Antitumor Drugs

Synthesis and Conformational Analysis of (*E*)-9,10-Dehydroepothilone B: A Suggestive Link between the Chemistry and Biology of Epothilones**

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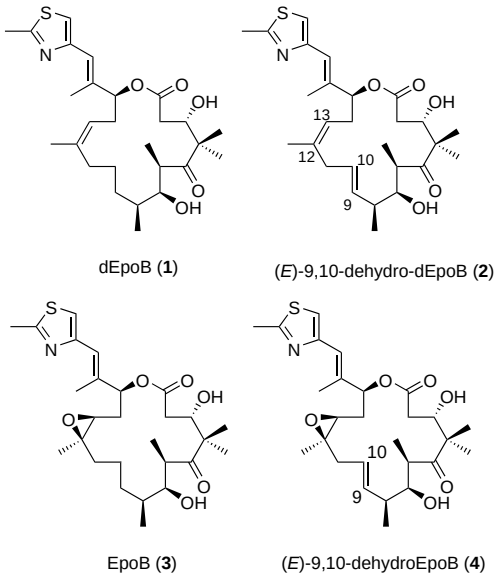
The substantial multidisciplinary interest in epothilones, fueled by their emergence as promising anticancer drug candidates, has led to a worldwide effort to synthesize new analogues[1] and to establish their structure–activity relationships with a view to identifying and developing later-generation agents for clinical evaluation.[2] Human clinical trials of several epothilones as drugs, seeking to assess issues of toxicity, optimal dosage, and efficacy, are well underway. For example, 12,13-desoxyepothilone (**1**, dEpoB),[3] which was initially developed in our laboratory by total synthesis, is now undergoing human clinical trials after showing promising activity in in vivo tests.[4]

In our early studies we had found that epothilone B (**3**, EpoB),[5] which contains an epoxide at C12–C13, is significantly more cytotoxic than is its 12,13-desoxy analogue (**1**, dEpoB).[6] However, from the perspective of therapeutic index, the desoxy compound seemed to us to be much more promising.[7] More recently, we reported the total synthesis of (*E*)-9,10-dehydro-12,13-desoxyepothilone B (**2**), using a stereoselective ring-closing metathesis reaction as a key step.[8] The incorporation of unsaturation at C9–C10 over and above the usual *Z*-12,13 double bond (see compound **2**) resulted in a marked increase in in vitro potency of the drug. The beneficial effects of the incremental C9–C10 unsaturation have been extended to in vivo experiments in xenograft mice. Moreover, compound **2** enjoys major pharmacokinetic advantages over dEpoB (**1**). These twin benefits allowed a decrease in the dosing levels for **2** relative to **1** in xenograft experiments by an order of magnitude.[8a] Accordingly, we wondered if the incorporation of a double bond at C9–C10 in epothilone B, with the epoxide intact (i.e. (*E*)-9,10-dehydroEpoB (**4**)) would alter the biological profile of the drug in the same manner.



In practice, epoxidation of **2** with 2,2′-dimethyldioxirane (DMDO) proceeded with high chemoselectivity at the more substituted C12–C13 double bond to give **4** (corresponding to the natural series in the configuration of the epoxide linkage) and its non-natural diastereomer (**5**) in 87% yield with a 1:2.6 ratio (Scheme 1). The stereochemical assignment of the epoxides was secured by selective reduction of the C9–C10 double bonds of **4** and **5** with diimide.[9] Examination of the spectral properties of these reduction products revealed that EpoB (**3**) had arisen from the minor epoxidation product of **2**, that is, **4**. Correspondingly, reduction of the major epoxidation product **5** afforded 12,13-bis-*epi*-EpoB (**6**). The preference for α epoxidation[10] in the case of **2** stands in striking contrast to the highly stereoselective epoxidation of dEpoB,[11,2b] which occurs from the β face,[10] leading to EpoB (**3**) (see below).[3b]

(*E*)-9,10-Dehydroepothilone B (**4**) was evaluated against a variety of cell types for an early assessment of its antitumor potential. As shown in Table 1, the compound exhibits high cytotoxic activity against a variety of sensitive and resistant tumor cell lines. A direct comparison of **4** with EpoB (**3**) indicates that this new analogue, **4**, is nearly three times as potent as **3** itself. Interestingly, **5** and **6** in the non-natural (13*R*)-α-epoxide series display much lower in vitro activity than **3** and **4**, respectively.

