ORGANIC LETTERS

2002
Vol. 4, No. 6
995−997

Synthesis, Conformational Analysis, and Bioassay of 9,10-Didehydroepothilone D

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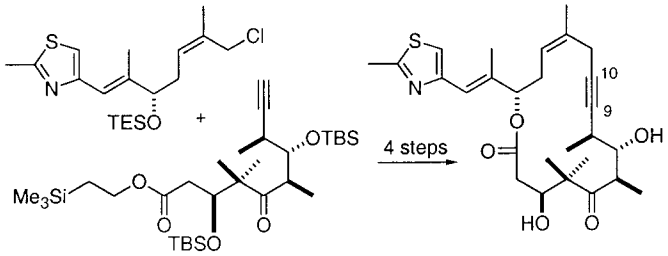
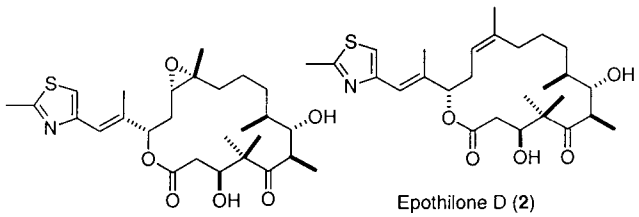
Received January 22, 2002

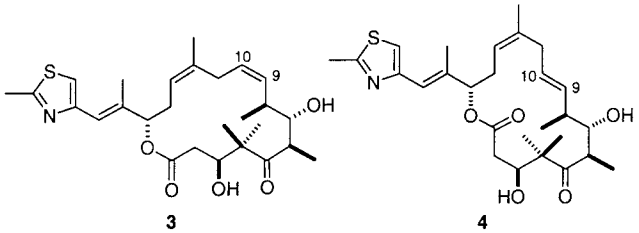
ABSTRACT



9,10-Didehydroepothilone D was synthesized, its conformation was studied, and its tubulin polymerization and antiproliferative activity were compared with that of epothilone D and certain analogues.

Naturally occurring epothilones present in the soil bacterium *Sorangium cellulosum* as well as synthetic analogues continue to be of much current interest as potential drug candidates for the treatment of various forms of cancer.[1] Epothilone B (**1**), epothilone D (**2**), and a macrolactam analogue of **1** have entered clinical trials, and it is likely that further variants of these structures will be evaluated clinically soon. The ability of **1** and **2** to bind to β-tubulin and to stabilize the microtubule assembly in a manner which inhibits cell division is a crucial aspect of the pharmacological profile of these compounds,[2] yet we have only a rudimentary knowledge of how this process occurs at the molecular level.[3] One approach to this problem is to impose constraints on portions of the epothilone framework which cause displacement of key components of the pharmacophore and thereby disrupt their interaction with complementary sites at the tubulin receptor.





Previous reports from these laboratories described syntheses of *cis*-[4] and *trans*-9,10-dehydroepothilone D,[5] **3** and **4**, respectively. Those structures were intended to represent

(1) For recent reviews and perspectives, see: (a) Nicolaou, K. C.; Ritzen, A.; Namoto, K. *Chem. Commun.* **2001**, 1523. (b) Mulzer, J. *Monatsh. Chem.* **2000**, *131*, 205. (c) Altmann, K.-H.; Wartmann, M.; O'Reilly, T. *Biochim. Biophys. Acta* **2000**, *1470*, M79. (d) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434.

(2) For a review of this aspect of epothilone pharmacology, see: Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2014.

(3) Attempts to obtain structural details of the interaction of epothilones with β-tubulin at atomic resolution have been unsuccessful thus far.

10.1021/ol020023d CCC: $22.00
Published on Web 02/27/2002

two extreme deformations about the C8−C11 portion of the epothilone perimeter, with the cis isomer **3** constraining these four atoms to a syn coplanar arrangement, whereas the trans isomer **4** places the array in an antiperiplanar orientation. According to isodesmic energy calculation based on a PM3 algorithm, the two conformations of epothilone D shown in Figure 1 which **3** and **4** were designed to mimic are both

