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Total Synthesis of Hypermodified Epothilone Analogs with Potent in Vitro Antitumor Activity

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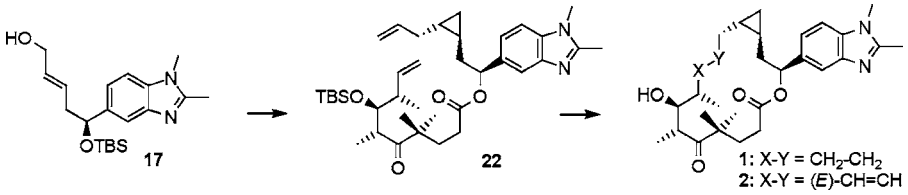
ABSTRACT



The convergent total synthesis of hypermodified epothilone analogs 1 and 2 has been achieved with the stereoselective cyclopropanation of allylic alcohol 17 and ring-closing olefin metathesis with diene 22 as the key steps. In spite of significant structural differences between these analogs and the natural epothilone scaffold, 1 and 2 are potent inducers of tubulin polymerization and inhibit the growth of human cancer cells in vitro with sub-nM IC_{50} values.

Over the past decade a variety of natural products have been identified that inhibit human cancer cell growth through the stabilization of cellular microtubules and the associated suppression of microtubule dynamics.[1,2] These compounds are functional analogs of the important clinical anticancer drugs taxol (paclitaxel, Taxol) and docetaxel (Taxotere) and thus represent important new lead structures for anticancer drug discovery. The most widely studied members of this group are the bacterial natural products epothilone A and B (Epo A/B) (Figure 1), for which an extensive body of structure−activity relationship (SAR) data has been accumulated based on the synthesis and biological evaluation of different types of semisynthetic derivatives and fully synthetic analogs.[3,4]

As a result of these efforts at least six epothilone analogs have entered clinical evaluation in humans (in addition to Epo B),[5,6] and one of these (BMS-247550, ixabepilone) has recently obtained FDA approval as an anticancer drug.[7] As for most epothilone analogs investigated to date, however, the structures of these clinical agents are closely related to the natural epothilone scaffold and few attempts have been

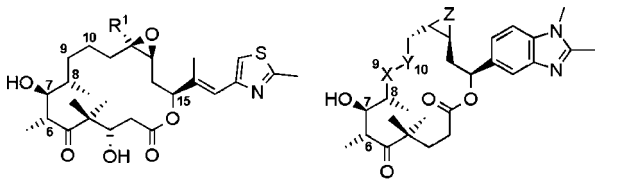


Figure 1. Structures of epothilones A and B and of target compounds **1** and **2**.

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(1) For recent reviews on microtubule-stabilizing agents, see: (a) Altmann, K.-H.; Gertsch, J. *Nat. Prod. Rep.* **2007**, *24*, 327−357. (b) Myles, D. C. *Ann. Rep. Med. Chem.* **2002**, *37*, 125−132. (c) Altmann, K.-H. *Curr. Opin. Chem. Biol.* **2001**, *5*, 424−431.
(2) Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253−265.

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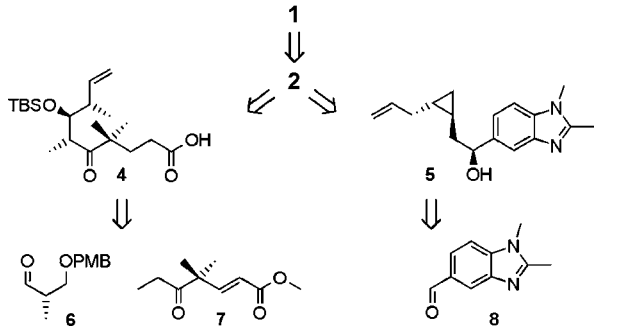
reported to create more rigorously modified analogs[8,9] that might perhaps provide a higher degree of pharmacological differentiation from the natural product leads. Our own research in the area of natural-product-based microtubule stabilizers has thus focused on the development of "hyper-modified" epothilone analogs (i.e., molecules with very limited, if any, structural similarity with the original epothilone scaffold) that would eventually represent new chemotypes for microtubule stabilization.[9] In this context we have previously reported the total synthesis of analog **3** (Figure 1), which exhibits potent tubulin-polymerizing and antiproliferative activity.[9c]

