Design, synthesis and biological evaluation of bridged epothilone D analogues†

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Six epothilone D analogues with a bridge between the C4-methyl and the C12-methyl carbons were prepared in an attempt to constrain epothilone D to its proposed tubulin-binding conformation. Ring-closing metathesis (RCM) was employed as the key step to build the C4–C26 bridge. In antiproliferative assays in the human ovarian cancer (A2780) and prostate cancer (PC3) cell lines, and also in tubulin assembly assay, all these compounds proved to be less active than epothilone D.

Introduction

The epothilones are a class of macrolide natural products isolated from the soil myxobacterium *Sorangium cellulosum*.[1] Epothilones A (**1**) and B (**2**) (epoA and B) (Fig. 1) were discovered in 1986 based on their antifungal activity,[1] but they attracted only moderate scientific interest until the report by Bollag *et al.* in 1995 that they had the same mechanism of action as paclitaxel, stabilizing the tubulin polymer and causing apoptotic cell death.[2] This report triggered a large amount of research on their biology and chemistry, which has been reviewed on several occasions.[3] At this time one epothilone derivative, the semisynthetic epoB analogue ixabepilone (**3**), has been approved for clinical use,[4] and at least six other epothilone analogues are in advanced clinical trials.[5] EpoB is in phase III trials and EpoD (**4**) is currently in Phase II clinical trials. Additional new and exciting analogues, such as fludelone,[6] have emerged in recent years.

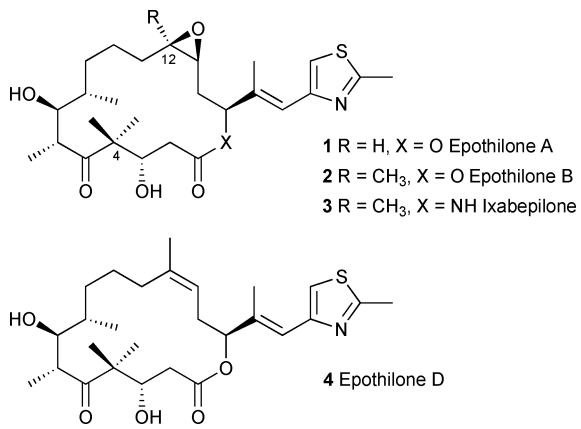

Fig. 1 Structures of epothilones A (**1**) and B (**2**), ixabepilone (**3**), and epothilone D (**4**).

The nature of the binding of the epothilones to tubulin has been extensively investigated. The early observation that epothilones can displace tubulin-bound paclitaxel suggested that both are bound by the same or overlapping binding sites.[7] A combined paclitaxel-epothilone hybrid construct has been prepared and shown to promote tubulin assembly 3-fold less effectively than paclitaxel, lending support to the hypothesis that paclitaxel and the epothilones share a common binding site and mechanism of action.[8] The X-ray structure of epoB has been published,[9] and two distinct solution conformations have been detected for epoA and epoB,[10] but neither result addressed the conformation of epothilone on the tubulin polymer. A conformation of tubulin-bound epothilone has been proposed based on solution NMR spectroscopy.[11] The NMR experiments were however performed on unassembled tubulin, and it is uncertain if the conformation of epoA bound to tubulin is the same as its conformation bound to microtubules. Finally, minireceptor modeling approaches led to 3D QSAR models of epothilone.[12]

Recently one of us proposed an entirely different epothilone A conformation based on crystallographic electron density maps of Zn-stabilized tubulin sheets that diffract to 2.9 Å, molecular modeling, and NMR-NAMFIS treatment.[13,14] The proposed binding conformation from this study explains most of the SAR studies generated for the epothilones, and also accounts for the drug resistance of mutated cell lines. Analysis of this Nettles *et al.* model for epoA reveals juxtaposition of a C4 methyl carbon and C12-H at a distance of 4.5 Å (Fig. 2). The corresponding separation between C4-Me and C12-Me (epoB) is 5.5 Å. To test the proposed binding modes, we designed epoD analogues with built-in conformational restrictions between these positions by introducing a bridge between C4 and C26.

Herein, we report the synthesis of six new bridged epothilone analogues and their antiproliferative activities towards

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† Electronic supplementary information (ESI) available: Experimental procedures for the synthesis of compounds **7**, **9**, **14a**, **14b**, **15**, **16**, **18**, **19a–e**, **20a**, **21–23**, **31**, **37–40**, **41a–d**, **42**, **43**, **54**, and **55**, and selected ^{1}H NMR spectra. CCDC reference numbers 699878. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b814823f

‡ These authors contributed equally to the synthetic work reported.

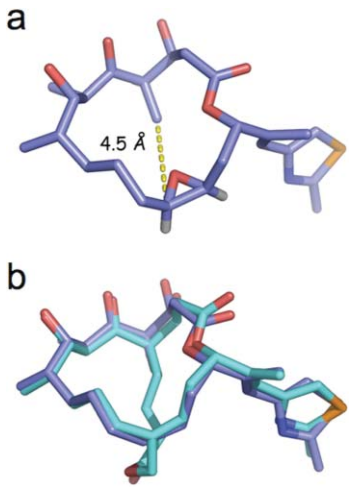


Fig. 2 (a) The epoA model proposed by Nettles *et al.*, based on EC density with juxtaposition of the C4-methyl and C12-H (methyl for epoB); r(C–C) = 4.5 Å. (b) MMFF-energy minimized structures of the EC template and the designed bridged epoD analogue **5**. (Overlaid with 3 point selection in PyMol).

human-ovarian (A2780) and prostate cancer (PC3) cell lines, as well as their tubulin-assembly activities.

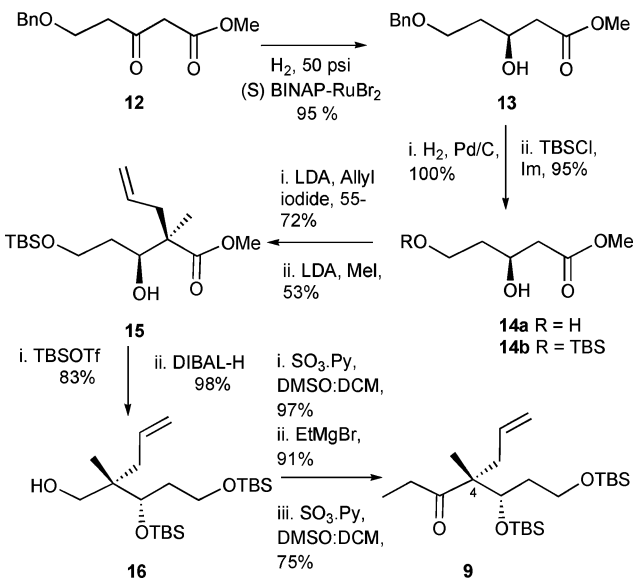
Results

Synthetic strategy

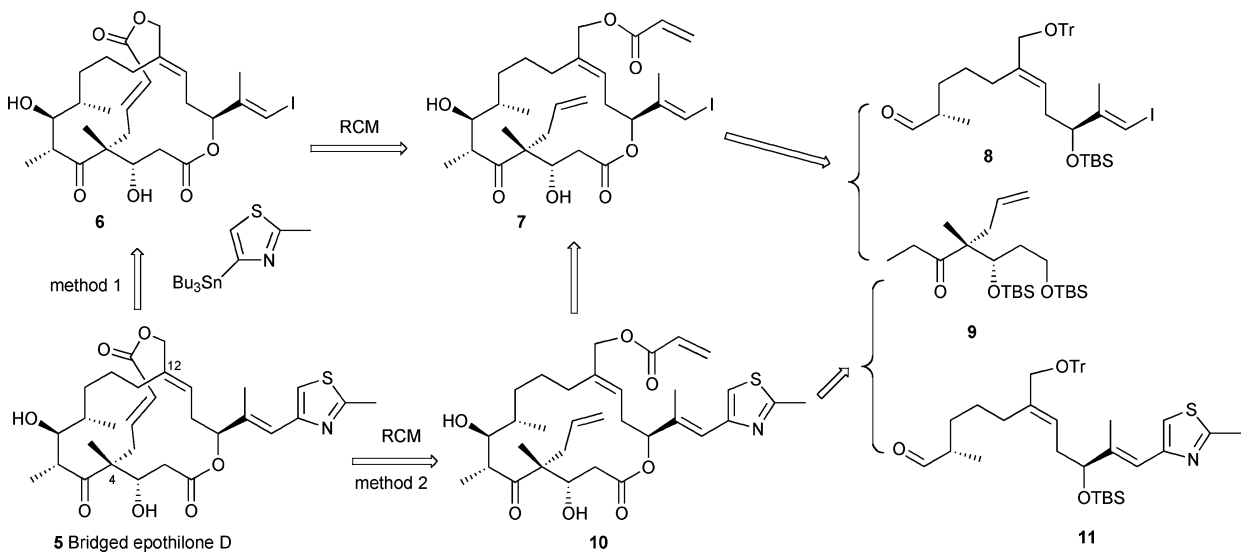
The introduction of a bridge between C4-Me and C26 in epothilone D was planned with a ring closing metathesis reaction as the key step, by analogy with our successful synthesis of bridged paclitaxel derivatives.[15] Thus, the synthesis of bridged analogue **5** was designed to proceed from diene precursors **7** or **10** (Scheme 1). The diene precursor **7** could be derived from fragments **8** and **9** in a convergent manner. Fragment **8** has previously been synthesized by us employing Nicolaou's method[16] as an intermediate in the synthesis of fluorescently labeled epothilone analogues.[17] The alternative diene precursor **10** could be synthesized from fragments **9** and **11**.

Synthesis

The synthesis of fragment **8** was carried out as previously described.[16,17] Ketone **9** was prepared as shown in Scheme 2. Treatment of methyl acetoacetate with benzyl chloromethyl ether provided diketone **12**,[18] which was subjected to catalytic asymmetric reduction to provide hydroxyester **13**.[19] The benzyl group was deprotected and the resulting diol **14a** was selectively protected with *tert*-butyldimethylsilyl chloride to give ester **14b** in 95% overall yield. Compound **14b** was treated with LDA and allyl iodide to provide an allyl derivative in excellent yield and diastereoselectivity, which was subjected to LDA and methyl iodide to provide compound **15** as the major diastereomer in 53% yield (dr. 3:1).



