Conformational Properties of Epothilone

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The epothilones are macrolide natural products, produced by myxobacterium *Sorangium cellulosum (So ce* 90*)*, which mimic the biological activity of the anticancer agent Taxol. These interesting compounds have been shown to bind tubulin, induce microtubule polymerization, and stabilize microtubule dynamics. Coupled with its biological activity, epothilone's relatively simple structure has made it an exciting target for total synthesis and analogue preparation. To date, only limited information has been reported with regard to epothilone's conformation in solution. We have used a combination of NMR studies and computational modeling to investigate the conformational properties of this exciting new lead in cancer chemotherapy. One- and two-dimensional ^{1}H NMR experiments as well as computational methods suggest that the epothilone A prefers to exist in two conformations in both CD_2Cl_2 and DMSO/D_2O. Knowledge of the solution conformation of epothilone in both organic and biological media may allow for the determination of the spatial arrangement of key functionality necessary for tubulin binding and the stabilization of microtubule dynamic structures. In addition, increased understanding of epothilone and its analogues' conformational properties may allow for the rational design of new compounds with microtubule-stabilizing properties.

Introduction

In June 1995, Merck reported the identification of a new class of 16-membered macrolide, epothilones A and B, that mimic all the biological effects of Taxol, both in vitro and in cultured cells.[1] The epothilones represent an important new lead in the search for improved chemotherapeutic agents, which has led to intense interest from the synthetic and biological community.[2] In fact, analogues of epothilone B have recently been shown to be >35 000 times more potent than Taxol in inhibiting multidrug-resistant cell lines.[3] Our interest in the development of the next generation of microtubule-stabilizing agents is focused not only on the determination of the structural features of epothilone (and Taxol) essential for tubulin binding but also on the determination of the necessary spatial arrangement of its functionality. While numerous papers have appeared presenting structure−activity relationships (SAR) for epothilone analogues, little discussion has been directed at the relationship between biological activity and conformation.[4,5]

In 1996, the absolute and relative stereochemistry of epothilone A and B were disclosed in a series of papers by Höfle et al.[6] The relative stereochemistry of epothilone B was confirmed by X-ray crystallographic analysis, Figure 1. In addition, the paper stated that nuclear magnetic resonance spectroscopy (NMR) supported the conclusion that the conformation of epothilones in solution is similar to that in the crystal via a combination of vicinal coupling constants and observed nuclear Overhauser effect measurements (NOEs). However, only limited spectroscopic data that supported this conclusion were presented.

Before the disclosure of the relative and absolute stereochemistry of the epothilones by Höfle et al., Georg recognized the importance of conformational analysis of the epothilone system and reported NMR and molecular modeling results aimed at determination of the relative stereochemistry.[7] Although unable to accurately assign the complete structure, the extensive NMR data reported as a part of this effort have set the stage for additional studies and interpretation.

Recently, we began a new program aimed at investigating the relationship between conformation and biological activity of a number of complex natural products. In this paper, we report the results of 2D NMR and computational studies directed at the determination of the conformational properties of the epothilone class of natural products.

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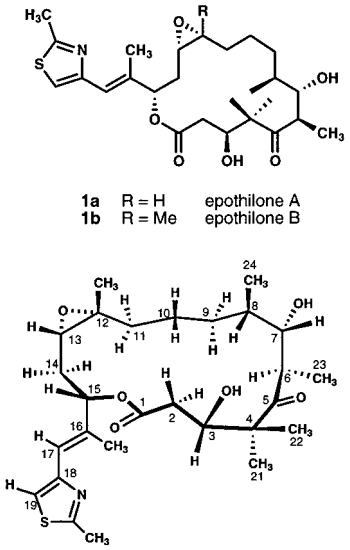

1a R = H epothilone A
1b R = Me epothilone B

Figure 1. Structures of epothilone A and B and a three-dimensional representation of the solid-state conformation of epothilone B as reported by Höfle et al. (ref 6).

