ORGANIC LETTERS

2006
Vol. 8, No. 4
685−688

Design, Total Synthesis, and Evaluation of Novel Open-Chain Epothilone Analogues

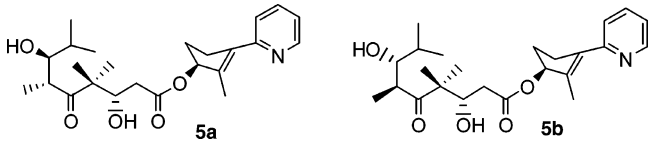
Mamoun M. Alhamadsheh,[†] Richard A. Hudson,[†,‡] and L. M. Viranga Tillekeratne*[,†,‡]

Department of Medicinal & Biological Chemistry, College of Pharmacy, and Department of Chemistry, College of Arts & Sciences, University of Toledo, Toledo, Ohio 43606

ltillek@utnet.utoledo.edu

Received November 28, 2005

ABSTRACT



The design, total synthesis, and biological evaluation of two open-chain analogues of epothilone incorporating the critical C1–C8 fragment and the aromatic side chain held together by a small molecular scaffold have been achieved. Biological evaluation revealed that further restraint between the flexible C1–C8 region and the molecular scaffold may be necessary for potent inhibition of cell proliferation.

Discovery of the anticancer agent paclitaxel[1] and the elucidation of its unique mechanism of action as a promoter of tubulin polymerization and microtubule stabilization[2] opened a new era in anticancer drug discovery research. Although clinically used for treatment of ovarian and breast cancers,[3] poor water solubility and susceptibility to multi-drug-resistant (MDR) cancer cells[4] limited its clinical use. In adddition, paclitaxel has a complex molecular architecture, which is intolerant to chemical modification, and attempts to synthesize active analogues of simpler structure have not been productive.[5]

A number of other natural products with a paclitaxel-like mechanism of action have been reported.[6−10] Prominent among them are the epothilones **1**−**4** (Figure 1), initially isolated from the cellulose-degrading myxobacterium *Sorangium cellulosum*.[6,10,11] Improved water solubility and demonstrated activity against MDR cell lines made them attractive potential alternatives to paclitaxel in cancer treatment.[11] The simpler molecular structure of the epothilones has facilitated the synthesis of new drug candidates.[11]

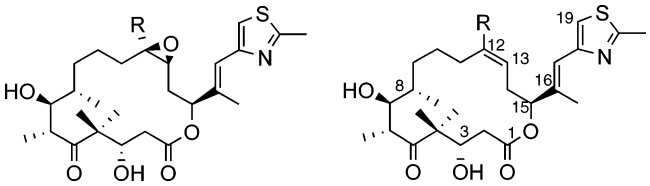


Figure 1. Structures of some natural epothilones.

[†] College of Pharmacy.
[‡] College of Arts & Sciences.

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggan, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
(2) (a) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665. (b) Schiff, P. B.; Horwitz, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1561. (c) Rowinsky, E. K.; Cazenave, L. A.; Donehower, R. C. *J. Natl. Cancer Inst.* **1990**, *82*, 1247.
(3) (a) Rowinsky, E. K.; Donehower, R. C. *New Engl. J. Med.* **1995**, *332*, 1004. (b) Cortes, J. E.; Pazdur, R. *J. Clin. Oncol.* **1995**, *13*, 2643. (c) Rowinsky, E. K. *Annu. Rev. Med.* **1997**, *48*, 353.
(4) (a) Horwitz, S. B.; Cohen, D.; Rao, S.; Ringel, I.; Shen, H. J.; Yang, C. P. *Monogr. Natl. Cancer Inst.* **1993**, *15*, 55. (b) Gupta, R. S. *J. Cell. Physiol.* **1983**, *114*, 137.
(5) Wang, M.; Cornett, B.; Nettles, J.; Liotta, D. C.; Snyder, J. P. *J. Org. Chem.* **2000**, *65*, 1059.