Scheme 2 Synthesis of ketone **9**.



Scheme 1 Retrosynthetic analysis for bridged epothilone **5**.

The configuration of allyl derivative **15** was initially assigned based on literature precedent;[19] this assignment was subsequently established by converting it to the ketal **17**. Selective irradiation of the C4-methyl group (Fig. 3) resulted in signal enhancement of the protons H_a-H_c on the adjacent carbons. Irradiation of proton H_a of the (*S*) chiral center gave signal enhancement of the C4-methyl group and the H_b and H_c protons, but not of the CH_2 protons of the allyl group.

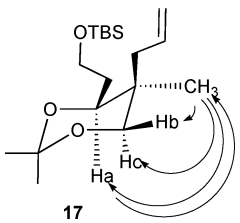


Fig. 3 Key NOE correlations of ketal **17**.

These results confirm the (*R*) configuration of the newly introduced center at the C4 carbon. The remaining transformations from **15** to **9** were straightforward and proceeded *via* alcohol **16** in 52% overall yield for the five steps.

Aldol coupling of aldehyde **8** and ketone **9** using LDA as the base furnished **18** in 85% yield and high diastereoselectivity (11:1). The stereochemistries at the C6 and C7 positions of the major and minor diastereomers were assigned by analogy to literature precedents.[20] Presumably this aldol coupling step proceeds *via* the favoured Cram/Felkin-Ahn transition state leading to a 6*R*,7*S* major diastereomer. Alcohol **18** was converted to hydroxyacid **19e** and then to lactones **20a** and then **21** in seven synthetic steps and 51% overall yield by methods similar to those described previously[16] (Scheme 3).

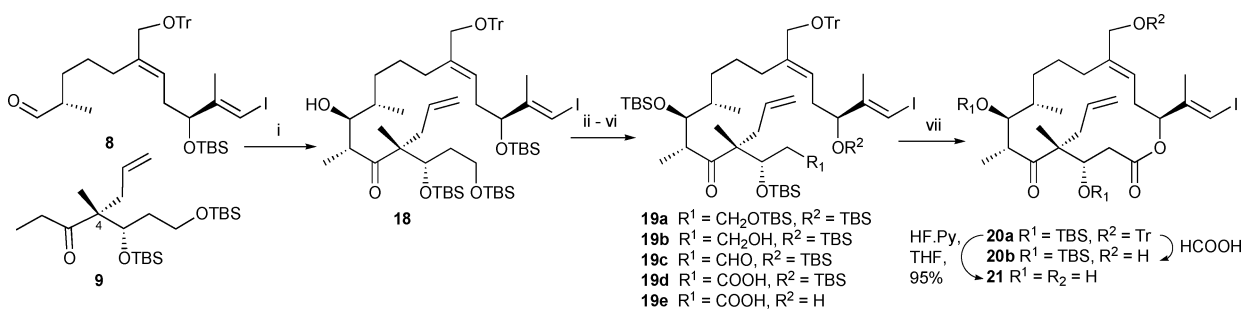
Having made the advanced C4-allyl-C4-demethyl-triol intermediate **21**, we acylated it separately with acryloyl chloride, 3-butenoyl chloride, and 4-pentenoyl chloride by a known protocol[18] to yield the diene precursors **7**, **22** and **23** in good yields (Scheme 4).

At this point, two options were available for the last two steps of the synthesis. The first was to attach the thiazole unit and then do the ring closing metathesis, while the second reverses the order of these steps. The second option was attractive, since it would generate the macrocyclic iodide building blocks **6**, **24**, or **25** that could be exploited by attaching various side chain units such as pyridine and benzothiazoles, in addition to the parent thiazole unit. Initially, diene **7** was subjected to ring closing metathesis[21] with either first- or second- or third-generation Grubbs catalyst and also the second-generation Hoveyda-Grubbs catalyst. The reaction was very sluggish, and when the reaction time was prolonged the starting material decomposed and no product **6** was obtained. Similar results were observed for diene **22**. Diene **23**, however, with the longest olefin chain, underwent ring closing metathesis with the second-generation Grubbs catalyst, albeit in low yield, to furnish the desired product **25** (Scheme 4).

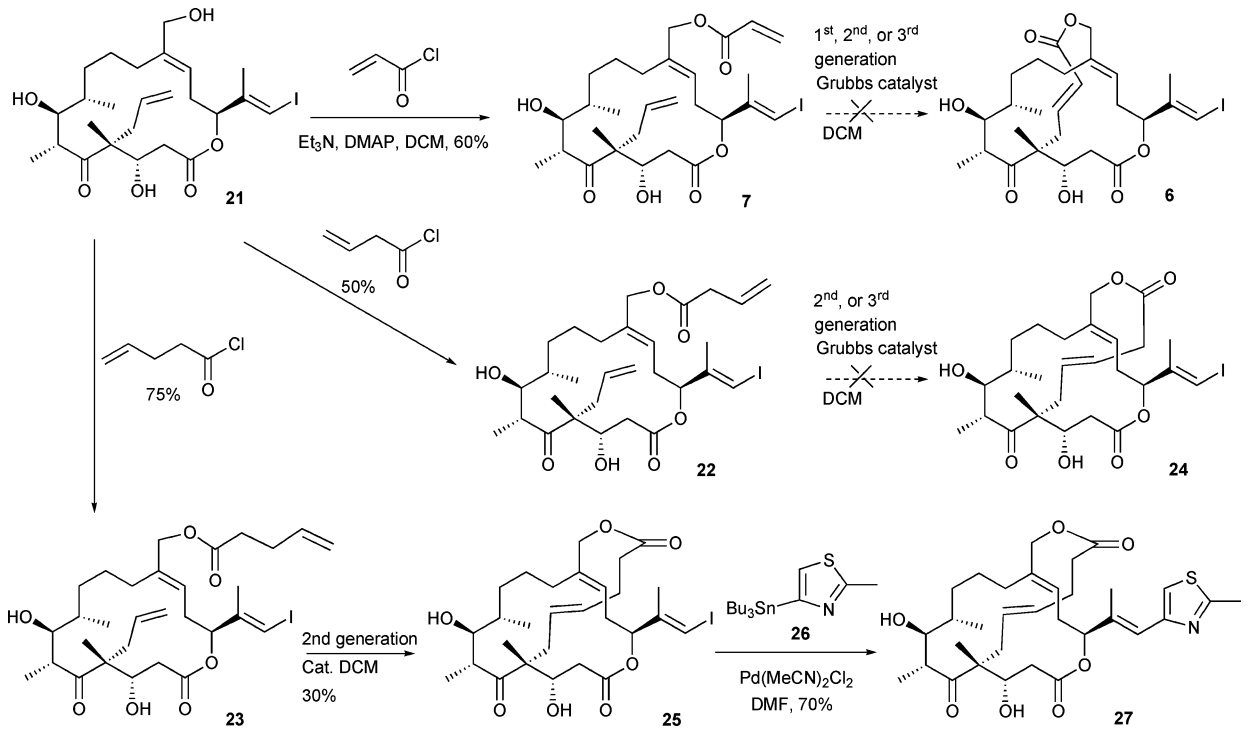
The configuration of the double bond in **25** was established as *trans* based on 1D NOE experiments. Selective irradiation of either of its olefinic bridge protons resulted in signal enhancement of the CH_2 protons adjacent to the other side of the double bond, thus confirming the *trans* orientation of double bond. The bridged iodide **25**, on coupling with the thiazolyl stannane **26** in the presence of $Pd(MeCN)_2Cl_2$, provided the final bridged epothilone D analogue **27** in 70% yield.

At this point we reconsidered option 1, introduction of the thiazole unit on the triol **21** followed by acylation at the C26 position and then ring closing metathesis, with the notion that replacing the iodide with the thiazole unit might bring the ring closing olefinic units together. Treatment of triol **21** with thiazolyl stannane **26** provided the intermediate **28**, which then was acylated with 3-butenoyl chloride to give **29**. Gratifyingly, when **29** was subjected to ring closing metathesis with second-generation Grubbs catalyst, the ring closed epothilone D analogue **30** was obtained, albeit in low yield (Scheme 5). In an effort to make the shorter bridged analogue **5** by this route, diene **31** was likewise prepared from **28**. However, this diene did not undergo normal ring closing metathesis with second-generation Grubbs catalyst to give the expected product **5**.

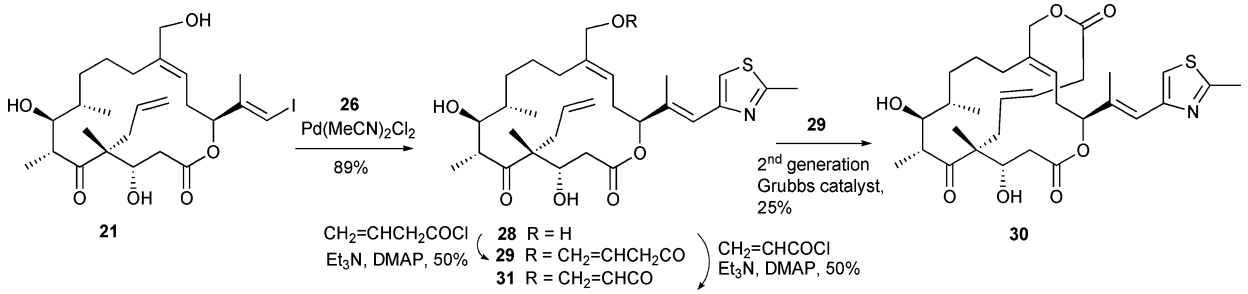
Since conjugation of double bonds with carbonyl groups reduces their reactivity towards ring-closing metathesis, we next investigated cyclization of the diene precursor **33** (Scheme 6) with a C26 allyloxy group instead of a C26 acryloyl group. The (4*R*)-4-allyl-4-demethyl-epothilone analogue **32** was prepared by selective deprotection of the trityl group of lactone **20** followed by coupling with thiazolyl stannane **26**. Alcohol **32** was then converted to its sodium alkoxide with sodium hydride in THF and allylated with allyl iodide to give diene **33** in 23% yield, together with 55% starting material **32**. The yield could not be improved by prolonging the reaction time, which only led to the formation of elimination byproduct. Diol **34** was prepared by standard desilylation of **33** with HF·Py complex for comparison of its bioactivity with that of the final cyclized product. Happily, ring closing metathesis of **33** with second-generation Grubbs catalyst gave the expected



