Chemical Synthesis and Biological Evaluation of *cis*- and *trans*-12,13-Cyclopropyl and 12,13-Cyclobutyl Epothilones and Related Pyridine Side Chain Analogues

K. C. Nicolaou,*,† Kenji Namoto,† Andreas Ritzén,† Trond Ulven,† Mitsuru Shoji,† Jim Li,† Gina D'Amico,‡ Dennis Liotta,‡ Christopher T. French,§ Markus Wartmann,⊥ Karl-Heinz Altmann,⊥ and Paraskevi Giannakakou§

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, Chemistry Department, Emory University, 1521 Pierce Drive, Atlanta, Georgia 30322, Winship Cancer Institute, Emory University School of Medicine, 1365-B Clifton Rd., Atlanta, Georgia 30322, and Novartis Pharma AG, TA Oncology Research, CH-4002, Basel, Switzerland

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Abstract: The design, chemical synthesis, and biological evaluation of a series of cyclopropyl and cyclobutyl epothilone analogues (**3**−**12**, Figure 1) are described. The synthetic strategies toward these epothilones involved a Nozaki−Hiyama−Kishi coupling to form the C15−C16 carbon−carbon bond, an aldol reaction to construct the C6−C7 carbon−carbon bond, and a Yamaguchi macrolactonization to complete the required skeletal framework. Biological studies with the synthesized compounds led to the identification of epothilone analogues **3**, **4**, **7**, **8**, **9**, and **11** as potent tubulin polymerization promoters and cytotoxic agents with (12*R*,13*S*,15*S*)-cyclopropyl 5-methylpyridine epothilone A (**11**) as the most powerful compound whose potencies (e.g. IC_{50} = 0.6 nM against the 1A9 ovarian carcinoma cell line) approach those of epothilone B. These investigations led to a number of important structure−activity relationships, including the conclusion that neither the epoxide nor the stereochemistry at C12 are essential, while the stereochemistry at both C13 and C15 are crucial for biological activity. These studies also confirmed the importance of both the cyclopropyl and 5-methylpyridine moieties in conferring potent and potentially clinically useful biological properties to the epothilone scaffold.

Introduction

With some members in clinical trials, the epothilones command special attention as potential anticancer agents of considerable promise. In addition to the several naturally occurring substances, an impressive array of epothilone analogues have been constructed and biologically evaluated.[1,2] In a preliminary communication,[3] we reported the chemical synthesis and biological data of 12,13-cyclopropyl and 12,13-cyclobutyl epothilones A (**3**−**6**, Figure 1) where the epoxide moiety of epothilone A (**1**, Figure 1) has been replaced by a small cycloalkane ring. Encouraged by the biological actions of **3** and **4** we have extended these investigations to other members of the 12,13-cycloalkane epothilone family (e.g. compounds **7**−**12**, Figure 1). In this article we report the details of these endeavors including chemical synthesis and biological activities of all analogues shown in Figure 1. Interestingly, these investigations revealed that compounds **3** and **4** exhibit comparable potencies to epothilone A (**1**) in cytotoxicity studies, supporting the notion that the overall shape of the epothilone scaffold is a most significant feature in these structures for biological activity, as opposed to the epoxide oxygen whose presence appears to be less relevant, at least for in vitro biological activity.[3,4] Remarkably, even the *trans* analogues **7**, **8**, and **11** show good

† The Scripps Research Institute and University of California, San Diego.
‡ Emory University.
§ Winship Cancer Institute, Emory University School of Medicine.
⊥ Novartis Pharma AG.

(1) For reviews from these laboratories, see: (a) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2014−2045. (b) Nicolaou, K. C.; Ritzén, A.; Namoto, K. *Chem. Commun.* **2001**, 1523−1535.

(2) (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*, 2801−2803. (b) 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*, 757−759. (c) Meng, D.; Bertinato, P.; Balog, A.; Su, D. S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073−10092. (d) Su, D.-S.; Balog, A.; Meng, D.; Bertinato, P.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2093−2096. (e) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 166−168. (f) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z.; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 525−527. (g) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268−272. (h) Schinzer, D.; Limberg, A.; Bauer, A.; Böhm, O. M.; Cordes, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 523−524. (i) May, S.; Grieco, P. A. *Chem. Commun.* **1998**, 1597−1598. (j) Chou, T.-C.; Zhang, X.-G.; Balog, A.; Su, D.-S.; Meng, D.; Savin, K.; Bertino J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642−9647. (k) White, J. D.; Sundermann, K. F.; Carter, R. G. *Org. Lett.* **1999**, *1*, 1431−1434. (l) Sinha, S. C.; Sun, J.; Miller, G.; Barbas, C. F., III; Lerner, R. A. *Org. Lett.* **1999**, *1*, 1623−1626. (m) Sawada, D.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 209−212. (n) Martin, H. J.; Drescher, M.; Mulzer, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 581−583. (o) Zhu, B.; Panek, J. S. *Org. Lett.* **2000**, *2*, 2575−2578. (p) Bode, J. W.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 3611−3612.

(3) Nicolaou, K. C.; Namoto, K.; Li, J.; Ritzén, A.; Ulven, T.; Shoji, M.; Zaharevitz, D.; Gussio, R.; Sackett, D. L.; Ward, R. D.; Hensler, A.; Fojo, T.; Giannakakou, P. *ChemBioChem* **2001**, *1*, 69−75.

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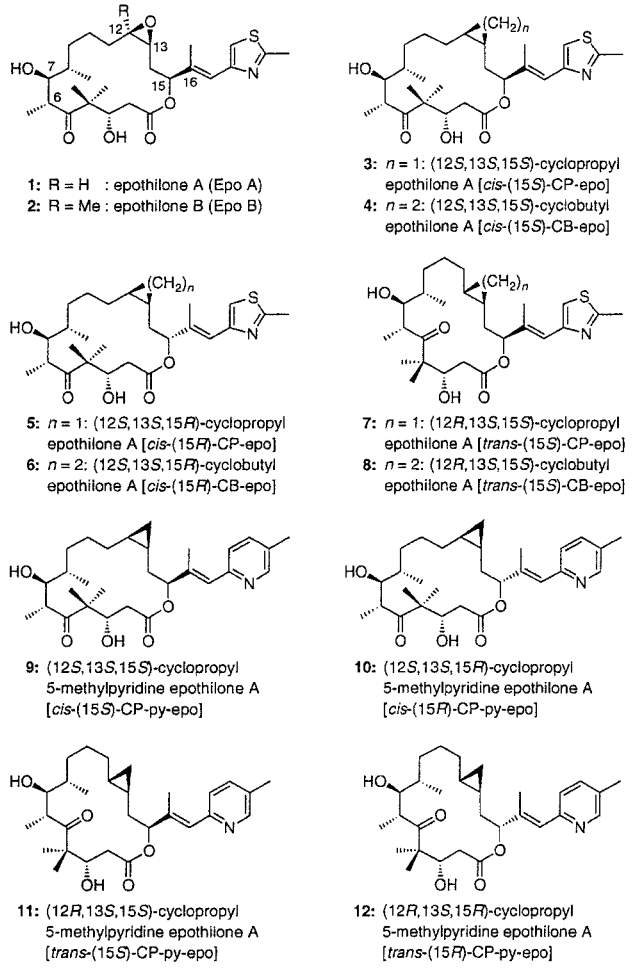


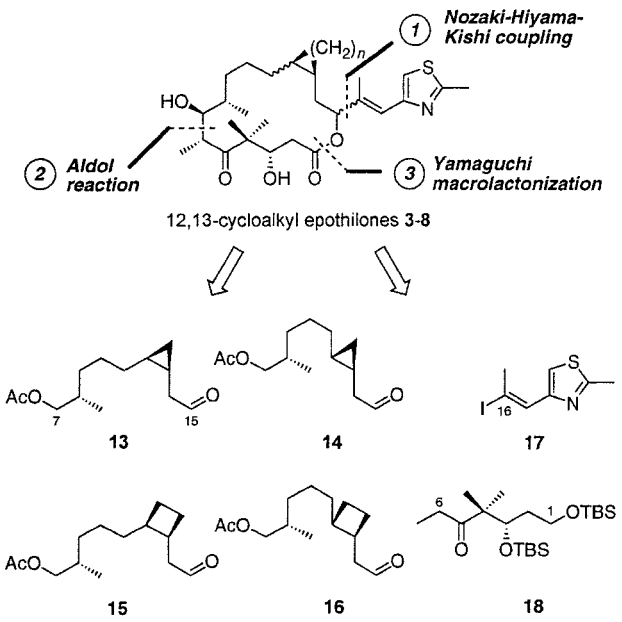
Figure 1. Epothilones prepared in this study.

to excellent activity, while all the other analogues, where the absolute configuration at C15 is *R* (**5**, **6**, **10**, and **12**), are virtually devoid of any cytotoxic activity. The most active analogue emerging from these endeavors was the 12,13-cyclopropyl 5-methylpyridine epothilone A (**11**), whose impressive cytotoxicity rivals that of epothilone B (**2**), the most potent naturally occurring epothilone.

Chemical Synthesis

Thiazole Epothilone Analogues. The chemical synthesis of the designed 12,13-cycloalkane thiazole epothilone analogues **3**−**8** was carried out according to a strategy derived from the retrosynthetic analysis shown in Scheme 1. Thus, a Nozaki−Hiyama−Kishi coupling,[5] an aldol reaction, and a Yamaguchi lactonization[6,7] were employed to disconnect the three strategic bonds as indicated, revealing building blocks **13**−**16**, **17**, and **18**. The assembly and elaboration of these building blocks to the final targets was to follow the order shown in

Scheme 1. Retrosynthetic Analysis and Key Fragments for Epothilone Analogues **3**−**8**



Scheme 1. Thus, coupling of the C7−C15 aldehyde fragment with the heterocyclic vinyl iodide, followed by elaboration and aldol reaction with the C1−C6 ketone segment, would lead, upon further elaboration, to the desired *seco*-hydroxy acid. Cyclization according to our Yamaguchi strategy[6,7] would then furnish, upon deprotection, the desired epothilone analogues.

The required building blocks **13**−**17** were synthesized as shown in Schemes 2−5, while ketone **18** was prepared by the previously described route.[7] The first required C7−C15 aldehyde (**13**) was constructed as shown in Scheme 2. Thus, Swern oxidation of optically active alcohol **19**[8] was followed by Wittig reaction and acid hydrolysis to afford the homologated aldehyde **20** in 85% overall yield. A second Wittig reaction employing commercially available phosphonium salt **21** led to a mixture of *cis* and *trans* olefins **22** (*cis*:*trans* ca. 20:1, 78% yield), which was reduced with diimide[9] to the saturated alcohol **23** (94% yield). Acetylation of the free hydroxyl group in **23** (100% yield) yielded acetate **24**, which upon hydrogenolysis of the benzyl ether afforded alcohol **25** (78% yield). Direct hydrogenation of **22** with palladium catalysts to simultaneously reduce the double bond and cleave the benzyl ether proved impractical, due to significant amounts of cyclopropyl ring-opened byproducts. Furthermore, although platinum catalysts cleanly reduced the double bond in **22**, they also reduced the aromatic ring of the benzyl group, rather than effecting hydrogenolysis of the C−O bond. Alcohol **25** was oxidized to the corresponding aldehyde (89% yield) with TPAP-NMO (for abbreviations of reagents and protecting groups, see legends in schemes), and then homologated to the desired aldehyde **13** via enol ether **26** by the two-step procedure described above for **20** (Wittig reaction followed by acidic hydrolysis), in 50% overall yield.

