

Synthesis of Epothilone 16,17-Alkyne Analogs by Replacement of the C13–C15(O)-Ring Segment of Natural Epothilone C

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Dedicated to Professor Henning Hopf on the occasion of his 62nd birthday

Keywords: Antitumor agents / Natural products / Metathesis / Macrocycles

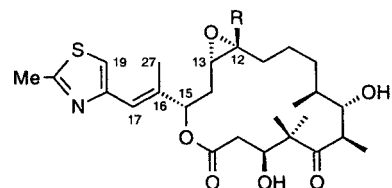
Ring-opening cross metathesis of epothilone C (**4a**) with ethylene, followed by silyl protection and ester hydrolysis, yielded an eastern ring segment C1–C12 as the carboxylic acid **10**. Separately, a western ring segment **12** carrying a C16–C17 triple bond was synthesized and coupled with **10** to form the ester **13**. Ring closure by olefin metathesis, deprotection, and then epoxidation, gave the 16,17-alkyne analogs

(**14b**, **3b**) of epothilone C and epothilone A. The identity of **3b** was proven by hydrogenation to (16Z)-epothilone A₈ (**17**) and comparison with an authentic sample prepared from natural epothilone A₈ (**18**). The biological activity of the new epothilones was determined.

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Introduction

The epothilones represent a unique group of antifungal and cytotoxic macrolides first isolated from the myxobacterium *Sorangium cellulosum*.^[1] Their exceptional biological activity is based on the same mechanism as that of taxol, namely the induction of tubulin polymerisation and suppression of microtubule dynamics.^[2,3] At nanomolar concentration it is the mitotic cells that are affected selectively by this process, leading to apoptosis of tumor cells. Epothilone B (**1b**), the most active of 39 structural variants produced by the bacterial culture,^[4] is a 6 to 25-fold more potent inhibitor of human cancer cell growth than is taxol.^[5] Moreover, in contrast to taxol, most epothilones also are highly active against multidrug-resistant tumor cells by evading the P-glycoprotein export system.^[2,6] For these and other favorable properties, epothilones have become popular synthetic targets and, within a few years of their discovery, numerous total syntheses of natural epothilones and a great variety of analogs had been published.^[7] Those groups who have had access to a supply of epothilones produced by fermentation have concentrated their efforts on chemical,^[8] microbial^[9] and enzymatic^[9a,10] transformations.

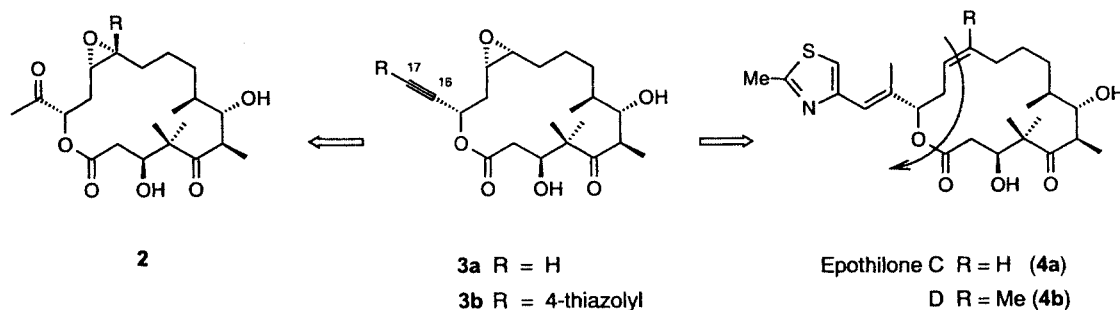


Epothilone A (**1a**) R = H
B (**1b**) R = Me

Whereas many functional groups of the macrocycle, including its ring size, and the thiazole unit have been modified extensively by derivatisations and total syntheses, the olefinic spacer element (C16, C17, C27) between these two moieties has attracted relatively little attention. After early work had shown that thiazole and phenyl residues attached directly to the macrocycle^[11] or by a one-atom-extended linker^[8e] lead to almost complete loss of activity, such modifications were not pursued any further. Similarly, spacers with a 16Z double bond^[12] or a C16–C17 single bond^[8a] were, more or less, inactive. Consequently, in subsequent synthetic work this moiety has been kept the same as it appears in the natural prototype. Conformational studies by NOE measurements have shown that in solution the side chain rotates around both single bonds of the linker,^[1,13] whereas it appears to be locked in a syn conformation with respect to the methyl group at C27 and thiazole H19 atom in the tubulin-bound state. This conclusion follows indirectly from the fact that substitution at C19^[8c] or C27,^[4,14] which interferes with this conformation, leads to a loss of biological activity. An elegant proof of this hypothesis came

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Scheme 1. Retrosynthetic analysis of epothilone alkyne analogs 3

from the Novartis^[15] and Schering^[16] groups by the synthesis of highly active benzimidazole and quinoline analogs in which the syn conformation is predetermined by the presence of the benzene ring. Another essential feature for biological activity seems to be the presence of a hydrogen bond acceptor in the side chain, such as an *N*-heterocycle or its *N*-oxide.^[8d,15–17] From a series of α -, β -, and γ -pyridyl analogs, first published by Nicolaou's group,^[17] we know that the nitrogen atom may be moved away from the macrocycle to a certain extent without loss of activity. Taking this information together, we hypothesized that the hitherto-unknown epothilone analogs with conformationally rigid alkyne spacer units were desirable synthetic targets.

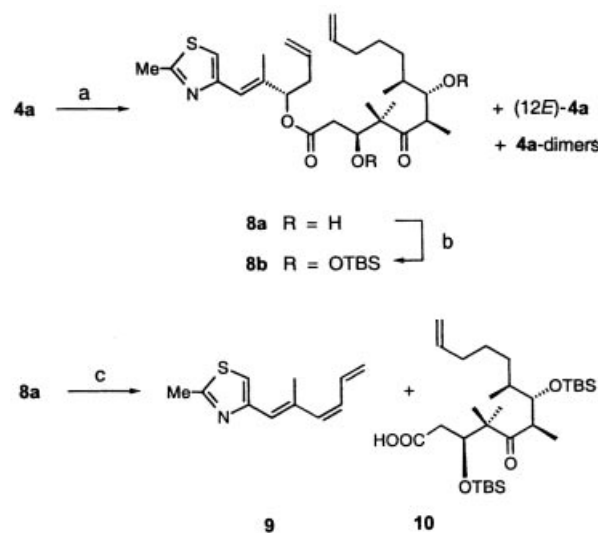
Results and Discussion

Following our well-established strategy,^[8c] first we investigated introducing the desired triple bond without touching the macrocycle and the C15 stereocenter. An obvious intermediate leading to compounds of type **3b** is a 15-alkynyl epothilone analog **3a** (R = H), which should undergo Sonogashira coupling^[18] with a variety of heterocyclic halides (Scheme 1). Attempts, however, to transform the acetyl group of **2**, which is easily obtained by ozonation of epothilones A and B, into an alkyne failed completely. Next we envisaged replacing the western ring segments of epothilones C and D (**4a,b**) with a synthetic building block carrying the desired C16–C17 triple bond. This strategy appeared particularly attractive when epothilones C and D became available in larger quantities by the fermentation of a *P*₄₅₀-deficient mutant of *Sorangium cellulosum*.^[9,19]