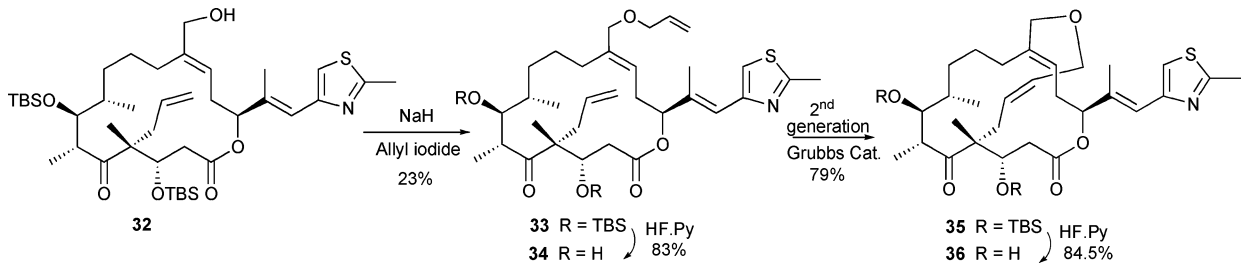
Scheme 3 Synthesis of lactone **21**. i. LDA, THF, –78 °C to –40 °C, 80%; ii. TBSOTf, 2,6-lutidine, 0 °C, 94%; iii. HF·Py in Py, 0 °C–RT, 86; iv. SO_3.Py, 87%; v. $NaClO_2$, NaH_2PO_4, 98%; vi. TBAF, THF, 0 °C–RT, 97%; vii. Et_3N, 2,4,6-trichlorobenzoyl chloride, DMAP, 78%.



Scheme 4 Synthesis of bridged epothilone D analogue **27**.



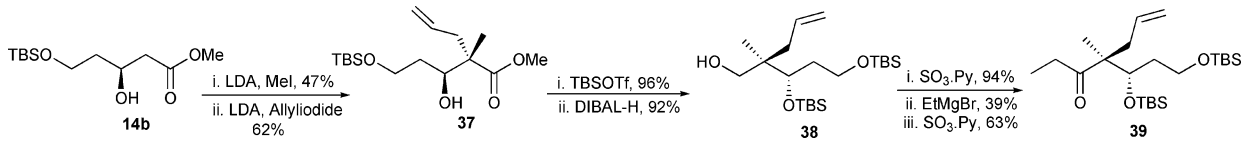
Scheme 5 Synthesis of bridged epothilone D analog **30**.



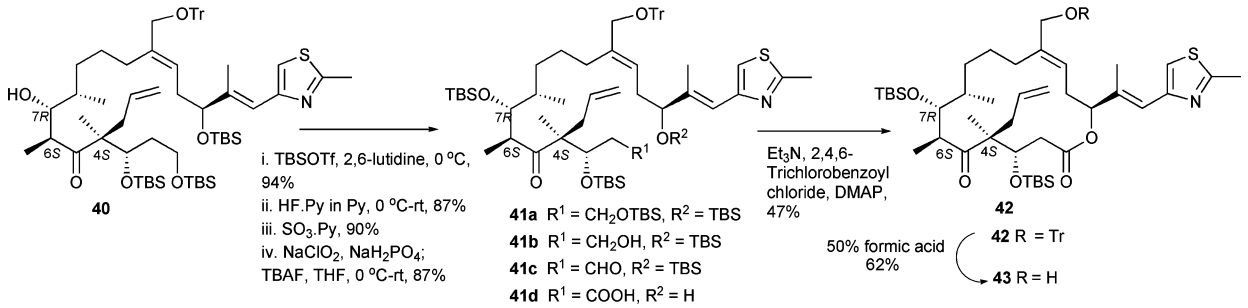
Scheme 6 Synthesis of bridged epothilone D analog **36**.

bridged epothilone analogue **35** in 79% yield. Desilylation of **35** with HF·Py complex in THF provided the final epothilone D analogue **36** in 84% yield, with a 5-atom bridge between C4 and C26. Interestingly, the ^{1}H NMR spectrum of the TBS ether **35** of the bridged analogue showed only one signal for each type of proton. However, the bridged analogue **36** with two free hydroxyl groups showed pairs of signals for each group of protons in its ^{1}H NMR spectrum in both $CDCl_3$ and CD_3CN. We attribute this pairing of signals to the existence of two atropisomers of **36**, and this conclusion was supported by the fact that some pairs of signals collapsed to single signals when the temperature was increased from 22 °C to 50 °C in $CDCl_3$.

We also investigated the effect of the stereochemistry at C4 on biological activity by preparing bridged epothilone analogues with the 4*S* stereochemistry. Compound **14b** was treated with LDA and methyl iodide to provide a methyl derivative, which was reacted with LDA and allyl iodide to furnish compound **37** as the major diastereomer in 62% yield. The remaining transformations from



Scheme 7 Synthesis of fragment C1-C6 with 4*S* stereochemistry.



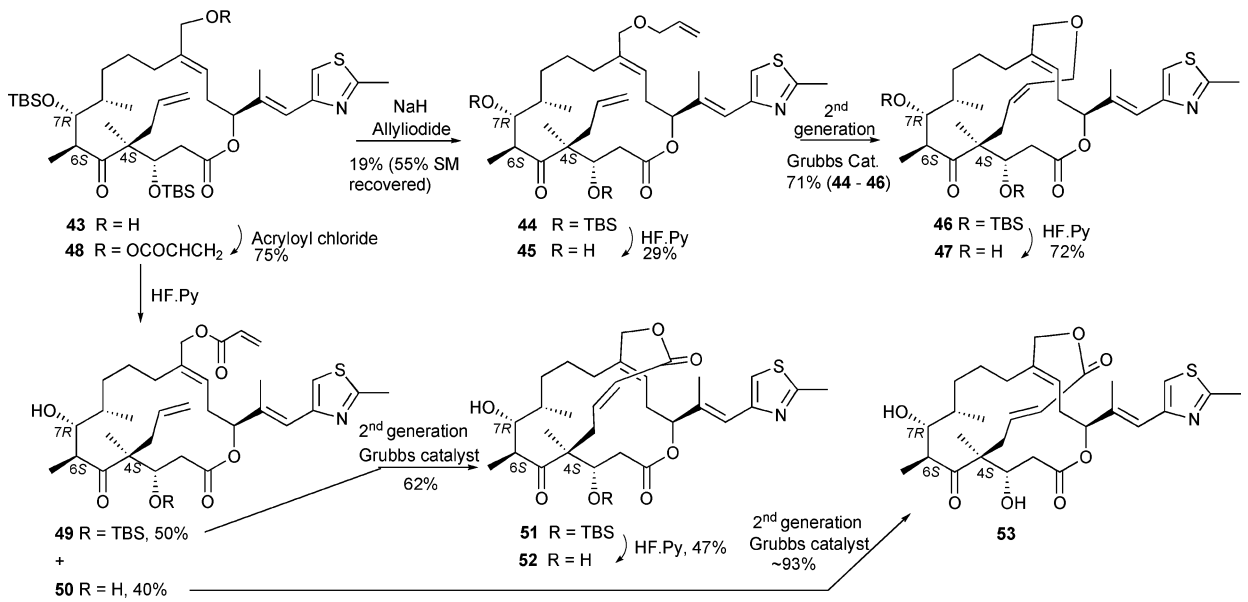
Scheme 8 Synthesis of allyl epothilone derivative **43**.

37 to **39** were straightforward and proceeded in 20% overall yield for the five steps (Scheme 7).

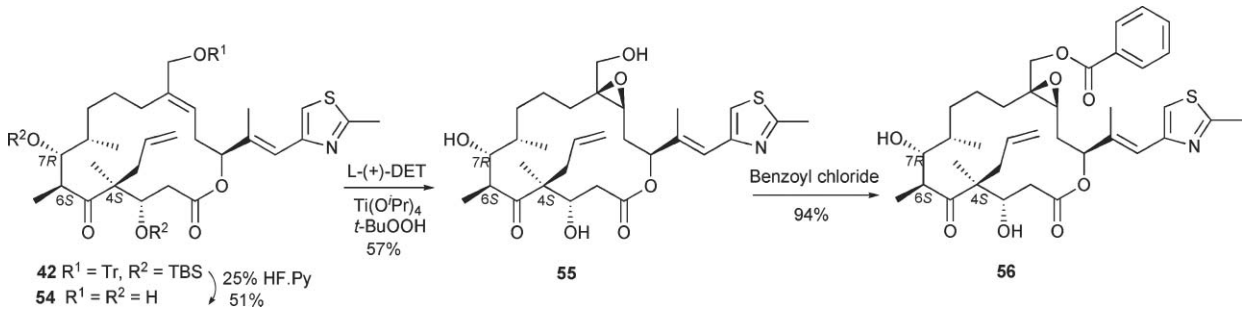
To our surprise, aldol coupling of aldehyde **11** and ketone **39** employing LDA as the base furnished the unexpected 6*S*,7*R* diastereoisomer **40** as the major product (46%), as well as two other diastereomers (28%) (Scheme 8).

The indicated stereochemistry of **40** was confirmed by X-ray crystallographic analysis of its derivative **56** described below. Alcohol **40** was converted to hydroxyacid **41d** and thence to lactones **42** and **43** in seven steps and 19% overall yield by methods similar to the preparation of compound **21** (Scheme 8). By the same procedures as used for the preparation of 4*R*,6*R*,7*S* bridged analogue **36**, and in similar yields, the 4*S*,6*S*,7*R* bridged analogue **47** was prepared from compound **43** *via* intermediates **44** and **46** (Scheme 9). The configuration of the bridging double bond in **47** was established as *cis* based in 1D NOE experiments. Selective irradiation of one of the olefinic bridge protons resulted in signal enhancement of the other olefinic bridge proton. The 4*S*,6*S*,7*R* bridged analogue **47** also exists as a pair of atropisomers, showing paired signals for each group of protons in its proton NMR spectrum in CD_3CN as solvent.

