

C6–C8 Bridged Epothilones: Consequences of Installing a Conformational Lock at the Edge of the Macrocycle

Weiqliang Zhan,^{*,[a]} Yi Jiang,^[a] Shubhada Sharma,^[b] Peggy J. Brodie,^[c] Susan Bane,^[b] David G. I. Kingston,^[c] Dennis C. Liotta,^[a] and James P. Snyder^{*,[a]}

Abstract: A series of conformationally restrained epothilone analogues with a short bridge between the methyl groups at C6 and C8 was designed to mimic the binding pose assigned to our recently reported EpoA–microtubule binding model. A versatile synthetic route to these bridged epothilone analogues has been successfully devised and implemented. Biological evalua-

tion of the compounds against A2780 human ovarian cancer and PC3 prostate cancer cell lines suggested that the introduction of a bridge between C6–C8 reduced potency by 25–1000 fold in

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comparison with natural epothilone D. Tubulin assembly measurements indicate these bridged epothilone analogues to be mildly active, but without significant microtubule stabilization capacity. Molecular mechanics and DFT energy evaluations suggest the mild activity of the bridged epo-analogues may be due to internal conformational strain.

Introduction

Epothilones are a family of cytotoxic polyketide natural products originally isolated from the bacterium *Sorangium cellulosum*.^[1] Epothilone A (EpoA, **1**) and epothilone B (EpoB, **2**) (Figure 1), two major representatives, were recognized to be potent inhibitors against breast and colon cancer cells shortly after their initial isolation.^[1] The mechanism of action of both EpoA and EpoB was established by the Merck group to be induction of tubulin polymerization in vitro resulting in the stabilization of microtubules under normally destabilizing conditions similar to the clinical anticancer drugs Taxol and docetaxel.^[2] While epothilones exert their antiproliferative action in a similar way to Taxol, the two classes of compounds are distinctly different in terms of their potency and ability to inhibit the growth of multidrug-resistant cancer cell lines.^[2–4] In contrast to Taxol, the epothilones are more efficacious promoters of cancer cell death with EpoB being the most active. Epothilones have also

been proven to be very poor substrates for the phosphoglycoprotein 170 (P-gp) efflux pump. Thus, they retain almost full activity against P-gp overexpressing, Taxol-resistant cell lines. Furthermore, epothilones are also active against cells with tubulin mutations which induce paclitaxel resistance.^[4a] This suggests that epothilone-derived drugs might be useful for treating certain drug resistant tumors. In addition, although EpoA and EpoB were the major products isolated from the myxobacterium, numerous other related structures of the epothilone class have been identified as minor components of the fermentation of myxobacteria, including, for example, epothilone C (EpoC, **3**) and D (EpoD, **4**). These compounds also exhibit potent anticancer properties (Figure 1).^[5] These exceptional biological advantages, com-

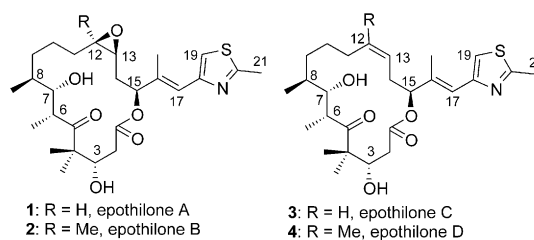


Figure 1. Structures of natural epothilones A–D.

[a] Dr. W. Zhan, Dr. Y. Jiang, Prof. D. C. Liotta, Dr. J. P. Snyder
Department of Chemistry, Emory University
1515 Dickey Drive, Atlanta GA 30322 (USA)
Fax: (+1) 404-712-8670
E-mail: weiqiang.zhan@emory.edu
jsnyder@emory.edu

[b] S. Sharma, Prof. S. Bane
Department of Chemistry, State University of New York
Binghamton, NY 13902-6016 (USA)

[c] P. J. Brodie, Prof. D. G. I. Kingston
Department of Chemistry
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061 (USA)

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the extensive structure–activity relationship (SAR) profiles of epothilones and to elucidating interactions between the ligands and microtubules.^[7–9] The tremendous efforts exerted to generate epothilone SAR profiles have greatly aided our understanding of the drug pharmacophore and the development of natural/unnatural analogues with improved biological activity and reduced toxicity. Significantly, these efforts have delivered at least seven compounds in advanced clinical trials, one of which recently won FDA approval for clinical use as an anti-cancer drug (Ixabepilone).^[10] Since the discovery of the microtubule-stabilizing properties of epothilones in 1995, the details of the binding poses for the structurally diverse taxanes and epothilones have been pursued in order to facilitate the rational design of improved and perhaps structurally simplified analogues.^[11–14] A variety of epothilone conformations and tubulin-binding modes have been proposed by pharmacophore mapping,^[11,12] solution NMR spectroscopy^[15,16] and the superposition of epothilones on taxanes in the electron crystallographic tubulin complex.^[13,14] By combining NMR spectroscopy, electron crystallography and molecular modeling, our group proposed a unique EpoA conformation and microtubule binding model that offers an alternative to the common pharmacophore model by describing the tubulin binding cavity as promiscuous.^[17] According to this model, epothilone and Taxol occupy the same gross binding pocket, but the tubulin–ligand binding is mediated through different sets of hydrogen bonds and hydrophobic interactions for the two compounds. The electron crystallographic structure of epothilone was superposed with that of Taxol bound to tubulin. The overlap suggested that the thiazole moiety of epothilone A and the benzyloxy phenyl of Taxol do not reside in the same region of the tubulin pocket. In addition, among the five oxygen-containing polar groups in epothilone, only the C7-OH falls near the similar C7-OH moiety in Taxol. In this comparison, the latter is the only common center between the two molecules. An unusual feature of the EpoA binding conformer derived by electron crystallography (EC) is the presence of a *syn*-pentane interaction between the methyl groups at C6 and C8. A conceivable test of the latter structural feature introduces a short bridge between the ring carbons to constrain the macrocycle to the EC model geometry. The cyclohexane rings depicted in **5–8** (Figure 2) illustrate one solution to the problem.

A potential liability of this strategy is that the small ring, expanding the volume of the epothilone, might introduce steric congestion with the tubulin residues lining the binding

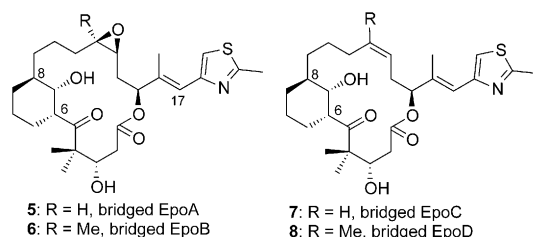


Figure 2. Structures of C6–C8 bridged epothilone analogues.

pocket. To examine specific geometric details of the corresponding structures, **5** and **6** in the proposed binding form were optimized by molecular mechanics to show that both reside in a stable local minimum. Subsequent docking of the latter into the β -tubulin taxoid site suggested that the additional CH₂ in the newly installed cyclohexane ring would not experience undue steric congestion with the protein (Figure 3). To our knowledge, modifications within the C6–

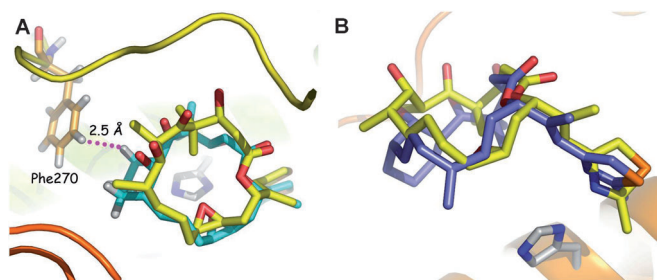


Figure 3. Docking poses of C6–C8 bridged epothilone analogues in the electron crystallography-determined tubulin binding site: A) Docking poses of **1** (yellow) and **5** (cyan); B) Docking poses of **1** (yellow) and **6** (blue). The shortest H–H contact between ligand and protein is 2.5 Å, an acceptable, though minimal, van der Waals separation.

C8 epothilone sector have received little previous attention (Figure 4).^[18–20] In a related study, however, Martin and co-workers introduced a three carbon bridge between C4 and C6 from the *pro-R* methyl at C4 in the EpoB framework (**9**, Figure 4).^[20] The compound had no effect when exposed to the MCF-7 tumor cell line. Since the EC binding conformer predicts the *pro-S* attachment to be the appropriate attachment direction, stereochemical inversion may be responsible for the lack of activity. Accordingly, bridged analogues **5–8** were selected as targets suitable for diagnostic tests of the EC epothilone binding model. In addition, ZK-EPO (Sagopilone, **10**) in which a vinyl moiety was introduced to the C6 methyl group and the C16–C21 side chain was replaced by a benzothiazole moiety, is currently undergoing advanced clinical development as the first fully synthetic epothilone candidate.^[18] Previously, we briefly communicated the synthesis of bridged **5**, and reported the compound to be only weakly active against the A2780 ovarian cancer cell line.^[19] In the present work, we describe the full synthetic details for **5–8**. All four analogues were further subjected to cytotoxicity assessment against human ovarian (A2780) and prostate cancer (PC3) cell lines, tubulin assembly and microtubule cold stabilization.

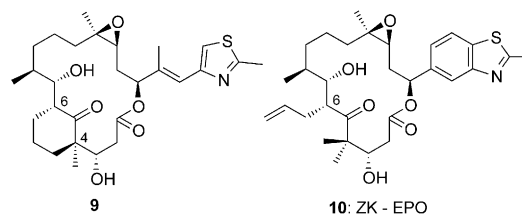
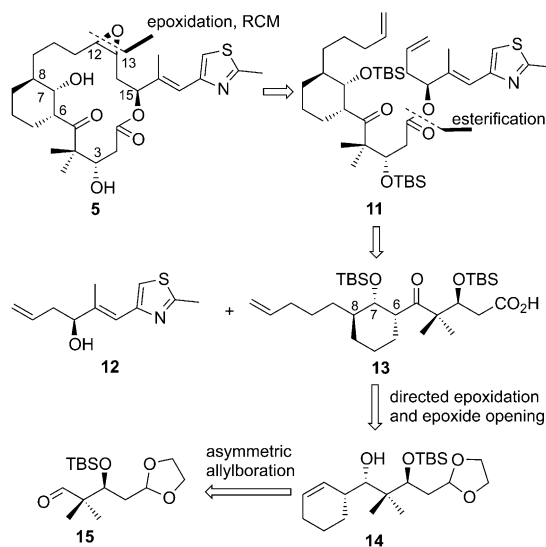


Figure 4. Selected epothilone analogues with modification around C6–C8.

