (London)* **1994**, *367*, 630. (b) Holton, R. A.; Somoza, C.; Kim, H.-B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1597. (c) Holton, R. A.; Kim, H.-B.; Somoza, C.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1599. (d) Masters, J. J.; Link, J. T.; Snyder, L. B.; Young, W. B.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1723. (e) Danishefsky, S. J.; Masters, J. J.; Link, J. T.; Young, W. B.; Snyder, L. B.; Magee, T. V.; Jung, D. K.; Isaacs, R. C. A.; Bornmann, W. G.; Alaimo, C. A.; Coburn, C. A.; DiGrandi, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2843. (f) Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Gränicher, C.; Houze, J. B.; Jänichen, J.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Mucciaro, T. P.; Mühlebach, M.; Natchus, M. G.; Paulsen, H.; Rawlins, D. B.; Satkofsky, J.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E.; Tomooka, K. *J. Am. Chem. Soc.* **1997**, *119*, 2755. (g) Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Houze, J. B.; Krauss, N. E.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Natchus, M. G.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E. *J. Am. Chem. Soc.* **1997**, *119*, 2757.

(8) The work described herein is based on the following disclosures from our laboratory: (a) Meng, D.; Sorensen, E. J.; Bertinato, P.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 7998. (b) Bertinato, P.; Sorensen, E. J.; Meng, D.; Danishefsky, S. J. *J. Org. Chem.* **1996**, *61*, 8000. (c) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2801. (d) Meng, D.; Su, D.-S.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733. (e) Su, D.-S.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757. (f) Balog, A.; Bertinato, P.; Su, D.-S.; Meng, D.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Tetrahedron Lett.* **1997**, *26*, 4529.

(9) (a) Nicolaou, K. C.; He, Y.; Vourloumis, D.; Vallberg, H.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2399. (b) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 166. (c) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 525. (d) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Glannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268.

(10) (a) Schinzer, D.; Limberg, A.; Böhm, O. M. *Chem. Eur. J.* **1996**, *2*, 1477. (b) Schinzer, D.; Limberg, A.; Bauer, A.; Böhm, O. M.; Cordes, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 523.

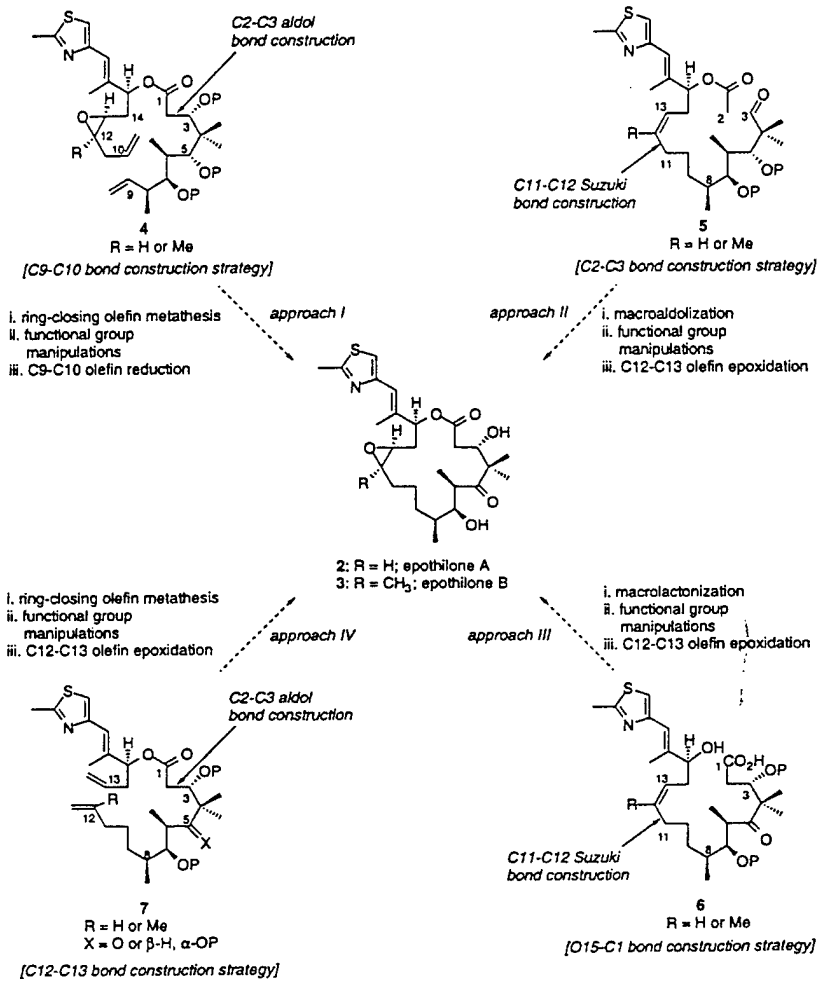
(11) For studies toward a total synthesis of the epothilones, see: (a) Mulzer, J.; Mantoulidis, A. *Tetrahedron Lett.* **1996**, *37*, 9179. (b) Claus, E.; Pahl, A.; Jones, P. G.; Meyer, H. M.; Kalesse, M. *Tetrahedron Lett.* **1997**, *38*, 1359. (c) Gabriel, T.; Wessjohann, L. *Tetrahedron Lett.* **1997**, *38*, 1363. (d) Taylor, R. E.; Haley, J. D. *Tetrahedron Lett.* **1997**, *38*, 2061. (e) De Brabander, J.; Rosset, S.; Bernardinelli, G. *Synlett* **1997** (July), 824.

(12) (a) Schrock, R. R.; Murdzek, J. S.; Bazan, G. C.; Robbins, J.; DiMare, M.; O'Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875. (b) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039. For reviews of ring-closing olefin metathesis, see: (c) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (d) Schmalz, H.-G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1833.

(13) For an outstanding demonstration of ring-closing olefin metathesis in the context of a natural product total synthesis, see: Houri, A. F.; Xu, Z.; Cogan, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1995**, *117*, 2943. For the first published application on a model basis of the olefin metathesis concepts to the epothilone problem see refs 9a and 8b disclosed in that order. For the disclosures of the total synthesis of epothilone A based on olefin metathesis see refs 9b and 8d disclosed in that order.

(14) For recent examples of ring-closing olefin metathesis, see: (a) Martin, S. F.; Chen, H.-J.; Courtney, A. K.; Liao, Y.; Pätzel, M.; Ramser, M. N.; Wagman, A. S. *Tetrahedron* **1996**, *52*, 7251. (b) Fürstner, A.; Langemann, K. *J. Org. Chem.* **1996**, *61*, 3942.

Scheme 1. Bond Construction Strategies for Total Synthesis of Epothilone A (2) and B (3)



Refining the matter still further, we directed our attentions to a construction in which the olefin metathesis bond would join carbons 9 and 10 (see 4). An alternative approach wherein carbons 10 and 11 would be joined seemed riskier. The latter construction would have involved substrates in which there was an allylic relationship between the epoxide and the C10−C11 unsaturation in both the starting material and the product. Accordingly, the olefin metathesis prospectus that we came to favor called for a precursor of the general type **4** (P = unspecified protecting group). The resultant olefin would comprise carbons 9 and 10 of the goal system. Reduction of the olefin, followed by appropriate functional group manipulations, would then lead to target systems **2** and **3**.

As will be shown, some surprising limitations in the ring-forming olefin metathesis reaction surfaced as we attempted to reduce this line of thinking to practice. When the full dimensions of the obstacles associated with a C9−C10 bond construction through ring-closing olefin metathesis were revealed, we came to focus on a fundamentally different assembly strategy wherein a double bond would be established between carbons 12 and 13 through ring-closing olefin metathesis. In this prospectus, the thiazole bearing chiral domain would display sp^3 asymmetry only at C-15. Carbons 12 and 13 would first be presented in the form of a *cis*-olefin, hopefully *en route* to a properly configured epoxide.

We also had occasion to contemplate an alternative synthetic logic, i.e. that of cross coupling, *wherein a bond would be fashioned between future carbons 11 and 12*. Specifically, we came to favor a *B*-alkyl Suzuki motif[15] to achieve this goal. In this line of reasoning, the fragments entering into this Suzuki coupling would be implied by a structure of the type **5**. From such a seco-compound, the 16-membered macrolide ring could be established through an intramolecular aldol addition,[16] giving rise to the C2−C3 bond (approach II). Alternatively, one could envision a post-Suzuki coupling structure of the type **6** which could set the stage for macrolide construction through macrolactonization[17] (approach III).

As will eventually be shown, the double bond at C-12 and C-13, either in the di- or trisubstituted series, could be very

(15) (a) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishi-Kawa, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314. (b) For an excellent review, see: Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457. (c) Johnson, C. R.; Braun, M. P. *J. Am. Chem. Soc.* **1993**, *115*, 11014. (d) Ohba, M.; Kawase, N.; Fujii, T.; Aoe, K.; Okamura, K.; Fathi-Afshar, R.; Allen, T. M. *Tetrahedron Lett.* **1995**, *36*, 6101.

(16) For a prior instance of a keto aldehyde macroaldolization, see: Hayward, C. M.; Yohannes, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 9345.

(17) For a review of methods for constructing macrolides, see: Paterson, I.; Mansuri, M. M. *Tetrahedron* **1985**, *41*, 3569. The first macrolactonization in this series was demonstrated (though unknown to us at the time of our experiments) by Nicolaou and associates (reference 9c).

effectively exploited for introduction of the β-epoxide required for the final targets. As this feasibility was established, we also directed efforts to establishment of the C12−C13 bond through a ring-closing olefin metathesis reaction. An assessment of this interesting possibility would require the construction of a diolefin of the general type **7** (see approach IV, Scheme 1). This subgoal was accomplished. We now proceed to describe the progress attained in pursuing each of these strategies.

The First Generation Ring-Closing Olefin Metathesis Strategy (Approach I, Scheme 1)

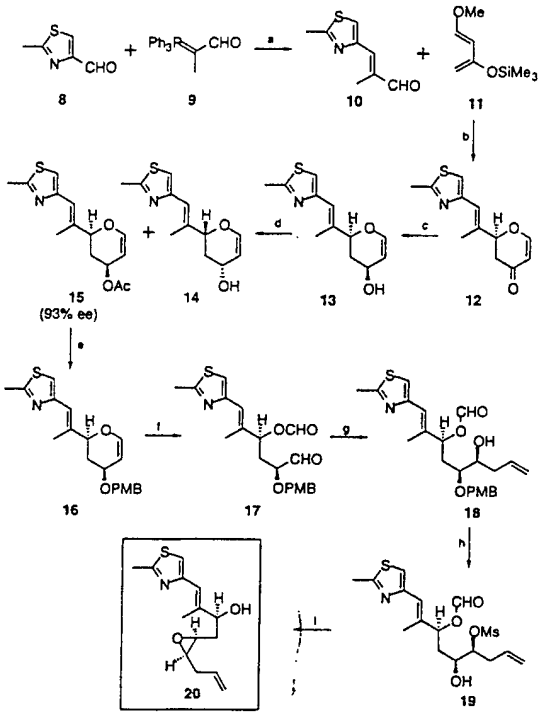
As was alluded to above, several options for constructing the 16-membered macrolide ring of the epothilones were considered as we contemplated strategies for a total synthesis. Initially, we favored a strategy wherein the C9−C10 would be fashioned during the course of the macrocyclization event. It was our hope that a structure of the general type **4** (see Scheme 1) could be induced to undergo a ring-closing olefin methathesis (RCM). Our considerations for favoring such an approach were several. In synthesizing and merging the subunits leading to **4**, all of the stereochemical problems associated with an epothilone total synthesis would have been overcome. The unsaturation at C9−C10 that would emerge from a successful ring-closing olefin metathesis could conceivably be removed by reduction *en route* to the epothilones, or used to introduce new functionality for the purpose of synthesizing novel analog structures.

As will be shown (*vide infra*), the guiding paradigm, *i.e.* the possibility of creating a C9−C10 double bond during the course of an intramolecular RCM process, was not reducible to practice in a subststrate having adequate functionality to reach the natural products. Nonetheless, many of our perceptions pertinent to the epothilone stereochemical problem and, indeed, some of the very compounds used in the pursuit of approach I, did find application to variations for the successful total syntheses. Hence, we describe here the findings pertinent to approach I, Scheme 1.

We defined goal system **20** (see Scheme 2) as a milestone compound under the approach I program. A structure of this genre would be joined to an acyl fragment (*vide infra*) to establish a precursor of the type hitherto generalized as **4**. Our path commenced with the known aldehyde **8**,[18] which was elongated in a Wittig-type construction with the commercially available phosphorane **9**, leading to **10** in 83% yield (see Scheme 2).[8a] At this stage, it was of interest to us to take advantage of a line of chemistry that our laboratory had innovated in the 1980s.[19] Thus, aldehyde **10** served as a "heterodienophile" in the context of a Lewis acid catalyzed diene−aldehyde cyclocondensation (LACDAC) reaction with the synergistic butadiene **11**.[20] The reaction proceeded quite smoothly, giving rise to the racemic dihydropyrone **12** in a yield of 65%.

Reduction of compound **12** via conditions that we had introduced some years ago for synthesizing artificial glycals bearing equatorial alcohols at C3 (glucose numbering),[21] led to racemic **13**. Here we were able to take advantage of more recently introduced methodology, wherein racemic glycals, derived by total synthesis rather than from carbohydrate sources, could be effectively resolved by lipase-mediated kinetic resolution.[22] In the event, we chose to carry out a kinetic resolution

Scheme 2[a]



[a] (a) C_6H_6, reflux (83%); (b) *trans*-1-methoxy-3-((trimethylsilyl)oxy)-1,3-butadiene (**11**), $BF_3{\cdot}OEt_2$, CH_2Cl_2; then CSA (65%); (c) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C → rt (99%); (d) Lipase-30, vinyl acetate, DME, rt, (−)-**15** (45%; 93% ee); (e) (i) K_2CO_3, MeOH, rt; (ii) PMBCl, NaH, DMF, 0 °C → rt (97% overall); (f) 3,3-dimethyldioxirane, K_2CO_3, CH_2Cl_2, 0 °C; then $NaIO_4$, H_2O/THF (92%); (g) allyl triphenylstannane, $SnCl_4$, CH_2Cl_2, −78 °C (98% of **18** + epimer (4:1)); (h) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (ii) DDQ, CH_2Cl_2/H_2O (20:1), 0 °C → rt (93% overall); (i) (i) $LiN(SiMe_3)_2$, THF, −78 → 0 °C; (ii) K_2CO_3, MeOH/H_2O (78% of the *cis* epoxide); PMB = *p*-$MeOC_6H_4CH_2$; Ms = SO_2CH_3.

through acetylation in the "forward sense". Following protocols of Wong,[23] the racemic alcohol was used in a transesterification experiment with vinyl acetate under the influence of Lipase-30 to afford alcohol **14** and acetate **15**. Of course, at this stage, we were in no position to assert the assignments of the absolute configuration to the antipodal glycal and glycal acetates with rigorous confidence. Rather, our tentative formulations arose from extensive precedents that had been garnered in our laboratory some years ago in this general area.[22] The presumed 3*S*-acetate **15** was subjected to deacetylation, giving rise to *ent*-**14**. The latter was subjected to the action of sodium hydride and *p*-methoxybenzyl chloride to afford **16**. In principle, it was initially supposed that compound **14** in the 3*R* series could be utilized in the synthesis program. However, as will be shown, an alternate route not requiring resolution to reach the desired 3*S* pyranoid series (cf. **16**) became available, and the possibility of recycling the 3*R* series available from the lipase chemistry was not pursued.

The next phase of the program called for disconnection of the C1−C2 bond of the artificial glycal **16** in such a fashion that C3 would emerge as C13 of the projected *cis*-epoxide **20**. We proceeded as follows. Drawing once again on chemistry that we had introduced in an earlier era for different purposes,[24]

(18) (a) Shafiee, A.; Mazloumi, A.; Cohen, V. I. *J. Heterocycl. Chem.* **1979**, *16*, 1563. (b) Schafiee, A.; Shahocini, S. *J. Heterocycl. Chem.* **1989**, *26*, 1627.

(19) (a) Danishefsky, S. J. *Aldrichim. Acta* **1986**, *19*, 59. (b) Danishefsky, S. J. *Chemtracts* **1989**, *2*, 273.

(20) Danishefsky, S. J.; Kitahara, T. *J. Am. Chem. Soc.* **1974**, *96*, 7807.

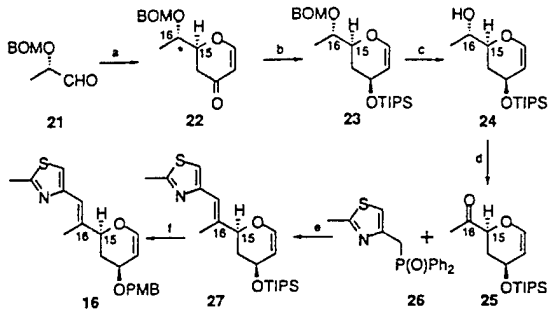
(21) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

(22) (a) Berkowitz, D. B.; Danishefsky, S. J. *Tetrahedron Lett.* **1991**, *32*, 5497. (b) Berkowitz, D. B.; Danishefsky, S. J. Schulte, G. K. *J. Am. Chem. Soc.* **1992**, *114*, 4518.

(23) Hsu, S.-H.; Wu, S.-S.; Wang, Y.-F.; Wong, C.-H. *Tetrahedron Lett.* **1990**, *31*, 6403.

(24) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1381.

Scheme 3[a]



[a] (a) *trans*-1-Methoxy-3-((trimethylsilyl)oxy)-1,3-butadiene (**11**), $MgBr_2 \cdot OEt_2$, THF, −10 °C; then AcOH, H_2O (93%); (b) (i) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C; (ii) TIPSCl, imidazole, DMF, 0 °C → rt (87% overall); (c) Na^0, NH_3 (l), THF, −78 °C; then MeOH, −78 → 25 °C (92%); (d) Dess–Martin periodinane, pyridine, CH_2Cl_2, rt (98%); (e) **26**, *n*-BuLi, THF, −78 °C; then **25**, THF, −78 °C → rt (87%); (f) (i) *n*-Bu_4NF, THF, rt; (ii) PMBCl, NaH, DMF, 0 °C → rt (97% overall); BOM = CH_2OCH_2Ph; TIPS = Si*i*-Pr_3; PMB = *p*-$MeOC_6H_4CH_2$.

reaction of **16** with 3,3-dimethyldioxirane gave rise to an intermediate epoxide which, on oxidative solvolysis with sodium metaperiodate, afforded a 92% yield of aldehyde **17**. It was hoped that the emergence of the future oxygen at C15 (epothilone numbering) in the form of the formate ester would serve to prevent hemiacetal formation with the aldehyde group. Such a complicating event would have been anticipated if the C15 oxygen were free. While there was considerable apprehension as to whether the formate protecting device would be equal to the challenges with which it would be confronted, in practice this group survived during reaction of compound **17** with allyltriphenylstannane.[25] There was obtained a 96% yield of a 4:1 mixture of diastereomers at the future C12. The major product was assumed (and demonstrated on the basis of subsequent events) to be **18** in the relative configurational sense. Compound **18** was then subjected to mesylation, followed by deprotection of the *p*-methoxybenzyl group to give the hydroxy mesylate **19**. Finally, the sequence was completed by cyclization of the hydroxy mesylate with lithium hexamethyldisilazide. In this way, goal system **20** was obtained. Since compound **20** was indeed a *cis*-epoxide, the assignments of relative stereochemistry to compounds subsequent to the intermediate **12** had been substantiated. As for the absolute configuration of **20**, this assignment was proven by a sharply modified route (*vide infra*), which was undertaken to avoid the need for any resolution.

The pursuit of this modified route was also motivated by the desire to demonstrate still another dimension to the cyclocondensation reaction.[19] The concept involved the leveraging of chirality of heterodienophiles in the LACDAC reaction to create a pyran matrix of defined absolute configurations. This accomplished, the original asymmetries of the hetereodienophile can be abrogated, depending on the needs of the synthesis.

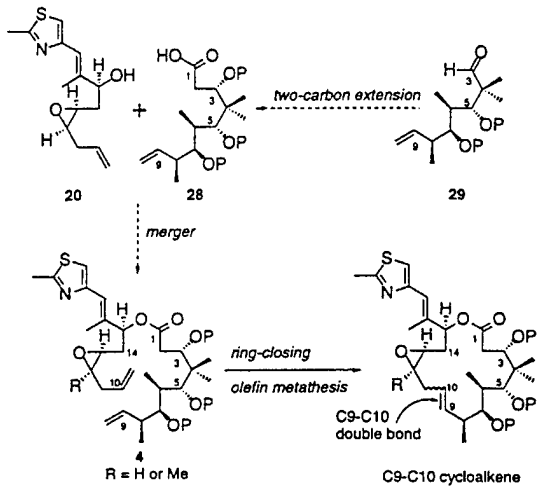
To teach this lesson, we proceeded as follows. Cyclocondensation of the known lactaldehyde derivative **21**[26] with **11** under mediation by magnesium bromide etherate gave rise to a 93% yield of a dihydropyrone (see Scheme 3). On the basis of earlier work,[27] we formulated this pyrone to be structure **22**. The importance of this assignment lies in the statement that it

(25) For a review of allylations, see: Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207.

(26) Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pirrung, M. C.; White, C. T.; VanDerveer, D. *J. Org. Chem.* **1980**, *45*, 3846.

(27) (a) Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F. *J. Am. Chem. Soc.* **1984**, *106*, 2455. (b) Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F.; Maring, C. J.; Springer, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1256.

Scheme 4. A C9–C10 Bond Construction through Ring-Closing Olefin Metathesis



makes about the absolute stereochemistry of the center destined to become C15. This center was presumed to be *S* as a consequence of α-chelation control in the cyclocondensation reaction. It will be appreciated that we had introduced an sp^3 chiral element which was *per se* unneeded, at the future trigonal C16 center (see the indicated carbon atom in **22**).

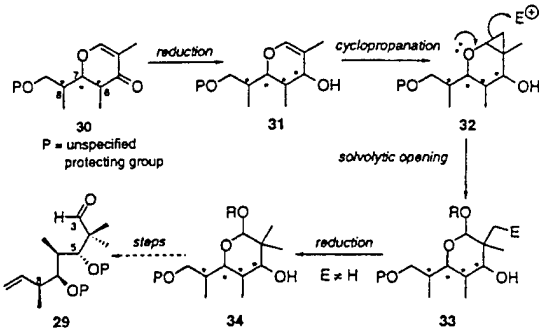
Compound **22** was integrated within the main synthetic pathway as follows. The ketone function of the dihydropyrone was reduced, as before, and the resultant alcohol at C3 (glycal numbering) was protected as its triisopropylsilyl ether (TIPS) derivative **23**. The benzyloxymethyl (BOM) group was discharged through the action of sodium in liquid ammonia and the alcohol function in the resultant **24** was subjected to oxidation using the Dess–Martin periodinane procedure.[28] This sequence provided **25** in 90% yield. This compound was, in turn, successfully condensed with phosphine oxide **26**[8a] in a Horner reaction,[29] thereby producing the elongated structure **27**. For purposes of stereochemical correlation, the silyl group was cleaved (*n*-Bu_4NF), and the resultant alcohol was reprotected as its *p*-methoxybenzyl ether. At this stage, we had achieved an alternate synthesis of compound **16** in a way that rigorously defined the relative stereochemistry as well as the absolute configuration. The conversion of intermediate **16** to **20** has already been discussed above.

With these successes as a platform, it was appropriate to focus on the construction of the acyl fragment projected for the olefin metathesis step. For this purpose, it would be appropriate to reach a carboxylic acid (cf. **28**, Scheme 4) for joining to alcohol **20** to reach system type **4**. We further presumed at the planning level that acid **28** would arise by a two carbon extension of a generic aldehyde (cf. **29**). In this section of the molecule, we would also be dealing with incorporation of the geminal methyl groups at the future C4 as well as the implementation of the appropriate chirality at carbons 6, 7, and 8. The chirality at C3 would have to be established during the course of the two carbon homologation alluded to above.

(28) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277. (c) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549. (d) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

(29) For examples, see: (a) Lythgoe, B.; Nambudiry, M. E. N.; Ruston, S.; Tideswell, J.; Wright, P. W. *Tetrahedron Lett.* **1975**, 3863. (b) Lythgoe, B. *Chem. Soc. Rev.* **1981**, 449. (c) Toh, H. T.; Okamura, W. H. *J. Org. Chem.* **1983**, *48*, 1414. (d) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098.

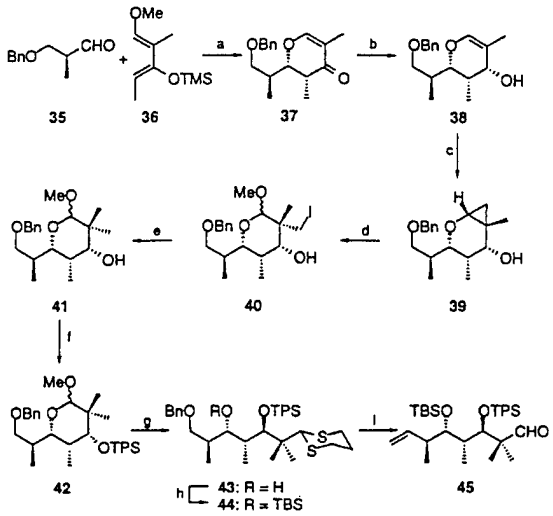
Scheme 5. General Strategy for a Synthesis of the Polypropionate Domain of the Epothilones



A central goal of our plan was the attainment of stereospecificity in the management of chirality at carbons 6, 7, and 8. As our thinking evolved (*vide infra*), it seemed that control over these issues could be facilitated if our scheme included a temporary chirality element at C5. Though C5 is destined to become a ketone, the temporary sp^3-associated chirality at this center would be very valuable in the management of stereochemistry in this region and in the introduction of the geminal methyl groups at C4. The possibility of using a dihydropyrone to address this problem presented itself.[8b] The thought was to translate the C-6, -7, and -8 domain of epothilone to correspond to dihydropyrone **30** (see Scheme 5). The latter could be accessed through cyclocondensation chemistry (*vide infra*). Hence, an artificial glycal (cf. **30**) was seen to be an exploitable intermediate *en route* to subgoal structure **29**. *More specifically, the thought was to utilize a C5 alcohol to facilitate and direct a cyclopropanation of the glycal double bond* (see **31** → **32**, Scheme 5).[30,31] A regiospecific solvolytic fragmentation of the cyclopropane ring in **32**[32] would then provide, in gross terms, an aldehyde equivalent of the type **33**. In the event that the crucial cyclopropane solvolysis step would be conducted in an oxidative sense (i.e. $E^- \neq H$), it would then be necessary to effect a reduction of **33** to reach a compound of the type **34**. The latter would then be advanced, as appropriate, to reach the desired aldehyde **29**. This general thinking is summarized in Scheme 5.

In practice, titanium-mediated cyclocondensation of the known and optically pure β-(benzyloxy)isobutyraldehyde **35**[33] with diene **36**,[34] following a protocol previously devised in our laboratory,[35] gave rise to dihydropyrone **37** (see Scheme 6).[8b] Reduction of this compound with lithium aluminum hydride in ether provided glycal **38**. The hydroxyl group was then used

Scheme 6[a]



[a] (a) $TiCl_4$, CH_2Cl_2, −78 °C; then CSA, PhH, rt (87%); (b) $LiAlH_4$, Et_2O, −78 °C (91%); (c) Et_2Zn, CH_2I_2, Et_2O, rt (93%); (d) NIS (7 equiv), MeOH, rt; (e) *n*-Bu_3SnH, AIBN (cat.), PhH, reflux (80% from **39**; (f) Ph_3SiCl, imidazole, DMF, rt (97%); (g) 1,3-propanedithiol, $TiCl_4$, CH_2Cl_2, −78 → −40 °C (78%); (h) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, −78 °C (98%); (i) (i) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2/H_2O (19:1), rt (89%); (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C → 0 °C (90%); (iii) CH_3PPh_3Br, $NaN(SiMe_3)_2$, $PhCH_3$, 0 °C → rt (76%); (iv) $PhI(OCOCF_3)_2$, $CH_2Cl_2/CH_3CN/H_2O$, rt (85%); Bn = CH_2Ph; TMS = $SiMe_3$; TPS = $SiPh_3$; TBS = Si-*t*-$BuMe_2$.

to direct a cyclopropanation under modified Conia–Simmons–Smith conditions[31a] to afford cyclopropano derivative **39**. Oxidative solvolytic fragmentation of this cyclopropane was accomplished through the agency of *N*-iodosuccinimide in methanol to provide methyl glycoside **40**.[8b] Reductive deiodination of this compound led to the branched artificial methyl glycoside **41**, after which triphenylsilylation afforded the protected derivative **42**.

At this stage, it was timely to cleave the pyran ring with a view toward liberating the future aldehyde corresponding to C3 of the epothilones. Advancement *en route* to this goal involved subjecting compound **42** to the combined action of 1,3-propanedithiol and titanium(IV) chloride. This protocol led to the formation of the dithioacetal **43**.[36] Protection of the future C7 alcohol as shown (see compound **44**) was followed by olefin formation and liberation of the aldehyde function. In this way the specific compound **45** was in hand.

The next stage in the pursuit of approach I involved a two-carbon expansion starting with aldehyde **45**. The goal was the production of an acylation partner for alcohol **20** *en route* to a competent substrate for ring-closing olefin metathesis. In an early experiment, aldehyde **45** was treated with the lithium enolate of *tert*-butyl acetate (Rathke anion, see Scheme 7).[37]

(30) (a) Winstein, S.; Sonnenberg, J. *J. Am. Chem. Soc.* **1961**, *83*, 3235. (b) Dauben, W. G.; Berezin, G. H. *J. Am. Chem. Soc.* **1963**, *85*, 468. (c) Furukawa, J.; Kawabata, N.; Nishimura, J. *Tetrahedron* **1968**, *24*, 53. (d) For recent examples of oxygen-directed cyclpropanations of glycals, see: Hoberg, J. O.; Bozell, J. J. *Tetrahedron Lett.* **1995**, *36*, 6831. (e) For an excellent review of substrate-directable chemical reactions, see: Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, *93*, 1307.

(31) For selected examples of glycal cyclopropanations, see: (a) Boeckman, R. K., Jr.; Charette, A. B.; Asberom, T.; Johnston, B. H. *J. Am. Chem. Soc.* **1991**, *113*, 5337. (b) Murali, R.; Ramana, C. V.; Nagarajan, M. *J. Chem. Soc., Chem. Commun.* **1995**, 217. (c) Henry, K. J., Jr.; Fraser-Reid, B. *Tetrahedron Lett.* **1995**, *36*, 8901. (d) Hoberg, J. O.; Claffey, D. J. *Tetrahedron Lett.* **1996**, *37*, 2533. (e) Timmers, C. M.; Leeuwenburgh, M. A.; Verheijen, J. C.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron: Asymmetry* **1996**, *7*, 49. (f) Scott, R. W.; Heathcock, C. H. *Carbohydr. Res.* **1996**, *291*, 205. (g) For an excellent review of cyclopropanes in synthesis, see: Wong, H. N. C.; Hon, M.-Y.; Tse, C.-H.; Yip, Y.-C.; Tanko, J.; Hudlicky, T. *Chem. Rev.* **1989**, *89*, 165.

(32) (a) Collum, D. B.; Mohamadi, F.; Hallock, J. S. *J. Am. Chem. Soc.* **1983**, *105*, 6882. (b) Collum, D. B.; Still, W. C.; Mohamadi, F. *J. Am. Chem. Soc.* **1986**, *108*, 2094. (c) For an important early study, see: Wenkert, E.; Mueller, R. A.; Reardon, E. J., Jr.; Sathe, S. S.; Scharf, D. J.; Tosi, G. *J. Am. Chem. Soc.* **1970**, *92*, 7428.

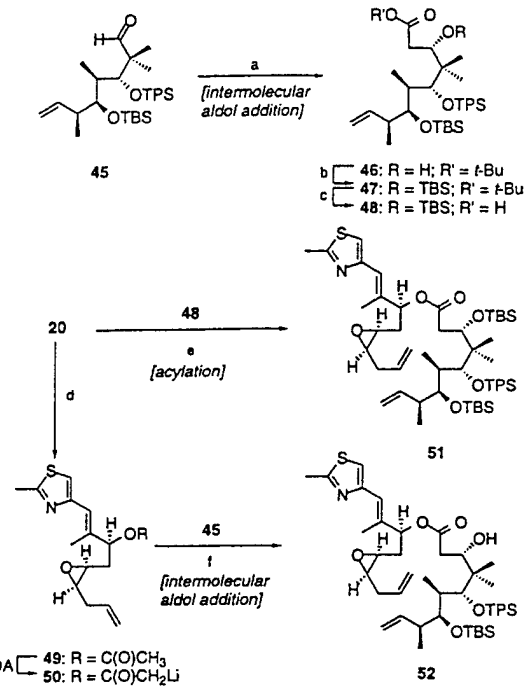
(33) (a) Cohen, N.; Eichel, W. F.; Lopresti, R. J.; Neukom, C.; Saucy, G. *J. Org. Chem.* **1976**, *41*, 3505. (b) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873. (c) Meyers, A. I.; Babiak, K. A.; Campbell, H. L.; Comins, D. L.; Fleming, M. P.; Henning, R.; Heuschmann, M.; Hudspeth, J. P.; Kane, J. M.; Reider, P. J.; Roland, D. M.; Shimizu, K.; Tomioka, K.; Walkup, R. D. *J. Am. Chem. Soc.* **1983**, *105*, 5015. (d) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348.

(34) Danishefsky, S. J.; Yan, C.-F.; Singh, R. K.; Gammill, R. B.; McCurry, P. M., Jr.; Fritsch, N.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101*, 7001.

(35) (a) Danishefsky, S. J.; Pearson, W. H.; Harvey, D. F.; Maring, C. J.; Springer, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1256. (b) Danishefsky, S. J.; Myles, D. C.; Harvey, D. F. *J. Am. Chem. Soc.* **1987**, *109*, 862.

(36) For an example, see: Egbertson, M.; Danishefsky, S. J. *J. Org Chem.* **1989**, *54*, 11.

Scheme 7[a]



[a] (a) *t*-BuOC(O)CH_2Li, THF, 0 °C (90%; *ca.* 2.5:1 mixture of C-3 epimers in favor of **46**); (b) *t*-BuMe_2SiOTf, 2,6-lutidine, CH_2Cl_2, rt; (c) TESOTf, 2,6-lutidine, CH_2Cl_2, rt (90% overall); (d) Ac_2O, Et_3N, 4-DMAP, CH_2Cl_2, rt (94%); (e) **48**, EDC, CH_2Cl_2, 4-DMAP, rt; then **20** (78%); (f) **45** + **49**, LDA, THF, −78 °C (2−6:1 mixture of C3 epimers in favor of **52**; 85%); TBS = Si*t*-BuMe_2; TPS = $SiPh_3$.

There was thus generated a mixture of diastereomeric alcohols at the carbon destined to become C3 of epothilone. The major product was shown to have the required 3*S* configuration. The alcohol function in compound **46** was successfully protected as the *tert*-butyldimethylsilyl (TBS) ether derivative (see compound **47**). At this stage, it was possible to cleave the *tert*-butyl ester function to generate the acid **48**.

The coupling of the previously described alcohol **20** with acid **48** was conducted under the influence of 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) to produce, at long last, the proposed metathesis substrate **51** (see Scheme 7). It was also of interest to investigate the possibility of a more concise construction of a possible metathesis substrate. This approach started with acetylation of compound **20** to provide **49**. This acetate, when treated with lithium diisopropylamide, generated a presumed lithium ester enolate (see proposed structure **50**). This enolate underwent successful union with aldehyde **45** to produce a mixture of C3 epimers with the desired compound **52** predominating. A particularly interesting and efficient method for conducting the coupling of **49** and **45** involved merger of the two units in a "Barbier" sense.[38] *In this mode, the aldehyde and the ester would be concurrently exposed to the action of lithium diisopropylamide.* It was felt that such a treatment might be successful since the aldehyde is nonenolizable. In the event, an 85% yield of a ca. 5:1 mixture of C3 epimers was obtained with the major product being the 3*S* compound shown. This experiment was to have significant implications for our intramolecular aldol addition strategy which will be discussed under approach II.

(37) Rathke, M. W.; Sullivan, D. F. *J. Am. Chem. Soc.* **1973**, *95*, 3050.
(38) For a prior instance of a Barbier aldol reaction, see: Linde, R. G., II; Jeroncic, L. O.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 2534.

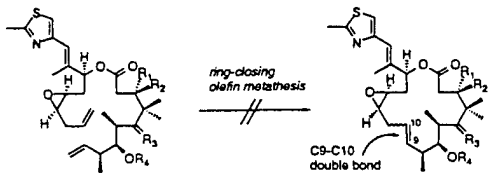
We now had in hand substrates **51** and **52**. Surprisingly, when these substrates were submitted to a range of conditions and catalysts designed to bring about ring-closing olefin metathesis, no convincing indication could be garnered for the presence of cyclized systems even to a small extent. While we could not exclude the possibility that trace amounts of desired products had been produced, the overwhelming bulk of the material was clearly of a very complicated nature, suggesting probable oligomerization. In any case, neither the protected system **51**, nor the structure bearing a free C3 alcohol in **52** served as usable substrates *en route* to epothilone A.

We did not take the setbacks in this early skirmishing with substrates **51** and **52** to necessarily establish the nonviability of the concept. We hoped that the feasibility of the RCM reaction could be sharply influenced by the nature and stereochemistry of the "decorating" substituents along the acyl chain. It seemed possible that certain substrates might be more amenable to cyclization in that conformational factors (in these variants) might help to predispose proximity between the terminal vinyl groups, or that properly selected substituents would be less conformationally obtrusive in the cyclic products which we were hoping to form on metathesis. In that spirit, we synthesized a wide variety of compounds as potential participants in the RCM reaction.[39] Unfortunately, none of these candidate substrates produced workable amounts of cyclized product. At best, mass spectrometric analysis indicated the possible formation of some desired materials. However, attempted isolation of traces of RCM products (assuming they were actually present) from very complex mixtures proved to be unsuccessful.

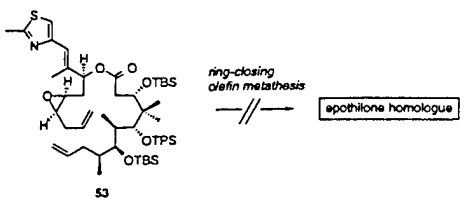
These facts, however disheartening, forced us to conclude that the row of substituents projecting from C3 through C8 had created an unmanageable problem of steric hindrance with respect to participation of the C9 double bond in the metathesis process. To probe this matter further, we went so far as to synthesize a compound which would, in itself, not constitute a promising intermediate for reaching epothilone. However, we thought that the study of the olefin metathesis possibility with this substrate could shed more light on the failures of the previously described entries. Accordingly, we synthesized compound **53**[40] in which the vinyl group on the acyl side was insulated from the secondary methyl group at C8 by another methylene group. Unhappily, homologous olefin **53** also failed to undergo ring-closing olefin metathesis.[40]

As described elsewhere,[8b] we went on to demonstrate to our satisfaction that the "culprit" in preventing the ring forming olefin metathesis reaction was the network of functionality between C3 and C8. There was nothing inherently unworkable

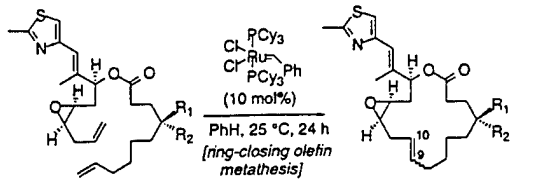
(39) For specific substrates tested in ring-closing olefin metathesis reactions, see Supporting Information.



(40) Compound **53** was not a successful substrate for ring-closing olefin metathesis.



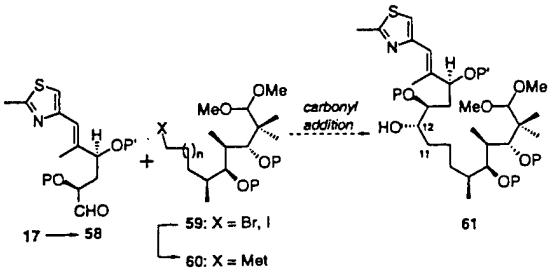
Scheme 8. Construction of the Compounds **56** and **57** through Ring-Closing Olefin Methathesis



54: R_1, R_2 = H
55: R_1, R_2 = CH_3

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

Scheme 9. Intermolecular Carbonyl Addition Strategy for the Construction of the C11−C12 Bond



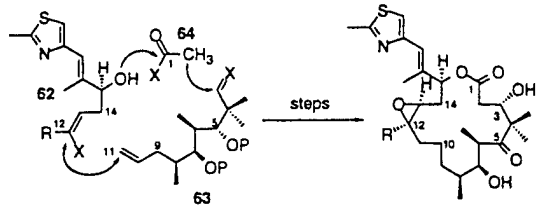
about inclusion of the homoallylic epoxide or the thiazolyl function, which were situated in the alkyl sector of the proposed premacrolide substrate. This "probable cause" argument was demonstrated by successful ring-closing metathesis reactions of substrates **54** and **55** (see Scheme 8). Unfortunately, while products **56** and **57**, respectively, could be obtained from such reactions in excellent yields, they did not display significant biological activity, either in tubulin binding or in cell-culture cytotoxicity studies.

We were now in the horns of what seemed to be an unsolvable dilemma. Those substrates in which olefin metathesis, leading to a C9−C10 cycloalkene (see Scheme 8), could be conducted provided products with nonuseful biological profiles. Conversely, those substrates which were functionalized in the spirit of serious potential epothilone precursors failed to undergo ring-closing olefin metathesis. It was when the full scope of this conundrum became clear that approach I was set aside in favor of other options.

Since we had demonstrated the ability to generate a protected aldehyde of the type **17** (Scheme 2), we turned to the possibility of a convergent coupling of this sort of system (generalized as **58**) with a conventional nucleophile in the form of a metallo derivative (generalized as **60**) (see Scheme 9). In the ideal scenario, **60** would be derived from **59**, in which case a product such as **61** could be anticipated.

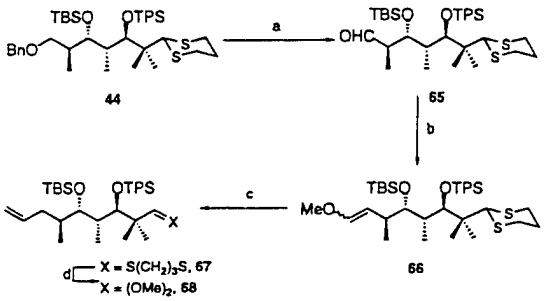
In practice, it proved possible to synthesize potential probe compounds for such metalation reactions (i.e. systems of the type **59**). Surprisingly, at no point were we able to accomplish the metalation of any such derivative to produce a competent organometallic nucleophile corresponding to **60**. In all cases, either unreacted aldehyde was recovered with the protonated metal species or decomposition took place. These failures were documented, not only in attempted couplings to the relatively sophisticated electrophile **17**, but even with much simpler electrophiles. We could garner little evidence to show that we had achieved metalation either in the series $n = 0$ or $n = 1$. These failures, suggesting problems in accessing external agents to the terminus of **59**, mirrored some of the difficulties which were soon to be encountered in the Suzuki coupling scheme (*vide infra*). Fortunately, this problem could be overcome in a novel way.

Scheme 10. C11−C12 Suzuki Bond Construction



2: R = H; epothilone A
3: R = CH_3; epothilone B

Scheme 11[a]



[a] (a) (i) DDQ, CH_2Cl_2/H_2O (89%); (ii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 → 0 °C (90%);(b) $MeOCH_2PPh_3Cl$, *t*-BuOK, THF, 0 °C → rt (86%); (c) (i) *p*-TSOH, dioxane/H_2O, 50 °C (99%); (ii) CH_3PPh_3Br, NaHMDS, $PhCH_3$, 0 °C → rt (76%); (d) $PhI(O-COCF_3)_2$, MeOH/THF, rt, 0.25 h (92%); Bn = CH_2Ph; TPS = $SiPh_3$; TBS = Si*t*-$BuMe_2$.

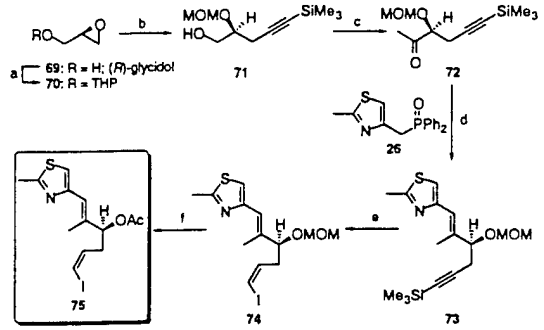
B-Alkyl Suzuki Strategy (Approaches II and III, Scheme 1)

We now report the results of the first successful syntheses of epothilones A and B which were achieved by the alkyl Suzuki method. The concept is generalized in Scheme 10 anticipating a synthesis of epothilones A and B. Through some as yet unspecified method, we envisioned a route to a *Z*-haloalkene **62**. Furthermore, it was expected that the chemistry would lend itself to construction of a terminal vinyl system in the context of a protected C3 substructure, generalized as system **63**. Construction of the C11−C12 bond would be the hallmark of the scheme and would be accomplished through a *B*-alkyl Suzuki coupling[15] (*vide infra*). Also to be dealt with would be a two carbon insert corresponding to carbons 1 and 2 of epothilone (see **64**). The appendage **64** could be incorporated in the scheme at several stages. If these carbon−carbon bond producing maneuvers were to be realized, there would also remain the need for introduction of the C12−C13 β-epoxide through the action of a suitable oxidizing agent. It was from these perceptions that our overall strategy for reaching epothilone A emerged. With suitable modification, a route to epothilone B was also embraced under this paradigm.

In practice, we turned to aldehyde **65** (Scheme 11). Compound **65** had been previously described as arising from **44** and had been converted to terminal vinyl compound **45** (Scheme 6). Now, the same aldehyde was successfully coupled to (methoxymethyl)triphenylphosphorane to give rise to **66**.[8c] The latter was then subjected to sequential hydrolysis and Wittig reactions to afford **67**. Finally, it proved possible to convert the dithiane linkage protecting C3 to the dimethyl acetal **68**. Here it was envisioned that the future C3 aldehyde could be revealed from the dimethyl acetal even in a mutlifunctionalized substrate.

With this chemistry in hand, we turned our attentions to the other projected Suzuki coupling partner. The specific version

Scheme 12[a]



[a] (a) Dihydropyran, PPTS, CH_2Cl_2, rt (73%); (b) (i) $Me_3SiCCLi$, $BF_3{\cdot}OEt_2$, THF, −78 °C (76%); (ii) MOMCl, *i*-Pr_2NEt, $Cl(CH_2)_2Cl$, 55 °C (85%); (iii) PPTS, MeOH rt (95%); (c) (i) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 → rt; (ii) MeMgBr, Et_2O, 0 °C → rt (85% for two steps); (iii) TPAP, NMO, 4 Å mol sieves, CH_2Cl_2, 0 °C → rt (93%); (d) **26**, *n*-BuLi, THF, −78 °C; then **72**, THF, −78 °C → rt (97%); (e) (i) *N*-iodosuccinimide, $AgNO_3$, $(CH_3)_2CO$ (64%); (ii) dicyclohexylborane, Et_2O, AcOH (65%); (f) (i) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt (86%); (ii) Ac_2O, pyr, 4-DMAP, CH_2Cl_2, rt (99%); PPTS = pyridinium *p*-toluenesulfonate; MOMCl = methoxymethyl chloride; TPAP = tetra-*n*-propylammonium perruthenate; NMO = *N*-methylmorpholine *N*-oxide.

of the generic structure **62** which was settled upon was the iodoacetate (see **75**, Scheme 12). Needless to say, it would be necessary to "deliver" this compound with the appropriate olefin geometry and in optically pure form for melding into the epothilone synthesis. In theory, depending on the coupling modality, we could utilize either enantiomeric version at C15; each enantiomer could be interfaced into the synthesis, subject to whether inversion or retention would be required at C15.

In our first route,[8c] we anticipated retention of configuration at this center (see Scheme 12). Accordingly, our program started with the commercially available *R*-(+)-glycidol (**69**).[41] The hydroxyl group was protected in the form of a THP ether (see compound **70**). In a defining step, the epoxide linkage was used to alkylate the lithium salt of (trimethylsilyl)acetylene, under the conditions described, to give rise to compound **71**. It will be recognized that the chiral center of glycidol is retained *en route* to coupling partner **75**.

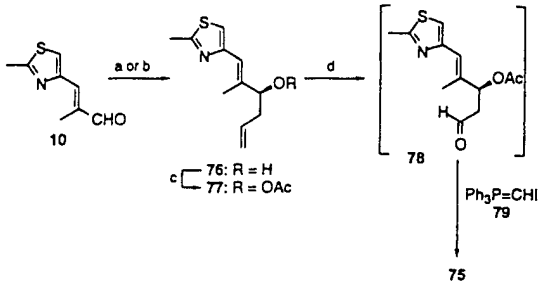
The next phase involved the classical transformation of the primary alcohol linkage to a methyl ketone. This was accomplished, as indicated, to provide ketone **72**. Drawing from an important precedent (see **25** + **26** → **27**, Scheme 3),[8a] we could accomplish the introduction of the thiazolyl nucleus through an Emmons reaction[29] of phosphine oxide **26** with methyl ketone **72**. In the concluding phase of this synthesis, silyl acetylene **73** was converted to the corresponding iodoalkyne which, upon reduction,[42] gave rise to the *cis*-iodoalkene **74**. Finally, cleavage of the MOM protecting group, as shown, followed by acetylation, produced the desired *cis*-vinyl iodide **75**.

When the general concept of the alkyl Suzuki coupling proved to be fruitful (*vide infra*), more concise syntheses of **75** were achieved. For this purpose, we returned to the enal **10**, a substance employed in an earlier stage of the synthesis.[8a] Allylation of this compound with tri-*n*-butylstannane in the presence of the (*S*)-BINOL enantiodirecting ligand, as described by Keck,[43] gave rise to the allylated product **76** in greater than

(41) For an excellent review of the chemistry of glycidol, see: Hanson, R. M. *Chem. Rev.* **1991**, *91*, 437.

(42) Corey, E. J.; Cashman, J. R.; Eckrich, T. M.; Corey, D. R. *J. Am. Chem. Soc.* **1985**, *107*, 713.

Scheme 13[a]



[a] (a) Allyltri-*n*-butylstannane, (*S*)-(−)-BINOL, $Ti(Oi\text{-}Pr)_4$, CH_2Cl_2, −20 °C (60%; >95% ee); (b) $[(-)\text{-Ipc}]_2BCH_2CHCH_2$, Et_2O, −100 °C; then 3 N NaOH, 30% H_2O_2 (83%; >95% ee); (c) Ac_2O, 4-DMAP, Et_3N, CH_2Cl_2 (96%); (d) (i) OsO_4, NMO, 0 °C; (ii) $NaIO_4$, THF/H_2O, rt (iii) **79**, THF, −78 → 0 °C (50% overall).

95% enantiomeric excess (see Scheme 13). Alternatively, we could effect an asymmetric allylation of enal **10** through the use of Brown's procedure.[44,9b] These transformations were generally faster and higher yielding but, of course, lacked the feature of implementation of chirality through catalytic means. The optical purity of allylic alcohol **76** prepared by allyl boration, was established by formation of the Mosher ester and subsequent analysis by ^1H and ^{19}F NMR spectroscopy. Eventually, the assignment and extent of optical purity were corroborated by interfacing this product with compound **75** derived from the glycidol route. Protection of carbinol **76** afforded acetate **77**. In a very delicate set of transformations, this homoallylic acetate was subjected to oxidative cleavage, as shown, to generate the putative β-acetoxy aldehyde **78**. That we had resorted, in the first instance, to the glycidol route reflected our fears that such a structure would be nonviable given its projected vulnerability to β-elimination of the cinnamyl-like acetoxy function. However, in practice, this difficulty could be managed. Wittig-type reaction with the known phosphorane **79**[45] gave rise to **75**. Certainly, this route proved to be more concise for reaching compound **75** than the *R*-glycidol based route shown in Scheme 12. The actual practicality of the process will depend on the feasibility of the scale up of the conversion of **77** to the vulnerable **78** *en route* to **75**.

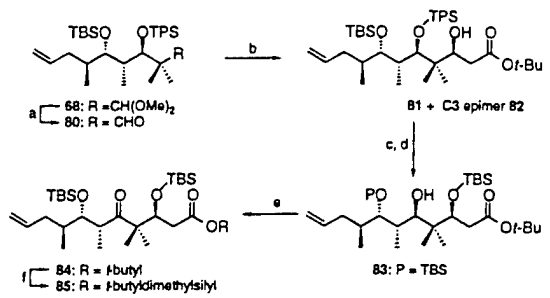
Even while this work was in progress, we were investigating a variation wherein the hypothetical Suzuki coupling would be conducted with a more advanced coupling partner, better positioned to reach epothilone itself. For this purpose, we returned to the acetal **68** which was deprotected to give rise to aldehyde **80** (see Scheme 14). This compound was condensed with lithio *tert*-butyl acetate[37] (i.e. the Rathke anion). There was produced a 63% yield of **81**, as well as its C3 stereoisomer (**82**, not shown here) in a ratio of approximately 2:1. The undesired C3 epimer could be oxidized to the corresponding ketone with Dess−Martin periodinane[28] and subsequently reduced in a stereoselective fashion to give the desired C3 alcohol exclusively. The major product **81** was subjected to the action of buffered HF·pyridine, whereupon the triphenylsilyl function was selectively cleaved from the C5 oxygen. It was further possible to selectively silylate the C3 alcohol through the combined action of TBS triflate and 2,6-lutidine, providing compound **83**. At this point, the C5 alcohol in compound **83** was oxidized to produce the ketone **84**. Finally, the *tert*-butyl

(43) Keck, G. E. Tarbet, K. H.; Geraci, L. S. *J. Am. Chem. Soc.* **1993**, *115*, 8467.

(44) Racherla, U. S.; Brown, H. C. *J. Org. Chem.* **1991**, *56*, 401.

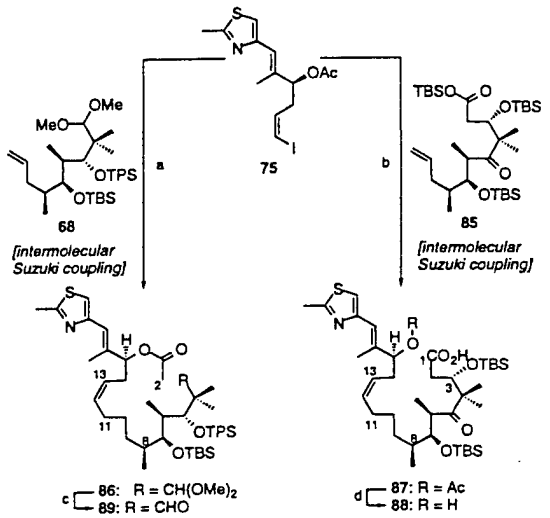
(45) (a) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 2173. (b) Stork, G.; Zhao, K. *J. Am. Chem. Soc.* **1990**, *112*, 5875. (c) Chen, J.; Wang, T.; Zhao, K. *Tetrahedron Lett.* **1994**, *35*, 2827.