Shown in Scheme 3 is the synthesis of the *trans*-cyclopropyl aldehyde **14**, which closely parallels that of its *cis* counterpart **13** described above. Thus, a Charette cyclopropanation[8] of allylic alcohol **27**[10] yielded the enantiomerically enriched cyclopropane **29** in 98% yield (ee >90% by Mosher ester analysis).[11] Oxidation (SO_3·py) followed by Wittig reaction afforded enol ether **30** (81% overall yield), whose desilylation (TBAF), benzylation (NaH, BnBr), and acid

(4) Johnson, J.; Kim, S.-H.; Bifano, M.; DiMarco, J.; Fairchild, C.; Gougoutas, J.; Lee, F.; Long, B.; Tokarski, J.; Vite, G. *Org. Lett.* **2000**, *2*, 1537−1540.

(5) (a) Takai, K.; Kitamura, T.; Kuroda, T.; Hiyama, T.; Nozaki, H. *Tetrahedron Lett.* **1983**, *24*, 5281−5284. (b) Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644−5646.

(6) (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989−1993. (b) Mulzer, J.; Mareski, P. A.; Bushmann, J.; Luger, P. *Synthesis* **1992**, 215−228. (c) Nicolaou, K. C.; Hepworth, D.; King, N. P.; Finlay, M. R. V.; Scarpelli, R.; Pereira, M. M. A.; Bollbuck, B.; Bigot, A.; Werschkun, B.; Winssinger, N. *Chem. Eur. J.* **2000**, *6*, 2783−2800.

(7) Nicolaou, K. C.; Ninkovic, S.; Sarabia, F.; Vourloumis, D.; He, Y.; Vallberg, H.; Finlay, M. R. V.; Yang, Z. *J. Am. Chem. Soc.* **1997**, *119*, 7974−7991.

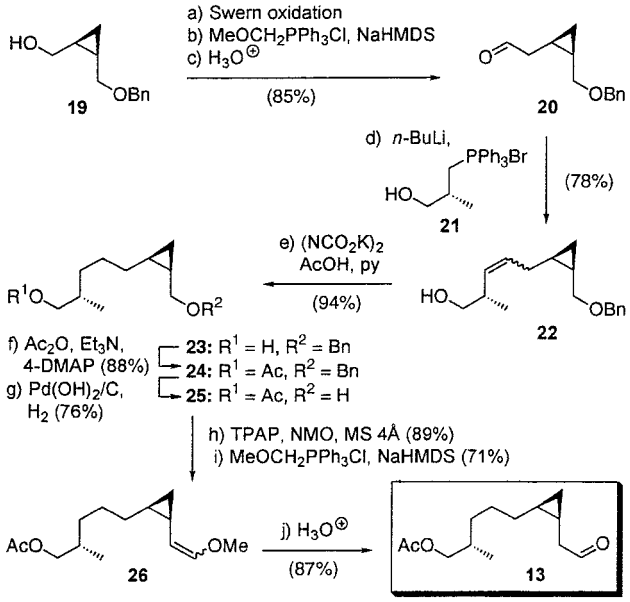
(8) Charette, A. B.; Juteau, H.; Lebel, H.; Molinaro, C. *J. Am. Chem. Soc.* **1998**, *120*, 11943−11952.

(9) Pasto, D. J.; Taylor, R. T. *Org. React.* **1991**, *40*, 92−155.

(10) (a) Azzena, F.; Calvani, F.; Crotti, P.; Gardelli, C.; Macchia, F.; Pinechi, M. *Tetrahedron* **1995**, *51*, 10601−10626. (b) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4743−4763.

(11) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549. (b) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

Scheme 2[a]



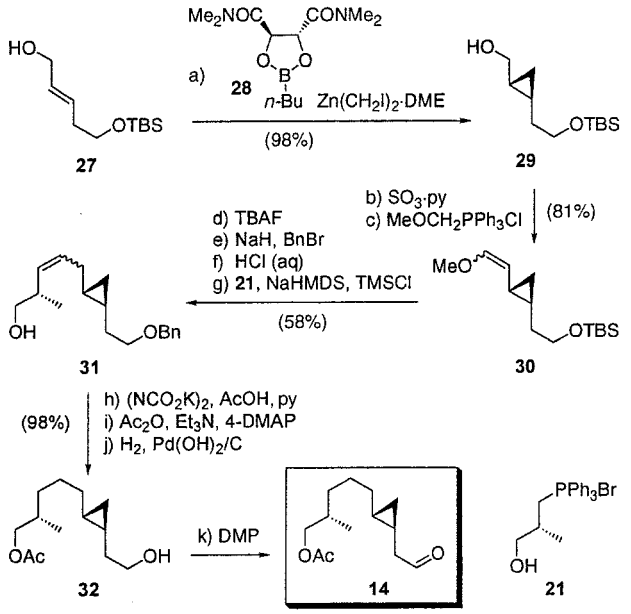
[a] Reagents and conditions: (a) $(COCl)_2$ (1.5 equiv) DMSO (2.0 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −78 °C; (b) $MeOCH_2PPh_3Cl$ (1.5 equiv), NaHMDS (1.4 equiv), THF, −78 °C; (c) catalytic HCl, acetone:water 9:1, 65 °C, 1 h, 85% over 3 steps; (d) **21** (1.5 equiv), *n*-BuLi (3.0 equiv), THF, −78 °C, 78%; (e) $(NCO_2K)_2$ (20 equiv), HOAc (40 equiv), MeOH, py, 25 °C, 48 h, 94%; (f) Ac_2O (1.1 equiv), Et_3N (1.2 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 0.5 h, 88%; (g) 20% $Pd(OH)_2/C$, H_2 (1 atm), EtOAc:EtOH 1:1 25 °C, 2 h, 76%; (h) TPAP (0.05 equiv), NMO (1.5 equiv), MS 4 Å, CH_2Cl_2, 25 °C, 1 h, 89%; (i) $MeOCH_2PPh_3Cl$ (1.2 equiv), NaHMDS (1.1 equiv), THF, 0 °C, 71%; (j) catalytic HCl, acetone:water 9:1, 55−60 °C, 2 h, 87%. 4-DMAP = 4-(dimethylamino)pyridine, NaHMDS = sodium hexamethyldisilazide, NMO = *N*-methylmorpholine *N*-oxide, py = pyridine, TPAP = tetra-*n*-propylammonium perruthenate.

hydrolysis led to the homologated aldehyde, which reacted with the ylide derived from phosphonium salt **21** to afford olefin **31** in 58% yield for the four steps. Diimide reduction, acetylation, and hydrogenolysis furnished alcohol **32** (98% overall yield). Dess−Martin oxidation then yielded the desired aldehyde **14**, which was not isolated, but rather used immediately for the subsequent Nozaki−Hiyama−Kishi coupling (vide infra).

The syntheses of the C12−C13-cyclobutyl aldehydes **15** and **16** were carried out as shown in Scheme 4. As these compounds are very closely related to the cyclopropane derivatives **13** and **14**, a similar synthetic route was again followed. Thus, starting from the monoacetate **33**, readily available through enzymatic group-selective saponification of the corresponding diacetate,[12] *cis*-aldehyde **34** was prepared by Dess−Martin periodinane oxidation (95% yield), while the corresponding *trans*-aldehyde **39** was conveniently available by base-catalyzed epimerization of **34** (88% from **33**). Following the route described for the cyclopropyl derivatives, **34** and **39** were homologated to **35** and **40**, respectively, and the latter compounds were coupled with the chiral phosphorane derived from enantiomerically pure phosphonium salt **21** and NaHMDS-TMSCl to yield olefins **36** and **41**, respectively. Hydrogenation of the double bond using a platinum catalyst [olefin **36** was initially reduced with diimide because it was done in parallel with **22**, where catalytic hydrogenation was not feasible, see discussion of **22** above; because the reduction was incomplete (see Supporting Information), further catalytic hydrogenation with Pt was necessary; it was later found that Pt hydrogenation alone worked for compound **41**] followed by standard protecting group manipulations afforded alcohols **38** and **43**, which were again homologated and protected, as summarized in Scheme 4, thus producing aldehydes **15** and **16**, respectively.

(12) (a) Laumen, K.; Schneider, M. *Tetrahedron Lett.* **1985**, *26*, 2073−2076. (b) Kasel, W.; Hultin, P. G.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1985**, 1563−1564.

Scheme 3[a]



[a] Reagents and conditions: (a) DME (2.2 equiv), Et_2Zn (2.2 equiv), CH_2I_2 (4.4 equiv), **28** (1.2 equiv), CH_2Cl_2, 98% yield, >90% ee; (b) Et_3N (6.0 equiv), SO_3·py (3.0 equiv), CH_2Cl_2:DMSO 4:1, 0 °C, 2 h; (c) $MeOCH_2PPh_3Cl$ (1.5 equiv), NaHMDS (1.3 equiv), THF, −40→25 °C, 12 h, 81% over 2 steps; (d) TBAF (1.5 equiv), THF 25 °C, 2 h; (e) NaH (1.5 equiv), BnBr (2.0 equiv), THF:DMF 5:1, 0→25 °C, 10 h; (f) catalytic HCl, acetone:water 9:1, 50 °C, 5 h; (g) **21** (1.5 equiv), NaHMDS (2.8 equiv), TMSCl (1.5 equiv), THF, 58% over 4 steps; (h) $(NCO_2K)_2$ (20 equiv), HOAc (40 equiv), MeOH, py, 25 °C, 7 h; (i) Ac_2O (2.0 equiv), Et_3N (5.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 20 min; (j) 20% $Pd(OH)_2/C$, H_2 (1 atm), EtOAc:EtOH 1:1 25 °C, 6 h, 98% over 3 steps; (k) DMP (1.2 equiv), CH_2Cl_2, 0→25 °C, 40 min. 4-DMAP = 4-(dimethylamino)pyridine, DME = dimethoxyethane, DMP = Dess−Martin periodinane, NaHMDS = sodium hexamethyldisilazide, py = pyridine, TBAF = tetrabutylammonium fluoride, TMSCl = chlorotrimethylsilane.