Results and Discussion

Synthesis of C4–C8 bridged epothilones: Retrosynthetic analysis: The first-generation synthetic plan for the C6–C8 bridged epothilones, based on ring closure metathesis (RCM) as a key step, is summarized in Scheme 1. Compound **5** is used as an example. Although RCM is known to give both *cis* and *trans* isomers during total syntheses of natural epothilones,^[21] it was applied as a key step here considering that isolation of both isomers could contribute to the structure–activity profile (SAR) for the compound series. Applying a general disconnection strategy to epothilones, the bridged target **5** could be traced back to alcohol **12** and the advanced intermediate keto acid **13** after retrosynthetic epoxidation, RCM and esterification. The preparation of keto acid **13** was conceived as the key step along this route, by which the cyclohexane core structure with three adjacent chiral centers would be constructed. First, the stereochemistry at C7 and C8 in **13** was contemplated by means of sequential substrate directed epoxidation^[22] and regiocontrolled epoxide opening from homoallylic alcohol **14**. Moving further along the retrosynthetic path, alcohol **14** was envisioned to arise from aldehyde **15** by utilizing Brown's asymmetric cyclohexenylboration strategy.^[23]

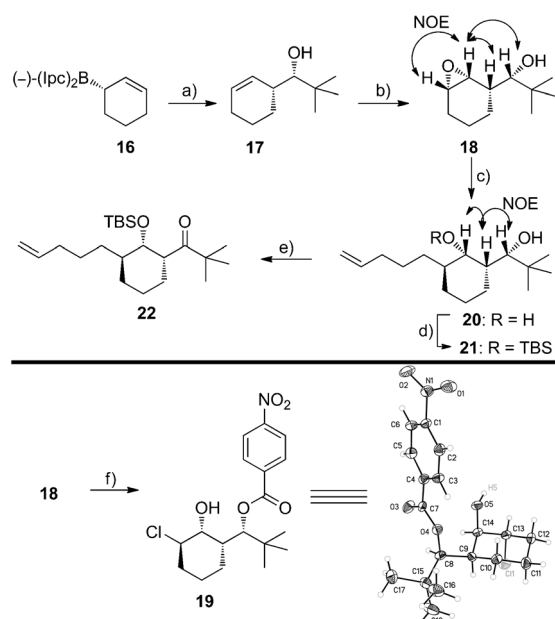


Scheme 1. Retrosynthetic analysis of C6–C8 bridged EpoA **5** by olefin metathesis.

Model study: To test the feasibility of the substrate-directed epoxidation and subsequent regio-controlled epoxide opening strategy, a simplified model system was studied as shown in Scheme 2. The model study started from (–)-*B*-2-cyclohexen-1-yl diisopinocampheylborane (**16**), prepared by treating cyclohexa-1,3-diene with diisopinocampheylborane derived from (+)- α -pinene at -25°C in tetrahydrofuran (THF) as described by Brown et al.^[23] The freshly prepared solution of borane **16** in THF was cooled to -100°C and treated with pivalaldehyde. After oxidation with H_2O_2 in the

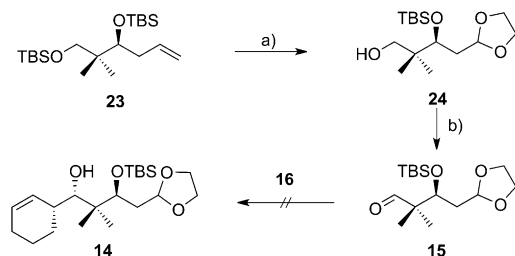
presence of NaHCO_3 , homoallylic alcohol **17** was obtained in 70 % yield (d.r. > 95 % by ^1H NMR).^[24] The highly stereoselective epoxidation of **17** was first achieved by a homoallylic alcohol-directed vanadium-catalyzed epoxidation strategy to afford hydroxy epoxide **18** in 88 % yield as a single isomer. Further study indicated that *m*CPBA-based epoxidation also delivered the desired epoxide **30** in 84 % yield. The relative configuration was confirmed by NOE. Considering the difference between the optical rotation value of **17** and that reported previously ($[\alpha]_{\text{D}}^{25} = -3.4$, $c = 1.0$, CHCl_3 , lit.:^[23b] $+6.83$, l 0.5, neat), the *p*-nitrobenzoyl derivative **19** was prepared. X-ray crystallography of **19** unambiguously confirmed the absolute configuration of alcohol **17** and hydroxy epoxide **18** (Scheme 2). The site of epoxide ring opening by chloride anion further supports the original plan for regioselective nucleophilic opening of hydroxy epoxide. With successful stereoselective epoxidation, the next key step in Scheme 2 is epoxide opening with an alkyl nucleophile in a regioselective manner. Fortunately, this transformation was successfully performed by treatment of **18** with freshly prepared 4-pentenylmagnesium bromide in the presence of CuCN (10 mol %). The desired diol **20** was obtained exclusively in 89 % yield. We concur with Flippin and co-workers^[25] that the regioselectivity of this metal catalyzed epoxide opening is not only controlled by the Fürst-Plattner rule favoring diaxial orientation, but also is most likely reinforced by a chelation process. Following the remarkable success of the two key steps in the model study, we turned our attention to probe the regioselective protection of the two secondary hydroxy groups in **20** and the subsequent oxidation of the sterically hindered secondary alcohol in the model system. Selective silylation of the sterically less hindered OH group in **20** was achieved by slow addition of *tert*-butyldimethylsilyl triflate (TBSOTf) to a solution of **20** in CH_2Cl_2 at -78°C in the presence of 2,6-lutidine to provide the mono-silyl ether **21** in 85 % yield. Surprisingly, no silylation of the sterically hindered hydroxyl group was detected even when 1.5 equiv of TBSOTf was added. At this stage, a NOESY analysis for silyl ether **21** supported the previously described regioselectivity of the oxirane opening and selective TBS protection (Scheme 2). Swern oxidation of the sterically hindered alcohol afforded the desired olefinic ketone **22** in quantitative yield.

Construction of building blocks: Encouraged by the results of the model studies, we proceeded to construct carboxylic acid **13**. In pursuit of this advanced intermediate, the previously reported silyl ether **23**^[19] was subjected to ozonolysis, followed by acid-catalyzed acetal protection with ethylene glycol and selective desilylation of the primary TBS silyl ether to afford primary alcohol **24** in 56 % yield over three steps (Scheme 3). Exposure of **24** to Swern oxidation produced the desired aldehyde **15** in quantitative yield. At this point, we were in a position to probe the feasibility of establishing the C5–C6 bond by Brown's protocol.^[23] Unfortunately, when aldehyde **15** was subjected to the standard Brown conditions, no workable amounts of product **14** could



Scheme 2. Model study. a) Pivaldehyde, Et₂O/THF, -100 °C, 70 %, d.r. > 95 %. b) *t*BuOOH, VO(acac)₂ (cat.), CH₂Cl₂, 88 %; or *m*CPBA, CH₂Cl₂, 84 %. c) CH₂=CH(CH₂)₃MgBr, CuCN (cat.), Et₂O, -60 → 0 °C, 89 %. d) TBSOTf, 2,6-lutidine, CH₂Cl₂, -78 °C, 85 %. e) (COCl)₂, Et₃N, DMSO, CH₂Cl₂, -78 °C → RT, quant. f) 4-Nitrobenzoyl chloride, pyridine, DAMP, THF, RT, 16 %.

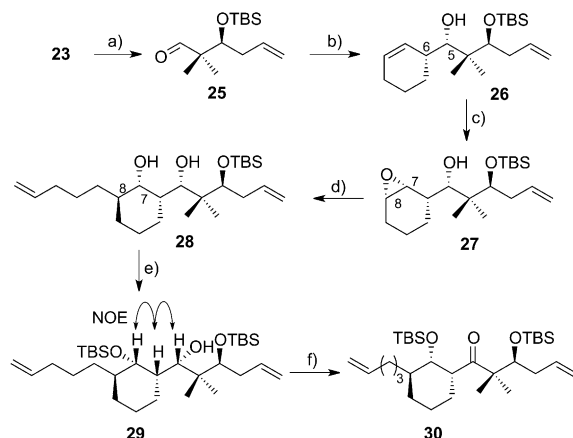
be separated (Scheme 3), while over 90 % of aldehyde **15** was recovered before the oxidative quench. Attempts to facilitate the reaction by increasing temperature and reaction



Scheme 3. a) 1) O₃, CH₂Cl₂, -78 °C, then PPh₃, RT; 2) ethylene glycol, PTSA (cat.), benzene, reflux; 3) HF/pyridine, THF, 0 °C → RT, 53 % (3 steps). b) (COCl)₂, Et₃N, DMSO, CH₂Cl₂, -78 °C → RT, quant.

time did not lead to satisfactory results. We presume the allylboration is disfavored not only by steric hindrance from the α-quaternary carbon of the aldehyde, but also by the coordination between borane and the acetal oxygen atoms, which in turn interrupts the interaction between borane and aldehyde. To address this problem, we turned our attention to an alternative aldehyde **25** (Scheme 4) in which a terminal olefin replaces the acetal in **15** and, thereby, avoids the potential coordination described above. Selective desilylation and subsequent Swern oxidation converted silyl ether **23** into the desired aldehyde **25**. Upon treatment of the modified aldehyde **25** with freshly prepared borane **16**, the