Scheme 14[a]



[a] (a) *p*-TsOH, dioxane/H_2O (5:1), 50 °C (81% overall); (b) *tert*-butyl acetate, LDA, THF, −78 °C, (95%; *ca.* 2:1 mixture of C-3 epimers); (c) HF·pyr, pyr, THF, rt (98%); (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (96%); (e) Dess−Martin periodinane, CH_2Cl_2, rt (89%); (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (93%); TPS = $SiPh_3$; TBS = Si*t*-$BuMe_2$.

Scheme 15[a]



[a] (a) **68**, 9-BBN, THF, rt; then **75**, $PdCl_2(dppf)_2$, Cs_2CO_3, Ph_3As, H_2O/DMF, rt (75%); (b) **85**, 9-BBN, THF, rt; then **75**, $PdCl_2(dppf)_2$, Cs_2CO_3, Ph_3As, H_2O/DMF, rt (56%); (c) *p*-TsOH, dioxane/H_2O, 50 °C (85%); (d) K_2CO_3, MeOH/H_2O (84%); 9-BBN = 9-borabicyclo-[3.3.1]nonane; dppf = 1,1′-bis(diphenylphosphino)ferrocene; TPS = $SiPh_3$; TBS = Si*t*-$BuMe_2$.

ester could be converted to the *tert*-butyldimethylsilyl ester (see compound **85**) through the agency of TBS triflate and 2,6-lutidine.

We were now in a position to probe the feasibility of establishing the C11−C12 bond by Suzuki coupling in several contexts. Compound **68** was subjected to the action of 9-BBN (see Scheme 15). Remarkably, even the hydroboration of this terminal olefin required surprisingly coercive conditions. This slowness of hydroboration is reminiscent of the difficulties previously discussed in Scheme 9 for metalation of systems of the type **59** as a route to **60**. Fortunately, the hydroboration of **68** could be conducted under more vigorous conditions (as demonstrated by independent oxidative quenching experiments that revealed the anti-Markovnikov hydration product).

Having convinced ourselves that compound **68** had indeed been successfully hydroborated, we now conducted the last phase of the Suzuki reaction under mediation by palladium(II) chloride−1,1′-bis(diphenylphosphino)ferrocene (dppf) in the presence of the (*Z*)-vinyl iodide **75** as shown (Scheme 15). Gratifyingly, there was obtained a 72% yield of the acetate **86**.[8c] Within the limits of our detection, there had been no loss of stereointegrity of the C11−C12 double bond. The remarkable versatility of the *B*-alkyl Suzuki reaction[15] was further demonstrated by successful coupling of keto ester **85** with **75**. Under these circumstances, the TBS ester was cleaved during the course of the reaction, and the acetate was subsequently cleaved through the action of K_2CO_3 in aqueous methanol to give rise to the hydroxy acid **88**.

Our attentions would next be directed to the construction of the 16-membered ring. It was felt that our chances for achieving a stereoselective epoxidation would be better if the framework of the ring system were already in place when the oxidation of the C12−C13 double bond would be conducted. In our first attempt at macrocyclization, we favored a bold possibility. *The thought was to close the ring by connecting the methyl group of the acetate ester (C2) with the aldehyde center (C3) in a construct to be derived from compound 86.* That such a prospect could be even considered, arose from the fact that the *gem*-dimethyl substitution at C4 blocks the possibility of deprotonation of the aldehyde function. It will be recalled that earlier, in converting compound **45** to **52** (Scheme 7), we had exploited this principle by conducting an ester enolate aldol coupling under Barbier-type conditions.[38] Here, we would be drawing from the same concept in a macroaldolization step. To set the stage for this interesting ring-forming possibility, the acetal function in compound **86** was cleaved, thus revealing the electrophilic C3 aldehyde (see **86** — **89**, Scheme 15). In the crucial event, deprotonation of compound **89** (see Scheme 16) was accomplished through the action of potassium hexamethyldisilazide in THF at −78 °C. Remarkably, these conditions allow a stereoselective macroaldolization, resulting in the selective formation (6:1) of the desired (*S*)-C3 alcohol **90**. In some small scale experiments, compound **90** was the *only* product noted at the analytical level. However, optimal results from the standpoint of yield were actually obtained when the aldolate intermediate derived from cyclization was quenched at 0 °C or even at room temperature. When the quenching experiment was conducted at lower temperature, greater amounts of the undesired epimer **91** were obtained with an increase in mass recovery. Apparently, aldolate equilibration favors the formation of the desired 3*S* alcohol, whereas conditions more nearly approximating kinetic control give rise to lower degrees of stereoselectivity. At higher temperatures, it would seem that there is virtually no kinetic control in the stereochemistry of the macroaldolization step; the diastereomeric ratios observed indicate that equilibration occurs. While it is interesting to ponder and sort out methods to control stereoselectivity, in practice, the undesired epimer **91** could be utilized in our synthesis. Thus, oxidation of **91** to the ketone **92** set the stage for a diastereoselective reduction with $NaBH_4$ to provide the desired epimer **90** in high yield. Presumably, this outcome reflects the directing effects of the C5-OTPS function.

Cleavage of the triphenylsilyl ether in **90** could be conducted selectively, producing the C3−C5 diol (see compound **93**). Selective protection of the C3 hydroxyl in this compound was readily achieved, thus exposing the C5 alcohol in **94** for oxidation to a ketone. There was then produced di-TBS C12−C13 desoxyepothilone (**95**). Cleavage of the two silyl protecting groups could be accomplished, giving rise to desoxyepothilone A (**96**).

The whole scheme was now at considerable risk as we approached the matter of epoxidation of the C12−C13 double bond. We had hoped that oxidation would occur from the desired β-face on the basis of local conformational preferences that rendered this face of the molecule more accessible (see substructure **96** in Figure 2).[46] We further hoped to maximize our opportunities for stereocontrol by conducting the reaction

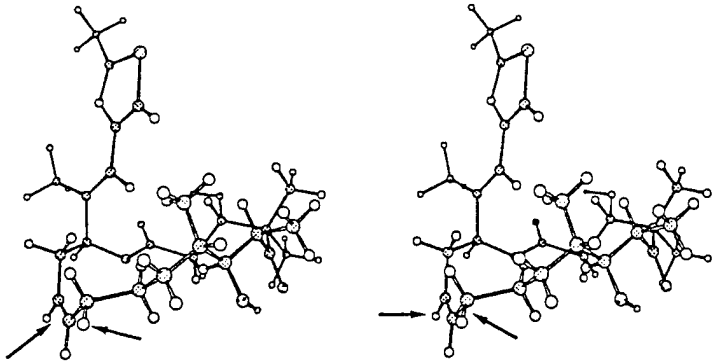


Figure 2. Macromodel minimized stereoview of desoxyepothilone A (**96**).

at low temperature. Given our many successful applications of the oxidizing powers of 3,3-dimethyldioxirane[24] it was not unnatural that we would turn to this reagent.

In practice, the major product of this reaction was the long sought after epothilone A (see **96** → **2**, Scheme 16), *confirmed by spectral and chromatographic comparisons of this material with authentic epothilone A kindly provided by Professor Höfle.* The first total synthesis of epothilone A had thus been accomplished. Two very minor products in the epoxidation were also isolated. One is the corresponding α-epoxide between C12 and C13. Still another product is one in which this epoxide linkage is present, but is accompanied by an epoxide functionality at C16–C17.

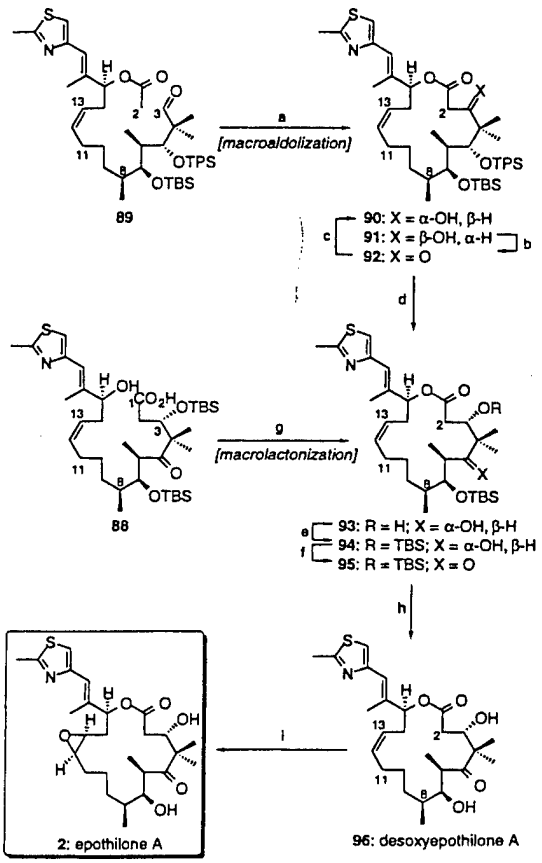
Although we had achieved the epoxidation reaction with high stereoselectivity, our reasoning based on model **96** can be questioned. Nicolaou and co-workers studied the use of the more conventional *m*-chloroperoxybenzoic acid.[9b,c] The use of this reagent produced mixtures of α and β epoxides. Hence, the factors controlling the facial sense of epoxidation are rather more subtle than is reflected in modeling of gross steric accessibility as suggested in **96**, since the course of oxygen delivery is strongly reagent dependent. However, we did have at our disposal a protocol for highly stereoselective epoxidation in the desired sense.

Having demonstrated the macroaldol route, we turned to the possibility of macrolactonization. This goal brought us back to compound **88** which, under Yamaguchi conditions,[47] led to the previously encountered **95**. Thus, we now had available to us two routes to enter the desoxyepothilone series in the form of compound **95** and, shortly thereafter, epothilone itself.

We next turn to our total synthesis of epothilone B (**3**) (see Scheme 17).[8c] We had hoped that this synthesis could be accomplished using, as much as possible, the chemistry that had served so well for the synthesis of epothilone A (**2**).[8c] Indeed, our route started with compound **77**, which was cleaved to the corresponding aldehyde **78**. Condensation of this aldehyde with the appropriate Wittig reagent[45c] gave rise to compound **97**, albeit in only 43% yield. Fortunately, the reaction was highly stereoselective, giving rise to the required *Z*-isomer as the only product. The stage was now set for the key Suzuki coupling. In this instance, we confined ourselves to acetal olefin **68**. Once again, hydroboration of **68** as before was followed by coupling of the resultant borane with (*Z*)-vinyl iodide **97**, giving rise to compound **98** in 77% yield. Cleavage of the acetal linkage led to aldehyde **99**. Once again, aldol condensation occurred in much the same manner as previously noted for compound **89**, thus allowing us to enter into the desoxyepothilone B series.

The protocols to reach desoxyepothilone B from cyclized material were already in hand from our synthesis of the A compound, **90**. Thus, in the case at hand, cleavage of the C5 TPS ether generated the diol **103**, which upon resilylation of

Scheme 16[a]

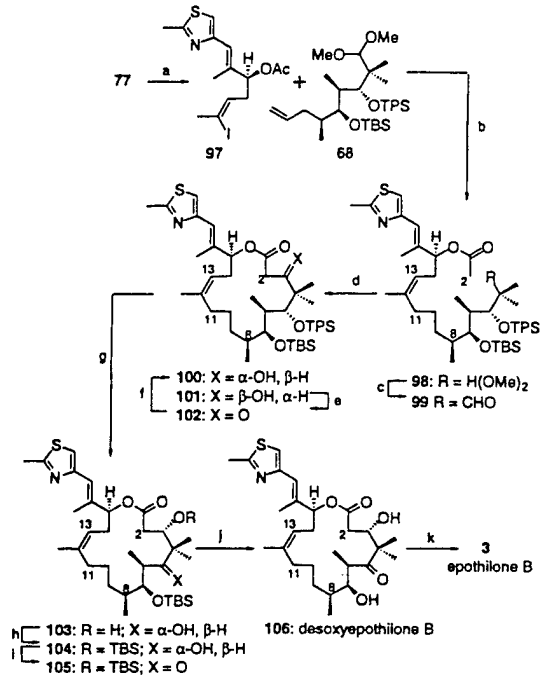


[a] (a) KHMDS, THF, −78 °C, 0.001M (51%, 6:1 α/β); (b) Dess–Martin periodinane, CH_2Cl_2, rt; (c) $NaBH_4$, MeOH, THF, −78 °C → rt (80% for two steps); (d) HF·pyridine, pyridine, THF, rt (99%); (e) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (93%); (f) Dess–Martin periodinane, CH_2Cl_2, rt (84%); (g) 2,4,6-trichlorobenzoyl chloride, TEA, 4-DMAP, toluene, rt (88%); (h) HF·pyridine, THF, rt (99%); (i) 3,3-dimethyldioxirane, CH_2Cl_2, −35 °C (49%; ≥16:1 mixture of diastereomers in favor of **2**); TPS = $SiPh_3$; TBS = Si*t*-BuMe$_2$.

(46) Molecular modeling was performed with MacroModel version 5.5; The MMZ force field was used with a Monte Carlo random walk conformational search.

(47) (a) Yamaguchi, M.; Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Mulzer, J.; Mareski, P. A.; Buschmann, J.; Luger, P. *Synthesis* **1992**, 215.

Scheme 17[a]



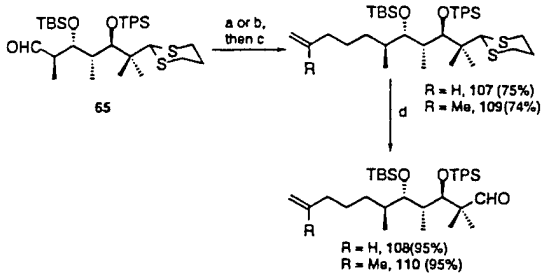
[a] (a) OsO_4, NMO, $(CH_3)_2CO/H_2O$, 0 °C; (ii) $Pb(OAc)_4$, Na_2CO_3, C_6H_6, 0 °C → rt; (iii) $Ph_3P{=}C(I)CH_3$, THF, −20 °C (43% from **77**; *Z* geometrical isomer only); (b) **68**, 9-BBN, THF, rt; then **97**, $PdCl_2(dppf)_2$, Cs_2CO_3, Ph_3As, DMF/H_2O, rt (77%); (c) *p*-TsOH, dioxane/H_2O, 55 °C (71%); (d) KHMDS, THF, −78 °C (60%; **100**:**101**/2.1:1); (e) Dess−Martin periodinane, CH_2Cl_2, rt; (f) $NaBH_4$, MeOH, rt (67% for two steps); (g) HF·pyridine, pyridine, THF, rt (94%); (h) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (89%); (i) Dess−Martin periodinane, CH_2Cl_2, rt (87%); (j) HF·pyridine, THF, rt (92%); (k) 3,3-dimethyldioxirane, CH_2Cl_2, −50 °C (97%; ≥20:1 mixture of diastereomeric *cis*-epoxides in favor of **3**); NMO = *N*-methylmorpholine *N*-oxide; 9-BBN = 9-borabicyclo[3.3.1]nonane; dppf = 1,1′-bis(diphenylphosphino)ferrocene; KHMDS = $KN(SiMe_3)_2$; TPS = $SiPh_3$; TBS = Si*t*-$BuMe_2$.

the exposed C3 hydroxyl group gave the product **104**. Oxidation of the C5 alcohol led to desoxyepothilone B *bis* (TBS) ether (compound **105**). Cleavage of the silyl blocking groups at C3 and C7 was accomplished, as shown, thereby allowing us to reach desoxyepothilone B (**106**). For obvious reasons, we turned to the use of dimethyl dioxirane in the oxidation of this compound. Happily, *this reaction was even more regio- and stereoselective, producing epothilone B (3) identical in all respects with an authentic sample, kindly provided by Professor Höfle.*

The Second Generation Ring-Closing Olefin Metathesis Strategy (Approach IV, Scheme 1)

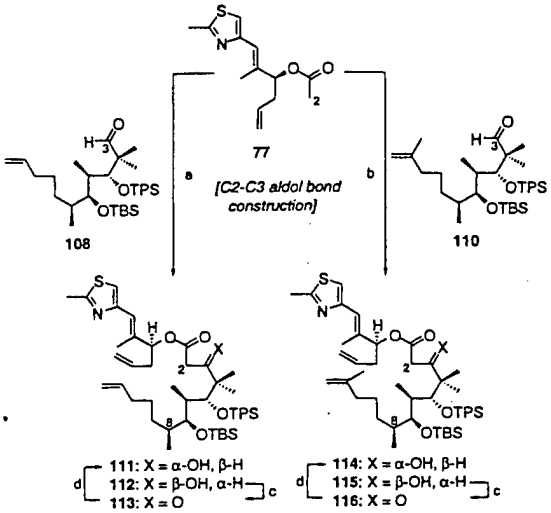
Even though we had accomplished our primary goals of synthesizing epothilones A and and B, it was still of interest to reinvestigate the possibility of intramolecular olefin metathesis.[8d] However, in this case we would be focusing on elaborating the C11−C12 double bond in the course of the metathesis reaction. Since we had known that epoxidation of this double bond could be conducted in a highly stereoselective and favorable direction, we were obviously more disposed to consider syntheses where this double bond would be elaborated in the decisive cyclization step. Toward this end, we returned to thioacetal aldehyde **65**. This compound was itself converted to product **107** through butenylation and deoxygenation at C9 (see Scheme 18). The C3 aldehyde function could be liberated by cleavage of the dithiane, as indicated, to provide aldehyde **108**.

Scheme 18[a]



[a] (a) 3-Butenylmagnesium bromide, Et_2O, −78 → 0 °C; (b) 4-iodo-2-methyl-1-butene, *t*-BuLi (2.1 equiv), Et_2O, −78 → −50 °C; then **65**, Et_2O, −78 → 0 °C; (c) (i) thiocarbonyl diimidazole, 4-DMAP, 95 °C; (ii) *n*-Bu_3SnH, AIBN, C_6H_6, 80 °C; (d) (i) $(CF_3CO_2)_2IC_6H_5$, MeOH/THF, rt; (ii) *p*-TsOH, dioxane/H_2O, 50 °C.

Scheme 19[a]



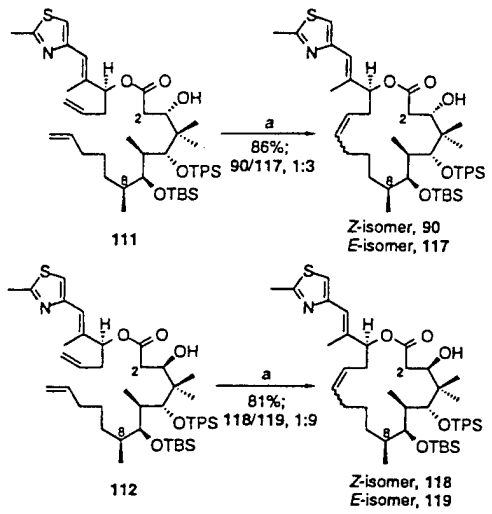
[a] (a) LDA, THF, −78 °C (65%; **111**:**112**/1:1); (b) LDA, THF, −78 °C (70%; **114**:**115**/*ca.* 1:1); (c) Dess−Martin periodinane, CH_2Cl_2, rt; (d) $NaBH_4$, MeOH, THF, −78 °C → rt (*ca.* 92% for two steps).

In order to probe the applicability of such a construction to the total synthesis of epothilone B, we returned to aldehyde dithiane **65**. The latter was converted to compound **109** by isobutenylation and deoxygenation. Once again, cleavage of the dithiane linkage provided aldehyde **110**.

With compounds **77** as well as **108** and **110** in hand, assembly of substrates for RCM were possible. To simplify the initial merger step, we turned, once again, to an intermolecular aldol condensation of the ester enolate, derived from **77**, with aldehydes **108** and **110** (see Scheme 19). In practice, the coupling could be readily conducted to give a mixture of stereoisomers at C3. The product, bearing the *S* configured alcohol, could be separated. The *R*-alcohol, in each case, was recycled through an oxidation/reduction sequence as shown.

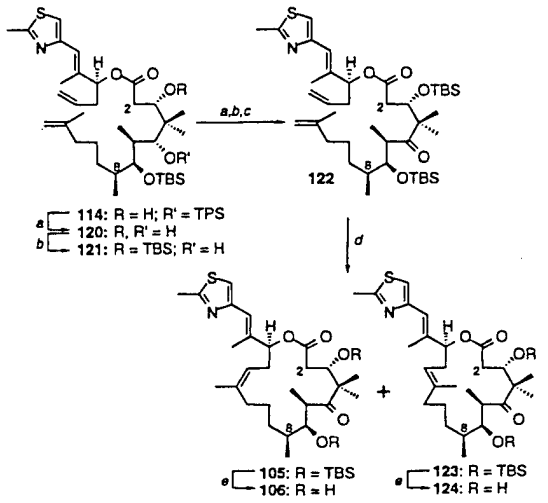
Indeed, with increased spacing between the C12 olefin and the branched positions of the polypropionate domain (see compound **111**), olefin metathesis chemistry proved to be successful.[8d] Cyclizations were conducted as described in Scheme 20 for compounds **111** and **112**. In these studies, we took recourse to both the ruthenium-based catalyst of Grubbs[12b] and the molybdenum-based catalyst of Schrock[12a] to mediate metathesis. In our work, the ruthenium-based system proved to be generally more efficacious for constructing the disubstituted double bonds with the properly configured (3*S*) alcohol.

Scheme 20[a]



[a] (a) $RuBnCl_2(PCy_3)_2$, 50 mol %, C_6H_6, 0.001 M rt, 24 h.

Scheme 21[a]

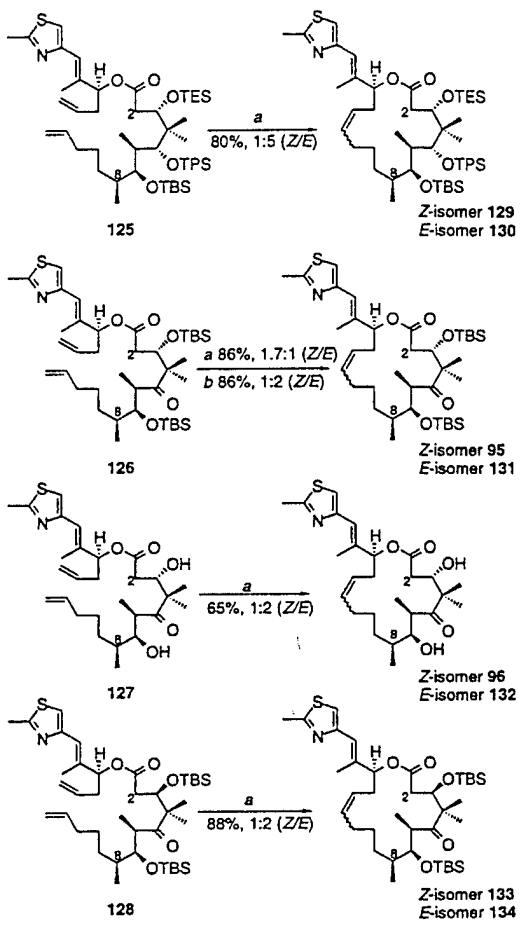


[a] (a) HF·pyr, pyr, THF, rt (93%); (b) TBSOTf, 2,6-lutidine, CH_2Cl_2, −30 °C (85%); (c) Dess−Martin periodinane, CH_2Cl_2 (94%); (d) $Mo(CHMe_2Ph)(N(2,6\text{-}(i\text{-}pr)_2C_6H_3))(OCMe(CF_3)_2)_2$ 20 mol %, C_6H_6, 0.001 M, 55 °C, 2 h, 86%, **105/123** 1:1; (e) HF·pyr, THF, rt, 2 h, 90%.

Although the yields of cyclized products were generally quite good, it was unfortunate that the resultant C12−C13 olefins were produced as a serious mixtures of *E*/*Z* isomers (**90**:**117**). The *Z* compounds could be correlated with earlier intermediates arising from the previously described *B*-alkyl Suzuki pathway. The *E* compounds were independently deprotected and converted to the corresponding *E* desoxyepothilone systems, as shown in Scheme 23 (*vide infra*).

Olefin metathesis was also attempted on compound **114** (see Scheme 21).[8c] In the event, this substrate failed to cyclize with the ruthenium-based system described by Grubbs,[12b] or the molybdenum based catalyst described by Schrock.[12a] However, when compound **122**, derived from **124** as shown, was treated with the Schrock catalyst, cyclization was successful producing a 1:1 mixture of *Z* and *E* isomers **105** and **123**.[8c] These products were independently processed, as shown, leading to the previously encountered *Z*-desoxyepothilone B (**106**) and *E*-desoxyepothilone B (**124**).

Scheme 22[a]

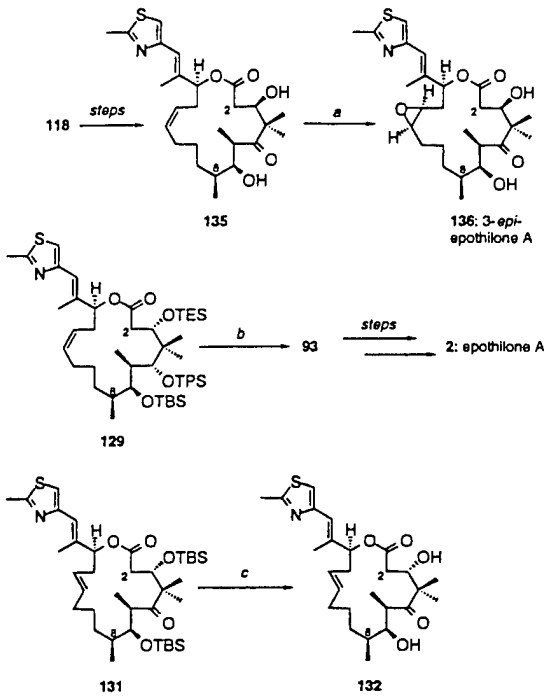


[a] (a) $RuBnCl_2(PCy_3)_2$, 50 mol %, C_6H_6, 0.001 M, rt, 4 h; (b) $Mo(CHMe_2Ph)(N(2,6\text{-}(i\text{-}pr)_2C_6H_3))(OCMe(CF_3)_2)_2$ 20 mol %, C_6H_6, 0.001 M, rt, 1 h, 86%.

As seen with substrates **111** and **112**, a point mutation of stereochemistry at C3 had tilted the process toward substantial stereoselectivity. Unfortunately, it was the 3-*epi* substrate (**112**) in which a high stereochemical margin was obtained, and, in fact, the unnatural *E*-double bond isomer was favored. We then mounted a considerable effort toward improving the stereoselectivity of the olefin metathesis reaction with the goal of reaching the natural *Z* series from the "natural" 3*S* carbinol precursor.

Unfortunately, no hypothesis emerged to guide our experiments in crafting the remote functions in the C3−C7 sector. Accordingly, we took recourse to intermediates that were accessible from the synthetic studies already in place. In this respect, we had occasion to prepare compounds **125** to **128** and to study their olefin metathesis.[8d] Using our collection of substrates, we were able to observe effects on the stereochemical course of olefin metatheses as a function of the nature of the substituents along the acyl chain. However, the only decisive perturbations favoring significant stereoselectivity were in the transformations of **112** to **118** and **119** (Scheme 20) as well as **125** to **129** and **130** (Scheme 22), each of which resulted in the predominant formation of the unnatural *E* compounds.

Scheme 23[a]



[a] (a) 3,3-Dimethyldioxirane, CH_2Cl_2, −30 °C, 70%, 3:1 β/α; (b) HF·pyr, THF, rt, 93%; (c) HF·pyr, pyr, THF, rt, 92%.

As is seen from the data, there is a sensitivity of olefin geometry to variation of substituents at even some distance from the terminal vinyl groups. Presumably, the consequence of these structural permutations reflect subtle effects on the sense of presentation of the vinyl group of one sector to the terminal metal−carbene complex[12] derived from the other sector. In this connection, it is also interesting that the olefin geometry ratio is also sensitive to the catalyst employed (see ratio of **95**:**131**) when this question was probed.

Compounds **118**, **129**, and **131** were processed as shown to afford 3-*epi*-epothilone A (**136**), epothilone A, and (*E*)-12,13-desoxyepothilone A (**132**) (Scheme 23). The results of evaluations of the biological profiles of these epothilone analogs have been published elsewhere[8d,e] and provide a basis for the development of new classes of structurally simpler and synthetically more accessible agents.

Summary

Several total syntheses of epothilones A and B have been described herein. These constructions involved union at either the C11−C12 bond (*B*-alkyl Suzuki coupling) or the C12−C13 bond (ring-forming olefin metathesis). Our routes differ from all others in several important respects. First, the stereochemistry of the polypropionate region accrues from the LACDAC reaction[19] and cyclic matrices elaborated therefrom. *Ultimately, all sterochemistry in this domain is induced from the single chiral center, readily derivable "Roche aldehyde" derivative 35 using sound principles formulated in our group many years ago.*[19]

Of particular importance is that C8 is incorporated in this dynamic. By contrast, the other syntheses have taken recourse to separate syntheses of α-methyl aldehydes, using chiral auxiliaries, to "deliver" the C8 chiral center to the synthetic pool. Furthermore, our syntheses uniquely provide strict control over the geometry of the double bonds of epothilone B as well as A, through adaptations of the B-alkyl Suzuki reaction. Recourse to the separation of *E*:*Z* isomer mixtures arising from olefin metathesis is, at least in our hands, seriously disabling in terms of throughput of significant amounts of material.

A stereoselective conversion of desoxyepothilone A and B to the epoxides in the natural series has been accomplished with the use of dimethyldioxirane (see conversion of **96** → **2** and **106** → **3**). Also illustrated in these studies were the power of catalytic asymmetric allylation (**10** → **76**), the flexibility of glycidol as a multifacated member of the chiral pool (see **69** → **75**), and the power of the LACDAC reaction in assembling polypropionate frameworks (see **35** + **36** → **45**, **68** and **85**). The rather novel cyclopropanation of a glycal (**38** → **39**) followed by oxidative solvolysis (**39** → **40**) and reduction (**40** → **41**) as a route to the introduction of quaternary branching is also deserving of attention. Given the generality of the issues which have been addressed, it is likely that the lessons garnered here would find application to other problems in organic synthesis.

Experimental Section

General. All commercial materials were used without further purifications unless otherwise noted. The following solvents were distilled under positive pressure of dry nitrogen immediately before use: THF and diethyl ether from sodium/potassium−benzophenone ketyl, CH_2Cl_2, toluene, and benzene from CaH_2. All the reactions were performed under N_2 atmosphere. NMR (^1H, ^{13}C) spectra were recorded on Bruker AMX-400 MHz, Bruker Avance DRX-500 MHz, referenced to TMS (^1H-NMR, δ 0.00) or $CDCl_3$ (^{13}C-NMR, δ 77.0) peaks unless otherwise stated. LB = 1.0 Hz was used before Fourier transformation for all of the ^{13}C-NMR. IR spectra were recorded with a Perkin-Elmer 1600 series-FTIR spectrometer, and optical rotations were measured with a Jasco DIP-370 digital polarimeter using 10 cm pathlength cell. Low-resolution mass spectral analysis were performed with a JEOL JMS-DX-303 HF mass spectrometer. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate−ammonium molybdate solution followed by heating. Flash column chromatography was performed using the indicated solvent on E. Merck silica gel 60 (40−63 mm) or Sigma H-Type silica gel (10−40 mm). Melting points are obtained with Electrothermal melting point apparatus (series no. 9100) and are uncorrected.

Preparation of Compound 68. A solution of (methoxymethyl)-triphenylphosphonium chloride (2.97 g, 8.55 mmol) in THF (25 mL) at 0 °C was treated with KO^{t}Bu (8.21 mL, 1 M in THF, 8.1 mmol). The mixture was stirred at 0 °C for 30 min. Aldehyde **65** (3.10 g, 4.07 mmol) in THF (10 mL) was added, and the resulting solution was allowed to warm to rt and stirred at this temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (40 mL), and the resulting solution was extracted with Et_2O (3 × 30 mL). The combined Et_2O fractions were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel eluting with 5% Et_2O in hexanes to yield the methyl enol ether **66** (2.83 g, 86%) as a colorless foam.

To a solution of the methyl enol ether **66** (2.83 g, 3.50 mmol) in dioxane/H_2O (9:1, 28 mL) was added pTSA·H_2O (1.0 g, 5.30 mmol), and the resulting mixture was heated to 50 °C for 2 h. After cooling to rt, the mixture was diluted with Et_2O (50 mL) and washed successively with saturated aqueous $NaHCO_3$ (15 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated to provide the corresponding aldehyde (2.75 g, 99%) as a colorless foam: $[\alpha]_D$ = +1.74 (*c* = 0.77, $CHCl_3$); IR (film) 2929, 1725, 1428, 1253, 1115, 1039 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.31 (d, *J* = 3.4 Hz, 1 H), 7.68 (dd, *J* = 7.8, 1.4 Hz, 6 H), 7.45−7.37 (band, 9 H), 4.60 (d, *J* = 6.8 Hz, 1 H), 4.20 (s, 1 H), 3.51 (d, *J* = 6.7 Hz, 1 H), 2.68 (d, *J* = 14.0 Hz, 1 H), 2.60 (d, *J* = 13.5 Hz, 1 H), 2.37 (m, 1 H), 2.24 (m, 1 H), 1.90 (m, 1 H), 1.81 (m, 2 H), 1.68 (m, 2 H), 1.52 (m, 1 H), 1.32 (s, 3 H), 1.14−1.03 (band, 6 H), 0.86 (s, 9 H), 0.75 (d, *J* = 6.9 Hz, 3 H), −0.03 (s, 3 H), −0.06 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.8, 136.0, 134.6, 130.1, 128.0, 77.6, 76.2, 59.5, 45.1, 44.7, 43.8,

31.8, 30.9, 30.5, 26.3, 25.9, 22.5, 20.9, 18.6, 17.9, 14.7, −3.0, −3.5; HRMS calcd for $C_{39}H_{56}O_3S_2Si_2$: 692.3210; found: 731.2828 (M + K).

Methyltriphenylphosphonium bromide (1.98 g, 5.54 mmol) in THF (50 mL) at 0 °C was treated with lithium bis(trimethylsilyl)amide (5.04 mL, 1 M in THF, 5.04 mmol), and the resulting solution was stirred at 0 °C for 30 min. The aldehyde (2.00 g, 2.52 mmol), prepared above, in THF (5.0 mL) was added, and the mixture was allowed to warm to room temperature and stirred at this temperature for 1 h. The reaction was quenched with saturated aqueous NH_4Cl (15 mL) and extracted with Et_2O (3 × 20 mL). The combined Et_2O fractions were washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel eluting with 5% Et_2O in hexanes to afford compound **67** (1.42 g, 76%) as a colorless foam.

A solution of the dithiane **67** (1.0 g, 1.34 mmol), prepared above, in MeOH/THF (2:1, 13 mL) was treated with [bis(trifluoroacetoxy)-iodobenzene] (0.865 g, 2.01 mmol) at rt. After 15 min, the reaction was quenched with saturated aqueous $NaHCO_3$ (25 mL). The mixture was extracted with Et_2O (3 × 25 mL), and the combined Et_2O fractions were washed once with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. Purification of the residue by flash chromatography on silica gel eluting with 5% Et_2O in hexanes provided compound **68** (0.865 g, 92%) as a colorless foam: $[\alpha]_D = +1.74$ ($c = 0.77$, $CHCl_3$); IR (film) 1428, 1252, 1114, 1075, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.61 (dd, $J = 7.9$, 1.4 Hz, 6 H), 7.38 (s, 9 H), 5.47 (m, 1 H), 4.87 (d, $J = 10.0$ Hz, 1 H), 4.76 (d, $J = 15.9$ Hz, 1 H), 4.30 (d, $J = 3.7$ Hz, 1 H), 3.95 (s, 1 H), 3.56 (dd, $J = 7.5$, 1.4 Hz, 1 H), 3.39 (s, 3 H), 2.84 (s, 3 H), 2.02 (m, 1 H), 1.64 (m, 2 H), 1.34 (m, 1 H), 1.11 (s, 3 H), 1.02 (d, $J = 7.4$ Hz, 3 H), 0.90 (s, 3 H), 0.85 (s, 9 H), 0.62 (d, $J = 6.8$ Hz, 3 H), −0.04 (s, 3 H), −0.05 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.3, 135.8, 135.0, 129.9, 127.8, 114.9, 110.5, 60.1, 55.6, 46.5, 43.9, 36.8, 34.2, 26.3, 19.6, 18.6, 17.1, 16.16, 13.9, −2.9, −3.8; HRMS calcd for $C_{39}H_{58}O_4Si_2$: 646.3873; found: 685.3491 (M + K).

Preparation of Compound 76. A mixture of (*S*)-(−)-1,1′-bi-2-naphthol (0.259 g, 0.91 mmol), $Ti(O\text{-}i\text{-}Pr)_4$ (261 mL; 0.90 mmol), and 4 Å sieves (3.23 g) in CH_2Cl_2 (16 mL) was heated at reflux for 1 h. The mixture was cooled to rt, and aldehyde **10** was added. After 10 min, the suspension was cooled to −78 °C, and allyltri-*n*-butyltin (3.60 mL, 11.6 mmol) was added. The reaction mixture was stirred for 10 min at −78 °C and then placed in a −20 °C freezer for 70 h. Saturated aqueous $NaHCO_3$ solution (2 mL) was added, and the mixture was stirred for 1 h, poured over Na_2SO_4, and then filtered through a pad of $MgSO_4$ and Celite. The crude material was purified by flash chromatography (hexanes/ethyl acetate, 1:1) to give alcohol **76** as a yellow oil (1.11 g, 60%): $[\alpha]_D = -15.9$ (c 4.9, $CHCl_3$); IR (film) 3360, 1641, 1509, 1434, 1188, 1017, 914 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 25 °C) δ 6.92 (s, 1 H), 6.55 (s, 1 H), 5.82 (m, 1 H), 5.13 (dd, $J = 17.1$, 1.3 Hz, 1 H), 5.09 (d, $J = 10.2$ Hz, 1 H), 4.21 (t, $J = 6.0$ Hz, 1 H), 2.76 (br s, 1 H), 2.69 (s, 3 H), 2.40 (m, 2 H), 2.02 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 164.5, 152.6, 141.5, 134.6, 119.2, 117.6, 115.3, 76.4, 39.9, 19.0, 14.2; HRMS calcd for $C_{11}H_{15}NOS$: 209.0874 found: 209.0872 (M + H).

Preparation of Compound 77. To a solution of alcohol **76** (0.264 g; 1.26 mmol) in CH_2Cl_2 (12 mL) were added 4-DMAP (0.015 g, 0.098 mmol), Et_3N (0.45 mL; 3.22 mmol), and Ac_2O (0.18 mL; 1.90 mmol). After 2 h, the reaction was quenched by the addition of H_2O (20 mL) and extracted with EtOAc (4 × 20 mL). The combined organic extracts were dried with $MgSO_4$, filtered, and concentrated. Flash chromatography on SiO_2 (EtOAc/hexanes, 1:3) afforded acetate **77** as a yellow oil (0.302 g; 96%): $[\alpha]_D = -40.0$ (c 7.3, $CHCl_3$); IR (film) 1738, 1505, 1436, 1370, 1236, 1019 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 25 °C) δ 6.95 (s, 1 H), 6.52 (s, 1 H), 5.72 (m, 1 H), 5.33 (t, $J = 6.6$ Hz, 1 H), 5.10 (ddd, $J = 17.1$, 3.1, 1.5 Hz, 1 H), 5.07 (ddd, $J = 10.2$, 3.3, 1.7 Hz, 1 H), 2.70 (s, 3 H), 2.48 (dt, $J = 5.9$, 1.3 Hz, 2 H), 2.08 (s, 3 H), 2.07 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 170.1, 164.5, 152.4, 137.0, 133.4, 120.6, 117.6, 116.2, 77.9, 37.5, 21.1, 19.1, 14.7; HRMS calcd for $C_{13}H_{17}O_2NS$: 251.0980; found: 251.0983 (M^-).

Preparation of Compound 75. To a solution of acetate **77** (0.099 g; 0.39 mmol) in acetone (10 mL) at 0 °C were added H_2O (4 drops), OsO_4 (2.5% wt in butyl alcohol; 0.175 mL; 0.018 mmol), and *N*-methylmorpholine *N*-oxide (0.069 g; 0.59 mmol). The mixture was stirred at 0 °C for 2 h and then quenched with saturated aqueous Na_2SO_3 solution (10 mL). The solution was poured into H_2O (10 mL) and extracted with EtOAc (8 × 10 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated.

To a solution of the crude diol in THF/H_2O (4 mL, 3:1) was added $NaIO_4$ (0.260 g; 1.22 mmol). After 1.25 h, the reaction mixture was quenched with H_2O (10 mL) and concentrated. The aqueous layer was extracted with EtOAc (5 × 10 mL) and dried over $MgSO_4$. Flash chromatography (SiO_2, EtOAc/hexanes, 1:1) on a short pad of silica gave the crude aldehyde **78** as a yellow oil (0.080 g) which contained unidentified byproduct(s). This mixture was used without further purification.

To a solution of $(Ph_3P^-CH_2I)I^-$ (0.100 g; 0.19 mmol) in THF (0.25 mL) at rt was added sodium bis(trimethylsilyl)amide (1 M soln in THF, 0.15 mL, 0.15 mmol). To the resulting solution at −78 °C were added HMPA (0.022 mL; 0.13 mmol) and the crude aldehyde **78** from the previous step (0.016 g) in THF (0.25 mL). The reaction mixture was then stirred at rt for 30 min. After the addition of saturated aqueous NH_4Cl (10 mL), and the solution was extracted with EtOAc (4 × 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by preparative thin-layer chromatography (prep-TLC) (EtOAc/hexanes, 2:3) to give the vinyl iodide **75** as a yellow oil (0.014 g; 50% for three steps).

Preparation of Compound 80. The acetal **68** (0.930 g, 1.44 mmol) was dissolved in dioxane/H_2O (9:1, 20 mL), and *p*TSA·H_2O (0.820 g, 4.32 mL) was added. The mixture was heated at 55 °C for 2 h. After cooling to rt, the solution was poured into Et_2O (200 mL) and washed once with saturated aqueous $NaHCO_3$ solution (30 mL) and once with brine (30 mL) and dried over anhydrous $MgSO_4$. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (9:1) gave 0.702 g (81%) of the aldehyde **80** as a white foam: $[\alpha]_D = -12.8$ ($c = 3.4$, $CHCl_3$); IR (film) 2929, 1722, 1472, 1429, 1256, 1115, 1059 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.76 (s, 1 H), 7.71 (d, $J = 6.4$ Hz, 6 H), 7.42 (m, 9 H), 5.49 (m, 1 H), 4.87 (m, 1 H), 4.75 (m, 1 H), 4.08 (d, $J = 1.6$ Hz, 1 H), 3.56 (dd, $J = 1.6$, 8.7 Hz, 1 H), 2.18 (m, 1 H), 1.70 (m, 1 H), 1.46 (m, 2 H), 1.10 (s, 3 H), 0.89 (d, $J = 6.4$ Hz, 3 H), 0.79 (s 9 H), 0.60 (d, $J = 6.5$ Hz, 3 H), −0.8 (s, 3 H), −0.83 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.5, 137.6, 135.6, 134.2, 130.0, 127.9, 115.4, 80.7, 76.4, 51.7, 43.5, 38.2, 34.9, 26.1, 20.9, 20.1, 18.5, 15.5, 12.9, −3.3, −3.9; HRMS calcd for $C_{37}H_{52}O_3S_2$: 600.3455; found: 623.3333 (M + Na).

Preparation of Compound 81. The aldehyde **80** (0.702 g, 1.17 mmol) was dissolved in THF (50 mL), and *tert*-butyl acetate (1.26 mL, 9.36 mmol) was added. The solution was cooled to −78 °C, and LDA (2.0 M soln, 3.51 mL, 7.02 mmol) was added. After 20 min, the reaction was quenched with MeOH (10 mL) and H_2O (100 mL). The mixture was extracted with Et_2O (3 × 100 mL). The combined organics were washed once with brine (30 mL) and dried over anhydrous $MgSO_4$. The crude mixture contained a 2:1 ratio (**81**:**82**) of diastereomers. Purification was done by flash chromatography on silica gel eluting with hexanes/ethyl acetate (19:1) to give 0.527 g (63%) of the desired isomer **81** as a white foam: $[\alpha]_D = -11.2$ ($c = 1.4$, $CHCl_3$); IR (film) 3493, 2929, 1710, 1429, 1153, 1115, 1045 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35 (dd, $J = 1.2$, 7.6 Hz, 6 H), 7.38 (m, 9 H), 5.50 (m, 1 H), 4.86 (d, $J = 9.5$ Hz, 1 H), 4.73 (d, $J = 17.1$ Hz, 1 H), 4.09 (d, $J = 3.5$ Hz, 1 H), 3.93 (br d, $J = 10.0$ Hz, 1 H), 3.75 (d, $J = 7.1$ Hz, 1 H), 3.34 (d, $J = 2.6$ Hz, 1 H), 2.29 (dd, $J = 2.5$, 16.4 Hz, 1 H), 2.18 (m, 2 H), 1.67 (m, 2 H), 1.44 (m, 1 H), 1.41 (s, 9 H), 1.05 (d, $J = 7.4$ Hz, 3 H), 0.95 (s, 3 H), 0.86 (s, 12 H), 0.64 (d, $J = 6.7$ Hz, 3 H), −0.03 (s, 3 H), −0.05 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 138.0, 136.7, 134.8, 129.8, 127.6, 115.0, 81.2, 78.8, 75.9, 71.3, 44.4, 43.5, 38.0, 37.2, 34.9, 28.0, 26.3, 21.2, 20.5, 18.8, 16.0, 14.1, −3.0, −4.1; HRMS calcd for $C_{43}H_{64}O_5Si_2$: 572.4293; found: 573.4390 (M + H).

Preparation of Compound 83. The alcohol **81** (0.110 g, 0.0153 mmol) was treated with pyridine buffered HF·pyridine solution (3.0 mL) (stock solution was prepared from 20 mL of THF, 11.4 mL of pyridine, and 4.2 g of hydrogen fluoride−pyridne (Aldrich Co.)) at rt and stirred for 2 h. The reaction mixure was poured into saturated aqueous $NaHCO_3$ (50 mL) and extracted with ether (3 × 50 mL). The organic layer was washed in sequence with saturated aqueous $CuSO_4$ (3 × 10 mL) and saturated aqueous $NaHCO_3$ (10 mL) and then dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by

flash chromatography on silica gel eluting with hexanes/ethyl acetate (7:1) to give the corresponding diol (0.070 g, 98%) as a white foam.

The diol (0.231 g, 0.48 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and cooled to −30 °C. 2,6-Lutidine (0.168 mL, 1.44 mmol) was added followed by TBSOTf (0.131 mL, 0.570 mmol). After 1 h at −30 °C, the reaction was poured into Et_2O (300 mL), washed once with 1 N HCl (50 mL), once with saturated aqueous $NaHCO_3$ (50 mL), and once with brine (30 mL), and dried over anhydrous $MgSO_4$. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (20:1) gave alcohol **83** (0.276 g, 96%) as a clear oil: $[\alpha]_D = -5.4$ (c = 0.9, $CHCl_3$); IR (film) 3466, 2930, 1728, 1462, 1369, 1254, 1156 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.76 (m, 1 H), 4.99 (m, 2 H), 4.15 (t, J = 4.4 Hz, 1 H), 3.91 (d, J = 4.0 Hz, 1 H), 3.60 (bs, 1 H), 3.75 (dd, J = 3.3, 7.8 Hz, 1 H), 2.81 (dd, J = 4.7, 17.3 Hz, 1 H), 2.41 (m, 1 H), 2.22 (dd, J = 4.2, 17.3 Hz, 1 H), 1.88 (m, 1 H), 1.73 (m, 2 H), 1.45 (s, 9 H), 0.94 (d, J = 7.0 Hz, 3 H), 0.92 (s, 3 H), 0.90 (s, 9 H), 0.89 (s, 12 H), 0.85 (s, 3 H), 0.08 (s, 3 H), 0.07 (s, 3 H), 0.06 (s, 3 H), 0.05 (s, 3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 138.2, 115.5, 80.8, 77.7, 74.4, 46.3, 43.7, 40.9, 38.7, 37.9, 39.7, 28.1, 26.2, 26.0, 19.5, 19.2, 18.5, 18.1, 16.9, 15.1, −3.9, −4.1, −4.2, −4.9; HRMS calcd for $C_{31}H_{64}O_5Si_2$: 572.4293; found: 573.4390 (M + H).

Preparation of Compound 85. The alcohol **83** (0.275 g, 0.46 mmol) was dissolved in CH_2Cl_2 (5.0 mL), and Dess−Martin periodinane (0.292 g, 0.690 mmol) was added. After 2 h, a 1:1 mixture of saturated aqueous $NaHCO_3$/saturated aqueous $Na_2S_2O_3$ (2.0 mL) was added. After 10 min, the mixture was poured into Et_2O (40 mL), and the organic layer was washed with brine (3.0 mL) and dried over anhydrous $MgSO_4$. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (19:1) gave ketone **84** (0.244 g, 89%) as a clear oil.

The olefin **84** (0.420 g, 0.76 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated successively with 2,6-lutidine (1.75 mL, 15 mmol) and TBSOTf (1.72 mL, 7.5 mmol). After 7 h, the reaction was poured into Et_2O (150 mL), washed successively with 0.2 N HCl (25 mL) and brine (20 mL), and dried over anhydrous $MgSO_4$. The residue was purified by flash chromatography on a short pad of silica gel with fast elution with hexanes/ethyl acetate (20:1) to give TBS ester **85** (0.611 g, 93%) as a clear oil. The purification must be done quickly to avoid hydrolysis of the silyl ester: $[\alpha]_D = -35.4$ (c = 0.4, $CHCl_3$); IR (film) 2930, 1730, 1692, 1472, 1367, 1253, 1155, 1084 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.73 (m, 1 H), 4.97 (m, 2 H), 4.33 (dd, J = 3.8, 5.5 Hz, 1 H), 3.78 (dd, J = 1.9, 7.3 Hz, 1 H), 3.19 (m, 1 H), 2.53 (dd, J = 3.8, 17.3 Hz, 1 H), 2.25 (m, 2 H), 1.85 (m, 1 H), 1.40 (m, 1 H), 1.24 (s, 3 H), 1.07 (s, 3 H), 1.04 (d, J = 6.8 Hz, 3 H), 0.92 (s, 12 H), 0.91 (s, 9 H), 0.87 (s, 9 H), 0.26 (s, 3 H), 0.26 (s, 3 H), 0.10 (s, 3 H), 0.06 (s, 6 H), 0.05 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 217.1, 170.6, 137.2, 115.0, 79.8, 77.1, 73.2, 52.9, 45.0, 40.8, 37.5, 34.7, 27.5, 25.6, 25.4, 22.4, 19.7, 17.9, 17.6, 17.3, 14.9, −4.2, −4.3, −4.9, −5.4.

Preparation of Compound 87. To a solution of olefin **85** (0.053 g, 0.081 mmol) in THF (0.8 mL) was added 9-BBN (0.5 M soln in THF, 0.323 mL, 0.162 mmol). In a separate flask, the iodide **75** (0.036 g, 0.097 mmol) was dissolved in DMF (1.0 mL). Cs_2CO_3 (0.053 g, 0.162 mmol) was then added with vigorous stirring followed by sequential addition of Ph_3As (0.0025 g, 0.0081 mmol), $PdCl_2(dppf)_2$ (0.0067 g, 0.0081 mmol), and H_2O (0.052 mL, 2.91 mmol). After 4 h, the borane in THF was added to the iodide mixture in DMF. The reaction quickly turned dark brown in color and slowly became pale yellow after 2 h. The reaction was then poured into saturated aqueous NH_4Cl (10.0 mL) and extracted with $CHCl_3$ (3 × 30 mL). The combined organics were washed with H_2O (2 × 50 mL) and once with brine (50 mL) and dried over anhydrous $MgSO_4$. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (4:1 → 3:1) gave 0.036 g (56%) of the coupled product **87** as a pale yellow oil: $[\alpha]_D = -29.2$ (c = 0.3, $CHCl_3$); IR (film) 3500−2600, 1738, 1710, 1691, 1236, 988 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.95 (s, 1 H), 6.59 (s, 1 H), 5.49 (m, 1 H), 5.31 (m, 1 H), 5.25 (dd, J = 5.7, 7.7 Hz, 1 H), 4.38 (dd, J = 3.8, 6.5 Hz, 1 H), 3.78 (dd, J = 1.9, 6.7 Hz, 1 H), 3.13 (m, 1 H), 2.71 (s, 3 H), 2.49 (m, 2 H), 2.42 (m, 1 H), 2.31 (dd, J = 6.5, 16.3 Hz, 1 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 1.95 (m, 1 H), 1.89 (m, 2 H), 1.48 (m, 3 H), 1.21 (s, 3 H), 1.15 (m, 2 H), 1.12 (s, 3 H), 1.06 (d, J = 6.9 Hz, 3 H), 0.90 (s, 12 H), 0.88 (s, 9 H), 0.10 (s, 3 H), 0.07 (s, 3 H), 0.06 (s, 3 H), 0.05 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 217.9, 176.7, 170.2, 164.9, 152.2, 137.6, 132.7, 123.9, 120.3, 115.9, 78.4, 73.6, 53.5, 45.0, 40.1, 38.8, 31.0, 30.6, 27.9, 27.7, 26.2, 26.0, 23.5, 21.9, 21.2, 19.2, 18.9, 18.4, 18.1, 17.5, 15.7, 14.9, −3.7, −3.9, −4.3, −4.7; HRMS calcd for $C_{40}H_{71}O_6NSSi_2$: 765.4490; found: 766.4571 (M + H).

Preparation of Compound 88. The acetate **87** (0.035 g, 0.044 mmol) was dissolved in MeOH/H_2O (2:1, 1.5 mL), and K_2CO_3 (0.050 g) was added. After 3 h, the reaction was diluted with saturated aqueous NH_4Cl (5.0 mL) and extracted with $CHCl_3$ (5 × 10 mL). The hydroxy acid **88** was purified by flash chromatography on silica gel eluting with hexanes/ethyl acetate (4:1 → 2:1) to give the pure hydroxy acid **88** (0.030 g, 84%): $[\alpha]_D = -19.8$ (c = 16.5, $CHCl_3$); IR (film) 3600−2450, 1710, 1700, 1472, 1253, 988 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.95 (s, 1 H), 6.64 (s, 1 H), 5.55 (m, 1 H), 5.42 (m, 1 H), 4.39 (dd, J = 3.6, 6.4 Hz, 1 H), 4.15 (t, J = 6.7 Hz, 1 H), 3.79 (dd, J = 1.7, 6.9 Hz, 1 H), 3.13 (m, 1 H), 2.71 (s, 3 H), 2.49 (dd, J = 3.6, 16.4 Hz, 1 H), 2.40 (m, 2 H), 2.31 (dd, J = 6.5, 16.4 Hz, 1 H), 2.11 (m, 1 H), 2.01 (s, 3 H), 1.38 (m, 3 H), 1.20 (s, 3 H), 1.15 (m, 2 H), 1.13 (m, 3 H), 1.12 (s, 3 H), 1.05 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.87 (s, 9 H), 0.10 (s, 3 H), 0.06 (s, 6 H), 0.05 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 218.0, 176.2, 165.0, 152.5, 141.8, 133.2, 124.9, 118.9, 115.2, 73.5, 53.6, 44.9, 40.1, 38.9, 33.3, 30.8, 28.0, 27.9, 26.2, 26.0, 23.5, 19.2, 18.9, 18.5, 18.2, 17.4, 15.9, 14.5, −3.7, −3.8, −4.2, −4.6; HRMS calcd for $C_{38}H_{69}O_6NSSi_2$: 723.4384; found: 746.4285 (M + Na).