The requisite vinyl iodide **17** was constructed from aldehyde **44**[7] via a sequence involving (i) a modified Corey−Fuchs protocol[13] with in situ methylation of the intermediate acetylenide via intermediates **45** (88%) and **46** (97%), (ii) stereoselective hydrostannylation[14] (84%), and (iii) iodine−tin exchange (99%), as shown in Scheme 5. This sequence represents a significant improvement, regarding both simplicity and yields, over the preliminary route previously disclosed.[3]

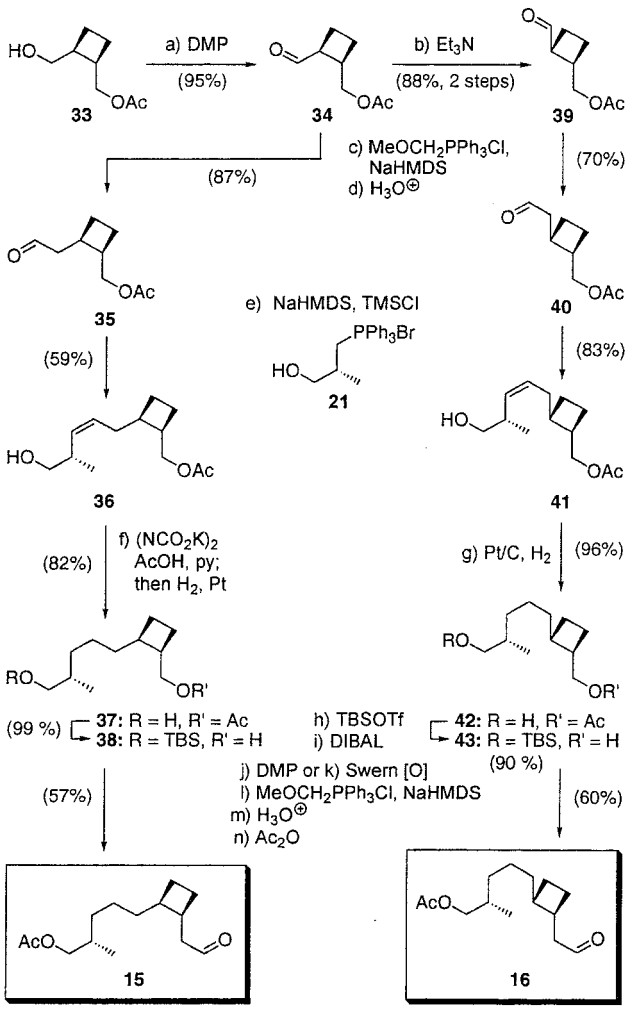
With all the building blocks in hand, final assembly of epothilone analogues **3**−**8** could begin. The cyclopropyl analogues **3**, **5**, and **7** were synthesized as shown in Scheme 6. Aldehyde **13** was coupled with vinyl iodide **17** by the Nozaki−Hiyama−Kishi procedure employing $CrCl_2$-$NiCl_2$,[5] furnishing a diastereomeric mixture of alcohols **48** (ca. 1:1 ratio, 56% yield, unoptimized). This mixture was taken through the sequence until chromatographic separation of the two isomers became feasible upon Yamaguchi macrolactonization (vide infra).[6] Silylation of **48** (TBSOTf-2,6-lutidine, 100% yield) furnished silyl ether **49,** which was deacetylated (DIBAL, 99% yield) to yield the advanced intermediate alcohol **50**. In a similar way, *trans*-aldehyde **14** was coupled with iodide **17**, but this time, oxidation (DMP) of the resulting mixture of epimeric secondary alcohols led to ketone **56** in 75% overall yield. Stereoselective reduction of **56** with (−)-DIPCl[15] afforded alcohol **57** as a single stereoisomer (by ^1H NMR spectroscopy) in 84% yield, thus demonstrating the flexibility of this route to generate either one

(13) Grandjean, D.; Pale, P.; Chuche, J. *Tetrahedron Lett.* **1994**, *35*, 3529−3530.

(14) Betzer, J.-F.; Ardisson, J.; Lallemand, J.-Y.; Pancrazi, A. *Tetrahedron Lett.* **1997**, *38*, 2279−2282.

(15) (a) Brown, H. C.; Ramachandran, P. V. *Acc. Chem. Res.* **1992**, *25*, 16−24. (b) Brown, H. C.; Park, W. S.; Cho, B. T.; Ramachandran, P. V. *J. Org. Chem.* **1987**, *52*, 5406−5412.

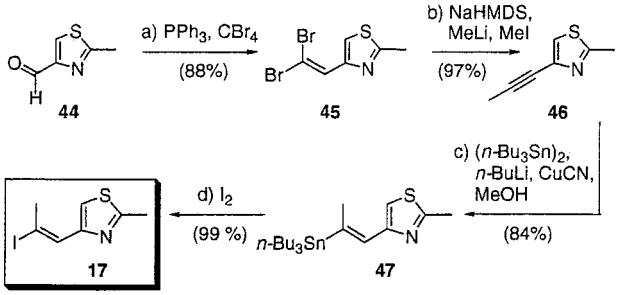
Scheme 4[a]



[a] Reagents and conditions: (a) DMP (1.2 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 25 °C, 3 h, 95%; (b) starting with alcohol **33**: $(COCl)_2$ (1.1 equiv), DMSO (2.2 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −78 °C; then Et_3N, 25 °C, 5 days, 88% over 2 steps; (c) $MeOCH_2PPh_3Cl$ (1.15 equiv), NaHMDS (1.10 equiv), THF, −78→25 °C, 89%; (d) 0.12 N HCl (aq):acetone (1:9), reflux, 1 h, 98% (**35**), 94% (**40**); (e) **21** (2.0 equiv), NaHMDS (3.8 equiv), THF, 0 °C, 2 h; then TMSCl (2.0 equiv), 25 °C, 20 min; then **35** (or **40**), THF, −78→25 °C, 20 h, 59% (**36**), 83% (**41**); (f) $(NCO_2K)_2$ (20 equiv), AcOH (40 equiv), py:MeOH (5:1), 25 °C, 48 h; then PtO_2 (0.05 equiv), H_2 (1 atm), MeOH, 25 °C, 20 min, 82%; (g) 10 wt % Pt on carbon (0.02 equiv), H_2 (1 atm), EtOAc, 25 °C, 8 h, 96%; (h) TBSOTf (1.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78→0 °C, 20 min; (i) DIBAL (2.0 equiv), CH_2Cl_2, −78 °C, 5 min, 99% (**38**), 90% (**43**) for 2 steps; (j) DMP (1.2 equiv), $NaHCO_3$ (5.1 equiv), CH_2Cl_2, 25 °C, 3 h, 94% (**38**); (k) $(COCl)_2$ (1.1 equiv), DMSO (2.2 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −78→25 °C, 97% (**43**); (l) $MeOCH_2PPh_3Cl$ (1.15 equiv), NaHMDS (1.10 equiv), THF, −78→25 °C; (m) 0.12 N HCl (aq):acetone (1:9), reflux, 1 h; (n) Ac_2O (1.1 equiv), Et_3N (2.5 equiv), 4-DMAP (0.02 equiv), CH_2Cl_2, 0 °C, 20 min, 60% (**15**), 62% (**16**) for 3 steps. DIBAL = diisobutylaluminum hydride, 4-DMAP = 4-(dimethylamino)pyridine, DMP = Dess−Martin periodinane, NaHMDS = sodium hexamethyldisilazide, py = pyridine, TMSCl = chlorotrimethylsilane.

or both C15 epimers. The 15*S* stereochemistry was assumed based on the chirality of the reducing agent. Compound **57** was protected as a TBS ether (**58**, TBSOTf, 2,6-lutidine, 91% yield), which was then deacetylated with DIBAL to provide the desired alcohol **59** in 93% yield. The stage was now set for the stereoselective aldol coupling which was employed to simultaneously create the C6−C7 bond and set the stereochemistry at these stereocenters. To this end, DMP oxidation of **50** and **59**, respectively, was immediately followed by aldol addition of the previously described C1−C6 ketone **18**[7] using LDA according

Scheme 5[a]



[a] Reagents and conditions: (a) PPh_3 (4.0 equiv), CBr_4 (2.0 equiv), CH_2Cl_2, 0 °C, 4 h, 88%; (b) NaHMDS (1.0 equiv), MeLi (2.0 equiv), MeI (5.0 equiv), −78→25 °C, 12 h, 97%; (c) *n*-BuLi (4.0 equiv), $(n\text{-}Bu_3Sn)_2$ (4.0 equiv), CuCN (2.0 equiv), MeOH (110 equiv), THF, 87%; (d) I_2 (1.1 equiv), CH_2Cl_2, 0 °C, 99%. NaHMDS = sodium hexamethyldisilazide.

to our optimized protocol.[6c] In this manner, aldols **51** (63%) and **60** (70%) were generated and isolated with complete control of the C6−C7 stereochemistry (as determined by ^1H NMR spectroscopy). Protection of the secondary hydroxyl groups as the TBS ethers **52** and **61**, followed by a two-step oxidation of the C1 position (liberated selectively by the action of HF·py) and selective cleavage of the C15 TBS ether (TBAF), afforded the hydroxy acids **53** and **62**, respectively. Yamaguchi macrolactonization[6] of **53** gave a 69% combined yield of the protected epothilone derivatives **54** and **55**, which were chromatographically separated [**54** (42%); **55** (27%)]. Analogously, macrolactonization of **62** yielded bis-silyl ether **63** (53% from **61** after 5 steps). Desilylation of **54**, **55**, and **63** with 20% TFA in CH_2Cl_2 finally afforded the desired epothilone analogues **3**, **5**, and **7**, respectively. The 15*S* configuration of the *trans* analogue **7** was now further corroborated by comparison of the ^1H NMR spectrum of **7** with those of the *cis* isomers **3** and **5**, where the spectrum of **7** is more similar to that of **3** than to that of **5**, particularly considering the signals from the protons attached to C2 and C15 (see Supporting Information).