In a next step we are now exploring whether specific structural changes in the Northern part of **3** will have similar effects on biological activity as have been observed for natural epothilones. Among the most relevant of these modifications are the replacement of the epoxide moiety by a cyclopropane ring (leading to target structure **1**, Figure 1) and the incorporation of an *E*-double bond between C9 and C10 (target structure **2**, Figure 1). Both types of modifications are known to preserve or even increase the antiproliferative activity of Epo B or D, while simultaneously providing distinct advantages at the pharmacological level.[10,11]

Compared to our synthesis of **3**, which had relied on the late stage stereoselective epoxidation of a C12−C13 *E*-double bond after macrolactonization-based ring closure,[9c] the presence of an additional double bond in **2** clearly mandated the installment of the cyclopropane moiety to occur at an earlier stage. This led to a completely different overall strategy for the synthesis of **1** and **2** (compared to **3**), which is outlined in Scheme 1. Thus, while target structure **1** was

Scheme 1. Retrosynthetic Analysis for Target Structures **1** and **2**



envisioned to be accessible from **2** through simple hydrogenation, the latter was foreseen to be assembled via an esterification−RCM sequence from building blocks **4** and **5**. The synthesis of **4** would take advantage of the commercially available (*S*)-Roche ester **9** as a precursor for aldehyde **6**,[12] whose aldol reaction with γ-keto ester **7**[9c] would set the stereocenters at C6, C7, and C8.

The Eastern part of the molecule would be derived from dimethyl benzimidazole aldehyde **8**[9b] via enantioselective allylation,[13] homologation, and subsequent stereoselective cyclopropanation of an allylic alcohol[14] as the key step.

As illustrated in Scheme 2, the aldol reaction between aldehyde **6** and γ-keto ester **7** produced two *syn* products in a ratio of 1.4:1 in favor of **10**. Separation of these diastereomers by flash chromatography was straightforward, and the stereochemistry of the major isomer **10** was established by Mosher ester analysis.[15] In light of the ease of isolation of pure **10**, no attempts were made at this point to improve the selectivity of the reaction. TBS protection of the newly formed secondary hydroxyl group followed by catalytic hydrogenation of the double bond and oxidative cleavage of the terminal PMB protecting group with DDQ then furnished hydroxy ester **11**. The latter was homologated by TPAP/NMO oxidation[16] and subsequent Wittig methylenation to furnish olefin **12**. Finally, ester hydrolysis gave building block **4** in quantitative yield (from **12**).

(3) For reviews on the total synthesis of epothilones, see: (a) Watkins, E. B.; Chittiboyina, A. G.; Avery, M. A. *Eur. J. Org. Chem.* **2006**, *18*, 4071−4084. (b) Altmann, K.-H. *Org. Biomol. Chem.* **2004**, *2*, 2137−2152. (c) Nicolaou, K. C.; Ritzén, A.; Namoto, K. *Chem. Commun.* **2001**, 1523−1535. (d) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434−8456. (e) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2015−2045.

(4) For recent reviews on the epothilone SAR, see: (a) Altmann, K.-H.; Pfeiffer, B.; Arseniyadis, S.; Pratt, B. A.; Nicolaou, K. C. *ChemMedChem* **2007**, *2*, 396−423. (b) Höfle, G.; Reichenbach, H. *Anticancer Agents from Natural Products*; CRC Press: Boca Raton, 2005; pp 413−450.

(5) Larkin, J. M. G.; Kaye, S. B. *Expert Opin. Invest. Drugs* **2006**, *15*, 691−702.

(6) Altmann, K.-H. *Curr. Pharm. Design* **2005**, *11*, 1595−1613.

(7) http://www.cancer.gov/cancertopics/druginfo/fda-ixabepilone.

(8) For examples, see: (a) Alhamadsheh, M. M; Gupta, S.; Hudson, R. A.; Perera, L.; Tillekeratne, L. M. V. *Chem. Eur. J.* **2008**, *14*, 570−581. (b) Alhamadsheh, M. M.; Hudson, R. A.; Tillekeratne, L. M. V. *Org. Lett.* **2006**, *8*, 685−688. (c) Winkler, J. D.; Holland, J. M.; Kasparec, J.; Axelsen, P. H. *Tetrahedron* **1999**, *55*, 8199−8214.