Preparation of Compound 89. To a solution of the the olefin **68** (0.680 g, 1.07 mmol) in THF (8.0 mL) was added 9-BBN (0.5 M soln in THF, 2.99 mL, 1.50 mmol). In a separate flask, the iodide **75** (0.478 g, 1.284 mmol) was dissolved in DMF (10.0 mL). Cs_2CO_3 (0.696 g, 2.14 mmol) was then added with vigorous stirring followed by sequential addition of Ph_3As (0.034 g, 0.111 mmol), $PdCl_2(dppf)_2$ (0.091 g, 0.111 mmol), and H_2O (0.693 mL, 38.5 mmol). After 4 h, the borane solution was added to the iodide mixture in DMF. The mixture quickly turned dark brown in color and slowly became pale yellow after 2 h. The reaction was then poured into H_2O (100 mL) and extracted with Et_2O (3 × 50 mL). The combined organics were washed with H_2O (2 × 50 mL) and once with brine (50 mL) and dried over anhydrous $MgSO_4$. Purification by flash chromatography on silica gel eluting with hexanes/EtOAc (7:1) gave 0.630 g (75%) of the coupled product **86** as a pale yellow oil. This compound could not be separated completely from residual borane impurities and was taken forward in impure form.

The acetate **86** (0.610 g, 0.770 mmol) was dissolved in dioxane/H_2O (9:1, 15 mL), and pTSA·H_2O (0.442 g, 2.32 mmol) was added. The mixture was then heated to 55 °C. After 3 h, the mixture was cooled to rt and poured into Et_2O. This solution was washed once with saturated $NaHCO_3$ (30 mL) and once with brine (30 mL) and dried over anhydrous $MgSO_4$. Purification by flash chromatography on silica gel eluting with hexanes/EtOAc (7:1) gave 0.486 g (85%) of the aldehyde **89** as a pale yellow oil: $[\alpha]_D = -18.7$ (c = 0.53, $CHCl_3$); IR (film) 1737, 1429, 1237, 1115, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.74 (s, 1 H), 7.61 (dd, J = 7.8, 1.2 Hz, 6 H), 7.38 (m, 9 H), 6.94 (s, 1 H), 6.53 (s, 1 H), 5.39 (m, 1 H), 5.31 (m, 1 H), 5.29 (t, J = 6.9 Hz, 1 H), 4.61 (d, J = 4.3 Hz, 1 H), 3.50 (dd, J = 5.2, 2.6 Hz, 1 H), 2.70 (s, 3 H), 2.48 (m, 2 H), 2.14 (m, 1 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 1.83 (m, 2 H), 1.41 (m, 1 H), 1.18 (m, 1 H), 1.01 (s, 3 H), 0.99 (s, 3 H), 0.91 (d, J = 7.4 Hz, 3 H), 0.85 (s, 9 H), 0.69 (m, 1 H), 0.58 (d, J = 6.8 Hz, 3 H), −0.05 (s, 3 H), −0.06 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.46, 170.01, 164.49, 152.46, 137.10, 135.60, 134.22, 132.55, 130.65, 127.84, 123.82, 120.66, 116.19, 81.09, 78.47, 76.73, 51.66, 43.14, 38.98, 30.99, 30.42, 27.63, 26.10, 21.15, 20.92, 20.05, 19.15, 18.49, 15.12, 14.70, 12.75, −3.25, −4.08; HRMS calcd for $C_{50}H_{69}O_5NSSi_2$: 851.4435; found: 890.4100 (M + K).

Preparation of Compounds 90 and 91. To a solution of the acetate−aldehyde **89** (0.084 g, 0.099 mmol) in THF (99 mL) at −78 °C was added potassium bis(trimethylsilyl)amide (0.5 M in toluene, 1.0 mL, 0.5 mmol) dropwise. The resulting solution was stirred at −78 °C for 30 min. Then the reaction mixure was transfered via cannula to a short pad of silica gel and washed with Et_2O. The residue was purified by flash chromatography (silica, 12% EtOAc in hexane) to give the 3*S* product **90** and the 3*R* product **91** in a 6:1 ratio in 51% combined yield:

Compound 90: $[\alpha]_D = -39.4$ (c 0.52, $CHCl_3$); IR (film) 3508, 1733, 1428, 1254, 1113, 1034 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 60 °C) δ 7.85 (dd, J = 7.3, 2.1 Hz, 6 H), 7.22 (m, 9 H), 6.54 (s, 1 H), 6.49 (s, 1 H), 5.53 (d, J = 6.0 Hz, 1 H), 5.42 (m, 2 H), 4.22 (d, J =

5.9 Hz, 1 H), 4.19 (d, J = 3.5 Hz, 1 H), 4.17 (br s, 1 H), 2.58 (m, 1 H), 2.45 (m, 2 H), 2.31 (s, 3 H), 2.29 (m, 2 H), 2.13 (s, 3 H), 2.00 (m, 2 H), 1.81 (m, 1 H), 1.56 (m, 1 H), 1.35−1.28 (band, 2 H), 1.24 (d, J = 7.1 Hz, 3 H), 1.20 (m, 1 H), 1.11 (s, 3 H), 1.07−0.92 (band, 13 H), 0.88 (d, J = 6.7 Hz, 3 H), 0.12 (s, 6 H); ^{13}C NMR (125 MHz, C_6D_6, 60 °C) δ 171.1, 164.3, 153.7, 137.4, 136.6, 136.0, 130.1, 128.5, 125.5, 120.2, 116.7, 78.5, 75.8, 72.8, 44.6, 41.6, 38.3, 32.7, 31.8, 30.1, 28.5, 27.9, 26.6, 22.2, 21.3, 18.9, 18.8, 15.8, 15.7, 1.30, −2.76, −3.66; HRMS calcd for $C_{50}H_{69}O_5NSSi_2$: 851.4435; found: 852.4513 (M + H).

Compound 91: $[\alpha]_D$ = −53.9 (c 0.37, $CHCl_3$); IR (film) 2927, 1734, 1428, 1114, 1036 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6, 60 °C) δ 7.82 (m, 6 H), 7.22−7.19 (band, 9 H), 6.59 (s, 1 H), 6.53 (s, 1 H), 5.58−5.53 (band, 2 H), 5.49 (m, 1 H), 4.39 (d, J = 9.7 Hz, 1 H), 3.98 (d, J = 4.7 Hz, 1 H), 3.86 (dd, J = 1.9, 5.6 Hz, 1 H), 3.59 (br s, 1 H), 2.49−2.43 (band, 3 H), 2.34−2.26 (band, 6 H), 2.18 (s, 3 H), 1.98 (m, 2 H), 1.51 (m, 1 H), 1.50−1.30 (band, 2 H), 1.21−1.19 (band, 6 H), 1.03−0.99 (band, 10H), 0.85 (d, J = 5.4 Hz, 3 H), 0.78 (s, 3 H), 0.10 (s, 3 H), 0.07 (s, 3 H); HRMS calcd for $C_{50}H_{69}O_5NSSi_2$: 851.4435; found: 852.4489 (M + H).

Preparation of Compound 93. The lactone **90** (0.032 g, 0.0376 mmol) was treated with pyridine-buffered HF·pyridine solution (1 mL) (stock solution was prepared from 20 mL of THF, 11.4 mL of pyridine, and 4.2 g of hydrogen fluoride−pyridne (Aldrich Co.)) at room temperature for 2 h. The reaction mixure was poured into saturated aqueous $NaHCO_3$ (15 mL) and extracted with Et_2O (3 × 30 mL). The organic layer was washed in sequence with saturated aqueous $CuSO_4$ (3 × 10 mL) and saturated aqueous $NaHCO_3$ (10 mL) and then dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (silica, 25% EtOAc in hexane) to give diol **93** (0.022 g, 99%) as a white foam: $[\alpha]_D$ = −111.7 (c = 0.7, $CHCl_3$); IR (film) 3463, 2928, 1729, 1253 cm^{-1}; ^{1}H NMR (C_6D_6, 500 MHz, 60 °C) δ 6.65 (s, 1 H), 6.57 (s, 1 H), 5.50 (dd, J = 2.58, 9.42 Hz, 1 H), 5.39 (m, 2 H), 4.34 (s, 1 H), 4.07 (dd, J = 2.2, 9.9 Hz, 1 H), 3.47 (t, J = 4.5 Hz, 1 H), 3.11 (br s, 1 H), 2.69 (m, 2 H), 2.57 (m, 2 H), 2.31 (s, 3 H), 2.18 (m, 6 H), 2.0 (m, 1 H), 1.78 (m, 1 H), 1.53 (m, 2 H), 1.27 (m, 1 H), 1.09 (d, J = 4.5 Hz, 3 H), 0.98 (m, 15H), 0.91 (s, 3 H), 0.14 (s, 3 H), 0.13 (s, 3 H); ^{13}C NMR (C_6D_6, 125 MHz, 60 °C) δ 171.4, 164.3, 153.8, 137.8, 133.2, 128.5, 125.8, 120.3, 116.6, 83.2, 78.7, 75.8, 73.9, 42.6, 40.9, 39.4, 35.5, 34.4, 32.3, 28.1, 26.3, 22.5, 22.4, 18.8, 18.6, 17.0, 16.5, 15.5, −3.6, −4.4; LRMS calcd for $C_{32}H_{55}O_5NSSi$: 593.4; found: 616.3 (M + Na).

Preparation of Compound 94. To a cooled (−30 °C) solution of diol **93** (0.029 g, 0.048 mmol) and 2,6-lutidine (0.017 mL, 0.147 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added TBSOTf (0.015 mL, 0.065 mmol). The resulting solution was then stirred at −30 °C for 30 min. The reaction was quenched with 0.5 M HCl (10 mL) and extracted with Et_2O (15 mL). The ether layer was washed with saturated aqueous $NaHCO_3$ (5 mL), dried (Na_2SO_4) and concentrated in vacuo. Purification of the residue by flash chromatogrphy (silica, 8% EtOAc in hexane) afforded TBS ether **94** (0.032 g, 93%) as white foam: $[\alpha]_D$ = −21.7 (c 0.35, $CHCl_3$); IR (film) 3471, 2928, 1742, 1253, 1076 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6, 38 °C) δ 6.62 (s, 1 H), 6.53 (s, 1 H) 5.49−5.46 (band, 3 H), 4.41 (br s, 1 H), 4.10 (br s, 1 H), 3.49 (br s, 1 H), 2.70−2.64 (band, 2 H), 2.44 (dd, J = 16.1, 6.5 Hz, 1 H), 2.34 (d, J = 15.5 Hz, 1 H), 2.28 (s, 3 H), 2.22−2.15 (band, 5H), 2.02 (m, 1 H), 1.81 (m, 1 H), 1.68 (m, 1 H), 1.50 (m, 1 H), 1.34 (m, 1 H), 1.18 (s, 3 H), 1.14 (d, J = 20.9 Hz, 3 H), 1.02−0.98 (band, 23 H), 0.90 (s, 3 H), 0.16−0.15 (band, 9 H), 0.10 (s, 3 H); ^{13}C NMR (125 MHz, C_6D_6, 43 °C) δ 171.3, 164.2, 153.9, 137.9, 133.0, 127.9, 120.1, 116.6, 78.9, 75.1, 44.7, 41.0, 32.8, 31.9, 27.9, 27.6, 26.4, 26.2, 25.9, 21.7, 18.9, 18.51, 18.49, 17.4, 15.53, −3.4, −3.6, −4.3, −4.4; HRMS calcd for $C_{38}H_{69}O_5NSSi_2$: 707.4435; found: 746.4062 (M + K).

Preparation of Compound 95. To a solution of alcohol **94** (0.030 g, 0.0424 mmol) in CH_2Cl_2 (2.0 mL) at 25 °C was added Dess−Martin periodinane (0.036 g, 0.0848 mmol) in one portion. The resulting solution was then allowed to stir at 25 °C for 1.5 h. The reaction was quenched by the addition of 1:1 saturated aqueous $NaHCO_3$:$Na_2S_2O_3$ (10 mL) and stirred for 5 min. The mixture was then extracted with Et_2O (3 × 15 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, 8% EtOAc in hexane) provided ketone **95** (0.025 g, 84%) as white foam: $[\alpha]_D$ = −21.93 (c = 1.4, $CHCl_3$); IR (film): 2928, 1745, 1692, 1254, 1175, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.97 (s, 1 H), 6.57 (s, 1 H), 5.53 (dt, J = 3.4, 11.1 Hz, 1 H), 5.37 (dd, J = 16.4, 9.9 Hz, 1 H), 5.00 (d, J = 10.3 Hz, 1 H), 4.02 (d, J = 9.7 Hz, 1 H), 3.89 (d, J = 8.7 Hz, 1 H), 3.00 (m, 1 H), 2.82 (d, J = 6.5 Hz, 1 H), 2.71 (m, 5H), 2.36 (q, J = 10.7 Hz, 1 H), 2.12 (, 3 H), 2.07 (dd, J = 8.2 Hz, 1 H), 1.87 (bs, 1 H), 1.49 (m, 3 H), 1.19 (m, 5H), 1.14 (s, 3 H), 1.08 (d, J = 6.8 Hz, 3 H), 0.94 (m, 12 H), 0.84 (s, 9 H), 0.12 (s, 3 H), 0.10 (s, 3 H), 0.07 (s, 3 H), −0.09 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 218.7, 170.1, 164.5, 152.6, 137.9, 133.9, 124.8, 119.6, 115.9, 72.7, 53.2, 43.9, 41.0, 40.3, 32.9, 32.3, 28.4, 27.1, 26.3, 26.1, 26.0, 19.2, 19.1, 18.3, 18.2, 17.1, 16.0, 15.2, 14.3, −4.2, −4.4, −4.6, −4.8; HRMS calcd for $C_{38}H_{67}O_5NSSi_2$: 705.4315, found: 706.4357 (M + H).

Macrolactonization To Produce Compound 95. To a solution of hydroxy acid **88** (0.094 g, 0.133 mmol) in THF (1 mL) were added Et_3N (0.11 mL, 0.79 mmol) and 2,4,6-trichlorobenzoyl chloride (0.104 mL, 0.66 mmol). The mixture was stirred at rt for 0.25 h, diluted with toluene (15 mL), and added dropwise over a period of 3 h to a solution of DMAP (0.167 mg, 1.37 mmol) in toluene (50 mL). After complete addition, the mixture was stirred for additional 0.5 h and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel eluting with hexanes/ethyl acetate (9:1) gave 0.081 g (88%) of the previously described lactone **95**.

Preparation of Compound 96. To a solution of TBS ether **95** (0.027 g, 0.038 mmol) in THF (1.0 mL) at 25 °C in a plastic vial was added dropwise HF·pyridine (0.5 mL). The resulting solution was allowed to stir at 25 °C for 2 h. The reaction mixture was diluted with chloroform (2 mL) and very slowly added to saturated aqueous $NaHCO_3$ (20 mL). The mixture was extracted with $CHCl_3$ (20 mL × 3). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, 30% EtOAc in hexane) provided diol **96** (0.018 g, 99%) as white foam: $[\alpha]_D$ = −84.7 (c = 0.85, $CHCl_3$); IR (film): 3493, 2925, 1728, 1689, 1249 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.96 (s, 1 H), 6.59 (s, 1 H), 5.44 (dt, J = 4.3, 10.4 Hz, 1 H), 5.36 (dt, J = 5.1, 10.2 Hz, 1 H), 5.28 (dd, J = 1.7, 9.8 Hz, 1 H), 4.11 (d, J = 7.2 Hz, 1 H), 3.74 (s, 1 H), 3.20 (d, J = 4.5 Hz, 1 H), 3.14 (dd, J = 2.2, 6.8 Hz, 1 H), 3.00 (s, 1 H), 2.69 (m, 4 H), 2.49 (dd, J = 11.3, 15.1 Hz, 1 H), 2.35 (dd, J = 2.5, 15.1 Hz, 1 H), 2.27 (m, 1 H), 2.05 (m, 1 H), 2.04 (s, 3 H), 2.01 (m, 1 H) 1.75 (m, 1 H), 1.67 (m, 1 H), 1.33 (m, 4 H), 1.21 (s, 1 H), 1.19 (m, 2 H), 1.08 (d, J = 7.0 Hz, 3 H), 1.00 (s, 3 H), 0.93 (d, J = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 226.5, 176.5, 171.1, 158.2, 144.7, 139.6, 131.1, 125.7, 122.0, 84.6, 80.2, 78.6, 59.4, 47.9, 45.4, 44.6, 38.5, 37.9, 33.7, 33.6, 28.7, 25.1, 25.0, 21.9, 21.7, 19.6; HRMS calcd for $C_{26}H_{39}O_5NS$ 477.2549, found 478.2627 (M + H).

Preparation of Epothilone A (2). To a cooled (−50 °C) solution of desoxyepothilone A (**96**) (0.009 g, 0.0189 mmol) in dry CH_2Cl_2 (1 mL) was added freshly prepared 3,3-dimethyldioxirane (0.95 mL, 0.1 M in acetone). The resulting solution was allowed to warm to −30 °C for 2 h. A stream of nitrogen was then bubbled through the solution to remove excess dimethyldioxirane. The residue was purified by flash chromatography (silica, 40% EtOAc in hexane) and afforded epothilone A (**2**) (0.0046 g, 49%) as colorless solid and 0.0003 g of the *cis*-epoxide diastereomer: $[\alpha]_D$ = −41.6 (c = 0.51, MeOH); IR (film): 3464, 2926, 1737, 1689, 978, 755 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 7.00 (s, 1 H), 6.56 (s, 1 H), 5.39 (dd, J = 9.2, 2.0 Hz, 1 H), 4.16 (br d, J = 10.0 Hz, 1 H), 3.73 (dd, J = 8.6, 4.2 Hz, 1 H), 3.59 (br s, 1 H), 3.21 (m, 1 H), 2.99 (m, 1 H), 2.87 (m, 1 H), 2.68 (s, 3 H), 2.50−2.44 (band, 2 H), 2.38 (dd, J = 15.0, 3.2 Hz, 1 H), 2.14−2.08 (band, 4 H), 1.86 (m, 1 H), 1.75−1.66 (band, 3 H), 1.55 (m, 1 H), 1.41 (m, 4 H), 1.35 (s, 3 H), 1.14 (d, J = 6.9 Hz, 3 H), 1.06 (s, 3 H), 0.98 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 220.2, 170.9, 165.6, 152.4, 138.1, 120.2, 116.7, 77.2, 74.9, 73.4, 57.8, 55.1, 53.7, 39.5, 36.8, 32.1, 30.9, 30.1, 27.7, 23.8, 22.0, 20.2, 19.3, 17.3, 15.6, 14.3; HRMS calcd for $C_{26}H_{39}O_6NS$: 493.2498, found: 494.2578 (M + H).

Preparation of Compound 97. To a suspension of ethyltriphenylphosphonium iodide (0.250 g, 0.60 mmol) in THF (6 mL) was added *n*BuLi (2.5 M soln in hexanes, 0.24 mL, 0.60 mmol) at rt. After disappearance of the solid material, the solution was added to a mixture of iodine (0.152 g, 0.60 mmol) in THF (4 mL) at −78 °C. The resulting suspension was vigorously stirred for 5 min at −78 °C and then warmed to −20 °C and treated with sodium bis(trimethylsilyl)amide (1 M soln in THF, 0.56 mL, 0.56 mmol). The resulting red solution was stirred for 5 min followed by the slow addition of aldehyde **78** (0.074 g, 0.30

mmol). The mixture was stirred at −20 °C for 40 min, diluted with pentane (50 mL), filtered through a pad of Celite, and concentrated in vacuo. Purification of the residue by flash column chromatography (hexanes/ethyl acetate, 85:15) gave 0.141 g (43% overall from acetate **77**) of the vinyl iodide **97** as a yellow oil: $[\alpha]_D = -20.7$ (*c* 2.45, $CHCl_3$); IR (film) 2920, 1738, 1369, 1234 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.98 (s, 1 H), 6.53 (s, 1 H), 5.43 (t, J = 6.5, 5.4 Hz, 1 H), 5.35 (t, J = 6.6, 6.5 Hz, 1 H), 2.71 (s, 3 H), 2.58−2.50 (band, 5H), 2.08 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 164.6, 152.4, 136.9, 130.3, 120.7, 120.6, 116.4, 103.6, 40.3, 33.7, 19.2, 19.1, 14.9; HRMS calcd for $C_{14}H_{18}O_2NIS$: 391.0103; found: 392.0181 (M + H).

Preparation of Compound 98. To a solution of olefin **68** (0.977 g, 1.55 mmol) in THF (3 mL) was added 9-BBN (0.5 M soln in THF, 3.4 mL, 1.7 mmol). In a separate flask, iodide **97** (0.749 g, 1.92 mmol) was dissolved in DMF (5 mL). Cs_2CO_3 (1.154 g, 3.54 mmol) was then added with vigorous stirring followed by sequential addition of $PdCl_2(dppf)_2$ (0.162 g, 0.198 mmol), Ph_3As (0.061 g, 0.20 mmol), and H_2O (0.42 mL, 23.4 mmol). After 5 h, the borane solution was added to the iodide mixture in DMF. The reaction quickly turned dark brown in color and slowly became pale yellow after 3 h. The solution was then poured into H_2O (10 mL) and extracted with Et_2O (3 × 15 mL). The combined organic layers were washed with H_2O (3 × 15 mL), brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash column chromatography (hexanes/ethyl acetate, 9:1) gave the coupled product **98** (1.073 g; 77%) as a yellow oil: IR (film) 2931, 1738, 1429, 1239, 1072, 709 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 15 H), 6.98 (s, 1 H), 6.59 (s, 1 H), 5.30 (t, J = 6.9 Hz, 1 H), 5.15 (t, J = 6.7 Hz, 1 H), 4.38 (d, J = 3.5 Hz, 1 H), 4.02 (s, 1 H), 3.60 (m, 2 H), 3.41 (t, J = 6.9 Hz, 2 H), 2.88 (s, 3 H), 2.79 (s, 3 H), 2.41 (m, 2 H), 2.16−2.10 (band, 6 H), 1.80 (m, 2 H), 1.70 (s, 3 H), 1.30−1.01 (band, 6 H), 0.85 (s, 15H), 0.73 (d, J = 6.8 Hz, 3 H), 0.62 (d, J = 6.7 Hz, 3 H), 0.00 (br s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 164.5, 152.7, 138.6, 137.5, 135.8, 135.3, 129.9, 127.8, 120.6, 119.1, 116.2, 110.6, 110.5, 78.9, 77.8, 63.3, 60.4, 60.2, 55.5, 46.6, 43.9, 43.4, 38.0, 32.3, 31.7, 30.4, 26.2, 26.1, 26.0, 23.6, 21.3, 19.6, 19.4, 19.2, 18.6, 17.1, 15.8, 15.4, 14.8, 14.2, 13.6, −2.9, −3.9, −4.1; HRMS calcd for $C_{53}H_{77}O_6NSSi_2$ 911.5010, found 950.4613 (M + K).

Preparation of Compound 99. The acetal **98** (0.069 g, 0.077 mmol) was dissolved in dioxane/H_2O (9:1, 1 mL), and *p*TSA·H_2O (0.045 g, 0.237 mmol) was added. The mixture was then heated to 55 °C. After 3 h, the mixture was cooled to rt, poured into saturated aqueous $NaHCO_3$, and extracted with Et_2O (4 × 15 mL). The combined ether extracts were washed with saturated aqueous $NaHCO_3$ (1 × 30 mL), brine (1 × 30 mL), dried over $MgSO_4$, filtered, and concentrated. Flash column chromatography (hexanes/EtOAc, 3:1) gave aldehyde **99** (0.046 g, 71%) as a pale yellow oil: $[\alpha]_D = -13.3$ (*c* 0.95, $CHCl_3$); IR (film) 3070, 2929, 2856, 1737, 1429, 1238, 1116, 1056 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.79 (s, 1 H), 7.71−7.69 (band, 6 H), 7.52−7.44 (band, 9 H), 7.01 (s, 1 H), 6.60 (s, 1 H), 5.31 (t, J = 6.6 Hz, 1 H), 5.12 (t, J = 6.9 Hz, 1 H), 4.85 (d, J = 4.5 Hz, 1 H), 3.58 (br s, 1 H), 2.77 (s, 3 H), 2.47 (m, 2 H), 2.14 (s, 3 H), 2.13 (s, 3 H), 1.87 (m, 2 H), 1.72 (s, 3 H), 1.49 (m, 1 H), 1.30 (m, 2 H), 1.10 (m, 1 H), 1.09 (s, 3 H), 1.06 (s, 3 H), 0.98 (d, J = 1.5 Hz, 3 H), 0.92 (s, 9 H), 0.80 (m, 2 H), 0.65 (d, J = 6.7 Hz, 3 H), 0.02 (s, 3 H), 0.00 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.7, 170.2, 164.6, 152.6, 138.4, 137.4, 135.7, 135.1, 134.9, 134.3, 130.1, 127.9, 120.6, 119.2, 116.2, 81.2, 78.9, 51.7, 43.1, 39.1, 32.3, 31.7, 30.9, 26.1, 26.0, 23.6, 21.3, 20.8, 20.2, 19.2, 18.6, 14.9, 14.8, 12.8, 0.00, −3.2, −4.0; HRMS calcd for $C_{51}H_{71}O_5NSSi_2$ 865.4592, found 904.4201 (M + K).

Preparation of Compounds 100 and 101. To a solution of aldehyde **99** (0.290 g, 0.341 mmol) in THF (34 mL) at −78 °C was added potassium bis(trimethylsilyl)amide (0.5 M soln in toluene, 3.4 mL, 1.7 mmol). The solution was stirred at −78 °C for 1 h and then quenched with saturated aqueous NH_4Cl and extracted with Et_2O (3 × 15 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated. Flash column chromatography (hexanes/EtOAc, 7:1) gave 0.120 g of the desired α-alcohol **100** and 0.055 g of β-alcohol **101** (60% combined yield) as pale yellow oils.

Conversion of Compound 101 to Compound 100. To a solution of β-alcohol **101** (0.075 g, 0.088 mmol) in 0.5 mL of CH_2Cl_2 at rt was added Dess−Martin periodinane (0.188 g, 0.44 mmol). The resulting solution was stirred at rt for 1 h and then treated with Et_2O (2 mL) and saturated aqueous $NaHCO_3$:$Na_2S_2O_3$ (3 mL, 1:1), poured into H_2O (20 mL), and extracted with Et_2O (4 × 10 mL). The combined ether solutions were washed with H_2O (1 × 30 mL), brine (1 × 30 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. To a solution of crude ketone **102** in MeOH/THF (2 mL, 1:1) at −78 °C was added $NaBH_4$ (0.017 g, 0.447 mmol). The resulting solution was stirred at rt for 1 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with Et_2O (3 × 15 mL). The organic layers were dried with $MgSO_4$, filtered, and concentrated. Flash column chromatography (hexanes/ethyl acetate, 9:1) gave 0.052 g (67%) of the α-alcohol **100** as a pale yellow oil and 0.009 g of β-alcohol **101**.

Compound 100: $[\alpha]_D = -45.4$ (*c* 1.0, $CHCl_3$); IR (film) 3490, 2929, 2856, 1729, 1428, 1114, 1037, 708 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 60 °C) δ 7.85 (m, 6 H), 7.22 (m, 9 H), 6.55 (s, 1 H), 6.49 (s, 1 H), 5.56 (br d, J = 6.9 Hz, 1 H), 5.25 (m, 1 H), 4.24 (br s, 1 H), 4.17 (m, 2 H), 2.60 (m, 1 H), 2.47 (m, 2 H), 2.29−2.22 (band, 6 H), 2.15 (s, 3 H), 2.08 (m, 2 H), 1.96 (m, 1 H), 1.84 (br s, 1 H), 1.68 (s, 3 H), 1.43 (m, 1 H), 1.32 (m, 2 H), 1.24 (d, J = 7.1 Hz, 3 H), 1.11 (s, 6 H), 1.04 (s, 9 H), 0.91 (d, J = 6.3 Hz, 3 H), 0.12 (s, 6 H); HRMS calcd for $C_{51}H_{71}O_5NSSi_2$: 865.4591; found: 866.4716 (M + H).

Preparation of Compound 103. The silyl ether **100** (0.210 g, 0.247 mmol) was dissolved in HF·pyridine/pyridine/THF (15 mL). The solution was stirred at rt for 2 h and then diluted with Et_2O (1 mL), poured into a mixture of Et_2O/saturated $NaHCO_3$ (20 mL, 1:1), and extracted with Et_2O (4 × 10 mL). The Et_2O extracts were washed with saturated aqueous $CuSO_4$ (3 × 30 mL), saturated aqueous $NaHCO_3$ (1 × 30 mL), and brine (1 × 30 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. Flash column chromatography (hexanes/EtOAc, 9:1) gave diol **103** (0.141 g, 94%) as a pale yellow oil: $[\alpha]_D = -41.7$ (*c* 0.65, $CHCl_3$); IR (film) 3460, 2928, 1728, 1252, 1037, 757 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 58 °C) δ 6.69 (s, 1 H), 6.56 (s, 1 H), 5.51 (dd, J = 9.4, 3.0 Hz, 1 H), 5.20 (m, 1 H), 4.30 (br s, 1 H), 4.10 (dd, J = 9.7, 2.6 Hz, 1 H), 3.50 (br s, 1 H), 3.07 (br s, 1 H), 2.74 (t, J = 9.6, 5.7 Hz, 1 H), 2.70 (dd, J = 16.0, 3.5 Hz, 1 H), 2.52 (dd, J = 16.0, 9.7 Hz, 1 H), 2.31−2.25 (band, 4 H), 2.24 (s, 3 H), 2.22−2.18 (band, 3 H), 1.91 (m, 1 H), 1.81 (m, 1 H), 1.61−1.58 (band, 4 H), 1.50 (m, 1 H), 1.37 (m, 2 H), 1.11 (d, J = 6.9 Hz, 3 H), 1.04 (d, J = 7.0 Hz, 3 H), 0.99 (s, 12 H), 0.93 (s, 3 H), 0.16 (s, 3 H), 0.14 (s, 3 H); ^{13}C NMR (125 MHz, C_6D_6, 58 °C) δ 171.3, 164.3, 153.9, 138.2, 137.6, 122.1, 120.4, 116.6, 82.8, 79.2, 73.8, 42.7, 39.5, 34.0, 32.9, 31.8, 26.3, 22.4, 21.9, 18.8, 18.6, 17.0, 15.5, −3.5, −4.5; LRMS calcd for $C_{33}H_{57}O_5NSSi$ 607.4, found 608.4 (M + H).

Preparation of Compound 104. To a solution of diol **103** (0.0066 g, 0.011 mmol) in 0.5 mL of CH_2Cl_2 at −78 °C were added 2,6-lutidine (7 μL, 0.060 mmol) and TBSOTf (5 μL, 0.022 mmol). The resulting solution was stirred at −30 °C for 0.5 h and then quenched with H_2O (5 mL) and extracted with Et_2O (4 × 10 mL). The ether solutions were washed with 0.5 M HCl (1 × 10 mL) and saturated aqueous $NaHCO_3$ (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated. Flash column chromatography (hexanes/EtOAc, 93:7) gave alcohol **104** (0.0070 g, 89%) as a pale yellow oil: $[\alpha]_D = -2.6$ (*c* 3.15, $CHCl_3$); IR (film) 3453, 2929, 1726, 1252, 1030 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 60 °C) δ 7.15 (s, 1 H), 6.66 (s, 1 H), 6.58 (s, 1 H), 5.54 (t, J = 6.8, 6.7 Hz, 1 H), 5.21 (m, 1 H), 4.64 (br s, 1 H), 3.80 (br s, 1 H), 2.99 (m, 1 H), 2.84 (dd, J = 14.0, 6.7 Hz, 1 H), 2.45 (dd, J = 14.1, 1.5 Hz, 1 H), 2.37 (s, 3 H), 2.29 (s, 3 H), 2.17−2.08 (band, 4 H), 1.80 (m, 1 H), 1.70−1.60 (band, 4 H), 1.49 (m, 1 H), 1.12−1.08 (band, 5H), 1.04 (d, J = 6.8 Hz, 3 H), 1.00−0.90 (band, 25H), 0.18 (s, 3 H), 0.12 (s, 3 H), 0.11 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (125 MHz, C_6D_6) δ 171.2, 164.1, 154.2, 138.8, 121.4, 120.8, 116.6, 80.1, 77.6, 74.7, 44.7, 41.8, 33.5, 32.5, 31.9, 27.1, 26.4, 26.8, 23.3, 20.2, 18.9, 18.4, 18.3, 17.4, 15.1, −3.8, −4.3, −4.4; LRMS calcd for $C_{39}H_{71}NO_5SSi_2$: 721.5; found: 722.7 (M + H).

Preparation of Compound 105. To a solution of alcohol **104** (0.107 g, 0.148 mmol) in CH_2Cl_2 (3 mL) at rt was added Dess−Martin periodinane (0.315 g, 0.743 mmol). The resulting solution was stirred at rt for 2 h, treated with Et_2O (1 mL) and saturated aqueous $Na_2S_2O_3$/saturated aqueous $NaHCO_3$ (2 mL, 1:1), poured into H_2O (20 mL), and extracted with Et_2O (4 × 10 mL). The ether solution was washed with saturated aqueous $NaHCO_3$ (1 × 20 mL), dried with $MgSO_4$, filtered, and concentrated. Flash column chromatography (hexanes/EtOAc, 9:1) gave ketone **105** (0.092 g, 87%) as a pale yellow oil: $[\alpha]_D = -18.7$ (*c* 3.65, $CHCl_3$); IR (film) 2931, 2856, 1742, 1696, 1463, 1255, 835 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.85 (s, 1 H), 6.46 (s,

1 H), 5.06 (t, J = 8.4, 7.7 Hz, 1 H), 4.86 (d, J = 10.0 Hz, 1 H), 3.92 (d, J = 9.3 Hz, 1 H), 3.78 (d, J = 8.9 Hz, 1 H), 2.92 (m, 2 H), 2.69 (d, J = 15.5 Hz, 1 H), 2.60−2.55 (band, 1 H), 2.36 (m, 1 H), 2.06 (d, J = 3.5 Hz, 1 H), 2.00−1.93 (band, 5H), 1.63−1.58 (band, 6 H), 1.44 (m, 2 H), 1.15 (s, 3 H), 1.08 (s, 3 H), 1.03 (s, 3 H), 0.99 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.9 Hz, 3 H), 0.85−0.73 (band, 12 H), 0.00−0.06 (band, 9 H), −0.21 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.1, 171.2, 164.5, 152.5, 140.6, 138.8, 119.3, 119.1, 115.9, 79.8, 76.2, 53.4, 39.1, 32.5, 31.9, 31.4, 29.2, 27.4, 26.3, 26.1, 24.5, 24.3, 23.0, 19.1, 18.7, 18.6, 17.8, 15.3, −3.3, −3.7, −5.6; LRMS calcd for $C_{39}H_{69}O_5NSSi_2$: 719.4; found: 720.6 (M + H).

Preparation of Compound 106. To a solution of ketone **105** (0.092 g, 0.128 mmol) in THF (4.5 mL) at 0 °C was added HF·pyridine (2.25 mL) dropwise. The solution was stirred at rt for 2 h, diluted with $CHCl_3$ (2 mL), poured into saturated aqueous $NaHCO_3$/$CHCl_3$ (20 mL, 1:1) slowly, and extracted with $CHCl_3$ (4 × 10 mL). The combined $CHCl_3$ layers were dried with $MgSO_4$, filtered, and concentrated. Flash column chromatography (hexanes/EtOAc, 3:1) gave desoxyepothilone B (**106**)-(0.057 g, 92%) as a pale yellow oil: $[\alpha]_D$ = −61.4 (c 2.85, $CHCl_3$); IR (film) 3465, 2969, 1735, 1691, 1377, 1181, 1148 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1 H), 6.58 (s, 1 H), 5.21 (d, J = 9.3 Hz, 1 H), 5.15 (dd, J = 10.7, 5.5 Hz, 1 H), 4.28 (br d, J = 9.2 Hz, 1 H), 3.80−3.50 (band, 3 H), 3.17 (m, 1 H), 3.16 (br s, 1 H), 2.69 (s, 3 H), 2.66−2.61 (band, 3 H), 2.46 (dd, J = 14.6, 3.4 Hz, 1 H), 2.34−2.22 (band, 3 H), 2.18 (s, 3 H), 2.07 (s, 3 H), 1.88 (m, 1 H), 1.75 (m, 1 H), 1.34 (s, 3 H), 1.02 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 220.6, 210.7, 170.4, 164.9, 151.9, 139.1, 138.4, 120.8, 119.3, 115.6, 78.9, 74.1, 72.3, 69.4, 53.7, 53.4, 41.7, 39.6, 38.3, 32.5, 31.7, 29.2, 22.9, 19.0, 18.0, 15.7; HRMS calcd for $C_{27}H_{41}NO_5NS$: 491.2705; found $C_{27}H_{42}NO_5S$: 492.2782 (M + H).

Preparation of Epothilone B (3). To a solution of desoxyepothilone B (**106**) (0.090 g, 0.183 mmol) in CH_2Cl_2 (1.8 mL) at −78 °C was added freshly prepared dimethyldioxirane (0.087 M soln in acetone, 3.60 mL, 0.313 mmol) dropwise. The resulting solution was warmed to −50 °C for 1 h, and another portion of dimethyldioxirane (1.0 mL, 0.087 mmol) was added. After stirring at −50 °C for additional 1.5 h, any excess dimethyldioxirane and solvent were removed by a stream of N_2 at −50 °C. The crude reaction mixture was determined to be >20:1 ratio of diastereomeric *cis*-epoxides by ^{1}H NMR spectroscopy. The resulting residue was purified by flash column chromatography (hexanes/EtOAc, 1:1) to give epothilone B (**3**) (0.090 g, 97%) as a white solid: $[\alpha]_D$ = −31.0 (c 0.045, $CHCl_3$); mp 93.6−94.7 °C; IR (film) 3454, 2962, 1727, 1690, 1263, 978 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (s, 1 H), 6.52 (s, 1 H), 5.43 (dd, J = 7.7, 2.4 Hz, 1 H), 4.22 (m, 2 H), 3.78 (t, J = 4.3 Hz, 1 H), 3.30 (m, 1 H), 2.81 (dd, J = 7.5, 4.6 Hz, 1 H), 2.70 (s, 3 H), 2.54 (m, 1 H), 2.37 (d, J = 12.7 Hz, 1 H), 2.09 (s, 3 H), 1.93 (m, 1 H), 1.72 (m, 2 H), 1.49 (m, 2 H), 1.43 (m, 3 H), 1.37 (s, 3 H), 1.32 (s, 3 H), 1.17 (d, J = 6.8 Hz, 3 H), 1.08 (s, 3 H), 1.01 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.5, 170.5, 165.1, 151.7, 137.5, 119.6, 116.1, 74.1, 72.8, 69.5, 61.6, 61.3, 53.7, 53.0, 42.9, 39.1, 36.3, 32.2, 32.0, 31.7, 30.6, 29.2, 22.7, 22.3, 19.6, 19.0, 17.1, 15.7, 13.7; LRMS calcd for $C_{27}H_{41}O_5NS$ 507.3, found 508.4 (M + H).

Preparation of Compound 109. To a solution of *tert*-butyllithium (3.6 mL of a 1.7 M solution in Et_2O; 6.08 mmol) in Et_2O (5 mL) at −78 °C was added a solution of 4-iodo-2-methyl-1-butene (0.596 g; 3.04 mmol) in Et_2O (20 mL). After 0.5 h, a solution of aldehyde **65** (1.03 g; 1.52 mmol) in Et_2O (5 mL) was added. After 5 min at −78 °C, the cooling bath was removed, and the solution was allowed to warm to 0 °C and stirred for 0.5 h. The reaction mixture was then poured into saturated aqueous NH_4Cl solution (100 mL) and extracted with Et_2O (2 × 100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (silica, 5−7% Et_2O/hexanes) to give a 3:1 mixture of diastereomeric alcohols (1.01 g; 89%) as a white foam.

A solution of the mixture of alcohols prepared above (1.01 g, 1.35 mmol), thiocarbonyl diimidazole (0.801 g, 4.05 mmol), and 4-DMAP (0.164 g, 1.35 mmol) in THF (10 mL) was heated to 90 °C. A stream of N_2 was then used to evaporate the THF completely. The sticky residue was maintained at 90 °C for 2 h, cooled to rt, and diluted with CH_2Cl_2 (5 mL). Purification of the residue by flash chromatography (SiO_2, 20% EtOAc:hexane) provided an epimeric mixture of thionoimidazolides (1.25 g, 99%) as a pale yellow foam.

A solution of the mixture of thionoimidazolides prepared above (1.25 g, 1.34 mmol), n-Bu_3SnH (0.66 mL, 2.01 mmol), and AIBN (0.022 g, 0.13 mmol) in benzene (14 mL) was refluxed for 1 h. The reaction mixture was cooled to rt and diluted with Et_2O (14 mL). To this solution, DBU (0.32 mL, 2.01 mmol) was added, and the resulting mixture was titrated with a solution of iodine in Et_2O until the solution color turned yellow and white precipitate formed. This slurry was filtered through a 5 cm thick pad of silica gel and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 25% CH_2Cl_2:hexanes) to give the dithiane **109** (0.823 g, 84%) as a white foam: $[\alpha]_D$ = 5.97 (c 15.9, $CHCl_3$); IR (film) 2931, 1428, 1252, 1114, 1057 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (dd, J = 7.8, 1.3 Hz, 6 H), 7.45−7.38 (band, 9 H), 4.69 (s, 1 H), 4.64 (s, 1 H), 4.56 (d, J = 3.9 Hz, 1 H), 4.16 (s, 1 H), 3.53 (d, J = 5.2 Hz, 1 H), 2.71 (d, J = 13.4 Hz, 1 H), 2.54−2.43 (band, 2 H), 2.08 (m, 1 H), 1.79−1.73 (band, 4 H), 1.73 (s, 3 H), 1.66−1.61 (band, 2 H), 1.35 (s, 3 H), 1.32 (m, 1 H), 1.16 (s, 1 H), 1.03 (d, J = 7.3 Hz, 3 H), 0.91−0.85 (band, 11 H), 0.65 (d, J = 6.8 Hz, 3 H), −0.03 (s, 3 H), −0.05 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.3, 135.9, 134.9, 129.8, 127.8, 109.3, 77.4, 77.1, 38.0, 37.8, 31.0, 30.4, 29.6, 26.2, 25.9, 25.5, 22.6, 22.4, 20.9, −3.1, −4.0; HRMS calcd for $C_{43}H_{64}O_2S_2Si_2$: 732.3886; found: 755.3784 (M + Na).

Preparation of Compound 110. A solution of compound **109** (0.95 g, 1.3 mmol) in MeOH/THF (2:1, 18 mL) was treated with [bis-(trifluoroacetoxy)iodobenzene] (1.118 g, 2.6 mmol) at rt. After 15 min, the reaction was quenched with saturated aqueous $NaHCO_3$ (25 mL). The mixture was extracted with Et_2O (3 × 25 mL), and the organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in dioxane/water (5:1, 12 mL) and treated with pTSA·H_2O (0.74 g, 3.9 mmol), and the resulting mixture was heated at 50 °C for 2 h. After cooling to rt, the mixture was diluted with Et_2O (50 mL) and washed successively with aqueous $NaHCO_3$ (15 mL) and brine (20 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated. Purification of the residue by flash chromatography (SiO_2, 25% CH_2Cl_2:hexanes) afforded aldehyde **110** (0.79 g, 95%) as a white foam: $[\alpha]_D$ = −15.2 (c 11.8, $CDCl_3$); IR (film) 2931, 1722, 1472, 1429 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1 H), 7.64 (d, J = 6.6 Hz, 6 H), 7.46−7.37 (band, 9 H), 4.69 (s, 1 H), 4.59 (s, 1 H), 4.04 (d, J = 4.2 Hz, 1 H), 3.52 (dd, J = 5.3, 2.5 Hz, 1 H), 2.16 (m, 1 H), 1.77 (m, 2 H), 1.70 (s, 3 H), 1.54 (m, 1 H), 1.44−1.26 (band, 3 H), 1.00 (s, 6 H), 0.92−0.80 (band, 15H), 0.62 (d, J = 6.8 Hz, 3 H), 0.08 (s, 3 H), 0.02 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.6, 146.0, 135.7, 134.4, 130.1, 127.9, 109.6, 81.1, 76.9, 51.7, 43.3, 38.9, 38.0, 30.4, 26.2, 25.6, 22.4, 21.1, 20.1, 18.6, 15.1, 12.8, −3.2, −4.0; HRMS calcd for $C_{40}H_{58}O_3Si_2$ 642.3925, found 665.3842 (M + Na).

Preparation of Compound 114. To a solution of acetate **77** (0.285 g, 1.16 mmol) and aldehyde **110** (0.49 g, 0.772 mmol) in THF (1 mL) at −78 °C was added LDA (2 M soln in THF, 0.772 mL, 1.54 mmol). The yellow mixture was stirred at −78 °C for 40 min and then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was extracted with Et_2O (3 × 15 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (SiO_2, 6% EtOAc:hexanes) provided α-alcohol **114** (0.239 g, 35%) and β-alcohol **115** (0.238 g, 35%).

To a solution of β-alcohol **115** (0.238 g, 0.27 mmol) in CH_2Cl_2 (3 mL) was added Dess−Martin periodinane (0.689 g, 1.62 mmol) at rt. The resulting solution was stirred for 1 h and then quenched by the addition of 1:1 saturated aqueous $NaHCO_3$:$Na_2S_2O_3$ (10 mL). The mixture was extracted with Et_2O (3 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting C-3 ketone **116** was *immediately* dissolved in a mixture of MeOH (4 mL) and THF (2 mL) and cooled to −78 °C. Sodium borohydride (0.102 g, 2.7 mmol) was then added, and the mixture was allowed to warm to rt and stirred for 1 h. The reaction was then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was extracted with ether (3 × 15 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography (SiO_2, 8% EtOAc:hexanes) provided α-alcohol **114** (0.230 g, 92%): $[\alpha]_D$ = −45.3 (c 0.29, $CHCl_3$); IR (film) 3502, 2927, 1715, 1428 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 6.5 Hz, 6 H), 7.45−7.30 (band, 9 H), 6.93 (s, 1 H), 6.50 (s, 1 H), 5.65 (m, 1 H), 5.31 (t, J = 6.7 Hz, 1 H), 5.05 (m, 2 H), 4.69 (s, 1 H), 4.63 (s, 1 H), 4.13 (d, J = 3.5 Hz, 1 H), 4.00 (m, 1 H), 3.73 (d, J = 4.5 Hz, 1 H), 3.25 (d, J = 2.5 Hz, 1 H), 2.71 (s, 3 H), 2.42 (m,

4 H), 2.06 (m, 1 H), 2.02 (s, 3 H), 1.87−1.78 (band, 2 H), 1.75 (s, 3 H), 1.70 (m, 1 H), 1.31−1.20 (band, 2 H), 1.12 (m, 1 H), 1.02 (d, J = 8.0 Hz, 3 H), 0.96 (s, 3 H), 0.91 (s, 3 H), 0.79 (s, 9 H), 0.60 (d, J = 7.0 Hz, 3 H), −0.06 (s, 3 H), −0.07 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 131.1, 164.6, 152.4, 146.2, 136.4, 135.7, 134.9, 133.2, 129.8, 127.7, 121.4, 117.9, 116.5, 109.4, 79.0, 78.7, 76.3, 71.8, 44.2, 43.0, 38.7, 38.0, 37.4, 36.5, 30.4, 26.2, 26.1, 25.7, 22.5, 21.0, 20.5, 19.2, 18.5, 15.2, 14.5, 13.4, −3.1, −4.3; HRMS calcd for $C_{53}H_{75}O_5NSSi_2$: 893.4904, found: 932.4529 (M + K).

Preparation of Compound 120. The aldol product **114** (0.219 g, 0.27 mmol) was treated with buffered HF·pyridine in THF (8.0 mL) at rt (the stock solution was prepared from 20 mL THF, 11.4 mL pyridine and 4.2 g hydrogen fluoride−pyridne (Aldrich Co.)). After 2 h, the reaction was poured into saturated aqueous $NaHCO_3$ and extracted with Et_2O. The organic layer was washed in sequence with saturated aqueous $CuSO_4$ (3 × 30 mL) and saturated aqueous $NaHCO_3$ (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (silica, 10 → 20% EtOAc in hexane) to give diol **120** (0.15 g, 93%) as a white foam: $[\alpha]_D = -40.5$ (c 3.8, $CDCl_3$); IR (film) 3457, 2930, 1732, 1472, 1386, 1252 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s, 1 H), 6.51 (s, 1 H), 5.72 (m, 1 H), 5.35 (t, J = 6.7 Hz, 1 H), 5.08 (m, 2 H), 4.68 (s, 1 H), 4.65 (s, 1 H), 4.07 (d, J = 10.0 Hz, 1 H), 3.92 (br s, 1 H), 3.80 (br s, 1 H), 3.49 (br s, 1 H), 2.68 (s, 3 H), 2.61−2.45 (band, 4 H), 2.07 (d, J = 1.2 Hz, 3 H), 2.01 (br s, 1 H), 1.69 (s, 3 H), 1.55 (m, 1 H), 1.36 (m, 1 H), 0.99 (d, J = 6.9 Hz, 3 H), 0.88 (s, 9 H), 0.80 (s, 3 H), 0.09 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 164.5, 152.5, 145.8, 136.8, 133.3, 120.9, 117.7, 116.3, 109.8, 78.5, 41.5, 38.0, 37.5, 37.1, 32.9, 25.9, 25.5, 22.3, 21.1, 19.1, 18.2, 16.1, 14.6, −4.4; LRMS calcd for $C_{35}H_{61}O_5NSSi$: 635.4; found: 658.5 (M + Na).

Preparation of Compound 121. To a cooled (−30 °C) solution of diol **120** (0.110 g, 0.173 mmol) and 2,6-lutidine (0.121 mL, 1.04 mmol) in anhydrous CH_2Cl_2 (2 mL) was added TBSOTf (0.119 mL, 0.519 mmol). The resulting solution was then stirred at −30 °C for 30 min. The reaction was quenched with 0.5 M HCl (50 mL) and extracted with Et_2O (150 mL). The Et_2O layer was washed with saturated aqueous $NaHCO_3$ (50 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification of the residue by flash chromatogrphy (silica, 5 → 8% EtOAc in hexane) afforded TBS ether **121** (0.112 g, 85%) as white foam: $[\alpha]_D = -33.7$ (c 1.6, $CHCl_3$); IR (film) 3478, 2929, 1737, 1471, 1253 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.93 (s, 1 H), 6.50 (s, 1 H), 5.71 (m, 1 H), 5.30 (t, J = 6.8 Hz, 1 H), 5.08 (m, 2 H), 4.67 (s, 1 H), 4.65 (s, 1 H), 4.20 (m, 1 H), 3.81 (br s, 1 H), 3.40 (br s, 1 H), 2.90 (dd, J = 17.2, 3.9 Hz, 1 H), 2.69 (s, 3 H), 2.47 (m, 2 H), 2.35 (dd, J = 17.2, 5.4 Hz, 1 H), 2.07 (s, 3 H), 2.01 (m, 2 H), 1.99 (m, 1 H), 1.69−1.60 (band, 4 H), 1.54−1.48 (band, 2 H), 1.25 (m, 1 H), 0.94 (d, J = 7.0 Hz, 3 H), 0.91 (s, 3 H), 0.89−0.87 (band, 12 H), 0.86 (s, 3 H), 0.83 (s, 9 H), 0.07 (s, 3 H), 0.02 (s, 3 H), 0.01 (s, 3 H), −0.05 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 164.5, 152.5, 146.2, 136.7, 133.4, 121.2, 117.8, 116.4, 109.7, 78.7, 78.1, 74.4, 43.4, 39.8, 38.1, 37.5, 32.9, 26.0, 25.9, 25.6, 22.4, 19.9, 19.2, 18.2, 18.1, 14.2, −4.0, −4.2, −4.22, −4.9; HRMS calcd for $C_{41}H_{75}O_5NSSi_2$: 749.4904, found: 788.4518 (M + K).

Preparation of Compound 122. To a solution of alcohol **121** (0.110 g, 0.147 mmol) in CH_2Cl_2 (2.0 mL) at rt was added Dess−Martin periodinane (0.249 g, 0.583 mmol) in one portion. The resulting solution was then allowed to stir at 25 °C for 1.5 h. The reaction was quenched by the addition of 1:1 saturated aqueous $NaHCO_3$:$Na_2S_2O_3$ (10 mL) and stirred for 5 min. The mixture was then extracted with Et_2O (3 × 15 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (silica, 6% EtOAc in hexane) provided ketone **122** (0.099 g, 94%) as white foam: $[\alpha]_D = -55.1$ (c 0.85, $CHCl_3$); IR (film) 2929, 1737, 1695, 1253 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1 H), 6.49 (s, 1 H), 5.70 (m, 1 H), 5.29 (t, J = 6.8 Hz, 1 H), 5.05 (m, 2 H), 4.68 (s, 1 H), 4.65 (s, 1 H), 4.34 (dd, J = 5.9, 3.4 Hz, 1 H), 3.72 (d, J = 5.4 Hz, 1 H), 3.15 (m, 1 H), 2.70 (s, 3 H), 2.53−2.42 (band, 3 H), 2.28 (dd, J = 17.0, 6.1 Hz, 1 H), 2.06 (s, 3 H), 1.99 (m, 2 H), 1.69 (s, 3 H), 1.41 (m, 1 H), 1.33−1.29 (band, 3 H), 1.25−1.21 (band, 4 H), 1.04−1.02 (band, 6 H), 0.92−0.83 (band, 21 H), 0.10 (s, 3 H), 0.05 (s, 3 H), 0.03 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 164.5, 152.5, 146.0, 136.7, 133.4, 121.1, 117.8, 116.4, 109.8, 78.6, 77.7, 74.0, 53.3, 45.2, 40.3, 38.7, 38.3, 37.5, 31.6, 26.2, 26.0, 25.6, 23.1, 22.4, 20.3, 19.3, 18.5, 18.2, 17.1, 15.5, 14.5, −3.6, −3.8, −4.3, −4.7; HRMS calcd for $C_{41}H_{73}O_5NSSi_2$: 747.4748; found: 786.4362 (M + K).

Preparation of Compound 124. To a solution of diene **122** (0.015 g; 0.02 mmol) in dry, degassed benzene (20 mL) at rt was added Schrock's metathesis catalyst [Mo(CHMe$_2$Ph)(*N*-(2,6-(*i*-Pr)$_2$C$_6$H$_3$))-(OCMe(CF$_3$)$_2$)$_2$] (0.0038 g; 0.005 mmol) in a dry box. The reaction mixture was then warmed to 55 °C and stirred for 2 h. The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography (silica, 4% EtOAc/hexanes) to give an inseparable 1:1 mixture of stereoisomeric alkenes **105** and **123** (0.013 g; 86%).