In an additional effort to investigate the effect of the 4*S*,6*S*,7*R* stereochemistry on metathesis of a C26 acryloxyl group, the C26 acryloyl derivative **48** was prepared from **43**. Diene **48** did not undergo ring closing metathesis with second-generation Grubbs catalyst. However, dienes **49** and **50**, with one or two free hydroxyl groups, underwent ring closing metathesis with second-generation Grubbs catalyst to furnish the bridged products **51** (62%) and **53** (93%). The latter compound was contaminated by a minor epothilone impurity which could not be removed by normal chromatography; the major compound was estimated to be 86.5% pure by ^{1}H NMR spectroscopy (Scheme 9).



Scheme 9 Synthesis of bridged epothilone D analog **47**, **52**, and **53**.



Scheme 10 Synthesis of 4*S*,6*S*,7*R* epothilone D derivative **56**.

Desilylation of **51** with HF·Py complex in THF provided the bridged analogue **52** with the 4*S*,6*S*,7*R* stereochemistry. The configurations of the double bonds in **51** and **52** were established as *cis* based on the coupling constant ($J = 11.5$ Hz) of the olefinic proton adjacent to the carbonyl group of the newly made bridge. The coupling constant ($J = 17.0$ Hz) of the corresponding olefinic proton of **53** indicated that the configuration of its bridged double bond was *trans*.

Assignment of the stereochemistry of the C6 and C7 chiral centers of the 4*S* series of compounds could not readily be achieved by conventional NMR methods, and so the benzoyloxy derivative **56** was prepared from **42** *via* intermediate **54** and **55** (Scheme 10).

Global deprotection of **42** with HF·Py complex yield triol **54** in 51% yield. Then using the Sharpless conditions [L-(+)-DET, Ti(*i*PrO)$_4$, *t*BuOOH],[22] allylic alcohol **54** was converted to 12β,13β-epoxide **55** (d.e. > 95% as judged by ^{1}H NMR spectroscopy). The primary hydroxyl group at C26 of triol **55** was selectively acylated with benzoyl chloride to yield the benzoyloxy derivative **56** as colorless needles from hexanes-acetone. Single crystal X-ray crystallography of **56** confirmed its absolute stereochemistry as C3(*S*), C4(*S*), C6(*S*), C7(*R*), C8(*S*), C12(*S*), C13(*S*) and C15(*S*) (Fig. 4).[23]

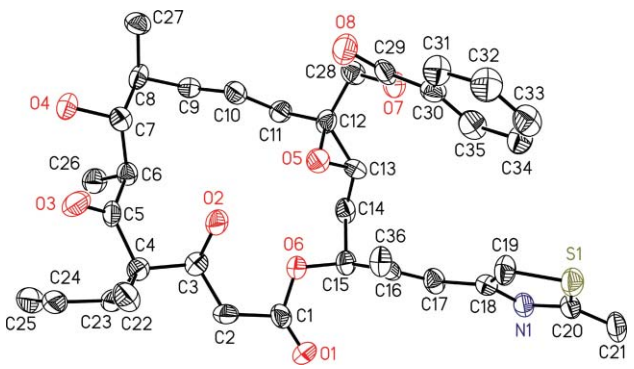


Fig. 4 Displacement ellipsoid drawing (50%) of compound **56**.

Bioactivity

The in vitro antiproliferative activities of the open and constrained epothilone analogues were determined by employing the A2780 ovarian cancer and PC-3 prostate cancer cell lines (Table 1). The activities of the constrained analogues **27**, **30** and **52** were comparable to those of their respective open chain analogues, but they were significantly less than that of epothilone D. The effect of some of the analogues on tubulin assembly was also investigated. The trend in the ability of the analogue to induce microtubule assembly correlated with the trends in antiproliferative activities (Table 1).

Table 1 Bioactivity data of epothilone D and its analogues

Cmpd	IC_{50}, A2780 (μM)	IC_{50}, PC3 (μM)	EC_{50}, tubulin assembly (μM)
EpoD	0.04	0.016 ± 0.001	0.44 ± 0.02
7	19.3 ± 2.5	ND	ND
23	25.6 ± 7.0	ND	ND
25	22.0 ± 1.1	ND	ND
27	15.6 ± 3.0	2.1 ± 0.15	6.9 ± 0.9
28	2.1 ± 1.1	1.2 ± 0.4	1.3 ± 0.1
29	4.7 ± 0.3	1.2 ± 0.1	0.88 ± 0.06
30	3.2 ± 0.1	0.93 ± 0.06	2.4 ± 0.1
31	0.24 ± 0.02	ND	0.58 ± 0.06
34	0.10 ± 0.04	0.077 ± 0.016	0.54 ± 0.07
36	3.9 ± 0.5	1.2 ± 0.3	1.62 ± 0.13
45	6.2 ± 0.8	ND	ND
47	22.9 ± 0.9	ND	ND
50	5.2 ± 0.2	ND	ND
52	4.9 ± 0.3	ND	ND
53	31.2 ± 1.2	ND	ND

Molecular modeling and discussion

The bridged epothilone D analogues **27**, **30** and **36** were docked into the tubulin binding site of epothilone using the Glide docking program.[24] Neither **27** nor **30** adopts a docking pose similar to that of epothilone A in the electron crystallographic (EC) binding model.[13] This is most likely due to the longer and less compatible length of the cross-ring bridges in **27** and **30**. As expected, the shorter bridge in **36** permits the analogue to readily adopt the epothilone A EC binding model in the binding site (Fig. 5). In the cell-free tubulin assembly assay, the reduction in EC_{50} compared to EpoD correlates with bridge length, **36** < **30** < **27**, while the most active analog is only 3–4 fold less active than EpoD (Table 1). One possible explanation for the lack of potency improvement in the bridged series is that the bridge alters the conformational profile of the ligands to introduce a greater degree of global conformational strain relative to the monocyclic standard, thereby decreasing K_a and increasing DG_{bind}.

The cell-based data shows another activity pattern. That is, the EC_{50} values for all three compounds in both A2780 and PC3 cytotoxicty assays are 75–390 fold greater than that for EpoD, while the corresponding tubulin polymerization range is 4–15 fold

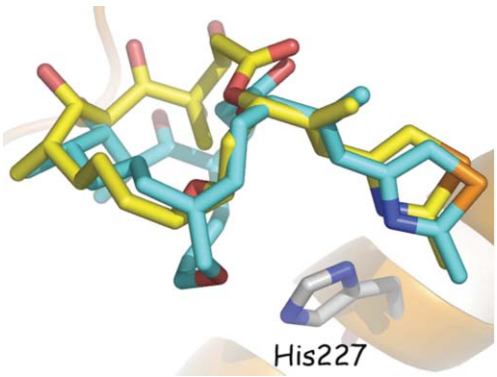


Fig. 5 Docking poses of **1** (yellow) and **36** (cyan) in the EC determined tubulin binding site.

(Table 1). We attribute the differences provisionally to physical property variations associated with membrane permeability.

Two additional observations can be made from the data. The diastereomeric compounds **47**, **52**, and **53** were all significantly less active in the A2780 antiproliferative assay than compounds with the normal epothilone stereochemistry. This finding, while unsurprising, confirms the importance of stereochemistry in the bioactivity of the epothilones. A second observation is that the open chain 4-allyl analogue **34** is the most active of all the analogues prepared, and approaches the activity of epothilone D in the tubulin assembly assay. It is likewise only 2–5 fold less cytotoxic than EpoD (Table 1). Epothilones with additional substitution at C4 are suggested as a class of analogs that is worth investigating further.

Experimental

Genaral synthetic procedures

Optical rotations were recorded on a Perkin-Elmer 241 Polarimeter. Infrared spectra were measured using a SENSIR ATR on MIDAC M2004 Series spectrometer. NMR spectra were obtained on a JEOL Eclipse 500, a Varian Unity 400, or a Varian Inova 400 spectrometer in $CDCl_3$ or CD_3CN. The chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. High-resolution FAB mass spectra were obtained on a JEOL HX110 Double Focusing Mass Spectrometer. THF was distilled from sodium-benzophenone and dichloromethane was distilled from calcium hydride. Other reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using flash silica gel (32–63 μ). Preparative thin-layer chromatography (PTLC) separations were carried out on 500 μ or 1000 μ Uniplate thin layer chromatography plates. All reactions were carried out under a nitrogen atmosphere unless otherwise noted.

Bridged macrolactone iodide 25. To a solution of diene **23** (13 mg, 0.02 mmol) in dichloromethane (3 mL) was added second-generation Grubbs catalyst (6 mg, 0.007 mmol) at room temperature for 3 h and the resulting reaction mixture was stirred for 3 d. The dichloromethane was evaporated to give crude product, which was subjected to preparative thin layer chromatography over silica gel, eluting with 20% ethyl acetate in hexanes, to furnish **25** (3.7 mg, 30%). $[\alpha]_D$ −68.6 (*c* 0.035, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (s, 1H), 6.00 (m, 1H), 5.82 (m, 1H), 5.43 (s, 1H), 5.42 (t, J = 6.0 Hz, 1H), 5.00 (d, J = 14.0 Hz, 1H), 4.40 (m, 1H), 4.07 (d, J = 14.0 Hz, 1H), 3.49 (d, J = 2.4 Hz, 1H), 3.18 (dq, J = 5.8, 2.4 Hz, 1H), 2.88 (d, J = 2.8 Hz, 1H), 2.70–2.58 (m, 3H), 2.50–2.38 (m, 6H), 2.30 (m, 2H), 2.10 (m, 2H), 1.88 (s, 3H), 1.84 (m, 1H), 1.70 (m, 1H), 1.40 (m, 1H), 1.27 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.0, 172.6, 169.1, 143.7, 137.3, 134.4, 128.2, 118.0, 78.2, 77.4, 76.0, 75.6, 67.9, 63.8, 57.0, 42.7, 39.9, 39.1, 38.6, 32.0, 29.9, 29.8, 28.8, 27.7, 26.9, 24.0, 22.8, 16.9, 14.4, 14.3; HRFABMS: calcd for $C_{28}H_{42}O_7I$ (M + H) 617.1975, found 617.1982.