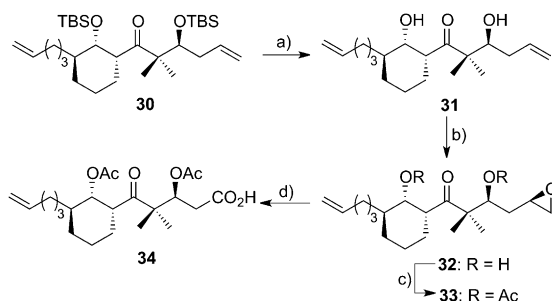
desired homoallylic alcohol **26** was obtained in excellent yield and selectivity (96 %, d.r. > 20:1 by ¹H NMR) as shown in Scheme 4. Surprisingly, both the C–C bond formation and oxidative cleavage of the B–O bond were unexpectedly sluggish, taking about three weeks. Stereochemistry at C5 and C6 was assigned on the basis of the model study (Scheme 2). With this chemistry in hand, the next phase involved a crucial stereoselective epoxidation and subsequent regioselective oxirane ring opening. Following the successful strategy achieved in the model study, alcohol **26** underwent chemoselective epoxidation by vanadium catalysis to provide hydroxy epoxide **27** in 93 % yield (d.r. > 20:1 by ¹H NMR), followed by copper-catalyzed epoxide opening with Grignard reagent to furnish diol **28** in 90 % yield as the sole diastereomer (Scheme 4). It is worth noting that an excess of Grignard reagent (8–9 equiv) was required to minimize formation of the bromohydrin side product.^[26] Selective silylation of the sterically less hindered OH group in **28** furnished silyl ether **29** (85 % yield). The relative stereochemistry of compound **29** was confirmed on the basis of NOESY experiments. In practice, the conversion from **26** to **29** could be completed in 93 % yield over three steps without purification of the intermediates **27** and **28**. To complete Scheme 4, Swern oxidation converted the secondary alcohol



Scheme 4. a) 1) HF/pyridine, THF, 0 °C → RT, 88 %; 2) (COCl)₂, Et₃N, DMSO, CH₂Cl₂, -78 °C → RT, 94 %. b) **16**, THF, -78 °C, then H₂O₂, NaHCO₃, 40 °C, 92 %, d.r. > 20:1. c) *t*BuOOH, VO(acac)₂ (cat.), CH₂Cl₂, 93 %, d.r. > 20:1. d) CH₂=CH(CH₂)₃MgBr (8–9 equiv), CuCN (cat.), Et₂O, -55 → 0 °C, 90 %. e) TBSOTf, 2,6-lutidine, CH₂Cl₂, -78 °C, 85 %. f) (COCl)₂, Et₃N, DMSO, CH₂Cl₂, -78 °C → RT, 99 %.

to ketone **30** in quantitative yield. The stereochemical assignment for ketone **30** was confirmed subsequently by comparison with its analogue **50** (see Scheme 9), the stereochemistry of which was determined by X-ray crystallography of a derivative.^[19] It is clear that aldehyde **25** has obvious advantages over aldehyde **15** in the context of cyclohexenylboration. However, application of **25** raised a second challenging problem, namely differentiation between the two terminal olefins with high structural similarity in **30**. As will be shown, the terminal olefin homoallylic to OTBS could be se-

lectively converted to a carboxylic acid by taking advantage of the masked homoallylic alcohol. Thus, we turned our attention to the hydroxy-directed epoxidation to give a carboxylic acid precursor. To pursue this strategy, desilylation of **30** with trifluoroacetic acid afforded diol **31** in 78 % yield (Scheme 5). Subsequently, vanadium catalyzed chemoselective epoxidation of **31**, as expected, led to β -hydroxy epoxide **32** in 89 % total yield as a mixture of two diastereomeric epoxides (ca. 10:1 by ^1H NMR). The stereochemistry of the epoxide has been tentatively assigned in accord with the proposed model by Mihelich and co-workers.^[27] In view of the subsequent cleavage of the epoxide, the diastereomeric epoxides were subjected to the next step without separation. At this stage, we initially attempted to reinstall the TBS silyl ether onto **32** in order to pursue the original synthetic plan (Scheme 1). However, all attempts with classical conditions failed to give satisfactory results.^[28] Fortunately, diacetyl epoxide **33** was cleanly obtained in 93 % yield by treatment of alcohol **32** with acetic anhydride and 4-dimethylaminopyridine (DMAP). At this point, it appeared timely to transfer the primary epoxide to the carboxylic acid. The conversion was accomplished with a three step sequence. The epoxide first underwent tetrabutylammonium bisulfate catalyzed hydrolysis,^[29] followed by NaIO_4 cleavage of the resulting diol to furnish an aldehyde intermediate. Purification of the aldehyde by silica gel flash column chromatography was unmanageable due to the instability of this intermediate. Thus, Pinnick oxidation of the crude aldehyde with NaClO_2 in the presence of 2-methyl-2-butene and NaH_2PO_4 in $t\text{BuOH}/\text{H}_2\text{O}$ provided the desired carboxylic acid **34** in 45 % yield over the three-step procedure (Scheme 5).



Scheme 5. a) TFA, CH_2Cl_2 , $-20 \rightarrow 0^\circ\text{C}$, 78 %. b) $t\text{BuOOH}$, $\text{VO}(\text{acac})_2$ (cat.), CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 89 %, d.r. 10:1 c) Ac_2O , DMAP, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 93 %. d) 1) $n\text{Bu}_4\text{NHSO}_4$ (cat.), $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 50°C ; 2) NaIO_4 , THF, RT; 3) NaClO_2 , NaH_2PO_4 , 2-methyl-2-butene, $t\text{BuOH}/\text{H}_2\text{O}$, 45 % (3 steps).

Assembly of building blocks and synthesis of 12,13-*trans*-6,8-bridged epothilone via olefin metathesis: With the key building block **34** in hand, our attention was directed to the feasibility of the olefin metathesis strategy. Therefore, the coupling between alcohol **12** and carboxylic acid **34** was performed under the influence of 1-ethyl-3-((dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and DMAP to furnish the proposed metathesis precursor **35** in 58 % yield as depicted in Scheme 6. In this coupling reaction, serious β -

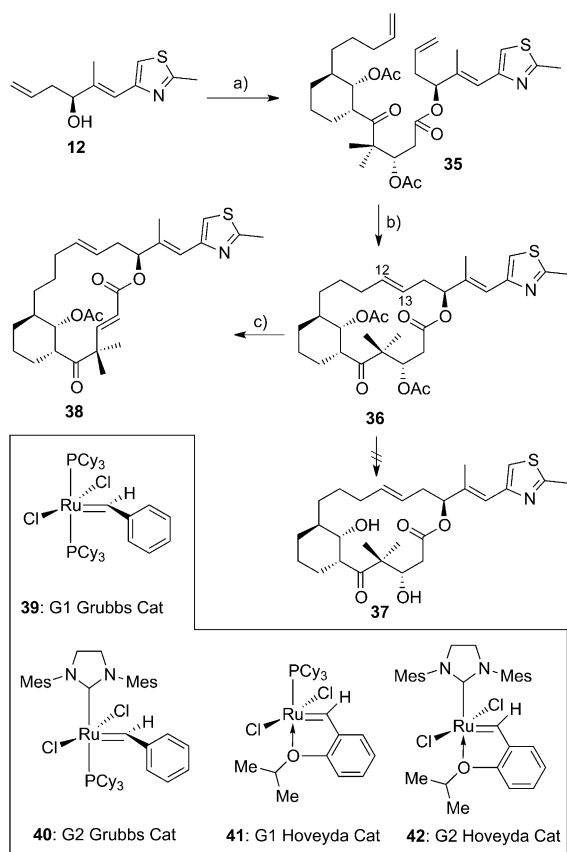
elimination from the β -acetate of the carboxylic acid was responsible for the modest yield. However, β -elimination was completely suppressed by a modified Yonemitsu–Yamaguchi protocol,^[30] giving keto ester **35** in 86 % yield. Exposure of **35** to metathesis catalysts **39–42** under highly dilute conditions resulted in clean formation of a single *trans*-macrocyclic olefin **36** ($J_{\text{H12-H13}} = 14.4$ Hz). Grubbs catalysts **39** and **40** as well as the Hoveyda catalyst **41** gave the *trans*-product in high yields, while the reaction was unresponsive to Hoveyda catalyst **42** (entry 5, Scheme 6). It is widely known that the *E/Z* selectivity of the ring closure metathesis depends on many factors including substrate, solvent, temperature and concentration.^[31] In this specific case, attempts to modify the geometric outcome of the reaction by choosing solvents and temperatures as recorded in Table 1 were unsuccessful. The

Table 1. Ring closure metathesis of **35** gives *trans*-olefin **36** exclusively.

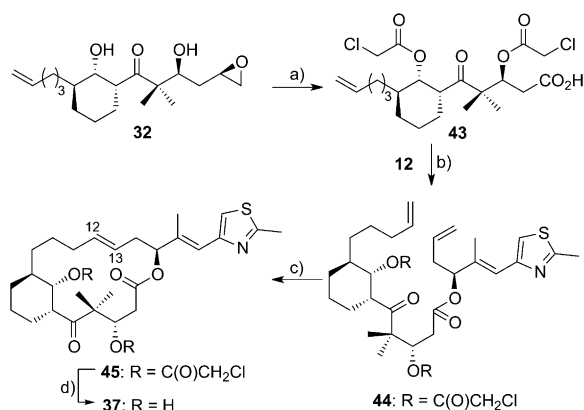
Entry	Cat. (10 mol %)	Conditions	Yield [%]	<i>E/Z</i> ^[a]
1	39	CH_2Cl_2 , RT, 12 h	84	>20:1
2	39	toluene, 80°C , 12 h	76	>20:1
3	40	CH_2Cl_2 , RT, 12 h	quant	>20:1
4	40	toluene, 80°C , 12 h	quant	>20:1
5	41	CH_2Cl_2 , RT, 12 h	<5	ND
6	41	toluene, 80°C , 12 h	95	>20:1

[a] The *E/Z* ratio was judged from crude ^1H NMR.

disheartening geometric results from olefin metathesis temporarily directed our attention to the 12,13-*trans*-6,8-bridged epothilone **C** (**37**, Scheme 6). Considering previous SAR studies suggesting that the non-natural epothilone analogue 12,13-*trans*-epothilone **C** is only slightly less active than the natural epothilone **C** (**3**),^[32] the C6–C8 bridged analogue **37** was regarded as potentially providing valuable structural information for the project. With this in mind, we turned to the deacylation of **36** (Scheme 6). Surprisingly, none of the reaction conditions applied was able to accomplish deprotection to produce the dihydroxyl lactone **37**. In all cases, either unreacted acetate was recovered or decomposition took place. One of the major side reactions arising from attempted deacylation was β -elimination leading to lactone **38**, which could be alternatively prepared from **36** in 96 % yield by treatment with 8-diazabicyclo[5.4.0]undec-7-ene (DBU). Such a process has been documented to suggest that the approximate 180° torsion angle around C2–C3 might be responsible for the elimination.^[3,33] We presume the unsuccessful deacylation could also arise from the competition between hydrolysis and elimination of the β -acetate. In this specific case, β -elimination might be much faster than hydrolysis of the acetate. To facilitate deacylation, we envisioned introducing a substituent to the acetyl moiety which could increase the acetyl hydrolysis rate, while not significantly altering its leaving group character. With this scenario in mind, the chloroacetyl group was introduced, recognizing that it might be 350–700 fold more quickly hydrolyzed than acetyl depending on the nature of the intermediates.^[34] As shown in Scheme 7, the required chloroace-



Scheme 6. a) **34**, EDCI, DMAP, CH_2Cl_2 , RT, 58%; or 2,4,6-trichlorobenzoylchloride, DMAP, Et_3N , toluene, $-78 \rightarrow 0^\circ\text{C}$, 86%. b) RCM, see text. c) DBU, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 96%.