To a solution of the mixture of alkenes prepared above (0.013 g; 0.018 mmol) in THF (1 mL) was added HF·pyridine (0.5 mL), and the resulting solution was stirred for 1.5 h. The reaction mixture was then poured into saturated aqueous $NaHCO_3$ solution (50 mL) and extracted with chloroform (3 × 30 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (silica, 30% EtOAc/hexanes) to give an equimolar mixture of stereoisomeric alkenes **106** and **124** (0.008 g; 90%). The mixture was separated by preparative thin-layer chromatography (2% MeOH/CH_2Cl_2, four elutions). **124**: IR (film) 3484, 2922, 1732, 1693, 1464, 1260 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98 (s, 1 H), 6.57 (s, 1 H), 5.30 (m, 1 H), 5.11 (t, J = 6.7 Hz, 1 H), 4.32 (d, J = 10.0 Hz, 1 H), 3.67 (m, 1 H), 3.29−3.25 (band, 3 H), 2.70 (s, 3 H), 2.65−2.45 (band, 5 H), 2.16 (m, 1 H), 2.07 (s, 3 H), 1.98 (m, 1 H), 1.73−1.61 (band, 3 H), 1.60 (s, 3 H), 1.31 (m, 1 H), 1.28 (s, 3 H), 1.17 (d, J = 6.8 Hz, 3 H), 1.05 (s, 3 H), 0.98 (d, J = 7.0 Hz, 3 H); HRMS calcd for $C_{27}H_{41}O_5NS$: 491.2705; found: 492.2795 (M + H).

Acknowledgment. This research was supported by the National Institutes of Health (grant number: CA-28824). Postdoctoral Fellowship support is gratefully acknowledged by E.J.S. (NSF, CHE-9504805), A.B. (NIH, CA-GM 72231), P.B. (NIH, CA 62948), and T.K. (NIH AI-09355). A U.S. Department of Defense Predoctoral Fellowship is gratefully acknowledged by D.M. (F33 US ARMY DAMD 17-97-1-7146). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses. Professor Dr. G. Höfle of the Gesellschaft für Biotechnologische Forschung is gratefully acknowledge for providing natural epothilone A and epothilone B for comparative analysis. We also thank Professor Gunda Georg of the University of Kansas for bringing this problem to our attention.

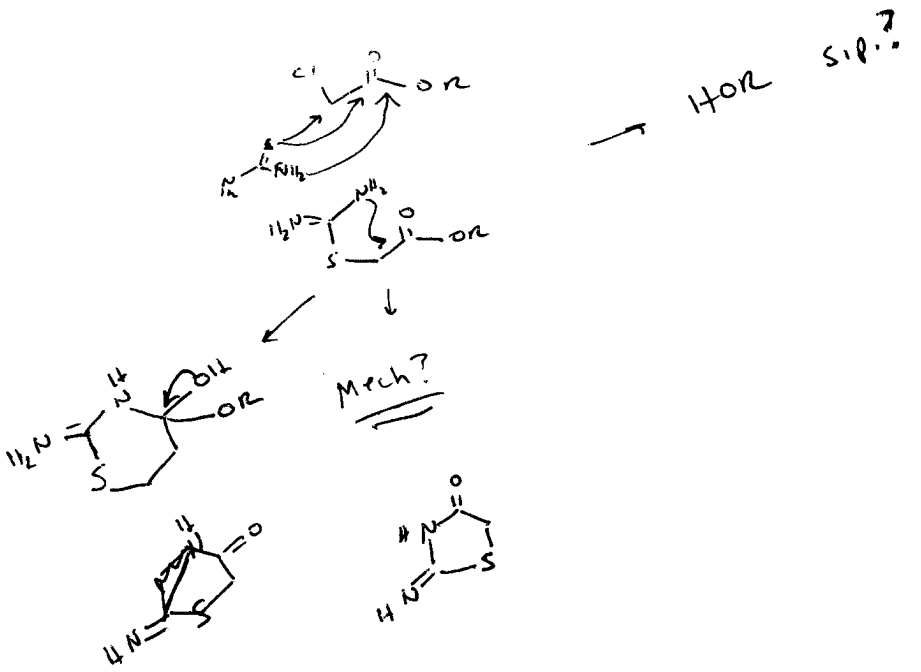
Supporting Information Available: Experimental procedures for the preparation of compounds **10**, **12**, **13**, **15**, **16**−**20**, **22**, **24**, **27**, **37**−**44**, **55**−**57**, **65**, **70**−**75**, **107**, **108**, **111**, **112**, **117**−**119**, and **125**−**136** as well as full characterization data are included (60 pages). See any current masthead page for ordering and Internet access instructions.

JA971946K

ANGEWANDTE CHEMIE

International Edition in English

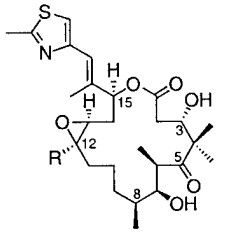
Reprint



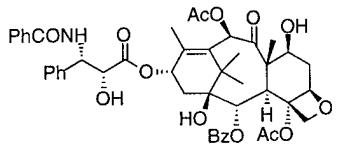
Structure–Activity Relationships of the Epothilones and the First In Vivo Comparison with Paclitaxel**

Dai-Shi Su, Aaron Balog, Dongfang Meng, Peter Bertinato, Samuel J. Danishefsky,* Yu-Huang Zheng, Ting-Chao Chou, Lifeng He, and Susan B. Horwitz

The discovery and characterization of the bacterial natural products epothilones A (**1**) and B (**2**) have evoked a great deal of interest.[1] Their high levels of cytotoxicity and their potent stabilization of microtubules are reminiscent of the biological activity of paclitaxel (**3**), a clinically valuable resource in cancer



1, R = H: epothilone A
2, R = Me: epothilone B



3: paclitaxel

chemotherapy.[2] Paclitaxel (**3**), which has been in use for the treatment of ovarian and breast carcinomas, is also being evaluated against a variety of other tumors. Nonetheless, its application is hampered by difficulties in formulation and susceptibility to multiple drug resistance (MDR). Though a massive amount of analog synthesis based on the paclitaxel structure has been accomplished, the framework appears to be intolerant to major simplification with maintenance of biological activity. To the best of our knowledge, no compound which is of a significantly lower level of structural complexity than paclitaxel has been of clinical interest as a replacement for the parent drug.

Since the epothilones are more water-soluble than paclitaxel and, in preliminary in vitro studies, seemed to perform better against several MDR cell lines, this series warranted evaluation. Our laboratory and several others have attacked the problem of the total synthesis of **1** and **2**.[3–9] This goal, in the case of **1**, was first accomplished in a stereocontrolled fashion by using a boron-alkyl Suzuki coupling strategy to establish the C11–C12

[*] Prof. S. J. Danishefsky,[+] Dr. D.-S. Su, Dr. A. Balog, D. Meng,[+] Dr. P. Bertinato
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: Int. code +(212)772-8691

Dr. Y.-H. Zheng, Dr. T.-C. Chou
Laboratory for Preclinical Pharmacology
Sloan-Kettering Institute, New York, NY (USA)

L. He, Dr. S. B. Horwitz
Department of Molecular Pharmacology
Albert Einstein College of Medicine, Bronx, NY (USA)

[+]Additional address:
Columbia University, Department of Chemistry
Havemeyer Hall, New York, NY 10021 (USA)

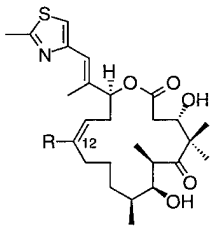
[**] This research was supported by the National Institutes of Health (grants to S. J. D. (CA-28824) and S. B. H. (CA-39821) and postdoctoral fellowships to A. B. (CA-GM 72231) and P. B. (CA-62948)). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectrometric analyses. Professor Dr. G. Höfle, of the Gesellschaft für Biotechnologische Forschung, Braunschweig (Germany), is gratefully acknowledged for providing natural epothilone A and B for comparative analysis. We also thank Professor Gunda Georg of the University of Kansas, Lawrence, KS (USA), for bringing the epothilone problem to our attention.

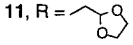
  0570-0833/97/3619-2093 $ 17.50+.50/0

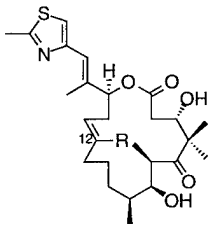
bond.[3c, e] Our total synthesis was followed by three reports of total syntheses involving nonstereoselective ring-forming olefin metathesis reactions to establish the C12–C13 bond.[3d, 4, 5] More recently, a stereoselective Wittig reaction to establish the C12–C13 bond en route to **1** has also been described.[4c]

The first synthesis of epothilone B (**2**) was accomplished in our laboratory, again, through the use of a highly stereoselective boron-alkyl Suzuki coupling reaction to form the trisubstituted olefin.[3e] A Wittig-based route, generating a mixture of olefin isomers leading to **2**, was recently reported.[4d] An important advance in the field was the stereocontrolled epoxidation of the C12–C13 double bond in desoxyepothilones A (**4**) and B (**5**) through the agency of 3,3-dimethyldioxirane.[3c–e]

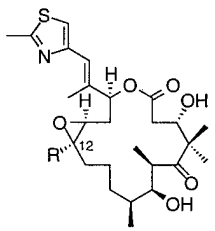
While none of our syntheses described thus far are practical for the full-scale pharmaceutical development of an epothilone, they have provided more than ample quantities for biological evaluations of the natural products both in vitro and in vivo, and for early evaluations of a range of fully synthetic analogs. Presently, we are examining compounds obtained by obvious explorations of key intermediates in the total syntheses of **1** and **2**. At this writing, we have not yet generated analogs derived from extended operations on the completed natural products themselves. We have focused on structure–activity relationships (SAR) to determine zones of the molecule that are tolerant or intolerant of structural change. Such insights would be valuable in focusing studies on molecular modeling. Furthermore, it was hoped to gather in vivo data for a direct comparison of epothilone B and paclitaxel.



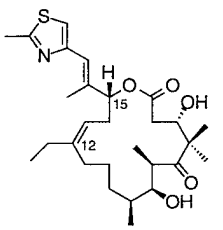
4, R = H
5, R = methyl
8, R = ethyl
10, R = propyl
11, R = 



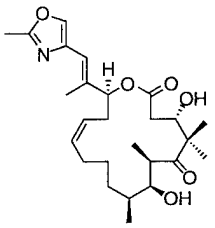
6, R = H
7, R = methyl
9, R = propyl



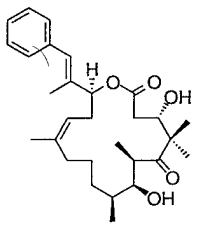
12, R = ethyl
13, R = propyl
14, R = hexyl



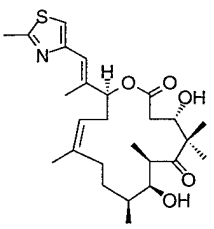
15, C15-epimer



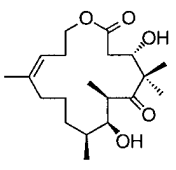
16



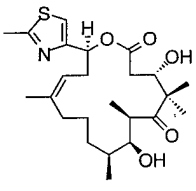
17



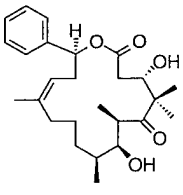
18



19



20



21

For the definition of the SAR of the epothilones, it is convenient to divide the structure of the drug into an acyl sector (shown arbitrarily as C1–C8), an O-alkyl sector (C9–C15), and a pendant aryl sector projecting from C15. In our preliminary SAR studies on epothilones we found that the acyl sector is rather intolerant of modification. For instance, inversion of stereochemistry at C3 ($S \rightarrow R$), or reduction at C5 results in serious abrogation of activity. Wholesale deletion of functionality at C3, C5, C6, C7, and C8 results in a loss of cytoxicity and of activity in the tubulin/microtubule system.[10] Indeed, as we recently demonstrated, a single permutation—deletion of the 8-methyl group—has a highly deleterious effect on biological function.[11] We now report that deletion of the "C9" methylene group (see the 15-membered macrolide **18**) also results in a major loss of activity in the tubulin polymerization/depolymerization assays, which have correlated rather closely with other indicators of biological function.[12]

Turning to the O-alkyl sector, we have expanded on the original[13] and subsequent findings to the effect that epothilone B (**2**) is more potent than epothilone A (**1**) with regards to tubulin polymerization/depolymerization and cytotoxicity (Figure 1 and Table 1). It was also found that the (*Z*)-desoxy compounds **4** and **5** retain a function closely comparable to their natural counterparts, **1** and **2**, respectively. Once again, the "B" precursor, compound **5**, is more active than the "A" precursor, compound **4**. Even the (*E*)-desoxy compounds **6** and **7** maintain significant biological activity, although less than that of the (*Z*) analogs, **4** and **5**, respectively.[3d, e, 4d] We now report that epothilone B is approximately 3400 times more active than paclitaxel against the resistant human leukemic cell line CCRF-CEM/VBL in cell-culture cytotoxicity studies.

The alkyl sector has been studied in greater detail.[14] Thus, substitution of an ethyl group at C12 in epothilone B is well tolerated (see compounds **8** and **12**, the ethyl versions of **5** and **2**, respectively) as are the higher homologues **10**, **13** and **14**.[15] Interestingly, biological activity is not lost in compound **11** bearing the polar acetal functionality on C12, nor in the *E*-desoxy compound **9**.[16] Further, activity is still retained, though in attenuated form, upon inversion of stereochemistry at C15 (see compound **15**). In summary, the O-alkyl sector is remarkably tolerant of modification with basic maintenance of in vitro function.

We also investigated the aryl sector.[14] Our studies indicate that this sector is also quite tolerant to permutation. For instance, the substitution of oxygen for sulfur was well tolerated. Thus, compound **16** (an oxazole rather than a thiazole) is equipotent with **4** in the tubulin polymerization assay (Figure 1)[17] as well as in cytotoxicity assays (Table 1).[18] We then studied a more drastic variant of the aryl domain with phenyl in place of the thiazolyl unit, and found that compound **17** retains 60% of the activity of **2** in the tubulin polymerization assay. Though there is a loss of one order of magnitude in the cytotoxicity assay, compound **17** is still highly cytotoxic.

  0570-0833/97/3619-2094 $ 17.50+.50/0

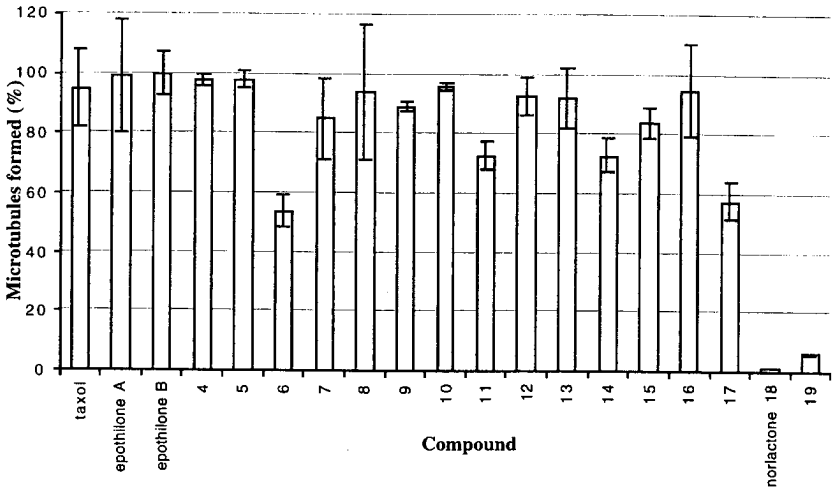


Figure 1. Formation of microtubules in the presence of 10 μM of the tested compounds. (Microtubules formed in the presence of 10 μM epothilone B defined as 100%.)

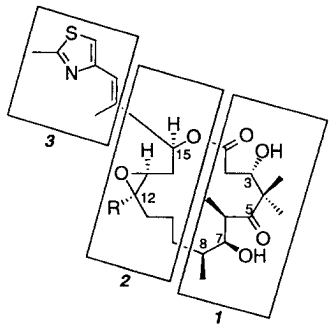
Table 1. Relative efficacy of epothilone compounds against drug-sensitive and -resistant CCRF-CEM cell lines[18].

Compound	CCRF-CEM IC_{50} [μM]	CCRF-CEM/VBL IC_{50} [μM]
paclitaxel (**3**)	0.002	4.140
epothilone A (**1**)	0.003	0.020
epothilone B (**2**)	0.0002	0.001
4	0.022	0.012
5	0.009	0.017
6	0.052	0.035
7	0.090	0.262
8	0.021	0.077
9	0.090	0.254
10	0.039	0.067
11	0.003	0.009
12	0.001	0.007
13	0.004	0.006
14	0.027	0.049
15	0.055	0.197
16	0.030	0.049
17	0.098	0.146
18	>10.0	8.95
19	>10.0	>10.0
20	3.52	1.20
21	1.80	>5.00

Given these data, we wondered whether the aryl sector is even necessary. Could it be replaced by a single hydrogen at C15? Toward this end, we prepared compound **19**. However, it has very low cytotoxicity (IC_{50} > 10.0 μM) and shows almost no activity in tubulin polymerization/depolymerization assays. We then asked whether the olefinic spacer element connecting the aromatic rings with C15 is needed. Therefore, we prepared compounds **20** and **21**. These substances showed a major loss of cytotoxicity. Accordingly, the aryl sector can now be defined with greater precision: it requires an olefinic spacer linking the lactone at C15 to an aromatic subsection which is substantially tolerant to modification.

Of course, it will be necessary to prepare and evaluate many more compounds to provide a definitive mapping of the SAR profile of this series with single carbon atom resolution. However, it is already clear that the acyl sector constitutes a "hot spot" with great sensitivity to structural change (Scheme 1). By contrast, the O-alkyl and aryl sectors exhibit significant tolerance, both in the tubulin assays and in cytotoxicity screens.

We have also initiated in vivo evaluations of the epothilones. As a first line of inquiry, we conducted a direct comparison of fully synthetic epothilone B (**2**) with paclitaxel (**3**). Since the susceptibility to drug resistance is one of the vulnerabilities of paclitaxel, we scrutinized the in vivo efficacy of the two drugs against resistant tumor tissues.[19] In the initial studies, CCRF-CEM/VBL tumor tissue was implanted subcutaneously in SCID mice. The tumor-bearing mice were treated periodically, according to a set protocol, with epothilone B (**2**, 0.7 mg kg^{-1}) and paclitaxel (**3**, 2 mg kg^{-1}). Although the concentration of paclitaxel used was higher than that of epothilone B (ca. 1.7:1 molar ratio), the reduction in tumor size was substantially greater with epothilone B at each interval of measurement (Table 2).



Scheme 1. The three arbitrarily defined sectors of the epothilones: aryl (1), alkyl (2), and acyl (3) sectors. Sector 1 is very sensitive to modifications; sectors 2 and 3 are more tolerant.

Table 2. Chemotherapeutic effect of daily treatments with epothilone B and paclitaxel in CB-17 SCID mice bearing drug-resistant human CCRF-CEM/VBL xenografts[a].

Drug	Dose [mg kg^{-1}][c]	Average tumor volume (T/C)[b] day 7	day 12	day 17	day 22
control	0	1.0	1.0	1.0	1.0
epothilone B	0.7	1.0	0.32	0.40	0.33
paclitaxel	2.0	1.0	0.60	0.58	0.70

[a] Multidrug resistant CCRF-CEM/VBL tumor tissue, 50 μL per mouse, was implanted subcutaneously on day 0. Solutions of the drugs in DMSO were injected intraperitoneally on days 7, 8, 9, 10, 14, and 15. There were seven CB-17 SCID mice in each treated group and in the control group. [b] The tumor volumes for each group on day 7 were about 1 mm^3. The average tumor volumes of the control group on days 12, 17, and 22 were 35, 107 and 278 mm^3, respectively. [c] On day 12, the average decreases in body weight due to treatment with epothilone B and paclitaxel were 2.7 and 3.4%, respectively.

In a subsequent experiment, epothilone B and paclitaxel were administered weekly (Table 3).[22] In these experiments both the sensitive (CCRF-CEM) and resistant (CCRF-CEM/VBL) tumor tissues were implanted in SCID mice. Epothilone B was given both intraperitoneally (H_2O) and intravenously (DMSO) for comparison. Epothilone B and paclitaxel show similar reduction of tumor size in treatment of the sensitive tumor (CCRF-CEM). Against the resistant tumor (CCRF-CEM/VBL), epothilone B shows a significant advantage over paclitaxel. It is also interesting to note that epothilone B is more

  0570-0833/97/3619-2095 $ 17.50+.50/0

Table 3. Chemotherapeutic effect of weekly treatments with epothilone B and paclitaxel in CB-17 SCID mice bearing human CCRF-CEM and CCRF-CEM/VBL xenografts[a].

Tumor	Drug	Route	Dose [mg kg^{-1}][b]	Average tumor volume (T/C) [c] day 10	day 15	day 20	day 25
CCRF-CEM	control	i.p.	0	1.0	1.0	1.0	1.0
	epothilone B	i.p.	1.5	0.40	0.40	0.37	0.34
		(H_2O)	3.0[d]	0.41	0.35	0.38	0.50
	epothilone B	i.v.	1.5	0.38	0.34	0.42	0.37
		(DMSO)	3.0[e]	0.28	0.38	0.29	0.24
	paclitaxel	i.p.	20.0[f]	0.33	0.28	0.31	0.34
		(DMSO)	30.0[g]	0.43	0.25	0.23	0.25
CCRF-CEM/ VBL	control	i.p.	0	1.0	1.0	1.0	1.0
	epothilone B	i.p.	1.5[h]	0.23	0.34	0.27	0.26
		(H_2O)	3.0[i]	0.26	0.25	0.20	0.22
	epothilone B	i.v.	1.5	0.21	0.19	0.27	0.26
		(DMSO)	3.0	0.24	0.27	0.14	0.20
	paclitaxel	i.p.	20.0[j]	0.77	0.51	0.60	0.59
		(DMSO)	30.0[k]	0.58	0.46	0.42	0.61

[a] CCRF-CEM and CCRF-CEM/VBL tumor tissue, 50 µL per mouse, implanted subcutaneously on day 0. The drugs were administered on day 5, 12, 19 (i.p. = intraperitoneally, i.v. = intravenously). There were five CB-17 SCID mice in each group. [b] On day 20 the average decrease in body weight due to epothilone B doses of 1.5 and 3.0 mg kg^{-1} was 5.0 and 6.6%, respectively. [c] The tumor volumes for each group on day 5 was about 8 mm^3. The average tumor volumes in the CCRF-CEM control group on days 10, 15 and 20 were 57, 145, 335 mm^3; those of the CCRF-CEM/VBL control group were 62, 173, and 386 mm^3, respectively. [d]–[k] A number of mice died of drug toxicity: two on day 23 (d), two on day 19 (e), three on days 13, 21, and 21 (f), three on days 6, 6, and 21 (g), one on day 24 (h), two on day 24 (i), two on day 13 (j), and four on day 6 (k).

effective against the resistant tumor tissues than sensitive tissues in these experiments.

At present, none of our analogs are more potent in vitro than the naturally occurring epothilone B (**2**), although the ethyl compound **12** is fully competitive in all comparisons thus far available. However, we have established zones of the molecule that are most likely to be responsive to molecular modification (see Scheme 1). Also, early in vivo data suggest a potentially valuable margin of advantage of epothilone B (**2**) relative to paclitaxel (**3**) against a highly resistant MDR tumor implant. In all in vivo experiments conducted so far, epothilone B and paclitaxel were equally effective in reducing the size of sensitive (CCRF-CEM) tumor tissues. While it is far too early to argue that we have demonstrated a superior alternative to paclitaxel, the data already in hand mark the epothilones as worthy of continuing interest. Our present focus is on selecting the most promising compounds for further development, and on a major restructuring of our synthesis for the preparation of any desirable compound in the series in an eminently practical fashion.

Received: July 8, 1997 [Z 10647 IE]
German version: *Angew. Chem.* **1997**, *109*, 2178–2181

Keywords: antitumor agents · epothilones · in vivo testing · natural products · structure–activity relationships

[1] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomberg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567.

[2] a) "Taxane Anticancer Agents, Basic Science and Current Status": *ACS Symp. Ser.* **1995**, 583; b) "The Chemistry and Pharmacology of Taxol® and its Derivatives": *Pharmacochem. Libr.* **1995**, *22*; c) K. C. Nicolaou, W.-M. Dai, K. Guy, *Angew. Chem.* **1994**, *106*, 38; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15.

[3] Model reactions: a) D. Meng, E. J. Sorensen, P. Bertinato, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 7998; b) P. Bertinato, E. J. Sorensen, D. Meng, S. J. Danishefsky, *ibid.* **1996**, *61*, 8000; Total syntheses: c) A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108*, 2976; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801; d) D. Meng, D.-S. Su, A. Balog, P. Bertinato, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *J. Am. Chem. Soc.* **1997**, *119*, 2733; e) D.-S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T. -C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757.

[4] Model reactions: a) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* **1996**, *108*, 2554; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2399; Total syntheses: b) Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *ibid.* **1997**, *109*, 170 and **1997**, *36*, 166; c) K. C. Nicolaou, F. Sarabia, S. Ninkovic, Z. Yang, *ibid.* **1997**, *109*, 539 and **1997**, *36*, 525; d) K. C. Nicolaou, N. Winssinger, J. Pasto, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Glannakakout, E. Hamel, *Nature (London)* **1997**, *387*, 268.

[5] Model reactions: a) D. Schinzer, A. Limberg, O. M. Böhm, *Chem. Eur. J.* **1996**, *2*, 1477; Total synthesis: b) D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1994**, *106*, 543; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 523.

[6] T. Gabriel, L. Wessjohann, *Tetrahedron Lett.* **1997**, *38*, 1363.

[7] E. Claus, A. Pahl, P. G. Jones, H. M. Meyer, M. Kalesse, *Tetrahedron Lett.* **1997**, *38*, 1359.

[8] J. Mulzer, A. Mantoulidis, *Tetrahedron Lett.* **1996**, *37*, 9179.

[9] R. E. Taylor, J. D. Haley, *Tetrahedron Lett.* **1997**, *38*, 2061.

[10] Unpublished results using compounds described in refs. [3a,b].

[11] A. Balog, P. Bertinato, D.-S. Su, D. Meng, E. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Tetrahedron Lett.* **1997**, *38*, 4529.

[12] Identification of the deleted carbon in compound **18** as C9 is arbitrary; it could also be looked upon as deletion of C10 or C11. For the purpose of this discussion, we look upon this compound as a "C9 deletion" corresponding to an alteration of the substituents at C8.

[13] a) D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325; b) R. J. Kowalski, P. Giannakakou, E. Hamel, *J. Biol. Chem.* **1997**, *272*, 2534.

[14] All new analogs described here were prepared by extensions of our previously published methodology: D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, in press.

[15] The (*Z*)-desoxy compound with *n*-hexyl at C12 was also found to have significant biological activity; however, the solubility of this compound in DMSO is very low. Therefore, the results are not included in Figure 1.

[16] Compound **9** was treated with 3,3-dimethyldioxirane to create the *trans*-epoxide, which was found to have significant biological activity. However, the absolute configuration of the *trans*-epoxide has not been determined.

[17] Microtubule protein (MTP) was purified from calf brains by two cycles of temperature-dependent assembly and disassembly [20]. The concentration of tubulin in the preparation was approximately 85%. Assembly experiments were done in the presence or absence of 10 µM drug plus MTP (1 mg mL^{-1} diluted in assembly buffer containing 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES), 1 mM 1,2-di(2-aminoethoxy)ethane-*N*,*N*,*N*′,*N*′-tetracetic acid (EGTA), 0.5 mM $MgCl_2$, and 3 M glycerol, pH 6.6). This concentration of drug gives an approximate 1:1 ratio to tubulin dimer. Assembly was followed spectrophotometrically at 350 nm, 37 °C for 40 min, by monitoring changes in turbidity as a measure of polymer mass [21]. Aliquots (200 µL) were taken from each assembly reaction and centrifuged at 28000 rpm, 27 °C for 30 min. The amount of protein remaining in the supernatant was determined, allowing the protein concentration in the microtubule pellet to be calculated. Controls with assembly buffer or DMSO were included. The value obtained for microtubules formed with 10 µM epothilone B was defined as 100%.

[18] The cytotoxicities of test compounds were determined by the growth of human lymphoblastic leukemic cells CCRF-CEM or their sublines resistant to vinblastine and taxol (CCRF-CEM/VBL). XTT-microculture tetrazolium/fromazan assays were used. The IC_{50} values were calculated based on five or six assays at various concentrations; a median-effect plot was generated using computer software [23].

[19] The multidrug resistant CCRF-CEM/VBL tumor tissue was implanted subcutaneously to CB-17 SCID mice, and the tumor-bearing mice were treated on days 7, 8, 9, 10, 14, and 15 with 0.7 mg kg^{-1} epothilone B intraperitoneally. The average tumor sizes were reduced by 68, 60, and 67% on days 12, 17, and 22, respectively. In the parallel experiments, 2 mg kg^{-1} taxol reduced tumor size by 40, 42, and 30%, respectively.

[20] R. C. Weisenberg, *Science* **1972**, *177*, 1104.

[21] F. Gaskin, C. R. Cantor, M. L. Shelanski, *J. Mol. Biol.* **1974**, *89*, 737.

[22] Experiments were also conducted with daily (0.7 and 1.0 mg kg^{-1}) and twice daily (0.15 and 0.25 mg kg^{-1}) treatments of epothilone B. The efficacy of once and twice daily treatments with epothilone B was similar to the weekly treatments, but the drug toxicity was slightly higher.

[23] J. Chou, T. C. Chou, IBM-PC Software "Dose-Effect Analysis with Microcomputers", Biosoft, Cambridge, **1987**.

  0570-0833/97/3619-2096 $ 17.50+.50/0

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 1369–1374, February 1998
Applied Biological Sciences

Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones

DONGTANG MENG

MARK M. MOASSER*, LAURA SEPP-LORENZINO*, NANCY E. KOHL†, ALLEN OLIFF†, AARON BALOG‡, DAI-SHI SU‡, SAMUEL J. DANISHEFSKY‡, AND NEAL ROSEN*§¶

*Department of Medicine, §Program in Cell Biology, and ‡Program in Molecular Pharmacology and Therapeutics, Sloan–Kettering Institute, Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; and †Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486

Contributed by Samuel J. Danishefsky, December 1, 1997

ABSTRACT **An important class of cellular proteins, which includes members of the p21ras family, undergoes posttranslational farnesylation, a modification required for their partition to membranes. Specific farnesyl transferase inhibitors (FTIs) have been developed that selectively inhibit the processing of these proteins. FTIs have been shown to be potent inhibitors of tumor cell growth in cell culture and in murine models and at doses that cause little toxicity to the animal. These data suggest that these drugs might be useful therapeutic agents. We now report that, when FTI is combined with some cytotoxic antineoplastic drugs, the effects on tumor cells are additive. No interference is noted. Furthermore, FTI and agents that prevent microtubule depolymerization, such as taxol or epothilones, act synergistically to inhibit cell growth. FTI causes increased sensitivity to induction of metaphase block by these agents, suggesting that a farnesylated protein may regulate the mitotic check point. The findings imply that FTI may be a useful agent for the treatment of tumors with wild-type *ras* that are sensitive to taxanes.**

Potent and specific peptidomimetic inhibitors of farnesyl transferase (FTIs) have been synthesized and characterized by several laboratories (1–4). These compounds originally were conceived as potential anti-neoplastic drugs because the Ras family of proteins is farnesylated. Members of the *ras* family of protooncogenes are mutated in 30% of human cancers, and the Ras protein plays an important role in the development and progression of many human cancers. Ras is isoprenylated through the addition of a C15 farnesyl moiety. This modification confers association with the plasma membrane. Mutants of Ras that do not become membrane-associated are not transforming, and FTIs cause the reversion of transformation of fibroblasts that express the *Ha-ras* gene (reviewed in ref. 5).

FTIs also inhibit the growth of a majority of human tumor cells in culture. In a variety of animal systems, including *v-H-ras* transgenic mice and xenograft models, FTIs inhibit tumor growth, causing complete tumor regression in some murine models (6, 7). However, it is not clear that the key defarnesylated target protein is Ras. Human tumor cells without *ras* mutation often are quite sensitive to FTIs (8). The membrane association of Ki-ras and N-ras proteins is much less sensitive than is that of Ha-ras, yet tumor cells containing mutated *Ki-ras* can be quite sensitive to the drug (7, 9). Remarkably, even though FTI affects the processing of wild-type Ras protein, the drug has little discernible toxicity in animals at doses that have major anti-tumor effects (6).

These data do not rule out the possibility that Ras inhibition plays an important role in FTI action, but they suggest that other targets may be involved (10). A number of other proteins are known to be farnesylated, including RhoB and Rap2, lamins A and B, phosphorylase kinase, rhodopsin kinase, cyclic GMP phosphodiesterase, and the γ subunit of transducin (5). Whatever the mechanism of inhibition of tumor cell growth, FTIs are novel drugs with wide therapeutic index in animals. Their role in the treatment of cancer patients has not been defined, but their low toxicity in animals, especially the absence of myelosuppression, suggests that they could be used effectively in combination with conventional chemotherapeutic agents. However, FTIs are cytostatic in some experimental models and could conceivably interfere with the effects of cytotoxic agents. We now have tested the effects of combinations of FTI and a variety of commonly used anti-cancer agents on human tumor cells in culture. FTI in combination with many of these agents causes potent and additive cell killing. Moreover, the effect of FTI in combination with taxol or an epothilone, agents that stabilize microtubule polymerization, is synergistic. Analysis of the mechanism of this interaction suggests that FTI enhances the mitotic block caused by exposure to these agents.

MATERIALS AND METHODS

Cell Culture and Growth Assays. MCF-7 and MDA-MB-468 breast cancer cells were obtained from the American Type Culture Collection and maintained in a 1:1 mixture of DME-to-F12 media supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 4 mM glutamine, and 10% heat-inactivated fetal bovine serum and incubated at 37°C at 5% CO_2. Growth assays were performed by seeding 5,000 or 10,000 cells per well in 6-well clusters and incubating for 24 h before drug treatments. Various drug treatments then were administered as outlined for individual experiments, and cells were incubated for 8–10 days, at which time they were harvested by trypsinization and counted with a Coulter counter. Doxorubicin (Pharmacia), cisplatin (Bristol–Meyers), and taxol (Bristol–Meyers) were diluted appropriately in media to achieve the desired experimental conditions. The FTI L-744832 [Merck (6)] was dissolved in PBS, desoxyepothilone A was dissolved in dimethyl sulfoxide, and appropriate dilutions were made in media to achieve desired experimental conditions. Cells were exposed to chemotherapy for 4 h to approximate *in vivo* exposure of tumors to these drugs. FTI is used in continuous culture because preclinical studies indicate tumor regrowth upon cessation of therapy (6).

Cell Cycle Analysis. Cell cycle distribution was studied in cells harvested by trypsinization, taking care to preserve the suspended and adherent cell populations. After washing in cold PBS, cell nuclei were prepared by the method of Nusse

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FTI, farnesyl transferase inhibitor; FACS, flow-assisted cell sorter.

¶To whom reprint requests should be addressed. e-mail: rosenn@mskcc.org.

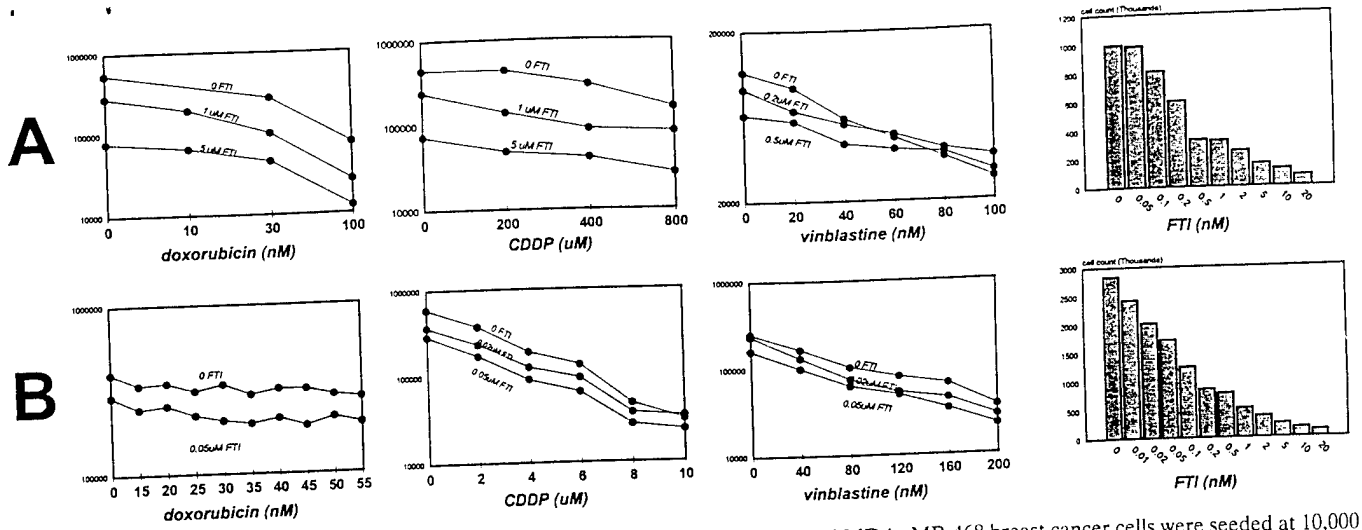


FIG. 1. Growth inhibition assays of FTI and chemotherapy combinations. MCF-7 and MDA- MB-468 breast cancer cells were seeded at 10,000 and 20,000 cells per well and the following day were exposed to the stated chemotherapeutic agent for 4 h. Cells then were washed and placed in media containing the FTI L-744832 [Merck (6)] and incubated for 7–10 days. Cells then were harvested by trypsinization and counted in a Coulter counter. The assays were performed at doses of chemotherapy and FTI that show only modest growth inhibition by themselves to best evaluate additive effects. Combinations of FTI with doxorubicin, cisplatin, and vinblastine are shown for MDA-MB-468 (*A*) and MCF-7 (*B*) cells. The growth-suppressive effects of FTI alone also are depicted on the right for comparison.

(11), and cell cycle distribution was determined by flow cytometric analysis by using red fluorescence of 488 nm of excited ethidium bromide-stained nuclei as a measure of DNA content. Linear displays of fluorescence emissions were used to study and compare cell cycle phases whereas logarithmically displayed emissions were used to best quantitate the cells with degraded sub-G_1 DNA content characteristic of apoptotic cells.

To quantitate mitotic indices, cells were harvested as described above, fixed in 3% paraformaldehyde for 10 min at room temperature, and stained in 24 μg/ml bis-benzimide (Hoechst Pharmaceuticals 33258). An aliquot of cells was placed on a glass slide and viewed under fluorescence microscopy. Mitotic cells were identified by characteristic chromatin condensation, and mitotic indices were quantitated by counting 1,000 cells manually.

Taxol Uptake Studies. To study taxol uptake, cells were treated with HPLC purified ^{3}H-labeled taxol (Moravek Biochemicals, Brea, CA). After 24 h, cells were washed rapidly with PBS and lysed in 1% Nonidet P-40 lysis buffer. Lysates were counted in scintillation fluid. Experimental arms and appropriate controls all were performed in triplicate.

Immunohistochemistry. MCF-7 cells were seeded on fibronectin-treated glass slides and 24 h later exposed to experimental arms as described. At the time of characterization, cells were fixed in 20% methanol at −20°C for 20 min and double-stained with anti-tubulin mouse mAbs (Sigma) and anti-centromere human serum (gift of K. Elkon, Cornell University, Ithaca, NY). Subsequent staining by using fluorescein isothiocyanate- and rhodamine-conjugated secondary antibodies was performed such that red fluorescence identified tubulin and green fluorescence identified centromeres.

RESULTS

Growth Inhibition by Combinations of Chemotherapy and FTI. Treatment of cultured breast cancer cells containing wild-type *ras* with FTI causes a dose-dependent growth inhibition that is additive with the dose-dependent cytotoxicity of several chemotherapeutic agents. These include the DNA-intercalating antibiotic doxorubicin, the DNA-crosslinking agent cisplatin, the microtubule blocking agent vinblastine, and the DNA and RNA synthesis inhibitor fluorouracil (Fig. 1 and data not shown). The experiments shown here were performed at the lowest doses that show growth inhibitory activity for each agent so as to best illustrate additive and potentially synergistic activities. However, higher concentrations of FTI and of chemotherapeutic agents produce complete growth inhibition. In contrast to the additive effects seen with most chemotherapeutic drugs, when FTI is given in combination with the microtubule-stabilizing agent taxol, the combination is synergistic. Addition of FTI steepens the taxol

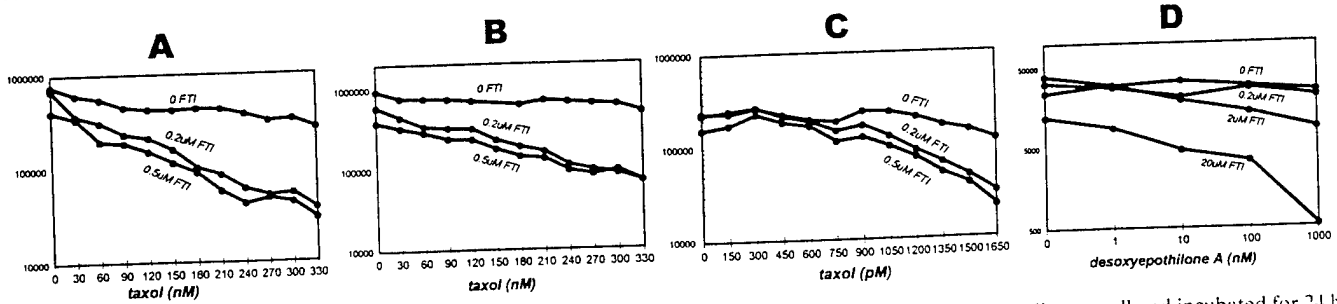


FIG. 2. Growth inhibition assays of FTI and taxol combination. MDA-MB-468 cells were seeded at 10,000 cells per well and incubated for 24 h, then treated in three scheduling variations. In *A*, cells were treated in a sequential schedule. Cells were exposed to taxol for 4 h, washed, and subsequently incubated in media containing FTI for 7–10 days. In *B*, cells were treated with FTI before taxol exposure; 24 h after initiating FTI treatment, cells also were exposed to taxol for 4 h and washed off, and FTI treatment continued until days 7–10. In *C*, the time of two drug exposures was maximized to determine whether the degree of synergy could be increased. Cells were placed in media containing both FTI and taxol and incubated for 7–10 days, exposing cells to both agents continuously. In *D*, cells were exposed to desoxyepothilone A for 4 h, then placed in media containing FTI for 7 days.

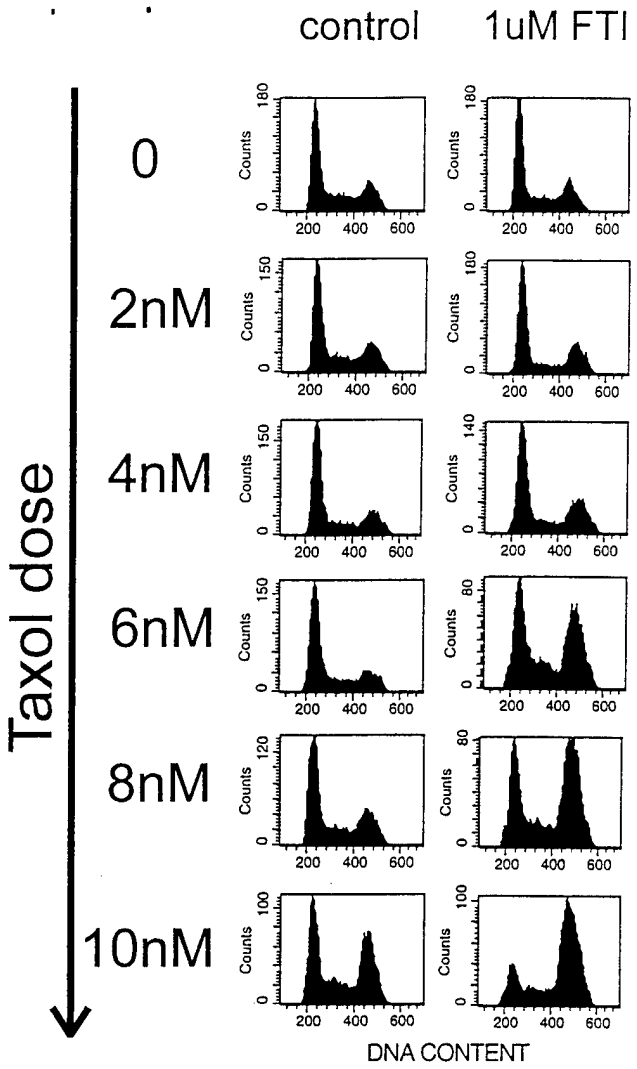


FIG. 3. The sensitivity of M phase progression to taxol in the presence and absence of FTI. MCF-7 cells were seeded at 1 million/10-cm dish and placed the following day in varying concentrations of taxol in the presence of 1 μM FTI or vehicle control (PBS). After 24 h of taxol exposure, cells were harvested and cell cycle distribution was determined as described in *Materials and Methods*. Increasing the concentration of FTI to 10-μM does not increase the twofold sensitization of M phase progression to taxol seen here (data not shown), indicating that the effect is maximal at 1 μM FTI. In MDA-MB-468 cells, taxol causes an M phase block with a much higher degree of apoptotic cell death and shows a similar FTI-induced sensitization of mitosis to taxol.

dose–response curve (Fig. 2*a*). Although treatment with up to 300 nM taxol alone caused moderate growth inhibition in a 4-h treatment, the addition of <1 μM FTI produced an almost one and one-half log growth inhibition. Mathematical analyses performed on cell counts from the above growth assays at several degrees of growth inhibition by using the combination index of Chou and Talalay reveal indices that confirm synergistic effects (12). These effects are maintained when FT inhibition is initiated before taxol, indicating that the synergistic effect is independent of the sequence of exposure (Fig. 2*b*). In addition, increasing the duration that cells are exposed to both agents does not potentiate the synergy, indicating that the effect is saturated at these conditions (Fig. 2 *a–c*). Analysis of other cell lines shows that taxol and FTI have similar synergistic effects in T47D breast cancer and DU-145 prostate cancer cells and, to relatively lesser degrees, in MDA-MB-231 and MCF-7 breast cancer cells (data not shown). Thus, there is a mechanistic relationship between these two classes of agents that confers favorable anti-neoplastic effects. Taxol alters tubulin dynamics to promote and stabilize microtubules and arrests mitotic cells in metaphase (13, 14).

Table 1. Mitotic index analysis of Fig. 3

Taxol dose, nM	Control, %	1 μM FTI, %
4	3.3	12.3
6	8.8	16.9
8	20.5	27.6
10	30.0	36.0

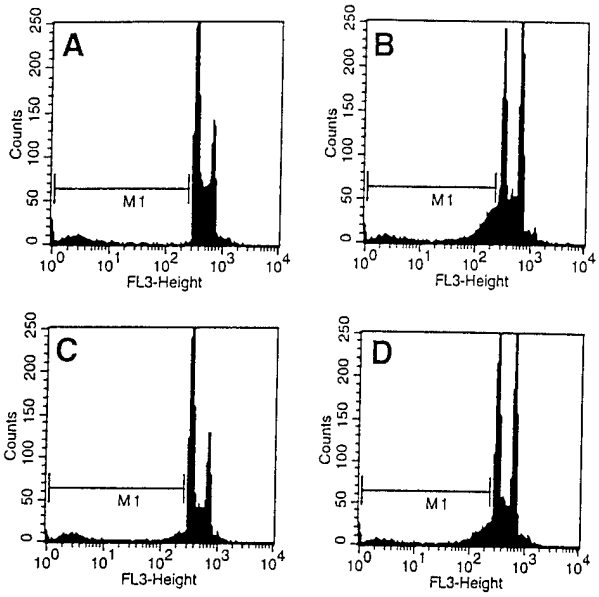


FIG. 4. Apoptotic DNA degradation induced by taxol in the presence or absence of FTI. The logarithmic fluorescence intensity is shown for several arms of the experiment described in Fig. 3 to highlight cells with sub-G_1 DNA content due to apoptotic DNA degradation (indicated by the M1 gate). The histograms correspond to *A* (0 taxol, 0 FTI) and *B* (8 nM taxol, 0 FTI), showing the characteristic DNA degradation seen with taxol-induced apoptosis in these cells. Histograms *C* (4 nM taxol, 0 FTI) and *D* (4 nM taxol, 1 μM FTI) demonstrate cells that become blocked in mitosis largely because of the addition of FTI, and this block also is characterized by apoptotic DNA degradation.

To examine whether the FTI synergy is unique to taxol or is shared by other agents that stabilize microtubules, we tested the effects of epothilones. The epothilones are a newly discovered class of compounds that bear little structural similarity to taxol. However, they have been found to stabilize microtubules and compete with taxol for binding to tubulin (15). They are potent inhibitors of mitosis and are active in cells that are resistant to taxol because of the multi-drug-resistant phenotype (15). The combination of FTI with desoxyepothilone A shows synergistic characteristics similar to the taxol–FTI combination (Fig. 2*d*), confirming that there is a mechanistic relationship between FTI and microtubule-stabilizing agents. FTIs previously have not been described to affect mitotic progression or microtubule dynamics. FTI treatment of tumor cells in monolayer does not produce an immediate cell cycle block but rather a gradual decline in proliferative rate over 5–7 days and eventual arrest with a multiphasic cell cycle distribution, including both G_1 and G_2/M phases (data not shown). The mechanism of growth retardation induced by FTIs is unclear but may involve disruption of signals mediated by farnesylated proteins such as Ras.

Potential Mechanism of Synergy. We examined whether the mechanism of synergy involves an effect of taxol on Ras-mediated signal transduction. Taxol treatment of MCF-7 and

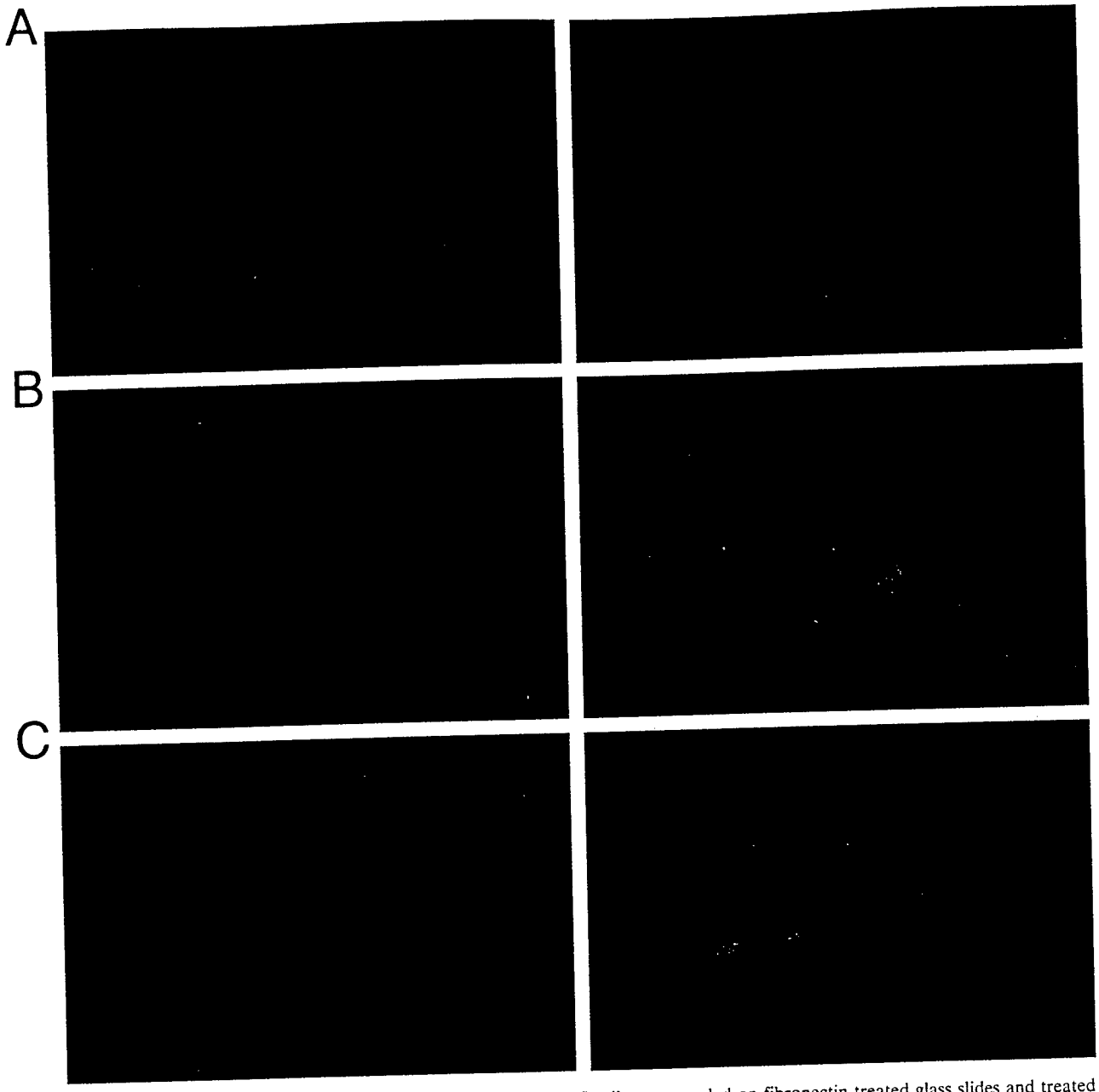


FIG. 5. Immunofluorescence microscopy of mitotic progression. MCF-7 cells were seeded on fibronectin-treated glass slides and treated the following day simultaneously with the experiment in Fig. 3 and in the same experimental arms. After 24 h, cells were washed and fixed in cold methanol and double-stained with anti-tubulin and anti-centromere antibodies as described in *Materials and Methods*. Conjugated secondary antibody staining was done such that red fluorescence identifies tubulin (*Left*) and green fluorescence identifies centromeres (*Right*) in the same microscopic view. *A* (0 taxol, 0 FTI) shows normal mitosis in progression. *B* (10 nM taxol, 0 FTI) shows cells blocked in metaphase due to taxol alone. *C* (6 nM taxol, 1 μM FTI) shows cells blocked in metaphase largely caused by the addition of FTI. The cells are blocked in a metaphase characterized by chromosome alignment but disorderly spindle formation similar to that of *B*.

MDA-468 cells has no effects on the expression of the EGFR, HER2, Ras, ERK1, or ERK2 proteins. Although others have reported that taxol interferes with lipid incorporation into Ras (16), our studies of Ras processing by gel migration analysis do not corroborate this (data not shown). In addition, the EGF-induced activation of MAP kinase is not affected by taxol (data not shown). We proceeded to study whether FTI potentiates the anti-mitotic activity of taxol. In a 24-h assay, taxol causes mitotic arrest in these cells, an effect that becomes apparent at concentrations of 8–10 nM. The concentration dependence of this anti-mitotic effect is altered by the addition of FTI. This difference is most prominent in MCF-7 cells, the cells in which the growth assays show marginal synergy. In this cell line, FTI has no effects on mitosis, nor does 6 nM taxol. However, the combination causes a majority of the cells to block in G_2/M (Fig. 3).

Table 2. Effect of FTI on cellular taxol uptake

Experimental arm	Counts ± SD
1) no [^{3}H]-taxol - control	13 ± 5
2) 7nM [^{3}H]-taxol - control - no cells	182 ± 33
3) 7nM [^{3}H]-taxol - control - no FTI	16,956 ± 767
4) 7nM [^{3}H]-taxol - 1 μM FTI	17,290 ± 924

Further Characterization of the Synergistic Block. The block induced by FTI and taxol was found to be in M phase as determined by mitotic index analysis. Table 1 shows that, in the presence of FTI, mitotic accumulation begins at lower doses of

taxol. Cells inhibited by the FTI and taxol combination at the lowest doses undergo DNA degradation characteristic of apoptosis similar to that seen with taxol alone at higher doses (Fig. 4). Immunohistochemical analysis with anti-tubulin and anti-centromere antibodies revealed that the FTI–taxol mitotic block is characterized by an abnormal chromosome alignment and disordered spindle apparatus that is consistent with metaphase arrest and indistinguishable from that induced by higher doses of taxol alone (Fig. 5). Occasional cells have disordered separation of chromatids that suggests abnormal progression into anaphase.