5–7 was investigated.^[21–25] With equimolar amounts of Grubbs' catalyst **5**,^[26] a 46% conversion of **4a** to **8a** was obtained after 20 h. However, with catalytic amounts of **5** (30 and 15 mol %) conversion stopped, even with prolonged reaction times, at 24 and 14%, respectively (exp. 1–3, Table 1). The second-generation Grubbs' catalysts **6**^[27] and **7**,^[28] which feature *N*-heterocyclic carbene ligands, proved

Table 1. Cross metathesis of epothilone C (**4a**) and ethylene with ruthenium catalysts **5–7** in dichloromethane

Exp	Catalyst	mg/mL		Yield (%)		
		[4a]	4a	8a	After 20 hours (12 <i>E</i>)- 4a	4a -dimers
1	100 mol % 5	1.9	51	46	—	3
2	30 mol % 5	1.9	74	24	—	2
3	15 mol % 5	1.9	85	14	—	≤ 1
4	15 mol % 6	1.9	20	66	2	12
5	15 mol % 7	1.9	11	76	3	10
After 8 hours						
6	10 mol % 7	1.6	31	59	2	8
7	10 mol % 7	4.8	17	62	2	19
8	10 mol % 7	14.4	10	60	2	28

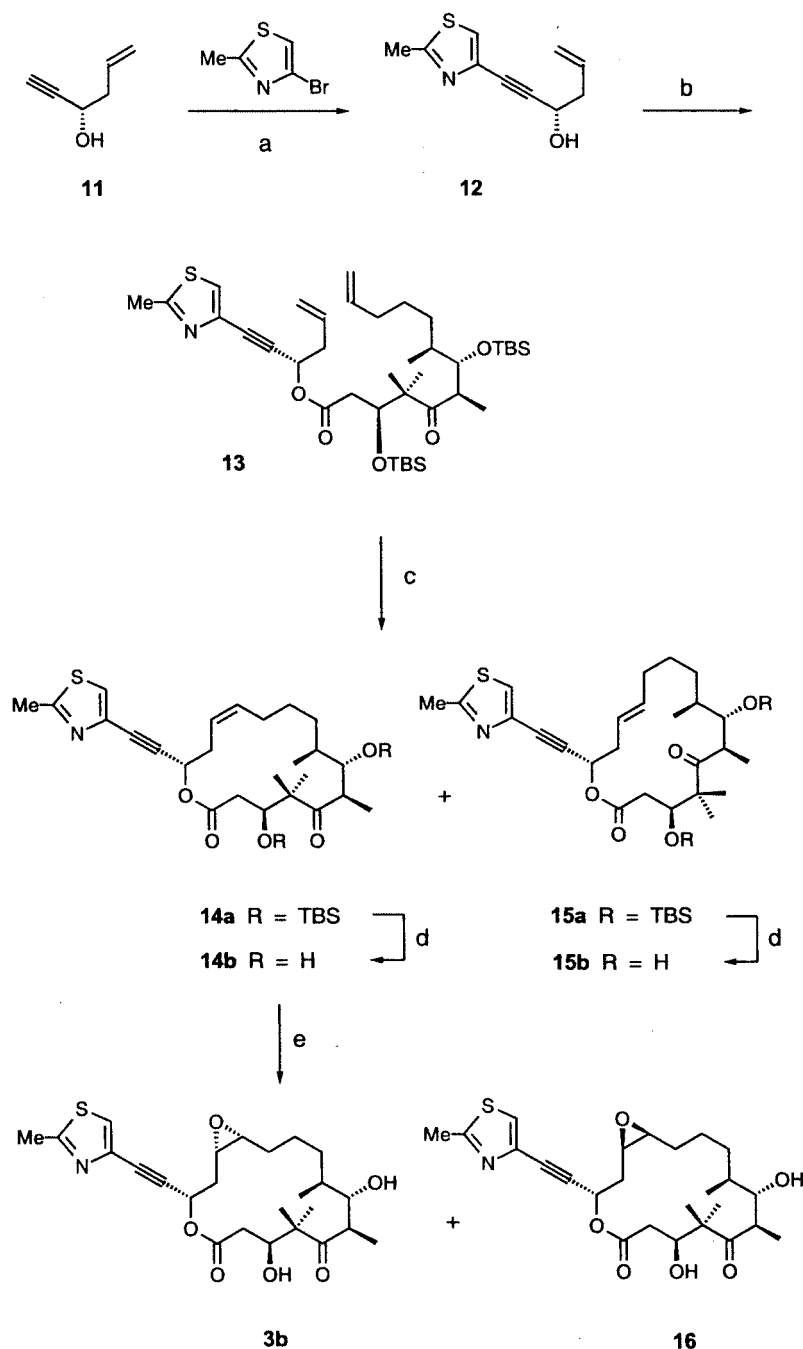
Scheme 2. Degradation of epothilone C (**4a**) to building block **10**. a) Metathesis catalysts **5–7** (0.14 equiv.), CH₂Cl₂, room temp., 44 h; b) 4 equiv. TBSOTf, 6 equiv. 2,6-lutidine, CH₂Cl₂, –20 °C, 24 h; c) 5 equiv. TBSOTf, 7 equiv. 2,6-lutidine, 30 °C, 2 h

Instead of cleaving the C12–C13 double bond of epothilone C (**4a**) by ozonolysis, ring-opening olefin metathesis with an excess of ≥ 6 equiv.^[20] of ethylene and catalysts

to be far more reactive and stable. With 15 mol % of **6** and **7**, conversions of **4a** of 66 and 76%, respectively, were achieved under standard conditions. In addition to the desired cleavage product **8a** (Scheme 2), the *trans* isomer of epothilone C (12*E*)-**4a** (2–3%) and dimers of **8a** resulting from cross metathesis (10–12%) were formed to a greater extent when using the second-generation catalysts than with catalyst **5** (exp. 4 and 5). As expected, the yield of dimers and some oligomers of **8a** increased significantly with higher substrate concentrations (exp. 6–8). Metathesis was

performed on a preparative scale with catalyst **7** (16 mol %) added in two portions during 44 h, and **8a** was isolated by silica gel chromatography in 83% yield based on recovered starting material. As a result of this one-step transformation, C12 is liberated and conserved in an unreactive functional group that is ready for ring-closing olefin metathesis towards the end of the synthesis.