Results and Discussion

Computational Methods. Since the epothilone conformation while bound to microtubules may not be similar to the solution structure or the reported X-ray structure, it is important to use computational modeling methods to gain insight into the available conformational space. To accomplish this, we have used internal coordinate Monte Carlo searching available in MacroModel (ver. 5.5).[8] This method has recently been shown to be an effective method for locating the minima of flexible systems.[9] To ensure an exhaustive search of the potential surface, 10 000 conformations were generated and minimized using the MM2* force field with the generalized Born/surface area (GB/SA) water model. Multiple searches were carried out using different starting geometries as well as different ring-closure bond choices. For the epothilone system, we have determined that 10 000 structures are sufficient to reproduce search convergence from different starting geometries. After minimization, a conformer was only saved if its steric energy was within 30 kJ/mol of the instant global minimum (lowest energy structure known during an incomplete conformational search) and did not duplicate a previously stored conformer. After completion of the search, the output files from the conformational searches were then analyzed using the *Filtr* command available in the analysis submode of Macromodel. Spreadsheets were created in which the fields contained a listing of conformer number, energy, and the specific dihedral angle for all rotatable bonds.

We have found that sets of polar coordinate maps provide an effective graphical method for presentation and analysis of data generated from conformational searches. As an example, the dihedral angle distribution [within 20 kJ/mol (4.8 kcal/mol) of the global min] for the C1−C9 polypropionate portion of epothilone A is displayed in Figure 2. We have focused our attention on this region of epothilone since analogues with structural modifications in this region have greatly diminished biological activity.[2] The radius of each plot is relative energy(kJ/mol) with the global minimum centered at the origin. Each data point (◆) represents a single conformer ordered with the lowest energy conformation at the origin. The set of the plots represents a model of the potential energy surface for this portion of the molecule. Although it is impossible to be certain, the fairly large 20 kJ/mol (4.8 kcal/mol) energy window, which contains 187 different conformers, should be sufficient to ensure that the conformation of epothilone while bound to microtubules is included. As a specific example, the polar map for torsion C6−C7−C8−C9 reveals that within 20 kJ/mol of the global minimum conformations exist with dihedral preferences of ∼60°, 180°, or ∼−60°, a classic butane-like distribution. In contrast, torsion C4−C5−C6−C7 prefer dihedral angles in a range between 90° and 160°. It is important to make clear that a polar map for torsion C4−C5−C6−C7 that includes conformations greater than 20 kJ/mol above the global minimum display larger angle distributions. Analysis of the set of these maps suggests that while some of the torsions are relatively flexible others are more restricted.

In Figure 3, the identical data used to create Figure 2 have been Boltzmann-averaged (298 K) to emphasize conformations closest to the global minimum. In this set of graphs, each data point (◆) represents a single conformation with the lowest energy conformation farthest from the origin. Qualitative analysis of the set of these graphs (Figure 3) suggests that the polypropionate portion of epothilone A is fairly rigid within 6.3 kJ/mol (1.5 kcal) of the global minimum. We have rationalized this rigidity by considering the presence of the methyl (and *gem*-dimethyl) substituents on alternating carbon centers at C4, C6, and C8. Thus, the rigidity of this region of epothilone is, at least in part, due to the minimization of *syn*-pentane interactions. Hoffman has previously recognized the conformational "rigidity" of apparently flexible molecules (acyclic), which he describes as having *specific preferred conformations*.[10] In addition, the presence of three β-hydroxycarbonyl relationships [C3−OH−C1(C═O), C3−OH−C5(C═O), and C7−OH−C5(C═O)] can also impart structural rigidity through six-membered hydrogen-bonded chelates.

Closer inspection of these graphs reveals that, within 1.5 kcal/mol of the global minimum, several torsions [(C2−C3−C4−C5), (C3−C4−C5−C6), (C5−C6−C7−C8), and (C6−C7−C8−C9)] actually have two preferred dihedral angles. Plotting each of these torsions against one another (Ramachandran map, not shown) has revealed that epothilone prefers to exist in only two conformations, Figure 4, with a calculated difference in steric energy of roughly 1 kcal/mol. Interestingly, the lower energy conformer A is qualitatively identical to the solid-state conformation. These two conformations (A and B) are essentially identical in the epoxide region, as well as the relationship between the thiazole side chain and the plane of the 16-membered ring. Differences between the two conformations are localized within two specific regions, (1) the relationship of C3-hydroxyl to the macrolide plane and (2) the dihedral angle of the C5−C6−C7−C8 torsion [A(60°) and B(180°)].

