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Original article

Epothilone D and its 9-Methyl analogues: Combinatorial syntheses, conformation, and biological activities

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ABSTRACT

Epothilone D (Epo D) and its 9-Methyl conformational analogues were synthesized through a highly efficient combinatorial approach. The fragment E was synthesized in 11 total steps with 6 longest linear steps, and each aldehyde B was prepared via a 3-step sequence. Starting from the common precursor E and a suitable aldehydes B, each target molecule were obtained in only 4 steps. The 9-(S)-epo D and 9-(R)-epo D demonstrated significant difference in inhibition activities against cancer cell lines and in conformational analysis.

1. Introduction

Compared to palitaxel, epothilones share similar mechanism of tubulin depolymerization, while show enhanced inhibition activities against various human cancer cells, especially against multi-drug-resistant cancer cell lines. The promising bioactivities of epothilones have attracted vast research on syntheses, conformational analysis and SAR (structure–activity relationships) studies of epothilones [1–6]. However, the bioactive conformation of the flexible C8–C11 region of epothilones remains to be fully recognized. For example, the 9-oxy epo D (Fig. 1) is thirty times less active than Epo D [4,5], while the synthetic 9,10-dehydroepothilone D (Fig. 1) prepared by Danishefsky et al. is more potent than Epo D [6], and hence entered clinical trial as anti-cancer drug candidate. Taylor applied the strategy of conformationally restricted analogues to explore the bioactive conformation in region of C10–C14, which not only provided insights into the biologically active conformation, but also resulted in the discovery of a few highly active rational designed analogues including 14-(R)-Me epo D [7] and 14-(S)-MeO epo D [8]. Herein we report the total syntheses of Epo D and its two conformationally restricted 9-Me analogues, and the analysis their conformational changes and biological activities.

2. Results and discussion

2.1. Chemistry

According to the retrosynthetic analysis (Scheme 1), all the three target molecules can be disconnected into three fragments: fragment A, B and D. And two approaches to synthesize these three compounds were proposed. Fragment C (R = H) [14] in approach 1, a known compound, was used to test our new synthetic strategy in establishing the C6, C7 and C8 chiral centers.

We started the synthesis with compound **1a** (Scheme 2) [9]. The 1,4 conjugate addition of Grignard reagent to compound **1a** provided compound **2a** with good stereoselectivity (dr >10:1) [10]. Aldehyde **3a** was obtained by reduction with DIBAL-H [11]. In this step, aldehyde **3a** is not only highly volatile but also prone to epimerize. Fortunately, through aqueous quenching of the reaction, followed by simple separation of organic phase and precipitation of the sultam with hexane, the aldehyde **3a** was obtained in a hexane

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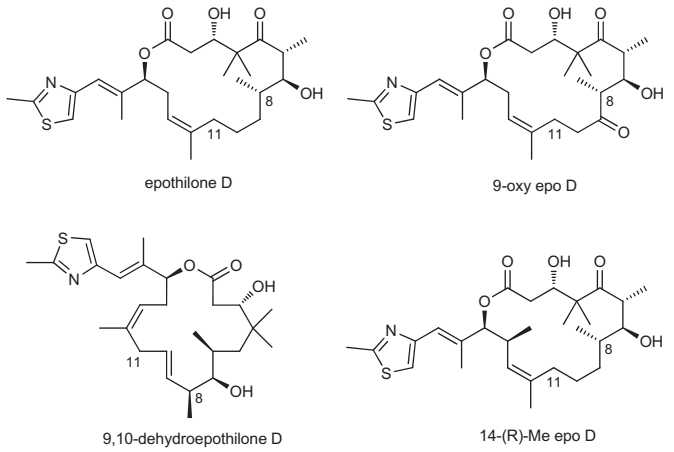


Fig. 1. Epo D, 9-oxy epo D, 9,10-dehydroepothilone D and Taylor's C14-Me epo D.