Bridged epothilone D 27. A solution of iodide **25** (3 mg, 0.0048 mmol), and $Pd(MeCN)_2(Cl)_2$ (2 mg) were added to a degassed solution of stannane **26** (8 mg, 0.02 mmol, 4 eq) in DMF (0.8 mL) at 25 °C and the resulting solution was stirred over 24 h. The reaction mixture was filtered off through a short plug of silica gel eluting with ethyl acetate. The filtrate was concentrated and the residue was subjected to preparative thin layer chromatography over silica gel, eluting with 25% ethyl acetate in hexanes, to furnish **27** (2 mg, 70%). $[\alpha]_D$ −68.2 (*c* 0.08, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (s, 1H), 6.59 (s, 1H), 6.06 (m, 1H), 6.00 (m, 1H), 5.67 (t, J = 10.8 Hz, 1H), 5.39 (br.s, 1H), 4.88 (d, J = 13.6 Hz, 1H), 4.55 (d, J = 10.8 Hz, 1H), 4.20 (d, J = 13.6 Hz, 1H), 3.54 (br.s, 1H), 3.36 (m, 1H), 3.22 (q, J = 6.8 Hz, 1H), 2.99 (br.s, 1H), 2.82 (m, 1H), 2.44 (s, 3H), 2.56–2.42 (m, 6H), 2.28 (m, 2H), 2.16 (m, 2H), 2.06 (s, 3H), 1.88 (m, 2H), 1.38 (m, 2H), 1.27 (d, J = 6.8 Hz, 3H), 1.25 (s, 3H), 0.99 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.0, 173.0, 169.6, 164.8, 152.1, 136.5, 133.8, 128.3, 120.1, 119.5, 115.2, 77.4, 75.8, 75.7, 67.2, 64.9, 57.4, 42.5, 40.0, 39.2, 38.8, 32.0, 30.1, 29.9, 28.7, 28.1, 26.5, 24.2, 19.1, 17.1, 16.9, 14.4, 14.1; HRFABMS: calcd for $C_{32}H_{46}NO_7S$ (M + H) 588.2995, found 588.2960.

(4*R*)-4-Allyl-4-demethyl-26-hydroxyepothilone 28. Iodide **21** was converted to **28** (33 mg, 89%) by a similar procedure to that described previously for the conversion of **25** to **27**. $[\alpha]_D$ −79 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.62 (s, 1H), 5.63 (m, 1H), 5.44 (dd, J = 9.8, 4.8 Hz, 1H), 5.30 (d, J = 8.4 Hz, 1H), 5.13 (dd, J = 17.2, 1.6 Hz, 1H), 5.10 (dd, J = 10.4, 1.6 Hz, 1H), 4.45 (d, J = 11.2 Hz, 1H), 4.06 (d, J = 13.2 Hz, 1H), 4.00 (d, J = 13.2 Hz, 1H), 3.90 (br.s, 1H), 3.66 (d, J = 6.0 Hz, 1H), 3.22 (qt, J = 6.8, 1.2 Hz, 1H), 3.13 (br.s, 1H), 2.67 (s, 3H), 2.60 (m, 3H), 2.50 (m, 1H), 2.38–2.30 (m, 3H), 2.05 (s, 3H), 1.96 (m, 3H), 1.80 (m, 1H), 1.62 (m, 2H), 1.45 (m, 2H), 1.30 (m, 2H), 1.17 (d, J = 6.8 Hz, 3H), 1.02 (s, 3H), 1.00 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.0, 170.5, 165.3, 152.0, 142.1, 139.2, 133.5, 122.2, 119.2, 119.1, 115.8, 78.5, 73.3, 71.6, 66.5, 58.0, 41.5, 40.2, 39.8, 37.4, 31.6, 28.2, 27.0, 24.7, 17.7, 15.7, 14.5, 13.8; HRFABMS: calcd for $C_{29}H_{44}NO_6S$ (M + H) 534.2889, found 534.2925.

(4*R*)-4-Allyl-4-demethyl-26-(3-butenoyloxy)epothilone D 29. Epothilone derivative **28** was acylated to provide **29** (10 mg, 50%) by a similar procedure to that described for the conversion of **21** to **22** (ESI). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.58 (s, 1H), 5.90 (m, 1H), 5.66 (m, 1H), 5.46 (dd, J = 10.2, 5.2 Hz, 1H), 5.30 (d, J = 9.6 Hz, 1H), 5.17 (dd, J = 17.2, 1.2 Hz, 1H), 5.15 (dd, J = 10.4, 1.2 Hz, 1H), 5.14 (dd, J = 17.2, 10.4 Hz, 1H), 5.12 (dd,

J = 10.4, 1.2 Hz, 1H), 4.55 (dd, J = 12.4 Hz, 1H), 4.46 (d, J = 12.4 Hz, 1H), 4.44 (m, 1H), 3.67 (d, J = 5.6 Hz, 1H), 3.62 (d, J = 5.2 Hz, 1H), 3.20 (q, J = 5.6 Hz, 1H), 3.12 (d, J = 7.2 Hz, 1H), 3.09 (dt, J = 7.2, 1.2 Hz, 1H), 2.69 (s, 3H), 2.62–2.42 (m, 3H), 2.40–2.22 (m, 3H), 2.06 (s, 3H), 2.02 (m, 1H), 1.80 (m, 1H), 1.64 (m, 1H), 1.42 (m, 2H), 1.30 (m, 2H), 1.14 (d, J = 6.8 Hz, 3H), 1.03 (s, 3H), 1.00 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.8, 170.4, 165.4, 152.0, 139.0, 137.1, 133.5, 130.3, 125.3, 119.4, 119.1, 118.9, 115.9, 78.2, 73.4, 71.7, 67.9, 57.9, 41.4, 40.2, 39.8, 39.3, 37.7, 32.5, 31.6, 28.3, 24.7, 19.2, 16.1, 15.7, 14.6, 12.2; HRFABMS: calcd for $C_{33}H_{48}NO_7S$ (M + H) 602.3152, found 602.3141.

Bridged epothilone D 30. A silimar procedure was employed to prepare bridged epothilone D (3 mg, 25%) as that described previously for the conversion of **23** to **25**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (s, 1H), 6.89 (s, 1H), 5.90 (m, 1H), 5.68 (t, J = 10.8 Hz, 1H), 5.32 (d, J = 4.8 Hz, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.48 (d, J = 10.8 Hz, 1H), 4.20 (d, J = 11.2 Hz, 1H), 3.46 (br.s, 1H), 3.36 (m, 1H), 3.00 (m, 2H), 2.90 (br.s, 1H), 2.80 (m, 1H), 2.62–2.45 (m, 3H), 2.30 (d, J = 15.6 Hz, 1H), 2.17–2.00 (s, 3H), 2.10 (s, 3H), 1.96 (s, 3H), 1.81 (m, 2H), 1.65 (m, 1H), 1.22 (m, 3H), 1.08 (d, J = 6.8 Hz, 3H), 0.99 (s, 3H), 0.86 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.1, 171.0, 170.4, 169.5, 145.2, 144.3, 137.0, 129.1, 126.6, 125.4, 114.7, 112.8, 74.5, 74.1, 71.3, 66.1, 57.7, 42.8, 39.0, 38.9, 38.4, 38.3, 31.8, 30.4, 29.7, 28.9, 25.9, 17.2, 16.4, 15.2, 13.6; HRFABMS: calcd for $C_{31}H_{44}NO_7S$ (M + H) 574.2838, found 574.2848.

(4*R*)-4-Allyl-4-demethyl-26-hydroxyepothilone D bis-TBS ether (32). To a solution of macrolactone **20a** (28 mg, 0.027 mmol) in ether (0.56 mL) at −5 °C was added formic acid (0.56 mL) and the reaction was allowed to proceed with stirring for 5 h at −5 °C. Water (10 mL) and then solid $NaHCO_3$ were sequently added until cessation of effervescence. The mixture was extracted with ether (10 mL × 3), the combined organic extracts were dried (Na_2SO_4), and the solvents were removed *in vacuo.* Purification of the crude product *via* preparative thin layer chromatography eluting with 50% ether in hexanes to yield lactone iodide **20b** (15 mg, 71%). $[\alpha]_D$ −21.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H), 6.39 (s, 1H), 5.69–5.59 (m, 1H), 5.43 (dd, J = 9.6, 6.0 Hz, 1H), 5.17–5.05 (overlap, 1H), 5.10 (d, J = 10.4 Hz, 1H), 5.05 (d, J = 17.2 Hz, 1H), 4.17 (d, J = 8.8 Hz, 1H), 4.09 (br.d, J = 12.4 Hz, 1H), 3.96 (d, J = 13.2 Hz, 1H), 3.89 (d, J = 8.8 Hz, 1H), 3.09–3.05 (m, 1H), 2.75–2.56 (m, 3H), 2.48–2.28 (m, 3H), 2.15–1.92 (m, 2H), 1.88 (s, 3H), 1.76–1.62 (m, 1H), 1.56–1.43 (m, 1H), 1.32–1.15 (m, 7H), 1.12 (d, J = 6.8 Hz, 3H), 1.11 (s, 3H), 0.96 (d, J = 6.8 Hz, 3H), 0.93 (s, 9H), 0.83 (s, 9H), 0.09 (two singlet, 9H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.0, 170.4, 146.0, 132.9, 118.7, 79.8, 77.7, 74.1, 66.3, 56.7, 40.9, 31.9, 31.1, 29.6, 28.1, 27.1, 26.3, 26.1, 20.5, 18.6, 18.5, −3.3, −3.5, −3.6, −5.3. HRFABMS: calcd for $C_{37}H_{68}O_6Si_2I$ (M + H) 791.3599, found 791.3676. Iodide **20b** was converted to **32** by a similar procedure to that described previously for the conversion of **25** to **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.51 (s, 1H), 5.58 (m, 1H), 5.43 (m, 1H), 5.00–4.97 (m, 3H), 3.94 (dd, J = 13.2, 4.4 Hz, 1H), 4.10–4.05 (m, 2H), 3.86 (dd, J = 8.4, 4.8 Hz, 1H), 3.04 (m, 1H), 2.76–2.53 (m, 3H), 2.67 (s, 3H), 2.46–2.20 (m, 3H), 2.18–1.92 (m, 3H), 2.08 (s, 3H), 1.75–1.55 (m, 4H), 1.06 (s, 3H), 0.89 (s, 9 H), 1.20–0.74 (m, 7 H), 0.77 (s, 9H), 0.03 (s, 9H), −0.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.4, 171.0, 164.8, 152.5, 143.9, 138.5, 133.0, 120.7, 119.6, 118.9, 116.2, 79.5, 74.6, 66.7, 60.7, 57.0, 48.2, 41.0, 39.2, 37.8, 32.6, 31.5, 28.3, 27.4, 26.6, 26.4, 19.6, 19.4, 18.9, 15.5, 14.4, −3.0, −3.25, −3.4, −5.3; HRFABMS: calcd for $C_{41}H_{72}NO_6SSi_2$ (M + H) 762.4619, found 762.4654.