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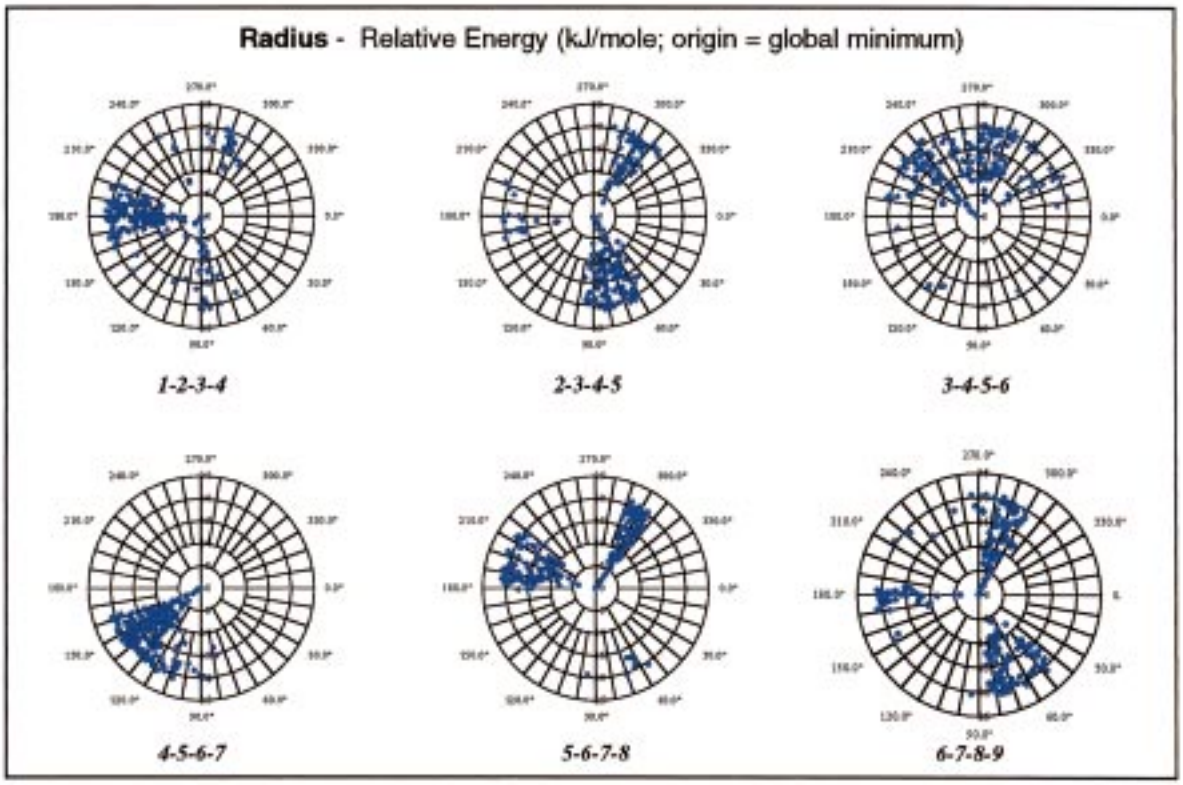

Figure 2. Epothilone A dihedral angle distribution for Monte Carlo conformational search. Each ◆ represents a single conformer. All conformations within 20 kJ/mol of the global minimum are included. The global minimum is at the origin.