Role of Drug Transport. The interaction observed between FTI and agents that stabilize tubulin polymerization could be based on an effect of FTI on the intracellular accumulation of these drugs. Synergy is not detectable between FTI and other drugs that are transported through a multi-drug-resistant, dependent process such as doxorubicin and vinblastine. We found that FTI had no significant effect on the cellular accumulation of [^{3}H]-taxol (Table 2). We conclude that the mechanism of FTI enhancement of the effects of taxol is probably not caused by effects on cellular transport. The synergy observed between epothilones and taxol is consistent with this conclusion. Multi-drug-resistant cells remain sensitive to epothilones, so these agents are likely to be transported by a different mechanism than taxol (15).

DISCUSSION

FTIs are novel anti-cancer agents that were designed to inhibit tumor growth by interfering with Ras processing. They suppress the growth of a broad range of tumor cell lines whether or not they contain mutations in the *ras* gene. Preclinical studies of FTI in animal models show that the drug has little toxicity at concentrations that have potent anti-tumor effects. These and other results imply that the FTI may affect multiple targets within the cell and that the antineoplastic activity may not be mediated via a Ras-dependent mechanism. They also suggest that FTI is a potentially useful anticancer agent and that, given its low toxicity, it might be particularly effective when given in combination with traditional cytotoxic drugs. We now show that FTI has additive effects on tumor cell lines in combination with several commonly used chemotherapeutic agents. In addition, FTI and taxol or epothilones act synergistically to inhibit tumor cell growth and arrest cells in metaphase.

These findings suggest a role for a farnesylated protein in regulating the mechanism whereby these drugs kill cells. The effect does not seem to occur at the level of drug transport. FTI could affect the interaction of taxol or epothilones with microtubules, regulate the sensitivity of the mitotic checkpoint, or directly inhibit mitotic progression. The last is unlikely because neither MCF-7 nor MDA-468 cells accumulate in mitosis when treated with high doses of FTI. FTI could affect certain aspects of mitosis through effects on MAP kinase, which is required for mitotic checkpoint function (17), or lamins A and B, two farnesylated proteins in the nuclear envelope (8), or RhoB, which is involved in regulating the actin cytoskeleton (10). However, the lack of obvious synergy of FTI with nocodazole (data not shown) suggests that these general mechanisms are less likely and that the effects of FTI may be related to mechanisms specific to the depolymerization of microtubules required for progression through mitosis.

We have hypothesized that synergistic growth inhibition might be secondary to synergistic induction of mitotic block. For technical reasons, the assay for enhancement of mitosis is done differently than the assay for growth synergy, each under conditions that optimize the effect. Enhancement of mitotic block was measured in cells treated with both drugs for 24 h, whereas growth experiments were assayed after 7–10 days of incubation. The synergistic block in M phase is easily demonstrable in MCF-7 cells, but the effect on cell number is subtle. The reverse is true in MDA-468 cells, in which potent synergistic cytotoxicity is observed but enhanced mitotic accumulation is marginal. It is possible that the synergistic mitotic block and growth inhibition are unrelated synergistic findings. The more likely explanation relates to the observation we have made that, in MDA-468 cells, mitotic block is associated with rapid apoptosis, whereas MCF-7 cells can arrest in M in a relatively stable fashion (data not shown). Thus, it is very difficult to measure an increased accumulation of MDA-468 cells in M, but the killing effects of the combination are easy to observe in growth assays. These findings also suggest the likelihood that the cellular response to FTI or to FTI-containing combinations also will be dependent on the constellation of other mutated genes in the cell.

These data have potentially important clinical implications. FTI is effective *in vitro* and in animal models in a variety of tumor types that are sensitive to cytotoxic chemotherapeutic agents. Preclinical toxicity profiles imply that FTIs do not exacerbate the toxicity of these agents. These characteristics suggest that the addition of FTI to chemotherapeutic agents could improve their therapeutic index. The identification of an agent that synergizes with taxol without obvious overlapping toxicities defines a new potentially potent combination of anti-cancer drugs. Because taxol is one of the most broadly active agents in use, our findings suggest new strategies for clinical testing that may affect the treatment of many types of human tumors. In addition, comparable synergy is seen with other microtubule-stabilizing drugs such as epothilones. The epothilones are a new class of water-soluble compounds that are more potent and more specific than taxol *in vitro* (15, 18) and may prove to be clinically superior to taxol. The data shown here were from a breast cancer model. Breast cancer cells rarely contain *ras* mutations, yet in these experiments they are inhibited by FTI and are exquisitely sensitive to the taxol-FTI combination, further supporting the notion that FT inhibition has applicability well beyond the realm of the mutant *ras* genotype.

This work was supported by National Institutes of Health Breast Cancer Specialized Programs of Research Excellence (SPORE) Grant P50CA68425-02 (N.R.). M.M. is supported by the American Society of Clinical Oncology Career Development Award. L.S.L. is supported by the National Institutes of Health Breast Cancer SPORE Career Development Award.

1. James, G. L., Goldstein, J. L., Brown, M. S., Rawson, T. E., Somers, T. C., McDowell, R. S., Crowley, C. W., Lucas, B. K., Levinson, A. D. & Marsters, J. C., Jr. (1993) *Science* **260,** 1937–1942.
2. Garcia, A. M., Rowell, C., Ackermann, K., Kowalczyk, J. J. & Lewis, M. D. (1993) *J. Biol. Chem.* **268,** 18415–18418.
3. Kohl, N. E., Mosser, S. D., deSolms, S. J., Giuliani, E. A., Pompliano, D. L., Graham, S. L., Smith, R. L., Scolnick, E. M., Oliff, A. & Gibbs, J. B. (1993) *Science* **260,** 1934–1937.
4. Nigam, M., Seong, C. M., Qian, Y., Hamilton, A. D. & Sebti, S. M. (1993) *J. Biol. Chem.* **268,** 20695–20698.
5. Kohl, N. E., Conner, M. W., Gibbs, J. B., Graham, S. L., Hartman, G. D. & Oliff, A. (1995) *J. Cell. Biochem.* **22,** Suppl., 145–150.
6. Kohl, N. E., Omer, C. A., Conner, M. W., Anthony, N. J., Davide, J. P., deSolms, S. J., Giuliani, E. A., Gomez, R. P., Graham, S. L., Hamilton, K., *et al.* (1995) *Nat. Med.* **1,** 792–797.
7. Kohl, N. E., Wilson, F. R., Mosser, S. D., Giuliani, E., deSolms, S. J., Conner, M. W., Anthony, N. J., Holtz, W. J., Gomez, R. P., Lee, T. J., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9141–9145.
8. Sepp-Lorenzino, L., Ma, Z., Rands, E., Kohl, N. E., Gibbs, J. B., Oliff, A. & Rosen, N. (1995) *Cancer Res.* **55,** 5302–5309.
9. Whyte, D. B., Kirschmeier, P., Hockenberry, T. N., Nunez-Oliva, I., James, L., Catino, J.J., Bishop, W. R. & Pai, J. K. (1997) *J. Biol. Chem.* **272,** 14459–14464.
10. Lebowitz, P. F., Davide, J. P. & Prendergast, G. C. (1995) *Mol. Cell. Biol.* **15,** 6613–6622.
11. Nusse, M., Beisker, W., Hoffmann, C. & Tarnok, A. (1990) *Cytometry* **11,** 813–821.
12. Chou, T. C. (1991) in *The Median Effect Principle and the Combination Index for Quantitation of Synergism and Antagonism,*

eds. Chou, T. C. & Rideout, D. C. (Academic, San Diego), Vol. 2, pp. 61–102.
13. Jordan, M. A., Toso, R. J., Thrower, D. & Wilson, L. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9552–9556.
14. Rowinsky, E. K., Donehower, R. C., Jones, R. J. & Tucker, R. W. (1988) *Cancer Res.* **48,** 4093–4100.
15. Bollag, D. M., McQueney, P. A., Zhu, J., Hensens, O., Koupal, L., Liesch, J., Goetz, M. X., Lazarides, E. & Woods, C. M. (1995) *Cancer Res.* **55,** 2325–2333.
16. Danesi, R., Figg, W. D., Reed, E. & Myers, C. E. (1995) *Mol. Pharmacol.* **47,** 1106–1111.
17. Minshull, J., Sun, H., Tonks, N. K. & Murray, A. W. (1994) *Cell* **79,** 475–486.
18. Muhlradt, P. F. & Sasse, F. (1997) *Cancer Res.* **57,** 3344–3346.

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 9642–9647, August 1998
Pharmacology

Desoxyepothilone B: An efficacious microtubule-targeted antitumor agent with a promising *in vivo* profile relative to epothilone B

TING-CHAO CHOU*†, XIU-GUO ZHANG*, AARON BALOG‡, DAI-SHI SU‡, DONGFANG MENG‡, KENNETH SAVIN‡, JOSEPH R. BERTINO*†, AND SAMUEL J. DANISHEFSKY*‡§

*Molecular Pharmacology and Therapeutics Program and the ‡Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, and †Cornell University Graduate School of Medical Sciences, 1275 York Avenue, New York, NY 10021

Contributed by Samuel J. Danishefsky, June 2, 1998

ABSTRACT **A new class of 16-membered macrolides, the epothilones (Epos), has been synthesized and evaluated for antitumor potential *in vitro* and *in vivo*. Recent studies in these and other laboratories showed that epothilones and paclitaxel (paclitaxel) share similar mechanisms of action in stabilizing microtubule arrays as indicated by binding-displacement studies, substitution for paclitaxel in paclitaxel-dependent cell growth, and electron microscopic examinations. The present study examined cell growth-inhibitory effects in two rodent and three human tumor cell lines and their drug-resistant sublines. Although paclitaxel showed as much as 1,970-fold cross-resistance to the sublines resistant to paclitaxel, adriamycin, vinblastine, or actinomycin D, most epothilones exhibit little or no cross-resistance. In multidrug-resistant CCRF-CEM/VBL$_{100}$ cells, IC$_{50}$ values for EpoA (1), EpoB (2), desoxyEpoA (3) (dEpoA), desoxyEpoB (4) (dEpoB), and paclitaxel were 0.02, 0.002, 0.012, 0.017, and 4.14 μM, respectively. *In vivo* studies, using i.p. administration, indicated that the parent, EpoB, was highly toxic to mice and showed little therapeutic effect when compared with a lead compound, dEpoB. More significantly, dEpoB (25–40 mg/kg, Q2Dx5, i.p.) showed far superior therapeutic effects and lower toxicity than paclitaxel, doxorubicin, camptothecin, or vinblastine (at maximal tolerated doses) in parallel experiments. For mammary adenocarcinoma xenografts resistant to adriamycin, MCF-7/Adr, superior therapeutic effects were obtained with dEpoB compared with paclitaxel when i.p. regimens were used. For ovarian adenocarcinoma xenografts, SK-OV-3, dEpoB (i.p.), and paclitaxel (i.v.) gave similar therapeutic effects. In nude mice bearing a human mammary carcinoma xenograft (MX-1), marked tumor regression and cures were obtained with dEpoB.**

The isolation of the naturally occurring macrolides epothilone A and epothilone B (EpoA and EpoB) from the myxobacteria *Sorangium cellulosum* (1, 2) and the subsequent demonstration of their ability to stabilize microtubule arrays *in vitro* have elicited considerable interest in this class of compounds (3, 8–11). We and others (4–8) recently have conducted total syntheses of these natural products (Fig. 1). In our lab more than 45 related analogs (12–14) have been prepared to investigate their chemical structure–biological activity relationships (5). Our studies allowed us to dissect the epothilone structure into three zones. Thus, in the C-1~8 acyl sector, structural changes are not tolerated in terms of *in vitro* cytotoxicity and microtubule stabilizing ability. This stands in contrast to the C-9~15 O-alkyl sector and the C-15 pendant aryl sectors wherein considerable modification of structures is tolerated (5, 12). In the present study, we describe results of *in vitro* and *in vivo* experiments on the *Z*-12,13-desoxy version of EpoB (see dEpoB, **4**).

It has been shown that the natural epothilones **1** and **2** have a similar mechanism of action to paclitaxel (paclitaxel), although the agents differ a great deal in their structures (5, 9, 15, 16). Paclitaxel, isolated from the Pacific yew tree (*Taxus brevifolia*), has been widely used clinically to treat a variety of solid cancers including neoplasms of ovary, breast, colon, and lung (16–20). Epothilones A and B as well as paclitaxel stabilize microtubule assemblies as demonstrated by binding displacement, substitution for paclitaxel in paclitaxel-dependent cell growth, and electron microscopic examinations (3). The epothilones are more water soluble than paclitaxel, thereby offering potentially distinct advantages for formulation. Furthermore, the Epos are more potent than paclitaxel in inhibiting cell growth, especially against cells expressing P-glycoprotein (Pgp) that are multidrug-resistant (MDR), including cross-resistance to paclitaxel (3, 5).

MATERIALS AND METHODS

Chemicals. All epothilones used in this study were obtained in our laboratory through total syntheses as described in previous publications (4, 5, 9, 12, 13). For *in vitro* studies, paclitaxel (paclitaxel), etoposide (VP-16), teniposide (VM), camptothecin (CPT), actinomycin D (AD), and vinblastine sulfate (VBL) were purchased from Sigma. All stock solutions of the above (except VBL in saline) were prepared by using dimethyl sulfoxide (DMSO) as a solvent and were further diluted to desired concentrations for experimental use. The final concentration of DMSO in tissue culture was 0.25% (vol/vol) or less to avoid solvent cytotoxicity. For *in vivo* studies, paclitaxel (paclitaxel) in Cremophor-EtOH was obtained from Bristol-Myers Squibb and further diluted with DMSO as needed. Vinblastine sulfate (Velban) (Eli Lilly) and doxorubicin (Adriamycin) HCl (DX or Adr) (Pharmacia) in saline were diluted with DMSO as needed. DMSO was used as a vehicle for epothilones. Each mouse received ≤40 μl DMSO in all experiments.

Cell Lines. The CCRF-CEM human T cell acute lymphoblastic leukemia cell line and its vinblastine-resistant (CCRF-CEM/VBL$_{100}$) and teniposide-resistant (CCRF-CEM/VM$_1$) sublines (19, 20) were obtained from W. T. Beck, University of Illinois, Chicago. CCRF-CEM/paclitaxel was developed in this laboratory (T.-C.C.) after continuous exposure of CCRF-CEM cells with increasing concentrations of paclitaxel (at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Epo, epothilone; dEpoB, desoxyepothilone B; MDR, multidrug resistant; DX, doxorubicin (Adriamycin); VBL, vinblastine; VM, teniposide; CPT, camptothecin; AD, actinomycin D.

§To whom reprint requests should be addressed. e-mail: dshfsky@ski.mskcc.org.

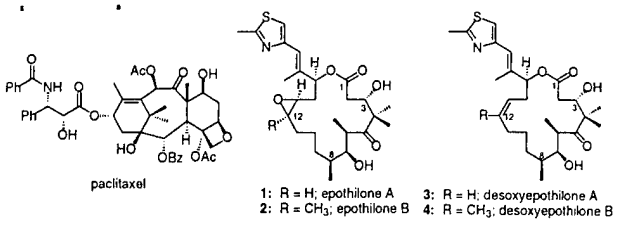


FIG. 1. Chemical structures of paclitaxel, epothilones A and B, and desoxyepothilones A and B.

IC_{50}–IC_{90}) for 10 months. The fresh medium with paclitaxel was replenished every week. The CCRF-CEM/paclitaxel cell lines exhibited 57-fold resistance to paclitaxel (IC_{50} = 0.12 μM) when compared with original CCRF-CEM cells at the beginning of the experiment (IC_{50} = 0.0021 μM, see Table 1). The DC-3F hamster lung fibroblast cell line and its actinomycin D-selected resistant sublines (DC-3F/ADII and DC-3F/ADX) were obtained from J. L. Biedler of the Memorial Sloan–Kettering Cancer Center. The murine leukemic P388/0 and its doxorubicin-selected subline (P388/DX) as well as human neuroblastoma SK-N-As and its doxorubicin-selected subline (SK-N-FI/Adr) were obtained from F. A. Schmid of the Memorial Sloan–Kettering Cancer Center.

The drug-resistant cell lines were cultured continuously in the presence of the selecting agent, AD, DX, VBL, or VM to maintain the drug-resistant phenotypes. Each sub-cell line was cultured for one to two passages in an appropriate concentration (e.g., IC_{50}) of the drug, which was then removed from the media, and the cells were resuspended in fresh media for a minimum of 4 days before each assay. All cells were cultured in RPMI 1640 medium/10%FBS at 37°C, 5% CO_2 (see below).

Cytotoxicity Assays. The cells were cultured at an initial density of 5×10^4 cells/ml. They were maintained in a 5% CO_2-humidified atmosphere at 37°C in RPMI 1640 medium (GIBCO/BRL) containing penicillin (100 units/ml), streptomycin (100 μg/ml) (GIBCO/BRL), and 10% heat-inactivated fetal bovine serum. Cytotoxicity studies for cells in suspension (such as for CCRF-CEM, P388, and sublines) were performed by the XTT-microculture tetrazonium method (21) in duplicate in 96-well microtiter plates. 2′,3′-bis(methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) was prepared at 1 mg/ml in prewarmed (37°C) medium without serum. Phenazine methosulfate (PMS) and fresh XTT were mixed together to obtain 0.025 mM PMS-XTT solution (25 μl of the stock 5 mM PMS was added per 5 ml of 1 mg/ml XTT). After a 72-h incubation, 50 μl of the assay aliquots was added to each well of the cell culture. After incubation at 37°C for 4 h, absorbance at 450 and 630 nm was measured with a microplate reader (EL340, Bio-Tek, Wincoski, VT).

The cytotoxicity of the drug toward the monolayer cell cultures (such as DC-3F, MCF-7, SK-N-As, and sublines) was determined in 96-well microtiter plates by the SRB method as described by Skehan and coworkers (22) for measuring the cellular protein content. Cultures were fixed with trichloroacetic acid and then stained for 30 min with 0.4% sulforhodamine B dissolved in 1% acetic acid. Unbound dye was removed by acetic acid washes, and the protein-bound dye was extracted with an unbuffered Tris base [tris(hydroxymethyl)aminomethane] for determination of absorbance at 570 nm in a 96-well microtiter plate reader. The experiments were carried out in duplicate. Each run entailed six to seven concentrations of the tested drugs. Data were analyzed with the median-effect plot (23) by using a previously described computer program (24).

***In Vivo* Antitumor Effects.** Athymic nude mice (nu/nu) were used for MX-1, MCF-7/Adr, and SK-OV3 human mammary and ovarian carcinoma xenografts. Mice were obtained from Taconic Farms (outbred, Swiss background). Male mice, 6–8 weeks old, weighing 20–25 g, were used. All studies were conducted in accordance with the guidelines of the National Institutes of Health "Guide for the Care and Use of Animals," and after protocol review by the Memorial Sloan–Kettering Cancer Center Institutional Care and Use Committee. For the humane treatment of tumor-bearing animals, mice were euthanized when tumors reached ≥10% of their total body weight.

RESULTS

***In Vitro* Comparisons of Structure–Activity Relationships.** To extend our recent study on structure–activity relationships of epothilones (5), we examined the susceptibility of CCRF-CEM leukemic cells and the respective drug-resistant sublines CCRF-CEM/VBL_{100} (Pgp-MDR cells) (19) and CCRF/CEM/VM_1 (cells with a mutated topo II enzyme) (20) to epothilones **1** and **2** and desoxyepthilones (**3, 4**) (Table 1). Although/VBL_{100} is 527-fold resistant to VBL and 1,971-fold resistant to paclitaxel, the epothilones (**1, 2**) exhibited only 6.1- to ~7.4-fold resistance, whereas desoxyepothilones (**3, 4**) evidenced only 0.6- to ~1.8-fold resistance. Using paclitaxel as the selecting agent, a cell line was obtained (CCRF-CEM/paclitaxel) that was 57-fold resistant to paclitaxel and found to be 10.9-fold resistance to VBL. By contrast, DX, AD, and VP-16 showed only 2.3- to 4.5-fold resistance, and, interestingly, **1** and **2** showed very little resistance (i.e., 1.4- to ~3.1-fold) and compounds **3** and **4** displayed almost no resistance (i.e., 0.7- to ~1.7-fold) (Table 1). It is also of interest to note that CCRF-CEM/VM_1 cells that were 117-fold resis-

Table 1. Susceptibility of CCRF-CEM and its drug-resistant sublines to epothilone derivatives

Compound	(A) CCRF-CEM	(B) CCRF-CEM/VBL_{100}	(C) CCRF-CEM/paclitaxel	(D) CCRF-CEM/VM_1	(B)/(A)	(C)/(A)	(D)/(A)
	IC_{50}, μM*						
1	0.0027	0.020	0.0037	0.0061	7.4	1.4	2.3
2	0.00035	0.0021	0.0011	0.0013	6.1	3.1	3.6
3	0.0220	0.012	0.0150	0.013	0.55	0.7	0.59
4	0.0095	0.017	0.0162	0.014	1.8	1.7	1.5
Paclitaxel	0.0021	4.140	0.120	0.0066	1,971	57	3.1
Vinblastine	0.00063	0.332	0.0069	0.00041	527	10.9	0.7
Etoposide	0.290	10.30	1.32	34.4	35	4.5	117
Adriamycin	0.036	1.74	0.082	0.128	48	2.3	3.6
Actinomycin D	0.00035	0.038	0.0013	0.00027	109	3.7	0.8

*Cell growth inhibition was measured by XTT tetrazonium assay (21) after 72-h incubation for cell growth as described previously. The IC_{50} values were determined with six to seven concentrations of each drug using a computer program (23, 24).

Table 2. Comparison of *in vitro* growth inhibition potency of epothilone derivatives against various parent and drug-resistant tumor cell lines

Compound	DC-3F	DC-3F/ ADX	P388/0	P388/ Adr	SK-N- As	SK-N- FI	MCF-7	MCF- 7/Adr
	IC_{50}, μM*							
1	0.0037	0.053 (14.5×)	0.0018	0.0010 (5.3×)	0.012	0.023 (1.9×)	0.0030	0.0094 (3.1×)
2	0.0006	0.017 (28×)	0.00029	0.0016 (5.5×)	0.004	0.010 (25×)	0.0005	0.0027 (5.4×)
3	0.011	0.042 (3.9×)	0.0213	0.0125 (0.59×)	0.073	0.223 (3.1×)	0.032	0.144 (4.5×)
4	0.00097	0.00091 (0.9×)	0.0068	0.0042 (0.62×)	0.021	0.046 (2.2×)	0.0029	0.0071 (2.4×)
Paclitaxel	0.095	32.0 (338×)	0.0029	0.326 (111×)	0.0016	0.130 (80×)	0.0033	0.150 (46×)
Actinomycin D	0.00044	0.572 (13,000×)	0.00015	0.0012 (8×)	0.00085	0.0119 (14×)	0.00068	0.00167 (2.5×)
Adriamycin	0.018	2.236 (124×)	0.0055	2.65 (482×)	0.077	1.42 (18.4×)	0.057	0.216 (3.8×)

Numbers in parentheses are folds of resistance based on the IC_{50} ratio when compared with the corresponding parent cell lines except for P388/0 and P388/Adr, and XTT assay (21) was used.

*Cell growth inhibition was measured by protein-staining SRB assay (22) after 72-h incubation as described previously. The IC_{50} values were determined with six to seven concentrations of each drug using a computer program. (23, 24).

tant to etoposide were sensitive to all Epos or dEpos listed in Table 1 with only 0.6- to 3.6-fold resistance.

***In Vitro* Effects Against Various Tumor Sublines.** Further susceptibility evaluations were conducted for EpoA, EpoB, dEpoA, and dEpoB in four additional tumor cell lines and four of their drug-resistant sublines (Table 2). Hamster lung tumor cells, DC-3F/ADX, which were selected 13,000-fold resistant to AD, were found to be 337-fold resistant to paclitaxel and 124-fold resistant to DX when compared with the parent cell line (DC-3F). In contrast, compounds **1**, **2**, and **3** showed only 3.9- to ~28-fold resistance, and compound **4** showed no cross-resistance (0.9-fold resistance).

Murine leukemic P388/Adr cells that were 482-fold resistant to DX, were found to be 111-fold resistant to paclitaxel. However, compounds **1** and **2** showed less than 6-fold resistance, and for **3** and **4** there was no cross-resistance (0.6-fold resistance).

Human neuroblastoma cells, SK-N-F1, that were selected as 18-fold resistant to DX were found to be 80-fold resistant to paclitaxel. By contrast, EpoB (**2**) was 25-fold resistant, whereas the resistance of **1**, **3**, and **4** was only between 1.9 and 3.1.

Human mammary carcinoma cells, MCF-7/Adr, that were selected 3.8-fold resistant to DX were found to be 46-fold resistant to paclitaxel. In contrast, compounds **1**, **2**, and **3** were 3.1- to ~5.4-fold resistant, and dEpoB (**4**) showed only 2.4-fold resistance.

Overall, dEpoB **4** was the least cross-resistant among Epos and dEpos in various drug-resistant tumor sublines. By contrast, paclitaxel suffers from marked cross-resistance in tumor cells that were selected to be resistant to VBL, DX, or AD. In three out of five cell lines studied, cross-resistance to paclitaxel was even greater than that of the selecting agents.

A Study of the Toxicity of dEpoB and EpoB. The toxicity of EpoB and dEpoB was compared in normal athymic nude mice on the daily i.p. schedule (see Table 3). EpoB (**2**) at 0.6 mg/kg, QDX4, i.p. led to lethality in all eight mice. In contrast, in the group treated with dEpoB (**4**) 25 mg/kg, QDx5, i.p., none of six mice died. It was also observed that the vehicle-treated control group showed a steady increase in body weight and the dEpoB treated mice maintained approximately the same average body weight, whereas the EpoB treated group showed steady decreases in body weight until death. These results indicated a higher toxicity for EpoB given daily than in tumor-bearing nude mice when the treatment was given every other day, i.p. (see Table 4). In the preliminary studies, for the non-tumor-bearing nude mice receiving EpoB 0.6 mg/kg or dEpoB 25 mg/kg, QDx4, i.p., there were no apparent changes in hematological cell counts or blood chemistry parameters except for a 43% decrease in lymphocytes. Similar leukopenia was found with paclitaxel. Some obstructive fecal mass in the large intestine was noted after Epo treatments in the preliminary study. No gross pathological abnormalities were observed in other organs. Further studies are being organized and will be described in due course.

Table 3. Toxicity of epothilone B and desoxyepothilone B in normal nude mice

Group	Dose, schedule, and route of administration	Mice, *n*	Mice that died, *n*
Control		4	0
2	0.6 mg/kg, QD × 4, i.p.	8	8*
4	25 mg/kg, QD × 4, i.p.	6	0

*Mice died of toxicity on days 5, 6, 6, 7, 7, 7, 7, and 7.

Therapeutic Effects Against MX-1 Xenografts. Our *in vitro* results suggested that the naturally occurring Epo B (**2**) was the most potent of the epothilone drugs. However, early *in vivo* probes (5) pointed to a worrisome toxicity that raised serious concerns as to the tolerability of this drug. Therefore, we wondered whether a less potent but still highly *in vitro* active congener such as 12,13-desoxyepo B (**4**) might provide a more useful therapeutic index. Accordingly, we launched a direct comparison of the *in vivo* performance of these two fully synthetic drugs. To evaluate these Epo agents in a broader context, we included paclitaxel in our comparisons as well as two mechanistically different chemotherapeutic agents, VBL and CPT.

Therapeutic effects of the various drugs were evaluated in athymic nude mice bearing human mammary adenocarcinoma MX-1 xenografts (Table 4). Compound **4** at a 15 mg/kg dose i.p. on days 7, 9, 11, 13, and 15 produced a 50–60% tumor volume reduction when compared with the control group. A higher dose of drug, 25 mg/kg, produced as much as 96% average tumor volume reduction measured 2 days after the last drug treatment (i.e., on day 17). These effects were achieved with no lethality nor significant body weight reduction. Furthermore, with a 25-mg/kg dose, one of six mice was tumor-free on day 35 after tumor implantation (i.e., on day 35). In contrast, after treatment with EpoB (0.3 mg/kg or 0.6 mg/kg, i.p., on days 7, 9, 11, 13, and 15), the average body weight

Table 4. Therapeutic effect of desoxyepothilone B, epothilone B, paclitaxel, vinblastine, and camptothecin in nude mice bearing human MX-1 xenograft

Drug	Dose, mg/kg	Average body weight change, g					Average tumor size, T/C				Toxicity death	*n*
		Day 7	11	13	15	17	Day 11	13	15	17		
Control		27.2	+0.8	+1.1	+1.9	+0.6	1.00	1.00	1.00	1.00	0/8	8
4	15	27.1	+0.8	+1.1	+1.6	+1.5	0.65	0.46*	0.49*	0.41**	0/6	6
	25†	27.0	+0.4	+0.7	+1.0	+0.7	0.38*	0.11**	0.05***	0.04****	0/6	6
2	0.3	26.9	+0.5	+0.4	−0.3	−1.2	1.00	0.71	0.71	0.84	0/7	7
	0.6‡	27.4	−0.3	−1.3	−2.1	−2.1	1.08	0.73	0.81	0.74	3/7‖	7
Paclitaxel	5	26.9	−0.1	+0.4	+1.1	+1.2	0.54	0.46	0.40*	0.45**	0/7	7
	10§	27.6	−2.7	−1.1	−0.3	+2.2	0.43	0.37	0.12	0.11	4/7‖	7
Vinblastine	0.2	25.7	+0.6	+1.4	+2.3	+2.9	0.65	0.54	0.56	0.88	0/7	7
	0.4¶	26.4	+0.8	+0.5	+1.9	+2.1	0.80	0.56	0.83	0.88	1/7‖	7
Campothecin	1.5	27.4	−0.9	−0.7	−0.4	+1.0	0.61	0.45*	0.32*	0.36**	0/7	7

MX-1 tissue, 50 μl/mouse, was implanted s.c. on day 0. Every other day in treatments were given on days 7, 9, 11, 13, and 15. The average tumor volumes of the control group on day 11, 13, 15, and 17 were 386 ± 120, 915 ± 245, 1,390 ± 324, and 1,903 ± 319 mm^3 (mean ± SEM), respectively; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.001$.

†One of six mice with no detectable tumor on day 35.

‡Three mice died of drug toxicity on day 17.

§Four mice died of drug toxicity on days 13, 13, 13, and 15.

¶One mouse died of drug toxicity on day 15.

‖*P* values were not shown because of toxic lethality.

decreased over 1 gm and 2 gm, respectively. In the case of 0.6 mg/kg treatment, three of seven mice died of toxicity. Despite the apparent toxicity at these doses, EpoB appeared to have only marginal therapeutic effect, as only 16–26% tumor volume reduction was observed (Table 4). The parallel experiments for paclitaxel in the i.p. setting led to a lower therapeutic effect. In animals treated with paclitaxel, 5 mg/kg i.p., there was 55% reduction in tumor volume and no decrease in average body weight. At a dose of 10 mg/kg i.p., paclitaxel showed a 89% tumor reduction; however, four of seven mice died of toxicity. In this survey, we also included doxorubicin (2–3 mg/kg) and camptothecin (1.5–3 mg/kg), i.e., near the maximal tolerated doses, and found that inferior results were obtained (see Table 4). Thus, even well below the maximal tolerated dose, dEpoB had the best therapeutic effect among the five compounds studied under these experimental conditions.

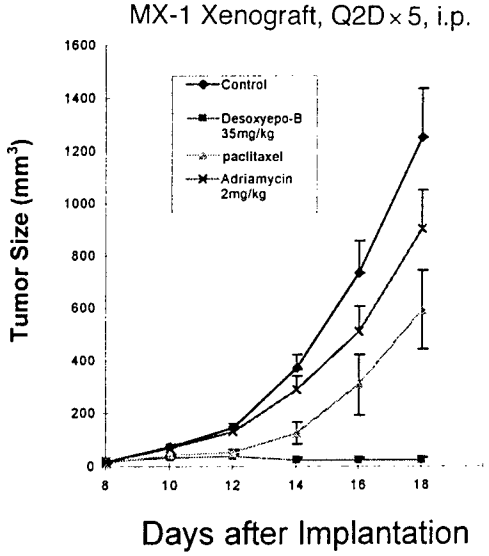


FIG. 2. Therapeutic effect of dEpoB, paclitaxel, and Adriamycin in nude mice bearing the human mammary carcinoma MX-1 xenograft. MX-1 tissue preparation, 100 μl per mouse, was implanted s.c. on day 0. Every other day i.p. treatments were given on days 8, 10, 12, 14, and 16 with 35 mg/kg dEpoB (■), 5 mg/kg paclitaxel (▲), 2 mg/kg Adriamycin (X), and vehicle (DMSO, 30 μl)-treated control (◆). For paclitaxel, 2 of 10 mice died of toxicity on day 18. For Adriamycin, 1 of 10 mice died of toxicity on day 22. For dEpoB, 10 of 10 mice survived and were subjected to the second cycle of treatment at 40 mg/kg on days 18, 20, 22, 24, and 26. This led to 3 of 10 mice tumor-free up to day 80, whereas 7 of 10 mice were with markedly suppressed tumors and were sacrificed on day 50.

We note, however, that paclitaxel was not evaluated in the optimal context (i.e., i.v. in a Cremophor formulation). We carried out our study in the i.p. mode because Epos **2** and **4** had exhibited considerable toxicity in i.v. injection. Only in the i.p. regime do the epothilones manifest useful therapeutic effects. Accordingly, the comparison reported herein is not intended to address the ultimate promise of epothilones versus paclitaxel in their respective optimal clinical settings.

In a separate experiment, MX-1 xenograft-bearing mice were treated with dEpoB (**4**) 35 mg/kg, Q2Dx5, i.p. beginning on day 8 after tumor implantation (Fig. 2). On day 16, 2 of 10 mice had no detectable tumor. These 10 mice were further treated with compound **4**, 40 mg/kg, Q2Dx5 beginning on day 18. At the end of treatment on day 26, 5 of 10 mice had no detectable tumor, and three remained tumor-free on day 60. There was modest body weight reduction during treatments, but no lethality occurred.

In a parallel experiment, 10 mice were treated with paclitaxel 5 mg/kg, Q2Dx5, i.p. from day 8 to day 16, followed by a second cycle of treatment in the same manner from day 18 to day 26. The tumor sizes were reduced but continued to grow during treatment, and, by day 24, the average tumor size was 2,285 ± 597 mm^3 (n = 10). In a further experiment, DX was given 2 mg/kg, Q2Dx5, i.p. (Fig. 2). The therapeutic effect was much weaker when compared with dEpoB or paclitaxel. It should be noted that in Fig. 2, no data after day 18 are shown because the tumor burden in the control group was excessive and the mice in this group were sacrificed.

Therapeutic Effects Against MCF-7/Adr Xenografts. The therapeutic effects of dEpoB also were evaluated in nude mice bearing xenografts of human mammary adenocarcinoma resistant to DX (MCF-7/Adr) (Table 5). For reference purposes, paclitaxel, DX, and CPT also were included in this study. The background findings for this work were the *in vitro* data shown in Table 2. Thus, MCF-7/Adr cells selected to be 3.8-fold resistant to DX had been found to be 46-fold resistant to paclitaxel and only 2.4-fold resistant to dEpoB (**4**). In the *in vivo* studies, each drug was given Q2Dx5 i.p. beginning on day 8 after tumor implantation. Paclitaxel (12 mg/kg) and DX (3 mg/kg) were highly toxic to the nude mice with 3/7 and 3/6 lethality, respectively. CPT 3 mg/kg led to moderate toxicity without lethality. By contrast, 35 mg/kg dEpoB showed neg-

Table 5. Therapeutic effects of desoxyepothilone B, paclitaxel, adriamycin, and camptothecin in nude mice bearing MDR human MCF-7/Adr tumor

Drug	Dose, mg/kg	Average body weight change, g					Average tumor size, T/C				Toxicity death	*n*
		Day 8	11	13	15	17	Day 11	13	15	17		
Control	0	25.0	+2.0	+2.6	+3.1	+3.7	1.00	1.00	1.00	1.00	0/8	8
dEpoB	35	25.0	0.3	+0.7	+0.6	+0.8	0.31**	0.27***	0.30***	0.34*	0/8	8
Paclitaxel	6	25.3	+1.7	+1.8	+0.8	+0.9	0.57	0.66	0.85	0.90	0/7	7
	12	24.5	+0.7	−1.3	−2.4	0	0.50	0.51	0.32	0.40	3/7	7†
Adriamycin	2	25.6	+0.2	−0.4	−0.6	−0.4	0.70	0.68	0.84	0.78	0/8	8
	3	24.6	+0.5	−1.3	−3.2	−1.6	0.66	0.83	0.57	0.53	3/6	6†
Campothecin	1.5	24.4	+1.1	+0.9	+1.7	+1.4	1.08	0.72	0.61	0.72	0/8	8
	3	24.5	−0.6	−0.4	−0.8	−0.9	0.95	0.76	0.61	0.43*	0/6	6

MCF-7/Adr cell 3×10^6/mouse was implanted s.c. on day 0. Every other day i.p. treatments were given on days 8, 10, 12, 14, and 16. The average tumor size of control group on days 11, 13, 15, and 17 was 392 ± 84, 916 ± 210, 1,499 ± 346, and 2,373 ± 537 mm^3, respectively (mean ± SEM). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.
†*P* values were not shown because of toxic lethality.

ligible toxicity as shown by minimal body weight changes (Table 5).

In these studies it was found that 6 mg/kg paclitaxel and 2 mg/kg DX produced only slight growth suppression of this drug-resistant tumor, which was not significantly different from the control group (see Table 5). However, dEpoB at 35 mg/kg significantly suppressed tumor size by 66–73% when compared with the control group ($P < 0.005$–0.05), and CPT at 3 mg/kg reduced 57% of tumor size on day 17 ($P < 0.05$ when compared with control group). Thus, dEpoB (**4**) stands out as the superior drug prospect among the four agents tested against this drug-resistant tumor.

Therapeutic Effects Against SK-OV3 Ovarian Adenocarcinoma and a Comparison of i.p. and i.v. Administration. Nude mice bearing human ovarian adenocarcinoma, SK-OV3, were treated with dEpoB, both i.p. (DMSO as solvent) and i.v. (Cremophor and EtOH, 1:1) injections. For comparison, paclitaxel, i.p. and i.v. (clinical samples in Cremophor and EtOH as specified by the manufacturer), and EpoB, i.v. (Cremophor and EtOH, 1:1), also were included in this experiment. As shown in Table 6, for Q2Dx5 schedule, dEpoB, i.p. (35 mg/kg), and paclitaxel, i.v. (15 mg/kg), both yield significant therapeutic effects against SK-OV3 with the tumor size on day 21, treated/control = 0.28 in both cases. By contrast, dEpoB, i.v. (15 mg/kg), paclitaxel, i.p. (5 mg/kg), and EpoB, i.v. (0.6 mg/kg), showed more toxicity and less therapeutic effect.

Further studies of dEpoB, both i.p. and i.v., were conducted with the MX-1 adenocarcinoma (see Table 7). In the Q2Dx5 schedule, dEpoB, i.p. (35 mg/kg), and paclitaxel, i.v. (15 mg/kg), gave potent therapeutic effects, as shown earlier. However, dEpoB, i.v. (15 mg/kg), and paclitaxel, i.p. (5 mg/kg), again showed high toxicity and little therapeutic value against the MX-1 tumor. Thus, dEpoB showed the best results when given i.p. and paclitaxel gave the best results when given i.v. (Cremophor and EtOH, 1:1). Attempts will be made to explore optimal formulations of the epothilones so that the i.v. route can be used routinely.

DISCUSSION

Two classes of naturally occurring compounds, Epos and paclitaxel, which apparently are structurally dissimilar, show similar mechanisms of action in stabilizing microtubule assemblies (3, 5, 9–14). These similarities include binding tubulin, substitution for paclitaxel in maintaining paclitaxel-dependent cell growth in a resistant cell line, and similar morphologic changes as determined by electron microscopic examination of the drug–microtubule complex. There are, however, differences between the two classes of compounds. These differences are most strikingly exhibited by the lack of cross-resistance in cytotoxicity between the Epos and paclitaxel even in CCRF-CEM/paclitaxel cells (Table 1). Furthermore, CCRF/CEM/VBL_{100}, which are 527-fold resistant to vinblastine and 1,971-fold resistant to paclitaxel, were only 6.1-fold resistant to EpoB and 1.8-fold resistant to dEpoB (Table 1). In DC-3F/ADX cells, there was 13,000-fold resistance to actinomycin D and 338-fold resistance to paclitaxel. However, these cells were only 28-fold resistant to EpoB and had no resistance to dEpoB (i.e., 0.9-fold resistant or collateral sensitivity) (Table 2). It is of interest to note that paclitaxel showed a higher degree of cross-resistance in these cell lines than other MDR drugs such as doxorubicin, actinomycin D, vinblastine, or etoposide. In some cases, the degrees of resistance to paclitaxel were even greater than those of the resistance-selecting agent (e.g., CCRF-CEM/VBL_{100} in Table 1 and SK-N-FI and MCF/7-Adr in Table 2). In contrast, among all compounds tested, dEpoB showed the least cross-resistance in several drug-resistant cell lines (e.g., DC-3F/Adr).

It should be noted that in this study we performed parallel cancer chemotherapeutic studies for EpoB, dEpoB, paclitaxel, and other drugs under the same experimental conditions (i.e.,

Table 6. Therapeutic effects of desoxyepothilone B, Epo B, and paclitaxel in nude mice bearing SK-OV-3 tumors using different vehicles and different routes of administration

Drug/route	Dose, mg/kg	Average body weight change, g					Average tumor size, T/C				Tumor disappearance	Toxicity death
		Day 11	15	17	19	21	Day 15	17	19	21		
Control	0	26.4	−0.2	−0.4	+0.2	+0.8	1.00	1.00	1.00	1.00	0/6	0/6
4/i.p.	35	27.8	−1.7	−2.1	−2.1	−2.4	0.57	0.33	0.35	0.28	0/6	0/6
4/i.v.	15	27.0	0	−0.6	−1.1	−2.6	0.86	0.56	0.50	0.44	0/6	4/6*
2/i.v.	0.6	27.0	−0.9	−0.5	−3.3	−3.4	0.75	0.69	0.88	0.77	0/6	0/6
Paclitaxel/i.p.	5	27.4	−1.1	−2.0	−1.0	−0.6	0.69	0.60	0.49	0.40	0/6	0/6
Paclitaxel/i.v.	15	27.2	−0.6	−0.8	−0.8	−0.9	0.97	0.67	0.42	0.28	0/6	0/6

Fifty-microgram tumor tissue was implanted s.c. on day 0. Every other day i.p. or i.v. treatments were given on days 11, 13, 15, 17, and 19. The average tumor size of control group (right side) on day 15, 17, 21, and 23 was 170, 392, 659, 1,003, and 1,280 mm^3, respectively. i.p. route used DMSO, and i.v. route used Cremophor + EtOH (1:1) as vehicles.
*Four of six mice died of drug toxicity on days 23, 23, 23, and 25.

Table 7. Therapeutic effects of desoxyepothilone B, Epo B, and paclitaxel in nude mice bearing MX-1 tumors using different vehicles and different routes of administration

Drug/ route	Dose, mg/kg	Average body weight change, g					Average tumor size, T/C				Tumor disappearance	Toxicity death
		Day 9	13	15	17	19	Day 13	15	17	19		
Control	0	26.4	−0.2	−0.4	+0.2	+0.8	1.00	1.00	1.00	1.00	0/6	0/6
4/i.p.	35	27.8	−1.7	−2.1	−2.1	−2.4	0.35	0.14	0.04	0.02	3/6	0/6
4/i.v.	15	27.0	0	−0.6	−1.1	−2.6	0.47	0.30	0.10	0.04	0/6	4/6*
2/i.v.	0.6	27.0	−0.9	−0.5	−3.3	−3.4	0.67	0.63	0.61	0.51	0/6	0/6
Paclitaxel/i.p.	5	27.4	−1.1	−2.0	−1.0	−0.2	0.59	0.72	0.59	0.55	0/6	0/6
Paclitaxel/i.v.	15	27.2	−0.6	−0.8	−0.08	−0.9	0.36	0.13	0.04	0.01	3/6	0/6

Fifty-microgram tumor tissue was implanted s.c. on day 0. Every other day i.p. or i.v. treatments were given on days 9, 11, 13, 15, and 17. The average tumor size of control group (left side) on days 13, 15, 17, and 19 was 274, 378, 677, and 1,139 mm^3, respectively. i.p. route used DMSO, and i.v. route used Cremophor + ETOH (1:1) as vehicles.
*Four of six mice died of drug toxicity on days 23, 23, 23, and 25.

treatment schedule, Q2D; solvent vehicle, DMSO; and route of administration, i.p.), except for the studies shown in Tables 6 and 7, where the i.p. and i.v. routes were compared directly. Further studies using different schedules, different vehicles, and different routes of administration with different tumors are being organized and will be described in due course.

In summary, our results already indicate that even though EpoB **2** is the most potent of the epothilones *in vitro,* it is by no means the optimal candidate for cancer therapy in terms of therapeutic index (i.e., the therapeutic efficacy at tolerable dosage, or the ratio of toxic dose vs. the therapeutic dose). Compound **4**, lacking the epoxide functionality, exhibited far superior therapeutic results *in vivo* as compared with the more potent EpoB **2**. Similarly, the present therapeutic results for dEpoB (**4**) in MX-1 xenografts were far better than those for EpoB (**2**), paclitaxel, doxorubicin, vinblastine, or camptothecin when these drugs were administered i.p. In addition, the effects of **4** on MCF-7/Adr xenografts were significantly better than those for paclitaxel, doxorubicin, and camptothecin. Desoxyepothilone B (**4**) also showed moderate therapeutic effect, similar to paclitaxel, against the human ovarian adenocarcinoma, SK-OV3.

Thus far, we regard dEpoB (**4**) as our lead compound for potential development. Further evaluations of other fully synthetic epothilone analogs are planned. Indeed, some modified epothilones available by total synthesis are evidencing rather promising early *in vitro* results. Through the experiments reported herein, it was found that i.p. administration of dEpoB (**4**) is far better tolerated than the i.v. method. We currently are surveying methods of improving i.v. administration of the epothilones. In view of the finding that Epos have little or no cross-resistance against MDR tumor cells *in vitro*, the special therapeutic advantage of such compounds might be beneficial against MDR tumors.

This research was supported by the National Institutes of Health [Grants CA-28824 (S.J.D.) and CA-GM 72231 (A.B.)]. D.M. gratefully acknowledges an Army Breast Cancer Predoctoral Fellowship (F33 US ARMY DAMD 17-97-1-7146).

1. Höfle, G., Bedorf, N., Steinmetz, H., Schumburg, D., Gerth, K. & Reichenbach, H. (1996) *Angew. Chem. Int. Ed. Engl.* **35,** 1567–1569.
2. Gerth, K., Bedorf, N. Höfle, G., Irschik, H. & Reichenbach, H. (1996) *J. Antibiot.* **49,** 560–563.
3. Bollag, D. M., McQuency, P. A., Zhu, J., Hensens, O., Koupal, L., Liesch, J., Goetz, M., Lazarides, E. & Woods, C. M. (1995) *Cancer Res.* **55,** 2325–2333.
4. Balog, A., Meng, D., Kamenecka, T., Bertinato, P., Su, D.-S., Sorensen, E. J. & Danishefsky, S. J. (1996) *Angew. Chem. Int. Ed. Engl.* **35,** 2801–2803.
5. Su, D.-S., Balog, A., Meng, D., Bertinato, P., Danishefsky, S. J., Zheng, Y.-H., Chou, T.-C., He, L. & Horwitz, S. B. (1997) *Angew. Chem. Int. Ed. Engl.* **36,** 2093–2096.
6. Nicolaou, K. C., Winssinger, N., Pastor, J. A., Ninkovic, S., Sarabia, F., He, Y., Vourloumis, D., Yang, Z., Li, T., Giannakakou, P. & Hamel, E. (1997) *Nature (London)* **387,** 268–272.
7. Ynag, Z., He, Y., Vourloumis, D., Vallberg, H. & Nicolaou, K. C. (1997) *Angew. Chem. Int. Ed. Engl.* **36,** 166–168.
8. Schinzer, D., Limberg, A., Bauer, A., Böhm, O. M. & Cordes, M. (1997) *Angew. Chem. Int. Ed. Engl.* **36,** 523–524.
9. Meng, D., Su, D.-S., Balog, A., Bertinato, P., Sorensen, E. J., Danishefsky, S. J., Zheng, Y.-H., Chou, T.-C., He, L. & Horwitz, S. B. (1997) *J. Am. Chem. Soc.* **119,** 2733- 2734.
10. Muhlradt, P. F. & Sasse, F. (1997) *Cancer Res.* **57,** 3344–3346.
11. Service, R. E. (1996) *Science* **274,** 2009.
12. Meng, D., Bertinato, P., Balog, A., Su, D.-S., Kamenecka, T., Sorensen, E. J. & Danishefsky, S. J. (1997) *J. Am. Chem. Soc.* **119,** 10073–10092.
13. Su, D.-S., Meng, D., Bertinato, P., Balog, A., Sorensen, E. J., Danishefsky, S. J., Zheng, Y.-H., Chou, T.-C., He, L. & Horwitz, S. B. (1997) *Angew. Chem. Int. Ed. Engl.* **36,** 757–759.
14. Chou, T.-C., Zhang, X.-G. & Danishefsky, S. J. (1998) *Proc. Am. Assoc. Cancer Res.* **39,** 163–164.
15. Schiff, P. B., Fant, J. & Horwitz, S. B. (1979) *Nature (London)* **277,** 665–667.
16. Landino, L. M. & MacDonald, T. L. (1995) in *The Chemistry and Pharmacology of paclitaxel and Its Derivatives,* ed. Favin, V. (Elsevier, New York), Chapter 7, p. 301.
17. Rose, W. C. (1992) *Anti-Cancer Drugs* **3,** 311–321.
18. Rowinsky, E. K., Eisenhauer, E. A., Chaudhry, V., Arbuck, S. G. & Donehower, R. C. (1993) *Seminars Oncol.* **20,** 1–15.
19. Cass, C. E., Janowska-Wieczorek, A., Lynch, M. A., Sheinin, H., Hindenburg, A. & Beck, W. T. (1989) *Cancer Res.* **49,** 5798–5804.
20. Danks, M. K., Yalowich, J. C. & Beck, W. T. (1987) *Cancer Res.* **47,** 1297–1301.
21. Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monks, A., Tierney, S., Nofziger, T. H., Currens, M. J., Seniff, D. & Boyd, M. R. (1988) *Cancer Res.* **48,** 4827–4833.
22. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenny, S. & Boyd, M. R. (1990) *J. Natl. Cancer Inst.* **82,** 1107–1112.
23. Chou, T.-C. & Talalay, P. T. (1984) *Adv. Enzyme Reg.* **22,** 27–55.
24. Chou, J. & Chou T.-C. (1987) *Dose-Effect Analysis with Microcomputers: Quantitation of ED_{50}, Synergism, Antagonism, Low-Dose Risk, Reception-Ligand Binding and Enzyme Kinetics,* IBM-PC software and manual (Biosoft, Cambridge, U.K.).

Prostate Cancer and Prostatic Diseases (1998) 5, 1–12

http://www.stockton-press.co.uk/pcan

The microtubule-stabilizing agents epothilones A and B and their desoxy-derivatives induce mitotic arrest and apoptosis in human prostate cancer cells

L Sepp-Lorenzino[1,2], A Balog[3], D-S Su[3], D Meng[3], N Timaul-Holmes[1,2], HI Scher[2], SJ Danishefsky[3] & N Rosen*[1,2]

Program in [1]Cell Biology, Department of [2]Medicine and Laboratory of [3]Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10021, USA

Epothilones are a new class of natural products that bind to tubulin and prevent the depolymerization of microtubules, although they have no structural similarity to paclitaxel. Taxanes are only marginally effective in the treatment of disseminated prostate cancer, although they may have useful activity when administered in combination with estramustine. Unlike paclitaxel, epothilones are not substrates for P-glycoprotein and are active in multidrug resistant cells. Epothilones A and B (EA, EB) have recently been synthesized *in toto*. In this report, we examine the effects of synthetic epothilones and their desoxy derivatives, as well as paclitaxel, on prostate cancer cell lines. EB was the most active of these compounds in tissue culture (IC_{50}: 50–75 pM), four to ten-fold more potent than paclitaxel. EA and the desoxyderivatives of EA and EB (dEA, dEB) were also active, but less potent than EB. Each of these compounds causes mitotic block followed by apoptotic cell death. The relative potencies for cell cycle arrest and cytotoxicity directly correlate with the ability of the drugs to bind microtubules, stabilize mitotic spindles and induce the formation of interphase microtubule bundles. Therefore, synthetic epothilones are potent inhibitors of prostate cancer cell lines and work in a fashion similar to paclitaxel. Recently, we showed that farnesyl transferase inhibitors sensitize tumor cells to paclitaxel-induced mitotic arrest. We now have extended these observations to show that paclitaxel and the epothilones synergize with FTI to arrest the growth of prostate cancer cells. Moreover, this occurs in DU145, a cell line that is not particularly sensitive to the FTI. The combination of FTI and epothilone represent a new potential clinical strategy for the treatment of advanced prostatic cancer.

Keywords: epothilones; taxanes; microtubules; apoptosis; cell cycle arrest

Introduction

Metastatic prostate cancer is difficult to treat and is relatively refractory to chemotherapeutic agents.[1,2] Progress in the treatment of this disease could well benefit from development of new agents.[3] Epothilones are 16-membered macrolides isolated from the myxobacterium *Sorangium cellulosum*.[4,5] These compounds were originally described as antifungal agents, however, their use was limited because of an associated herbicidal activity. The tumor cytotoxicity of these drugs was revealed when they were tested in the NCI 60-cell line screen. Epothilones inhibited the growth of a variety of human tumor cell lines with an IC_{50} in the 100 pM range.[4,6,7]

The mechanism whereby epothilones exert their cellular effects is similar to that of paclitaxel.[6,7] Epothilones bind to tubulin and cause the hyperstabilization of microtubules with subsequent mitotic arrest and apoptotic cell death.[6,7] Both epothilones and taxanes bind α,β-tubulin

Correspondence: *Dr N Rosen, Program in Cell Biology and Department of Medicine, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, Box 271, New York, NY 10021, USA.
Received 23 October 1998; revised and accepted 28 October 1998

heterodimers in a 1:1 stoichiometric ratio and epothilones are capable of displacing bound, labeled paclitaxel at concentrations, and with kinetics similar to those displayed by paclitaxel itself.[6–8] Despite these mechanistic similarities, the structures of the two drugs are quite different and no obvious and convincing homology modeling has yet established a common pharmacophore (Figure 1). Furthermore, whereas taxanes are substrates for P-glycoprotein, epothilones are not. Cells with the mdr phenotype that are resistant to taxanes remain sensitive to epothilones.[6–10] This ability of the epothilones to outperform paclitaxel in mdr contexts constitutes a potentially important advantage for this family of agents relative to the taxanes. Furthermore the epothilones may provide advantages relative to paclitaxel in terms of the ease of formulatability.[5] Moreover, paclitaxel is not considered to be an effective agent for prostate cancer, although it has recently been shown to have activity when administered with estramustine.[11–15] The reasons that prostate cancers are resistant to paclitaxel are unknown, although the high levels of P-glycoprotein and Bcl-2 expression observed in advanced tumors may play a role.[2,16] Recently, the total synthesis of epothilones A and B (EA, EB) and their desoxy counterparts (dEA, dEB) was accomplished in these laboratories[10,17] and others.[18–20] We now report potent *in vitro* cytotoxicity effects of these synthetically derived epothilones on prostate cancer cells. Each epothilone caused stabilization of microtubules that caused cells to become growth arrested in mitosis and undergo programmed cell death, EB was the most active agent. Epothilones had synergistic activity when given in combination with a farnesyl:protein transferase inhibitor, even in prostate cells that are relatively insensitive to both drugs. We conclude that epothilones, a class of compounds that stabilize microtubules and are P-glycoprotein independent, are active against prostate cancer cell lines and may comprise a new treatment modality for this disease.