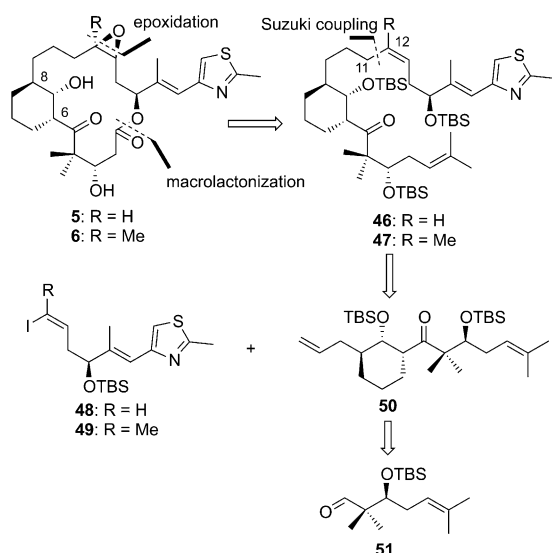
Scheme 7. a) 1) $(\text{ClCH}_2\text{CO})_2\text{O}$, DMAP, pyridine, CH_2Cl_2 , 0°C , quant.; 2) $\text{NaIO}_4/\text{H}_5\text{IO}_6$, $\text{THF}/\text{H}_2\text{O}$; 3) NaClO_4 , NaH_2PO_4 , 2-methyl-2-butene, $t\text{BuOH}/\text{H}_2\text{O}$, 72% (2 steps). b) 2,4,6-trichlorobenzoylchloride, DMAP, Et_3N , toluene, $-78 \rightarrow -35^\circ\text{C}$, 49%. c) RCM, cat. **42**, CH_2Cl_2 , RT, 77%. d) $\text{NH}_4\text{OH}/\text{MeOH}$, 0°C then NH_3/MeOH , 0°C , 57%.

tate was prepared from epoxy alcohol **32** in quantitative yield. Subsequent exposure to $\text{NaIO}_4/\text{H}_5\text{IO}_6$ mediated cleavage of the terminal epoxide to generate an aldehyde which was subsequently subjected to Pinnick oxidation to furnish the carboxylic acid **43** in 72% overall yield. Esterification of

keto acid **43** by alcohol **12** was achieved in modest yield with the modified Yonemitsu–Yamaguchi protocol.^[35] Without complication, the following olefin metathesis led cleanly to *trans*-product **45** ($J_{\text{H12-H13}} = 14.8 \text{ Hz}$). For example, a 77% yield of **45** was achieved with Hoveyda second-generation metathesis catalyst **42**. After screening various conditions for the crucial deacylation step, we discovered that it could be successfully performed by careful treatment of **45** with ammonium hydroxide in methanol (1:10, v/v) followed by treatment with ammonia in methanol to afford the 12,13-*trans*-6,8-bridged EpoC analogue **37** in 57% yield (Scheme 7). To bring the synthesis to a close, we initially attempted to isomerize the C12–C13 *trans* double regioselectively to the desired *cis* geometry. Unfortunately, following attempts such as photoirradiated isomerization,^[18] iodine-catalyzed free radical isomerization^[36] and Vedejs isomerization,^[37] no observable amounts of the isomerized product could be separated (assuming isomerization occurred). Either unreacted *trans*-olefin was recovered or decomposition took place.

Second-generation synthesis via Suzuki coupling: As discussed above, some surprising limitations surfaced in the ring forming olefin metathesis reaction. Although there is still much opportunity to optimize reaction conditions and employ alternative methods such as the molybdenum-based Schrock catalyst,^[38] the epothilone literature teaches that the stereochemical outcome of the RCM process is highly substrate dependent.^[21a,39] With such an ambiguous precedent, it was imperative to select a reliable method for accessing the desired *Z* stereochemistry. An important alternative to introduce the *Z*-double bond at C12–C13 is Danishefsky's *B*-alkyl Suzuki coupling strategy.^[40] Given the widespread application of this strategy in epothilone synthesis, we elected to explore its utility for our targets. The retrosynthesis for bridged epothilones **5** and **6** via the second-generation Suzuki coupling strategy is summarized in Scheme 8.^[41] The key disconnection for this route is at the C11–C12 bond, leading to vinyl iodides **48/49** and olefin **50** as the Suzuki coupling partners. The keto diene **50** derives from aldehyde **51** following a sequence similar to that for the synthesis of dienyl ketone **30** in Scheme 4. In contrast to keto diene **30**, a *gem*-dimethyl moiety was introduced at the right-side terminal olefin of **50** in order to easily differentiate the two olefins.

Construction of building partners for Suzuki coupling: To pursue this modified route, the preparation of Suzuki coupling precursor **50** is illustrated in Scheme 9.^[19] The modified aldehyde **51** was first obtained by a four-step sequence from **23** in 58% yield, followed by allylboration of aldehyde **51** with freshly prepared (–)-*B*-2-cyclohexen-1-yl diisopinocampheylborane (**16**) to give homoallylic alcohol **52** in 96% yield (d.r. >20:1 by $^1\text{H NMR}$). Then, using the previously validated vanadium-catalysis strategy, alcohol **52** was converted to epoxy alcohol **53** in 93% yield (d.r. >20:1 by $^1\text{H NMR}$). Reaction of epoxide **53** with allylmagnesium bro-

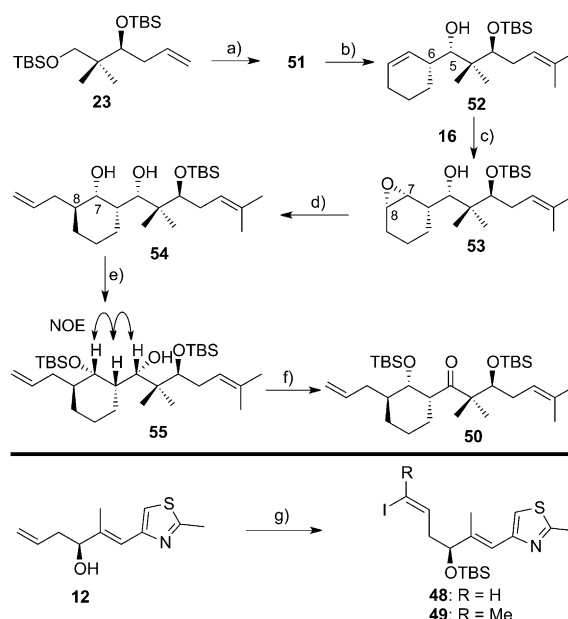


Scheme 8. Retrosynthesis of C6–C8 bridged EpoA/B by Suzuki coupling.

mide in a copper-catalyzed fashion furnished epoxide-opened product **54** (85% yield) along with a trace of the C7-alkylated isomer and bromohydrin. The sterically less hindered hydroxyl group from **54** was selectively converted to TBS silyl ether **55** in 97% yield. At this point, a NOESY analysis was executed to confirm the relative stereochemistry (Scheme 9). Finally, the sterically hindered secondary alcohol in diene **55** was transformed to dienyl ketone **50** by Swern oxidation in 85% yield. The absolute configuration of dienyl ketone **50** has been verified by X-ray crystal structure analysis of a derivative.^[19] Preparation of the other Suzuki coupling partners, vinyl iodides **48/49**, was accomplished from alcohol **12** in a four-step sequence (Scheme 9).^[39c,42]

Completion of the synthesis of bridged epothilones A–D:

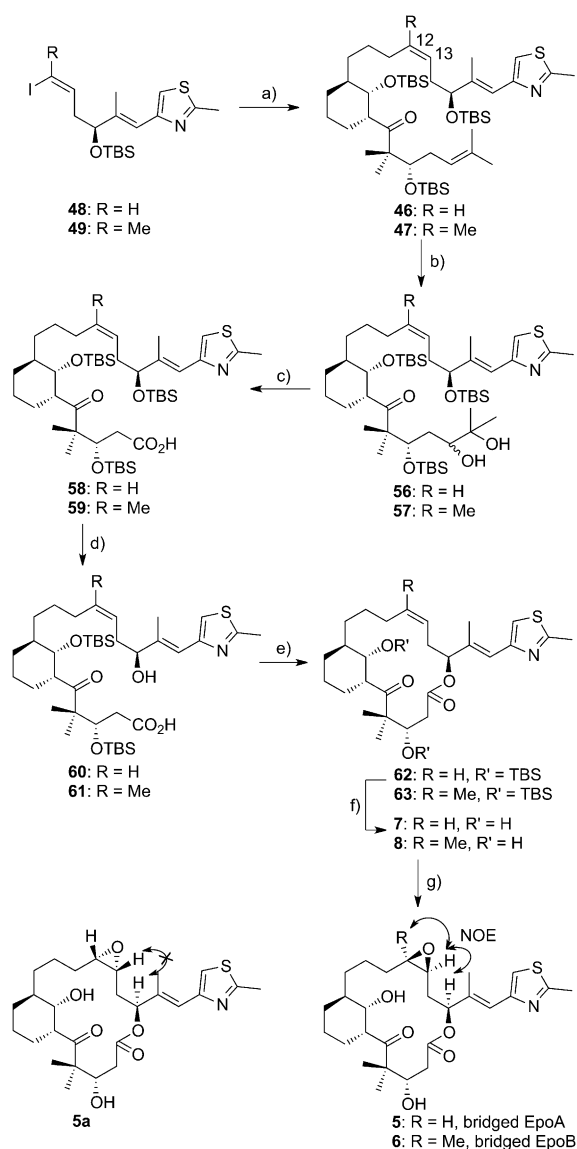
With the requisite coupling precursors in hand, the final steps in the synthesis of bridged epothilones **5/6** were carried out as depicted in Scheme 10. After regioselective hydroboration with 9-BBN, olefin **50** was coupled with vinyl iodides **48/49** in accordance with an approach reported by Danishefsky to furnish *cis*-olefins **46/47** ($J_{\text{H12-H13}} = 10.8 \text{ Hz}$, **46**) in 92 and 57% yield, respectively.^[40a] The following crucial regioselective dihydroxylation of trienes **46/47** was performed under Sharpless asymmetric dihydroxylation conditions^[43] to selectively convert the *gem*-dimethyl olefin to diols **56/57** as a mixture of diastereomers (**56**: 42% yield, 86% brsm, ca. 5:1 ratio by $^1\text{H NMR}$; **57**: 42% yield, 87% brsm, ca. 4:1 ratio by $^1\text{H NMR}$). The stereochemistry of the hydroxyl group was undefined. Without separation, the resulting mixture of diols was subjected to NaIO_4 mediated glycol cleavage and subsequent Pinnick oxidation to furnish the corresponding carboxylic acids **58** and **59** in 78 and 58% yield, respectively, after two steps (Scheme 10). As shown in Scheme 10, final steps in the synthesis of the target compounds **5/6** involved conversion of keto acids **58/59** to dihy-



Scheme 9. a) See ref. [19]. b) **16**, THF, -78°C , then H_2O_2 , NaHCO_3 , 40°C , 96%, d.r. $>20:1$. c) *t*BuOOH, $\text{VO}(\text{acac})_2$ (cat.), CH_2Cl_2 , 93%, d.r. $>20:1$. d) AllylMgBr (8.0 equiv), CuCN (cat.), Et_2O , $-55 \rightarrow 0^\circ\text{C}$, 85%. e) TBSOTf, 2,6-lutidine, CH_2Cl_2 , -78°C , 97%. f) $(\text{COCl})_2$, Et_3N , DMSO, CH_2Cl_2 , $-78^\circ\text{C} \rightarrow \text{RT}$, 85%. g) See ref. [39c, 42].

droxy lactones **60/61** by employing a procedure utilized by Nicolaou et al. in the total synthesis of epothilones A and B.^[44] Selective desilylation with tetra-*n*-butylammonium fluoride (TBAF), followed by Yamaguchi lactonization (**62** in 51% yield, and **63** in 60% yield) and global desilylation in the presence of a freshly prepared trifluoroacetic acid (TFA) solution in CH_2Cl_2 (v/v, 1:4) gave C6–C8 bridged epothilone C (**7**, 88% yield) and epothilone D (**8**, 91% yield). Finally, we were pleased to obtain the C6–C8 bridged epothilone A as a mixture of **5** and its *cis*-epoxide diastereomer **5a** (84% total yield, ca. 2:1 ratio, $^1\text{H NMR}$) by treatment with 3,3-dimethyldioxirane (DMDO) as described by Danishefsky et al.^[40a] Fortunately, these two diastereomers were separable by preparative thin-layer chromatography. In a similar fashion, the C6–C8 bridged epothilone D analogue **8** was converted to the bridged epothilone B analogue **6** in 52% yield (d.r. $>20:1$ by $^1\text{H NMR}$). The stereochemistry of the epoxide of these bridged epothilones was determined by 1D and 2D NOE analysis (Scheme 10).

Bioactivity: Microtubule assembly and cytotoxicity: The C6–C8 bridged epothilone analogues **5a**, **5–8**, **36–38** were exposed to A2780 ovarian cancer and PC3 prostate cancer cell lines to evaluate their antiproliferative properties (Table 2). In general, these C6–C8 bridged compounds are significantly less potent than the corresponding open chain analogues. Against the A2780 cell line, compound **38** exhibited the highest potency with an $\text{IC}_{50} = 1.1 \mu\text{M}$, but it is still 27-fold less potent than EpoD. C6–C8 Bridged Epo B **6** exhibited the highest potency against the PC3 prostate cancer cell line with a 206-fold potency loss by comparison with the



Scheme 10. a) **50**, 9-BBN, Cs_2CO_3 , AsPh_3 , $[\text{PdCl}_2(\text{dppf})]$, DMF/ H_2O , RT, **46**: 92%, **47**: 57%. b) $(\text{DHQD})_2\text{PHAL}$, K_2CO_3 , $\text{K}_3\text{Fe}(\text{CN})_6$, $\text{CH}_3\text{SO}_2\text{NH}_2$, $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$, $t\text{BuOH}/\text{H}_2\text{O}$, $0^\circ\text{C} \rightarrow \text{RT}$, **56**: 42% (86%, BRSM), d.r. 5:1; **57**: 42% (87%, brsm), d.r. 4:1. c) 1) NaIO_4 , THF/ H_2O , 0°C ; 2) NaClO_4 , NaH_2PO_4 , 2-methyl-2-butene, $t\text{BuOH}/\text{H}_2\text{O}$, **58**: 78% (2 steps), **59**: 58% (2 steps). d) TBAF, THF, $0^\circ\text{C} \rightarrow \text{RT}$, **60**: 95%, **61**: 90%. e) 2,4,6-Trichlorobenzoylchloride, Et_3N , DMAP, toluene, RT, **62**: 51%, **63**: 60%. f) TFA, CH_2Cl_2 , $-20 \rightarrow 0^\circ\text{C}$, **7**: 88%, **8**: 91%. g) DMDO, CH_2Cl_2 , $-50 \rightarrow -30^\circ\text{C}$, **5** (**5a**) 84%, d.r. 2:1; **6**: 52%, d.r. >20:1.

activity of EpoD. The ability of C6–C8 bridged epothilones to promote tubulin assembly and stabilize the microtubules against cold induced disassembly was also studied using $50 \mu\text{M}$ of the compounds in 4% (v/v) DMSO. The results were compared to that of $10 \mu\text{M}$ PTX under identical conditions (Figure 5). Compounds **37** and **5a** displayed the least effect on tubulin assembly. Compounds **6** and **8** were the most active, however, less so than PTX. None of the C6–C8

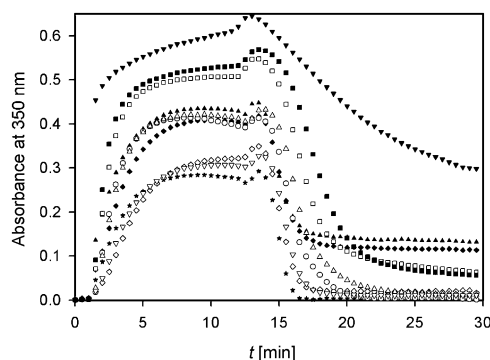


Figure 5. Tubulin assembly and microtubule cold stabilizing activity of bridged epothilones and PTX ($\blacktriangledown$), **5a** ($\blacktriangle$), **5** ($\blacklozenge$), **6** ($\diamond$), **7** ($\circ$), **8** ($\triangle$), **36** (∇), **37** ($\square$), **38** ($\blacksquare$). Tubulin ($10 \mu\text{M}$) was incubated with $50 \mu\text{M}$ of a bridged epothilone or $10 \mu\text{M}$ PTX in the presence of 1 mM GTP and 4% DMSO in PME. Assembly was monitored in terms of apparent absorption at 350 nm. The arrow indicates temperature drop from 37 to 4°C . For reference, a tubulin sample in the absence of promoter ($\star$) was also included.

bridged epothilones showed significant ability to stabilize the microtubules against cold induced disassembly.

Molecular mechanics and DFT energy evaluations: Obviously, these biological outcomes are not consistent with our having installed a C6–C8 bridge that constrains **5–8** to an efficacious bioactive epothilone pose. There are several possible explanations for this: 1) the C6–C8 bridge has retained the target conformation of the epothilone ring, but altered the binding mode to prevent effective coordination with the protein; 2) incorporation of the C6–C8 bridge has raised the energy of the bound target conformations of **5–8** making them inaccessible to microtubules; 3) the molecules have been constrained as expected (Figure 3), but the target conformation is not the de facto bound form as proposed. Concerning 1), there are precedents for loss of activity resulting from internal bridge building in taxanes. In two cases, NMR evidence supported the conclusion that the ligand conformations appear to be retained, but the additional tether interferes sterically with the tubulin binding site, lifts the molecule higher in the pocket and thereby reduces ligand binding.^[45] In one of these reports involving cyclic constraints in the C13 side chain, the differences in tubulin binding and MCF7 cell cytotoxicity (5–10 fold and 37–120 fold, respectively) arose from expansion of ring size from five members to six.^[50a] Thus, in spite of our initial assessment that a six-membered ring in **5–8** would cause little steric congestion (Figure 3), the degree of crowding may have been underestimated. With respect to 2), we have examined the conformational landscapes of **1** and **5** to determine the energy differences between the respective bound conformations (Fig-

Table 2. Cytotoxicity of epothilone B, D and C6–C8 bridged epothilone analogues ($\text{IC}_{50} [\mu\text{M}]$).