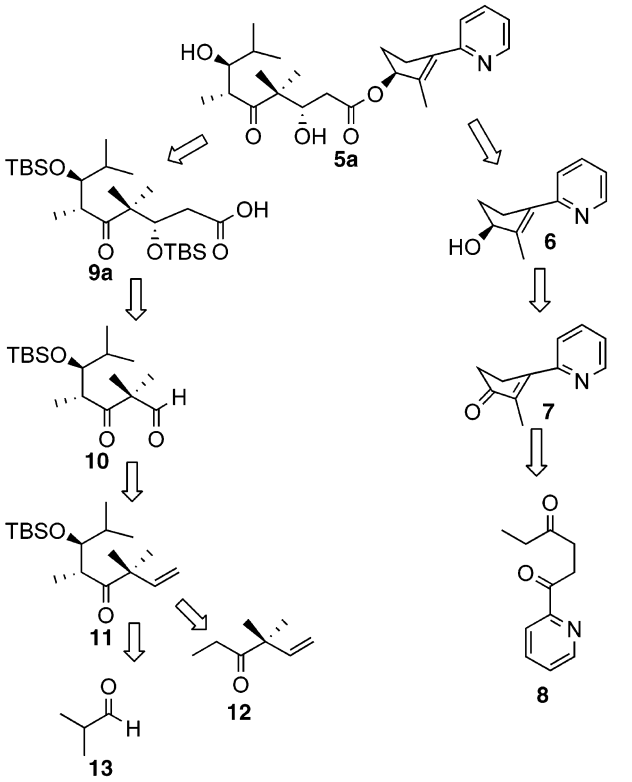
10.1021/ol0528787 CCC: $33.50
Published on Web 01/26/2006

Extensive SAR studies have shed light on key functionalities of the molecule critical for biological activity.[11−13] These studies have shown that the C1−C8 sector constitutes a critical part of the molecule for biological activity and is not amenable to change.[11−13] The aromatic side chain at C15, though necessary, can tolerate some modification;[11−13] a nitrogen heterocycle connected to the macrolactone ring by an olefinic spacer at the position *ortho* to the nitrogen atom is the minimum requirement. Nicolaou et al. showed that replacing the thiazole ring with methylpyridines generated potent analogues.[14] In the tubulin-bound conformation of epothilone A, as determined by NMR studies in aqueous solution, the 16-Me and 19-H have been shown to adopt a syn orientation as in the crystal structure obtained from methanol/water.[15,16] This makes the nitrogen atom of the aromatic ring more accessible for hydrogen bonding with receptor functional groups. Active analogues incorporating substantial modification in the C9−C15 sector have been synthesized. Analogues with cis and trans C12/C13 olefinic bonds[17,18] as well as C12/C13 cyclopropyl moieties[19] retain substantial activity, pointing to a role for oxirane function in **1** and **2** as a stabilizer of the bioactive conformation, rather than serving as an electrophilic center or a hydrogen bond acceptor.[20] Contrary to earlier suggestions of an exo-oreintation of the epoxide ring, Nettles et al., by a combination of NMR, electron crystallography, and molecular modeling studies, proposed that tubulin-bound epothilone A adopts a conformation in which the epoxide ring is in an endo-orientation, with the C10−C15 fragment folded beneath the macrocycle.[21] Danishefsky's group has shown that the presence of a C-9−C10 trans double bond is well tolerated,[22] whereas the corresponding cis-isomer had diminished activity.[23] These findings are consistent with the tubulin-bound conformation of epothilone A reported by Carlomagno et al.,[15] in which the C8−C10 fragment adopts an antiperiplanar conformation, analogous to that in the X-ray crystal structure.[6b] Thus, the C9−C15 region of the molecule may play an important role, inter alia, in conformational stabilization necessary for receptor binding. This may be mainly in setting the critical relative geometry between the aromatic side chain and the C1−C8 region. Recent studies on the bioactive conformation of epothilone underscore the importance of conformation−activity relationships, in addition to classical SAR.[15,16,20−24]

In view of the presumptive role played by the C9−C15 segment in maintaining the conformational stability necessary for receptor binding, we speculated that a molecule in which the critical C1−C8 region and the aromatic side chain of epothilones are held in position by a small molecular scaffold may satisfy such conformational requirements for receptor binding and would also add to the repository of molecules available for SAR comparison.