Materials and methods

Materials

Epothilones A and B and their desoxy counterparts were synthesized according to References 10 and 17. Stock solutions were prepared in dimethylsulfoxide (DMSO), fractionated and stored at −80°C. One-thousand-fold concentrated working solutions were similarly prepared and stored. The farnesyl:protein transferase inhibitor (FTI) L-744,832 was from Merck & Co. (West Point, PA). FTI was dissolved in PBS and stored −80°C.

Cell lines

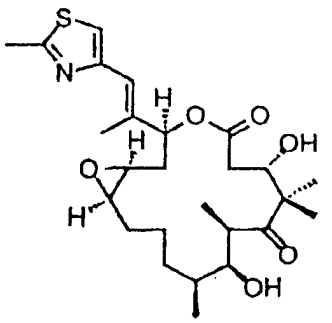
The human prostate carcinoma cell lines, DU145 and TSU-Pr1, were obtained from the American Type Culture Collection and from Dr D Nanus (Genitourinary Service, MSKCC), respectively. Both cell lines were grown in DME:F12 (1:1) medium supplemented with 10% FBS, 2 mM glutamine and 50 units/ml each of streptomycin and penicillin. Cultures were grown at 37°C in 10 cm dishes in a humidified incubator with 5% CO_2/air.

Immunofluorescence

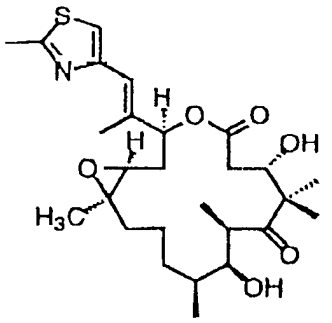
The effects of epothilones on the microtubule network were studied in cell cultures treated with dimethylsulfoxide (DMSO) or epothilones for 24 h. Cells floating in the media were collected by centrifugation and attached cells were trypsinized. These populations were combined and cell number was determined with a Coulter counter. Aliquots of 10^6 cells were washed with phosphate-buffered saline (PBS) and fixed with 100% methanol for 10 min at −20°C. Alternatively, cells were grown and treated on fibronectin-coated slides placed in multiwell plates. Fixed cells were rehydrated in PBS for 10 min at room temperature and exposed for 30 min at 37°C to a blocking solution consisting of 2% bovine serum albumin (BSA), 10% normal goat serum and 0.05% Tween-20 in PBS. Tubulin was detected by immunostaining with a monoclonal anti-tubulin-α antibody (clone 5B-152 from Sigma.) Cells and slides were incubated with a 1:1000 dilution of the primary antibody in blocking buffer for 1 h at room temperature, followed by an incubation with fluorescein isothiocyanate (FITC)-coupled anti-mouse IgG goat secondary antibodies (Molecular Probes) (1:100 dilution in blocking buffer). Cells were washed three times with 1 ml of 0.5% BSA in 0.05% Tween-20 in PBS between and after incubations with primary and secondary antibodies. DNA was stained with bisbenzimide, which was included in the secondary antibody solution (3 μg/ml final concentration). Cells grown on coverslips were mounted on glass slides with Vectashield (Vector), to prevent quenching of fluorescence. For cells stained in suspension, a 1:1 mixture of the cell suspension with Vectashield was prepared. After equilibrating the mixture at room temperature for 5 min, 7 μl were placed under a 22 × 22 mm coverslip. Immunofluorescence was detected with a Zeiss epifluorescence microscope, at 40× and 100×, using appropriate filters for detection of FITC and bisbenzimide. Each field was photographed using both filters, slides were also analyzed via confocal microscopy.

Cell cycle analysis and determination of apoptosis

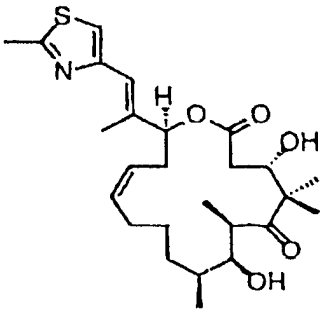
Nuclear and whole cell samples were prepared for cell cycle analysis according to References 21 and 22. Ethidium iodide-stained nuclei and propidium iodide-labeled cells were analyzed with a Becton Dickinson fluorescence-activated cell sorter (FACS). Ungated data was employed to calculate the percentage of cells containing less than 2n DNA content, which connotes the presence of apoptotic cells. Apoptosis was confirmed by morphological evaluation of the samples via quantitative fluorescence microscopy. For this purpose, 10^5 cells were fixed in 3% paraformaldehyde in PBS and stored at 4°C until further analysis. Cells were then washed with PBS and resuspended in 25 ml of PBS containing 3 μg/ml of bisbenzimide. Over 400 cells were counted on duplicate slides to



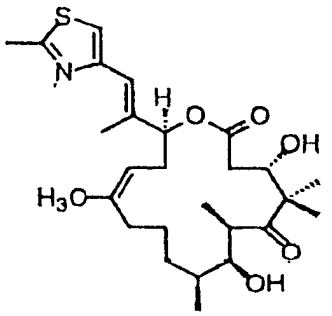
Epothilone A
$C_{26}H_{39}NO_6S$
Mol. Wt.: 493.66



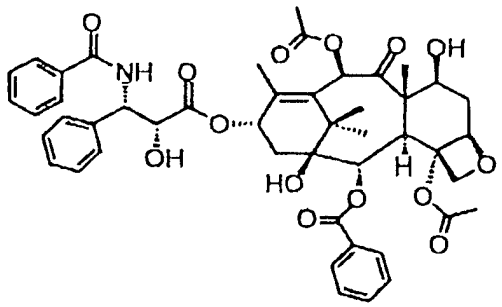
Epothilone B
$C_{27}H_{41}NO_6S$
Mol. Wt.: 507.68



Desoxyepothilone A
$C_{26}H_{39}NO_5S$
Mol. Wt.: 477.66



Desoxyepothilone B
$C_{27}H_{41}NO_5S$
Mol. Wt.: 491.68



Paclitaxel
$C_{47}H_{51}NO_{14}$
Mol. Wt.: 853.92

Figure 1 *Structure of epothilones A and B and desoxy derivatives.*

obtain a statistically relevant number, and the experiment was repeated at least twice.

Cell proliferation assay

To investigate the effect of epothilones on the proliferation of human tumor cell lines, 6-well plates were seeded at 10 000 cells per well and treated for 4 h with increasing epothilone concentrations. Control cultures were exposed to 0.1% DMSO. Cultures were then washed twice with PBS and grown for 5–7 d, depending on the density of vehicle-treated controls. In experiments in which epothilones were combined with the farnesylation inhibitor, cells were treated with the epothilone for 4 h then the FTI was added for the length of the experiment. Cell number was determined with a Coulter counter following trypsinization of the monolayers. Each condition was

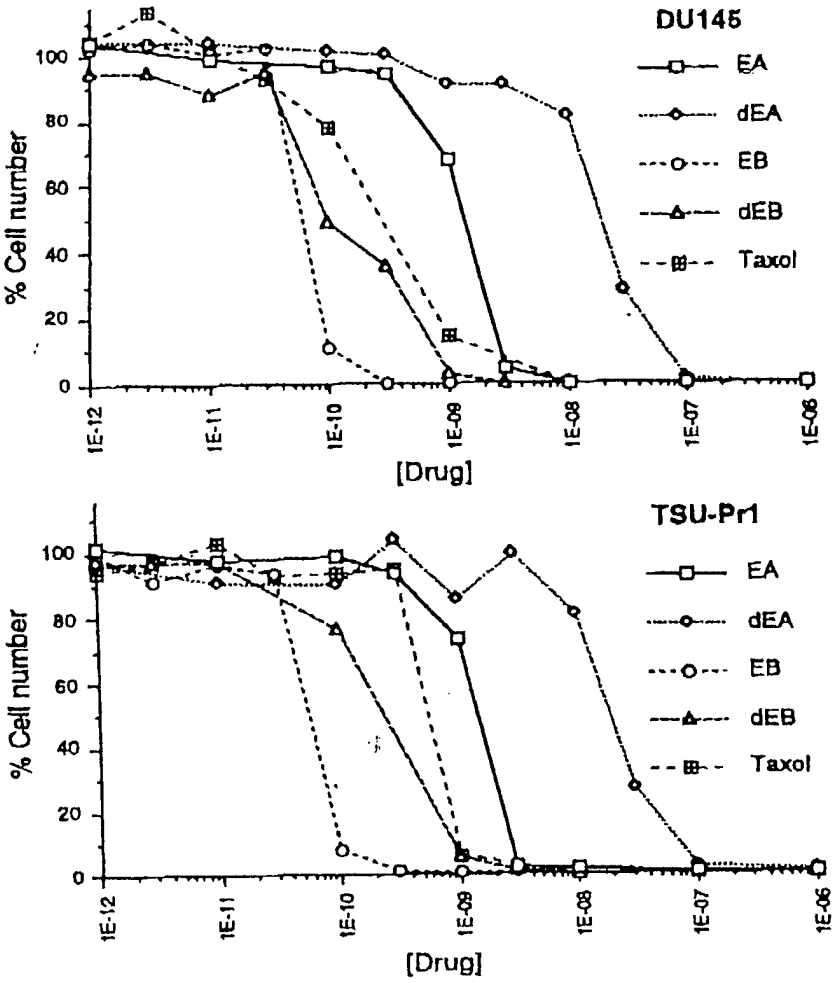


Figure 2 *Effect of epothilones and paclitaxel on human prostate cancer cell growth. TSU-Pr1 and DU145 cultures were treated continuously for 5–7 d in the presence of increasing drug concentrations. DMSO-treated control cultures remained in logarithmic growth throughout the experiment. EA = epothilone A, EB = epothilone B, dEA = desoxyepothilone A, dEB = desoxyepothilone B.*

Table 1 IC_{50}s for inhibition of anchorage-dependent growth of TSU-Pr1 and DU145 human prostate cancer cells

Drug	TSU-Pr1	DU145
Epothilone A	1.2 × 10−9M	1.3 × 10−9M
Desoxyepothilone A	2 × 10−8M	2 × 10−8M
Epothilone B	5 × 10−11M	6 × 10−11M
Desoxyepothilone B	2.5 × 10−10M	1.7 × 10−10M
Paclitaxel	6 × 10−10M	2.2 × 10−10M

done in duplicate and each type of experiment was repeated at least three times.

Results

Effect of epothilones on anchorage-dependent growth of human prostate cancer cell lines

We determined the effects of synthetic epothilones and desoxyepothilones (Figure 1) on the growth of TSU-Pr1 and DU145 human prostate cancer cells. Continuous treatment with these drugs had a profound antiproliferative effect (Figure 2 and Table 1). EB was the most active epothilone, approximately 7- to 10-fold more active than paclitaxel in inhibiting the anchorage-dependent growth of human prostate cancer cells (0.05–0.06 nM for EB *vs* 0.22–0.6 nM for Paclitaxel) (Figure 2 and Table 1). Among the epothilones, the order of potency was EB, followed by dEB, EA and dEA, which were 5-, 23- and 360-fold less potent, respectively, than the parent EB (Figure 2 and Table 1).

Microtubule stabilization and induction of apoptosis

Microscopic examination of the cultures treated with epothilones revealed an increase in the number of detached cells and an increase in cellular debris. The cells that remained attached exhibited clear cytoskeletal changes. As shown in the confocal micrographs of TSU-Pr1 cells stained for tubulin (green) and DNA (blue) (Figure 3), increasing concentrations of EB resulted in

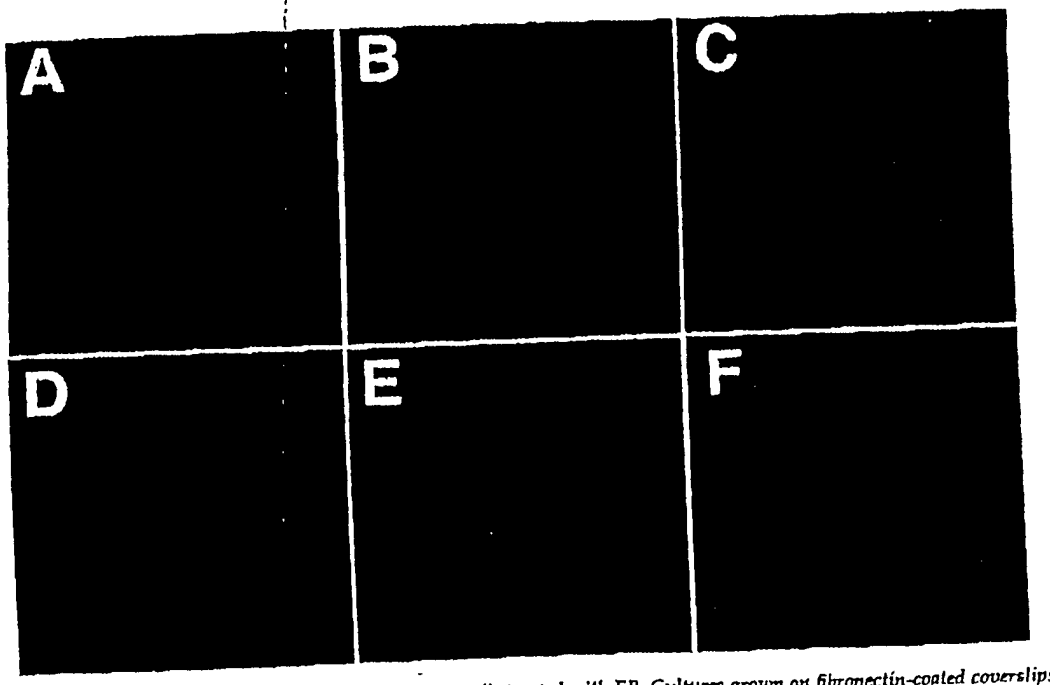


Figure 3 *Confocal micrographs of TSU-Pr1 human prostate cancer cells treated with EB. Cultures grown on fibronectin-coated coverslips were treated for 24 h with increasing concentrations of epothilone B (EB). Cells were immunostained for tubulin (green) and for DNA (blue). (a) Untreated, (b) 0.1 nM EB, (c) 1 nM EB, (d) 10 nM EB, (e) 100 nM EB, (f) 1 μM EB.*

the formation of microtubule aggregates in the interphase cells. An extensive microtubule network was observed in cultures treated with 1 nM EB for 24 h. At 0.1 and 1 μM EB, thick fibers of microtubules were clearly seen in the cytoplasm of TSU-Pr1 (Figure 3e and f). However, a large proportion of cells were not attached to the substratum. Analysis of these cells indicated that they were either in mitosis (open arrows) or undergoing apoptosis (closed arrows) (Figure 4). Mitotic cells presented mitotic spindles and condensed chromosomes aligned in the metaphase plate, on the other hand, apoptotic cells showed heavily stained nuclear fragments (Figure 4).

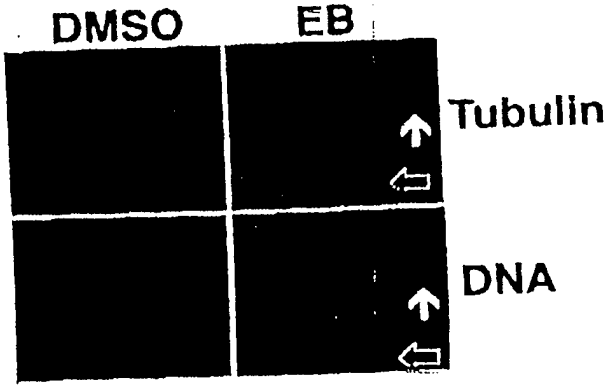


Figure 4 *Mitotic arrest and apoptosis induced by epothilones in TSU-Pr1 cells. TSU-Pr1 cells were treated or not (DMSO) with 1 μM EA or dEA for 24 h. Floating and attached cells were combined and immunostained for tubulin and DNA. Open arrows indicate cells undergoing mitosis and closed arrows cells undergoing apoptosis.*

Effect of epothilones on cell cycle dynamics of human prostate cancer cell lines

We conducted flow cytometric analysis to investigate the effects of these drugs on cell cycle progression and cell death. A 24 h treatment with epothilones induced a dose-dependent increase in the proportion of cells with less than a diploid DNA content. A representative ungated FACS analysis profile for TSU-Pr1 cells treated with or without 10 nM EB for 24 h is shown in Figure 5. The appearance of a 'sub-G1' population correlates with the DNA fragmentation that occurs during cell death by apoptosis. As described below, the epothilones induced programmed cell death of human prostate cancer cells. Figure 5 shows a graphic representation of the changes in the sub-G1 population induced by epothilones and paclitaxel. The relative potencies for DNA cleavage correlate with the IC_{50}s obtained for inhibition of cell growth.

FACS analysis was also employed to investigate changes in cell cycle distribution. As shown for Figure 6, epothilones induced a dose-dependent cell cycle arrest of cells at the G2/M stage (4n DNA content), at the expense of cells in G1. Changes in the percentages of cells in each cell cycle phase are presented in graphic form in Figure 6. Paclitaxel and EB were the most potent in affecting the cell cycle, whereas dEB, EA and dEA were 3- to 10-fold less effective. We analyzed the distribution of cells in interphase, mitosis and apoptosis by using a combination of FACS analysis and immunostaining. As

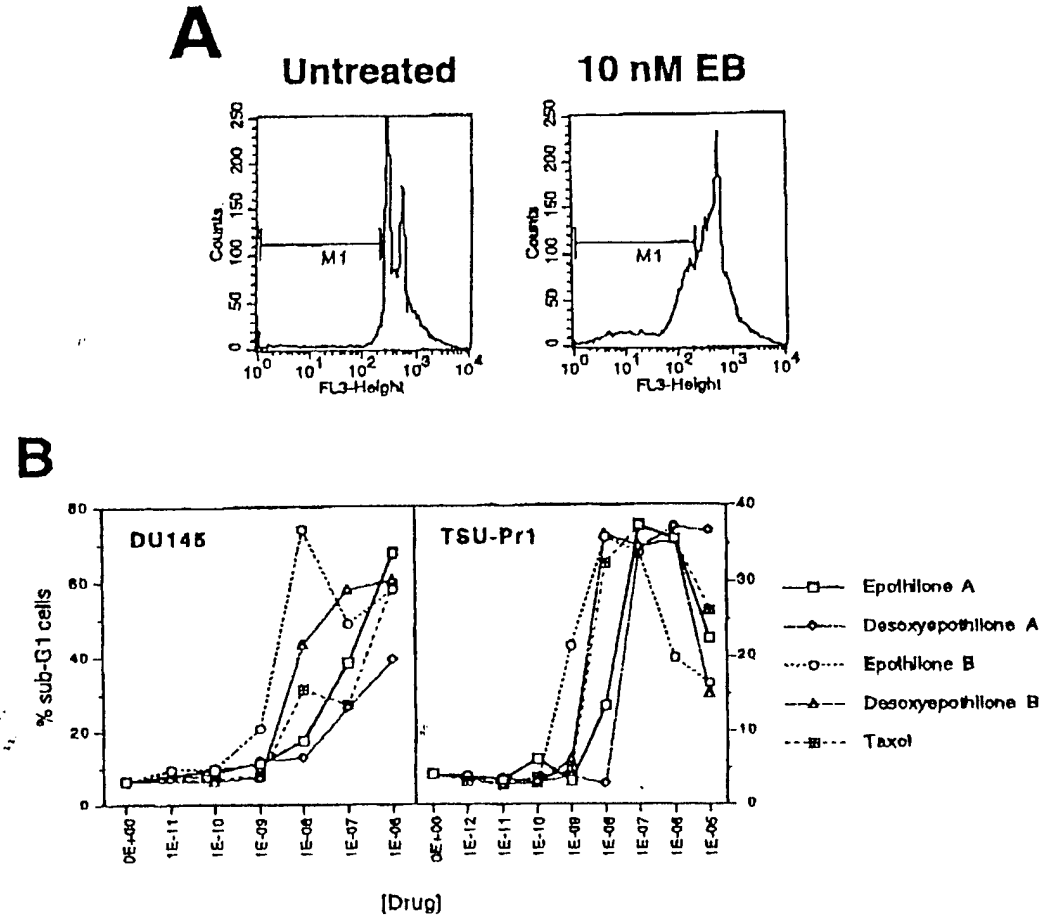


Figure 5 *DNA fragmentation induced by epothilones. Ungated FACS analysis of propidium iodide-stained whole cells demonstrate the appearance of cells with less than 2n DNA content (sub-G1) in epothilone-treated cultures in a dose-dependent manner. (a) A representative plot is known for TSU-Pr1 cells treated or not for 24 h with 10 nM EB. The sub-G1 population is shown in the M1 gate. (b) Quantification of the proportion of TSU-Pr1 and DU145 cells having fragmented DNA as a function of drug concentration.*

shown in Figure 7, treatment with epothilones induced mitotic arrest and death by apoptosis.

Synergistic effect of epothilones and farnesyl transferase inhibitors for growth inhibition

These data demonstrate that prostate carcinoma cells are sensitive to epothilones and that they undergo mitotic arrest and apoptosis in response to the drugs. We have recently discovered that paclitaxel synergizes with a protein farnesylation inhibitor (FTI) to inhibit the growth of human breast and prostate cancer cells. We explored the possibility that epothilones would also enhance the antiproliferative effect of the FTI. As shown in Figure 8, pretreatment of DU145 human prostate cancer cells with epothilones sensitized the cells for a subsequent treatment with FTI. DU145 cells are particularly resistant to the FTI as a single agent ($IC_{50} > 20\,\mu M$), however, pretreatment with epothilones increased its effectiveness by over two orders of magnitude. An algorithm was used to calculate whether these drugs were synergistic.[23] Two drugs are considered synergistic if the surviving fraction of the combination is lower than the product of the surviving fractions for each agent alone. Based on this criterium, FTI and microtubule-stabilizing agents were synergistic in inhibiting the growth of DU145 human prostate cancer cells.

Discussion

Taxanes bind to microtubules and prevent their depolymerization.[24] This causes growth arrest of tumor cells in the mitotic and G1 phases of the cell cycle and leads to subsequent apoptosis.[25–27] These compounds have potent antitumor activity and already have great utility in the treatment of several human cancers, including breast, ovarian, esophageal, lung and other tumors.[28–36] However, even in cases of metastatic disease that are very sensitive to taxanes, drug resistance eventually supervenes, and many human tumors are resistant to the drug *de novo*.[2,34,37]

These findings have stimulated a search for taxane derivatives or analogs with different spectra of antitumor activities. Remarkably, recent work has shown that

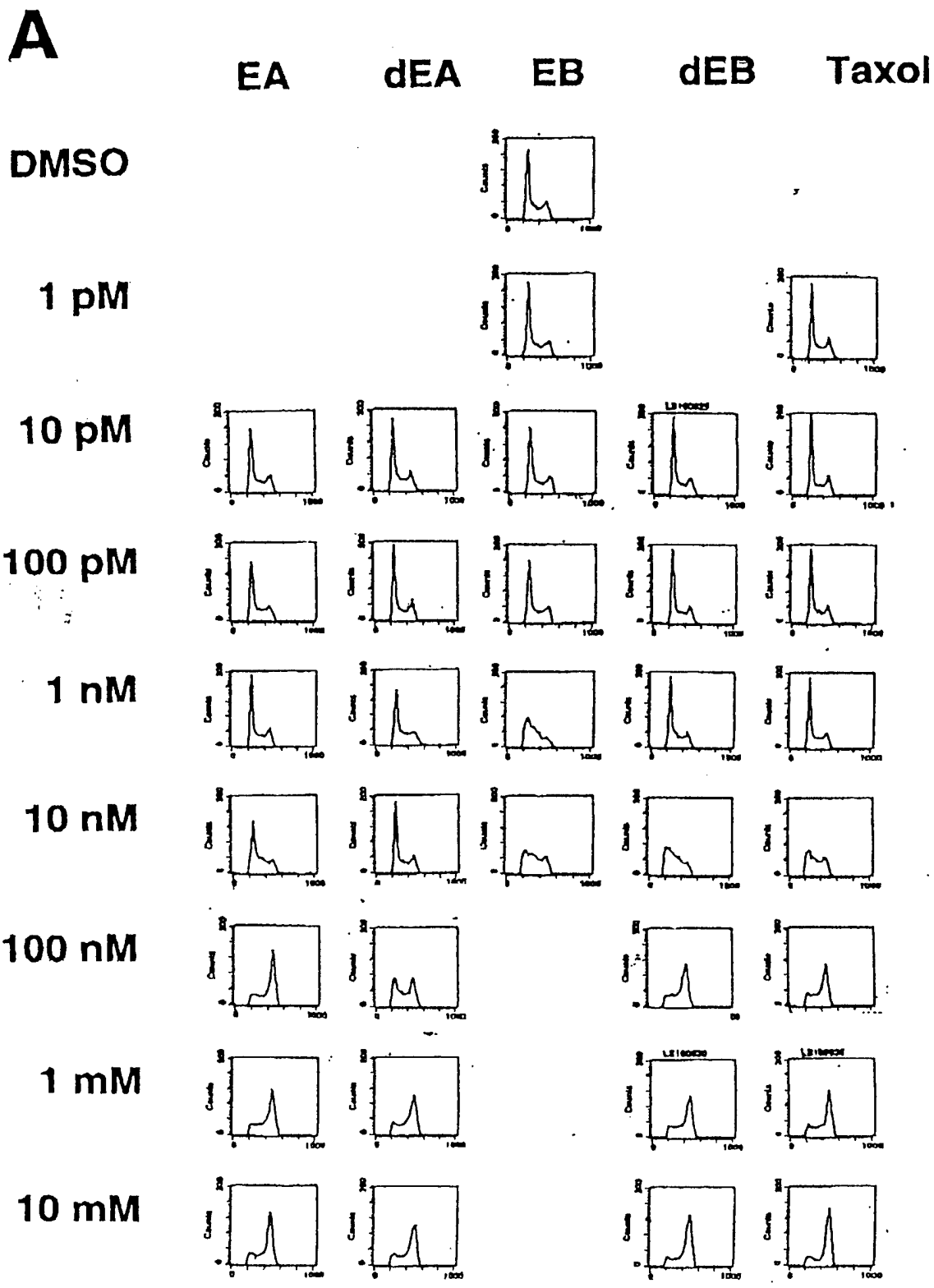


Figure 6 *Changes in cell cycle distribution induced by epothilones. TSU-Pr1 cells were grown in the presence or absence of epothilones or paclitaxel for 24 h. Cells were fixed, stained with propidium iodide and analyzed by FACS. Histograms were obtained and the percentages of cells in each cell cycle stage were calculated with Becton Dickinson FACS software. (a) Linear FACS plots of number of cells vs DNA content. (b) Graphic representation of dose-dependent changes in cell cycle distribution.*

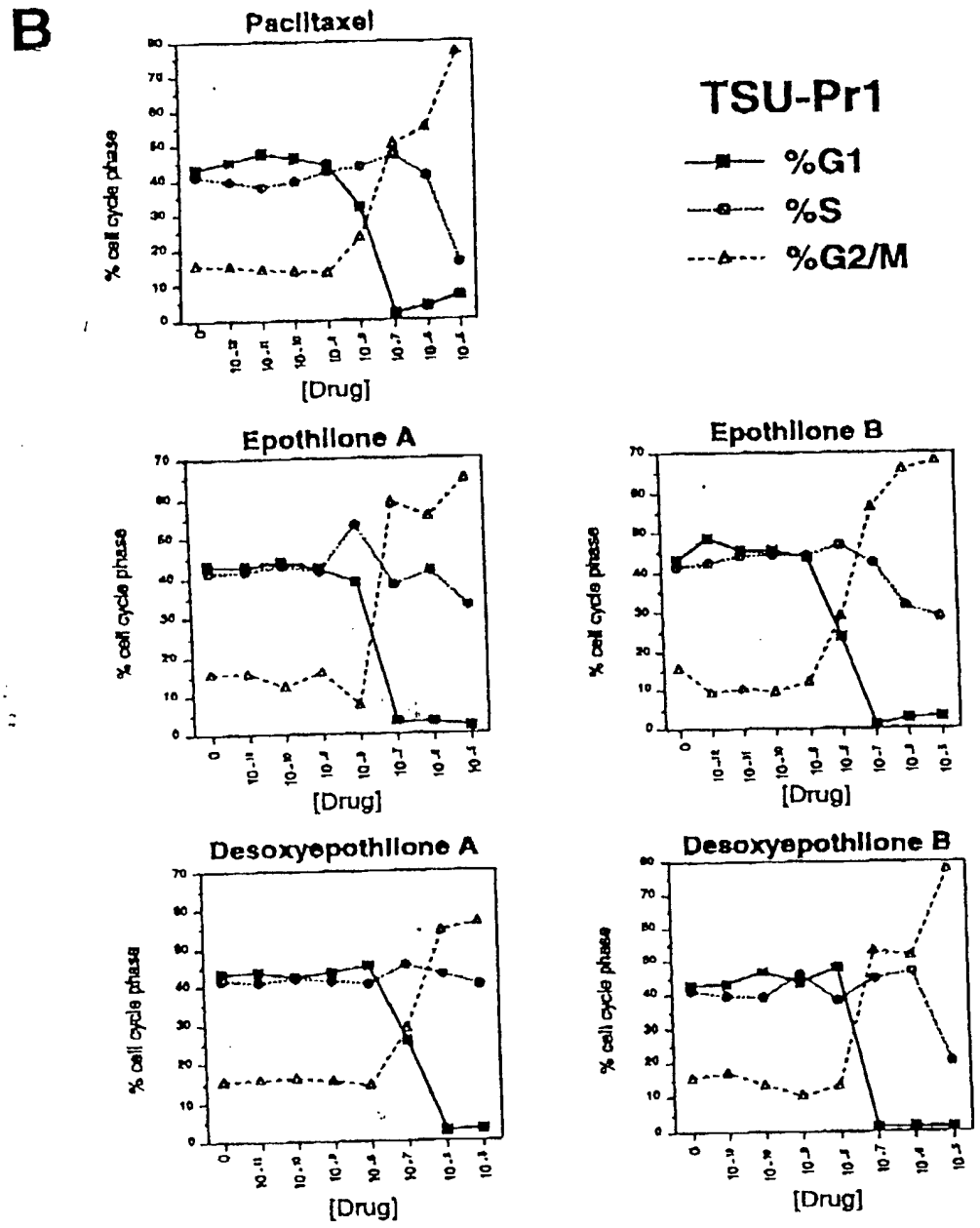


Figure 6 *(Continued)*

another class of natural products, the epothilones, bind to microtubules and exert effects on cells almost identical to those of paclitaxel. In addition, epothilones are not substrates for P-glycoprotein and are more water-soluble than taxanes.[5,7–10] These properties suggest that epothilones may have different clinical uses as well. Recently, the total synthesis of epothilones A and B was accomplished in these laboratories.[10,17] These successes allowed for the synthesis of a variety of epothilone derivatives as well. We have studied the biologic effects of BA, EB, dEA and dEB on prostate cancer cell lines and found that they all inhibit growth potently (Figure 2 and Table 1). There is no indication in this system that the effects of epothilones on cells are qualitatively different from those of paclitaxel. Drug administration is associated with hyperpolymerization of microtubules, the formation of microtubular fibers in G1, and mitotic arrest (Figures 3, 4, 6, and 7). Cells then undergo an apoptotic cell death (Figures 5 and 7). The drugs do vary in potency, EB is by far the most active compound with an IC_{50} of 50–75 pM compared to IC_{50} of 200–700 pM for paclitaxel. The other compounds are all active, but somewhat less potent than the parent EB (dEB, IC_{50} 250 pM; EA IC_{50} 2 nM, dEA IC_{50} 20 nM.) The relative potencies for mitotic arrest and cytotoxicity were

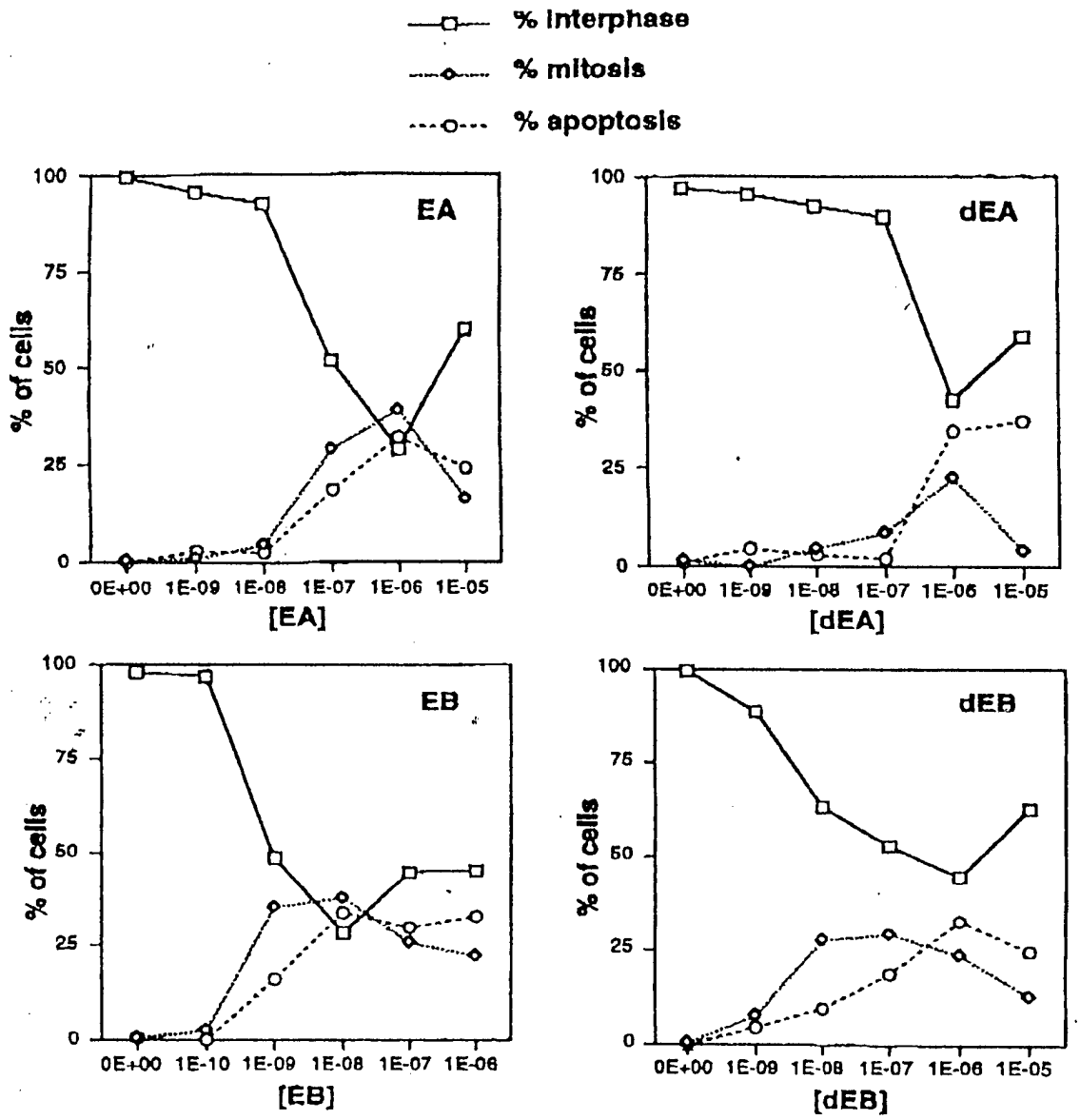


Figure 7 *Mitotic arrest and apoptosis in response to epothilones. Graphical representation of quantitative microscopy data indicating percentages of cells in interphase, mitosis and apoptosis and cells exhibiting alterations in microtubules.*

directly related to the ability of the drugs to cause stabilization of the mitotic spindle and to form microtubule bundles in interphase. Therefore, epothilones and paclitaxel probably cause their cellular effects by very similar mechanisms.

What then are the potential advantages of epothilones as anticancer drugs? Firstly, they are more soluble in water than paclitaxel, perhaps leading to a much greater ease of administration.[5] Secondly, mdr cells are sensitive to epothilones.[7–10] Although it is unlikely that acquired resistance to paclitaxel is mediated often by increases in P-glycoprotein expression, the mdr phenotype could play an important role in the endogenous resistance of many types of carcinoma. Thirdly, synthesis of epothilones is now possible.[10,17,18] This will allow the generation of multiple derivatives, some of which may have increased potency, bypass resistance mechanisms or have decreased toxicity. The similar binding and biologic effects of the very dissimilar taxane and epothilone structures suggests that synthesis of derivative drugs with altered properties will be possible.

The inadequacy of current treatments for hormone-independent prostatic cancer is characteristic of the situation for most advanced adenocarcinomas.[1–3] Paclitaxel is essentially inactive as a single agent in this disease, although it has been recently shown that the combination of paclitaxel and estramustine is effective.[11–15] Both drugs bind to microtubules and cause kinetic stabilization of microtubules[12,38,39] the molecular basis for the synergy is still unknown. It has been proposed that the two drugs use complementary mechanisms for microtubule stabilization, however, estramustine might also sensitize cells to

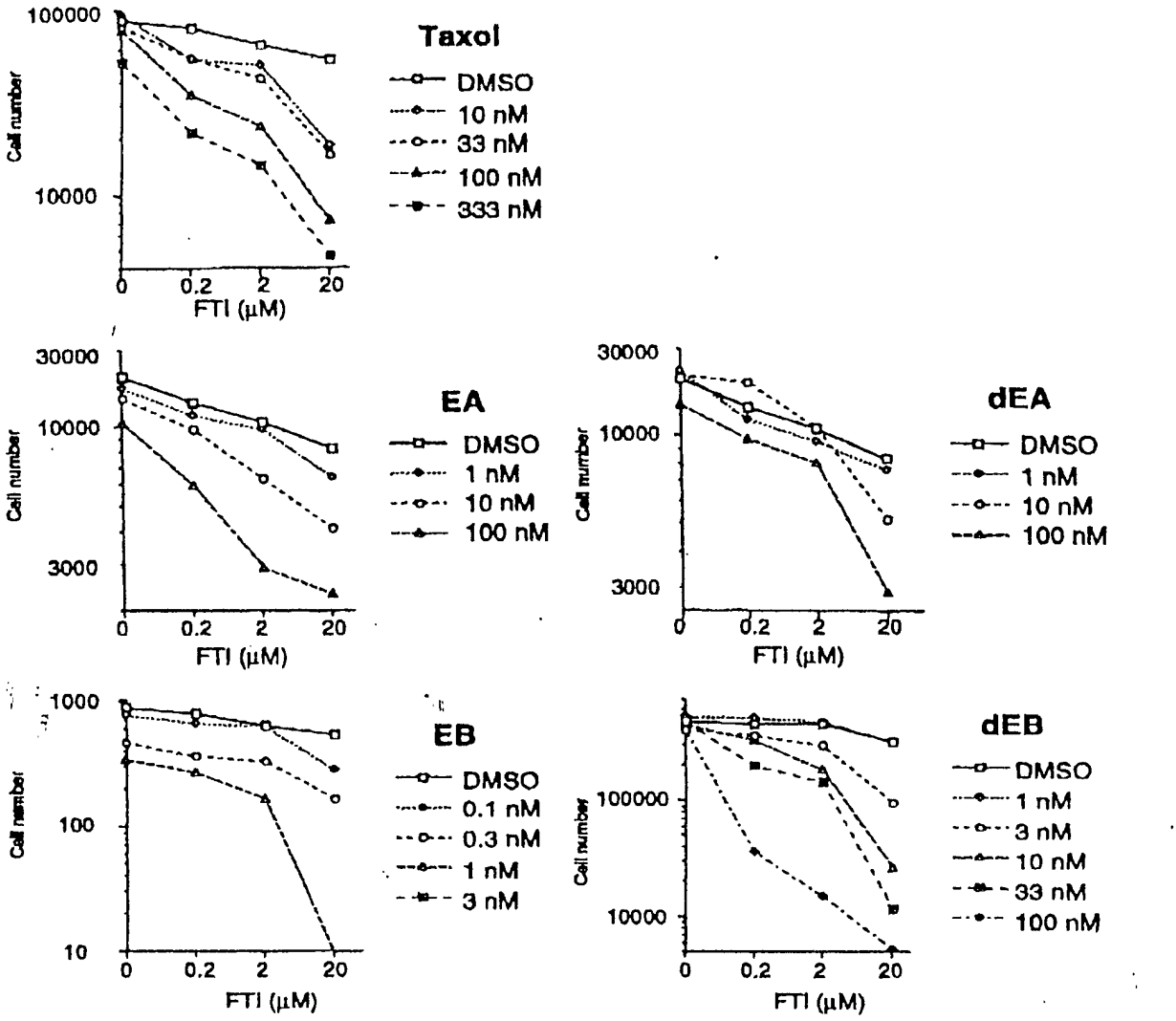


Figure 8 *Pretreatment with epothilones enhances the antiproliferative response of human prostate cancer cell lines to a farnesylation inhibitor (FTI). DU145 cell cultures were pretreated with drug or vehicle for 4 h prior to incubation for 5–7 d in the presence or absence of increasing FTI concentrations.*

paclitaxel by antagonizing the function of P-glycoprotein. We show in this report that prostate carcinoma cells can be quite sensitive to epothilones. The expression of P-glycoprotein has been reported to be elevated in some prostate carcinoma cell lines and in tumor tissue. If these data are confirmed, our data suggest that epothilone may be more effective in this disease than paclitaxel. Experiments in animal models of prostate cancer are now underway to test this assertion.

We have recently shown that farnesyl transferase inhibitors inhibit the growth of tumor with wild type Ras, including prostate, breast and small cell lung carcinomas.[40] As these drugs are generally non-toxic, we went on to test whether they would be effective when given in combination with traditional cytotoxic drugs. We found that farnesyl transferase inhibitors sensitized cells to the mitotic block induced by paclitaxel and, in combination, the two drugs cause synergistic arrest of cell growth and apoptosis[41,42] in this report we extend these observations. Epothilones or paclitaxel in combination with farnesyl transferase inhibitors display marked synergy in inhibiting the growth of the prostate cell line DU145 (Figure 8). Furthermore, this cell line is not particularly sensitive to either drug alone, suggesting that this combination may sensitize moderately resistant tumors. Animal experiments with these combinations are now in progress, preparatory to planning clinical trials in patients with advance prostate cancer.

Conclusions

Epothilones are natural products that bind to tubulin and prevent the depolymerization of microtubules. They have no structural similarities to taxol and they are active in

multidrug resistant cells. Epothilones and various derivatives thereof have now been synthesized. In this report we compared the antitumor cell activity of these synthetic epothilones and of taxol in prostate cancer cell lines. We showed that epothilones potently inhibit the growth of metastatic human prostate carcinoma cells, several of the epothilones are more active than taxol, they cause mitotic arrest and apoptosis. Furthermore, epothilones and farnesyl transferase inhibitors have synergistic antiproliferative effects, including a cell line that is particularly refractory to the farnesylation inhibitor when given singly. Epothilones may constitute a class of drugs with taxol-like action but with a different spectrum of antitumor activity. Therefore, they may be useful in certain adenocarcinomas that are relatively resistant to taxol, such as prostate cancer.

Acknowledgements

The authors are grateful to Ms D Domingo (SKI) for FACS analysis, to Dr K Manova from the Molecular Cytology Core Facility (SKI) for expert advice on fluorescence microscopy, and to Ms S Krueger from the Center for Biomedical Imaging Technology, University of Connecticut Health Center, for confocal microscopy. This work was supported by CaPCure, The PepsiCo Foundation, Merck & Co, the National Institutes of Health (CA-28824 to SJD, CA-GM-72231 to A.B.). N.R. and L.S.-L. received a NCI Breast SPORE program P50CA68425-02 grant and career development award, respectively. D.M. was supported by an Army Breast Cancer Predoctoral Fellowship (P33 US ARMY DAMD 17-97-1-7146).

References

1 Scher HI, Mazumdar M, Kelly WK. Clinical trials in relapsed prostate cancer. *J Natl Cancer Inst* 1996; 88: 1623–1634.
2 van Brussel JP, Mickisch GH. Circumvention of multidrug resistance in genitourinary tumors. *Int J Urol* 1998; 5: 1–15.
3 Slovin SF *et al*. Targeted therapy for prostate cancer: the Memorial Sloan-Kettering Cancer Center approach. *Semin Oncol* 1996; 23: 41–48.
4 Gerth K *et al*. Epothilones A and B: antifungal cytotoxic compounds from *S. cellulosum* (myxobacteria). *J Antibiot* 1996; 49: 560–563.
5 Höfle G *et al*. Epothilones A and B- novel 16-membered macrolides with cytotoxic activity: isolation, crystal structure, and conformation in solution. *Angew Chem Int Ed Engl* 1996; 35: 1567–1569.
6 Bollag DM *et al*. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res* 1995; 55: 2325–2333.
7 Kowalski RJ, Giannakakou P, Hamel E. Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel (Taxol(R)). *J Biol Chem* 1997; 272: 2534–2541.
8 Giannakakou P *et al*. Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization. *J Biol Chem* 1997; 272: 17118–17125.
9 Balog A *et al*. Stereoselective syntheses and evaluation of compounds in the 8-desmethylepothilone A series: some surprising observations regarding their chemical and biological properties. *Tetrahedron Lett* 1997; 38: 4529–4532.
10 Su D-S *et al*. Total synthesis of (—)-epothilone B: an extension of the Suzuki coupling method and insights into structure-activity relationships of the epothilones. *Angew Chem Int Ed Engl* 1997; 36: 757–759.
11 Hudes GR *et al*. Paclitaxel plus estramustine in metastatic hormone-refractory prostate cancer. *Semin Oncol* 1995; 41–45.
12 Hudes GR *et al*. Phase II trial of 96-hour paclitaxel plus oral estramustine phosphate in metastatic hormone-refractory prostate cancer. *J Clin Oncol* 1997; 15: 3156–3163.
13 Keren RS, Muggia FM. Response to estramustine phosphate and paclitaxel in patients with advanced breast cancer: a phase I study. *Semin Oncol* 1997; S26–S29.
14 Pienta KJ, Naik H, Lehr JE. Effect of estramustine, etoposide, and taxol on prostate cancer cell growth *in vitro* and *in vivo*. *Urology* 1996; 48: 164–170.
15 Pienta KJ, Smith DC. Paclitaxel, estramustine, and etoposide in the treatment of hormone-refractory prostate cancer. *Semin Oncol* 1997; S1572–S1577.
16 Haldar S, Basu A, Croce CM. Bcl2 is the guardian of microtubule integrity. *Cancer Res* 1997; 57: 229–233.
17 Balog A *et al*. Total synthesis of (—)-epothilone A. *Angew Chem Int Ed Engl* 1996; 35: 2801–2803.
18 Nicolaou KC *et al*. Synthesis of epothilones A and B in solid and solution phase. *Nature* 1997; 387: 268–272.
19 Schinzer D *et al*. Total synthesis of (—)-epothilone A. *Angew Chem Int Ed Engl* 1997; 36: 523–524.
20 Yang Z *et al*. Total synthesis of epothilone A: the olefin metathesis approach. *Angew Chem Int Ed Engl* 1997; 36: 166–168.
21 Giaretti W, Nusse M. Light scatter of isolated cell nuclei as a parameter discriminating the cell-cycle subcompartments. *Methods Cell Biol* 1994; 41: 389–400.
22 Nusse M *et al*. Flow cytometric detection of mitotic cells using bromodeoxyuridine/DNA technique in combination with 90 degrees and forward scatter measurements. *Cytometry* 1990; 11: 813–821.
23 Chou TC, Motzer RJ, Tong Y, Bosl GJ. Computerized quantitation of synergism and antagonism of taxol, topotecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design. *J Natl Cancer Inst* 1994; 86: 1517–1524.
24 Schiff BP, Horwitz SB. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc Natl Acad Sci USA* 1980; 77: 1561–1565.
25 Dorr RT. Pharmacology of the taxanes. *Pharmacotherapy* 1997; 96S–104S.
26 Horwitz SB. Taxol (paclitaxel): mechanisms of action. *Ann Oncol* 1994; S3–S6.
27 Sorger PK, Dobles M, Tournebize R, Hyman AA. Coupling cell division and cell death to microtubule dynamics. *Curr Opin Cell Biol* 1997; 9: 807–814.
28 Belani CP. Paclitaxel/carboplatin in the treatment of non-small-cell lung cancer. *Oncology (Huntingt)* 1998; 74–79.
29 Bunn PJ. Defining the role of paclitaxel in lung cancer: summary of recent studies and implications for future directions. *Semin Oncol* 1997; S12153–S12162.
30 Valero V. Docetaxel as single-agent therapy in metastatic breast cancer: clinical efficacy. *Semin Oncol* 1997; S1311–S1318.
31 Sweeney CJ, Sandler AB. Treatment of advanced (stages III and IV) non-small-cell lung cancer. *Curr Probl Cancer* 1998; 22: 83–132.
32 Rowinsky EK. The development clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu Rev Med* 1997; 48: 353–374.
33 Khayat D, Antoine E. Docetaxel in combination chemotherapy for metastatic breast cancer. *Semin Oncol* 1997; S1319–S1326.
34 Goldspiel BR. Clinical overview of the taxanes. *Pharmacotherapy* 1997; 110S–125S.
35 D'Andrea GM, Seidman AD. Docetaxel and paclitaxel in breast cancer therapy: present status and future prospects. *Semin Oncol* 1997; S1327–S1344.
36 Coukos G, Rubin SC. Chemotherapy resistance in ovarian cancer: new molecular perspectives. *Obstet Gynecol* 1998; 783–972.
37 Kearns CM. Pharmacokinetics of the taxanes. *Pharmacotherapy* 1997; 105S–109S.

38 Panda D, Miller HP, Islam K, Wilson L. Stabilization of microtubule dynamics by estramustine by binding to a novel site in tubulin: a possible mechanistic basis for its antitumor action. *Proc Natl Acad Sci USA* 1997; 94: 10560–10564.

39 Laing N *et al*. Interaction of estramustine with tubulin isotypes. *Biochemistry* 1997; 36: 871–878.

40 Sepp-Lorenzino L *et al*. A peptidomimetic inhibitor of farnesyl:-protein transferase blocks the anchorage-dependent and -independent growth of human tumor cell lines. *Cancer Res* 1995; 55: 5302–5309.

41 Moasser MM *et al*. Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones. *Proc Natl Acad Sci USA* 1998; 95: 1369–1374.

42 Sepp-Lorenzino L *et al*. Farnesyl:protein transferase inhibitors (FTIs) as potential agents for the management of prostate cancer. 1998; (submitted).

Pergamon

Tetrahedron 54 (1998) 12721–12736

TETRAHEDRON

A useful α, α' - annulation reaction of enamines

Alison J. Frontier,[a] Samuel J. Danishefsky,[a,b*] Gary A. Koppel,[c] Dongfang Meng[a,b]

Received 2 June 1998; accepted 11 August 1998

Abstract: The reactions of a series of enamines generated from a range of cyclic ketones with chloromethyl and iodomethyl vinyl ketone have been studied. The principal products are bridged ring diketones. The four carbon bridge, bearing a 2-oxobutyl function, spans the α and α' carbons of the original cyclanone. Presumptive evidence as to the pathway of this novel one step bridging annulation is provided.

Keywords: Addition Reactions: Annulation; Cyclisation

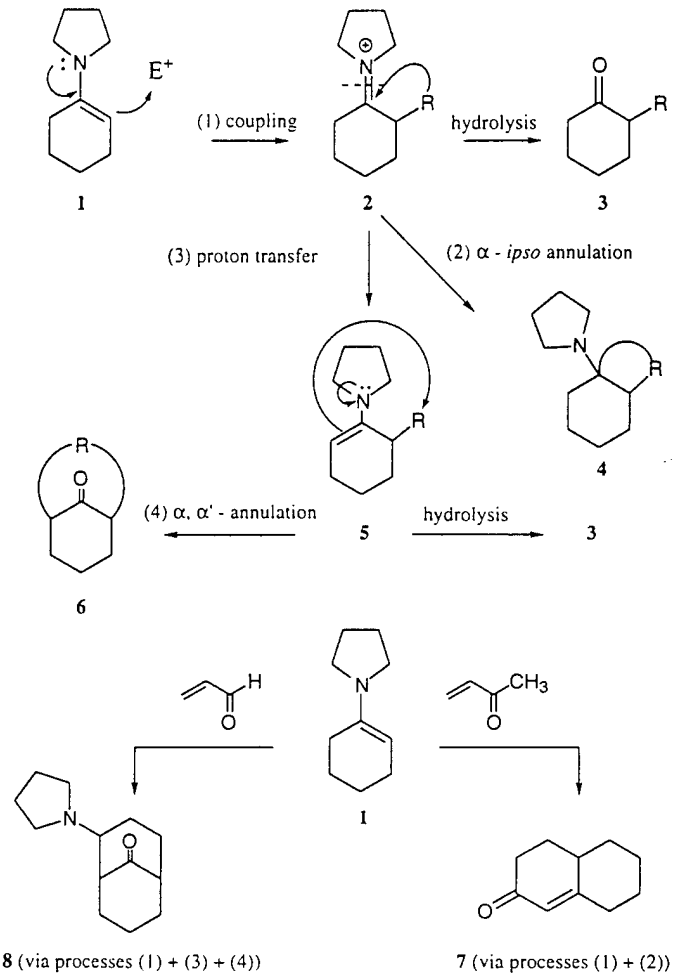
Enamines were introduced by Gilbert Stork and associates[1,2] into organic synthesis owing to their capacity to function as virtual enolates. Through recourse to enamines, many of the complications associated with the generation and use of actual carbanionic agents can be obviated. Scheme I summarizes the logic of the method. The events subsequent to attack of the general electrophile (see E^+) upon **1**, shown here for convenience as the pyrrolidene enamine derived from cyclohexanone, can take several forms. In the simplest situation, the product iminium species **2** survives until hydrolytic workup, whereupon **3** (R is derived from E^+) is obtained. In another variation, a new nucleophilic center is generated *within R* during the alkylation. The nucleophile may then react with the iminium moiety (see formation of **4**, Scheme I). We refer to this process as an *α-ipso annulation*. The adaptability of enamines to the Robinson annulation reaction[2] (see product **7**) is an instance of an *α-ipso annulation*.