Silyl protection of the hydroxyl groups gave **8b** and ester cleavage with lithium hydroxide furnished building block **10**, which had been synthesized previously by Danishef-



Scheme 3. Synthesis of epothilone alkyne analog **3b**. a) 0.07 equiv. $\text{PdCl}_2(\text{PPh}_3)_2$, 11.5 equiv. NEt_3 , 0.23 equiv. CuI , 15 min at room temp., 6 h at 80 °C, 1.1 equiv. alcohol **11**; b) **10**, 1.3 equiv. DCC, 0.24 equiv. DMAP, CH_2Cl_2 , 0 °C–room temp., 16 h; c) 0.2 equiv. metathesis catalyst **5**, CH_2Cl_2 , room temp., 48 h, (*Z*:*E* = 1:1); d) TFA/ CH_2Cl_2 (6:1), 0 °C, 2 h; e) dimethyl dioxirane, CH_2Cl_2 /acetone, –20 °C, 2 h

sky's^[29] and Schinzer's^[30] groups. Remarkably, in a diversionary side reaction, ester **8b** was cleaved using TBSOTf/lutidine through ester carbonyl activation and fragmentation to yield triene **9** and the TBS ester of **10**. With a five-fold excess of reagents at 30 °C the ester was cleaved entirely and **10** was obtained from **8a** in a single step, after acidic workup, in 80% yield.

The western ring segment **11** incorporating the triple bond was constructed from propargylic aldehyde by stereoselective allylation with tri-*n*-butylallylstannane in the presence of (*S*)-BINOL, as described previously by Keck et al.^[31] Sonogashira coupling^[18] of **11** with 4-bromo-2-methylthiazole gave building block **12** whose enantiomeric purity was determined as 9:1 by ¹H NMR spectroscopy of the Mosher ester (Scheme 3). With building blocks **10** and **12** at hand we proceeded along a similar synthetic route as those described in Nicolaou's^[32] and Schinzer's^[30] total syntheses of epothilone A. Esterification of carboxylic acid **10** with alcohol **12** using the DMAP/DCC protocol,^[33] followed by olefin metathesis with catalyst **5**, gave a 1:1 mixture of the *E/Z* isomers **14a** and **15a** after silica gel flash chromatography. After deprotection by treatment with trifluoroacetic acid and silica gel chromatography, pure isomers (*Z*)-**14b** and (*E*)-**15b** were obtained in 43% yield each. The stereochemical assignments of **14b** and **15b** were based on the chemical shifts of the allylic carbon atoms C11 and C14 in ¹³C NMR spectra. Because of steric effects in (*Z*)-**14b** the signals of these carbon atoms were found at δ = 27.6 and 32.9 ppm, whereas in (*E*)-**15b** they were shifted relatively downfield to δ = 31.3 and 37.7 ppm. These trends are in good agreement with chemical shifts^[4] of related compounds.

Epoxidation of lactone (*Z*)-**14b** with dimethyl dioxirane at –20 °C gave, after HPLC separation, a 32% yield of the α -epoxide **3b** (natural configuration) and β -epoxide **16** (66%). This assignment of configurations was based on NMR chemical shifts; NOE experiments, however, gave ambiguous results, and the magnitude of optical rotations indicated the opposite assignment. To solve this problem by direct comparison with a natural epothilone, the putative α -epoxide **3b** was hydrogenated with Lindlar catalyst to give the 16*Z*-isomer **17** (Scheme 4) of epothilone A₈ (**18**)^[4]. Meanwhile, natural epothilone A₈ (**18**) was isomerised photochemically^[12] to its (16*Z*)-isomer **17**. Both of these samples of **17** displayed identical spectroscopic and analytical properties, whereas the product [(16*Z*)-12,13-epiepothi-

lone A₈, **19**] obtained by partial hydrogenation of β -epoxide **16** was significantly different from **17**.

Both alkyne analogs **14b** and **3b** showed only marginal cytotoxicities of IC₅₀ $\geq$ 500 ng/ml for the standard mouse fibroblast cell line L929.^[34]

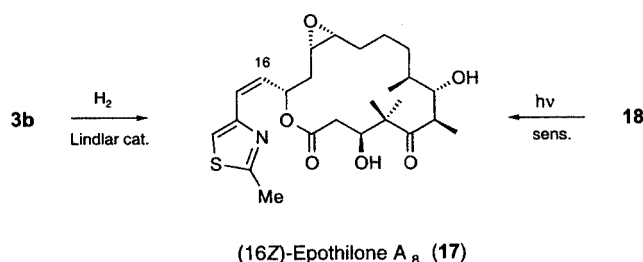
Conclusion

Inspection of molecular models of the alkyne analog **3b** and biologically active epothilones indicates that there is no significant difference in the volume of space occupied by the side chains. It is proposed, therefore, that the exact positioning of the nitrogen atom in the side chain plays a decisive role in biological activity of these compounds. When entering the tubulin binding site, the orientation of the side chain of epothilones can be adjusted by only two parameters: the angle of inclination of the side chain with respect to the macrocycle, and its rotation around the C15–C16 bond. On comparing the alkyne analogs with epothilones **1**, there appears to be no difference with respect to the possible angle of inclination of the side chain, and only a slightly increased distance of the nitrogen atom from the C15 atom of the macrocycle (5.0 Å in **3b** and 4.8 Å in **1a**). There is a great difference, however, in the conformational space in which the nitrogen atom can move on rotation around the C15–C16 bond. In case of epothilone A (**1a**), the nitrogen atom rotates in a circle with a radius of 2.9 Å, but in the alkyne analog **3b** this radius is only 1.2 Å. This difference in range is large enough to abolish a strong hydrogen bond on going from **1a** to **3b**. The loss of this hydrogen bond apparently is responsible for the reduced activity of **3b** relative to **1a** by a factor of over 100.

To the best of our knowledge, opening of macrocyclic natural products by olefin cross metathesis has not been described before, although the proof of principle has been demonstrated by the cleavage of an unsubstituted 16-membered lactone with tetradeuteroethylene.^[25] Preliminary experiments indicate that other macrolides also may be opened up, and linear unsaturated natural products fragmented, by olefin cross metathesis with ethylene. Not surprisingly, the outcome of these reactions depends strongly on the other functional groups present, and cannot be predicted. The products obtained may either be used as synthetic building blocks or, in case of modified natural products, used for structural and configurational analysis. In contrast to oxidative degradation, olefin metathesis produces lipophilic fragments that are more easily isolated by column chromatography or directly analysed by gas chromatography.