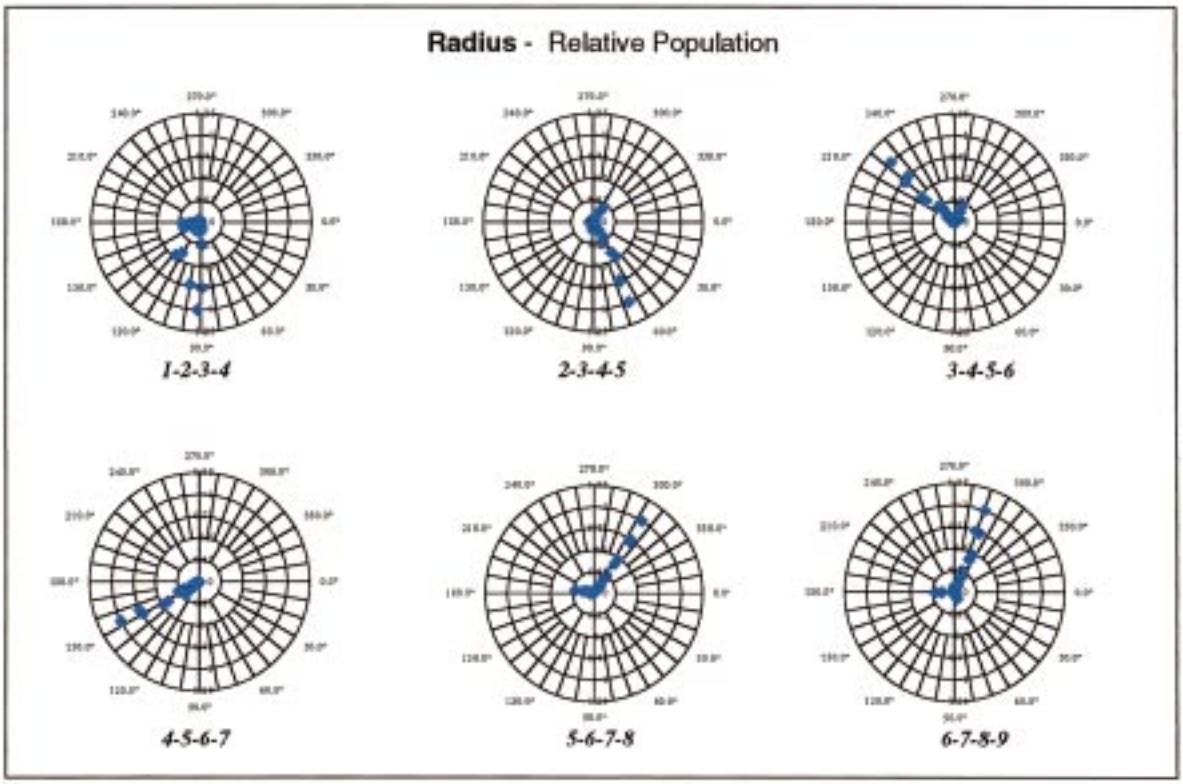

Figure 3. Epothilone A dihedral angle distribution. The identical data plotted in Figure 2 have been Boltzmann averaged. Each ◆ represents a single conformer. The lowest energy conformations are farthest from the origin.

In the lower energy conformer A, the C3-hydroxyl is hydrogen-bonded to the C5 ketone carbonyl. In contrast, the C3-hydroxyl of conformer B resides in the plane of the macrolide ring and is hydrogen-bonded to the C1 ester carbonyl. The other qualitative difference between these two low energy conformations is the dihedral angles