Alternatively, **2** may undergo proton transfer to produce a new enamine such as trisubstituted **5**.[2,3] The ensuing sequence of events depends on the character of **5**. It could suffer hydrolysis upon workup to give the previously mentioned **3**, or, it could participate in another enamine-electrophile reaction. Were this second coupling to occur in an intramolecular fashion, the product, following hydrolysis, would be of the type **6** (Scheme I). We categorize the transformation of **1** into **6** *via* **5** as an *α, α'-annulation*. The acrolein bridging annulation reaction of enamines, though mechanistically complicated (see product **8**),[4] is an example of such an annulation.

E-mail: S-Danishefsky@ski.mskcc.org

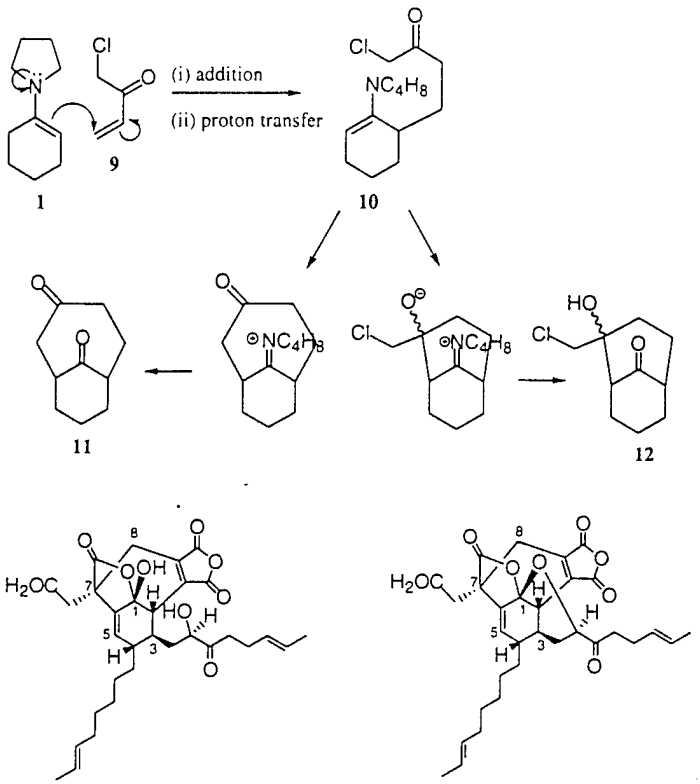
0040-4020/98/$ - see front matter
PII: S0040-4020(98)00788-1

Scheme I



Some years ago,[5] we wondered about the likely course of reaction of simple enamines (cf. **1**) with chloromethyl vinyl ketone **9** (see Scheme II).[6] In practice, the experiment produced virtually a 1:1 mixture of **11** and **12** in a combined yield of 40 - 50%. In terms of transformation type, we classified both of these products as having arisen from an α, α'-annulation sequence (*i. e.* steps 1 + 3 + 4, Scheme I). It seemed that the final outcome was a result of events following the formation of intermediate **10** *via* proton transfer. Nucleophilic attack at the sp^3 carbon, with expulsion of chloride, would ultimately lead to **11**. Alternatively, cyclization in an aldol sense would eventually provide **12**.

Scheme II

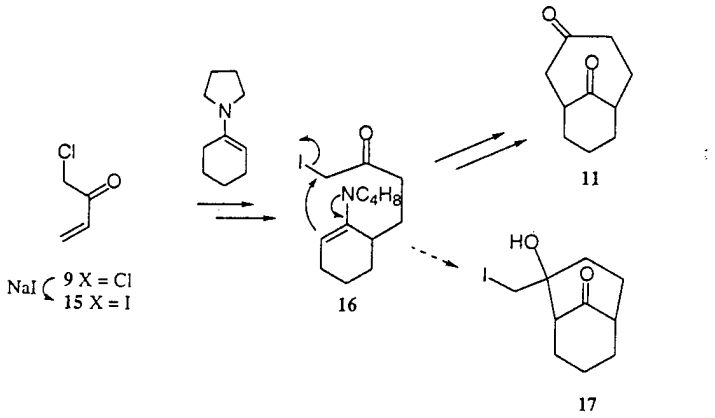


Following the recent publications of Kaneko and co-workers,[7,8] the issues associated with this α, α' annulation process leading to **11** assumed added interest. Thus, the Pfizer workers had isolated two metabolites and demonstrated their structures to be **13** and **14**. Aside from the intrinsic challenges posed to the field of synthesis by these fascinating displays of the inventiveness of biosynthesis, the demonstrated biological activities of the CP compounds as farnesyl transferase and squalene cyclase inhibitors have, not surprisingly, attracted additional notice.[9,10,11] The possibility of gaining viable access to bridged bicyclo [4.3.1]-octane substructures (cf. **11**), prompted a further study of the α, α'-annulation reaction, first reported from our laboratory 30 years ago.[5]

While we had certainly not proven that the divergence point of the reaction pathways leading to **11** and **12** follows formation of **10**, the proposal did not seem unreasonable. Since we were most interested in obtaining the [4.3.1]-bridged ring system (*cf.* **11**, **13** and **14**), the possibility of optimizing its formation relative to the aldol derived product (*cf.* **12**) was investigated. With this in mind, we considered the use of iodomethyl vinyl ketone **15** (see Scheme III) as an alternative. It was hoped that in the terminal phase of the annulation sequence, displacement of iodine would be more competitive with the aldol reaction than the

corresponding replacement of chlorine in **9**. The somewhat unstable iodomethyl compound was readily synthesized from **9** by reaction with sodium iodide in acetone. Treatment of **15** with enamine **1** in benzene at room temperature, followed by a brief period of reflux gave, upon hydrolysis, the bridged ring product **11** in 49% yield. We presume that annulation occurred via intermediate **16**. The hypothetical aldol product **17** was not detected in the crude reaction mixture. We screened a variety of solvents and discovered that when the reaction is conducted in THF with iodomethyl enone **15** as the annulating agent, the yield of **11** rises to 59-63%.

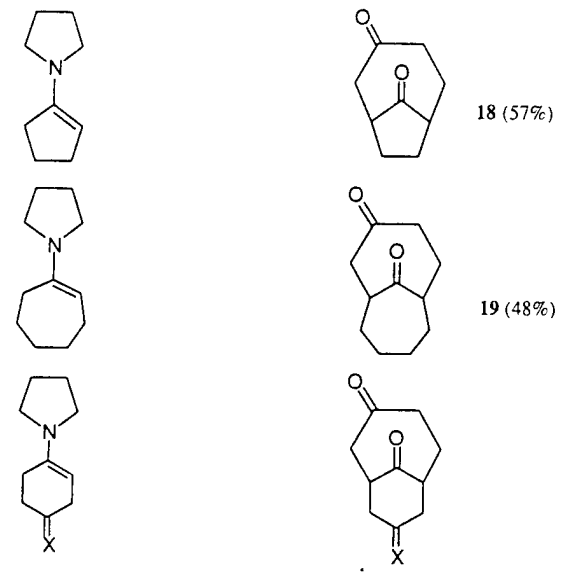
Scheme III



We investigated the generality of the process by surveying its applicability to other cyclic enamines. The data appear in Table I. Inspection of the results reveals that the desired α, α'-annulation can be achieved in workable yields with unsubstituted enamines. The procedure can be conducted on large scale and purification, in most cases, is greatly simplified by filtration of the insoluble iminium salt. Thus, even when the yields are disappointing, the product is practically devoid of side products after hydrolysis and is thus readily purified.

Incorporation of alkyl groups at C_4 is tolerated (see **20** and **21**), though with some loss in yield. However, substitution of C_4 with electron withdrawing groups (see **23** and **24**) results in serious attrition of yields. This diminution may reflect the higher energy associated with the conjugate addition step as a consequence of electron withdrawal in an inductive sense. In contrast, the process does tolerate a 4-methylene group (see **22**). Presumably this *exo* unsaturation can be exploited as an implementation site for further functionalizations.

Table I

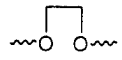


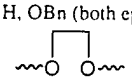
18 (57%)

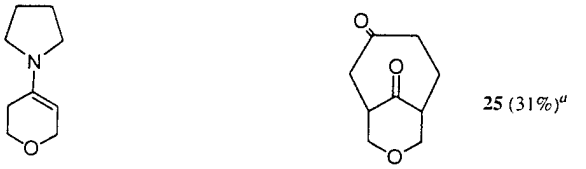
19 (48%)

X = H; Et
X = Me; Me
X = CH_2
X = H, OBn

20 X = H; Et (both epimers) 46%
21 X = Me; Me 31%[b]
22 X = CH_2 55%
23 X = H, OBn (both epimers) 21%[b]

X = 

24 X =  < 5%

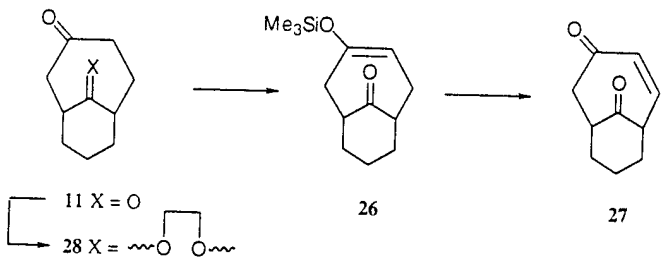


25 (31%)[a]

a) Yield was calculated from tetrahydropyran-4-one; the corresponding enamine was used directly in the annulation
b) Crude enamine was used in the annulation

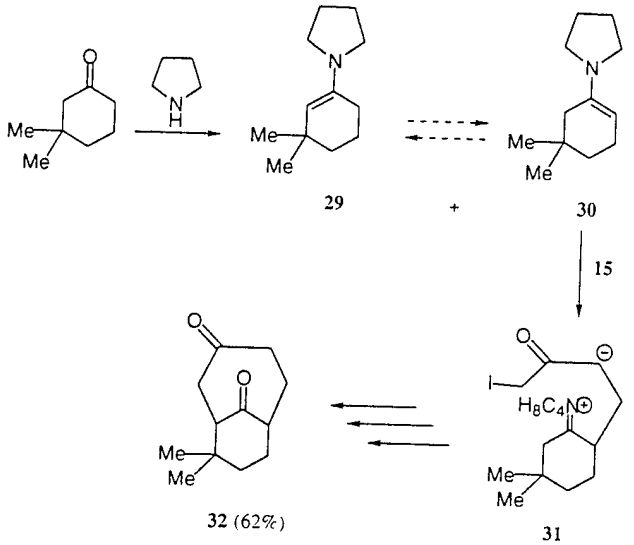
With a view towards an eventual route to the CP compounds **13** and **14**, we carried out some preliminary chemical investigations into the chemistry of diketone **11** (Scheme IV). This compound lent itself to conversion to silyl enol ether **26** and thence, following Saegusa reaction,[12] to enone **27**. Compound **13** also responded to selective ketalization at the C_1 bridged ketone (see compound **28**).

Scheme IV



At this point it seemed prudent to explore the regiochemistry of this kind of α, α'-annulation reaction with an unsymmetrical enamine. We examined the case of 3,3-dimethylcyclohexanone (see Scheme V). The

Scheme V



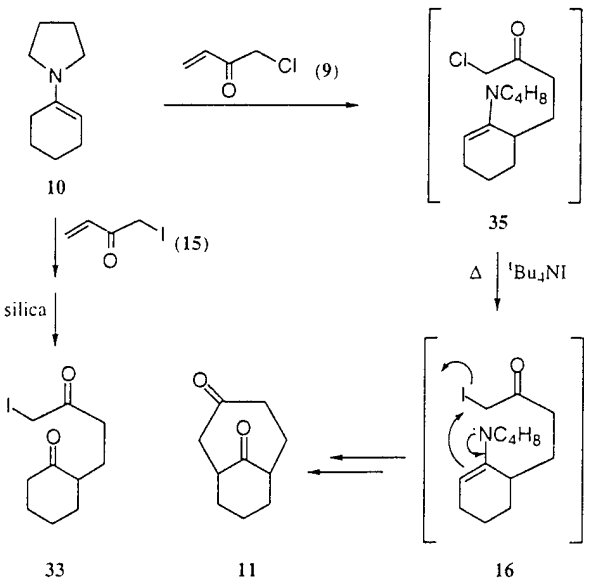
latter was converted to a 1:2 mixture of **29** and **30** under standard enamine forming conditions. Reaction of this material with **15** provided a 62% yield of **32**. We attributed this result to the participation of enamine **30** in an initial conjugate addition. Adduct **31**, produced from the Michael reaction, apparently suffers proton transfer and iodine displacement to lead, after hydrolysis, to **32**. Therefore, it seems that isomer **29** does not react under these conditions. Furthermore, it is possible that equilibration occurs between enamines **29** and **30**

during the reaction. The failure of **29** itself to enter into the annulation sequence is not *per se* surprising, since the initial alkylation step would result in the formation of a secondary neopentyl center.

Further evidence that the initial step in the annulation sequence is conjugate addition was obtained by a detailed study of the reaction of enamine **1** and enone **15**. In the initial phase of the process, the enamine and enone are mixed at room temperature and thin-layer chromatography indicates immediate conversion to a new product *distinct* from **11**. When the mixture is heated, this material disappears and α, α'-annulation product **11** is the only compound isolated from the reaction. It was not possible to isolate the transient material by conventional methods; it seemed to decompose upon workup. However, when the contents of this reaction were subjected directly to silica gel chromatography, we isolated a 1:1 adduct resulting from hydrolysis. The spectral data for this material were consistent with its formulation as **33** (Scheme VI), the hydrolysis product arising from initial conjugate addition.

Given the finding that the Michael addition step can be decoupled from the cyclization phase, we returned to the use of the chloromethyl enone **9**. Once again, reaction with enamine **1** was conducted in tetrahydrofuran at room temperature. Subsequent treatment of the presumed **16** with tetra-*n*-butyl ammonium iodide followed heating at 70 °C produced a 60-75% yield of **11** (Scheme VI). Since no aldol derived products were discerned, we assumed that **16** had indeed undergone Finkelstein type displacement, thereby producing **36** *in situ*. The latter, upon cyclization in the now expected fashion, gave rise to **11**. Hence, we can utilize the more readily accessible chloromethyl compound **9**, and derive the benefits of starting with **15** by generating **36** in situ. Although purification by filtration of the iminium salt becomes difficult under this protocol, avoiding the need to synthesize **15** in a separate step makes this procedure an attractive alternative.

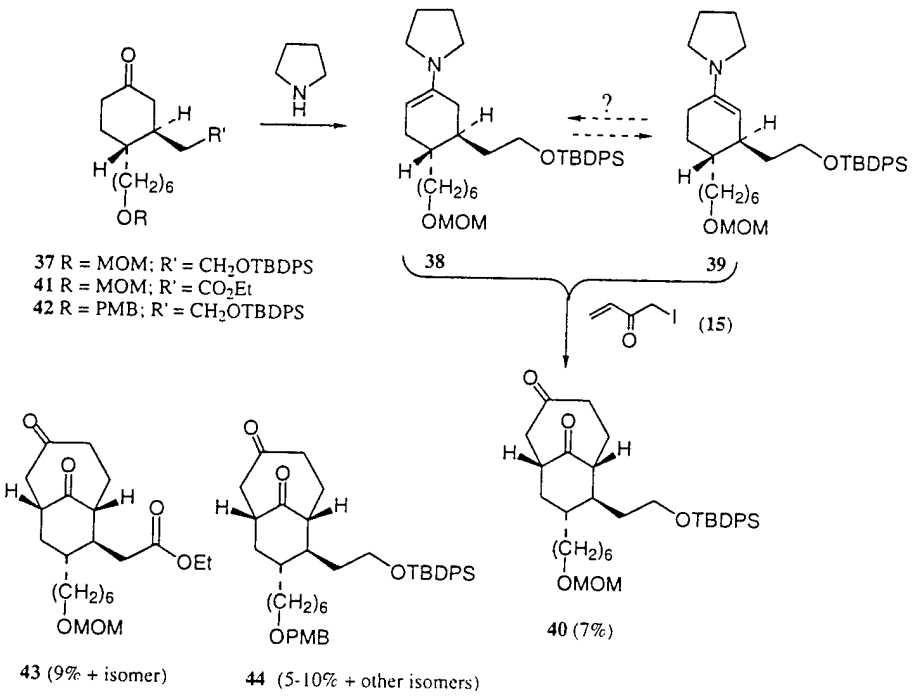
Scheme VI



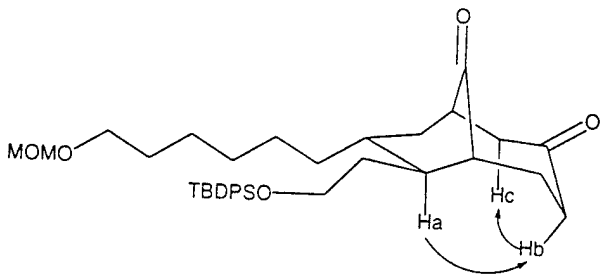
We then examined substrates that might be more realistic for an eventual synthesis of **13** and **14**. Compound **37** (see Scheme VII), available from a parallel but unrelated study,[13] upon reaction with pyrrolidine gave rise to a 1:1 mixture of enamines (**38** and **39**). Treatment with **15** gave a complex mixture. The major component was isolated and identified as **40**, in only 5-10% yield. This assignment was made on the basis of COSY and NOESY experiments. The skeletal structure shown in Scheme VIII was established by proton correlation, and irradiation of H_b (see Scheme VIII) enhanced the signals of H_a and H_c, indicating *trans*-stereochemistry.

We also studied the annulation of ketones **41** and **42** via their enamine mixtures corresponding to the **38**, **39** mixture shown for **37**. In each of these instances, an array of closely related products was obtained (ca. 50%). Processing of these materials led to the isolation of **43** (from **41**) and **44**[14] (from **42**), again in only 5 - 10% yields. It will be noted that the annulation sequences on ketones **37**, **41** and **42** correspond in their regiochemistry to having arisen from enamines of the type **39**. However, given the very low yields obtained for the bridged products, and the diversity of products produced, we do not attribute any significance to these results.

Scheme VII



Scheme VIII



In summary, though the route described above is quite interesting for the one step construction of many bridged ring systems from enamines, its application to the synthesis of the CP compounds must await advances in the attainment of synthetically viable control in complex settings.

Experimental

General. Reactions were carried out under an argon atmosphere. THF was distilled from sodium-benzophenone ketyl under an argon atmosphere. Enamines were distilled immediately before use and used directly in the annulations as described in the general procedure below unless otherwise noted. The ketones used in the enamine syntheses were purchased from Aldrich and used without purification. Those ketones that were not commercially available were prepared in one or two steps by literature procedures from starting materials obtained from Aldrich. Chlorotrimethylsilane was distilled. Analytical thin layer chromatography was performed on E. Merck silica gel 60 F_{254} plates (0.25 mm). Flash chromatography was performed using the indicated solvent on silica gel 60 (40-63 mm). Proton NMR spectra were obtained on a Bruker AMX-400 MHz referenced to $CDCl_3$ (δ 7.26) and the ^{13}C on a Bruker 300 MHz referenced to $CDCl_3$ (δ 77.0). Low- and high resolution mass spectral analyses were obtained on a JEOL JMS-DX-303HF mass spectrometer.

Preparation of 4-methylenecyclohexanone

Methyl triphenylphosphonium bromide (21.4 g, 59.8 mmol) was dissolved in anhydrous benzene (100 mL) at 25° C and potassium *tert*-butoxide was added in a single portion. The resultant solution became bright yellow after stirring at 25° C for 1 h. 1,4-cyclohexanedione *mono*-ethyleneketal (4.15 g, 26.6 mmol) in anhydrous benzene (200 mL) was added *via* cannula. The reaction mixture stirred for 1 h at 25° C and was quenched with sat. NH_4Cl (40 mL). It was then diluted with water (200 mL), the layers were separated and the aqueous layer was extracted with ether (4 x 60 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica (20% ethyl acetate/hexane) gave 4.1 g (100%) of 4-methylenecyclohexanone ethyleneketal.

4-Methylenecyclohexanone ethyleneketal (4.1 g, 26.6 mmol) was taken up in acetone (225 mL) and H_2O (20 mL) was added followed by pyridinium*p*-toluene sulfonate (6.7 g, 26.6 mmol) and the resultant solution was heated to reflux for 6 h. The reaction mixture was cooled to 25° C and concentrated to a small volume (*ca.* 40 mL) *in vacuo*. It was diluted with water (150 mL) and extracted with CH_2Cl_2 (3 x 75 mL). The combined organics were washed successively with sat. aq. $NaHCO_3$ (75 mL), brine (75 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give 4-methylenecyclohexanone (2.7 g, 92%) of sufficient purity for conversion to its enamine. Spectral data was in agreement with that previously reported for 4-methylenecyclohexanone.[15]

General preparation of enamines

A 1.0M solution of ketone (1.0 equiv) in benzene was stirred at 25° C in a flask equipped with a Dean-Stark trap and a condenser. Pyrrolidine (1.1 equiv) was added via syringe and the mixture was heated at reflux 12 h, at which point water separation had occurred. The solvent was removed *in vacuo* and the residue was distilled for immediate use in the annulation sequence.

Preparation of 1-iodo-but-3-en-2-one (15).

1-Chloro-but-3-en-2-one[16] **9** (1.35 g, 12.9 mmol) was dissolved in 120 mL acetone at 25° C. Sodium iodide (5.8 g, 39.0 mmol) was added in a single portion. White precipitate appeared immediately and after 5 minutes, the solution was diluted with 300 mL ether and successively washed with water (100 mL), sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude residue was used immediately in the annulation sequence as it rapidly decomposes. ^1H NMR ($CDCl_3$, 400 MHz) δ 6.48 (dd, J=17.5, 10.3, 1H), 6.32 (d, J=17.5, 1H), 5.87 (d, J=10.4, 1H), 3.93 (6s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.4, 132.4, 130.6, 3.6; HRMS (EI) *m/e* calcd for C_4H_5OI 195.9385, found 195.9385 (M^+).

General procedures for the α-α' annulation of enamines

Representative procedure A for annulation with 1-iodo-but-3-en-2-one.

A 0.1-0.2 M solution of freshly distilled enamine (1.0 equiv) in anhydrous THF was stirred at 25° C, and a 1.0 M solution of crude 1-iodo-but-3-en-2-one in THF (prepared from 1.1 equiv 1–chloro-but-3-en-2-one and 1.65 equiv sodium iodide) was added *via* cannula. After consumption of starting material by TLC analysis (typically 0.5-1.0 h at 25° C) the mixture was heated to reflux for 1-2 h, until consumption of intermediate was evident by TLC analysis. The reaction was cooled to 25° C and the insoluble iminium salt was separated by filtration and dried briefly *in vacuo*. (note: some iminium salts were partially soluble in THF- in these cases the filtrate as well as the iminium salt is subjected to the hydrolysis) A 0.2-0.5M solution of iminium salt THF/1N HCl (3:1) was stirred at 25° C for 4 h. The reaction mixture is diluted with 1 N NaOH and extracted repeatedly with CH_2Cl_2 (note: some compounds were extremely resistant to extraction from the aqueous layer). The combined organic layers were washed successively with 10% $Na_2S_2O_4$ and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Crude products were purified by flash chromatography on SiO_2.

Representative procedure B for annulation of enamines with 1-chloro-but-3-en-2-one and tetrabutylammonium iodide.

Preparation of Bicyclo[4.3.1]decane-3,10-dione (11).

Freshly distilled 1-pyrrolidino-1-cyclohexene **1** (310 mg, 2.0 mmol) was dissolved in 20 mL of anhydrous THF at 25° C. 1-Chloro-but-3-en-2-one (236 mg, 2.3 mmol) in 2 mL anhydrous THF was introduced *via* cannula. Tetrabutylammonium iodide (2.2 g, 6.0 mmol) was then added in one portion. The solution was heated to reflux for 2 h and then cooled to 25° C. 3 N HCl (3.0 mL) was added to the reaction mixture and stirred an additional 5 h. The mixture was diluted with 50 mL 1 N NaOH and extracted with CH_2Cl_2 (6 X 20 mL). The combined organic layers were washed successively with $Na_2S_2O_4$ (20 mL), brine (20 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on SiO_2 (30% ethyl acetate/ hexane) to give 203 mg (60%) of **11**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.89 (m, 1H), 2.60-2.79 (m, 3H), 2.52 (ddd, J=16.0, 5.7, 3.9, 1H), 2.40 (ddd, J=16.1, 12.4, 4.1, 1H), 2.13 (m, 1H), 1.80-2.10 (m, 6H), 1.59 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.6, 210.9, 47.7, 44.6, 44.5, 40.7, 32.7, 32.2, 25.5, 15.7; HRMS (EI) *m/e* calcd for $C_{10}H_{14}O_2$ 166.0994, found 166.1000 (M^+).

Isolation of 2(4-Iodo-3-oxo-butyl)-cyclohexanone (33)

Immediately after the addition of enone **15** to 1-pyrrolidino-1-cyclohexene **1** (as in procedure A), the reaction mixture was passed quickly through a pad of silica and rinsed with 30% EtOAc in hexane. Removal of solvent in vacuo gave iodide **33**. ^{1}H NMR($CDCl_3$, 400MHz) δ 3.80 (s, 2H), 2.79 (m, 2H), 2.41 - 2.20 (band, 3H), 2.14 - 1.93 (band, 3H), 1.86 (m, 1H), 1.69 - 1.50 (band , 3H), 1.40 (m, 1H); ^{13}C NMR (125MHz, $CDCl_3$) δ 212.9, 203.1, 49.6, 42.2, 37.0, 34.5, 28.1, 25.1, 22.3, 6.2; LRMS m/e calcd for $C_{10}H_{15}O_2I$ 294.0, found 295.1($M+H^+$)

Preparation of Bicyclo[4.2.1]nonane-3,9-dione (18)

Reaction of 1-pyrrolidino-1-cyclopentene (275 mg, 2.0 mmol) and 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (230 mg, 2.2 mmol) and sodium iodide (495 mg, 3.3 mmol)] via procedure A gave an iminium salt that was partially soluble in THF. Hydrolysis gave a crude product which was purified by flash chromatography on SiO_2 (40% ethyl acetate/hexane) to give 173 mg (57%) of diketone **18**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.65 (dd, J=14.4, 5.7, 1H), 2.55 (m, 4H), 2.32 (dd, J=14.4, 3.3, 1H), 2.23 (m, 2H), 2.13 (dt, J=20.1, 6.5, 1H), 1.61-1.88 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 220.7, 210.3, 45.4, 44.8, 41.0, 40.1, 29.6, 25.83, 25.79; HRMS (EI) *m/e* calcd for $C_9H_{12}O_2$ 152.0837, found 152.0837 (M^+).

Preparation of Bicyclo[4.4.1]undecane-3,11-dione (19)

Reaction of 1-pyrrolidino-1-cycloheptene (330 mg, 2.0 mmol) with 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (230 mg, 2.2 mmol) and sodium iodide (495 mg, 3.3 mmol)] was carried out as described in procedure A. Hydrolysis at 60° C (3.5h) gave a crude product which was purified by flash chromatography on SiO_2 (40% ethyl acetate/hexane) to give 172 mg (48%) of diketone **19**. ^{1}H NMR ($CDCl_3$,

400 MHz) δ 2.89 (m, 1H), 2.69 (dt, J=16.7, 5.9, 1H), 2.52 (m, 3H), 2.31 (t, J=12.1, 1H), 2.04 (m, 1H), 1.76 (m, 4H), 1.60 (m, 3H), 1.38 (m, 1H), 1.14 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 217.1, 209.4, 54.5, 49.1, 46.1, 41.9, 29.9, 28.4, 26.3, 25.4, 23.0; HRMS (EI) *m/e* calcd for $C_{11}H_{16}O_2$ 180.1150, found 180.1145 (M^+).

Preparation of 8-ethyl-bicyclo[4.3.1]decane-3,10-dione (20)

Reaction of 1-pyrrolidino-4-ethyl-cyclohexene (900 mg, 5.1 mmol) with 1-iodo-but-3-en-2-one (1.0 g, 5.1 mmol) was carried out as in procedure A. The iminium salt was not isolated before hydrolysis. Purification by flash chromatography on SiO_2 (5% ether/CH_2Cl_2) gave 450 mg (46%) of a diastereomeric mixture of diketone **20**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.82 (m, 1H), 2.65 (m, 3H), 2.48 (m, 1H), 2.37 (m, 1H), 2.13 (m, 1H), 1.65-1.98 (m, 4H), 1.54 (dquintets, J=26.7, 5.0, 2H), 1.25 (m, 2H) 0.88 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 216.0, 211.2, 47.2, 45.6, 44.1, 41.0, 39.1, 38.6, 28.7, 28.3, 26.5, 11.6; HRMS (EI) *m/e* calcd for $C_{12}H_{18}O_2$ 194.1307, found 194.1305 (M^+).

Preparation of 4,4-methyl-bicyclo[4.3.1]decane-3,10-dione (21)

Reaction of *crude* 1-pyrrolidino- 4,4-dimethylcyclohexene[17] (358 mg, 2.0 mmol) with 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (230 mg, 2.2 mmol) and sodium iodide (495 mg, 3.3 mmol)] was carried out as in procedure A. Purification by flash chromatography on SiO_2 (25% ethyl acetate/hexane) gave 136 mg (37%) of diketone **21**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.81 (m, 1H), 2.62-2.75 (m, 3H), 2.37-2.52 (m, 2H), 2.12 (m, 1H), 1.93 (ddd, J=14.1, 9.6, 2.1, 1H), 1.73-1.87 (m, 3H), 1.69 (dd, J=14.1, 3.4, 1H), 1.03 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 218.6, 210.1, 45.5, 43.1, 41.9, 40.6, 40.1, 32.1, 29.8, 29.5, 26.8; HRMS (EI) *m/e* calcd for $C_{12}H_{18}O_2$ 194.1307, found 194.1306 (M^+).

Preparation of 4-methylene-bicyclo[4.3.1]decane-3,10-dione (22)

Reaction of 1-pyrrolidino-4-methylene cyclohexene (1.75 g, 10.8 mmol) with 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (1.35 g, 12.9 mmol) and sodium iodide (5.8 g, 39.0 mmol)] was carried out as in procedure A. Purification by flash chromatography on SiO_2 (25% ethyl acetate/hexane) gave 1.05 g (55%) of diketone **22**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.08 (dd, J=6.3, 1.7, 2H), 2.87 (m, 2H), 2.43-2.79 (m, 7H), 2.34 (ddd, J=18.4, 10.6, 4.1, 1H), 2.03 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.0, 209.9, 138.9, 117.0, 48.0, 44.7, 43.8, 40.1, 39.9, 39.5, 25.1; HRMS (EI) *m/e* calcd for $C_{11}H_{14}O_2$ 178.0994, found 178.1002 (M^+).

Preparation of 8-benzyloxy-bicyclo[4.3.1]decane-3,10-dione (23)

Reaction of *crude* 1-pyrrolidino-4-benzyloxycyclohexene[18] (684 mg, 2.8 mmol) with 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (326 mg, 3.1 mmol) and sodium iodide (693 mg, 1.65 mmol)] was carried out as in procedure A. Purification by flash chromatography on SiO_2 (5% ether/CH_2Cl_2) gave 150 mg (21%) of diketone **23** as a diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (m, 5H), 4.54 (m, 2H), 3.91 (m, 1H), 2.94 (m, 1H), 2.82 (m, 1H), 2.70 (m, 1H), 2.33-2.62 (m, 3H), 2.02-2.25 (m, 5H), 1.81

(m, 1H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.2, 210.6, 128.39, 128.35, 127.6, 127.41, 127. 39, 127,37, 70.4, 69.1, 45.7, 45.3, 41.3, 40.8, 36.4, 35.9, 27.7; HRMS (EI) *m/e* calcd for $C_{17}H_{20}O_3$ 272.1412, found 272.1404 (M^+).

Preparation of 8-ethyleneketal-bicyclo[4.3.1]decane-3,10-dione (24)

Reaction of 1-pyrrolidino-4-ethyleneketal cyclohexene (418 mg, 2.0 mmol) and 1-iodo-but-3-en-2-one (196 mg, 2.0 mmol) was carried out as in procedure A. The expected annulation intermediate was detected by TLC analysis but did not convert efficiently to **24**. Hydrolysis was accomplished by addition of H_2O to the reaction mixture and heating to reflux 12 h. Purification by flash chromatography on SiO_2 (25-50% ethyl acetate/hexane) gave 20 mg (<5%) of diketone **24** and two other compounds in minimal yield; these were not characterized. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.03 (t, J=6.3, 2H), 3.83 (t, J=6.3, 2H), 3.55 (dd, J= 10.8, 5.2, 1H), 2.38-2.52 (m, 3H), 2.32 (m, 2H), 2.11-2.21 (m, 5H), 2.02 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 217.1, 208.8, 106.8, 64.7, 63.6, 47.4, 44.6, 44.44, 44.37, 42.8, 28.4, 25.5; HRMS (EI) *m/e* calcd for $C_{12}H_{16}O_4$ 224.1049, found 224.1047 (M^+).

Preparation of 8-oxa-bicyclo[4.3.1]decane-3,10-dione (25)

The crude enamine [prepared as usual from tetrahydro-4*H*-pyran-4-one (220 mg, 2.2 mmol) and pyrrolidine (235 mg, 3.3 mmol)] was dried under high vacuum before being used directly in the annulation. Reaction of this enamine with 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (230 mg, 2.2 mmol) and sodium iodide (495 mg, 3.3 mmol)] was carried out as described in procedure A . Purification by flash chromatography on SiO_2 (50% ethyl acetate/hexane) gave 114 mg (31%, 2 steps from tetrahydro-4*H*-pyran-4-one) of diketone **25**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.14 (dd, J=37.1, 12.0, 2H), 3.82 (dd, J=27.8, 11.9, 2H), 2.95 (ddd, J=18.0, 12.2, 5.5, 1H), 2.67 (m, 4H), 2.52 (dt, 18.1, 4.9, 1H), 2.18 (m, 1H), 2.06 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.4, 209.7, 71.4, 71.3, 48.8, 48.0, 43.2, 39.1, 25.7; HRMS (EI) *m/e* calcd for $C_9H_{12}O_3$ 168.0786, found 168.0786 (M^+).

Preparation of 9,9-dimethyl-bicyclo[4.3.1]decane-3,10-dione (32)

Reaction of 1-pyrrolidino-3,3-dimethylcyclohexene[19] (523 mg, 2.9 mmol) with 1-iodo-but-3-en-2-one [prepared from 1-chloro-but-3-en-2-one (336 mg, 3.2 mmol) and sodium iodide (723 mg, 4.8 mmol)] was carried out as described in procedure A. Purification by flash chromatography on SiO_2 (40% ethyl acetate/hexane) gave 350 mg (62%) of diketone **32**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.93 (dd, J=21.1, 13.0, 1H), 2.64 (m, 1H), 2.44 (ddd, J=19.1, 6.0, 3.5, 1H), 2.35 (dd, J=13.0, 8.5, 1H), 2.28 (ddd, 19.0, 11.9, 3.8, 1H), 2.17 (t, J=8.3, 1H), 1.88-2.03 (m, 4H), 1.52 (m, 1H), 1.23 (m, 1H), 0.92 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.8, 210.3, 56.2, 46.7, 41.2, 39.3, 38.3, 28.3, 27.0, 26.8, 26.3, 24.2; HRMS (EI) *m/e* calcd for $C_{12}H_{18}O_2$ 194.1307, found 194.1307 (M^+).

Preparation of Bicyclo[4.3.1]dec-4-ene-3,10-dione (27)

Bicyclo[4.3.1]decane-3,10-dione (**11**) (244 mg, 1.47 mmol) was dissolved in anhydrous THF (12 mL) and cooled to -78° C. Lithium hexamethyldisilazane (1.0M in THF, 1.65 mL) was added via syringe. The resultant mixture stirred at -78° C for 5 minutes, and triethylamine (615 mL, 4.41 mmol) and TMSCl (317µl, 2.25mmol) was added *via* syringe. The reaction mixture warmed to 0° C over *ca.* 0.5 h and was quenched with sat. aq. $NaHCO_3$ (1.0 mL). It was diluted with ethyl acetate (75 mL), washed successively with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL), dried ($Na_2S_2O_4$), filtered, and concentrated *in vacuo* to give the crude silyl enol ether **26**, which was used in the next reaction without further purification.

Silyl enol ether **26** was azeotroped from benzene (3x10 mL) and dissolved in anhydrous acetonitrile (10 mL) at 25° C. Palladium(II) acetate (363 mg, 1.62 mmol) was added in a single portion and the reaction mixture stirred 12 h at 25° C. The solution was filtered through a pad of celite and concentrated *in vacuo*. Purification by flash chromatography on SiO_2 (30% ethyl acetate/hexane) gave 158 mg (66%) of enone **27**. ^1H NMR ($CDCl_3$, 400 MHz) δ 6.31 (dd, J=12.2, 7.6, 1H), 6.19 (d, J=12.3, 1H), 3.47 (m, 1H), 2.84 (m, 1H), 2.63, (m, 2H), 1.84-2.02 (m, 4H), 1.59 (m, 2H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.5, 201.9, 139.2, 133.0, 52.6, 45.8, 44.6, 32.0, 30.7, 15.4 ; HRMS (EI) *m/e* calcd for $C_{10}H_{12}O_2$ 164.0837, found 164.0843 (M^+).

Preparation of 10-ethyleneketal-bicyclo [4.3.1] decan-3-one (28)

Bicyclo[4.3.1]decane-3,10-dione (**11**) (62 mg, 0.37 mmol) was dissolved in anhydrous benzene (10 mL) and ethylene glycol (2.1 mL, 37 mmol) was added followed by *p*-toluenesulfonic acid (7 mg, 0.04 mmol). The solution was heated to reflux and monitored by TLC. After 7 days, TLC analysis indicated the consumption of starting material. The reaction mixture was cooled to 25° C and the solvent was removed *in vacuo*. The residue was partitioned between water (15 mL) and CH_2Cl_2 (15 mL), the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3x10 mL). The combined organic fractions were washed successively with sat. aq. $NaHCO_3$ (10 mL), brine (10 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by flash chromatography on SiO_2 (20% ethyl acetate/hexane) gave 54 mg (69%) of ketal **28**. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.94 (dd, J=13.4, 5.5, 4H), 2.85 (dd, J=13.7, 5.8, 1H), 2.50-3.50 (m, 2H), 2.39 (dd, J=13.6, 5.6, 1H), 2.23 (m, 1H), 1.99 (m, 4H), 1.68 (m, 3H), 1.41 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.1, 111.2, 64.4, 64.1, 44.9, 40.9, 38.5, 37.6, 27.8, 27.6, 24.6, 17.2; HRMS (EI) *m/e* calcd for $C_{12}H_{18}O_3$ 210.1256, found 210.1263 (M^+).

Preparation of 7-[2-(*tert*-butyl-diphenyl-silanyloxy)-ethyl]-8-(6-methoxy-hexyl)-bicyclo[4.3.1]decane-3,10-dione (40)

Enamines **38** and **39** were prepared from ketone **37** (131 mg, 0.25 mmol) and pyrrolidine (89 mg, 1.25 mmol) in the presence of catalytic *p*-toluenesulfonic acid, isolated as usual and dried under high vacuum. Reaction of the resultant crude mixture of enamines **38** and **39** with 1-iodo-but-3-en-2-one (49 mg, 0.25 mmol) was carried out as described in procedure A. The iminium salt was hydrolyzed without isolation. Proton NMR of the crude mixture after workup showed diketone **40** and a small amount of a diastereomer. Purification by flash chromatography on SiO_2 (5% ether/CH_2Cl_2 and then 25% ethyl acetate/hexane) gave 10

mg (7%, 2 steps from ketone **37**) of diketone **40**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (m, 4H), 7.40 (m, 6H), 4.62 (s, 2H), 3.62-3.73 (m, 2H), 3.51 (t, J=6.6, 2H), 3.36 (s, 3H), 2.70 (dd, J=14.7, 4.9, 1H), 2.53 (m, 1H), 2.46 (m, 3H), 2.35 (dd, J=12.9, 2.2, 1H), 2.06 (m, 1H), 1.98 (ddd, J=14.2, 8.5, 3.3, 1H), 1.68-1.81 (m, 3H), 1.60 (m, 5H), 1.11-1.50 (m, 8H), 1.09 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 218.8, 210.3, 135.6, 135.5, 133.6, 129.7, 127.7, 96.4, 67.8, 61.0, 55.1, 52.8, 45.9, 44.2, 43.0, 41.9, 39.6, 37.7, 34.7, 32.5, 29.7, 28.9, 26.8, 26.2, 19.2; HRMS (EI) *m/e* calcd for $C_{41}H_{47}O_4Si$ 592.3584, found 631.3244 (M^++K).

Preparation of [8-(6-methoxymethoxy-hexyl)-3,10-dioxo-bicyclo[4.3.1]dec-7-yl]acetic acid ethyl ester (43)

Enamine was prepared from ketone **41** (150 mg, 0.46 mmol) and pyrrolidine (164 mg, 2.30 mmol) in the presence of catalytic *p*-toluenesulfonic acid, isolated as usual and dried under high vacuum. Reaction of the resultant crude mixture of enamines with 1-iodo-but-3-en-2-one (49 mg, 0.25 mmol) was carried out as described in procedure A. The iminium salt was not isolated before hydrolysis. Proton NMR of the crude mixture after workup showed diketone **43** and a small amount of a diastereomer. These diastereomers could not be separated by conventional flash chromatography. Purification by flash chromatography on SiO_2 (5% ether/CH_2Cl_2 and then 35% ethyl acetate/hexane) gave 16 mg of diketone **43** contaminated with an unidentified diastereomer, (9%, 2 steps from ketone **41**). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.61 (s, 2H), 4.12 (q, J=7.1, 2H), 3.51 (t, J=6.6, 2H), 3.36 (s, 3H), 2.81 (dd, J=13.5, 5.2, 1H), 2.58-2.73 (m, 2H), 2.48 (m, 1H), 2.29-2.41 (m, 2H), 2.11-2.28 (m, 2H), 2.02 (dd, J=16.4, 10.7, 1H), 1.85-1.97 (m, 1H), 1.61 (m, 1H), 1.60 (m, 2H), 1.20-1.43 (m, 8H), 1.26 (t, J=7.1, 3H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.4, 210.6, 172.3, 96.4, 67.7, 60.8, 55.1, 49.4, 47.2, 45.1, 42.4, 40.7, 34.5, 32.5, 32.2, 29.7, 29.5, 29.4, 27.3, 26.8, 26.1, 14.2; HRMS (EI) *m/e* calcd for $C_{22}H_{37}O_6$ 397.2590, found 397.2579 (M^+).

Preparation of 7-[2-(*tert*-butyl-diphenyl-silanyloxy)-ethyl]-8-(6-4-methoxy-benzyloxy)-hexyl)-bicyclo[4.3.1]decane-3,10-dione (44)

Enamine was prepared as usual from ketone **42** and pyrrolidine. The crude mixture of enamines (410 mg, 0.62 mmol) was dissolved in 6 mL of anhydrous THF at 25° C. A solution of 1–chloro-but-3-en-2-one (72 mg, 0.69 mmol) in 2 mL anhydrous THF was introduced *via* cannula. Tetrabutylammonium iodide (697 mg, 1.89 mmol) was then added in one portion. The solution was heated to reflux for 2 h and then cooled to 25° C. Aqueous HCl (0.5 N, 3.0 mL) was added to the reaction mixture and stirred an additional 1 h. The mixture was diluted with 20 mL aqueous saturated $NaHCO_3$ and extracted with CH_2Cl_2 (6 X 20 mL). The combined organic layers were washed successively with $Na_2S_2O_4$ (20 mL), brine (20 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on SiO_2 (10% ethyl acetate/ hexane) to give 47 mg (11%) of diketone **44** and 170 mg of unseparable isomers. ^{1}H NMR (500MHz, $CDCl_3$) δ 7.63 (m, 4H), 7.42 - 7.36 (band, 6H), 7.27 (d, J = 8.5Hz, 2H), 6.88 (d, J = 8.5Hz, 2H), 4.43 (s, 2H), 3.80 (s, 3H), 3.69 (m, 1H), 3.65 (M, 1H), 3.43 (t, J = 6.61Hz, 2H), 2.70 (dd, J = 14.8, 4.9Hz, 1H)2.58 - 2.35 (band, 4H), 2.08 (m,1H), 1.99 (m, 1H), 1.77 - 1.68 (band, 2H), 1.62 - 1.50 (band, 4H), 1.40 - 1.10 (band,

10H), 1.04 (s, 9H), 0.88(t, J = 6.7Hz, 1H); ^{13}C NMR (125Hz, $CDCl_3$) δ 218.8, 210.2, 159.1, 135.5, 133.6, 130.7, 129.7, 129.2, 127.7, 113.7, 72.5, 70.1, 61.0, 55.3, 52.8, 45.9, 44.2, 43.0, 41.8, 39.6, 37.8, 35.0, 32.8, 29.8, 29.7, 26.8, 26.2, 19.2; LRMS m/e calcd for $C_{42}H_{56}O_5Si$ 668.4, found 691.2 ($M + Na^+$)

Contribution from [a]The Department of Chemistry, Columbia University, Havemeyer Hall, New York, N.Y. 10027, [b]The Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Ave., Box 106, New York, N.Y. 10021, USA and The Department of Chemistry, Butler University, 4600 Sunset Avenue, Indianapolis, IA 46208.

[1] G. Stork, R, Terrell, J. Szmuszkovicz, *J. Am. Chem. Soc.* **1954**, *76*, 2029.

[2] G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, R. Terrell, *J. Am. Chem. Soc.* **1963**, *85*, 207.

[3] For a discussion on issues governing the disposition of isomeric enamines see: S. Danishefsky, M. Feldman, *Tetrahedron Lett.* **1965**, *34*, 1131.

[4] G. Stork, H. Landesman, *J. Am. Chem. Soc.* **1956**, *78*, 5129

[5] Ph.D. Thesis, Gary Koppel, University of Pittsburgh, 1969.

[6] J. R. Catch, D. F. Elliot, D. H. Hey, E. R. H. Jones, *J. Chem Soc.* **1948**, 278.

[7] T. Kaneko, *et al.*, *J. Am. Chem. Soc.* **1997**, *119*, 1594.

[8] T. Kaneko, *et al.*, *J. Anitbiot.* **1997**, *50*, 1.

[9] K. C. Nicolaou, *et. al.*, *Angew. Chem. Int. Ed. Eng.* **1997**, *36*, 1194, 2821.

[10] H. M. L. Davies, R. Calvo, G. Ahmed, *Tetrahedron Lett.* **1997**, *38*, 1737.

[11] P. W. M. Sgarbi, D. L. J. Clive, *Chem. Commun.* **1997**, 2157.

[12] Y. Ito, T. Hirao, T. Saegusa, *J. Org. Chem.*, **1978**, *43*, 1011.

[13] D'Amico, D. C., Deng, W., Kwon, O., Danishefsky, S. J. Unpublished results.

[14] The structures of **43** and **44** were assigned by comparison with **40**.

[15] J. Ishiyama, S. Maeda, K. Takahashi, Y. Senda, S. I. Maizumi *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1721.

[16] Prepared as in reference 7, with the modification for dehydrohalogenation introduced for chloroethylvinylketone (S. Danishefsky, B. H. Migdalof, *J. Am. Chem. Soc.* **1969**, *91*, 2806.)

[17] Prepared from 4,4-dimethylcyclohexanone (F. G. Bordwell, K. M. Wellman *J. Org. Chem.* **1963**, *28*, 1347) in the usual way.

[18] Prepared from 4-benzyloxycyclohexanone (M. Majewski, J. MacKinnon *Can. J. Chem.* **1994**, *72*, 1699) in the usual way.

[19] Prepared from 3,3-dimethylcyclohexanone (S.W. Pelletier, N. V. Mody *J. Org. Chem.* **1976**, *41*, 1069) in the usual way.

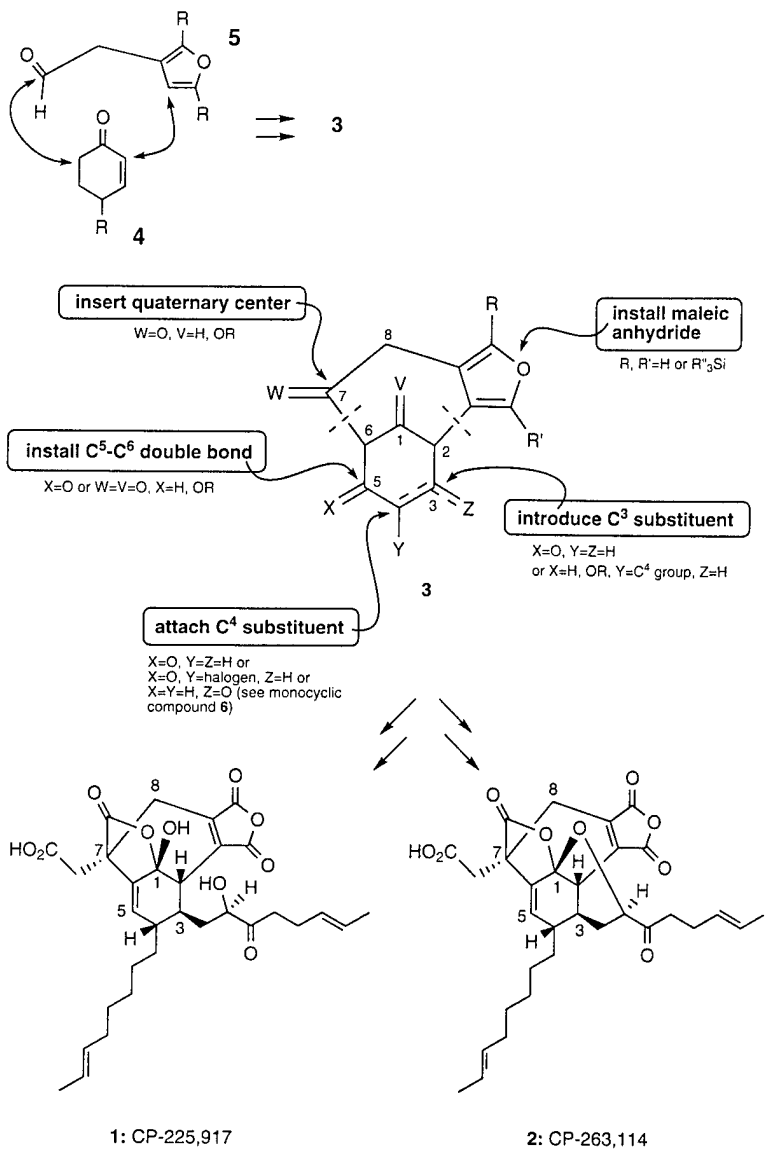
0040-4C
PII: S0C

Total Syntheses of CP-225,917 and CP-263,114: Creation of a Matrix Structure By Sequential Aldol Condensation and Intramolecular Heck Ring Closure**

Ohyun Kwon, Dai-Shi Su, Dongfang Meng, Wei Deng, Derin C. D'Amico, and Samuel J. Danishefsky*

Recently Kaneko and co-workers at the Charles Pfizer laboratories described the isolation and structure elucidation of compounds **1** and **2** (see Scheme 1).[1, 2] Apart from the curiosity provoked by their novel structures, these metabolites warrant additional attention as they are potent inhibitors of squalene synthase and farnesyl transferase. The potentialities of modulating the pathways regulated by these enzymes are of great interest to the pharmaceutical industry, though no agents operating through such mechanisms are in current usage.[3] Our notice of the Pfizer compounds arose from the inherent summons their syntheses pose to the ingenuity and capacities of organic chemistry.

Given the multifaceted problems which would have to be surmounted to complete such a goal in the case of **1** and **2**, it seems unlikely that achieving a total synthesis would impact on the availability of these natural products for detailed evaluation, let alone clinical application. However, since the structure–activity profiles of such compounds are not known, it is not inconceivable that useful biological function could be realized from rather simpler structures which might, in fact, be available in acceptable amounts through purely synthetic means. An exploration of the total syntheses of **1** and **2** might provide a valuable perspective to study these issues.



Scheme 1. Strategies for a matrix structure **3** in the total synthesis of **1** and **2**.

Several early and interesting initiatives directed to the synthesis of **1** and **2** have been reported by Nicolaou,[4] Davies,[5] Clive,[6] and Armstrong.[7] These previous thrusts directly addressed the introduction of the C5–C6 bridgehead double bond, which is present in both compounds. Progress toward this end was accomplished through bond-reorganization strategies (cycloadditions[8] or sigmatropic rearrangements).

The route we are exploring herein reflects a different view of the problem. Its main focus is on gaining rapid access to a 5,6-dihydro ring system, with provisions (albeit in less than mature form) for the introduction of all the functional groups needed to obtain **1** and **2**. The scheme provides for properly placed carbonyl groups and double bonds to serve these ends. Central to the plan is the presence of a furan moiety that contributes two carbon atoms to the four-carbon spanning element of coupling agent **5** (Scheme 1). The furan ring provides an annulation handle (see below) and, furthermore, is a locus of reasonable stability in the resultant cyclization product. Moreover, appropriate oxidation of the furan would lead to the maleic anhydride.[9] The optimal timing of this event would be ascertained as matters unfold. As for the introduction of the C5–C6 bridgehead double bond, we envisioned reliance on functionalities derivable from one or more keto groups at C7 and C1, where C5 is equipped with a leaving group.

[*] Prof. S. J. Danishefsky, O. Kwon, D. Meng, Dr. D. C. D'Amico
Department of Chemistry
Columbia University
Havemeyer Hall, New York, NY 10027 (USA)
Fax: (+1) 212-772-8691
Dr. D.-S. Su, Dr. W. Deng (and further address for Prof. S. J. Danishefsky, D. Meng)
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)

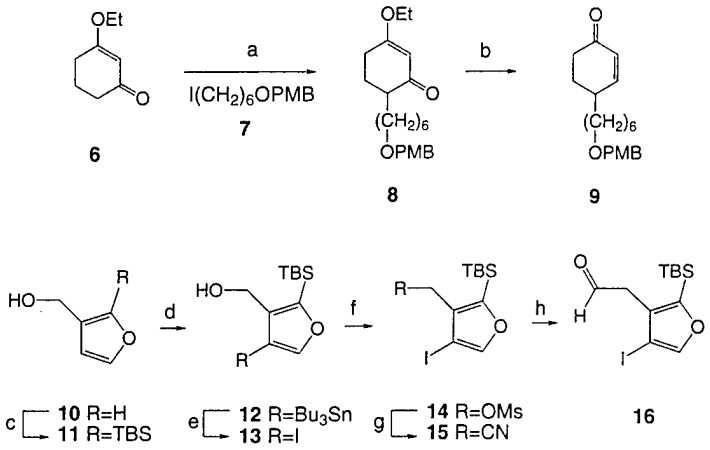
[**] This research was supported by the National Institutes of Health (grant number CA-28824 and HL 25848). Predoctoral fellowship support is gratefully acknowledged by O.K. (K.A.S.T.) and D.M. (U.S. Army). Postdoctoral fellowship support by the NIH is gratefully acknowledged by W.D. (grant number CA-67743) and D.C.D. (grant number F32 GM 17353).

  1433-7851/98/3716-1877 $ 17.50+.50/0

Our general thought process is summarized in formula **3** (Scheme 1). We emphasize that this picture is not intended to represent a specific compound. Rather, it conveys a perspective by which required functional groups are introduced through potential implementation sites in a matrix system. Left unaddressed in such a presentation are important subtleties of timing and orchestration.

Seeking proof of principle, we studied the feasibility of melding systems of types **4** and **5** as a route to the matrix entity **3**. In the particular variation we report here, the future substituent at C4 in **3** is introduced already in the monocyclic precursor **4**. In other constructions, we have introduced this substituent after formation of **3**.