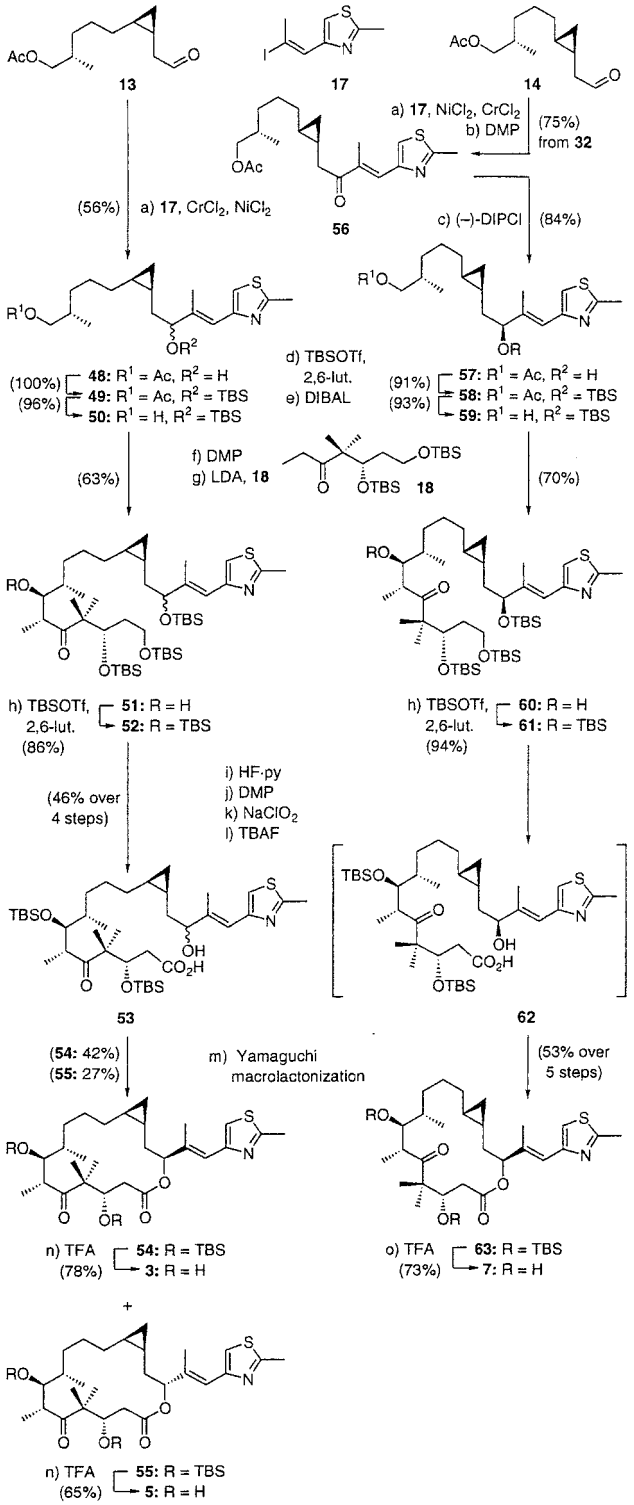
The *cis*-cyclobutyl thiazole epothilones **4** and **6** were assembled in an analogous fashion, as summarized in Scheme 7. A Nozaki−Hiyama−Kishi coupling between *cis*-aldehyde **15** and the side chain vinyl iodide **17** afforded the secondary alcohol **64** (89% yield) as a 1:1 mixture of C15 epimers. Protective group manipulations and oxidation yielded, via intermediates **65** and **66**, aldehyde **67**, which smoothly underwent the stereoselective aldol coupling reaction with ketone **18**, thus producing alcohol **68**. Further manipulation of protecting groups and oxidation of the C1 position yielded hydroxy acid **72**, which was cyclized by applying the Yamaguchi protocol to afford the two lactones **73** and **74**. At this point, the C15 epimers **73** and **74** were chromatographically separated and deprotected to yield the desired *cis*-cyclobutyl epothilones **4** and **6**, respectively, and in good overall yields.

The *trans*-cyclobutyl thiazole epothilone **8** was prepared by a similar sequence, as detailed in Scheme 8. Thus, after the Nozaki−Hiyama−Kishi coupling between aldehyde **16** and iodide **17**, the resulting alcohol was oxidized to the corresponding enone **75**, which was then stereoselectively reduced with (−)-DIPCl[15] to afford only the (15*S*)-epimer **76**. The remaining steps followed the same sequence described for the *cis* compounds (see Scheme 7), and proceeded smoothly and in similar yields, affording the targeted *trans*-cyclobutyl epothilone **8**.

Pyridine Epothilone Analogues. Some of the most active epothilone analogues prepared to date include within their structures a pyridine side chain as a replacement for the thiazole moiety of the naturally occurring substances.[16] Given the very promising preliminary results with cyclopropane epothilone analogue **3**, we reasoned that combining these two structural modifications might result in highly active compounds despite the absence of the epoxide oxygen. Such compounds (e.g. **9**−**12**, Figure 1) may be metabolically more stable leading to longer in vivo lifetime and lower toxicity. In an effort to improve the

(16) Nicolaou, K. C.; Scarpelli, R.; Bollbuck, B.; Werschkun, B.; Pereira, M. M. A.; Wartmann, M.; Altmann, K.-H.; Zaharevitz, D.; Gussio, R.; Giannakakou, P. *Chem. Biol.* **2000**, *7*, 593−599.

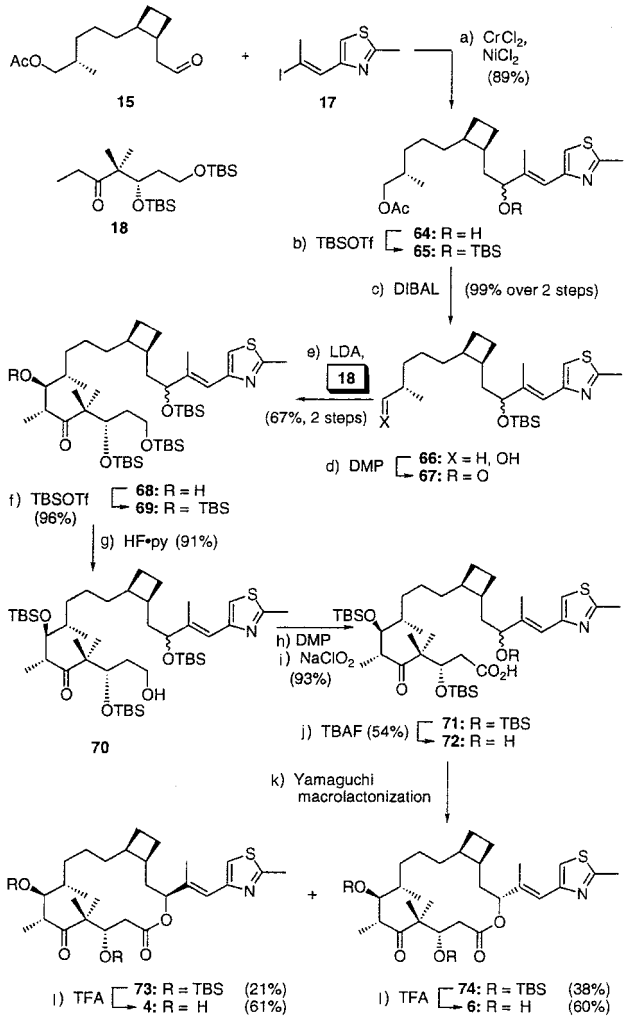
Scheme 6[a]



[a] Reagents and conditions: (a) **17** (1.5−2.0 equiv), $CrCl_2$ (10−13 equiv), $NiCl_2$ (0.02−0.13 equiv), DMSO, 25 °C, 6−12 h, 56% (**48**), 91% from **32**; (b) DMP (1.2 equiv), CH_2Cl_2, 0→25 °C, 0.5 h, 83%; (c) (−)-DIPCl (3.0 equiv), Et_2O, −15→25 °C, 18 h, 84%; (d) TBSOTf (1.1−2.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78 °C, 0.5−1 h, 91−100%; (e) DIBAL (2.0−3.1 equiv), CH_2Cl_2, −78 °C, 15 min to 1 h, 93−96%; (f) DMP (1.2 equiv), CH_2Cl_2, 25 °C, 1.5 h; (g) LDA (3.1 equiv), **18** (3.0 equiv), THF, −78 °C, 4 min, 63% (**51**), 70% (**60**); (h) TBSOTf (2.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −20→25 °C, 1.5−12 h, 86% (**52**), 94% (**61**); (i) HF·py, py, 0−25 °C, 3−4 h; (j) DMP (1.2−1.5 equiv), $NaHCO_3$ (1.5 equiv), CH_2Cl_2, 25 °C, 15 min to 2 h; (k) $NaClO_2$ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), NaH_2PO_4 (2.5 equiv), *t*-BuOH:H_2O 4:1, 25 °C, 10−20 min; (l) TBAF (12 equiv), THF, 25 °C, 16−26 h, 46% over 4 steps (**53**); (m) 2,4,6-trichlorobenzoyl chloride (2.4 equiv), Et_3N (6.0 equiv), THF, 0 °C, 1 h, then 4-DMAP (2.2 equiv), toluene, 75 °C, 3−11 h, 42% (**54**), 27% (**55**), 53% over 5 steps (**63**); (n) 20% TFA in CH_2Cl_2, 0 °C, 2 h, 78% (**3**), 65% (**5**); (o) 25% TFA in CH_2Cl_2, 25 °C, 7 h, 73%. DIBAL = diisobutylaluminum hydride, DIPCl = diisopinocampheyl chloroborane, 4-DMAP = 4-(dimethylamino)pyridine, DMP = Dess−Martin periodinane, LDA = lithium diisopropylamide, NaHMDS = sodium hexamethyldisilazide, py = pyridine, TBAF = tetrabutylammonium fluoride.

Footnote to Scheme 6 (continued)

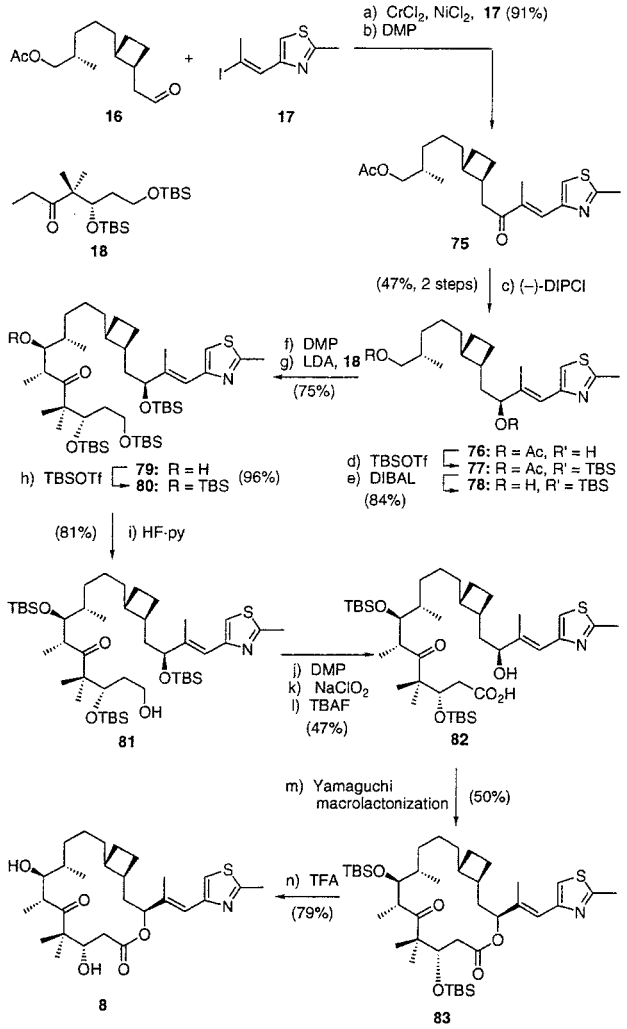
Scheme 7[a]