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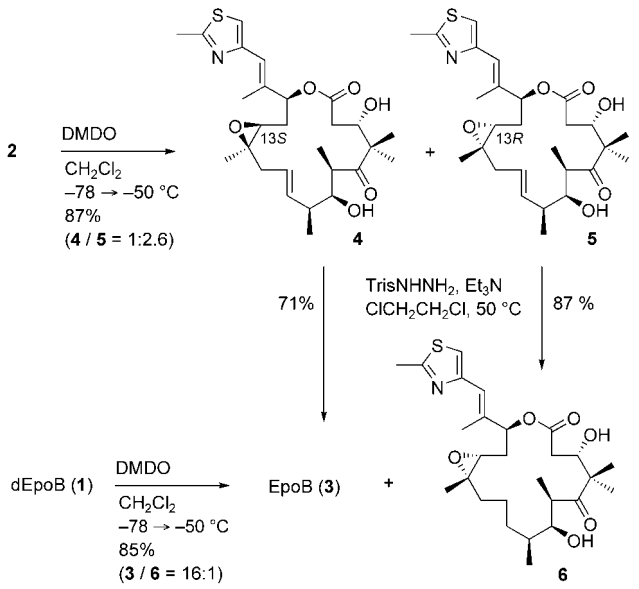

Scheme 1. Synthesis of **4** and EpoB (**3**).

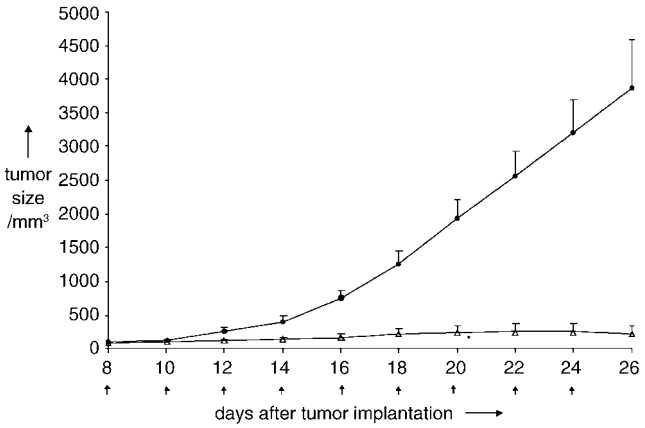

Figure 1. Therapeutic effect of (*E*)-9,10-dehydroEpoB (**4**) in nude mice bearing MX-1 xenograft (6 h intravenous infusion, $n = 4$). ● Control (sacrificed on day 26). △Dosage: 0.4 mg kg^{-1}, administered every other day, nine doses in total (25% of the mice had died by day 25, after 9 doses, 25% of the tumors had disappeared by day 34).

Table 1: In vitro cytotoxicities (IC_{50}) with tumor cell lines.[a]

Compounds	IC_{50} [μM][a]		
	CCRF-CEM	CCRF-CEM/VBL_{100}	CCRF-CEM/taxol
1 (dEpoB)	0.0036	0.016	0.0046
2	0.0009	0.0042	0.0012
3 (EpoB)	0.00062	0.0037	0.0011
4	0.00023	0.00032	0.00042
5	0.0134	0.0959	0.0802
6	0.083	0.4519	0.1507

[a] XTT assay following 72 h inhibition. CCRF-CEM is a human T cell acute lymphoblastic leukemia cell line. The CCRF-CEM/VBL and CCRF-CEM/taxol cell lines all overexpress P-glycoprotein and display a multidrug-resistance phenotype to MDR-associated oncolytics.[13]