(9) (a) Feyen, F.; Cachoux, F.; Gertsch, J.; Wartmann, M.; Altmann, K.-H. *Acc. Chem. Res.* **2008**, *41*, 21−31. (b) Feyen, F.; Gertsch, J.; Wartmann, M.; Altmann, K.-H. *Angew. Chem., Int. Ed.* **2006**, *45*, 5880−5885. (c) Cachoux, F.; Isarno, T.; Wartmann, M.; Altmann, K.-H. *Angew. Chem., Int. Ed.* **2005**, *44*, 7469−7473. (d) Cachoux, F.; Schaal, F.; Teichert, A.; Wagner, T.; Altmann, K.-H. *Synlett* **2004**, 2709−2712.

(10) For cyclopropane-based epothilone analogs, see: (a) Nicolaou, K. C.; Sasmal, P. K.; Rassias, G.; Reddy, M. V.; Altmann, K.-H.; Wartmann, M.; O'Brate, A.; Giannakakou, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 3515−3520. (b) Nicolaou, K. C.; Namoto, K.; Ritzén, 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*, 9313−9323. (c) 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*, 69−75. (d) 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.

(11) For 9,10-dehydro-epothilones, see: (a) Chou, T. C.; Dong, H. J.; Zhang, X. G.; Tong, W. P.; Danishefsky, S. J. *Cancer Res.* **2005**, *65*, 9445−9454. (b) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Chou, T. C.; Dong, H. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 10913−10922. (c) Yoshimura, F.; Rivkin, A.; Gabarda, A. E.; Chou, T. C.; Dong, H. J.; Sukenick, G.; Morel, F. F.; Taylor, R. E.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 2518−2521. (d) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Chou, T. C.; Dong, H. J.; Tong, W. P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 2899−2901. (e) Chou, T. C.; Dong, H. J.; Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Tong, W. P.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 4761−4767.

(12) Walkup, R. D.; Kahl, J. D.; Kane, R. R. *J. Org. Chem.* **1998**, *63*, 9113−9116.

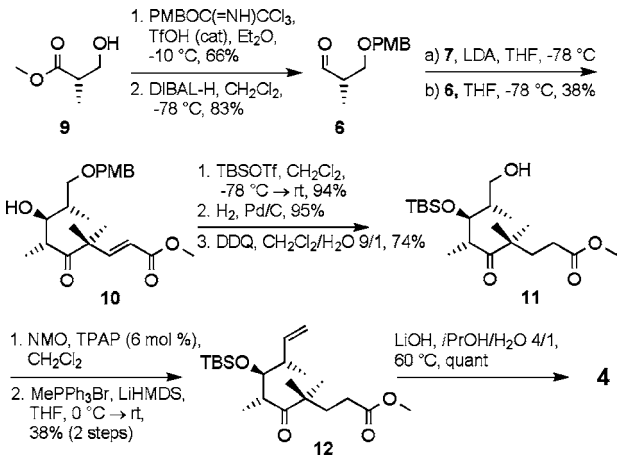
(13) Brown, H. C.; Ramachandran, P. V. *J. Organomet. Chem.* **1995**, *500*, 1−19.

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

(15) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

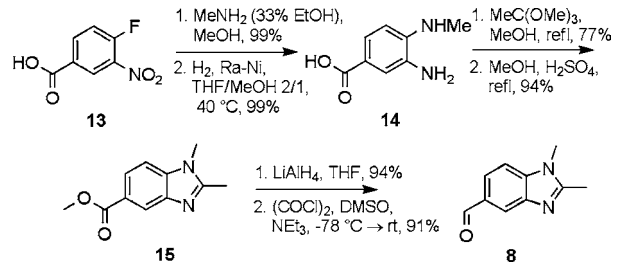
(16) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625−1627.

Scheme 2. Synthesis of Building Block **4**



As outlined above, the installment of the benzylic C15 stereocenter in building block **5** (epothilone numbering) relied on Brown allylation of aldehyde **8**, whose synthesis from commercially available **13** is summarized in Scheme 3.