[a] Reagents and conditions: (a) **17** (1.5 equiv), $CrCl_2$ (12.6 equiv), $NiCl_2$ (0.13 equiv), DMSO, 25 °C, 6 h, (89%, 2:3 mixture of C15 epimers); (b) TBSOTf (1.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78→0 °C, 20 min; (c) DIBAL (2.0 equiv), CH_2Cl_2, −78 °C, 5 min, 99% for 2 steps; (d) DMP (1.2 equiv), CH_2Cl_2, 25 °C, 1.5 h; (e) LDA (3.1 equiv), **18** (3.0 equiv), THF, −78 °C, 4 min, 67% for 2 steps; (f) TBSOTf (2.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −20→25 °C, 12 h, 96%; (g) HF·py, py, THF, 0→25 °C, 3 h, 91% (h) DMP (1.2 equiv), $NaHCO_3$ (1.5 equiv), CH_2Cl_2, 25 °C, 2 h; (i) $NaClO_2$ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), NaH_2PO_4 (2.5 equiv), *t*-BuOH:H_2O 4:1, 25 °C, 10 min, 93% for 2 steps; (j) TBAF (12 equiv), THF, 25 °C, 26 h, 54%; (k) 2,4,6-trichlorobenzoyl chloride (2.4 equiv), Et_3N (6.0 equiv), THF, 0 °C, 1 h, then 4-DMAP (2.2 equiv), toluene, 75 °C, 3 h, 21% (**73**), 38% (**74**); (l) 20 v/v% TFA in CH_2Cl_2, 0 °C, 2 h, 61% (**4**), 60% (**6**). DIBAL = diisobutylaluminum hydride, 4-DMAP = 4-(dimethylamino)pyridine, DMP = Dess−Martin periodinane, LDA = lithium diisopropylamide, py-pyridine, TBAF = tetrabutylammonium fluoride.

overall synthesis of these compounds, and to accommodate future preparation of other side chain-modified analogues via a convergent

Scheme 8[a]

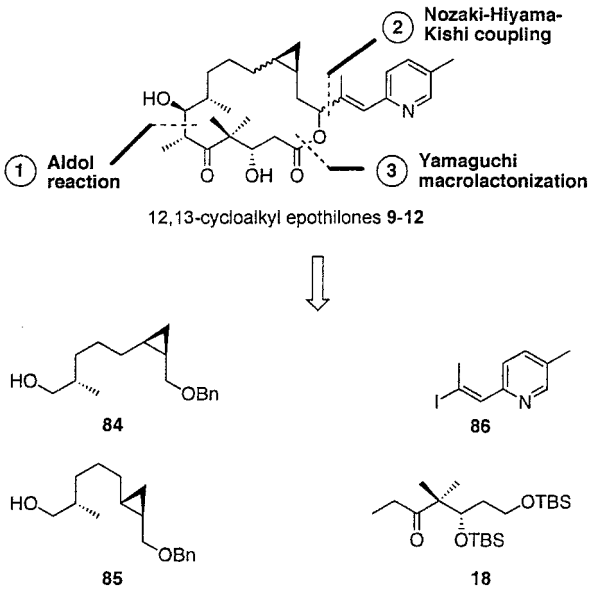


[a] Reagents and conditions: (a) **17** (1.5 equiv), $CrCl_2$ (12.6 equiv), $NiCl_2$ (0.13 equiv), DMSO, 25 °C, 6 h, 91%; (b) DMP (1.2 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 25 °C, 3 h; (c) (−)-DIPCl (3.0 equiv), Et_2O, −15→25 °C, 18 h, 47%, for 2 steps; (d) TBSOTf (1.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78→0 °C, 20 min; (e) DIBAL (2.0 equiv), CH_2Cl_2, −78 °C, 5 min, 84% for 2 steps; (f) DMP (1.2 equiv), CH_2Cl_2, 25 °C, 1.5 h; (g) LDA (3.1 equiv), **18** (3.0 equiv), THF, −78 °C, 4 min, 75% for 2 steps; (h) TBSOTf (2.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −20→25 °C, 12 h, 96%; (i) HF·py, py THF, 0→25 °C, 3 h, 81%; (j) DMP (1.2 equiv), $NaHCO_3$ (1.5 equiv), CH_2Cl_2, 25 °C, 2 h; (k) $NaClO_2$ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), NaH_2PO_4 (2.5 equiv), *t*-BuOH:H_2O 4:1, 25 °C, 10 min; (l) TBAF (12 equiv), THF, 25 °C, 26 h, 47% for 3 steps; (m) 2,4,6-trichlorobenzoyl chloride (2.4 equiv), Et_3N (6.0 equiv), THF, 0 °C, 1 h; then 4-DMAP (2.2 equiv), toluene, 75 °C, 3 h, 50%; (n) 20% TFA in CH_2Cl_2, 0 °C, 2 h, 79%. DIBAL = diisobutylaluminum hydride, DIPCl = diisopinocampheylchloroborane, 4-DMAP = 4-(dimethylamino)pyridine, DMP = Dess−Martin periodinane, LDA = lithium diisopropylamide, py = pyridine, TBAF = tetrabutylammonium fluoride.

strategy, a slightly different scheme for their total synthesis was designed based on the retrosynthetic analysis shown in Scheme 9. The devised strategy for the construction of the pyridine cycloalkane epothilones (**9**−**12**) is similar to that utilized for the total synthesis of their thiazole counterparts except for the reversal of the coupling order of the fragments. Thus, the aldol reaction of building blocks **84** and **85** with ketone **18** will now precede the Nozaki−Hiyama−Kishi coupling with vinyl iodide **86**.

The required building blocks **84** and **85** were prepared as shown in Scheme 10. A Wittig reaction between the ylide derived from the enantiomerically pure phosphonium salt **21** and NaHMDS-TMSCl, and the commercially available aldehyde **87** (68% yield), followed by

Scheme 9. Retrosynthetic Analysis and Key Fragments for Epothilone Analogs **9**−**12**



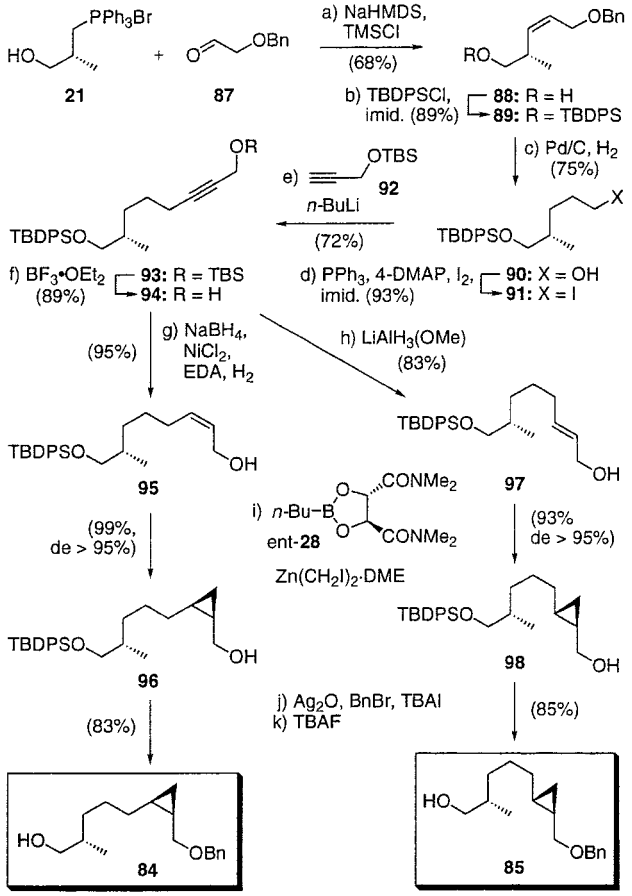
protection of the resulting alcohol **88** as its TBDPS ether (TBDPSCl-imid.), afforded alkene **89** in 89% yield. Hydrogenation of the double bond in **89** with concomitant cleavage of the benzyl ether gave primary alcohol **90** in 75% yield. This compound (**90**) was then converted into the corresponding iodide (**91**) in 93% yield by exposure to I_2/PPh_3. Coupling of **91** with alkyne **92** (*n*-BuLi, 72% yield), followed by removal of the TBS group ($BF_3 \cdot OEt_2$) from the resulting alkyne **93**, produced the propargylic alcohol **94** (89% yield). This compound was used as a common precursor to prepare both the *cis*- and the *trans*-cyclopropyl pyridine epothilone analogues (**9**−**12**). The synthesis of the *cis* series of compounds commenced with a nickel boride reduction[17] of alkyne **94** to furnish *cis* olefin **95** in 95% yield (Scheme 10), while the corresponding *trans* alkene (**97**) was prepared from the same intermediate (**94**) via reduction with $LiAlH_3(OMe)$[18] (83% yield). Charette cyclopropanation[8] of **95** and **97** smoothly afforded the cyclopropanes **96** (99% yield) and **98** (93%) in >95% de, as judged by ^1H NMR spectroscopic analysis of the corresponding Mosher esters.[11] Subsequent benzylation of the primary hydroxyl group, followed by removal of the silyl group at the other end of the molecule, led to the desired primary alcohols **84** and **85**, respectively.

The requisite side chain vinyl iodide **86** was synthesized as shown in Scheme 11. A Sonogashira coupling of 5-methyl-2-bromopyridine **99** with propyne[19] yielded alkyne **100** in 98% yield. This was then hydrostannylated, and the tin was exchanged for iodine (86% for two steps) by the same method as that employed to prepare the thiazole side chain precursor **17** (Scheme 5), thus yielding iodide **86** via stannane **101** (100% yield).

The final stages of the synthesis of the targeted pyridine analogues are depicted in Schemes 12 and 13. Oxidation of alcohols **84** and **85** with Dess−Martin periodinane was followed by the stereoselective aldol coupling with ketone **18**[7] previously employed (vide supra). This coupling was performed according to our general procedure,[6c] yielding aldols **102** (75% yield) and **108** (89% yield) with a dr of ca. 10:1 (by ^1H NMR spectroscopy) in both cases. Further elaboration of these compounds (**102** and **108**) involved TBS protection of their secondary alcohols, selective removal of the primary TBS group (HF·py), oxidation of the resulting primary alcohol (DMP; $NaClO_2$), and methylation of the so obtained carboxylic acid, leading to compounds

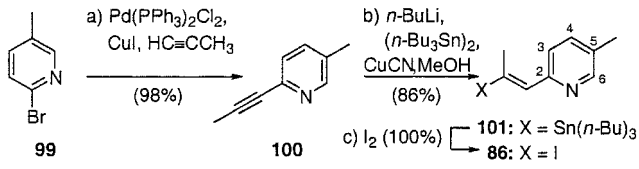
(17) Taber, D. F.; Herr, R. J.; Gleave, D. M. *J. Org. Chem.* **1997**, *62*, 194−198.
(18) Ashby, E. C.; Dobbs, F. R.; Hopkins, H. P., Jr. *J. Am. Chem. Soc.* **1975**, *97*, 3158−3162.
(19) Arcadi, A.; Cacchi, S.; Carnicelli, V.; Marinelli, F. *Tetrahedron* **1994**, *50*, 437−452.