Cpd.	EpoD	5a	5	6	7	8	36	37	38
A2780	0.04	24.3	8.5	3.6	19.0	5.1	5.6	9.6	1.1
PC3	0.016 ± 0.001	10.3 ± 1.6	11.6 ± 0.3	3.3 ± 0.2	16.3 ± 1.8	6.7 ± 0.2	7.6 ± 0.2	10.7 ± 0.6	9.8 ± 0.8

ure 3A) and their corresponding global minima (GM). Thus, individual 25000 step conformational searches for the two structures were performed with the MMFF/GBSA/H₂O protocol in Schrödinger's MacroModel.^[46] The GM were then re-optimized with the OPLS-2005, MM3 and AMBER molecular mechanics methods.^[46] The EpoA EC structure (**1**, Figure 3A) and that of **5** based on it were likewise optimized with the same four methods to put the structures (LM or local minimum forms) on the same energy scales. A comparison of the EC-related structures and the optimized variants demonstrated minimal average root mean square deviations (RMSD) of 0.098 to 0.35 Å for all heavy atoms. Superposition of the EC-based structures and the optimized OPLS-2005 conformers (average heavy-atom RMSD values of 0.26 and 0.14 Å, respectively) are provided in the Supporting Information to demonstrate essentially a perfect match between the EC bound and force-field optimized conformers. The energy differences between GM and LM for epoA and **5** for each method ($\Delta\Delta E((\text{GM-LM})^{\text{epoA}} - (\text{GM-LM})^5)$) were computed to show that epoA falls lower by 3.0 (OPLS-2005), 2.4 (AMBER), 1.1 (MMFF) and 0.8 (MM3) kcal mol⁻¹ (see the Supporting Information for details). Thus, in the molecular mechanics regime, the bridged structure **5** is predicted to require an additional 1.8 kcal mol⁻¹ on average relative to epoA to achieve the bound form on tubulin in the context of the EC structure. In order to avoid issues surrounding the variable parameterization of the force field protocols, we calculated the energies of the OPLS-2005 optimized GM and LM forms for EpoA (**1**) and **5** with the density functional protocol B3LYP/6-31G*. The $\Delta\Delta E = 5.5$ kcal mol⁻¹ reinforces the force field trend and suggests that the weaker binding for compound **5** may be a result of increased internal conformational strain energy relative to EpoA. The possibility remains that 3) is the underlying cause of the bio-data presented here. However, until a definitive structure of the tubulin–epothilone complex is determined, the current EC^[17] and NMR^[47] structures remain the front-line contenders for the bound conformation of epothilones to β -tubulin.

Conclusion

A series of conformationally restrained epothilone analogues with a short bridge between methyl groups at C6 and C8 was designed to mimic the binding pose derived for our recently reported EpoA–microtubule binding model. A versatile synthetic route to these bridged epothilone analogues has been successfully devised and implemented. The key stereochemistry within the bridged C6–C8 sector was controlled by asymmetric allylboration followed by hydroxy-directed epoxidation and regiocontrolled opening of the resultant epoxide. The *cis*-C12–C13 double was constructed via Suzuki coupling while the ring-closure metathesis exclusively gave *trans* selectivity. The C6–C8 bridged epothilones were evaluated for their biological activity against the A2780 human ovarian cancer and PC3 prostate cancer cell

lines. The cytotoxicity data implies that these epothilone analogues are considerably less potent than the natural epothilones. The tubulin assembly and microtubule cold stabilization assay reveal that compounds **6** and **8** inhibit tubulin assembly weakly, while none of the bridged epothilone analogues show significant microtubule stabilization against cold induced disassembly. Possible causes for the poor activity of the bridged epothilones include steric congestion between tubulin and the C6–C8 bridge, torsional strain in the flexible portion of the epothilone ring raising the energy requirement for binding and a mismatch with the empirical binding pose. Insights into the first two explanations are provided.

Experimental Section

General: Unless otherwise noted, commercial reagents and solvents were used as received unless otherwise noted. Flash column chromatography was performed by employing either Sorbent Technologies 200–400 mesh or Watersman 230–400 mesh silica gel 60. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 (0.25 mm thick) from EM Science. TLC plates were visualized by exposure to ultraviolet light (UV) and/or exposure to phosphomolybdic acid or potassium permanganate TLC stains followed by brief heating on a hot plate. Preparative TLC separation was performed on Analtech preparative plates pre-coated with silica gel 60 UV254 (0.5, 1.0 or 1.5 mm thick). Melting points (m.p.), determined on a MEL-TEMP Melting Point Apparatus from Laboratory Devices, are uncorrected. Optical rotations were measured on a Perkin–Elmer Model 341 digital polarimeter with a sodium lamp at room temperature. Infrared (IR) spectra were recorded on a Nicolet 370 with a diamond probe or an ASI ReactIR 1000 FI-IR Spectrophotometer with a silicone probe (wavenumbers (cm⁻¹)). Where noted “neat”, the sample was loaded as a thin film. Proton nuclear magnetic resonance (¹H NMR) spectra and carbon nuclear magnetic resonance (¹³C NMR) spectra were determined on an INOVA400 (¹H NMR: 400 MHz, and ¹³C NMR: 100 MHz) or INOVA600 (¹H NMR: 600 MHz, and ¹³C NMR: 150 MHz) instrument. Chemical shifts for ¹H NMR are reported in parts per million (δ =scale) with deuterated chloroform (CDCl₃) as the internal standard (7.26 ppm) and coupling constants are in Hertz (Hz). The following abbreviations are used for spin multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, brs=broad singlet. Chemical shifts for ¹³C NMR are reported in parts per million (δ =scale) relative to the central line of the triplet at 77.23 ppm for deuterated chloroform (CDCl₃). High resolution mass spectra (HRMS) were obtained on a JEOL JMS-SX102/SX102A/E or Thermo Finnigan LTQ-FTMS instrument. Experimental details, characterization data for all new compounds, NMR spectra of key intermediates, X-ray crystal structure data for **19** and results for energy calculations for **1** and **5** are available in the Supporting Information.

Molecular modeling and docking: The 3D structure of bridged epothilones **5** and **6** were constructed based on the electron crystallographic (EC) pose of EpoA bound to a β -tubulin.^[17] The resulting structure of **5** and **6** was then fully optimized with the MMFF/GBSA/H₂O force field to provide the nearest local minimum. The latter was flexibly Glide-docked^[48] into the electron crystallographic structure of EpoA–tubulin.^[17] The best docking pose was chosen on the basis of the Glide scoring function together with visualization to ensure a reasonable binding mode and match with the EC complex. Conformational analysis for **1** and **5** and energy evaluations are described in the Supporting Information.

trans-12,13-Macrolactone **36**

Procedure A: To a solution of diene **35** (7.5 mg, 0.0125 mmol, 1.0 equiv) in CH₂Cl₂ (12.5 mL, 0.001 M) was added Grubbs catalyst I (1.1 mg, 0.00125 mol, 10 mol%), and the reaction mixture was allowed to stir at 25°C for 12 h. After the completion of the reaction as established by

TLC, the solvent was removed under reduced pressure and the crude product was purified by preparative thin-layer chromatography (hexanes/ethyl acetate 3:1) to afford the *trans*-lactone **36** (6.0 mg, 84 %) as a white foam.

Procedure B: To a solution of diene **35** (15 mg, 0.0249 mmol, 1.0 equiv) in toluene (25 mL) was added with Grubbs catalyst I (2.1 mg, 0.00249 mol, 10 mol %), and the reaction mixture was allowed to stir at 80 °C for 12 h. After the reaction is complete, the mixture was worked up according to the procedure described in procedure A to furnish **36** (10.9 mg, 76 %).

Procedure C: Diene **35** (13 mg, 0.0216 mmol, 1.0 equiv) was converted to **36** (12.2 mg, 100 %) in accordance with the procedure described in procedure A except for the use of Grubbs catalyst II (1.8 mg, 0.0022 mol, 10 mol %).

Procedure D: Diene **35** (13 mg, 0.0216 mmol, 1.0 equiv) was converted to **36** (10.1 mg, 100 %) in accordance with the procedure described in procedure B except for the use of Grubbs catalyst II (1.8 mg, 0.0022 mol, 10 mol %).

Procedure E: Diene **35** (12 mg, 0.02 mmol, 1.0 equiv) was converted to **36** (10.8 mg, 95 %) in accordance with the procedure described in procedure A except for the use of Hoveyda–Grubbs catalyst II (0.6 mg, 0.002 mol, 5 mol %). The crude reaction mixtures in procedures A, B, C, D and E were determined to be >20:1 ratio of diastereomeric *trans*-olefin by ¹H NMR spectroscopy. $R_f=0.37$ (hexanes/ethyl acetate 2:1); $[\alpha]_D^{25} = -47.8$ ($c = 1.0$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = 6.97$ (s, 1H, SCH=C), 6.50 (s, 1H, CH=CCH₃), 5.87 (dd, $J = 7.2, 5.2$ Hz, 1H, CH₂CHOAc), 5.57 (ddd, $J = 14.4, 7.2, 7.2$ Hz, 1H, CH=CH), 5.47 (ddd, $J = 14.4, 7.2, 7.2$ Hz, 1H, CH=CH), 5.31 (dd, $J = 9.4, 1.9$ Hz, 1H, CHOC(O)CH₃), 5.07 (s, 1H, CHCHOAc), 3.29–3.23 (m, 1H, CHC(O)), 2.69 (s, 3H, N=C(S)CH₃), 2.71–2.68 (m, 1H, CH₂CO₂), 2.62–2.56 (m, 2H), 2.41 (dd, $J = 15.3, 4.9$ Hz, 1H), 2.21–2.15 (m, 1H), 2.10 (s, 3H, ArCH=CCH₃), 2.05 (s, 3H, CH₃CO₂), 2.03 (s, 3H, CH₃CO₂), 1.98–1.89 (m, 2H), 1.86–1.76 (m, 1H), 1.72–1.67 (m, 2H), 1.57–1.493 (m, 3H), 1.40–1.35 (m, 2H), 1.33–1.21 (m, 2H), 1.14 (s, 3H, C(CH₃)₂), 1.07 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta = 211.62, 170.71, 170.27, 169.21, 164.79, 152.88, 137.68, 132.67, 126.91, 119.64, 116.73, 79.62, 71.33, 70.48, 53.62, 41.94, 37.83, 37.71, 36.50, 31.30, 28.82, 26.94, 24.99, 24.09, 21.42, 21.24, 20.00, 19.89, 19.50, 18.87, 15.39$ ppm; IR (Nujol): $\tilde{\nu} = 2926, 2862, 1731, 1707, 1504, 1443, 1371, 1239, 1180, 1029, 972, 916, 731$ cm⁻¹; HRMS: m/z : calcd for C₃₁H₄₄NO₇S: 574.28385 [M–H]⁺; found 574.28292.