(4*R*)-4-Allyl-4-demethyl-26-(allyloxy)epothilone D bis-TBS ether 33. To a solution of **32** (20 mg, 0.026 mmol) in THF (1.5 mL) was added sodium hydride (6.3 mg, 60%, 0.158 mmol) at 0 °C, and the resultant mixture was allowed to stir at 25 °C for 30 min. Allyl iodide (48 μL, 0.525 mmol) was added to the above reaction mixture, and the reaction was allowed to proceed at 25 °C for additional 1.5 h. The reaction mixture was filtered off *via* a pad of siliga gel eluting with ether to provide crude product, which was subjected to preparative thin layer chromatography over silica gel, eluting with 30% ether in hexanes, to furnish allyl ether **33** (4.84 mg, 23%) and starting material (11 mg, 55%). Compound **33** was obtained as a colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.54 (s, 1H), 5.94–5.85 (m, 1H), 5.66–5.58 (m, 1H), 5.46 (dd, J = 6.8, 5.5 Hz, 1H), 5.26 (d, J = 13.6 Hz, 1H), 5.17 (d, J = 8.3 Hz, 1H), 5.04–5.01 (m, 3H), 4.16–4.08 (m, 1H), 4.04 (d, J = 9.4 Hz, 1H), 3.99–3.85 (m, 3H), 3.72 (d, J = 9.4 Hz, 1H), 3.10–3.04 (m, 1H), 2.85–2.64 (m, 3H), 2.70 (s, 3H), 2.45–2.31 (m, 3H), 2.19–1.98 (m, 3H), 2.11 (s, 3H), 1.83–1.76 (m, 1H), 1.32–1.02 (m, 2H), 1.11 (d, J = 6.8 Hz, 3H), 1.10 (s, 3H), 1.02–0.80 (m, 1H), 0.96 (d, J = 6.8 Hz, 3H), 0.95 (s, 9H), 0.82 (s, 9H), 0.10 (s, 3H), 0.07 (s, 6H), –0.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.8, 171.3, 165.1, 152.9, 138.9, 135.3, 133.4, 124.2, 119.8, 119.2, 117.5, 116.5, 79.8, 77.7, 74.9, 73.9, 71.3, 57.2, 48.7, 41.3, 40.2, 39.8, 37.8, 32.9, 31.8, 30.2, 28.7, 27.6, 26.8, 26.6, 19.7, 19.1, 19.0, 15.8, 0.49, −2.7, −2.96, −3.18, −5.00; HRFABMS: calcd for $C_{44}H_{76}NO_6SSi_2$ (M + H) 802.4932, found 802.4968.

(4*R*)-4-Allyl-4-demethyl-26-(allyloxy)epothilone D 34. A similar procedure was employed to prepare **34** (2.39 mg, 83%, colorless oil) as that described previously for the conversion of **20a** to **21** (ESI). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.58 (s, 1H), 5.93–5.85 (m, 1H), 5.68–5.59 (m, 1H), 5.43 (dd, J = 10.4, 4.4 Hz, 1H), 5.34 (d, J = 9.9 Hz, 1H), 5.25 (dt, J = 17.0, 1.6 Hz, 1H), 5.16 (br.d, J = 10.7 Hz, 1H), 5.13 (br.d, J = 17.9 Hz, 1H), 5.10 (br.d, J = 10.4 Hz, 1H), 4.44 (d, J = 10.4 Hz, 1H), 3.95–3.86 (m, 3H), 3.78 (d, J = 11.8 Hz, 1H), 3.66 (m, 2H), 3.21 (q, J = 6.8 Hz, 1H), 3.14 (br.s, 1H), 2.73–2.47 (m, 4H), 2.68 (s, 3H), 2.34–2.23 (m, 3H), 2.09–2.03 (m, 1H), 2.06 (s, 3H), 1.83–1.76 (m, 1H), 1.70–1.62 (m, 1H), 1.48–1.37 (m, 2H), 1.32–1.24 (m, 2H), 1.14 (d, J = 6.6 Hz, 3H), 1.02 (s, 3H), 1.01 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.0, 170.5, 165.4, 152.0, 139.4, 134.9, 133.5, 124.2, 119.3, 119.1, 117.2, 115.8, 78.6, 73.7, 73.4, 71.8, 71.2, 57.9, 41.4, 40.2, 39.8, 37.8, 32.6, 31.8, 28.3, 24.7, 19.2, 16.1, 15.7, 14.7, 12.2; HRFABMS: calcd for $C_{32}H_{48}NO_6S$ (M + H) 574.3202, found 574.3188.

Bridged epothilone D 35. To a solution of diene **33** (1.1 mg, 0.00137 mmol) in dichloromethane (3 mL) was added second-generation Grubbs catalyst (0.41 mg, 0.00048 mmol, 0.35 eq) in dichloromethane (2 mL) for a period of 1.5 h *via* syringe pump under N_2. The resulting reaction mixture was allowed to stir for 0.5 h at 25 °C, and then the dichloromethane was removed under reduced pressure. The residue obtained was subjected to preparative thin layer chromatography over silica gel, eluting with

30% ether in hexanes, to furnish **35** (0.84 mg, 79%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.46 (s, 1H), 5.62 (br.t, J = 10.4 Hz, 1H), 5.56–5.48 (m, 1H), 5.44 (br.s, 1H), 5.29 (d, J = 4.4 Hz, 1H), 4.61 (br.s, 2H), 4.49 (br.s, 2H), 4.23 (dd, J = 10.4, 2.5 Hz, 1H), 3.73 (d, J = 6.3 Hz, 1H), 3.32–3.26 (m, 1H), 3.10–3.04 (m, 1H), 2.71 (s, 3H), 2.70–2.65 (m, 1H), 2.54–2.45 (m, 2H), 2.16–2.03 (m, 4H), 2.15 (s, 3H), 1.70–1.62 (m, 1H), 1.46 (s, 3H), 1.42–1.26 (m, 2H), 1.26 (s, 3H), 1.14–1.02 (m, 1H), 1.07 (d, J = 6.8 Hz, 3H), 0.91, 0.87 (each s, 18H), 0.86 (overlap, 3H), 0.174 (s, 3H), 0.170 (s, 3H), 0.05 (s, 3H), 0.02 (s, 3H); HRFABMS: calcd for $C_{42}H_{72}NO_6SSi_2$ (M + H) 774.4619, found 774.4644.

Bridged epothilone 36. The epothilone D bridged analogue **36** was obtained from bridged epothilone D bis-TBS ether (**35**), according to the procedure described previously for the preparation of **21** (ESI), as a colorless oil in 84.5% yield.^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01, 7.00 (s, 1H), 6.67, 6.57 (s, 1H), 5.77–5.70 (m, 1H), 5.62–5.36 (m, 3H), 4.64 (br.s, 2H), 4.53 (br.s, 2H), 4.54–4.0, 4.15–4.11 (m, 1H), 3.65, 3.20 (br.s, 1H), 3.55, 3.39 (d, J = 9.5 Hz, 1H), 3.40–3.31 (m, 2H), 3.10–3.04 (m, ~0.5 H), 2.93 (br.d, J = 13.5 Hz, ~0.5H), 2.78, 2.71 (br.s, 1H), 2.74 (s, 3H), 2.75–2.46 (m, 3H), 2.25–2.06 (m, 3H), 2.15 (s, 3H), 1.85–1.80 (m, 1H), 1.51–1.40 (m, 4H), 1.26, 1.16 (s, 3H), 1.20, 1.11 (d, J = 6.5 Hz, 3H), 0.89, 0.86 (d, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.0, 170.2, 165.4, 152.3, 140.7, 132.0, 125.8, 119.2, 118.9, 116.6, 116.3, 77.4, 77.2, 76.2, 73.2, 72.6, 58.4, 44.3, 41.2, 35.6, 33.0, 32.8, 27.7, 25.0, 19.4, 16.9, 15.9, 15.79, 15.70; HRFABMS: calcd for $C_{30}H_{44}NO_6S$ (M + H) 546.2889, found 546.2914.

(4*S*)-4-Allyl-4-demethyl-26-(allyloxy)-6*S*,7*R*-epothilone D bis-TBS ether (44). Allyl ether **44** was synthesized as a colorless oil (4.0 mg, 19%), as well as 55% starting material was recovered, from primary alcohol **43** following the procedure described previously for the synthesis of allyl ether **33**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.33 (s, 1H), 5.91–5.82 (m, 1H), 5.75–5.62 (m, 1H), 5.35 (t, J = 8.8 Hz, 1H), 5.24–5.06 (m, 4H), 4.17 (d, J = 8.8 Hz, 1H), 3.99 (d, J = 2.8 Hz, 1H), 3.93–3.83 (m, 2H), 3.79 (d, J = 11.6 Hz, 1H), 3.47–3.35 (m, 1H), 2.70 (s, 3H), 2.65 (t, J = 6.8 Hz, 1H), 2.59–2.41 (m, 3H), 2.55–2.19 (m, 1H), 2.12 (s, 3H), 1.98–1.88 (m, 1H), 1.55–1.42 (m, 4H), 1.28–1.24 (m, 2H), 1.07 (d, J = 7.2 Hz, 3H), 0.96 (s, 3H), 0.92 (s, 9H), 0.90 (s, 9H), 0.88 (d, J = 6.4 Hz, 3H), 0.20 (s, 3H), 0.16 (s, 3H), 0.12 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.8, 170.5, 164.7, 152.7, 140.2, 136.7, 135.1, 134.1, 134.2, 122.2, 119.2, 118.1, 117.0, 116.3, 77.4, 75.0, 74.8, 73.8, 70.9, 56.6, 48.7, 42.4, 40.2, 38.9, 34.9, 31.1, 29.9, 28.2, 28.1, 26.5, 26.4, 19.4, 18.9, 18.8, 16.1, 14.3, –3.3, –3.8, –4.2, –4.6; HRFABMS: calcd for $C_{44}H_{76}NO_6SSi_2$ (M + H) 802.4932, found 802.4903.