Experimental Section

General: Analytical TLC: TLC aluminum sheets, silica gel Si 60 F₂₅₄, 0.2 mm (Merck), detection: UV absorption at 254 nm. Preparative TLC: precoated TLC plates, silica gel Si 60 F₂₅₄, 0.25, 0.5, and 1.0 mm layer thickness (Merck); solvent system A: dichloromethane/acetone/methanol, 88:10:2; B: petroleum ether/ethyl acetate,



Scheme 4. Comparison of synthetic and natural epothilones **3b** and **18** by conversion into epothilone A₈ [(16*Z*)-**17**]

85:15. Analytical HPLC: Nucleodur 125 $\times$ 2, 100-5 C18 (Macherey & Nagel), UV detection 254 nm, flow rate 0.4 mL/min; column B: ET 250/4 Nucleosil 100-7 (Macherey–Nagel), UV-detection 254 nm, flow rate 1.5 mL/min. Preparative HPLC: column C: Nucleosil RP-18-7-100, 250 $\times$ 20 mm (Macherey & Nagel), UV detection at 254 nm, flow rate 12 mL/min; column D: Nucleosil 100 (Knauer), 7 μ , 250 $\times$ 20 mm, UV detection at 254 nm, flow rate 15 mL/min. IR: FT-IR spectrometer 20 DXB (Nicolet). Column chromatography: silica gel (SiO₂, 15–25 μ m mesh, Merck). UV: spectrometer UV-2102 PC (Shimadzu), solvent: MeOH (Uvasol, Merck). Optical rotation: Perkin–Elmer instrument. NMR: Spectrometer WM-400 and AM-300 (Bruker), ¹H: 400 and 300 MHz, ¹³C: 100.6 and 75.5 MHz, CDCl₃ as solvent, standard δ = 7.25 ppm. MS: EI and DCI: spectrometer MAT 95 (Finnigan), resolution $M/\Delta M$ = 1000, high-resolution data from peak matching $M/\Delta M$ = 10000.

Cross-Metathesis of Epothilone C (4a) and Ethylene: a) Stock solutions of epothilone C and metathesis catalysts **5**, **6**, and **7** in dichloromethane were mixed and diluted with dichloromethane to give 1 to 3 mL samples which were immediately placed in a larger flask and stirred. The air was purged by evacuation and ethylene was added. The flask was kept at a slight overpressure of ethylene using a soft balloon. After 20 h at room temperature, samples were analysed by silica gel HPLC (solvent system: hexane/methyl *tert*-butyl ether/methanol, 80:19:1; **8a**: t_R = 4.4 min; **4a**: t_R = 9.1 min; (12*E*)-**4a**: t_R = 10.2; dimers of **4a**: t_R = 19–25 min. RP-18 HPLC/ESI-MS (acetonitrile/10 mM ammonium acetate buffer, pH 6.5, gradient 50:50 to 95:5 in 15 min) **4a** and (12*E*)-**4a**: t_R = 7.6 min, m/z = 478 [$M + H^+$]; **8a**: t_R = 11.5 min, m/z = 506 [$M + H^+$]; **4a**-dimers: t_R = 16.5–17.5 min, m/z = 983 [$M + H^+$]). The results are given in Table 1.

b) Technical grade epothilone (**4a**) (477 mg, 90% pure, 0.9 mmol) and **7** (44 mg, 0.07 mmol) were dissolved in dichloromethane (250 mL) and stirred. The air was replaced by ethylene, as described above, and stirring was continued under a slight overpressure of ethylene. After 20 h another portion of catalyst **7** (44 mg) was added. HPLC analysis after 44 h indicated the presence of **4a** (14%), **8a** (75%), (12*Z*)-**4a** (3%), and dimers of **4a** (9%). The reaction mixture was concentrated to 10 mL and applied to a column of silica gel (30 g). Elution with dichloromethane/methanol (200:1 to 100:1) gave **8a** [334 mg, 73% (83% based on recovered **4a**)] and **4a** (50 mg, 12%). From combined fractions from RP-18 and silica gel HPLC columns, (12*E*)-**4a** (7 mg, 1.6%) was obtained.

12,13-Bismethylene-12,13-secoepothilone C (8a): Tan coloured glass; R_f = 0.7 (solvent system A), blue spot on spraying with vanillin/sulfuric acid and heating to 135 °C; $[\alpha]_D^{25}$ = –75.4 (c = 3.5 in CHCl₃); ref.^[29] –76.2 (c = 0.37 in CHCl₃); ¹H and ¹³C NMR (CDCl₃, 300 and 75 MHz) signals within δ = $\pm$ 0.1 and $\pm$ 1 ppm, respectively, in relation to compound **127** in ref.^[29]

(12*E*)-Epothilone C: Colourless glass, R_f = 0.45 (solvent system A). ¹H NMR (CDCl₃): δ = 6.96 (s, 1 H), 6.55 (s, 1 H), 5.51 (dt, J = 15.3, 7.0 Hz, 1 H), 5.33–5.42 (m, 2 H), 4.18 (dt, J = 10.2, 3.5 Hz, 1 H), 3.74 (m, 1 H), 3.24 (dq, J = 6.8, 4.5 Hz, 1 H), 3.13 (d, J = 3.9 Hz, 1 H), 2.70 (s, 3 H), 2.68 (d, J = 4.6 Hz, 1 H), 2.54 (dd, J = 15.0, 10.2 Hz, 1 H), 4.47 (dd, J = 15.0, 3.2 Hz, 1 H), 2.44 (t, J = 6.8 Hz, 1 H), 2.12–2.23 (m, 1 H), 2.08 (s, 3 H), 1.90–2.02 (m, 1 H), 1.60–1.70 (m, 2 H), 1.47 (tt, J = 12.4, 4.4 Hz, 1 H), 1.28 (s, 3 H), 1.18 (d, J = 6.8 Hz, 3 H), 1.06 (s, 3 H), 0.98 (d, J = 7.0 Hz, 3 H) ppm.

Ester 8b. Silylation of 8a: Compound **8a** (18 mg, 36 μ mol) was dissolved in of dichloromethane (1 mL) and 2,6-lutidine (60 μ L,

0.53 mmol). The mixture was cooled to about –20 °C, TBSOTf (60 μ L, 0.26 mmol) was added with stirring, and then the mixture kept overnight at ca. –20 °C. The solvent was evaporated and then the residue was dissolved in ethyl acetate and extracted with 0.1 N HCl. The organic layer was evaporated to dryness and the residue separated by silica gel HPLC (hexane/*tert*-butyl methyl ether, 9:1) to give **8b** (19 mg, 75%) as colourless glass, R_f = 0.8 (solvent system A). $[\alpha]_D^{20}$ = +45.5 (c = 3.1 in CHCl₃); ref.^[30] +45.0 (c = 1 in CHCl₃). ¹H NMR (CDCl₃): δ = 6.92 (s, 19-H), 6.48 (s, 17-H), 5.79 (ddt, J = 16.8, 10.3, 6.7 Hz, 12-H), 5.71 (ddt, J = 16.6, 10.2, 7.0 Hz, 13-H), 5.29 (t, J = 6.7 Hz, 15-H), 5.09 (br d, J = 16.8 Hz, 13'-Ha), 5.03 (br d, J = 10.2 Hz, 13'-Hb), 4.98 (br d, J = 17.2 Hz, 12'-Ha), 4.92 (br d, J = 10.1 Hz, 12'-Hb), 4.33 (dd, J = 5.9, 3.7 Hz, 3-H), 3.73 (dd, J = 6.5, 4.3 Hz, 7-H), 3.14 (dq, J = 6.6, 7.0 Hz, 6-H), 2.69 (s, 21-H₃), 2.40–2.56 (m, 2-Ha, 14-H₂), 2.28 (dd, J = 16.8, 5.7 Hz, 2-Hb), 2.06 (q, J = 0.8 Hz, 27-H₃), 2.00 (m, 11-H₂), 1.29–1.51 (m, 8-H, 9-H, 10-H), 1.23 (s, 23-H₃), 1.05–1.22 (m, 9-H, 10-H), 1.04 (s, 22-H₃), 1.03 (d, J = 6.7 Hz, 24-H₃), 0.89 (d, J = 6.8 Hz, 25-H₃), 0.89, 0.87 (s, 2 Si*t*Bu), 0.10, 0.05, 0.03, 0.02 (s, 2 SiMe₂) ppm. ¹³C NMR (CDCl₃) signals identical to within about 1 ppm with those of **31a** in ref.^[30]

