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Design and Synthesis of C6–C8 Bridged Epothilone A

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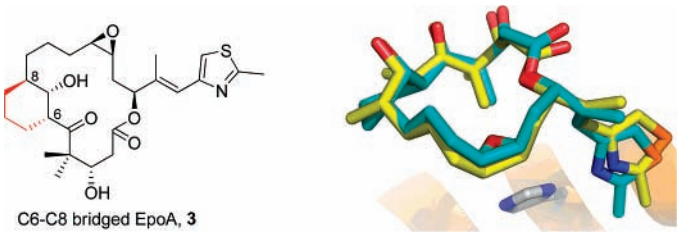
ABSTRACT



A conformationally restrained epothilone A analogue (3) with a short bridge between methyl groups at C6 and C8 was designed and synthesized. Preliminary biological evaluation indicates 3 to be only weakly active (IC_{50} = 8.5 μM) against the A2780 human ovarian cancer cell line.

Epothilones A (EpoA, **1**) and B (EpoB, **2**) (Figure 1) are polyketide macrolides isolated in 1993 from the myxobacterial strain *Sorangium celluosum* by Reichenbach, Höfle, and co-workers.[1] The intriguing biological activity[2] against a wide variety of cancer cell lines by stabilizing microtubules and populating the taxane binding site on β-tubulin was first established by Bollag et al.[3] In distinct contrast to paclitaxel, the epothilones possess improved water solubility and activity against drug-sensitive and multidrug-resistant human cancer cells both in vitro and in vivo.[4] These exceptional advantages, combined with the ease of synthesis by comparison with paclitaxel have evoked a vast research effort within academic and pharmaceutical research groups[5] that include numerous total and partial syntheses,[6] extensive structure−activity relationship (SAR) studies,[2,7] and conformational modeling.[8,9] Importantly, these contributions have resulted in at least seven compounds in advanced clinical trials, one of which

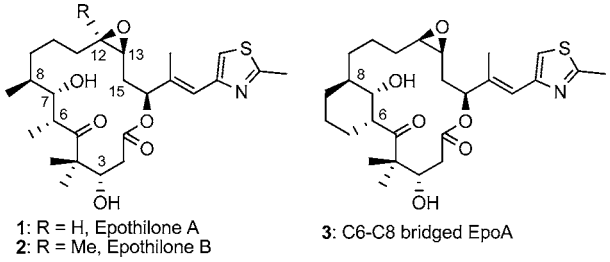


Figure 1. Structures of EpoA/B and C6−C8 bridged EpoA.

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has recently been approved by FDA as anti-cancer drug (Ixabepilone).[10]

Recently, our group proposed a unique EpoA conformation and microtubule binding model based on electron crystallography (EC), NMR conformer deconvolution, and SAR analysis.[9] A peculiar feature of the proposed binding conformer is the presence of a *syn*-pentane interaction between methyl groups at C6 and C8 that can be locked in place by incorporating the corresponding carbons in a six-membered ring (**3**, Figure 1). Optimization of **3** in the proposed binding form with OPLS2001[11] indicated it to be a stable local minimum (Figure 2). Furthermore, docking