Scheme 10[a]



[a] Reagents and conditions: (a) NaHMDS (2.1 equiv), TMSCl (1.1 equiv), THF, −78→25 °C, 6 h, 68%; (b) TBDPSCl (1.1 equiv), imidazole (2.0 equiv), DMF, 25 °C, 1 h, 89%; (c) 10% Pd/C, H_2 (1 atm), MeOH:THF 5:1, 50 °C, 10 h, 75%; (d) PPh_3 (1.4 equiv), 4-DMAP (0.01 equiv), I_2 (1.5 equiv), imidazole (2.0 equiv), MeCN/Et_2O, 5:3, 25 °C, 1 h, 93%; (e) *n*-BuLi (3.3 equiv), 3-(*tert*-butyldimethylsilyloxy)propyne (**92**), (3.5 equiv), THF/HMPA, 6:1, −78 to −30 °C, 2.5 h, 72%; (f) BF_3·OEt_2 (2.0 equiv), CH_2Cl_2, 25 °C, 1.5 h, 89%; (g) $NiCl_2$ (1.0 equiv), $NaBH_4$ (1.0 equiv), EDA (3.0 equiv), H_2 (1 atm), EtOH, 0 °C, 1 h, 95%; (h) $LiAlH_4$ (1.0 equiv), MeOH (1.0 equiv), THF, 50 °C, 0.5 h, 83%; (i) DME (2.2 equiv), Et_2Zn (2.2 equiv), CH_2I_2 (4.4 equiv), ent-**28** (1.2 equiv), CH_2Cl_2, −15→25 °C, 6 h, 99% (**96**), 93% (**98**); (j) Ag_2O (3.0 equiv), BnBr (2.6 equiv), TBAI (0.1 equiv), toluene, 24 h, 25→50 °C; (k) TBAF (5.0 equiv), THF, 25 °C, 4 h, 83% (**84**), 85% (**85**) over 2 steps. 4-DMAP = 4-(dimethylamino)pyridine, DME = dimethoxyethane, DMP = Dess−Martin periodinane, EDA = ethylenediamine, HMPA = hexamethylphosphoramide, NaHMDS = sodium hexamethyldisilazide, TBAF = tetrabutylammonium fluoride, TBAI = tetrabutylammonium iodide.

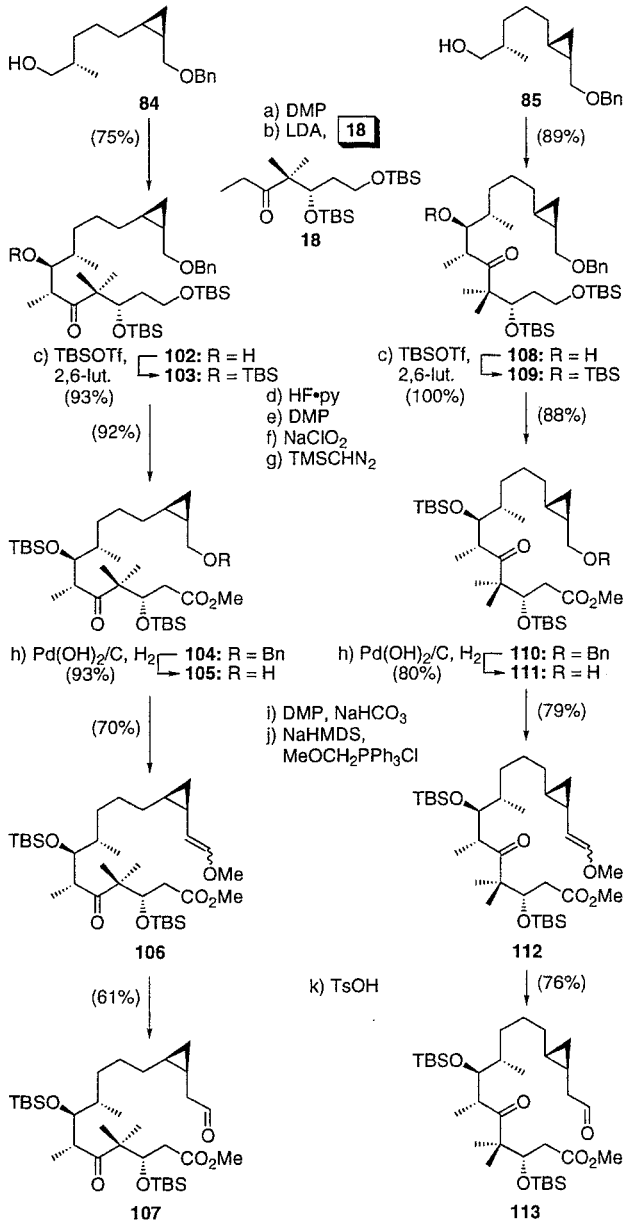
Scheme 11[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$ (0.01 equiv), CuI (0.02 equiv), propyne (1 atm), DMF/*i*-Pr_2NH, 6:5, 25 °C, 3 h, 98%; (b) *n*-BuLi (4.0 equiv), $(n\text{-}Bu_3Sn)_2$ (4.0 equiv), CuCN (2.0 equiv), MeOH (110 equiv), THF, −10 °C, 15 h, 86%; (c) I_2 (1.05 equiv), CH_2Cl_2, 25 °C, 5 min, 100%.

104 and **110**, as shown in Scheme 12. Hydrogenolysis of the benzyl ether from **104** and **110** was followed by oxidation of the resulting primary alcohols (**105** and **111**) to the corresponding aldehydes (DMP)

Scheme 12[a]

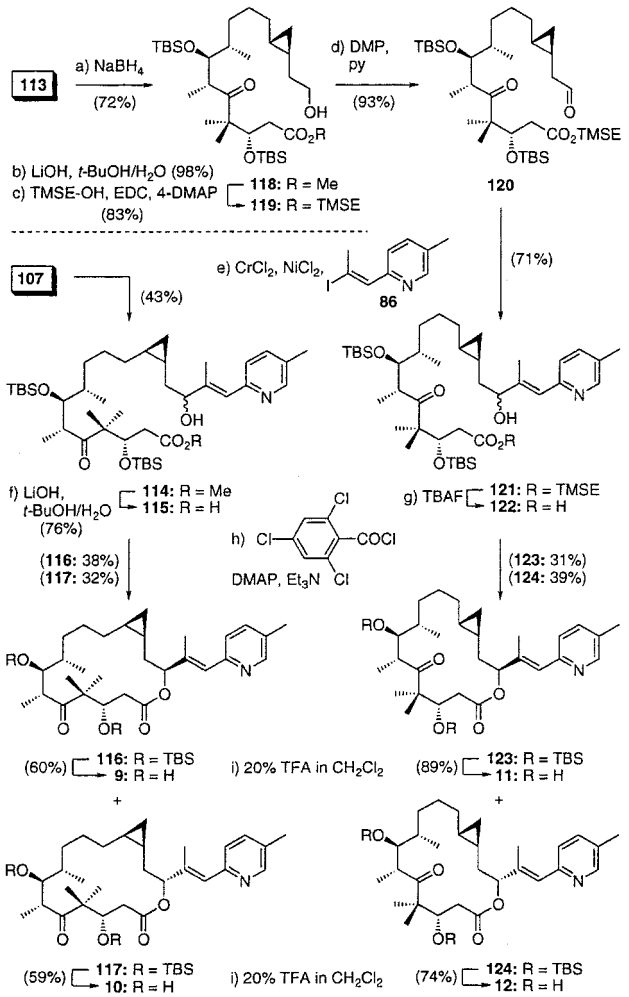


[a] Reagents and conditions: (a) DMP (1.2 equiv), CH_2Cl_2, 25 °C, 1.5 h; (b) LDA (2.5 equiv), **18** (2.4 equiv), THF, −78 °C, 4 min, 75% (**102**), 89% (**108**) over 2 steps, (c) TBSOTf (4.0 equiv), 2,6-lutidine (5.0 equiv), CH_2Cl_2, −20→25 °C, 1 h, 93% (**103**), 100% (**109**); (d) HF·py, py, 25 °C, 2 h; (e) DMP (1.2 equiv), $NaHCO_3$ (1.5 equiv), CH_2Cl_2, 25 °C, 6 h; (f) $NaClO_2$ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), NaH_2PO_4 (2.5 equiv), *t*-BuOH:H_2O 4:1, 25 °C, 10 min; (g) $TMSCHN_2$ (2.0 equiv), MeOH:benzene 1:1, 92% (**104**), 88% (**110**) over 4 steps; (h) 20% $Pd(OH)_2$/C, H_2 (1 atm), EtOAc:EtOH 1:1 25 °C, 6 h, 93% (**105**), 80% (**111**); (i) DMP (1.2 equiv), $NaHCO_3$ (1.5 equiv), CH_2Cl_2, 25 °C, 1.5 h; (j) $MeOCH_2PPh_3Cl$ (1.5 equiv), NaHMDS (1.3 equiv), THF, −40→25 °C, 70% (**106**), 79% (**112**); (k) TsOH (20 equiv), dioxane:H_2O 10:1, 50 °C, 5 h; then silylation as in (c), 61% (**107**), 76% (**113**). DMP = Dess−Martin periodinane, LDA = lithium diisopropylamide, NaHMDS = sodium hexamethyldisilazide, py = pyridine.

and homologation to install the C15 carbon atom, thus yielding aldehydes **107** and **113** via enol ethers **106** and **112**, respectively.

The *cis*-aldehyde **107** was then subjected to the Nozaki−Hiyama−Kishi coupling with vinyl iodide **86** to yield methyl ester **114** (43%, unoptimized), which was hydrolyzed to the corresponding acid (**115**) in 76% yield (Scheme 13). The ester hydrolysis (**114**→**115**) was extremely slow, requiring 4 days for completion. When the same

Scheme 13[a]



[a] Reagents and conditions: (a) $NaBH_4$ (1.1 equiv), CH_2Cl_2/EtOH, 7:3, −78 °C, 1 h, 72%; (b) LiOH, H_2O/*t*-BuOH, 1:1, 40 °C, 48 h, 98%; (c) EDC (2.0 equiv), 4-DMAP (0.5 equiv), TMSE-OH:CH_2Cl_2 2:1, 25 °C, 2 h, 83%; (d) DMP (2.5 equiv), py (10 equiv), CH_2Cl_2, 0 °C, 2.5 h, 93%; (e) **86** (2.0 equiv), $CrCl_2$ (10 equiv), $NiCl_2$ (0.02 equiv), DMSO, 25 °C, 12−36 h, 43% (**114**), 71% (**121**); (f) LiOH, H_2O:*t*-BuOH 2:3, 25 °C, 4 days, 76%; (g) TBAF (18 equiv), THF, 0 °C, 2 h; (h) 2,4,6-trichlorobenzoyl chloride (9.0 equiv), Et_3N (22 equiv), THF, 0 °C, 1 h, then 4-DMAP (3.0 equiv), toluene, 75 °C, 3 h, 38% (**116**), 32% (**117**), 31% (**123**), 39% (**124**); (i) 20% TFA in CH_2Cl_2, 25 °C, 2−22 h, 60% (**9**), 59% (**10**), 89% (**11**), 74% (**12**). 4-DMAP = 4-(dimethylamino)pyridine, DMP = Dess−Martin periodinane, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, py = pyridine, TBAF = tetrabutylammonium fluoride, TMSE = 2-(trimethylsilyl)ethyl.