Carboxylic Acid 10. Hydrolysis of Ester 8b: LiOH·H₂O (20 mg, 2 mmol) in H₂O (1.5 mL) was added to a solution of **8b** (36 mg, 49 μ mol) in 2-propanol (1 mL), and then the turbid solution was stirred for 16 h at 95 °C. The organic solvent was evaporated and the residue partitioned between diethyl ether and 0.1 N HCl. The organic layer was dried with MgSO₄, evaporated to dryness, and purified by HPLC (silica gel; eluent: hexane/*tert*-butyl methyl ether, 95:5) gave **10** as a colourless glass (25.0 mg, 94%). R_f = 0.4 (solvent system B), red spot after spraying with vanillin/sulfuric acid reagent and heating to 135 °C. RP-18 HPLC [methanol/10 mM ammonium acetate buffer (pH 6.5), 9:1]: t_R = 5.6 min. $[\alpha]_D^{20}$ = –33.9 (c = 2.3 in CHCl₃), ref.^[30] 31.7 (c = 1 in CHCl₃). ¹H and ¹³C NMR (CDCl₃): signals identical to **29** in ref.^[30]

Silylation and Ester Cleavage of 8a: 2,6-Lutidine (230 μ L, 2.0 mmol) and TBSOTf (230 μ L, 0.75 mmol) were added with stirring to a solution of **8a** (107 mg, 0.21 mmol) dissolved in dichloromethane (5 mL). After 2 h, TLC analysis (solvent system B, UV detection) indicated complete conversion of **8a** and the intermediate **8b** to a new compound **9**. The reaction mixture was concentrated in vacuo, the residue was dissolved in ethyl acetate, and then washed with 0.1 N HCl. The organic layer was dried with MgSO₄ and the solvents evaporated to give a colourless oil (375 mg). Purification by preparative RP-18 HPLC [solvent system acetonitrile/50 mM ammonium acetate buffer (pH 7), 8:2] gave **10** (80 mg, 70%) and **9** (25 mg, 60%).

(1*E*,3*Z*)-2-Methyl-4-(2-methylhexa-1,3,5-trienyl)thiazole (9): UV (MeOH): $\lambda_{\max}$ (ϵ) = 240 (210), 245 (220), 294 (10100), 305 (10200), 321 (7300) nm. ¹H NMR (CDCl₃): δ = 6.96 (s, 5'-H), 6.50 (s, 1-H), 6.35–6.50 (m, 3-H, 4-H, 5-H), 5.26 (dd, J = 16.2, 1.5 Hz, 6*E*-H), 5.11 (dd, J = 9.7, 1.5 Hz, 6*Z*-H), 2.70 (s, 2'-Me), 2.22 (s, 2-Me) ppm. ¹³C NMR (CDCl₃): δ = 164.7, 153.5, 138.3, 137.5, 136.8, 129.8, 124.8, 117.2, 116.4, 19.3, 14.1 ppm.

4-Bromo-2-methylthiazole: 2,4-Dibromothiazole (1 g, 4.11 mmol) was dissolved in anhydrous diethyl ether (25 mL) and the resulting solution stirred under a N₂ atmosphere at –78 °C. *n*BuLi (1.6 M solution in hexane, 2.82 mL, 4.52 mmol) was added and the stirring continued for 1 h before a solution of dimethylsulfate (1.16 mL, 12.34 mmol) in diethyl ether (1 mL) was added dropwise. After stirring for 4 h at –78 °C, the reaction mixture was warmed to room temperature and stirred for a further 14 h. The reaction mixture

was diluted with a saturated aqueous NaHCO_3 (10 mL). The aqueous layer was extracted with diethyl ether and the combined organic extracts were washed with brine and dried with MgSO_4 . Concentration under vacuum, and flash column chromatography (silica gel; petroleum ether/ethyl acetate, 10:1), yielded a yellow oil (0.52 g, 71%). ^1H NMR (CDCl_3): δ = 7.02 (s, 1 H), 2.71 (s, 3 H) ppm. ^{13}C NMR (CDCl_3): δ = 167.31, 124.18, 116.11, 19.40 ppm.

(S)-5-Hexen-1-yn-3-ol (11): $\text{Ti}(\text{O}-i\text{Pr})_4$ (150 μL , 0.51 mmol) was added to a mixture of (1S)-(-)-1,1'-bi-2-naphthol (280 mg, 0.975 mmol) in CH_2Cl_2 (13 mL) and oven-dried powdered 4-Å molecular sieves (2 g). The mixture was heated under reflux for 1 h and then the red-brown mixture cooled to room temperature. Propynal^[35] (263 mg, 4.87 mmol) was added and the mixture was stirred for 10 min. The mixture was cooled to -78°C , and tri-*n*-butylallylstannane (1.65 mL, 5.32 mmol) was added. After 30 min the flask was placed in a freezer at -20°C for 3 d. A saturated solution of NaHCO_3 (2 mL) was added, the mixture was stirred for 1 h before being dried (Na_2SO_4), filtered through a plug of Celite, and concentrated under vacuum. Purification by flash column chromatography (silica gel; petroleum ether/ethyl acetate, 6:1) yielded a yellow oil (370 mg, 70%). $[\alpha]_D^{25}$ = -30.9 (c = 1 in chloroform). The spectroscopic data for this compound matched that reported in the literature; $[\alpha]_D^{25}$ = -36.4 (c = 1.05 in chloroform).^[36]

(3S)-1-(2-Methylthiazol-4-yl)hex-5-en-1-yn-3-ol (12): A suspension of 4-bromo-2-methylthiazole (480 mg, 2.68 mmol), Et_3N (4 mL, 31 mmol), and $\text{PdCl}_2(\text{PPh}_3)_2$ (130 mg, 0.185 mmol) was stirred for 15 min under a N_2 atmosphere at room temperature. CuI (117 mg, 0.615 mmol) was added under a N_2 atmosphere followed by the dropwise addition of alcohol **11** (283 mg, 2.95 mmol) in Et_3N (1 mL, 0.71 mmol). The mixture was stirred for 15 min at room temperature, heated at 80°C for 6 h, and then concentrated under vacuum. Flash column chromatography (silica gel; petroleum ether/ethyl acetate, 3:2), yielded a yellow oil (0.29 g, 56%). $[\alpha]_D^{20}$ = -29.1 (c = 1 in chloroform). ^1H NMR (CDCl_3): δ = 7.26 (s, 1 H), 5.98–5.88 (m, 1 H), 5.23–5.16 (m, 2 H), 4.62 (dd, J = 11.9, 5.8 Hz, 1 H), 2.68 (s, 3 H), 2.58–2.54 (m, 2 H), 2.39 (d, J = 6.1 Hz, 1 H, OH) ppm. ^{13}C NMR (CDCl_3): δ = 165.8, 136.2, 133.1, 122.5, 118.9, 89.5, 79.0, 61.8, 41.9, 19.1 ppm.