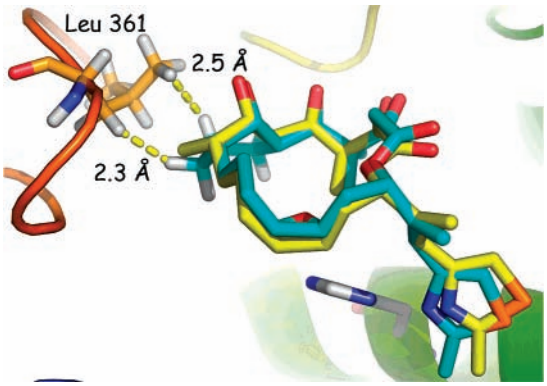


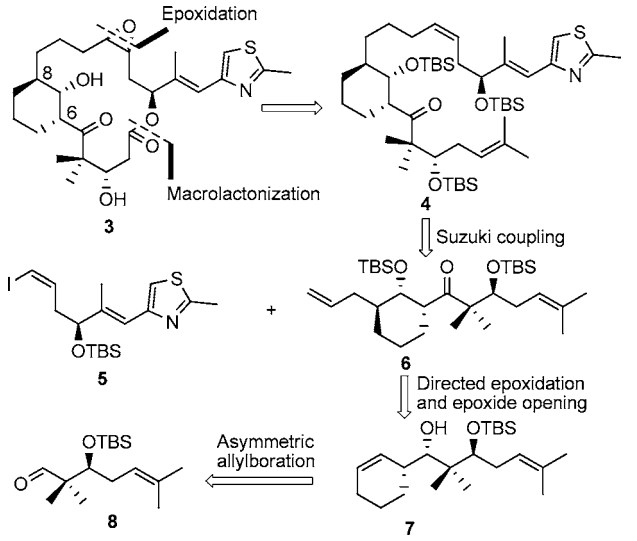
Figure 2. Docking poses of **1** (yellow) and **3** (cyan) in the EC-determined tubulin binding site. The shortest epo-tubulin H−H contact for **3** is 2.3 Å; the sum of the van der Waals radii.

the structure into β-tubulin suggested that the additional CH_2 in the newly installed cyclohexane ring would not experience steric congestion with the protein (Figure 2).

In addition, SAR studies have suggested that the C1−C8 sector is critical for maintenance of biological activity and is not amenable to significant change.[7] However, certain modifications within C1−C8 have yielded potent analogues.[12] An important data point is available from the work of Martin et al. who introduced a six-membered ring between C4−C6 from the *pro-R* methyl at C4 in the corresponding EpoB analogue.[13] The compound proved to be inactive against the MCF-7 tumor cell line. The electron crystallographic structure[9] suggests a *pro-S* attachment to be the compatible link. Stereochemical inversion might then be responsible for the lack of activity. In this context, EpoA analogue **3** was conceived as a potential diagnostic test of the electron crystallographic epothilone binding model.

The retrosynthesis of compound **3** is summarized in Scheme 1. The approach adopts a Suzuki−Miyaura coupling

Scheme 1. Retrosynthesis of **3**



strategy initially developed by Danishefsky for the synthesis of epothilones A and B.[14] The advanced intermediate **6**, in which the cyclohexane core structure has been constructed, was conceived to derive from **7** utilizing sequential substrate directed epoxidation and epoxide opening.[15] Homoallylic alcohol **7** is accessible from aldehyde **8** by Brown's method for preparing 1-(2-cyclohexenyl)-1-alkanols.[16]

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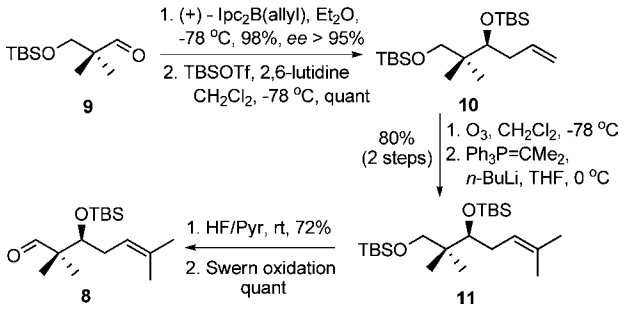
Our synthesis commenced with the known aldehyde **9**,[17] which was first converted to an enantiomerically enriched homoallylic alcohol intermediate (98% yield, ee > 95%, Mosher ester determination) by reaction with (+)-allyldiisopinocampheylborane prepared from (−)-chlorodiisopinocampheylborane and allylmagnesium bromide.[18] The homoallylic alcohol intermediate was subsequently subjected to silylation with TBSOTf to give silyl ether **10** in quantitative yield (Scheme 2). Ozonolysis of **10** followed by a Wittig