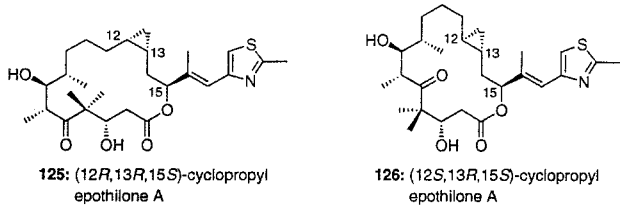


Figure 2. Cyclopropyl epothilones prepared in a previous study.[23]

sequence was applied to the *trans* compound **113**, it proved impossible to hydrolyze the corresponding methyl ester at a practical rate after the Nozaki−Hiyama−Kishi coupling. Clearly, another protecting group was needed for the C1 carboxylic acid, and we opted to try a trimethylsilylethyl (TMSE) ester instead of the methyl ester. In the event, the aldehyde **113** was reduced to the hydroxy ester **118** ($NaBH_4$, 72% yield), which could now be hydrolyzed to the corresponding

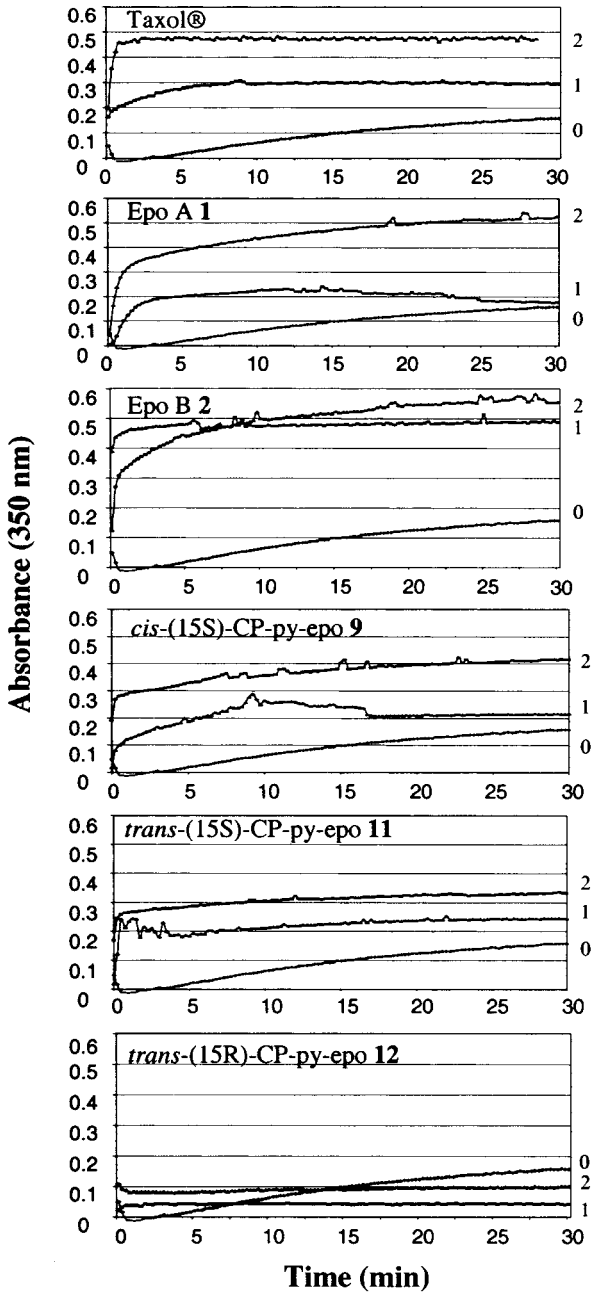


Figure 3. In vitro tubulin polymerization. Comparison of the abilities of epothilone A (**1**), *cis*-(15*S*)-CP-py-epo (**9**), *trans*-(15*S*)-CP-py-epo (**11**), and *trans*-(15*R*)-CP-py-epo (**12**) to induce tubulin polymerization in the absence of microtubule-associated proteins (MAPs). Polymerization reactions with epothilone B (**2**) and paclitaxel (Taxol) are included for comparison. In each assay 10 μM (1 mg/mL) purified rat brain tubulin in G-PEM buffer (80 mM PIPES, 1 mM GTP, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.9) was mixed with 3 μM (curve 1) or 10 μM (curve 2) compound and polymerization at 25 °C was followed for 30 min. The optical density at 350 nm (absorbance 350) was then recorded at 15 s intervals for 30 min. Curves 0 are included as controls, showing tubulin polymerization reactions under similar conditions in the absence of the respective compound.

hydroxy acid and protected (TMSE-OH, EDC, 4-DMAP), affording the TMSE ester **119** in 81% yield. Direct hydrolysis of aldehyde **113** was unsuccessful, which dictated the adoption of the above plan requiring reduction to the alcohol prior to hydrolysis. Reoxidation of **119** with Dess−Martin periodinane gave aldehyde **120** (93% yield), which smoothly underwent the Nozaki−Hiyama−Kishi coupling with **86** to furnish hydroxy ester **121** in 71% yield. Cleavage of the TMSE ester with TBAF now proceeded smoothly, affording hydroxy acid **122**

Table 1. Cytotoxicity of Epothilones **1** through **12** and Paclitaxel against 1A9 Human Ovarian Carcinoma Cells and β-Tubulin Mutant Cell Lines Selected with Paclitaxel or Epothilone A[a]

	cell line						
	1A9	A8 (β274)		PTX10 (β270)		PTX22 (β364)	
compound	IC_{50}	IC_{50}	RR	IC_{50}	RR	IC_{50}	RR
epothilone A (Epo A) **1**	2.37 ± 0.433	117 ± 27.01	49.3	23.35 ± 1.85	9.9	5.21 ± 0.344	2.2
epothilone B (Epo B) **2**	0.095 ± 0.007	2.14 ± 0.072	22.5	0.548 ± 0.156	5.8	0.163 ± 0.02	1.7
paclitaxel (Taxol)	1.77 ± 0.227	17.95 ± 3.08	10.14	52.75 ± 9.4	29.9	28.5 ± 2.75	16.1
cis-(15*S*)-CP-epo **3**	1.60 ± 0.124	23.43 ± 4.29	14.6	10.9 ± 1.4	6.8	2.6 ± 0.2	1.6
cis-(15*S*)-CB-epo **4**	8.8 ± 0.00	196 ± 0.00	22.2	62 ± 0.00	7.1	20 ± 0.00	2.3
cis-(15*R*)-CP-epo **5**[b]	225	>300 (inactive)	na	>300 (inactive)	na	>300 (inactive)	na
cis-(15*R*)-CB-epo **6**[b]	180	>300 (inactive)	na	>300 (inactive)	na	>300 (inactive)	na
trans-(15*S*)-CP-epo **7**	2.7 ± 0.100	48 ± 0.00	17.8	14.4 ± 0.00	5.3	3.7 ± 0.00	1.4
trans-(15*S*)-CB-epo **8**	25.5 ± 1.50	>300 (inactive)	>11.7	146 ± 0.00	5.7	63 ± 0.00	2.5
cis-(15*S*)-CP-py-epo **9**	1.40 ± 0.453	53.5 ± 14.57	38.2	8.15 ± 0.565	5.8	1.17 ± 0.94	0.84
cis-(15*R*)-CP-py-epo **10**	>300 (inactive)	>300 (inactive)	na	>300 (inactive)	na	>300 (inactive)	na
trans-(15*S*)-CP-py-epo **11**	0.625 ± 0.175	9.5	15.2	3.49 ± 0.00	5.6	0.39 ± 0.00	0.63
trans-(15*R*)-CP-py-epo **12**	>300 (inactive)	>300 (inactive)	na	>300 (inactive)	na	nd	na

[a] The antiproliferative effects of the tested compounds against the parental 1A9 and the paclitaxel- and epothilone-selected drug resistant clones (PTX10, PTX22, and A8, respectively) were assessed in a 72 h growth inhibition assay using the SRB (sulforhodamine-B) assay.[24] IC_{50} values for each compound are given in nM and represent the mean of 3−5 independent experiments ± standard error of the mean. Relative resistance (RR) is calculated as an IC_{50} value for each resistant subline divided by that for the parental cell line (1A9). [b] Data from ref 3. CP = cyclopropyl, CB = cyclobutyl, na = not applicable, nd = not determined, py = 5-methylpyridine side chain.