Mosher Ester of (3S)-1-(2-Methylthiazol-4-yl)hex-5-en-1-yn-3-ol: Pyridine (1 drop) was added to a solution of the alcohol **12** (5 mg, 0.025 mmol) and (S)-(+)-MTPA-Cl (13.3 mg, 0.05 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred overnight and then purified directly by thin layer chromatography (silica gel; petroleum ether/ethyl acetate, 3:2).

The diastereoisomeric ratio of 91:9 was determined by ^1H NMR spectroscopy.

Major diastereoisomer: ^1H NMR (CDCl_3 , 400 MHz): δ = 7.55–7.35 (m, 5 H, C_6H_5), 7.29 (s, 1 H, 1thiazol), 5.90–5.77 (m, 2 H, HCO and $\text{CH}=\text{CH}_2$), 5.22–5.16 (m, 2 H, CHCH_2), 3.55 (s, 3 H, OCH_3), 2.72–2.69 (m, 2 H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.68 (s, 3 H, CH_3 -thiazol) ppm.

(1S)-1-(2-Methylthiazol-4-ylethynyl)but-3-enyl (3S,6R,7S,8S)-3,7-Bis-*tert*-butyldimethylsiloxy-4,4,6,8-tetramethyl-5-oxo-12-tridecanoate (13): DCC (98.7 mg, 0.478 mmol) was added to a solution of acid **10** (200 mg, 0.368 mmol), alcohol **12** (78.6 mg, 0.405 mmol), and DMAP (12 mg, 0.09 mmol) in CH_2Cl_2 (10 mL) at 0°C . The mixture was stirred for 15 min at 0°C and for 16 h at room temperature, before being concentrated under vacuum. Flash column chromatography (silica gel; petroleum ether/ethyl acetate, 10:1) yielded a yellow oil (240 mg, 91%). $[\alpha]_D^{20}$ = -45.8 (c = 1 in

CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2929, 2856, 1742, 1697, 1641, 1472, 1253, 989 cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz): δ = 7.28 (s, 1 H, thiazole 5-H), 5.91–5.73 (m, 2 H, 12-H, 3'-H), 5.58 (t, J = 6.1 Hz, 1 H, 15-H), 5.20–4.90 (m, 4 H, 12'-H, 13'-H), 4.38 (dd, J = 6.3, 3.3 Hz, 1 H, 3-H), 3.74 (dd, J = 6.8, 2.2 Hz, 1 H, 7-H), 3.11 (dq, J = 6.8, 6.8 Hz, 1 H, 6-H), 2.67 (s, 3 H, thiazole CH_3), 2.60 (t, J = 6.6 Hz, 1 H, 2-H), 2.55 (dd, J = 16.7, 3.5 Hz, 1 H, 14-H), 2.29 (dd, J = 17.0, 6.3 Hz, 1 H, 14-H), 2.05–1.95 (m, 2 H, 11-H), 1.47–1.29 (m, 3 H), 1.17–1.08 (m, 3 H, 8-H, 9-H, 10-H), 1.21 (s, 3 H, 22-H), 1.05 (s, 3 H, 23-H), 1.03 (d, J = 6.6 Hz, 3 H, C_6 - CH_3), 0.89 (d, J = 6.6 Hz, 3 H, C_8 - CH_3), 0.88, 0.87(2s, 2×9 H, $\text{OSi}(\text{CH}_3)_3$), 0.089 [s, 3 H, $\text{OSi}(\text{CH}_3)_2$], 0.032, 0.028, 0.024 (3s, 3×3 H, $\text{OSi}(\text{CH}_3)_2$) ppm. ^{13}C NMR (CDCl_3 , 100.6 MHz): 217.6, 170.8, 165.6, 139.0, 136.1, 132.2, 123.2, 118.9, 114.4, 85.7, 78.0, 73.8, 63.8, 53.4, 45.2, 40.2, 39.1, 38.9, 34.4, 34.0, 30.5, 27.1, 26.3, 26.1, 25.7, 25.0, 23.4, 19.9, 18.6, 17.7, 15.5, -3.6 , -3.7 , -4.2 , -4.6 ppm. DCI-MS (120 eV, NH_3): 735 [$\text{M} + \text{NH}_4^+$], 718 [$\text{M} + \text{H}^+$]. HRMS (DCI): calcd. for $\text{C}_{39}\text{H}_{70}\text{N}_2\text{O}_5\text{Si}_2$ 735.4622, found 735.4675.

(4S,7R,8S,9S,16S)-4,8-Di-*tert*-butyldimethylsiloxy-5,5,7,9-tetramethyl-16-[2-(2-methyl-1,3-thiazol-4-yl)-1-ethynyl]-1-oxa-13-cyclohexadecen-2,6-dione, Mixture of the (13Z)- and (13E)-Isomers (14a and 15a): Bis(tricyclohexylphosphane)benzylideneruthenium dichloride (44 mg, 0.053 mmol) was added to a solution of diene **13** (190 mg, 0.264 mmol) in CH_2Cl_2 (66 mL) and the reaction mixture was stirred for 48 h at room temperature before being concentrated under vacuum. Flash column chromatography (silica gel; petroleum ether/ethyl acetate, 10:1) yielded **14a/15a** as yellow oil (95 mg, 52%).

(13Z)- and (13E)-(4S,7R,8S,9S,16S)-4,8-Dihydroxy-5,5,7,9-tetramethyl-16-[2-(2-methyl-1,3-thiazol-4-yl)-1-ethynyl]-1-oxa-13-cyclohexadecen-2,6-dione (14b and 15b): A solution of lactones **14a/15a** (95 mg, 0.137 mmol) in CH_2Cl_2 (12 mL) at -20°C was treated with trifluoroacetic acid (2 mL) and then stirred for 2 h at 0°C . After concentration under vacuum, the residue was diluted with ethyl acetate, washed with saturated NaHCO_3 solution, and then dried with MgSO_4 . Concentration under vacuum and separation of the residue by HPLC (hexane/*t*BuOMe/MeOH, 80:20:3) yielded hydroxy lactones (Z)-**14b** (27 mg, 43%) and (E)-**15b** (27 mg, 43%) as colourless foams.