The alkylation of **6** with **7** afforded **8** in 78% yield (Scheme 2).[10] Reduction of the vinylogous ester **8** and acidic

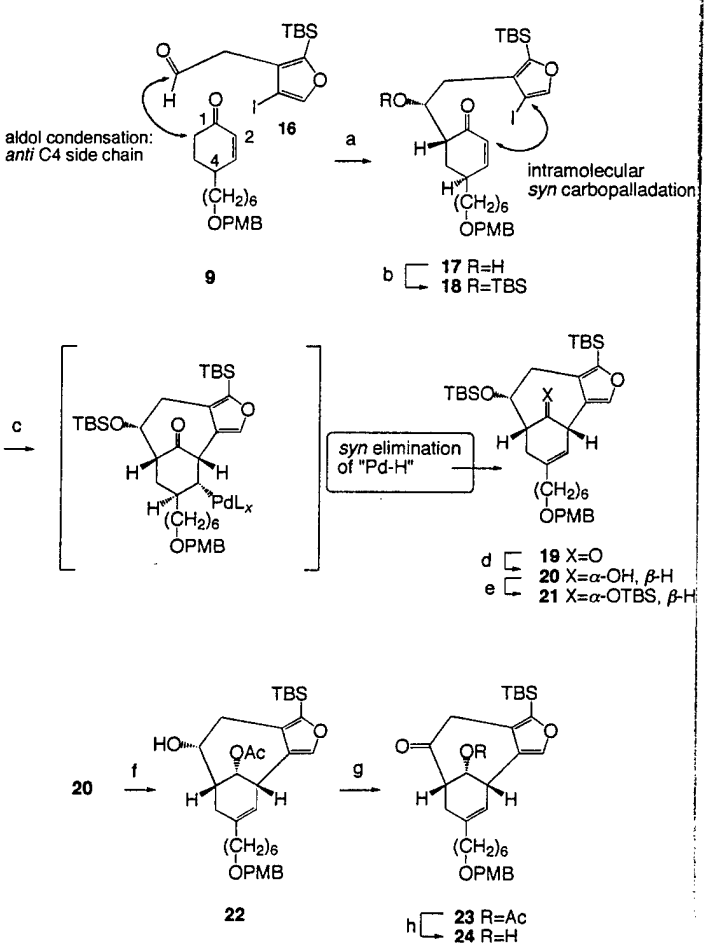


Scheme 2. Synthesis of monocycles **9** and **16**. a) LDA, HMPA, THF, −78 °C, then **7**, −78 °C → RT (room temperature), 16 h, 78%; b) $LiAlH_4$, Et_2O, 0 °C, 40 min, then 4 N HCl, RT, 0.5 h, 93%; c) 1. TBSCl, $NEt(iPr)_2$, CH_2Cl_2, RT, 1 h, 98%; 2. *n*BuLi, HMPA, THF, −78 °C → RT, 16 h, 89%; d) *n*BuLi, DME, −78 → 0 °C, 15 min, then Bu_3SnCl, 0 °C, 1 h, 80%; e) 1. I_2, CH_2Cl_2; 2. KF, Et_2O, RT, 16 h, 96%; f) MsCl, NEt_3, CH_2Cl_2, −78 °C, 2 h; g) KCN, DMF, RT, 16 h, 98% over two steps; h) DIBAlH, toluene, RT, 1 h, 92%. DIBAlH = diisobutyl aluminum hydride, DME = dimethoxyethane, HMPA = hexamethyl phosporamide, LDA = lithium diisopropylamide, Ms = methanesulfonyl, PMB = *p*-methoxybenzyl, TBS = *tert*-butyldimethylsilyl.

hydrolysis led, as anticipated,[11] to enone **9**, a specific example of the monocyclic precursor **4**. The synthesis of a furan of type **5** commenced with the commercially available **10**, which was converted into the corresponding *tert*-butyldimethylsilyl ether. Silyloxy-directed metalation at C2 followed by O → C migration of the silyl group afforded **11**. Next, we made use of hydroxyl-directed metalation at C3 to introduce a stannyl group (**12**). Destannylative iodination in the *ipso* sense provided **13**,[12,13] which was homologated (via mesylate **14**) to the furanylacetonitrile derivative **15**. Finally, controlled reduction of the nitrile functionality and careful hydrolysis afforded the somewhat labile aldehyde **16**.

With both coupling components in hand, we directed our attention to construction of a prototype corresponding to **3**. The plan involved an initial merger of the two components at the future C6–C7 bond through an aldol condensation. Formation of the bridging carbon–carbon bond would occur by an intramolecular Heck vinylation reaction.[14,15] Aldol reaction of the α'-enolate[16] derived from **9** with aldehyde **16** afforded with high stereoselectivity[17] **17** in 91% yield. At this point, it was prudent to protect the C7 alcohol in the form of its TBS derivative **18**.[18] The stage was now set for the critical intramolecular Heck reaction. In practice, cyclization occurred smoothly to afford **19**. Thus, a credible matrix compound for the projected synthesis is assembled in a remarkably straightforward way. The key to the successful Heck reaction on a side chain at C4 of cyclohexenone is that the aldol reaction at C6 occurs *anti* to the side chain. Thus, following protection and *syn* carbopalladation, *syn* elimination of palladium hydride is possible, which is central to the success of the enterprise.

Once we had compound **19**, we began to explore some possible functional-group modifications which could be helpful in reaching structures **1** or **2**. We found that reduction of **19** with diisobutyl aluminum hydride provides a single alcohol, **20** (Scheme 3). Silylation of **20** gives rise to **21**. Alternatively,

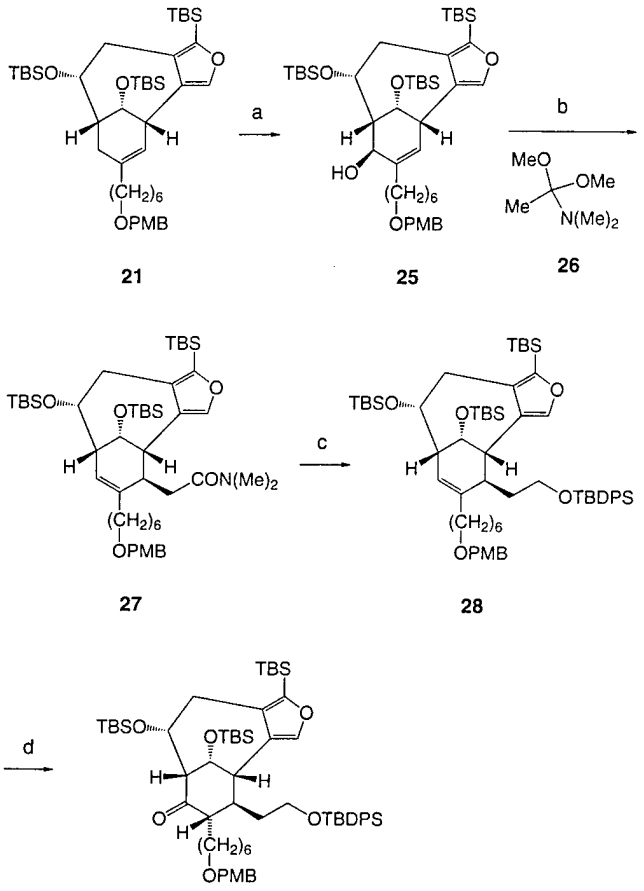


Scheme 3. Synthesis of tricycles **21** and **24**. a) **9**, LDA, THF, −78 °C, 1 h, then **16**, THF, −78 °C, 2 h, 91%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 63%; c) $[Pd(OAc)_2(PPh_3)_2]$, NEt_3, THF, Δ, 3 d, 85% or $[Pd(OAc)_2(PPh_3)_2]$, NEt_3, dioxane, Δ, 24 h, 77%; d) DIBAlH, CH_2Cl_2, −78 → −30 °C, 96%; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 16 h, 94%; f) 1. Ac_2O, NEt_3, DMAP, CH_2Cl_2, 16 h, 98%; 2. TBAF, THF, 45 °C, 2 h; g) TPAP, NMO, CH_2Cl_2, molecular sieves (4 Å), RT, 10 min; h) K_2CO_3, MeOH, RT, 1.25 h, 77% over three steps. DMAP = 4-(dimethylamino)-pyridine, NMO = *N*-methylmorpholin-*N*-oxide, TBAF = tetrabutylammonium fluoride, Tf = trifluoromethanesulfonyl, TPAP = tetrapropylammonium perruthenate.

  1433-7851/98/3716-1878 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **1998**, *37*, No. 13/14

acetylation of **20** followed by cleavage of the C7 silyl group led to **22**, which upon oxidation provided ketone **23**. The latter can be deprotected at C1 (**24**).

Another milestone was reached, albeit in a manner that currently[19] lacks tight regiochemical control,[20] through the action of selenium dioxide on **21**. This process produced **25** as well as a somewhat smaller amount of side chain oxidation products (Scheme 4). Reaction of **25** with **26** gave, upon [3,3] sigmatropic rearrangement, the γ,δ unsaturated amide **27**.[21] Reduction of the amide linkage and protection of the resulting primary alcohol led to **28**, which on hydroboration and oxidation provided **29**.[22]



21 **25** **26** **27** **28** **29**

Scheme 4. Synthesis of **29**. a) SeO_2, pyridine, 70 °C, 20 min, 31 % (+21 % side chain oxidation product); b) **26**, xylenes, 150 °C, 18 h, 91 %; c) 1. $LiEt_3BH$, THF, 0 °C → RT, 2 h, then 1N NaOH, H_2O_2, RT, 1.75 h, 91 %; 2. TBDPSCl, imidazole, DMAP, THF, RT, 2 h, 91 %; d) 1. $BH_3 \cdot THF$, 0 °C → RT, 16 h, then 1N NaOH, H_2O_2, RT, 1 h, 68 %; 2. Dess–Martin periodinane, CH_2Cl_2, RT, 2 h, 92 %. TBDPS = *tert*-butyldiphenylsilyl.

In principle, several compounds in this series contain promising functionality implements required to reach CP-225,917 and CP-263,114. This theme will be amplified in the following contribution.[23]

Received: March 4, 1998 [Z11553IE]
German version: *Angew. Chem.* **1998**, *110*, 1978–1981

Keywords: aldol reactions · Heck reactions · natural products · synthetic methods

[1] T. T. Dabrah, T. Kaneko, W. Massefski Jr., E. B. Whipple, *J. Am. Chem. Soc.* **1997**, *119*, 1594.
[2] T. T. Dabrah, H. J. Harwood Jr., L. H. Huang, N. D. Jankovich, T. Kaneko, J.-C. Li, S. Lindsey, P. M. Moshier, T. A. Subashi, M. Therrien, P. C. Watts, *J. Antibiot.* **1997**, *50*, 1.
[3] For a review on farnesyltransferase inhibitors, see J. B. Gibbs, S. L. Graham, G. D. Hartman, K. S. Koblan, N. E. Kohl, C. A. Omer, A. Oliff, *Curr. Opin. Chem. Biol.* **1997**, *1*, 197.
[4] K. C. Nicolaou, M. W. Harter, L. Boulton, B. Jandeleit, *Angew. Chem.* **1997**, *109*, 1243; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1194; K. C. Nicolaou, M. H. D. Postema, N. D. Miller, G. Yang, *Angew. Chem.* **1997**, *109*, 2922; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2821.
[5] H. M. L. Davies, R. Calvo, G. Ahmed, *Tetrahedron Lett.* **1997**, *38*, 1737.
[6] P. W. M. Sgarbi, D. L. J. Clive, *Chem. Commun.* **1997**, 2157.
[7] A. Armstrong, T. J. Critchley, A. A. Mortlook, *Synlett* **1988**, 552.
[8] S. L. Gwaltney, S. T. Sakata, K. J. Shea, *Tetrahedron Lett.* **1995**, *36*, 7177.
[9] For an interesting example of the use of a stable aromatic precursor to deliver a maleic anhydride in a total synthetic context, see G. Stork, J. M. Tabak, J. F. Blount, *J. Am. Chem. Soc.* **1972**, *94*, 4735.
[10] G. Stork, R. L. Danheiser, *J. Org. Chem.* **1973**, *38*, 1775.
[11] G. Stork, R. L. Danheiser, B. Ganem, *J. Am. Chem. Soc.* **1973**, *95*, 3414.
[12] D. E. Seitz, S.-H. Lee, R. N. Hanson, J. C. Bottaro, *Synth. Commun.* **1983**, *13*, 121.
[13] a) G. R. Martinez, P. A. Grieco, C. V. Srinivasan, *J. Org. Chem.* **1981**, *46*, 3761; b) E. J. Bures, B. A. Keay, *Tetrahedron Lett.* **1988**, *29*, 1247.
[14] For a review of the Heck reaction, see A. de Meijere, F. E. Meyer, *Angew. Chem.* **1994**, *106*, 2473; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2379.
[15] For an application of an intramolecular Heck reaction to the direct creation of a bridgehead olefin, see S. J. Danishefsky, J. J. Masters, W. B. Young, J. T. Link, L. B. Synder, T. V. Magee, D. K. Jung, R. C. A. Isaacs, W. G. Bornmann, C. A. Alaimo, C. A. Coburn, M. J. Di Grandi, *J. Am. Chem. Soc.* **1996**, *118*, 2843.
[16] cf. R. A. Lee, C. McAndrews, K. M. Patel, W. Reusch, *Tetrahedron Lett.* **1973**, *14*, 965.
[17] Early in our studies we conducted the same sort of aldol condensation of furan **16** (i.e., with **9** having a MOM rather than a PMB protecting group). The major product (ca. 83 %) corresponded to **17** (with MOM instead of PMB). However, the three other aldol products were also obtained (combined yield ca. 12 %), each in homogeneous form following chromotography. In the case of **17**, we could not find the corresponding minor products. This may reflect a subtle and unrecognized difference in experimental conditions. Alternatively, the difference may arise from a small sensitivity of the stereochemistry of the aldol reaction to the nature of the protecting group in the side chain at C4.
[18] In parallel experiments, the Heck reaction of the unprotected alcohol was also accomplished, but in much lower yield and with poor reproducibility. For another instance of the power of the sequential Heck–Aldol logic, see reference [15].
[19] We also conducted the allylic oxygenation under different condition, such as with SeO_2 in different solvent systems, with PCC,[15] and by Sharpless catalytic methods (M. A. Warpehoski, B. Chabaud, K. B. Sharpless, *J. Org. Chem.* **1982**, *47*, 2897). However, the experiments shown here provided the best results.
[20] In studies tangential to these we have learned how to circumvent this lack of acceptable regioselectivity in SeO_2 chemistry: D. Meng, Columbia University, unpublished results.
[21] a) A. E. Wick, D. Felix, K. Steen, A. Eschenmoser, *Helv. Chim. Acta* **1964**, *47*, 2425; b) D. Felix, K. Gschwend-Steen, A. E. Wick, A. Eschenmoser, *Helv. Chim. Acta* **1969**, 52, 1030.
[22] Selected physical properties for **29**: ^1H NMR (400 MHz, $CDCl_3$): δ = 7.5 (m, 4H), 7.35 (m, 6H), 7.19 (d, J = 8.6 Hz, 2H), 6.91 (s, 1H), 6.80 (dd, J = 6.7, 1.8 Hz, 2H), 4.35 (s, 2H), 4.32 (t, J = 6.4 Hz, 1H), 4.25 (dt, J = 8.6 , 3.3 Hz, 1H), 3.72 (s, 3H), 3.67 (m, 2H), 3.34 (t, J = 6.8 Hz, 2H), 2.72 (m, 3H), 2.47 (dd, J = 11.9, 3.0 Hz, 1H), 2.08 (m, 1H), 1.82

  1433-7851/98/3716-1879 $ 17.50+.50/0

(m, 1H), 1.50 (m, 4H), 1.18 (m, 8H), 0.90 (s, 9H), 0.86 (s, 9H), 0.79 (s, 9H), 0.64 (s, 9H), 0.16 (2s, 6H), −0.03 (s, 3H), −0.07 (s, 3H), −0.09 (s, 3H), −0.13 (s, 3H); FT-IR (neat): $\tilde{\nu}$ = 1714, 1512, 1470, 1250, 1104 cm^{-1}; MS: calcd for $C_{62}H_{98}O_7Si_4Na$: 1089.6, found: 1089.6 [*M*+Na].

[23] O. Kwon, D.-S. Su, D. Meng, W. Deng, D. D'Amico, S. J. Danishefsky, *Angew. Chemie.* **1998**, *110*, 1981–1983; *Angew. Chem. Int. Ed.* **1998**, *37*, 1880–1882.

  1433-7851/98/3716-1880 $ 17.50+.50/0

A Stereospecific Geminal Alkylation Scheme En Route To CP-225, 917 and CP-263,114**

Ohyun Kwon, Dai-Shi Su, Dongfang Meng, Wei Deng, Derin C. D'Amico, and Samuel J. Danishefsky*

Dedicated to Professor Barry M. Trost

In the previous contribution[1] we outlined a conceptual framework and encouraging results for assembling the ring systems of CP-225,917 (**1**) and CP-263,114 (**2**)[2, 3] (Figure 1).

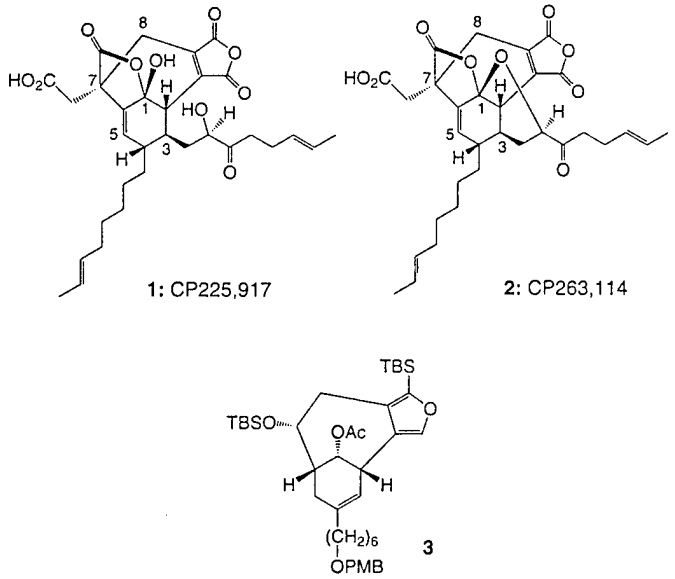
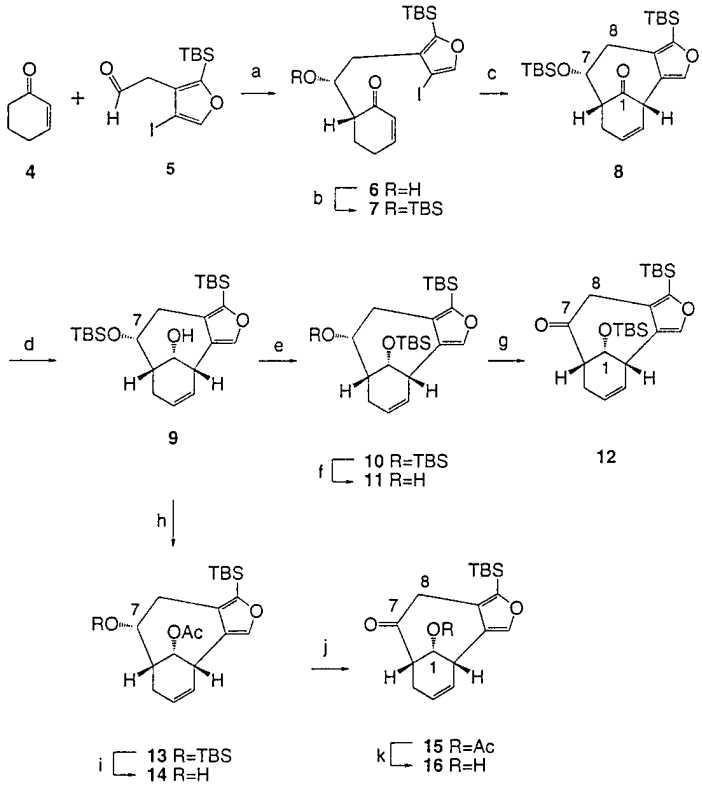


Figure 1. Structures of **1** and **2** as well as the central starting material **3**.

The key element of our synthetic program is the rapid construction of an intermediate lacking the C5–C6 bridgehead double bond (**3**) through the proper sequencing of aldol and intramolecular Heck-type bond formations using a 2,3,4-trisubstituted furan as a connecting device. We demonstrated how the resultant product of this sequence provides implementation sites through which the requisite functionality for the six-membered ring can be emplaced.[1]

Herein, we turn our attention to the more complex functionality found in the seven-membered ring of **1** and **2**. To explore our ideas concerning this sector of the natural products, we utilized the previously described **3**[1] as well as compound **8**, which lacks the substituent of C4 of the cyclohexenone ring (Scheme 1). Compound **8** was assembled through aldol condensation of cyclohexenone **4** with aldehyde **5**, which was also described in the previous article.[1] In this case, the stereospecificity was somewhat diminished relative to that encountered en route to **3**. The reaction provided **6** in 79% yield (as well an apparent diastereoisomer in 10% yield).[4] Protection of the secondary alcohol group gave rise to **7**. Intramolecular Heck vinlylation, as earlier,[1] provided **8** in 92% yield.



Scheme 1. Synthesis of ketones **12** and **16**. a) **4**, LDA, THF, −78 °C, 1 h, then **5**, THF, −78 °C, 2 h, 79% plus 10% diasteromer; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C→RT (room temperature), 1 h, 85%; c) $[Pd(OAc)_2(PPh_3)_2]$, NEt_3, THF, △, 4 d, 92%; d) DIBAlH, CH_2Cl_2, −78 → −30 °C; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C→RT, 16 h, 61% over two steps; f) EtOH/10% H_2SO_4 (10/1), RT, 31 h; g) Dess–Martin periodinane, CH_2Cl_2, RT, 0.5 h, 85% over two steps; h) Ac_2O, pyridine, DMAP; i) TBAF, AcOH, THF, RT; j) Dess–Martin periodinane, CH_2Cl_2, 93% over three steps; k) K_2CO_3, MeOH, RT, 88%. DIBAlH = diisobutylaluminum hydride, DMAP = 4-(dimethylamino)pyridine, LDA = ithium diisopropylamide, TBAF = tetrabutylammonium fluoride, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl.

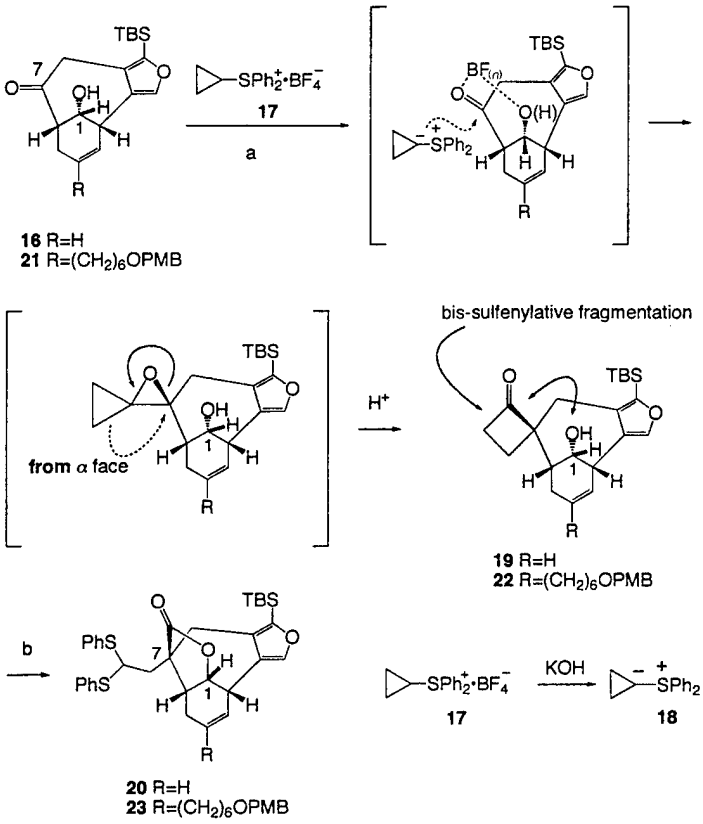
[*] Prof. S. J. Danishefsky, O. Kwon, D. Meng, Dr. D. C. D'Amico
Department of Chemistry
Columbia University
Havemeyer Hall, New York, NY 10027 (USA)
Fax: (+1) 212-772-8691
Dr. D.-S. Su, Dr. W. Deng (and further address for Prof. S. J. Danishefsky, D. Meng)
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)

[**] This research was supported by the National Institutes of Health (grant number CA-28824 and HL25848). Predoctoral fellowship support is gratefully acknowledged by O.K. (K.A.S.T.) and D.M. (U.S. Army). Postdoctoral fellowship support by the NIH is gratefully acknowledged by W.D. (grant number CA-67743) and D.C.D. (grant number F32 GM 17353).

  1433-7851/98/3716-1880 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **1998**, *37*, No. 13/14

We next sought to generate a unique ketone group at C7 so that we could pursue some interesting possibilities for the installation of the quaternary center. Toward this end, compound **8** was reduced with diisobutylaluminum hydride, and the resultant carbinol **9** was silylated to give **10**. Selective desilylation at C7 (→**11**) could be achieved because, in fact, the C1 silyloxy group proved to be difficult to cleave. Oxidation of **11** provided ketone **12**.[5] In a parallel series of reactions, **9** was acetylated, giving rise to **13**. Desilylation of the latter led to **14**. Oxidation provided **15**, and, following deacetylation, keto alcohol **16** was then in hand. Many attempts were made to deliver various carbon-based nucleophiles to the C7 ketone group of **12**.[6] Only marginal success was realized toward this goal. It seemed that a significant complicating feature was enolization of the ketone by deprotonation at C8.

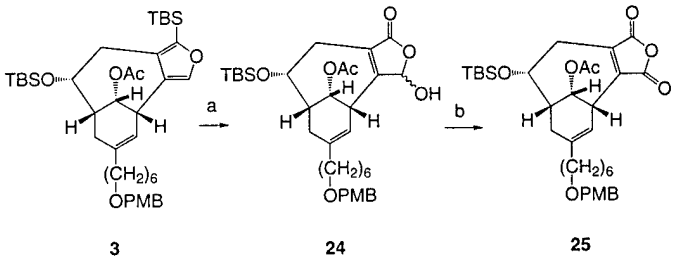
It was from this perspective that we turned to the use of the Trost sulfonium salt **17**.[7, 8] The hope was that the derived ylide **18**, being relatively nonbasic, would provide more favorable possibilities for C–C bond formation at C7 relative to enolization at C8 (Scheme 2). In practice, ketone **12** proved



Scheme 2. Synthesis of lactones **20** and **23**. a) **17**, KOH, DMSO, RT, 2.5 h, then aq HBF_4, Et_2O, RT, 56% for **19**, 46% for **22**; b) PhSSPh, NaOMe, MeOH, △, 5 d for **20**, 65 h for **23**, 81% for **20** based on 23% recovery of **19**, 35% for **23**. PMB = *p*-methoxybenzyl.

to be quite resistant to attack of **18** (generated from **17** through the action of potassium hydroxide).[8] We wondered about the possibility that the C7 ketone group of the hydroxyketone **16** might provide a more reactive electrophilic, site. Treatment of **16** with **18** occurred in a highly stereoselective fashion to provide, after acidic treatment, a spirocyclobutanone. From close analysis of the NMR spectrum, it seemed likely that **18** already had the stereochemistry indicated in **19**, wherein the spiroketone is on the β-face (i.e., *syn* to the C1 bridge). Such an outcome would be expected if formation of the carbon–carbon bond in the Trost reaction between **16** and **18** had occurred in the expected α sense (perhaps as a consequence of an emerging salt bridge between the oxido functions at C1 and C7). This surmise concerning the stereostructure of **19** was confirmed in a manner which itself augurs well for the synthesis of **1** and **2**: Under the Trost conditions[9] for bis-sulfenylation of the cyclobutanone, we obtained a cyclobutanone cleavage product in the form of lactone **20** in 62% yield.[10] In a similar way, **3** was converted into hydroxyketone **21**,[1] whose reaction with **18** and afforded **22** after workup with acid. Once again, under the conditions of bis-sulfenylative fragmentation of the cyclobutanone, compound **23** was obtained.

We next turned to validate the hypothesis concerning the use of the silylfuran as an entry to the maleic anhydride moiety. Encouragement toward this hope was secured upon photooxidation of **3** under the conditions shown in Scheme 3.



Scheme 3. Synthesis of anhydride **25**. a) O_2, rose bengal, $NEt(iPr)_2$, CH_2Cl_2, *hv*, 0 °C, 3.5 h, 93%; b) PCC, molecular sieves (4 Å), CH_2Cl_2, RT, 50 min, 83%. PCC = pyridinium chlorochromate.

This treatment gave rise to compound **24** as a mixture of diastereomers.[11, 12] Further oxidation with PCC afforded the anhydride **25**.[13]

Several problems await solution for the challenge of total syntheses of **1** and **2** to be surmounted. Foremost among these is the timing for introduction of the C5–C6 bridgehead double bond.[14] Nonetheless, the achievements accomplished, thus far in relatively few steps, encourage continued efforts directed at this goal.

Received: March 4, 1998 [Z11554IE]
German version: *Angew. Chem.* **1998**, *110*, 1981–1983

Keywords: alkylations · natural products · photooxidations · synthetic methods · ylides

[1] O. Kwon, D.-S. Su, D. Meng, W. Deng, D. C. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1978–1981; *Angew. Chem. Int. Ed.* **1998**, *37*, 1877–1880.

[2] T. T. Dabrah, T. Kaneko, W. Massefski, Jr., E. B. Whipple, *J. Am. Chem. Soc.* **1997**, 119, 1594.

  1433-7851/98/3716-1881 $ 17.50+.50/0

[3] T. T. Dabrah, H. J. Harwood, Jr., L. H. Huang, N. D. Jankovich, T. Kaneko, J.-C. Li, S. Lindsey, P. M. Moshier, T. A. Subashi, M. Therrien, P. C. Watts, *J. Antibiot.* **1997**, 50, 1.

[4] The ratio varied between 8:1 to 10:1 in favor of **6**. The Heck closure of the unprotected **6** was also accomplished, but in much lower yield and with poor reproducibility.

[5] a) D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, 48, 4155; b) R. E. Ireland, L. Liu, *J. Org. Chem.* **1993**, 58, 2899.

[6] Compound **12** was recovered after exposure to the conditions of the Wittig or Wadsworth–Emmons–Horner condensation. Similarly, it was unreactive toward the corresponding antimony ylide ($SbBu_3$, $N_2CH_2CO_2CH_3$, CuI (cat.), PhH, 50 °C). Addition of lithium ethoxyacetylide provided only a 21% yield of a tertiary alcohol (with 43% starting material recovered). The formation of the corresponding α,β-unsaturated ester from this product was not successful. A Peterson-type reaction with ethyl lithiotrimethylsilylacetate afforded only a 28% yield of the α,β-unsaturated ester (with 34% starting material recovered). By contrast, Reformatsky reaction provided almost complete transformation to a β-hydroxy ester. However, dehydration of this compound has thus far not been achieved. A modified Reformatsky reaction (with methyl dichloroacetate and Zn in the presence of diethylaluminum chloride: K. Takai, Y. Hotta, K. Oshima, H. Nozaki, *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1698) produced the α,β-unsaturated ester in 73% yield. Early attempts to deliver a one-carbon fragment to the enoate have not been productive. Given our success in spirobutanone formation, these studies have not yet been pursued in detail.

[7] B. M. Trost, M. J. Bogdanowicz, *J. Am. Chem. Soc.* **1973**, 95, 5311.

[8] B. M. Trost, M. J. Bogdanowicz, *J. Am. Chem. Soc.* **1973**, 95, 5321.

[9] B. M. Trost, J. H. Rigby, *J. Org. Chem.* **1976**, 41, 3217.

[10] At the present time we cannot be certain whether lactone formation occurs upon acidification of the product obtained upon opening of the four-membered ring (*seco*-cleavage product) or by the more interesting possibility wherein the C1 hydroxyl participates as the nucleophile in the cleavage step following bis-sulfenylation.

[11] M. R. Kernan, D. J. Faulkner, *J. Org. Chem.* **1988**, 53, 2773.

[12] S. R. Magnuson, L. Sepp-Lorenzino, N. Rosen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1998**, 120, 1615.

[13] Selected physical properties for **25**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.22 (d, $J = 8.6$ Hz, 2H), 6.84 (d, $J = 6.8$, 2.1 Hz, 2H), 5.34 (t, $J =$ 5.6 Hz, 1H), 5.12 (d, $J = 5.6$ Hz, 1H), 4.39 (s, 2H), 3.93 (dt, $J =$ 11.4 Hz, 1H), 3.77 (s, 3H), 3.58 (t, $J = 5.7$ Hz, 1H), 3.40 (t, $J =$ 6.5 Hz, 2H), 2.69 (dd, $J = 5.6$, 0.9 Hz, 1H), 2.53 (m, 3H), 2.31 (dd, $J = 10.2$ Hz, 1H), 2.02 (s, 3H), 1.58 (m, 4H), 1.28 (m, 6H), 0.85 (s, 9H), 0.039 (s, 3H), 0.007 (s, 3H); FT-IR (neat): 1771, 1698, 1558, 1508, 1248, 1091 cm^{-1}; LR-MS calcd for $C_{34}H_{48}O_5SiNa$: 635.3, found: 635.3 [M+Na].

[14] The construction of the C5–C6 bridgehead double bond has been accomplished recently: D. Meng, Columbia University, unpublished results.

  1433-7851/98/3716-1882 $ 17.50+.50/0

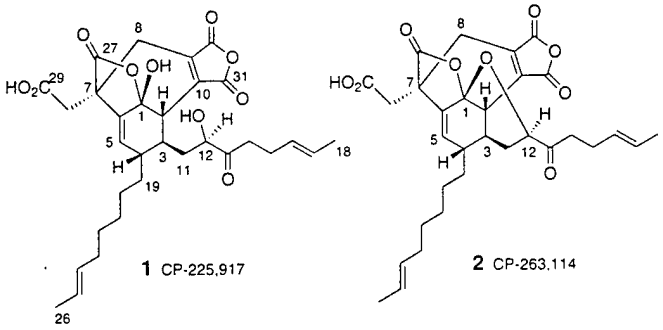
Stereospecific Sulfur-Mediated Cleavage of a Spirocyclobutanone: Synthesis of a Fully Functional Precursor to the CP Compounds**

Dongfang Meng and Samuel J. Danishefsky*

The screening of diverse sources of naturally occurring organic substances has many purposes. First, and no doubt foremost, is the hope that the activity profiles of some of the isolates may be of value for biomedical purposes. Compounds derived in this way may themselves become drugs, or may provide orienting leads for drug discovery. Indeed, some of the most important agents in the pharmaceutical industry began life in the context of natural products. Given major advances in separation sciences, structure determination by physical measurements, and the means for identifying specific cellular targets, the prospects for natural products as resources in drug discovery have never been better.[1, 2]

On rare occasion, compounds which arise from screening exercises are sufficiently striking that they emerge as milestone targets for the science of chemical synthesis. A mastery at the level of chemical synthesis of such systems may well result in analogue candidates for drug development. However, given enough architectural novelty, the milestone classification is not necessarily tied to any likely pharmaceutical advantage to be gained from chemical synthesis. Rather, the structure may pose an implicit challenge to the capabilities of synthesis. The vistas of synthesis grow from such confrontations, and the capacity of synthesis to contribute to projects with more likely benefit margins grows correspondingly.

Two recent compounds which well warrant the "milestone" designation are CP-225,917 and CP-263,114 (**1** and **2**, respectively) isolated at the Pfizer Laboratories.[3, 4] The derivations of these fascinating structures followed solely from spectroscopic measurements, and were not supported either by degradative or crystallographic validations. Although the compounds manifest two interesting forms of biological activity (inhibition of farnesyl transferase and squalene synthase), the great interest that they have engendered from the synthesis community can best be comprehended in terms of their novel molecular lattice work and the implicit challenge of such structures to chemists. Indeed, there is already a burgeoning literature describing highly imaginative approaches to assembling **1** and **2** in the laboratory.[5, 6] Clearly, any program which aspires to achieve a total synthesis of the CP compounds must transcend strictly regional issues and furnish provisions for all of the structural components of the natural products, including harmonization of competitive demands of various functional groups. Ideally, the synthesis program would be conducted with a view toward providing independent corroboration for the gross structure and configuration of **1** and **2**.



1 CP-225,917

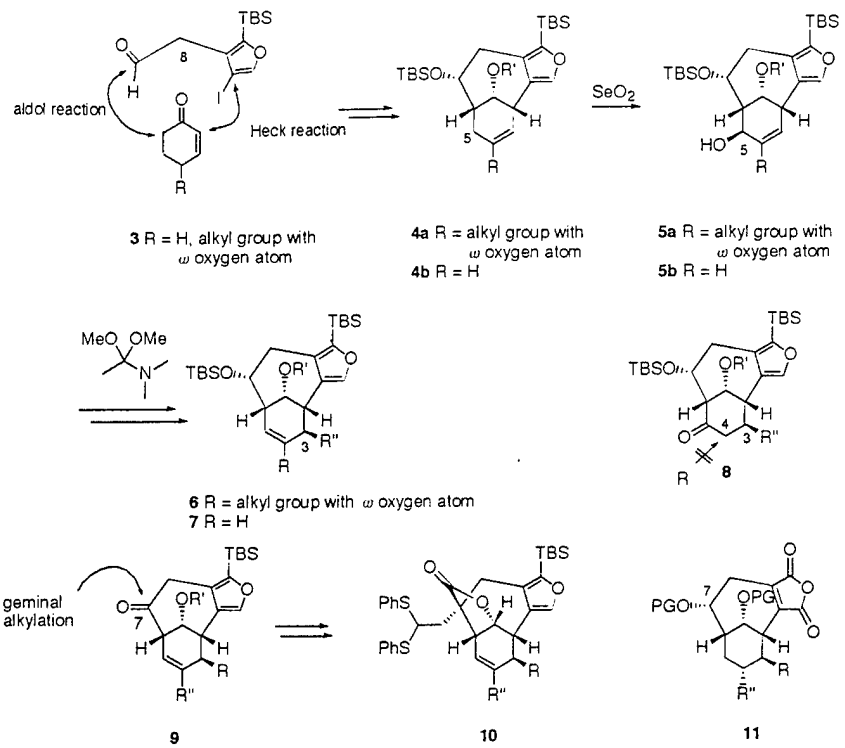
2 CP-263,114

Recently, we disclosed an approach to the CP problem using an initial intermolecular α'-aldol condensation of a cyclohexenone with furylacetaldehyde derivative **3** (Scheme 1). This merger was followed by a bridging intramolecular Heck ring closure, leading to a consensus matrix structure (**4**) which either did or did not contain a functional group at C4 (**4a** and **4b**, respectively). In this context we could functionalize C5 by selenium dioxide mediated hydroxylation and exploit the resultant C5 β-hydroxyl group to introduce a potentially useful structural implement at C3 (**5**→**6**). Unfortunately, in the **4a** series, the selenium dioxide functionalization step lacks any regiochemical preference between C5 and the allylic methylene substituent on the C4 side chain. By contrast, if the aldol-Heck sequence was conducted on the C4-unsubstituted system (i.e., cyclohexenone itself), the resulting product **4b**, lacking the C4 side chain, suffers clean functionalization at C5, setting the stage for the Claisen transposition (→**7**). Unfortunately, with the C3 handle in place, we were unable to introduce the C4 substituent from any structure derived from **7** (e.g. **8**). Clearly, these problems required careful attention.

In our earlier report, we had reached systems of the type **9**. The oxo function in these structures could be exploited to create a quarternary center at C7, albeit with a suboptimal level of oxidation in its branches (see lactone **10**).[6] Also, the furan ring was exploited, as planned, to provide a straightforward pathway to the 2,3-fused maleic anhydride appendage (→**11**).[6] Neither of the most advanced compounds **10** or **11** had the bridgehead (C5–C6) olefin in place. While it seemed possible that some of our chemical implements could be channeled to gain access to the C5–C6 olefin, the point had not been demonstrated in practice. Also to be addressed was the potentially serious question as to the extent to which a bridgehead double bond would be restrictive of other required chemistry. Here we describe a program wherein these difficulties have been resolved, and all required elements for a global synthesis of the CP compounds or analogues appear to be in place.

[*] Prof. S. J. Danishefsky, D. Meng
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-772-8691
E-mail: s-danishefsky@ski.mskcc.org
and
Department of Chemistry
Columbia University
Havemeyer Hall, New York, NY 10027 (USA)

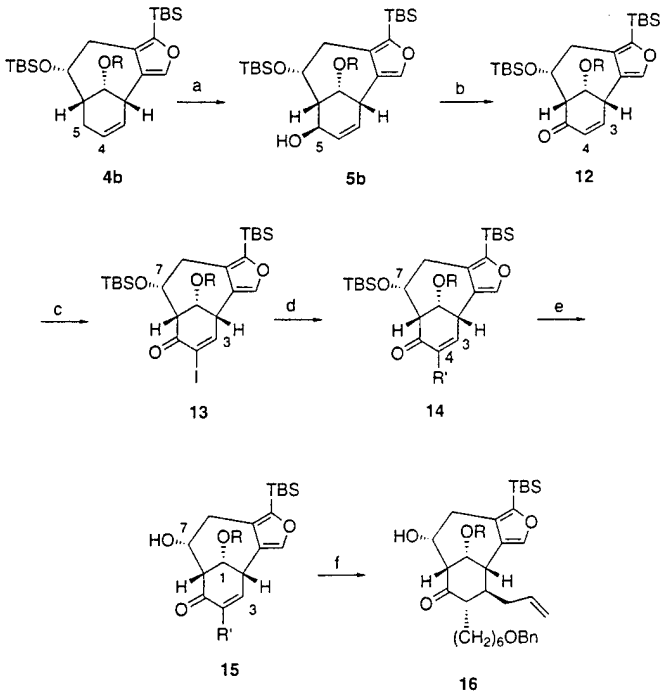
[**] This work was supported by the National Institutes of Health (grant numbers: HL 25848 and CA 28824 (S.J.D.) and CA-08748 (Sloan Kettering Institute Core Grant)). D.F.M. gratefully acknowledges the US Army for a predoctoral fellowship (grant number: F33 US Army DAMD 17-97-1-7146). We thank George Suckenick of the NMR Core Facility for mass spectral analyses.



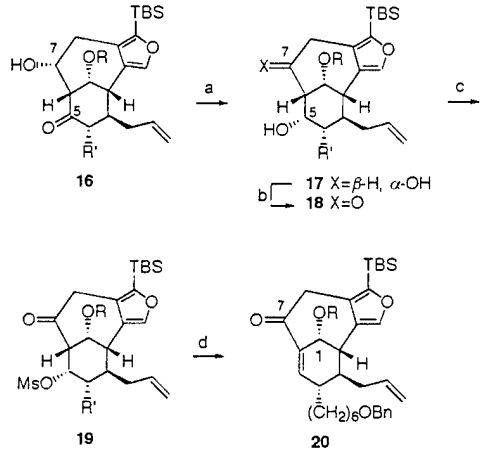
Scheme 1. Synthesis of the advanced CP precursors **10** and **11**.[6] TBS = *tert*-butyldimethylsilyl, PG = protecting group.

We returned to the previously described **4b**, which, upon oxidation with selenium dioxide and TPAP, afforded **12** (Scheme 2). The latter was converted into iodoenone **13** following the protocols of Johnson.[8] At this stage, we employed a *B*-alkyl Suzuki strategy[9, 10] to introduce the C4 side chain with the C5 oxo group already present (→**14**). In this way, the disastrous consequences of the regiorandom selenium-induced allylic dioxide hydroxylation of **4** were avoided. A variety of attempts to introduce a C3 substituent by conjugate addition to the enone linkage of **14** was to no avail. The logjam was broken as follows: A free alcohol functionality was exposed at C7 (→**15**). Presumably, this group plays a critical role in positioning the titanium tetrachloride derived Lewis acid moiety at the C5 oxo group. In the event, under these tightly defined circumstances, a Sakurai-type delivery of the β-oriented allyl group to C3 was accomplished.[12] Moreover, stereospecific β-protonation at C4 (either with HCl or on workup) afforded **16**. In this way, the stereochemical relationship assigned to the CP compounds at C3 and C4 had been simulated.

We turned next to installation of the C5–C6 double bond. Toward this end we could again exploit small but decisive differential reactivity margins. Thus, reduction of the C5 oxo group occurred from the β face (→**17**; Scheme 3). In a critical step, selective Swern



Scheme 2. Stereoselective introduction of side chains at C3 and C4 (R=TBS, R′ = $(CH_2)_6OBn$). a) SeO_2, 1,4-dioxane, 100 °C, 2 h, 90%; b) TPAP, NMO, CH_2Cl_2, 85%; c) I_2, pyridine, CH_2Cl_2, 95%; d) [$PdCl_2$(dppf)], Cs_2CO_3, $AsPh_3$, H_2O; R_3B, 70%; e) HF·pyridine, 90%; f) allyltrimethylsilane, $TiCl_4$, CH_2Cl_2, −78 °C, 75%. TPAP = tetrapropylammonium perruthenate, NMO = *N*-methylmorpholine *N*-oxide, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene, Bn = benzyl.



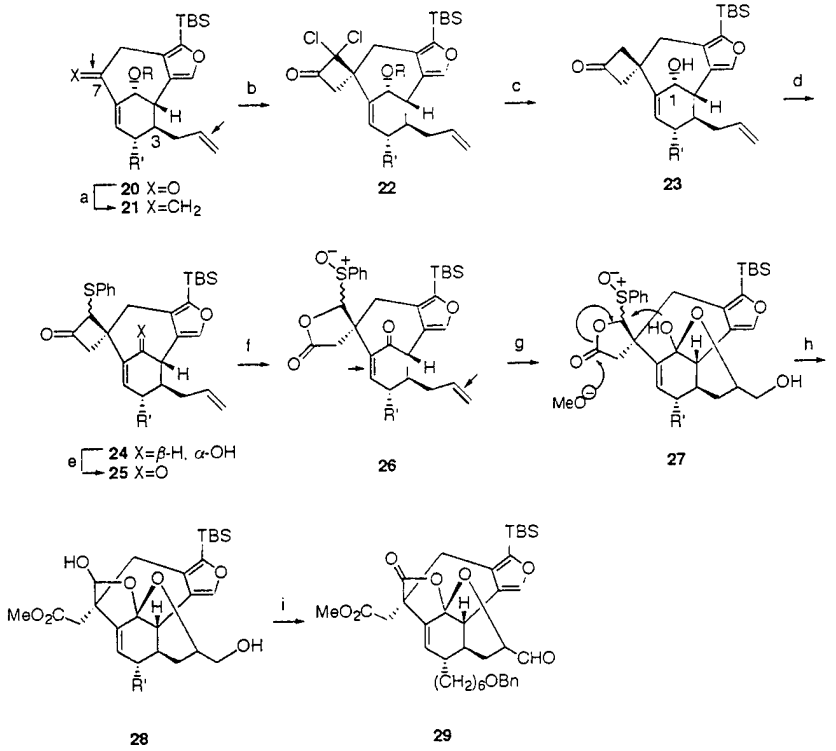
Scheme 3. Introduction of C5–C6 bridgehead double bond (R=TBS, R′ = $(CH_2)_6OBn$). a) $LiAlH_4$, Et_2O, 90%; b) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −78 °C; c) DMAP, CH_2Cl_2, MsCl, then Et_3N, MsCl, 70% for two steps; d) DBU, toluene, 80 °C, 90%. MsCl = methanesufonyl chloride, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

oxidation occurs at C7 to give monoketone **18**. At this stage, mesylation at C5 could be conducted (→**19**). Elimination of the mesyloxy functionality was achieved by the reaction of **19** with DBU, providing **20** with the C5–C6 (bridgehead) double bond in place.

It then transpired that with the bridgehead olefin moiety in nominal conjugation to the C7 oxo group,[13] the geminal alkylation methodology which had previously been employed

  1433-7851/99/3810-1486 $ 17.50+.50/0

to produce **10**[7] broke down completely due to the base lability of the system. A new program was thus devised in response to the very restricted terrain available for feasible chemical elaboration (Scheme 4). Fortunately, it did prove possible to conduct a Tebbe olefination of **20** (→**21**).[14]



Scheme 4. Construction of the quaternary center at C7 and the CP-263,114 building array (R = TBS, R' = $(CH_2)_6OBn$). a) Tebbe reagent, THF, −78 → −10 °C, 90%; b) trichloroacetyl chloride, Zn, Et_2O, DME, ultrasound, 85%; c) 1. Zn, NH_4Cl, MeOH, ultrasound, 80%; 2. TBAF, THF, 0 °C, 70%; d) PhSSPh, NaH/KH, THF, 80%; e) Dess–Martin periodinane, CH_2Cl_2, 90%; f) H_2O_2, MeOH, 70%; g) OsO_4,NMO, acetone/water, 70%; h) NaOMe, MeOH, 60%; i) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −78 °C, 70%. DME = dimethoxyethane, TBAF = tetrabutylammonium fluoride.

Remarkably, it was possible to realize a [2+2] cycloaddition of dichloroketene at the exocyclic double bond in the presence of the allyl group.[15] Moreover, and surprisingly, the reaction was highly stereoselective in the desired sense (→**22**). However, given the highly restricted menu of reactions feasible for the dichlorocyclobutanone functionality (it, too, is base labile), it was necessary to forgo the potential advantage of the geminal dichloro groups that are *syn* to the C1 bridge. Rather, both chlorine atoms were cleaved reductively and the C1 hydroxyl group was deprotected (→**23**). Possibly due to a directing effect of the free hydroxyl group, we could execute base-induced sulfenylation of **23** with diastereotopic specificity *syn* to the C1 bridge. At the kinetic level, it was possible to achieve stereoselectivity even within the required diastereotopic methylene group. However, in practice the configurational lability at this center was such that a diasterermeric mixture of **24** was employed in subsequent steps. Oxidation of **24** afforded **25**. While a regiospecific Baeyer–Villiger reaction, under the apparent control of the phenylsulfanyl group, could be conducted, the yield of the subsequent sequence of lactone cleavage and valence isomerization leading to **28** was rather low. This situation was much improved by further oxidation of the phenylsulfenyl lactone to the corresponding sulfoxide **26**. With the bridgehead double bond deactivated by its adjacency to the C1 oxo group, it proved possible to selectively dihydroxylate the terminal allyl group of **26**. Treatment of the crude hydroxylation product (see valence tautomer **27**)[16] with sodium methoxide in methanol afforded **28**, which on oxidation led to **29**.[17]

In various stages of these studies, we have demonstrated the viability of converting the furan moiety into the maleic anhydride unit. We have easily differentiated the aldehyde at C13 for relevant nucleophilic alkylation and, at an earlier stage, implemented the full CP side chain at C4. There remains now the still significant task of sequencing these capabilities in a fully harmonized program.

Received: January 7, 1999 [Z 12879 IE]
German version: *Angew. Chem.* **1999**, *111*, 1582–1585

Keywords: natural products · polycycles · synthetic methods

[1] H. Osada, *J. Antibiot.* **1998**, *51*, 973.
[2] S. L. Schreiber, *Bioorg. Med. Chem.* **1998**, *6*, 1127.
[3] T. T. Dabrah, T. Kaneko, W. Massefski, Jr., E. B. Whipple, *J. Am. Chem. Soc.* **1997**, *119*, 1594.
[4] T. T. Dabrah, H. J. Harwood, Jr., L. H. Huang, N. D. Jankovich, T. Kaneko, J.-C. Li, S. Lindsey, P. M. Moshier, T. A. Subashi, M. Therrien, P. C. Watts, *J. Antibiot.* **1998**, *50*, 1.
[5] Synthetic approaches to **1** and **2**: K. C. Nicolaou, M. W. Harter, L. Boulton, B. Jandeleit, *Angew. Chem.* **1997**, *109*, 1243; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1194; K. C. Nicolaou, M. H. D. Postema, N. D. Miller, G. Yang, *Angew. Chem.* **1997**, *109*, 2922; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2821; H. M. L. Davies, R. Calvo, G. Ahmed, *Tetrahedron Lett.* **1997**, *38*, 1737; P. W. M. Sgarbi, D. L. J. Clive, *Chem. Commun.* **1997**, 2157; A. Armstrong, T. J. Critchley, A. A. Mortlook, *Synlett* **1998**, 552; N. I. Waizumi, T. Ito, T. Fukuyama, *Tetrahedron Lett.* **1998**, *39*, 6015; C. Chen, M. E. Layton, M. D. Shair, *J. Am. Chem. Soc.* **1998**, *120*, 10784; M. M. Bio, J. L. Leighton, *J. Am. Chem. Soc.* **1999**, *121*, 890; K. C. Nicolaou, P. S. Baran, R. Jautelat, Y. He, K. C. Fong, H.-S. Choi, W. H. Yoon, Y.-L. Zhong, *Angew. Chem.* **1999**, *111*, 532; *Angew. Chem. Int. Ed.* **1999**, *38*, 549.
[6] O. Kwon, D.-S. Su, D. Meng, W. Deng, D. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1978; *Angew. Chem. Int. Ed.* **1998**, *37*, 1877; O. Kwon, D.-S. Su, D. Meng, W. Deng, D. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1981; *Angew. Chem. Int. Ed.* **1998**, *37*, 1880; A. J. Frontier, S. J. Danishefsky, G. A. Koppel, D. Meng, *Tetrahedron* **1998**, *54*, 12721.
[7] B. M. Trost, M. J. Bogdanowicz, *J. Am. Chem. Soc.* **1973**, *95*, 5311; B. M. Trost, M. J. Bogdanowicz, *J. Am. Chem. Soc.* **1973**, *95*, 5321; B. M. Trost, J. H. Rigby, *J. Org. Chem.* **1976**, *41*, 3217.
[8] C. R. Johnson, J. P. Adams, M. P. Braun, C. B. W. Senanayake, *Tetrahedron Lett.* **1992**, *33*, 917.
[9] Review: N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457.
[10] For a recent application of a *B*-alkyl Suzuki coupling in a complex context, see D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, *119*, 10073.
[11] The sequence started with hydroboration of 1-benxyloxy-5-hexene with diborane (alkene:diborane ≈ 4:1) in THF. The resultant solution was transferred to the reaction system for Suzuki coupling.
[12] A. Hosomi, H. Sakurai, *J. Am. Chem. Soc.* **1977**, *99*, 1673.

  1433-7851/99/3810-1487 $ 17.50+.50/0

[13] The extent of actual conjugation of the C7 oxo group with the bridgehead double bond is not clear. In NMR spectra, the chemical shift of the C5 proton in the enone suggests some conjugation.
[14] Review: S. H. Pine, *Org. React.* **1993**, *43*, 1.
[15] Review: J. A. Hyatt, P. W. Raynolds, *Org. React.* **1994**, *45*, 159.
[16] The osmylation product emerges as a complex mixture of ring and chain tautomers, which is further complicated by the existence of phenylsulfanyl epimers. Full purification is achieved only at the stage of **28** or **29**.
[17] Analysis of the NMR spectrum of aldehyde **29** suggests that the formyl group at C12 is in the α position. The stereochemical assignment for **2** presented the C13 oxo group as being in the β position. The stereochemical relationships between the two CP natural products at C12 have not been rigorously defined. These matters, as well as the order of thermodynamic stabilities at C12, will require further research.

  1433-7851/99/3810-1488 $ 17.50+.50/0