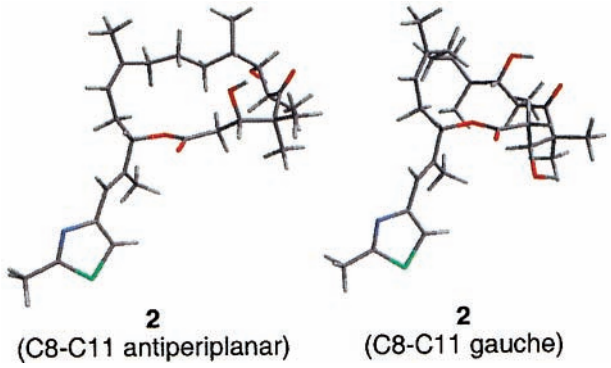
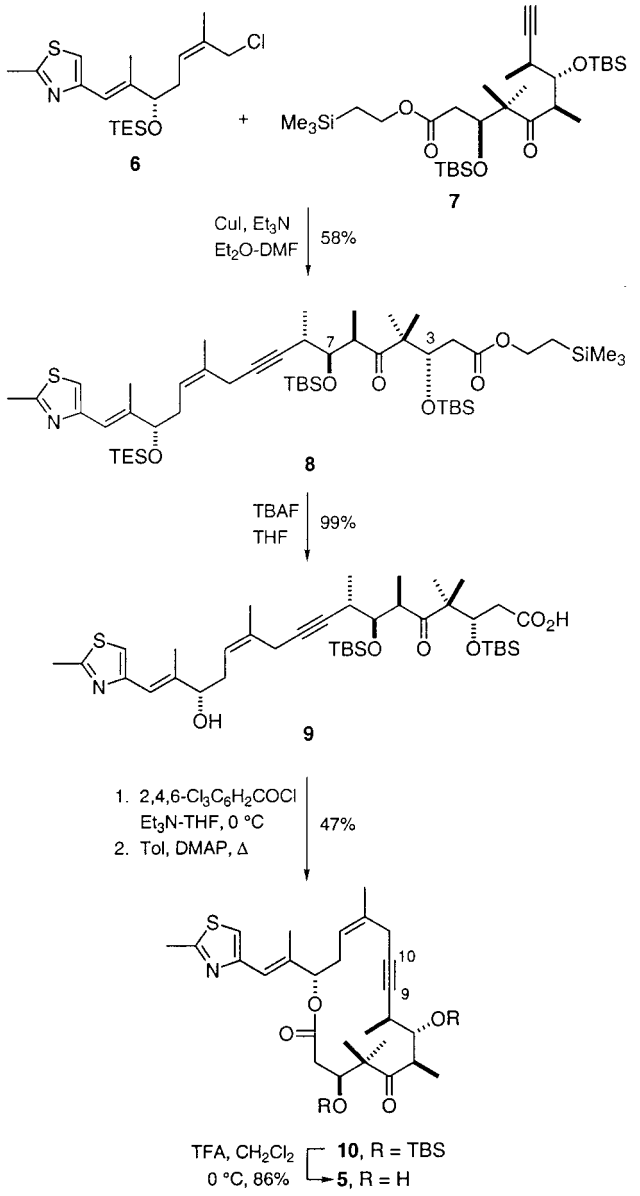


Figure 1. Energy-minimized conformations of epothilone D (**2**) calculated using Spartan (PM3). The antiperiplanar conformation corresponds to the major conformer of epothilone B (**1**) and the gauche conformation to the minor conformer of **1**.

energetically accessible.[6] Furthermore, the antiperiplanar conformer represented by **4** corresponds closely with the solid-state structure of epothilone B (**1**) determined by X-ray crystallography[7a] and also with the conformation of **1** shown by Höfle[7b] and by Taylor and Zajicek[8] on the basis of NOE experiments to predominate in solution. Interestingly, the syn coplanar structure of **3** approximates a minor C8−C11 gauche conformer of epothilone A observable in solution by NMR.[8] Tubulin polymerization assays as well as comparison of in vitro antiproliferative activities showed that **3** and **4** were only slightly less active than **2**,[5] suggesting that β-tubulin is quite catholic in its tolerance of different conformers in the C8−C11 region of the epothilone structure. A third data point could, in principle, be added to this set if a C9−C10 didehydro version of **2** were available, since the linear array that results from interposing an alkyne between C8 and C11 of the epothilone skeleton results in a displacement of this region different from either of the alignments shown in Figure 1. We describe herein the synthesis of 9,10-didehydroepothilone D (**5**) and report features of its conformation as well as its bioactivity in relation to that of **1**, **2**, **3**, **4**, and paclitaxel.

The allylic chloride **6** and the terminal alkyne **7**, each prepared previously in the course of our syntheses of **1** and **2**, were coupled in the presence of cuprous iodide in a modified Castro−Stephens reaction[9] to give the dienyne **8** (Scheme 1). Careful treatment of **8** with tetra-*n*-butyl-

Scheme 1



ammonium fluoride removed both the triethylsilyl ether and trimethylsilylethyl ester but left the *tert*-butyldimethylsilyl ethers at C3 and C7 in place. The resulting hydroxy acid **9** was lactonized under Yamaguchi conditions[10] to furnish **10**,

(4) (a) White, J. D.; Carter, R. G.; Sundermann, K. F.*J. Org. Chem.* **1999**, *64*, 684. (b) White, J. D.; Sundermann, K. F.; Carter, R. G. *Org. Lett.* **1999**, *1*, 1431.

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

(6) For an early study of epothilone conformation, see: Victory, S. F.; Vander Velde, D. G.; Jalluri, R. K.; Grunewald, G. L.; Georg, G. I. *Biorg. Med. Chem. Lett.* **1996**, *6*, 893.