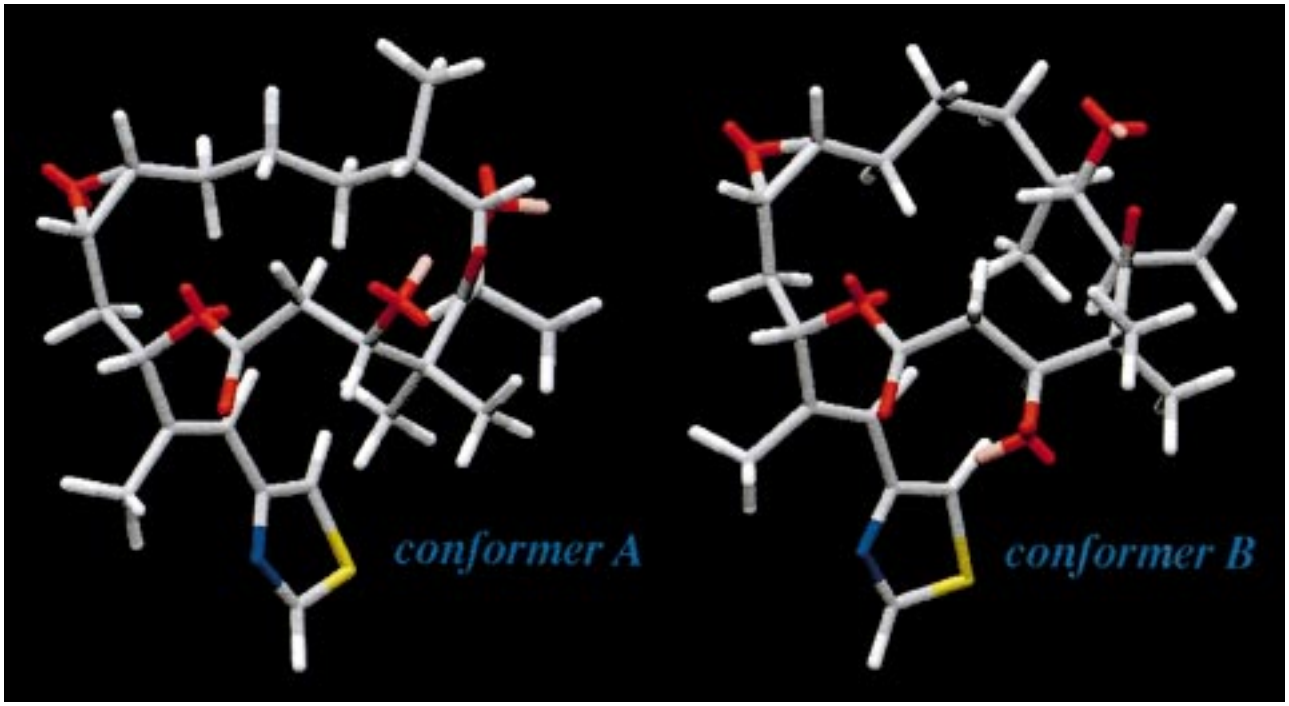

Figure 4.

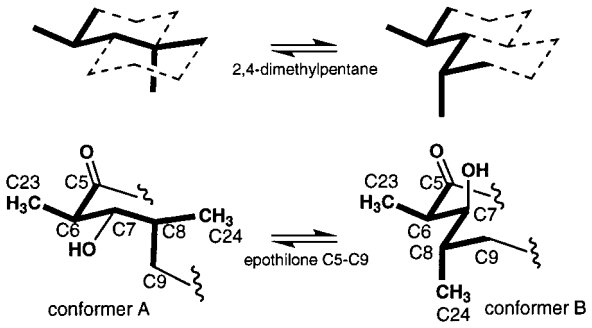

Figure 5. Conformational comparison between 2,4-dimethylpentane and epothilone (C5−C9).

of the torsions in the C6−C10 region. The differences in this region are reminiscent of Hoffmann's description of 2,4-dimethylpentane,[10] which has just two low-energy conformations because all other diamond lattice conformations suffer from *syn*-pentane interactions, Figure 5. Alternative combinations of these regional preferences were found to be >1.8 kcal/mol above the global minimum.

Similar computational studies and analysis has been conducted on epothilone B and the C12,C13-desoxy analogue of both compounds. Structure−activity relationships for this region of epothilone have been summarized.[2] We have found identical conformational preferences for these related structures. Although limited to computational experiments, it can be inferred that the biological activity differences between these analogues and epothilone A and B cannot be attributed to changes in conformational preferences.

NMR Spectroscopy. As a part of our initial effort in this area, we collected two-dimensional NOE spectroscopy (NOESY) and rotating-frame Overhauser effect spectroscopy (ROESY) data on epothilone A at various mixing times (600 MHz, τ_{mix} = 500, 300, 100 ms) in CD_2Cl_2 at room temperature. In this solvent, 19 NOEs were observed not including those whose assignments were obscured by spectral overlap or a potential two-dimensional correlation spectroscopy (COSY) interaction. Numerous additional NOEs were observed but not identified due to overlapping resonances particularly involving protons of the C9−C11 region. Many NOEs supported the presence of conformer A, Figure 6. In particular, a 60° dihedral angle for the C6−C7−C8−C9 torsion is supported by the observation of strong NOEs, H6−H9b and H6−H8.

Several strong NOEs were observed that violated the expected distance constraints based on conformer A, Table 1. However, each of these was easily accommodated by considering an alternative conformation, conformer B, Figure 6. Of particular significance were transannular NOE interactions H2−H7, H3−H6, and H3−H7. Additionally, the strong NOE H6−H24 supported a 180° dihedral angle for the C5−C6−C7−C8 torsion.