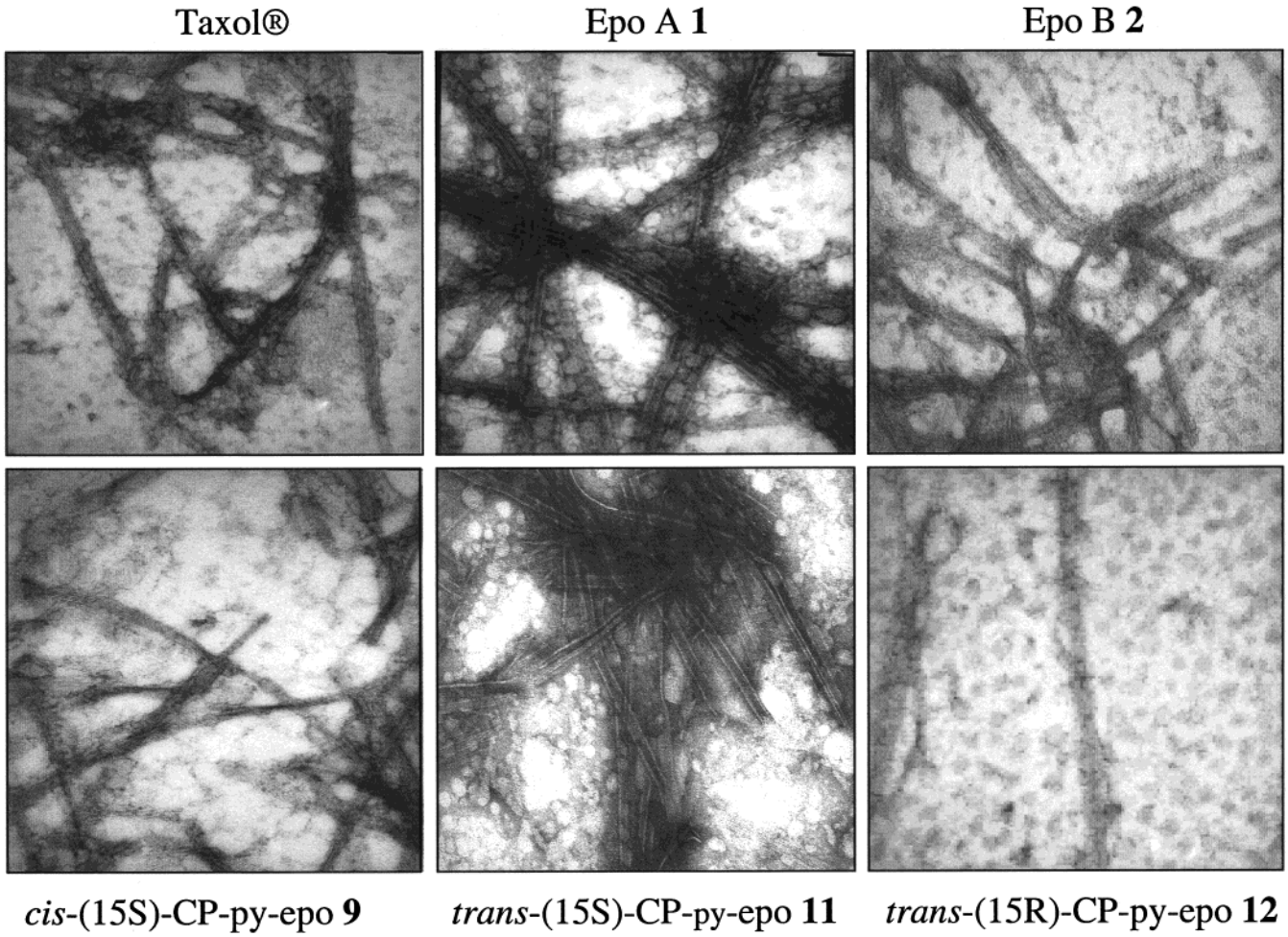


Figure 4. Electron microscopy of microtubules. Transmission electron microscopy of tubulin polymerization products induced by paclitaxel (Taxol), epothilone A (**1**), epothilone B (**2**), *cis*-(15*S*)-CP-py-epo (**9**), *trans*-(15*S*)-CP-py-epo (**11**) and *trans*-(15*R*)-CP-py-epo (**12**). Samples were removed from each polymerization reaction, placed on a 200 mesh Formvar-coated copper grid, stained with 0.5% uranyl acetate, and analyzed with use of a JEOL 1210 analytical transmission electron microscope at 90 kV.

in high yield. Both the *cis* and *trans* isomers **115** and **122** were cyclized by using the Yamaguchi protocol[6] (70% yield), after which the C15 epimers were chromatographically separated, yielding compounds **116**, **117**, **123**, and **124**. Desilylation of these compounds finally afforded the desired cyclopropyl epothilones **9**−**12** in excellent yields.

Chemical Biology

The biological activities of the synthesized epothilones were evaluated through cytotoxicity and tubulin polymerization assays. Cytotoxicity was first evaluated in a set of ovarian

Table 2. Tubulin Polymerization Potency[a] and Cytotoxicity[b] of Epothilones **1** through **12** and Paclitaxel against Human Epidermoid Cancer Cell Lines

compound	% TP[a]	KB-31[b]	KB-8511[b]
epothilone A (Epo A) **1**	69	2.15	1.91
epothilone B (Epo B) **2**	90	0.19	0.18
paclitaxel (Taxol)	49	2.92	626
cis-(15*S*)-CP-epo **3**	83	0.838	0.408
cis-(15*S*)-CB-epo **4**	79	60.7	29.7
cis-(15*R*)-CP-epo **5**[b]	26	159.5	66.7
cis-(15*R*)-CB-epo **6**[b]	29	378	156
trans-(15S)-CP-epo **7**	100	0.971	0.641
trans-(15*S*)-CB-epo **8**	82	23.1	11.5
cis-(15*S*)-CP-py-epo **9**	100	0.618	0.446
cis-(15*R*)-CP-py-epo **10**	6	>1000	>1000
trans-(15*S*)-CP-py-epo **11**	94	0.835	0.676
trans-(15*R*)-CP-py-epo **12**	<10	930	>1000

[a] % TP = percent tubulin polymerized after incubation of tubulin with a known concentration of compound (typically 3 μM). [b] Cytotoxicity toward human cancer cell lines as IC_{50} values given in nM. KB-31: epidermoid Taxol-sensitive, KB-8511: epidermoid Taxol-resistant (due to P-gp overexpression).

carcinoma cell lines, including a parental cell line (lA9) and three drug-resistant cell lines, namely the paclitaxel-resistant cell lines[20] lA9/PTX10 and lA9/PTX22 and the epothilone-resistant cell line[21] 1A9/A8. These resistant cell lines harbor distinct acquired β-tubulin mutations which affect drug−tubulin interaction and result in impaired taxane and epothilone-driven tubulin polymerization. The results of these biological investigations are summarized in Table 1. Further cytotoxicity studies were undertaken using a set of human epidermoid cancer cell lines, including a parent cell line (KB-31) and a paclitaxel-resistant (due to P-gp overexpression) cell line (KB-8511). The results of these studies are summarized in Table 2.

In agreement with previous reports,[3,4] we found that the cyclopropyl epothilone A (**3**) inhibits slightly more potently the 1A9 and KB-31 cell growth than the parent compound epothilone A (**1**). The 15*S*-cyclobutyl epothilone A (**4**) retains good activity but is less potent than either **1** or **3**. It is noteworthy that the 15*R*-isomers (**5** and **6**) of both compounds are inactive at low concentrations against the parental sensitive 1A9 and KB-31 cells. Interestingly, even the (12*R*,13*S*)-*trans*-substituted epothilones **7** and **8** showed good activity, again with the cyclopropyl analogue being the most potent. These results are in agreement with our previous report concerning *trans*-epoxide analogues of epothilones A and B.[22] In another study,[23] we found that (13*R*)-cyclopropyl epothilones **125** and **126** (see Figure 2), originally incorrectly assigned as (13*S*)-diastereomers, were inactive. Thus, we have now prepared and tested all four possible diastereomers of 12,13-cyclopropyl epothilone A, and on the basis of these results, it would appear that while the configuration at C12 has relatively little influence on the cytotoxicity, the 13*S* configuration is essential.

Remarkably, the *trans*-cyclopropyl pyridine analogue **11** showed outstanding activity against all of our cell lines, with IC_{50} = 0.6 nM in the 1A9 human ovarian carcinoma cell line. The *cis* analogue **9** was also highly active, but was a factor of 3 to 5 less active than **11**. Again, the 15*R* isomeric analogues (**10** and **12**) were inactive.

It is noteworthy that the active compounds (**3**, **4**, **7**, **8**, **9**, and **11**) display a similar cytotoxicity profile against the β-tubulin mutants compared to epothilone A (**1**) (see Table 1). In other words, these compounds lose some activity against the clones PTX10 (β270) and A8 (β274), suggesting that residues 270 and 274 are important for the binding of these analogues to tubulin. However, the most active analogue (**11**) still retains IC_{50} < 10 nM for all of these cell lines. Furthermore, we found in the current study, and in agreement with previous reports,[3,20,21] that the paclitaxel-selected clone PTX22 (β364) retains sensitivity to the epothilones, especially in the case of the most active analogues (**9** and **11**) where the relative resistance (RR) values are <1.

The cytotoxicity analysis was supplemented with data from two independent in vitro tubulin polymerization assays. In one assay, the fraction of tubulin polymerized into microtubules upon exposure to a given concentration of the respective compound was determined (see Table 2). In the other assay, tubulin polymerization kinetics upon exposure to the respective compounds was determined by using purified rat brain tubulin through measurement of the absorbance at 350 nm (see Figure 3). For this analysis, paclitaxel, epothilone A (**1**), and epothilone B (**2**) were used as controls while compounds **9**, **11**, and **12** were selected for in vitro analysis. Compound **12** had no in vitro activity consistent with the lack of cytotoxic activity for this compound (Table 1). Compounds **9** and **11** exhibited good in vitro activity although the maximum degree of tubulin polymerization induced by these compounds was smaller compared with that induced by epothilone A (**1**). However, the increased cytotoxic activity of compounds **9** and **11** relative to epothilone A (**1**) could potentially be explained by the faster kinetics of polymerization induced by compounds **9** and **11** [time to A_{350} = 0.25 is <1 min for compounds **9** and **11**, and 2 min for epothilone A (**1**)].

Finally, tubulin polymerization products of these compounds were examined by electron microscopy (Figure 4) to rule out the potential increase in absorbance due to the formation of nonmicrotubule polymers. As seen in Figure 4, all compounds tested induced the formation of microtubule polymers with the exception of compound **12** where no microtubules were observed.

Conclusion

In conclusion, we have constructed by total chemical synthesis seven 12,13-cyclopropyl and three 12,13-cyclobutyl epothilone analogues, and evaluated their biological activities against tubulin and a series of cancer cell lines. Among the several bioactive analogues, the novel, highly potent *trans*-cyclopropyl pyridine epothilone A (**11**) stands out. This compound is one of the most active epothilone analogues reported to date, with IC_{50} = 0.6 nM against the 1A9 ovarian carcinoma cell line. While the other 15*S* compounds synthesized also exhibited potent cytotoxicity against tumor cells, the 15*R* isomers were devoid of such actions, re-enhancing the view that while the oxygen atom at the C12−C13 site is not essential[2j,3,4] for biological activity, the proper configuration at C15 (15*S*) is crucial for such action. Continuing investigations in this area aim at further elucidation of the mechanism of action of the epothilones and their structure−activity relationships.

(20) Giannakakou, P.; Sackett, D. L.; Kang, Y.-K.; Zhan, Z.; Buters, J. T. M.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem.* **1997**, *272*, 17118−17125.

(21) Giannakakou, P.; Gussio, R.; Nogales, E.; Downing, K. H.; Zaharevitz, D.; Bollbuck, B.; Poy, G.; Sackett, D.; Nicolaou, K. C.; Fojo, T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904−2909.

(22) Nicolaou, K. C.; Vourloumis. D.; Li, T.; Pastor, J.; Winssinger, N.; He, Y.; Ninkovic, S.; Sarabia, F.; Vallberg, H.; Roschangar, F.; King, N. P.; Finlay, M. R. V.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, E. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2097−2103.

(23) Nicoloau, K. C.; Finlay, M. R. V.; Ninkovic, S.; King, N. P.; He, Y.; Li, T.; Sarabia, F.; Vourloumis, D. *Chem. Biol.* **1998**, *5*, 365−372.

(24) Skehan, P.; Storeng, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

Experimental Section

Full experimental details are provided as Supporting Information.

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Supporting Information Available: Full experimental details of all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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