Accordingly, we designed compound **5a** (Scheme 1), which embodies the critical C1−C8 fragment of natural epothilone, including all crucial stereocenters. The methyl-substituted olefinic spacer was incorporated in a cyclopen-

Scheme 1



(6) (a) Hofle, G.; Bedorf, N.; Gerth, K.; Reichenbach, H. (GFE), DE-B 4138042, 1993 [*Chem. Abstr.* **1993**, *120*, 52841]. (b) Hofle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Girth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567. (c) Gerth, K.; Bedorf, N.; Hofle, G.; Irschik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560. (d) 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) (a) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912. (b) Longley, E.; Caddigan, D.; Harmody, D.; Gunasekera, M.; Gunasekera, S. *Transplantation* **1991**, *52*, 650. (c) Ter Haar, E.; Kowalski, R.; Hamel, E.; Lin, C.; Longley, R.; Gunasekera, S.; Rosenkranz, H.; Day, B**.** *Biochemistry* **1996**, *35*, 243.

(8) (a) D'Ambrosio, M.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta* **1987**, *70*, 2019. (b) D'Ambrosio, M.; Guerriero, A.; Pietra, F. *Helv. Chim. Acta* **1988**, *71*, 964. (c) Ketzinel, S.; Rudi, A.; Schleyer, M.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **1996**, *59*, 873. (d) Lindel, T.; Jensen, P.; Fenical, W.; Long, B.; Casazza, A.; Carboni, J.; Fairchild, C. *J. Am. Chem. Soc.* **1997**, *119*, 8744.

(9) Corley, D.; Herb, R.; Moore, R.; Scheuer, P.; Paul, V. *J. Org. Chem.* **1988**, *53*, 3644.

(10) Altmann, K.-H. *Curr. Opin. Chem. Biol.* **2001**, *5*, 424.

(11) Wartmann, M.; Altmann, K.-H. *Curr. Med. Chem.*: *Anti-Cancer Agents* **2002**, *2*, 123.

(12) Nicolaou, K. C.; Roschanger, F.; Vourloumis, D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2014.

(13) 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.

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

(15) Carlomagno, T.; Blommers, M. J. J.; Meiler, J.; Jahnke, W.; Schupp, T.; Petersen, F.; Schinzer, D.; Altmann, K.-H.; Griesinger, C. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 2511.

(16) Heinz, D. W.; Schubert, W.-D.; Hofle, G. *Angew. Chem., Int. Ed. Engl.* **2005**, *44*,1298.

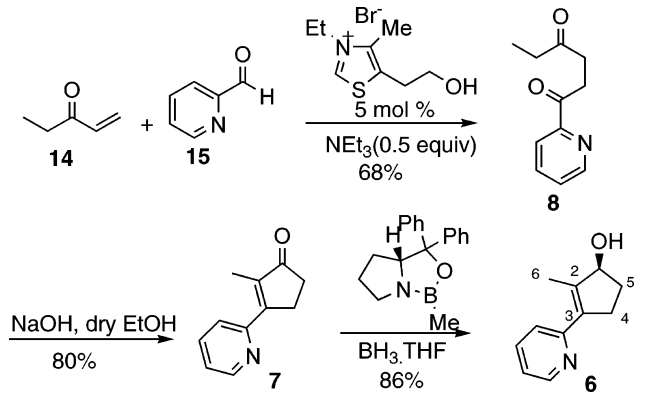
(17) (a) Meng, D.; Su, D. S.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y. H.; Chou, T. C.; He, L.; Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733. (b) Danishefsky, S. J.; Zheng, Y. H.; Chou, T. C.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757.

tenyl molecular scaffold, which carried a 2-pyridyl substituent as the aromatic moiety, in compliance with the requirement of an aromatic ring with a nitrogen atom ortho to the point of attachment of the ring to the macrocycle for maximal activity.[14]

The retrosynthesis for compound **5a** is shown in Scheme 1. We reasoned that the chiral alcohol **6** should be accessible via stereoselective reduction of ketone **7**, which in turn is easily derived from diketone **8**. The carboxylic acid **9a** can be synthesized from aldehyde **10** by Brown's allylation[25]—oxidation protocol. Stereoselective aldol coupling between the Mori's ketone **12**[26] and isobutyraldehyde **13** can be used to generate **11** as the precursor of **10**.