Scheme 2. Synthesis of Aldehyde **8**

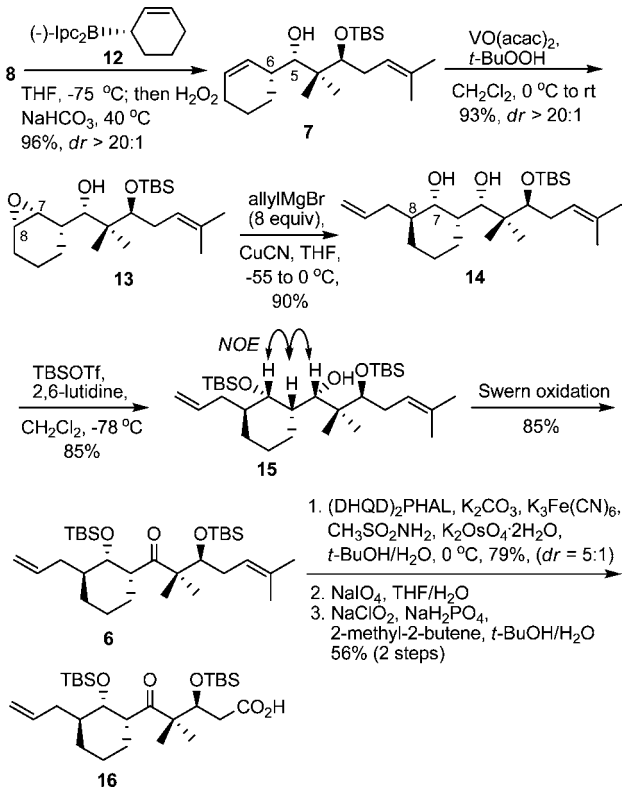


reaction furnished the desired *gem*-dimethyl olefin **11** in 80% yield (2 steps).[19] By exposure to HF/pyr, the primary silyl ether of **11** was selectively demasked in 72% yield,[20] and aldehyde **8** was achieved by subsequent Swern oxidation (quantitative yield).

Preparation of the Suzuki−Miyaura coupling precursor **6** was undertaken as shown in Scheme 3. Aldehyde **8** was combined with freshly prepared *B*-2-cyclohexen-1-yldiisopinocampheylborane **12** followed by oxidative cleavage of the B−O bond to provide intermediate **7** (96% yield, dr > 20:1 by ^{1}H NMR).[16] Surprisingly, both C−C bond formation and B−O bond cleavage by H_2O_2 in this reaction were unexpectedly sluggish (see Supporting Information), but nonetheless, the reaction gives satisfactory yield and selectivity. Stereochemistries at C5 and C6 were assigned on the basis of Brown's study.[16]

Homoallylic alcohol directed epoxidation of **7** was achieved by a vanadium-catalysis strategy[21] to provide the hydroxy

Scheme 3. Synthesis of Diene **6**



epoxide **13** in 93% yield (dr > 20:1 by ^{1}H NMR). The crucial regiocontrolled alkyl opening of the epoxide was successfully performed by treatment of **13** with allylmagnesium bromide in the presence of CuCN (10 mol %) to give the desired diol **14** (90% yield) along with a trace of C7-alkylated isomer and bromohydrin.[22] It is worth noting that an excess of Grignard reagent (8 equiv) was required to reduce the formation of bromohydrin. We reasoned that the regioselectivity of this metal-catalyzed epoxide opening was controlled not only by the Fürst−Plattner rule,[23] which favors a diaxial orientation, but also by stereoelectronic factors implicated in a chelation process.[15b,c] Selective silylation of the sterically less hindered OH group in **14**, followed by Swern oxidation afforded the desired keto diene **6** in 72% yield (2 steps). The relative configuration of **15** was confirmed by NOESY cross-peak analysis. To further confirm the absolute configuration, the conversion of olefin **6** to carboxylic acid **16** was carried out in three steps: (i) regioselective Sharpless asymmetric dihydroxylation[24] which led to a mixture of diastereometric diols (79% yield, ca. 5:1 ratio by ^{1}H NMR), (ii) cleavage of glycol to aldehyde with $NaIO_4$, and (iii) Pinnick oxidation[25] with $NaClO_2$ (56%, 2 steps). Single crystals of **16** were obtained from hexanes. X-ray crystallography confirmed that the desired stereochemistry has been maintained (see Supporting Information).