14b: t_R (RP-18 HPLC; acetonitrile/water, 35:65): 5.6 min. $[\alpha]_D^{20}$ = -123 (c = 1 in CH_2Cl_2). ^1H NMR (CDCl_3 , 300 MHz): δ = 7.30 (s, 1 H, 19-H), 5.65 (dd, J = 9.1, 2.9 Hz, 1 H, 15-H), 5.55–5.41 (m, 2 H, 12-H, 13-H), 4.20 (dd, J = 10.8, 2.7 Hz, 1 H, 3-H), 3.67–3.65 (m, 1 H, 7-H), 3.12 (dq, J = 6.6, 2.0 Hz, 1 H, 6-H), 2.88–2.77 (m, 1 H, 14-H), 2.70 (s, 3 H, 21-H), 2.51 (dd, J = 15.0 Hz, 10.9 Hz, 1 H, 2-H), 2.27 (dd, J = 15.2, 2.8 Hz, 1 H, 2-H), 2.18–2.00 (m, 3 H, 11-H, 14-H), 1.71–1.58 (m, 3 H, 8-H, 9-H, 10-H), 1.32 (s, 3 H, 22-H), 1.30–1.19 (3 H, 8-H, 9-H, 10-H), 1.18 (d, J = 6.7 Hz, 3 H, 24-H), 1.07 (s, 3 H, 23-H), 0.98 (d, J = 6.9 Hz, 3 H, 25-H) ppm. ^{13}C NMR (CDCl_3 , 75.5 MHz): δ = 220.8, 170.0, 164.4, 134.2, 134.3, 123.8, 123.0, 86.1, 80.0, 74.4, 72.0, 64.1, 53.3, 41.7, 39.4, 38.7, 32.9, 32.4, 27.6, 27.5, 22.7, 19.2, 18.4, 15.5, 13.7 ppm.

15b: $[\alpha]_D^{20}$ = -129 (c = 1 in CH_2Cl_2). ^1H NMR (CDCl_3 , 300 MHz): δ = 7.30 (s, 1 H, 19-H), 5.71 (t, J = 5.0 Hz, 1 H, 15-H), 5.53–5.48 (m, 2 H, 12-H, 13-H), 4.16 (dd, J = 9.6, 3.2 Hz, 1 H, 3-H), 3.64 (t, J = 3.5 Hz, 1 H, 7-H), 3.21–3.15 (m, 1 H, 6-H), 2.68 (s, 3 H, 21-H), 2.59 (bt, J = 5.2 Hz, 2 H, 14-H), 2.53 (dd, J = 15.2, 9.8 Hz, 1 H, 2-H), 2.38 (dd, J = 15.0, 3.2 Hz, 1 H, 2-H), 2.23–2.17 (m, 1 H, 11-H), 2.00–1.92 (m, 1 H, 11-H), 1.69–1.56 (m, 2 H, 9-H, 10-H), 1.46–1.35 (1 H, 9-H), 1.28 (s, 3 H, 22-H), 1.27–1.21 (m, 2 H,

8-H, 10-H), 1.13 (d, $J = 6.9$ Hz, 3 H, 24-H), 1.04 (s, 3 H, 23-H), 0.94 (d, $J = 6.9$ Hz, 3 H, 25-H) ppm. ^{13}C NMR (CDCl_3 , 75.5 MHz): $\delta = 220.7, 170.2, 166.0, 135.7, 135.2, 124.7, 123.1, 85.6, 80.2, 75.6, 72.2, 63.8, 53.0, 42.6, 38.9, 37.9, 37.7, 32.3, 31.3, 26.8, 21.0, 20.2, 19.2, 15.8, 14.2$ ppm.

Epoxidation of 14b: A solution of dimethyl dioxirane (2 equiv.) in acetone was added dropwise to a solution of olefin (**Z**)-**14b** (27 mg, 0.058) in CH_2Cl_2 (4 mL) at -20°C . Stirring was continued for 2 h at -20°C . Concentration under vacuum, and separation of the residue by HPLC (hexane/*tert*-BuOMe/MeOH, 80:20:3) yielded α -epoxide **3b** (17 mg, 61%) and β -epoxide **16** (9 mg, 32.5%) as colourless foams.

16-Demethyl-16,17-didehydroepothilone A (3b): t_{R} (silica gel HPLC; hexane/methyl *tert*-butyl ether/methanol, 77:20:3): 13.3 min. $[\alpha]_{\text{D}}^{20} = -34$ ($c = 1$ in CH_2Cl_2). IR (KBr): $\tilde{\nu} = 3453, 2958, 2850, 1744, 1690, 1500, 1467, 1376, 1290, 1261, 1147, 979, 775\text{ cm}^{-1}$. ^1H NMR (CDCl_3 , 400 MHz): $\delta = 7.32$ (s, 1 H, 19-H), 5.86 (dd, $J = 7.38, 3.3$ Hz, 1 H, 15-H), 4.18 (br d, $J = 9.1$ Hz, 1 H, 3-H), 4.04 (br s, 1 H, OH), 3.76 (t, $J = 4.3$ Hz, 1 H, 7-H), 3.76–3.21 (m, 2 H, 6-H, 13-H), 2.93 (dt, $J = 6.0, 4.2$ Hz, 1 H, 12-H), 2.69 (s, 3 H, 21-H), 2.57 H (dd, $J = 14.2, 10.1$ Hz, 1 H, 2-H), 2.38 (dd, $J = 14, 3.2$ Hz, 1 H, 2-H), 2.21–2.19 (ddd, $J = 14.8, 5.7, 3.4$ Hz, 1 H, 14-H), 2.07 (ddd, $J = 15.0, 7.5, 6.55$ Hz, 1 H, 14-H), 1.75–1.41 (m, 7 H, 8-H, 9-H, 10-H), 1.35 (s, 3 H, 22-H), 1.15 (d, $J = 7.1$ Hz, 3 H, 24-H), 1.07 (s, 3 H, 23-H), 0.98 (d, $J = 6.6$ Hz, 3 H, 25-H) ppm. ^{13}C NMR (CDCl_3 , 100.6 MHz): $\delta = 220.6, 170.2, 166.1, 135.5, 123.3, 85.0, 80.6, 75.1, 73.6, 62.7, 57.2, 53.8, 52.7, 43.7, 38.7, 36.0, 32.7, 29.7, 26.6, 23.6, 21.1, 20.5, 19.2, 17.1, 14.5$ ppm. EI-MS (70 eV): m/z (%) = 477 (27) $[\text{M} + \text{H}]^+$, 421 (14), 389 (19), 378 (100), 364 (28), 346 (27), 328 (15). HRMS (EI): calcd. for $\text{C}_{25}\text{H}_{35}\text{NO}_6\text{S}$, 477.2185; found, 477.2174.