trans-2,3-Keto lactone 38: A mixture of macrolactone **36** (21 mg, 0.0366 mmol, 1.0 equiv) in anhydrous CH₂Cl₂ (2 mL) was treated with DBU (55.7 mg, 0.366 mmol, 10.0 equiv) at room temperature. After being stirred for 3 h, no more **36** was detected from TLC. The solvent was removed under reduced pressure without further workup. The resultant residue was purified by preparative thin-layer chromatography (hexanes/ethyl acetate 4:1) to furnish product **38** (18.4 mg, 96 %) as a colorless oil. $R_f=0.51$ (hexanes/ethyl acetate 2:1); $[\alpha]_D^{25} = +17.4$ ($c = 1.68$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = 7.40$ (d, $J = 16.0$ Hz, 1H, CH=CHC(O)), 6.96 (s, 1H, SCH=C), 6.61 (s, 1H, CH=CCH₃), 6.07 (d, $J = 16.0$ Hz, 1H, CH=CHC(O)), 5.56 (dd, $J = 10.3, 2.2$ Hz, 1H, CHOC(O)CH₃), 5.53–5.37 (m, 2H, CH₂CH=CH), 4.86 (s, 1H, CHCHOAc), 3.01–2.98 (m, 1H, CHC(O)), 2.71 (s, 3H, N=C(S)CH₃), 2.52–2.49 (m, 1H), 2.44–2.39 (m, 1H), 2.19–2.14 (m, 1H), 2.11 (s, 3H, ArCH=CCH₃), 2.00 (s, 3H, CH₃CO₂), 2.04–1.95 (m, 1H), 1.93–1.85 (m, 1H), 1.69–1.62 (m, 2H), 1.58–1.52 (m, 1H), 1.50–1.37 (m, 3H), 1.26–1.12 ppm (m, 10H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 210.45, 170.59, 165.38, 164.88, 152.72, 152.39, 138.22, 132.55, 127.20, 121.98, 112.00, 116.39, 77.77, 71.34, 51.91, 43.51, 39.24, 36.58, 33.09, 28.54, 26.72, 23.64, 23.29, 23.00, 22.79, 21.38, 19.46, 15.61$ ppm; IR (Nujol): $\tilde{\nu} = 2929, 2861, 1713, 1645, 1503, 1444, 1379, 1362, 1294, 1242, 1177, 1048, 1017, 992, 970, 913, 879, 731$ cm⁻¹; HRMS: m/z : calcd for C₂₉H₄₀NO₅S: 514.26272 [M+H]⁺; found 514.26186.

trans-12,13-Hydroxy lactone 37: A solution of chlorolactone **45** (60 mg, 0.093 mmol) in methanol (10 mL) at 0 °C was treated with ammonium hydroxide (0.5 mL), and stirred at that temperature until the reaction was complete (ca. 12 h). The solvent was removed under reduced pressure to give white foam. Next, the white foam was dissolved in methanol (10 mL), and treated with amino methanol (1 mL, 7N in methanol) at

0 °C. After being stirred for 48 h, ¹H NMR suggested the reaction was complete. The solvent was removed under reduced pressure and the residue was purified by preparative thin-layer chromatography (hexanes/ethyl acetate 15:4) to afford hydroxy lactone **37** (26 mg, 57 %) as a white foam. $R_f=0.38$ (CH₂Cl₂/MeOH 15:1); $[\alpha]_D^{25} = -11.6$ ($c = 0.85$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = 6.96$ (s, 1H, SCH=C), 6.52 (s, 1H, CH=CCH₃), 5.48 (dd, $J = 9.8, 3.7$ Hz, 1H, CHOC(O)), 5.45–5.40 (m, 1H, CH=CH), 5.37–5.32 (m, 1H, CH=CH), 4.41 (dd, $J = 10.5, 1.8$ Hz, 1H, CHOHC(CH₃)₂), 4.16 (s, 1H, CHCHOH), 3.67 (d, $J = 1.5$ Hz, 1H, CHOHC(CH₃)₂), 3.65 (d, $J = 2.5$ Hz, 1H, CHCHOH), 2.81 (d, $J = 10.4$ Hz, 1H, CHC(O)), 2.70 (s, 3H, N=C(S)CH₃), 2.50–2.42 (m, 3H), 2.24–2.19 (m, 1H), 2.17 (d, $J = 17.0$ Hz, 1H, CH₂CO₂), 2.07 (s, 3H, CH=CCH₃), 1.93–1.83 (m, 3H), 1.73 (brs, 1H), 1.59–1.52 (m, 3H), 1.51–1.43 (m, 1H), 1.34–1.22 (m, 5H), 1.21–1.12 (m, 2H), 1.02 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta = 221.27, 173.30, 165.04, 152.42, 137.41, 134.02, 126.68, 120.60, 116.80, 79.52, 71.59, 71.53, 53.85, 43.83, 38.41, 37.97, 36.69, 31.70, 28.14, 27.45, 24.84, 24.27, 22.49, 20.64, 19.46, 16.05, 15.12$ ppm; IR (Nujol): $\tilde{\nu} = 3486$ (br), 2930, 2860, 1729, 1679, 1505, 1444, 1405, 1374, 1336, 1297, 1247, 1177, 1123, 1085, 1046, 984, 915, 865, 726, 676 cm⁻¹; HRMS: m/z : calcd for C₂₇H₄₀NO₅S: 490.26272 [M+H]⁺; found 490.26064.

Bridged epothilone C (7): A solution of Yamaguchi lactonization product **62** (89 mg, 0.124 mmol) in CH₂Cl₂ (0.1 mL) was treated with a freshly prepared CF₃CO₂H/CH₂Cl₂ (0.73 mL, v/v, 1:4) at –20 °C. The reaction mixture was allowed to reach 0 °C in 20 min and was stirred for additional 1 h at that temperature at which time all silyl ether disappeared from TLC plate. The mixture was diluted with CH₂Cl₂ (5 mL) and carefully neutralized by saturated aqueous NaHCO₃. After separation, the aqueous phase was further extracted with CH₂Cl₂ (2 × 5 mL). The combined organics were dried over MgSO₄, filtered and concentrated. The resulting residue was purified by preparative thin-layer chromatography (CH₂Cl₂/MeOH 20:1) to afford pure desired epothilone C analogue **7** (53.4 mg, 88 %) as a colorless oil. $R_f=0.36$ (CH₂Cl₂/MeOH 15:1); $[\alpha]_D^{25} = -86.8$ ($c = 1.0$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = 6.97$ (s, 1H, SCH=C), 6.62 (s, 1H, CH=CCH₃), 5.50 (ddd, $J = 10.5, 10.5, 5.0$ Hz, 1H, CH=CH), 5.40 (ddd, $J = 10.5, 10.5, 5.0$ Hz, 1H, CH=CH), 5.22 (d, $J = 8.3$ Hz, 1H, CHOC(O)), 4.42 (dd, $J = 11.5, 1.8$ Hz, 1H, CHOHC(CH₃)₂), 4.20 (s, 1H, CHCHOH), 3.89 (s, 1H, OH), 3.49 (s, 1H, OH), 2.98 (d, $J = 10.8$ Hz, 1H, CHC(O)), 2.76–2.63 (m, 4H), 2.50 (dd, $J = 14.7, 11.6$ Hz, 1H, CH₂CO₂), 2.32 (dd, $J = 14.7, 2.3$ Hz, 1H, CH₂CO₂), 2.30–2.24 (m, 1H), 2.18 (tt, $J = 10.7, 7.6$ Hz, 1H), 2.08 (s, 3H, CH=CCH₃), 2.04–1.85 (m, 3H), 1.84–1.74 (m, 1H), 1.66–1.43 (m, 4H), 1.36–1.29 (m, 2H), 1.28 (s, 3H, C(CH₃)₂), 1.26–1.16 (m, 2H), 1.06 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta = 220.74, 170.52, 165.35, 152.04, 139.43, 133.17, 125.23, 119.44, 115.82, 78.76, 73.05, 69.62, 54.22, 43.97, 39.79, 39.54, 31.91, 30.05, 28.83, 28.10, 25.17, 23.92, 23.58, 20.85, 19.25, 17.77, 16.26$ ppm; IR (Nujol): $\tilde{\nu} = 3478$ (br), 2928, 2860, 1736, 1678, 1507, 1443, 1409, 1291, 1248, 1187, 1084, 1046, 982, 913, 731 cm⁻¹; HRMS: m/z : calcd for C₂₇H₄₀NO₅S: 490.26272 [M+H]⁺; found 490.26144.

Bridged epothilone A (5) and (5a): To a solution of bridged epothilone C (**7**) (23 mg, 0.047 mmol, 1.0 equiv) in dry CH₂Cl₂ (2 mL) at –50 °C was added a freshly prepared dry solution of 3,3-dimethyldioxirane (1.18 mL, ca. 0.094 mmol, 0.08 M in acetone, 2.0 equiv). The resulting solution was allowed to warm to –30 °C for 2 h. A stream of argon was then bubbled through the solution to remove excess dimethyldioxirane. The crude mixture was determined to be a mixture of diastereomeric *cis*-epoxides (ca. 5:2 ratio by ¹H NMR). Preparative thin-layer chromatography (CH₂Cl₂/MeOH 20:1) to afford bridged epothilone A (**5**) (13.0 mg, 55 %) as a white foam and the *cis*-epoxide diastereomer **5a** (7.0 mg, 29 %) as a white solid. **5:** $R_f=0.34$ (CH₂Cl₂/MeOH 15:1); $[\alpha]_D^{25} = -26.9$ ($c = 0.87$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta = 6.98$ (s, 1H, SCH=C), 6.61 (s, 1H, CH=CCH₃), 5.35 (dd, $J = 9.9, 1.5$ Hz, 1H, CHOC(O)), 4.38 (d, $J = 10.3$ Hz, 1H, CHOHC(CH₃)₂), 4.31 (s, 1H, CHCHOH), 4.00 (s, 1H, OH), 3.83 (brs, 1H, CHCHOH), 3.03 (ddd, $J = 9.6, 3.0, 3.0$ Hz, 1H, CH₂CH-O(epoxide)CH), 2.98 (d, $J = 10.6$ Hz, 1H, CHC(O)), 2.96–2.93 (ddd, $J = 9.6, 3.0, 3.0$ Hz, 1H, CH₂CH-O(epoxide)CH), 2.68 (s, 3H, N=C(S)CH₃), 2.52 (dd, $J = 14.5, 11.3$ Hz, 1H, CH₂CO₂), 2.30 (dd, $J = 14.5, 2.5$ Hz, 1H, CH₂CO₂), 2.24–2.18 (m, 1H), 2.09 (s, 3H, CH=CCH₃), 2.08–2.02 (m, 1H), 1.95–1.84 (m, 3H), 1.80 (ddd, $J = 15.0, 9.9, 9.9$ Hz, 1H),