(4*S*)-4-Allyl-4-demethyl-26-(allyloxy)-6*S*,7*R*-epothilone D 45. A similar procedure was employed to prepare **45** (0.65 mg, 29%, colorless oil), together with 64% mono-TBS ether, from **44** as that described for the conversion of **20a** to **21** (ESI). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.51 (s, 1H), 5.95–5.86 (m, 1H), 5.64–5.57 (m, 2H), 5.47 (dd, J = 9.9, 3.5 Hz, 1H), 5.28 (dd, J = 17.0, 1.6 Hz, 1H), 5.19 (dd, J = 10.4, 1.6 Hz, 1H), 5.12 (d, J = 11.5 Hz, 1H), 5.11 (d, J = 15.4 Hz, 1H), 4.16 (br.d, J = 11.0 Hz, 1H), 3.96–3.94 (m, 2H), 3.91, 3.82 (ABq, J = 11.8 Hz, 2H), 3.58–3.50 (m, 2H), 3.31 (br.s, 1H), 2.92 (d, J = 3.6 Hz, 1H), 2.74–2.63 (m, 2H), 2.70 (s, 3H), 2.57 (dd, J = 16.2, 1.9 Hz, 1H), 2.48–2.20 (m, 4H), 2.09–2.00 (m, 2H), 2.08 (s, 3H), 1.49–1.38 (m, 2H), 1.09 (d, J = 6.9 Hz, 3H), 1.02 (d, J = 6.6 Hz, 3H), 0.95 (s, 3H), 0.95–0.82 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 222.0, 170.9, 165.1, 152.1, 138.7, 134.8, 132.5, 123.9, 120.3, 119.3, 117.0, 116.6, 78.4, 74.7, 73.1, 72.0, 71.1, 56.1, 42.4, 39.3, 38.6, 35.6, 33.3, 32.5, 29.7, 28.3, 24.2, 19.5, 16.4, 16.0, 15.6, 11.7; HRFABMS: calcd for $C_{32}H_{48}NO_6S$ (M + H) 574.3202, found 574.3223.

4*S*, 6*S*, 7*R*-Bridged epothilone D bis-TBS ether 46. Bridged analogue **46** was prepared as a colorless oil (2.4 mg, 71%) *via* ring closing metathesis reaction with second-generation Grubbs catalyst from terminal diene **44**, according to the procedure described previously for the synthesis of bridged compound **35**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (s, 1H), 6.50 (s, 1H), 5.43 (br.s, 1H), 5.50–5.32 (m, 2H), 5.29 (d, J = 5.2 Hz, 1H), 4.75 (dd, J = 11.2, 4.4 Hz, 1H), 4.61 (br.s, 2H), 4.48 (br.s, 2H), 3.93 (d, J = 9.6 Hz, 1H), 3.13–2.98 (m, 2H), 2.72 (s, 3H), 2.66 (dd, J = 14.0, 4.0 Hz, 1H), 2.45, 2.42 (ABq, J = 11.2 Hz, 2H), 2.20 (dd, J = 16.0, 12.0 Hz, 1H), 2.13 (s, 3H), 2.16–1.99 (m, 2H), 1.79–1.72 (m, 1H), 1.44 (s, 3H), 1.46–1.10 (m, 2H), 1.25 (s, 3H), 0.99 (d, J = 7.2 Hz, 3H), 0.90, 0.83 (each s, 18H), 0.80 (d, J = 6.8 Hz, 3H), 0.18 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.4, 169.9, 165.0, 152.9, 140.5, 135.7, 129.5, 124.2, 119.2, 119.1, 116.5, 77.4, 76.2, 74.7, 70.8, 57.0, 45.2, 40.8, 38.0, 35.3, 32.5, 29.9, 27.3, 26.4, 26.0, 19.5, 18.8, 18.2, 17.4, 16.7, 15.6, 13.0, –3.10, –3.31, –4.00, –5.28; HRFABMS: calcd for $C_{42}H_{72}NO_6SSi_2$ (M + H) 774.4619, found 774.4568.

Bridged epothilone 47. A similar procedure was used to prepare **47** (1.16 mg, 71.6%, colorless oil) from bis-TBS ether **46** based on the previously described method for the conversion of **20a** to **21** (ESI). ^{1}H NMR (500 MHz, CD_3CN) δ 7.17, 7.15 (s, 1H), 6.61, 6.55 (s, 1H), 5.65–5.27 (m, 4H), 4.62 (dd, J = 10.5, 4.0 Hz, 1H), 4.51 (br.s, 2H), 4.42 (br.s, 2H), 3.60–3.54, 3.40–3.35 (m, 2H), 3.32–3.28, 3.21–3.13 (m, 1H), 2.98–2.92 (m, 1H), 2.76 (dd, J = 10.5, 4.0 Hz, 1H), 2.66 (s, 3H), 2.56, 2.52; 2.41, 2.38 (ABq, J = 11.5 Hz, 2H), 2.27–2.05 (m, 4H), 2.15 (s, 3H), 1.58–1.36 (m, 6H), 1.27, 1.22 (s, 3H), 1.05, 1.04 (d, J = 7.0 Hz, 3H), 0.92, 0.86 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3CN) δ 218.6, 169.7, 165.0, 152.6, 140.7, 135.3, 129.3, 124.3, 119.0, 118.6, 116.7, 76.4, 75.4, 74.6, 74.2, 68.8, 56.8, 42.8, 39.2, 35.4, 33.3, 33.1, 26.9, 26.4, 24.7, 18.3, 16.3, 15.9, 13.5, 12.5; HRFABMS: calcd for $C_{30}H_{44}NO_6S$ (M + H) 546.2889, found 546.2908.

(4*S*)-4-Allyl-4-demethyl-26-(acryloyloxy)-6*S*,7*R*-epothilone D bis-TBS ether 48. Acryloyloxy derivative **48** was synthesized (6 mg, 56%) as a colorless oil from primary alcohol **43**, according to the procedure described previously for the preparation of **7** (ESI). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.36 (d, J = 17.3 Hz, 1H), 6.34 (s, 1H), 6.10 (dd, J = 17.3, 10.4 Hz, 1H), 5.77 (d, J = 10.4 Hz, 1H), 5.74–5.65 (m, 1H), 5.42 (t, J = 8.2 Hz, 1H), 5.16 (t, J = 4.1 Hz, 1H), 5.08 (d, J = 18.4 Hz, 1H), 5.07 (d, J = 9.6 Hz, 1H), 4.56, 4.51 (ABq, J = 12.6 Hz, 2H), 4.18 (br.d, J = 8.5 Hz, 1H), 3.98 (d, J = 3.3 Hz, 1H), 3.45–3.38 (m, 1H), 2.69 (s, 3H), 2.65 (dd, J = 14.5, 6.5 Hz, 1H), 2.56–2.43 (m, 4H), 2.25–2.20 (m, 2H), 2.11 (s, 3H), 1.95–1.87 (m, 1H), 1.60–1.44 (m, 5H), 1.07 (d, J = 7.1 Hz, 3H), 0.96 (s, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.86 (d, J = 6.3 Hz, 3H), 0.19 (s, 3H), 0.15 (s, 3H), 0.12 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 220.1, 170.4, 166.0, 164.6, 152.5, 137.9, 136.4, 134.1, 130.7, 128.5, 123.6, 119.4, 118.0, 116.3,

77.6, 74.8, 73.8, 68.5, 56.3, 48.6, 42.0, 40.2, 38.6, 34.8, 31.1, 28.3, 27.8, 26.3, 21.1, 19.3, 18.8, 18.6, 16.1, 15.8, 14.3, –3.5, –4.0, –4.3, –4.7; HRFABMS: calcd for $C_{44}H_{74}NO_7SSi_2$ (M + H) 816.4725, found 816.4722.