Scheme 3. Synthesis of Aldehyde **8**

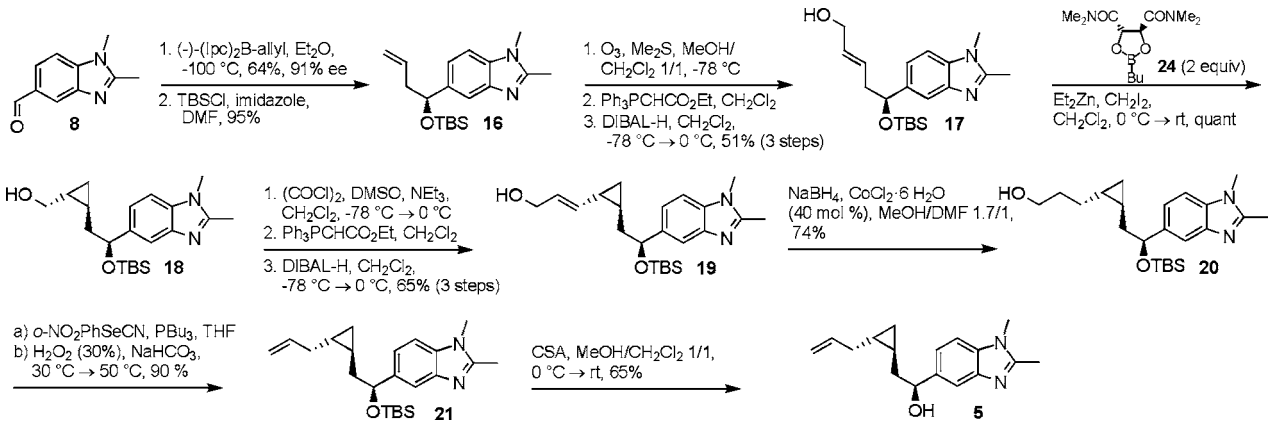


Allylation of **8** with (−)-(Ipc)$_2$B-allyl at −100 °C proceeded in acceptable yield (64%) and good selectivity (Scheme 4). After TBS protection (to provide **16**) and ozonolysis of the terminal double bond, the resulting aldehyde was subjected to Wittig olefination with $Ph_3P{=}CHCOOEt$. Subsequent DIBAL-H reduction of the α,β-unsaturated ester furnished *E* allylic alcohol **17** (51% yield, based on olefin **16**) as the precursor for a projected Charette cyclopropanation with Et_2Zn/CH_2I_2 in the presence of (+)-dioxaborolane **24**.[14]

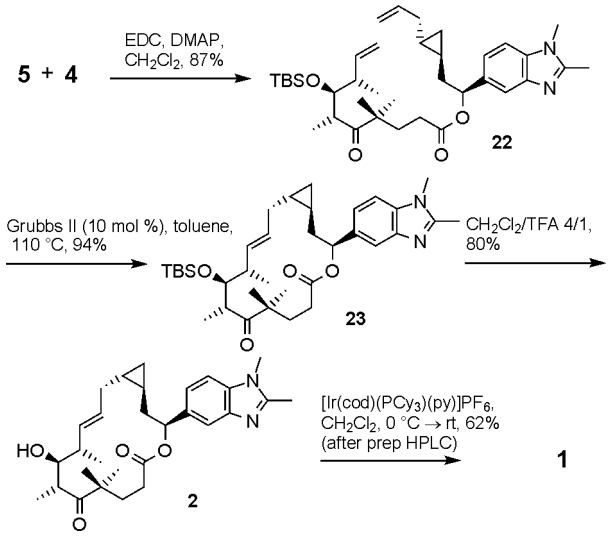
Gratifyingly, the cyclopropanation reaction was highly stereoselective, providing the desired cyclopropane **18** in quantitative yield as a single isomer.[17] After Swern oxidation of **18**, further homologation was carried out following the above olefination/DIBAL-H reduction protocol to give allylic alcohol **19** in 65% yield (based on **18**). Reduction of the olefinic double bond in **19** proved to be a significant challenge, as it was complicated by concomitant cyclopropane ring opening.[18] After extensive optimization we finally established the use of a Co-based reduction protocol ($CoCl_2 \cdot 6H_2O/NaBH_4$)[19] as the most efficient approach for the conversion of vinyl cyclopropane **19** to the saturated derivative **20**. The subsequent selenium oxide pyrolysis[20] of **20** and TBS deprotection of the secondary hydroxyl group in **21** proceeded smoothly, thus providing building block **5** in 59% yield (for the two-step sequence from **20**).