Thiazolium salt-catalyzed addition of 2-pyridinecarboxaldehyde **15** to the activated double bond of ethyl vinyl ketone **14** furnished the diketone **8**,[27] which in turn underwent an intramolecular aldol reaction to the cyclopentenone **7** (Scheme 2). Enantioselective reduction of **7** with BH_3-THF,

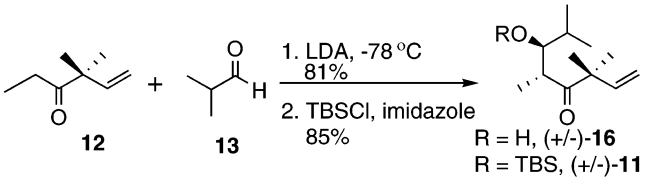
Scheme 2



using (*R*)-2-methyloxazaborolidine (CBS) catalyst,[28] gave the desired (*S*)-alcohol **6** in good yield (86%) and enantioselectivity (84% ee). The absolute stereochemistry and enantiomeric excess of alcohol **6** was confirmed by a modified Mosher ester method.[29,30]

The synthesis of carboxylic acid **9a** was undertaken as shown in Schemes 3 and 4. Mori's ketone **12** was prepared in two steps in 43% overall yield as reported.[26,31] By kinetically controlling the aldol reaction between the *Z*-enolate of **12** and isobutyraldehyde using LDA at −78 °C, we were able to form the desired syn aldol enantiomers (±)-**16** diastereoselectively (syn:anti 24:1)[26,32] (Scheme 3). No

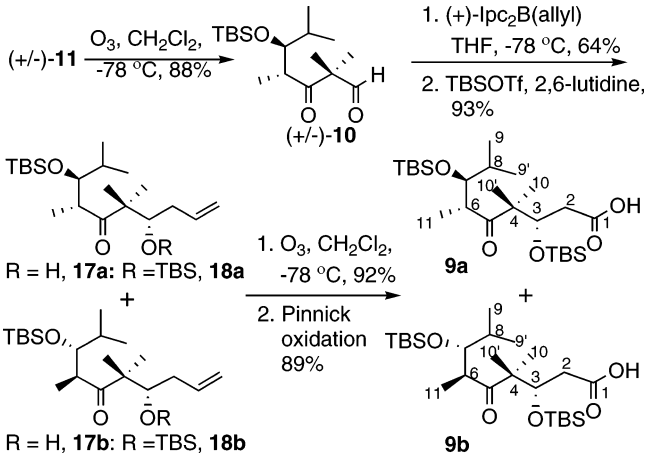
Scheme 3



attempt was made to resolve the racemic syn diols (±)-**16** at this stage.

Subsequent ozonolysis of the silylated alcohol (±)-**11** gave the aldehyde (±)-**10**, which was stereoselectively converted to the homoallylic alcohols **17a** and **17b** (84% de) by reaction with (+)-allyldiisopinocampheylborane (Scheme 4) prepared

Scheme 4



from (−)-*B*-methoxydiisopinocampheylborane and allylmagnesium bromide.[25] The (*S*)-configuration at the homoallylic

(18) (a) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabai, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giiannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268. (b) 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.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, E. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2097.

(19) (a) 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. (b) Nicolaou, K. C.; Namoto, K.; Li, J.; Ritzen, A.; Ulven, T.; Shoji, M.; Zaharevitz, D.; Gussio, R.; Sackett, D. L.; Ward, R. D.; Hensler, A.; Fojo, T.; Giannakakou, P. *ChemBioChem.* **2001**, *2*, 69. (c) Nicolaou, K. C.; Namoto, K.; Ritzen, A.; Ulven, T.; Shoji, M.; Li, J.; D'Amico, G.; Liotta, D.; French, C. T.; Wartmann, M.; Altmann, K.-H.; Giannakakou, P. *J. Am. Chem. Soc.* **2001**, *123*, 9313.