The presence of these two conformations in solution is also supported by $^1H-^1H$ vicinal coupling constants. Close inspection of the two conformers reveals a key dihedral angle difference between H6 and H7, Figure 6. The lower energy conformer has a 180° relationship between these two protons with a computationally predicted coupling, J_{calc} = 10.5 Hz. In contrast, the higher energy conformer B has a H6−H7 dihedral angle of ~66° (J_{calc} = 1.0 Hz). The observed coupling constant J_{obs} for H6−H7 was ~8.5 Hz in CD_2Cl_2. This intermediate value suggests a ~4:1 ratio of the two conformers at room temperature and ~0.8 kcal/mol difference in energy. In addition, the Boltzmann-averaged coupling constant calculated from the output of the Monte Carlo search discussed above was 8.9 Hz.

More recently, we have collected NOESY data in DMSO-d_6/25% D_2O at 5 °C. In this solvent, 41 NOEs were observed not including those whose assignments were obscured by spectral overlap or a potential COSY interaction. Under these conditions, we observed spectral information similar to that previously reported by Georg.[7]

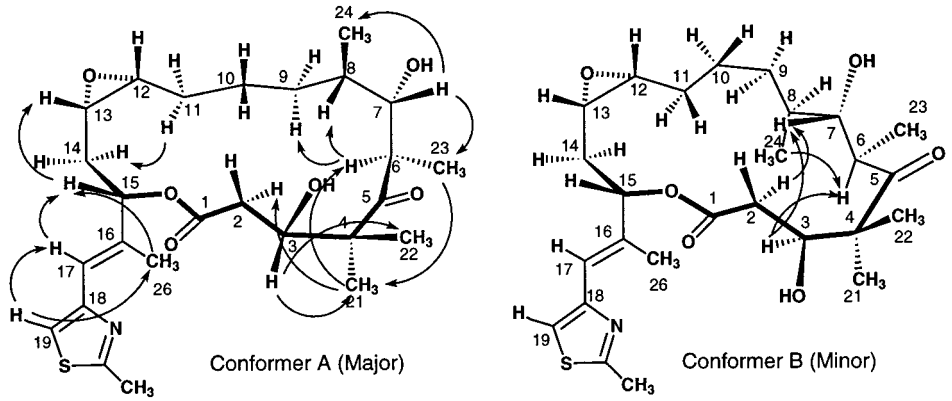

Figure 6. Solution conformations of epothilone A in CD_2Cl_2. Selected NOEs that support the presence of each conformations are included. The direction of the arrows is arbitrary.

Table 1. Selected Chemical Shifts, NOEs, and Calculated Distances for Epothilone A (s = Strong, m = Medium)

	^{1}H shift		atomic distance (Å)	
no.	(ppm, CD_2Cl_2)	NOE	conformer A	conformer B
2a	2.40	2a−7 (m)	4.49	2.25
		2a−21 (m)	2.63	4.43
3	4.18	3−6 (s)	4.75	2.24
		3−7 (m)	6.24	3.54
		3−21 (s)	3.53	2.48
		3−22 (s)	2.48	3.88
6	3.21	6−9a (m)	2.98	4.91
		6−21 (s)	2.25	2.41
		6−24 (s)	4.77	2.27
7	3.73	7−23 (s)	2.55	3.81
		7−24 (s)	2.38	2.69
8	1.70			
9a	1.40			
21	1.10	21−23 (s)	2.39	2.41
22	1.18			
23	1.37			
24	1.01			

A majority of the observed NOEs supported the preferred conformation as similar to the conformation in the solid state, conformer A. However, two observed NOEs support at least the minor presence of conformer B; H3−H6 and H3−H7. The presence of both conformations is also supported by the H6−H7 coupling constant of 9.0 Hz, essentially identical to the computationally predicted Boltzmann-averaged value.

In both solvent systems, NOEs (H13−H15, H11b−H14b) support the preferred conformation of the epoxide region to be exo to the macrolide ring. As expected, the thiazole-containing side chain showed no definite conformational preferences. Rapid rotation on the NMR time scale about the C15−C16 and C17−18 carbon−carbon bonds is supported by several observed NOEs.

Conclusions

From the present study, it is reasonable to conclude that the macrolide natural products epothilone A and B prefer two distinct conformations in solution. The somewhat unexpected rigidity of the polypropionate region has been rationalized by considering key structural features such as *syn*-pentane interactions. The results of this work enable a more detailed inspection of the structure−activity relationships of previously prepared epothilone analogues. In addition, the knowledge of the conformational preferences of this lead compound should assist in the rational design of related chemotherapeutic agents. Conformation-based rational design of epothilone analogues is currently underway in our laboratory.[11]

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