1.68 (ddd, $J=25.4$, 12.1, 5.3 Hz, 1H), 1.63–1.56 (m, 2H), 1.56–1.46 (m, 2H), 1.44–1.39 (m, 1H), 1.38–1.22 (m, 6H), 1.08 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta=220.87$, 170.45, 165.54, 151.68, 138.84, 120.16, 116.19, 76.96, 72.93, 68.08, 57.70, 55.75, 54.11, 43.80, 39.82, 39.65, 31.89, 30.90, 27.98, 25.34, 25.26, 24.74, 23.36, 21.04, 19.25, 17.96, 16.07 ppm; IR (Nujol): $\bar{\nu}=3462$ (br), 2930, 2864, 1731, 1679, 1509, 1444, 1413, 1390, 1293, 1258, 1181, 1154, 1085, 1046, 980, 919, 725 cm⁻¹; HRMS: m/z : calcd for C₂₇H₄₀NO₆S: 506.25763 [M+H]⁺; found 506.25654. *cis*-Epoxide diastereomer **5a**: $R_f=0.34$ (CH₂Cl₂/MeOH 15:1); [α]_D²⁵ = -58.9 ($c=1.0$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta=6.99$ (s, 1H, SCH=C), 6.65 (s, 1H, CH=CCH₃), 5.60 (t, $J=3.9$ Hz, 1H, CHOC(O)), 4.36 (dd, $J=11.1$, 1.9 Hz, 1H, CHOHC(CH₃)₂), 4.16 (s, 1H, CHCHOH), 3.99 (s, 1H, OH), 3.80 (brs, 1H, OH), 3.24 (ddd, $J=7.4$, 4.4, 4.4 Hz, 1H, CH₂CH-O(epoxide)CH), 3.11 (dd, $J=12.3$, 1.7 Hz, 1H, CHC(O)), 3.05–3.02 (m, 1H, CH₂CH-O(epoxide)CH), 2.69 (s, 3H, N=C(S)CH₃), 2.56 (dd, $J=14.7$, 11.2 Hz, 1H, CH₂CO₂), 2.37 (dd, $J=14.6$, 2.6 Hz, 1H, CH₂CO₂), 2.10 (s, 3H, CH=CCH₃), 2.09–1.96 (m, 3H), 1.93–1.82 (m, 3H), 1.66–1.46 (m, 6H), 1.39–1.18 (m, 6H), 1.08 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta=221.01$, 169.96, 165.32, 151.91, 136.90, 119.81, 116.12, 75.86, 72.88, 68.25, 57.23, 54.24, 53.79, 44.18, 39.78, 39.59, 30.83, 29.01, 28.28, 25.42, 25.06, 24.51, 22.20, 21.08, 19.27, 18.82, 16.31 ppm; IR (Nujol): $\bar{\nu}=3466$ (br), 2926, 2860, 1737, 1679, 1556, 1509, 1447, 1413, 1390, 1324, 1297, 1254, 1189, 1150, 1085, 1042, 1004, 984, 953, 919 cm⁻¹; HRMS: m/z : calcd for C₂₇H₄₀NO₆S: 506.25763 [M+H]⁺; found 506.25659. The direction of the epoxide was further determined by 1D and 2D NOE.

Bridged epothilone D (8): The desired C6–C8 bridged epothilone D (**8**) was prepared from bis(silyl ether) macrolactone **63** (205 mg, 0.28 mmol) by treatment with CF₃CO₂H according to the same procedure described above for the preparation of **7**, to obtain pure lactone **8** (137 mg, 91%) as a colorless oil or white foam. $R_f=0.46$ (CH₂Cl₂/MeOH 15:1); [α]_D²⁵ = -109.9 ($c=1.35$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta=6.95$ (s, 1H, SCH=C), 6.60 (s, 1H, ArCH=CCH₃), 5.13 (m, 2H, CHOC(O), CH₂CH=CCH₃), 4.62–4.39 (m, 1H, CHOHC(CH₃)₂), 4.28 (s, 1H, CHCHOH), 3.87 (s, 1H, OH), 3.69 (d, $J=5.9$ Hz, 1H, OH), 2.98 (dd, $J=12.4$, 2.2 Hz, 1H, CHC(O)), 2.67 (s, 3H, N=C(S)CH₃), 2.62 (ddd, $J=15.2$, 10.1, 10.1 Hz, 1H), 2.44 (dd, $J=14.2$, 11.4 Hz, 1H, CH₂CO₂), 2.35–2.30 (m, 1H), 2.26–2.21 (m, 2H), 2.06 (d, $J=1.1$ Hz, 3H, ArCH=CCH₃), 2.02–1.85 (m, 2H), 1.81–1.74 (m, 2H), 1.67 (s, 3H, CH=CCH₃CH₂), 1.62–1.44 (m, 5H), 1.38–1.19 (m, 6H), 1.04 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta=221.06$, 170.48, 165.33, 151.94, 140.02, 138.48, 121.17, 119.17, 115.61, 79.13, 73.02, 69.77, 54.28, 43.81, 40.03, 39.82, 32.89, 32.13, 29.93, 27.03, 25.26, 23.91, 23.88, 23.39, 20.89, 19.18, 17.02, 16.28 ppm; IR (Nujol): $\bar{\nu}=3435$ (br), 2934, 2864, 1733, 1679, 1509, 1447, 1413, 1378, 1336, 1293, 1251, 1185, 1143, 1081, 1046, 984, 938, 914, 849, 714, 683 cm⁻¹; HRMS: m/z : calcd for C₂₈H₄₂NO₅S: 504.27837 [M+H]⁺; found 504.27762.

C6–C8 bridged epothilone B (6): To a solution of bridged desoxyepothilone B (**8**) (0.20 mg, 0.04 mmol, 1.0 equiv) in CH₂Cl₂ (0.4 mL) at -78°C was added freshly prepared 3,3-dimethyldioxirane (1.0 mL, ca. 0.08 mmol, ca. 0.08 M in acetone, 2.0 equiv) dropwise. The resulting solution was warmed to -50°C for 1 h, and another portion of dimethyldioxirane (0.4 mL, 0.032 mmol) was added. After stirring at -50°C for additional 2.5 h, A stream of argon was then bubbled through the solution at -50°C to remove excess dimethyldioxirane and solvent. The crude reaction mixture was determined to be >20:1 ratio of diastereomeric *cis*-epoxides by ¹H NMR spectroscopy. The resulting residue was purified by preparative thin-layer chromatography (CH₂Cl₂/MeOH 30:1) to afford bridged epothilone B (**6**) (10.8 mg, 52%) as a white foam. $R_f=0.39$ (CH₂Cl₂/MeOH 15:1); [α]_D²⁵ = -57.8 ($c=1.0$, CHCl₃); ¹H NMR (600 MHz, CDCl₃): $\delta=6.98$ (s, 1H, SCH=C), 6.61 (s, 1H, ArCH=CCH₃), 5.33 (dd, $J=8.9$, 2.7 Hz, 1H, CHOC(O)), 4.43 (d, $J=10.0$ Hz, 1H, CHOHC(CH₃)₂), 4.37 (s, 1H, CHCHOH), 4.13 (s, 1H, OH), 4.00 (s, 1H, OH), 3.01 (dd, $J=12.1$, 1.8 Hz, 1H, CHC(O)), 2.79 (dd, $J=9.1$, 2.8 Hz, 1H, CH₂CH-O(epoxide)CH), 2.68 (s, 3H, N=C(S)CH₃), 2.50 (dd, $J=14.2$, 10.7 Hz, 1H, CH₂CO₂), 2.24 (dd, $J=14.3$, 2.6 Hz, 1H, CH₂CO₂), 2.16 (ddd, $J=15.1$, 2.8, 2.8 Hz, 1H), 2.08 (s, 3H, CH=CCH₃), 2.07–2.00 (m, 1H), 1.97–1.72 (m, 5H), 1.72–1.47 (m, 5H), 1.41–1.20 (m, 9H), 1.07 ppm (s, 3H, C(CH₃)₂); ¹³C NMR (100 MHz, CDCl₃): $\delta=221.45$, 170.43, 165.59, 151.62, 138.88, 120.07, 116.10, 77.02, 72.99, 68.27, 62.51, 61.72,

53.94, 43.76, 40.21, 39.76, 33.06, 32.68, 30.92, 25.47, 25.33, 24.77, 23.50, 23.00, 21.14, 19.23, 17.70, 16.18 ppm; IR (Nujol): $\bar{\nu}=3470$ (br), 2934, 2864, 1737, 1679, 1505, 1467, 1444, 1413, 1382, 1324, 1293, 1251, 1177, 1154, 1073, 1042, 984, 941, 880, 760 cm⁻¹; HRMS: m/z : calcd for C₂₈H₄₂NO₆S: 520.27328 [M+H]⁺; found 520.27228.

Tubulin purification: Tubulin was prepared by two cycles of temperature dependent assembly-disassembly as described by Williams and Lee.^[49] Determination of protein concentration was carried out as previously described.^[50]

Tubulin polymerization assay: Tubulin assembly was monitored by apparent absorption at 350 nm. Tubulin samples (10 μ M) were incubated at 37°C in PME buffer (100 mM PIPES, 1 mM MgSO₄, and 2 mM EGTA) containing 1 mM GTP in the spectrometer until a baseline was obtained. The ligand to be tested in DMSO was added a final concentration of 50 μ M (4% DMSO). After the assembly reached steady state, the temperature was dropped to 4°C. A tubulin sample (10 μ M) with 4% DMSO and no promoter was used as the reference.

Determination of cytotoxic activity: Cytotoxicity of bridged epothilones in PC3 cells was assessed with the SRB assay.^[51] The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.^[52]

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