(20) Altmann, K.-H. *Curr. Pharm. Des.* **2005**, *11*, 1595.

(21) Nettles, J. H.; Li, H.; Krahn, J. M.; Snyder, J. P.; Downing, K. H. *Science* **2004**, *305*, 866.

(22) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Chou, T.-C.; Dong, H.; Tong, W. P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 2899.

(23) (a) White, J. D.; Carter, R. G.; Sundermann, K. F. *J. Am. Chem. Soc.* **2001**, *123*, 5407. (b) White, J. D.; Carter, R. G.; Sundermann, K. F.; Waterman, M. [Erratum for *J. Am. Chem. Soc.* **2001**, *123*, 5407]. *J. Am. Chem. Soc.* **2003**, *125*, 3190.

(24) Taylor, R. E.; Chen, Y.; Galvin, G. M.; Pabba, P. K. *Org. Biomol. Chem.* **2004**, *2*, 127.

(25) Jadhav, P.; Bhat, K.; Perumal, P.; Brown, H. C. *J. Org. Chem.* **1986**, *51*, 432.

(26) Mori, I.; Ishihara, K.; Heathcock, C. *J. Org. Chem.* **1990**, *55*, 1114.

(27) Stetter, H.; Krasselt, J. *J. Heterocycl. Chem.* **1977**, *14*, 573.

(28) Corey, E. J.; Helal, C. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986.

(29) (a) Dale, J.; Mosher, H. *J. Am. Chem. Soc.* **1973**, *95*, 512. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

(30) $\Delta\delta = (\delta S - \delta R)$ values for the MTPA esters of compound **6**: H_3-6 (+0.155), H_2-5 (−0.14, −0.028), H_2-4 (−0.029, −0.068).

(31) Mayr, H.; Klein, H.; Sippel, E. *Chem. Ber.* **1983**, *116*, 3624.

(32) Heathcock, C.; Buse, C.; Kleschick, W.; Pirrung, M.; Sohn, J.; Lampe, J. *J. Org. Chem.* **1980**, *45*, 1066.

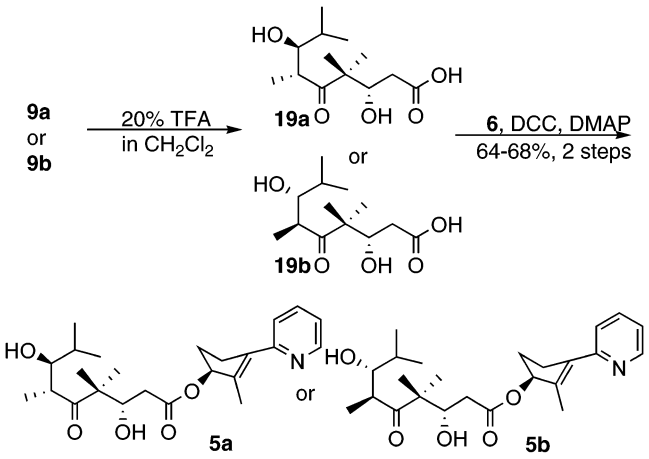
alcohol carbon was assigned from the discernible proton NMR signals of the MPTA esters and confirmed subsequently by Mosher ester analysis of the desilylated benzyl esters of **9a** and **9b** (vide infra).[29,34] Attempts to separate the two diastereomers by chromatography were unsuccessful. Silylation of the hindered secondary hydroxyl group of **17a**/**17b** was achieved with TBSOTf and 2,6-lutidine to give a mixture of **18a**/**18b** in excellent yield. After conversion to carboxylic acids **9a** and **9b** by ozonolysis followed by Pinnick oxidation,[33] the two products were conveniently separated by column chromatography. By converting the benzyl esters of desilylated **9a** and **9b** to the corresponding Mosher diesters, the stereochemistry at C7 was established as (*S*) and (*R*), respectively, while at the same time reconfirming the stereochemistry at C3 of both diastereomers as (*S*), thereby establishing the absolute configuration of **9a** and **9b** as shown.[34]