For the Suzuki−Miyaura cross coupling, vinyl iodide **5** was prepared from the previously reported aldehyde **17**[18b] (85% yield, *Z*/*E* = 10:1) using the Stork and Zhao olefination

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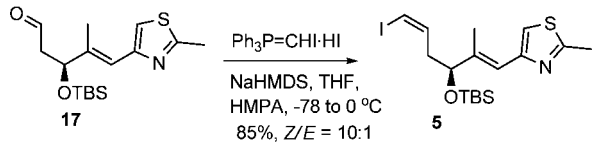
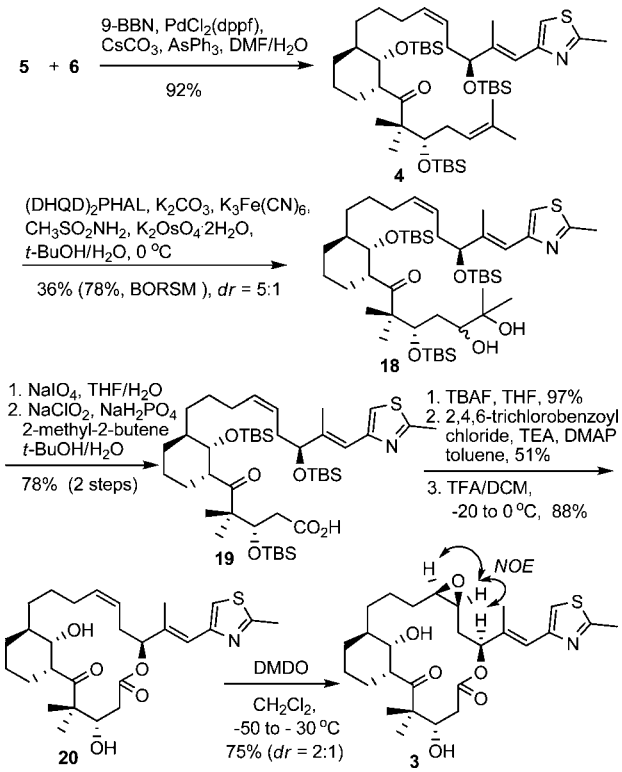
protocol (Scheme 4).[26] The geometry of the C═C was confirmed by ^{1}H NMR (^3J = 7.5 Hz).[26] With the requisite

Scheme 4. Synthesis of Vinyl Iodide **5**



coupling precursors in hand, the final steps in the synthesis of bridged epothilone **3** were carried out as depicted in Scheme 5. After regioselective hydroboration in the presence

Scheme 5. Complete Synthesis of **3**



of 9-BBN, olefin **6** was coupled with vinyl iodide **5** following an approach reported by Danishefsky et al.[14] to furnish *cis*-olefin **4** in 92% yield. The *gem*-dimethyl olefin of triene **4** was regioselectively dihydroxylated by the Sharpless protocol to give diol **18** as a mixture of diastereomers (36% yield, 78% BORSM, ca. 5:1 ratio by ^{1}H NMR). Diols **18** were cleaved to carboxylic acid **19** (78%, 2 steps) in a fashion similar to that utilized in the preparation of carboxylic acid **16**.

Completion of the synthesis of bridged epothilone **3** entailed the conversion of **19** to dihydroxy lactone **20** by employing a procedure used by Nicolaou et al.[18b] Selective desilylation with TBAF, followed by Yamaguchi lactonization and global desilylation in the presence of freshly prepared TFA/CH_2Cl_2 (v/v, 1:4) gave dihydroxy macrolactone **20** in 44% overall yield, which is a bridged epothilone C analogue.[27] Finally, we obtained the C6−C8 bridged epothilone **3** by treatment with 3,3-dimethyldioxirane (DMDO) as described by Danishefsky[14a] to afford a mixture of **3** and its *cis*-epoxide diastereomer **3′** in a ca. 2:1 ratio by ^{1}H NMR. Fortunately, these two diastereomers were separatable by preparative thin-layer chromatography. The stereochemistry of the epoxide was determined by NOESY analysis.

A preliminary evaluation of the potency of compound **3** was probed with the A2780 ovarian cancer cell line. Bridged EpoA **3** is only weakly active with an IC_{50} = 8.5 μM. This corresponds to a potency loss of 3900-fold in comparison with the activity of EpoA in the isogenic 1A9 cell line.[2] Syntheses of other conformationally restrained epothilone analogs are currently being pursued. If low potency against tumor cells for such epo-modifications persists, it may necessitate a re-examination of the electron crystallographic epothilone−tubulin binding representation.[9]

Acknowledgment. The work was supported in part by the NIH Grant No. CA-69571. We thank Kenneth Hardcastle (Emory University) for determination of the X-ray crystal structure of compound **16**.

Supporting Information Available: Molecular modeling and docking; experimental details, characterization data of all compounds, and NMR spectra of key intermediates; and X-ray crystallography data of **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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