A high-yielding EDC/DMAP-mediated esterification of **4** with **5** then gave diene **22**, which underwent smooth RCM in the presence of second generation Grubbs catalyst in refluxing toluene[11b,d] (Scheme 5). The use of toluene as a solvent resulted in significantly shorter reaction times compared with refluxing dichloromethane and gave *E* isomer **23** selectively and in excellent yield (94%; *E*/*Z* > 10/1). Deprotection of **23** with TFA furnished epothilone analog **2** as our first target structure. Initial attempts at the reduction of the C9−C10 double bond in **2** involved the use of in situ generated diimide (from $TrisNHNH_2$), which provided **1** in high yield. However, the product could not be obtained in pure form, even after HPLC purification (according to NMR analysis); in addition, long reaction times and a large excess of reducing agent were required to drive the reaction to completion. The conversion of **2** into the saturated target structure **1** was achieved most efficiently through catalytic hydrogenation with Crabtree's catalyst, which provided **1** in 62% yield after HPLC purification (note however that high

Scheme 4. Synthesis of Building Block **5**



Scheme 5. Assembly of Target Structures **1** and **2**



catalyst loading was required for complete turnover of the substrate). In contrast, the catalytic hydrogenation of **2** over Pd/C at elevated pressure (7 bar) led to mixtures of products, which have not been resolved at this point.

The hypermodified epothilone analogs **1** and **2** were tested for their ability to promote tubulin polymerization from soluble tubulin and to inhibit the growth of human cancer cells in vitro. The tubulin-polymerizing activity of both compounds is comparable with that of Epo B (data not shown) and higher than that of Epo A (Table 1). Likewise, **1** and **2** inhibit human cancer cell growth in vitro with low-nM IC_{50} values and are at least equipotent with Epo A (Table 1). Most remarkably, growth inhibition is significantly more pronounced for the multidrug-resistant KB-8511 cell line than the corresponding drug-sensitive KB-31 line.

Table 1. Tubulin-Polymerizing and Antiproliferative Activity of Epothilone Analogs **1** and **2**

		IC_{50} [nM]		
compound	EC_{50} [μM][a]	MCF-7[b]	KB-31[b]	KB-8511[b]
Epo A	4.3 ± 0.5	1.9 ± 0.6	2.15 ± 0.07[c]	1.91 ± 0.07[c]
1	3.2 ± 0.2	1.2 ± 0.2	0.43 ± 0.08	0.082 ± 0.03
2	2.9 ± 0.3	0.7 ± 0.3	0.25 ± 0.05	0.024 ± 0.004

[a] Concentration required to induce 50% of maximal tubulin polymerization achievable with the respective compound (10 μM of porcine brain tubulin). Tubulin polymerization was determined through turbidity measurements at 340 nm (A_{340}).[21] [b] IC_{50} values for human cancer cell growth inhibition. MCF-7: breast. KB-31, KB-8511: cervix. KB-8511 is a P-glycoprotein 170 (P-gp170)-overexpressing multidrug-resistant subline of the KB-31 parental line.[22a] Values represent the means of at least three independent experiments (± SD). For experimental details see refs 21 and 22. [c] Data from ref 9c.

Only minor differences are observed between **1** and **2**, which parallels previous findings with natural epothilones[11b] and suggests that efficient binding to tubulin requires an *anti*-periplanar conformation about the C9−C10 bond independent of the geometry of the C12−C13 bond (*cis* or *trans*).

Overall, the biological activity of compounds **1** and **2** makes them attractive candidates for broader in vitro and eventual in vivo profiling and further highlights the potential offered by natural epothilones to serve as leads for the development of new structural scaffolds for microtubule stabilization.

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

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(17) No diastereomeric impurities arising from incomplete stereoselectivity of the cyclopropanation reaction were detectable by 400 MHz NMR-spectroscopy. This assessment is based on the NMR-spectral comparison between **18** and the cyclopropanation product obtained from **17** in the presence of (−)-dioxaborolane *ent*-**24**.

(18) Barrett, A. G. M.; Tam, W. *J. Org. Chem.* **1997**, *62*, 7673−7678.

(19) He, R.; Deng, M. Z. *Tetrahedron* **2002**, *58*, 7613−7617.

(20) (a) Grieco, P. A.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1976**, *41*, 1485−1486. (b) Sharpless, K. B.; Young, M. W. *J. Org. Chem.* **1975**, *40*, 947−949.

(21) Hamel, E.; del Campo, A. A.; Lustbader, J.; Lin, C. M. *Biochemistry* **1983**, *22*, 1271−1279.

(22) (a) Meyer, T.; Regenass, U.; Fabbro, D.; Alteri, E.; Rösel, J.; Müller, M.; Caravatti, G.; Matter, A. *Int. J. Cancer* **1989**, *43*, 851−856. (b) Nicolaou, K. C.; Scarpelli, R.; Bollbuck, B.; Werschkun, B.; Pereira, M. M.; Wartmann, M.; Altmann, K.-H.; Zaharevitz, D.; Gussio, R.; Giannakakou, P. *Chem. Biol.* **2000**, *7*, 593−599.