Coupling between **9a** and alcohol **6** gave the corresponding ester. However, attempts to desilylate it to obtain the final product **5a** proved problematic. Stronger conditions such as 20% TFA in methylene chloride accomplished desilylation, but with concurrent cleavage of ester functionality giving **5a** in only 20% yield, along with the desilylated carboxylic acid **19a**. Milder conditions resulted in partial or no desilylation. As the difficulty in removing the protecting groups may be attributed to their highly hindered environment, we considered direct esterification of the desilylated carboxylic acids **19a** and **19b** with alcohol **6** (Scheme 5).

Treating compounds **9a** or **9b** with 20% trifluoroacetic acid in methylene chloride resulted in the formation of the fully deprotected intermediates **19a** or **19b**, respectively (Scheme 5). As expected, the carboxylic acids **19a** or **19b** reacted with alcohol **6** to give the target molecules **5a** or **5b**, respectively, as the sole esterification product in good yield (64% and 68%, respectively, over two steps). Neither of the hindered alcohols reacted either intramolecularly or intermolecularly with the carboxylic acid groups.

In preliminary cytotoxicity studies compound **5a** showed GI_{50} values of 21.9 and 45.1 μM against CNS cancer (SNB-75) and ovarian cancer (OVCAR-4) cell lines, respectively, in the NCI in vitro 60 cell line human tumor screen, but no activity was observed against any of the other cell lines. Compound **5b**, on the other hand, was not active against any of the cell lines. Interestingly, the C1−C8 fragment of **5a** mimicked the corresponding region, including absolute

Scheme 5



stereochemistry (3*S*,6*R*,7*S*), of natural epothilones, whereas **5b** was the 6*S*,7*R*-diastereomer. It should be noted that a meaningful conclusion about the level of cytotoxicity of **5a** cannot be drawn in the absence of a direct comparison with a natural epothilone. Nevertheless, these results suggest a modest level of selective activity and can be used as the basis for further exploration of this class of open-chain analogues, especially with emphasis on further conformational constraint with respect to the aromatic residue. In **5a** the methyl-substituted olefinic spacer of epothilone was incorporated in the cyclopentenyl molecular scaffold while the heteroaromatic ring was represented by a 2-pyridyl moiety, mimicking that of active pyridine epothilone analogues.[14] Low, but selective, in vitro cell antiproliferative activity of compound **5a** against CNS and ovarian cancer cell lines and the lack of activity of compound **5b** show that, while the molecular scaffold may establish some conformational restriction between the pyridyl group and the C1−C8 region, further restraint between the flexible elements of the C1−C8 region and the molecular scaffold may be necessary in establishing active analogues. Efforts in this direction are in progress in our laboratory.

Acknowledgment. This work was partly supported by a grant from the Elsa U. Pardee Foundation. We thank the NCI's Developmental Therapeutics Program for evaluation of the compounds.

Supporting Information Available: Experimental details, characterization data of all compounds, and ^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0528787

(33) Bal, B.; Childers, W. E.; Pinnick, H. *Tetrahedron* **1981**, *37*, 2091.

(34) $\Delta\delta = (\delta S - \delta R)$ values for the MTPA diesters of (a) **9a** benzyl ester: H_2-Bn (−0.01), H_2-2 (−0.07, −0.016), H_1-3 (−0.031), H_6-10,10′ (+0.076, +0.082), H_1-6 (+0.021), H_3-11 (+0.283), H_1-8 (+0.016), H_6-9,9′ (−0.014); (b) **9b** benzyl ester: H_2-Bn (−0.023), H_2-2 (−0.014, −0.036), H_1-3 (+0.011), H_6-10,10′ (+0.087, +0.108), H_1-6 (+0.189), H_3-11 (−0.224), H_1-8 (+0.03), H_6-9,9′ (+0.082)