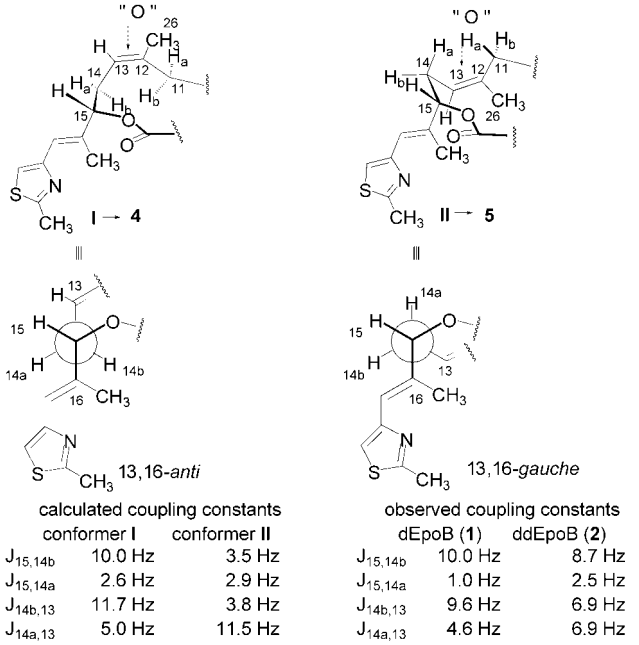

Figure 2. Conformation of the C11–C14 region of desoxy-epothilone.

The high cytotoxic activity of **4** encouraged us to determine its in vivo efficacy in nude mice bearing human tumor xenografts. As shown in Figure 1, compound **4** demonstrated remarkable potency in inhibiting the growth of implanted tumors at much lower dosages (0.4 mg kg^{-1}) relative to **3**. Compound **4** is, to our knowledge, the *most potent epothilone* of established in vivo efficacy.[12]

The dramatic difference in the stereochemistry of epoxidation of **2** relative to that observed in the case of **1** suggested that the conformational properties of these compounds in solution might be quite different. It was particularly intriguing to attempt to use the knowledge thus gained through a chemical perspective to gain insight into the conformational characteristics of the epothilones in solution.

To rationalize the stereochemistry of the epoxidation, we explored the conformation of **2** by both computational and NMR spectroscopy techniques.[14] Molecular-mechanics-based conformational analysis suggested that the C11–C14 region of **2** has a greater flexibility than dEpoB (**1**). The two extreme conformations for this region are depicted in Figure 2. In the 13,16-*anti* conformer (**I**), the C12–C13 double bond adopts an *anti* conformation with respect to the C16–C17 double bond. In contrast, in the 13,16-*gauche* conformer **II**, C12=C13 is in a *gauche* relationship with respect to C16=C17.

Our computational models projected the following characteristic coupling constants for these two boundary conformations: conformer **I**: $J_{15,14b} = 10.0$ Hz, $J_{15,14a} = 2.6$ Hz, $J_{14b,13} = 11.7$ Hz, $J_{14a,13} = 5.0$ Hz and conformer **II**: $J_{15,14b} = 3.5$ Hz, $J_{15,14a} = 2.9$ Hz, $J_{14b,13} = 3.8$ Hz, $J_{14a,13} = 11.5$ Hz. The observed ^{1}H NMR coupling constants for dEpoB (**1**) ($J_{15,14b} = 10.0$ Hz, $J_{15,14a} = 1.0$ Hz, $J_{14b,13} = 9.6$ Hz, $J_{14a,13} = 4.6$ Hz) and for (*E*)-9,10-dehydro-EpoB (**2**) ($J_{15,14b} = 8.7$ Hz, $J_{15,14a} = 2.5$ Hz, $J_{14b,13} = 6.9$ Hz, $J_{14a,13} = 6.9$ Hz), suggest that the population density of conformations converging toward **II** is higher in the case of **2** than in the case of **1**.