(4*S*)-4-Allyl-4-demethyl-26-(acryloyloxy)-6*S*,7*R*-epothilone analogues 49 and 50. A similar procedure was employed to prepare mono-TBS ether **49** (3.0 mg, 50%, colorless oil) and diol **50** (2.0 mg, 40%) from bis-TBS ether **48** as previously described for the conversion of **20a** to **21** (ESI). Compound **49**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.38 (d, J = 17.6 Hz, 1H), 6.33 (s, 1H), 6.10 (dd, J = 17.6, 10.0 Hz, 1H), 5.80 (d, J = 10.4 Hz, 1H), 5.55–5.45 (m, 2H), 5.32 (br.s, 1H), 5.10 (d, J = 9.2 Hz, 1H), 5.07 (d, J = 16.0 Hz, 1H), 4.63, 4.58 (ABq, J = 12.8 Hz, 2H), 4.17 (br.d, J = 8.8 Hz, 1H), 3.78–3.72 (m, 1H), 3.62 (d, J = 8.4 Hz, 1H), 3.54 (br.s, 1H), 2.78 (dd, J = 14.4, 6.4 Hz, 1H), 2.70 (s, 3H), 2.57–2.41 (m, 3H), 2.32–2.02 (m, 3H), 2.07 (s, 3H), 1.66–1.45 (m, 3H), 1.28–1.20 (m, 3H), 1.13 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 0.94 (s, 9H), 0.20 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 222.4, 170.4, 166.0, 164.8, 152.3, 137.0, 136.3, 132.8, 130.9, 128.4, 124.4, 119.1, 119.0, 116.2, 75.0, 73.8, 68.4, 67.6, 57.3, 44.2, 40.2, 39.5, 35.2, 33.5, 31.1, 29.7, 28.2, 26.4, 23.5, 19.5, 19.3, 18.6, 16.3, 15.7, 11.5, –3.5, –4.5; HRFABMS: calcd for $C_{38}H_{60}NO_7SSi$ (M + H) 702.3860, found 702.3855. Compound **50**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.51 (s, 1H), 6.43 (d, J = 17.3 Hz, 1H), 6.15 (dd, J = 17.5, 10.4 Hz, 1H), 5.87 (d, J = 10.4 Hz, 1H), 5.62–5.54 (m, 2H), 5.42 (t, J = 6.8 Hz, 1H), 5.13 (d, J = 18.1 Hz, 1H), 5.12 (d, J = 9.3 Hz, 1H), 4.67, 4.54 (ABq, J = 13.2 Hz, 2H), 4.20 (d, J = 11.3 Hz, 1H), 3.58 (q, J = 6.8 Hz, 1H), 3.53 (d, J = 9.3 Hz, 1H), 3.31 (br.s, 1H), 2.93 (d, J = 3.0 Hz, 1H), 2.73–2.66 (m, 1H), 2.70 (s, 3H), 2.55 (d, J = 15.9 Hz, 1H), 2.48 (dd, J = 14.2, 6.3 Hz, 1H), 2.42–2.30 (m, 4H), 2.09–2.00 (m, 1H), 2.07 (s, 3H), 1.66–1.40 (m, 3H), 1.26–1.22 (m, 1H), 1.09 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.3 Hz, 3H), 0.95 (s, 3H), 0.97–0.85 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 222.1, 170.7, 166.2, 165.2, 152.1, 136.8, 136.3, 132.5, 131.2, 128.3, 124.6, 120.3, 119.2, 116.7, 78.3, 74.7, 71.9, 70.3, 67.1, 56.2, 42.2, 39.0, 38.5, 35.6, 33.3, 32.6, 28.5, 24.3, 19.3, 16.0, 15.9, 15.5, 11.8; HRFABMS: calcd for $C_{32}H_{46}NO_7S$ (M + H) 588.2995, found 588.3037.

***Cis* bridged epothilone 51.** Compound **51** was prepared as a colorless oil with a *cis* double bond on the newly made bridge (2.8 mg, 62%) *via* ring closing metathesis with second-generation Grubbs catalyst from terminal diene **49**, according to the procedure described previously for the synthesis of bridged compound **35**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.58 (s, 1H), 6.09 (dt, J = 12.1, 3.0 Hz, 1H), 5.82 (dd, J = 11.5, 2.2 Hz, 1H), 5.78 (t, J = 6.8 Hz, 1H), 5.76 (d, J = 11.5 Hz, 1H), 4.72 (d, J = 8.5 Hz, 1H), 4.68, 4.33 (ABq, J = 11.8 Hz, 2H), 3.93 (t, J = 13.5 Hz, 1H), 3.42 (q, J = 6.6 Hz, 1H), 3.29 (d, J = 10.4 Hz, 1H), 2.88–2.73 (m, 2H), 2.71 (s, 3H), 2.59 (d, J = 11.0 Hz, 1H), 2.46–2.41 (m, 2H), 2.33 (t, J = 12.6 Hz, 1H), 2.15 (s, 3H), 1.99 (d, J = 14.3 Hz, 1H), 1.79–1.72 (m, 1H), 1.52–1.37 (m, 3H), 1.30–1.17 (m, 2H), 1.21 (s, 3H), 1.08 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.83 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 221.5, 169.9, 166.1, 165.1, 152.2, 141.6, 136.9, 136.1, 131.4, 123.1, 121.2, 117.1, 79.7, 74.9, 72.2, 68.1, 59.7, 40.0, 36.3, 32.7, 32.6, 31.1, 29.7, 26.7, 25.8, 19.4, 19.3, 18.2, 15.0, 10.4, 1.11, 0.09, –2.6, –3.8; HRFABMS: calcd for $C_{36}H_{56}NO_7SSi$ (M + H) 674.3547, found 674.3593.

***Cis* bridged epothilone 52.** A similar procedure was used to prepare **52** (1.36 mg, 47%, colorless oil) from mono-TBS ether **51** based on the previously described method for the conversion of **20a** to **21** (ESI). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (s, 1H), 6.56 (s, 1H), 6.25–6.15 (m, 1H), 5.90 (dd, J = 11.8, 1.9 Hz, 1H), 5.72 (d, J = 9.3 Hz, 1H), 5.65 (dd, J = 11.5, 1.7 Hz, 1H), 4.64, 4.45 (ABq, J = 11.4 Hz, 2H), 4.52–4.30 (m, 1H), 3.63–3.54 (m, 1H), 3.44–3.34 (m, 4H), 2.81–2.67 (m, 2H), 2.70 (s, 3H), 2.44–2.41 (m, 3H), 2.09 (s, 3H), 1.91–1.82 (m, 1H), 1.71–1.58 (m, 2H), 1.45–1.38 (m, 2H), 1.31–1.19 (m, 2H), 1.25 (s, 3H), 1.07 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 7.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 221.5, 169.9, 166.2, 165.1, 152.0, 141.8, 137.0, 136.8, 130.8, 123.1, 120.7, 116.9, 78.8, 76.8, 70.9, 70.7, 58.2, 41.1, 35.8, 33.2, 32.7, 31.6, 30.9, 29.7, 26.5, 22.7, 19.3, 15.3, 14.2, 10.8; HRFABMS: calcd for $C_{30}H_{42}NO_7S$ (M + H) 561.2760, found 560.2720.

***Trans* bridged epothilone 53.** Ring closing metathesis reaction of terminal diene **50** with second-generation Grubbs catalyst, according to the procedure described previously for the synthesis of bridged compound **35**, produced a mixture (0.86 mg, ~93% total yield) as a colorless oil. This mixture consists of 86.5% of **53** based on the integration of its ^{1}H NMR spectrum. Compound **53**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.55 (s, 1H), 5.94 (d, J = 17.0 Hz, 1H), 5.70 (dd, J = 17.0, 9.9 Hz, 1H), 5.46 (dd, J = 12.1, 3.0 Hz, 1H), 4.71 (t, J = 7.4 Hz, 1H), 4.59, 4.52 (ABq, J = 11.3 Hz, 2H), 3.43 (d, J = 10.4 Hz, 1H), 3.25 (q, J = 6.6 Hz, 1H), 2.82–2.71 (m, 1H), 2.70 (s, 3H), 2.55–2.31 (m, 6H), 2.09 (s, 3H), 2.08–2.02 (m, 1H), 1.75–1.65 (m, 1H), 1.50–1.20 (m, 3H), 1.23 (s, 3H), 1.06 (s, 3H), 1.03 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H), 0.97–0.85 (m, 1H); HRFABMS: calcd for $C_{30}H_{42}NO_7S$ (M + H) 560.2682, found 560.2593.

(4*R*)-4-Allyl-4-demethyl-26-(benzoyloxy)-6*S*,7*R*-epothilone B 56. 26-benzoyloxy-β-epoxide **56** was synthesized from β-epoxide triol **55** employing the same procedure as that described for compound **22** (ESI). Compound **56** was obtained as a colorless needles from hexanes-acetone in 94% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 7.2 Hz, 2H), 7.53 (t, J = 7.4 Hz, 1H), 7.35 (t, J = 7.4 Hz, 2H), 6.93 (s, 1H), 6.65 (s, 1H), 5.65 (br.s, 1H), 5.56–5.48 (m, 1H), 5.15–5.08 (overlap, 1H), 5.10 (d, J = 11.2 Hz, 1H), 5.09 (d, J = 16.0 Hz, 1H), 4.48–4.41 (m, 1H), 4.20 (br.s, 1H), 4.18 (d, J = 12.0 Hz, 1H), 3.79 (d, J = 9.0 Hz, 1H), 3.47 (br.s, 1H), 3.31 (q, J = 7.0 Hz, 1H), 3.25 (dd, J = 9.4, 3.3 Hz, 1H), 2.70 (s, 3H), 2.70–2.68 (m, 1H), 2.56 (d, J = 12.3 Hz, 1H), 2.52 (d, J = 9.1 Hz, 1H), 2.41 (dd, J = 14.3, 5.5 Hz, 1H), 2.25 (dt, J = 15.4, 3.8 Hz, 1H), 2.16 (s, 3H), 1.99–1.86 (m, 1H), 1.73–1.38 (m, 8H), 1.10 (d, J = 6.8 Hz, 3H), 1.02 (d, J = 6.4 Hz, 3H), 1.01 (s, 3H). Colorless needles of compound **56** ($0.03 \times 0.03 \times 0.80$ mm^3) were crystallized from hexanes/acetone at room temperature. The chosen crystal was centered on the goniometer of an Oxford Diffraction Gemini Ultra diffractometer equipped with a Sapphire 3 CCD detector and operating with CuKα radiation. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlis.[25] The Laue symmetry and systematic absences were consistent with the orthorhombic space group P212121. Structure solution was performed with the graphical user interface WinGX.[26] The structure was solved by direct methods using SIR92[27] and refined using SHELXTL-NT.[28] The asymmetric unit of the structure comprises one crystallographically independent molecule. The final refinement model

involved anisotropic displacement parameters for non-hydrogen atoms and a riding model for all hydrogen atoms. The absolute configuration was established from anomalous dispersion effects (Flack x = 0.01(6)). The configuration of chiral centers were assigned as C3(*S*), C4(*S*), C6(*S*), C7(*R*), C8(*S*), C12(*S*), C13(*S*) and C15(*S*).

Biological data. Tubulin was prepared by two cycles of temperature dependent assembly–disassembly as described by Williams and Lee.[29] ED_{50} values for tubulin assembly were determined as described in Chatterjee *et al.*[30]

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[31] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[32] The MTT assay[33] was used at SUNY for PC3 cells.

Molecular modeling and design. Structures of bridged epothilone analogues **27**, **30** and **36** were constructed in Maestro[34] by using the electron crystallographic structure of epo-A as template. The structures were subsequently optimized with the MMFF/GBSA/H_2O force field. The optimized structures of **27**, **30** and **36** were separately and flexibly docked into tubulin binding site by using the Glide docking protocol.[24] The best docking poses were chosen on the basis of the Emodel scoring function together with visualization to ensure reasonable binding modes.

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