12,13-Bisepi-16-demethyl-16,17-didehydroepothilone A (16): t_{R} (silica gel HPLC; hexane/methyl *tert*-butyl ether/methanol, 77:20:3): 13.8 min. $[\alpha]_{\text{D}}^{20} = -44.9$ ($c = 1$ in CH_2Cl_2). ^1H NMR (CDCl_3 , 400 MHz): $\delta = 7.31$ (s, 1 H, 19-H), 5.95 (dd, $J = 7.1, 3.0$ Hz, 1 H, 15-H), 4.12 (dd, $J = 10.4, 2.8$ Hz, 1 H, 3-H), 3.86 (t, $J = 3.8$ Hz, 1 H, 7-H), 3.38–3.32 (m, 1 H, 13-H), 3.27–3.21 (m, 1 H, 6-H), 3.00 (ddd, $J = 6.7, 4.7, 4.7$ Hz, 1 H, 12-H), 2.69 (s, 3 H, 21-H), 2.52 (dd, $J = 14.0, 10.3$ Hz, 1 H, 2-H), 2.39 (dd, $J = 14.0, 2.8$ Hz, 1 H, 2-H), 2.21 (ddd, $J = 15.1, 7.25, 7.25$ Hz, 1 H, 14-H), 2.13 (ddd, $J = 15.2, 6.1, 3.1$ Hz, 1 H, 14-H), 1.73–1.35 (m, 7 H, 8-H, 9-H, 10-H), 1.34 (s, 3 H, 22-H), 1.13 (d, $J = 7.1$ Hz, 3 H, 24-H), 1.06 (s, 3 H, 23-H), 0.95 (d, $J = 6.6$ Hz, 3 H, 25-H) ppm. ^{13}C NMR (CDCl_3 , 75.5 MHz): $\delta = 221.4, 170.0, 166.1, 135.7, 123.3, 85.1, 80.5, 73.2, 73.1, 62.2, 57.1, 55.3, 52.3, 42.9, 39.0, 37.5, 32.4, 31.8, 27.6, 27.0, 23.5, 20.6, 20.4, 16.4, 13.5$ ppm.

(16Z)-Epothilone A₈ (17): a) A solution of alkyne **3b** (5.0 mg) in methanol (1 mL) was stirred for 2 h with Lindlar catalyst (2 mg) under an atmosphere of H_2 . The catalyst was removed by centrifugation and the product (2.5 mg, 50%) was obtained by preparative HPLC (RP-18; acetonitrile/water, 40:60).

b) Epothilone A₈ (**18**, 20 mg, 42 μmol) and benzophenone (20 mg) were dissolved in benzene (2 mL) and irradiated with stirring from a distance of 4 cm with a 125 W high pressure mercury lamp at 0°C . After 10 min of irradiation, an equilibrium mixture of *E/Z* isomers **17** (44%) and **18** (56%) was obtained, which were recovered by preparative HPLC: **17** (7.0 mg, 35%), **18** (8.1 mg, 40%).

17: Colourless glass; t_{R} (RP-18 HPLC; acetonitrile/water, 40:60): 6.3 min. $[\alpha]_{\text{D}}^{25} = +24.9$ ($c = 2.6$ in CH_2Cl_2). UV (MeOH) λ_{max} (ϵ) = 210 (15900), 248 (15300) nm. ^1H NMR (CDCl_3 , 300 MHz):

$\delta = 7.03$ (s, 19-H), 6.61 (tdd, $J = 7.9, 2.7, 1.4$ Hz, 15-H), 6.37 (dd, $J = 14.8, 1.4$ Hz, 17-H), 5.67 (dd, $J = 14.8, 7.6$ Hz, 16-H), 4.08 (br d, $J = 9.5$ Hz, 3-H), 3.78 (br t, $J = 3.9$ Hz, 7-H), 3.24 (dq, $J = 5.6, 6.9$ Hz, 6-H), 3.19 (ddd, $J = 7.8, 5.2, 4.2$ Hz, 13-H), 2.91 (td, $J = 4.1, 6.9$ Hz, 12-H), 2.69 (s, 21-H₃), 2.49 (dd, $J = 15.4, 9.3$ Hz, 2-Ha), 2.43 (dd, $J = 15.4, 3.8$ Hz, 2-Hb), 2.21 (ddd, $J = 14.9, 5.3, 2.8$ Hz, 14a-H), 2.93 (td, $J = 7.9, 14.9$ Hz, 14b-H), 1.75–1.30 (m, 7 H), 1.35 (s, 22-H₃), 1.17 (d, $J = 6.9$ Hz, 24-H₃), 1.09 (s, 23-H₃), 1.00 (d, $J = 6.9$ Hz, 25-H₃) ppm. ^{13}C NMR (CDCl_3 , 75 MHz): $\delta = 220.2$ (s, C-5), 170.9 (s, C-1), 165.8 (s, C-20), 152.0 (s, C-18), 130.3 (s, C-16), 122.9 (d, C-17), 118.4 (d, C-19), 75.3 (d, C-15), 73.6 (d, C-7), 71.8 (d, C-3), 57.5 (d, C-12), 54.8 (d, C-13), 52.4 (s, C-4), 44.1 (d, C-6), 39.0 (t, C-2), 36.5 (d, C-8), 32.8 (t, C-14), 30.6 (t, C-9), 27.1 (t, C-11), 22.4 (t, C-10), 21.5 (q, C-23), 21.3 (q, C-22), 19.5 (q, C-21), 17.3 (q, C-25), 14.8 (q, C-24) ppm.

(16Z)-12,13-Epiepothilone A₈ (19): Alkyne **16** (3 mg, 6.3 μmol) was hydrogenated with Lindlar catalyst as described above, and **19** was isolated by RP-18 HPLC (acetonitrile/water, 4:6; $t_{\text{R}} = 6.9$ min); colourless amorphous solid. $[\alpha]_{\text{D}}^{25} = +35$ ($c = 0.6$ in CHCl_3). UV (MeOH) λ_{max} (ϵ) = 211 (17800), 249 (12500) nm. ^1H NMR (CDCl_3 , 300 MHz): $\delta = 7.04$ (s, 19-H), 6.78 (tt, $J = 7.9, 1.5$ Hz, 15-H), 6.34 (dd, $J = 11.7, 1.6$ Hz, 17-H), 5.75 (dd, $J = 11.7, 7.5$ Hz, 16-H), 3.99 (m, 3-H), 3.85 (br d, $J = 4.2$ Hz, 7-H), 3.41 (dt, $J = 9.4, 4.2$ Hz, 13-H), 3.34 (dq, $J = 2.7, 7.1$ Hz, 6-H), 2.97 (ddd, $J = 9.5, 4.2, 3.4$ Hz, 12-H), 2.69 (s, 21-H₃), 2.45 (dd, $J = 13.0, 10.3$ Hz, 2-Ha), 2.38 (dd, $J = 13.0, 2.9$ Hz, 2-Hb), 2.15 (ddd, $J = 14.9, 4.3, 1.7$ Hz, 14-Ha), 1.93 (dt, $J = 14.9, 8.8$ Hz, 14-Hb), 1.90–1.20 (m, 7 H), 1.35 (s, 22-H₃), 1.10 (d, $J = 6.9$ Hz, 24-H₃), 1.03 (s, 23-H), 0.94 (d, $J = 6.9$ Hz, 25-H₃) ppm.

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