Epoxidation of both boundary conformers **I** and **II** would be expected to occur *exo* to the macrolide ring, thus leading to epoxides **4** and **5**, respectively. Given Curtin–Hammett considerations,[15] it seems unlikely that a small increase in the population of conformer type **II** in (*E*)-9,10-dehydro-dEpoB (**2**) could, in itself, account for the shift to α selectivity[10] in the epoxidation reaction. It is more likely that the introduction of 9,10-unsaturation lowers the barrier of interconversion of **I** and **II**, with the latter being the more reactive conformer.

Computational studies in conjunction with NMR spectroscopic analysis prompt the notion that the incorporation unsaturation at C9–C10 leads to an increase in the flexibility of the C11–C14 region and has a substantial impact on the mean conformation of the polypropionate region of **2**. Analysis of the relevant ^{1}H NMR coupling constants also points to a significant difference in the H6–H7 dihedral angle of dEpoB (**1**) ($J_{6,7} = < 1.0$ Hz) and 9,10-dehydro-dEpoB (**2**) ($J_{6,7} = 6.5$ Hz). Significantly, the coupling constants in this region of the spectrum of **2** suggest an increase in conformational population in the direction observed in both the X-ray crystal structure of EpoB[5b] and in its solution NMR spectrum (Figure 3). Although we cannot be certain of the overall conformation of the macrolide while bound to tubulin, it

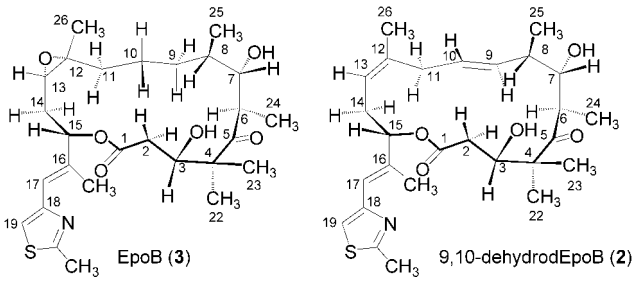

Figure 3. Preferred conformation of EpoB and 9,10-dehydro-dEpoB.

should be noted that the *trans* geometry of C9–C10 rigidifies the C8-C9-C10-C11 torsion angle to 180° and stabilizes the position of the C25 methyl group through a minimization of $A_{1,3}$ strain (Figure 3). These findings lead to the suggestion that the notable increase in biological activity of **2** relative to **1** reflects the impact of the double bond at C9–C10 on the conformation of the polypropionate region.

Furthermore, comparison of the H6–H7 coupling constants (**4** (3.8 Hz), **5** (1.8 Hz), **3** (4.2 Hz), **6** (< 1.0 Hz)) suggests that the long-range conformational guidance provided by the epoxides is in the same direction as that favored by the (*E*)-9,10 double bond of **2**. Moreover, the NMR spectroscopic data show that the long-range conformational guidance depends on the stereochemistry of the epoxides. Only the naturally configured β-epoxides (**3** and **4**) modulate the polypropionate region to populate the highly bioactive conformation. In contrast, long-range modulation by the non-natural α-epoxide series (**5** and **6**) is sharply attentuated. This finding nicely accounts for the higher cytotoxic activity in the natural EpoB series (**3** and **4**) relative to the non-natural α-epoxide series (**5** and **6**).

In summary, we have used molecular modeling and NMR spectroscopic analysis to account for the preferred (α) stereochemistry of epoxidation of the C12–C13 double bond of **2**. Through this epoxidation, we have synthesized the most active epothilone known, **4**, which demonstrated remarkable in vivo efficacy in xenografts. The arguments used to account for the stereochemistry of epoxidation of **2**, closely coordinated modeling, and NMR spectroscopic analysis serve to rationalize the remarkable potency-enhancing effect of the (*E*)-9,10 double bond in this series. We also account for the potency enhancement of the naturally configured β-epoxides (**3** and **4**) and lack of enhancement from the corresponding non-natural α-epoxides (**5** and **6**). While these studies are focused on the epothilones, they point to modalities in drug discovery of wider generality.

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