(7) (a) Gerth, K.; Bedorf, N.; Höfle, G.; Irachik, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 960. (b) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567.

(8) Taylor, R. E.; Zajicek, J. *J. Org. Chem.* **1999**, *64*, 7224.

(9) (a) Stephens, R. D.; Castro, C. E. *J. Org. Chem.* **1963**, *28*, 3313. (b) Mignani, G.; Chevalier, C.; Grass, F.; Allmang, G.; Morel, D. *Tetrahedron Lett.* **1990**, *31*, 5161.

(10) Inanaga, J.; Kirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

and subsequent cleavage of the remaining silyl ethers from **10** with trifluoroacetic acid gave 9,10-didehydroepothilone D (**5**).

Conformational analysis of **3**, **4**, and **5**, and a comparison of the strain energy of each with that of the antiperiplanar conformation of epothilone D (**2**) reveals that 9,10-didehydroepothilone D (**5**) is intermediate in strain between **3** and **4** (Figure 2). However, when the O−C1−C9 portion of

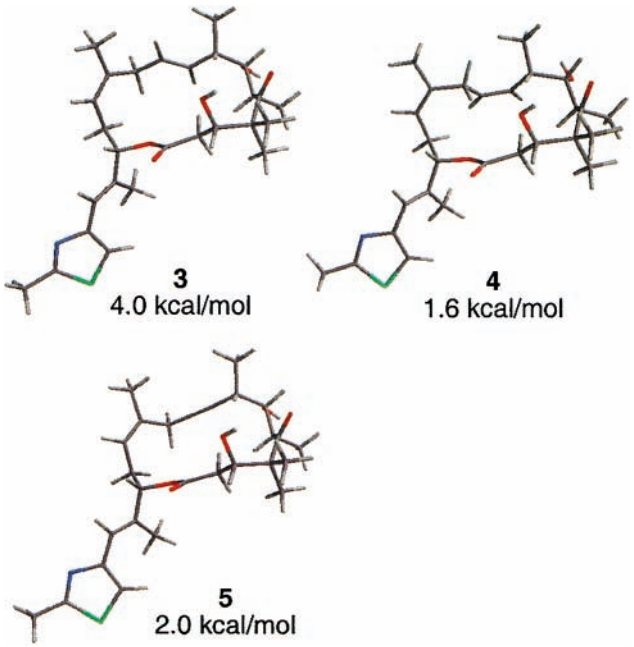


Figure 2. Energy-minimized conformations of **3**, **4**, and **5** calculated using Spartan (PM3). Strain energies are relative to that of the antiperiplanar conformation of **2** set at 0.0 kcal/mol.

5 is overlaid on the corresponding segment of **2**, there is misalignment at C10−C12 with both the antiperiplanar and gauche conformations of the latter structure. It was therefore of interest to compare the biological activity of **5** with that of **3** and **4** in the assays used previously for these analogues in order to determine whether the deformation present in the didehydro analogue is reflected in a diminished interaction with tubulin.

Tubulin polymerization activity as well as antiproliferative assays in two cell lines for **1**, **2**, **3**, **4**, **5**, and paclitaxel are shown in Table 1. The data reveal that **5** is indeed

Table 1. Comparison of Tubulin Polymerization Activity and Antiproliferative Activity of **1**, **2**, **3**, **4**, **5**, and Paclitaxel

compound	tubulin polymerization (%)	IC_{50} KB-31 (epidermoid) (nM)	IC_{50} KB-8511 (epidermoid) (nM)
epothilone B (**1**)	95	0.17	0.16
epothilone D (**2**)	88	1.94	1.00
9,10-*cis*-dehydro-epothilone D (**3**)	56	59.4	28.5
9,10-*trans*-dehydro-epothilone D (**4**)	36	103.7	70.4
9,10-didehydro-epothilone D (**5**)	<10	878.0	593.0
paclitaxel	53	2.67	841.8

significantly less active than either **3** or **4** and is also less active than paclitaxel except in a multi-drug resistant cell line (KB-8511). Thus, whereas rigidity at C8−C11 of the epothilone structure in either a syn coplanar or antiperiplanar arrangement can be accommodated by the tubulin binding pocket, it appears that a linear assembly across the C8−C11 domain cannot. The implications of this result in terms of the interaction of **2**, **3**, and **4** with β-tubulin should become clearer as more structural details of the epothilone binding site are revealed.

Acknowledgment. We are grateful to Professor Kevin P. Gable for valuable advice on computational aspects of this work. Financial support was provided by the National Institutes of Health (GM50574).

Supporting Information Available: Experimental procedures and characterization data for **5**, **8**, **9**, and **10**; protocols for tubulin polymerization and antiproliferative assays. This material is available free of charge via the Internet at http://pubs.acs.org.

OL020023D