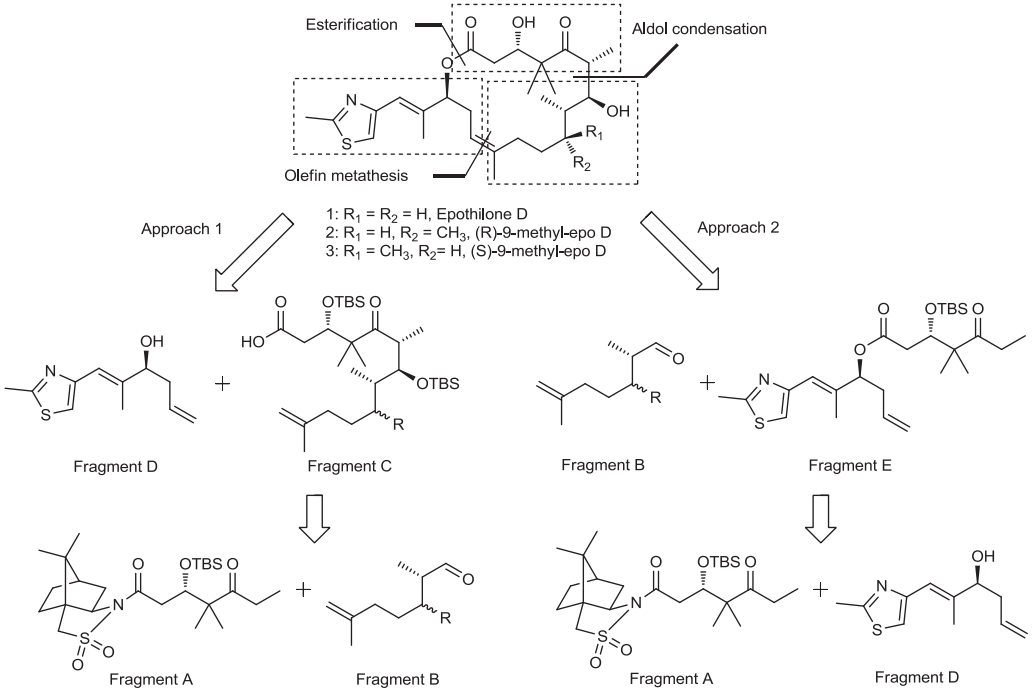
solution, and was used directly in the next aldol reaction. Then, the aldol reaction between **3a** and the Ti enolate of known ketone fragment **4** [12] resulted in the formation of **5** in high yield (81%) and 15:1 diastereoselectivity. Compound **5** was converted to known compound **7** through TBS protection and hydrolysis to remove the sultam auxiliary [13]. The structural analysis confirmed that carboxylic acid **7** is identical in all aspect to the same molecule in literature [14]. The synthesis of Epo D from compound **7** was also reported in the literature [14,15]. This sequence showed that the 1,4 conjugate addition, reduction of sultam derivatives with DIBAL-H to obtain aldehyde in hexane, and consequently aldol reaction with Ti enolate is a feasible and efficient strategy to establish the correct C6, C7 and C8 chiral centers, even with the volatile and unstable chiral aldehyde. With this strategy, the combinatorial strategy (approach 2) was applied to prepare Epo D and its 9-Me analogues.

The keto acid **8** was prepared from sultam **4** via hydrolysis with $LiOH/H_2O_2$ with 72.5% yield (Scheme 3) [16]. Coupling of fragments **8** and **9** with EDCI led to ester **10** in 85.5% yield [14,17], which is the common precursor for the synthesis of Epo D and its 9-Me analogues. Then, aldol reaction with Ti enolate of **10** and aldehyde **3a** in hexane resulted in the formation of compound **11** with 74% yield and over 15:1 diastereoselectivity [18], and the next TBS protection provided known compound **12**, which was converted to Epo D according to the methods in literature: RCM reaction and deprotection of TBS groups [14,17,19], and 33.7% yield was obtained when Zhan catalyst-1B was applied in the RCM step [20].

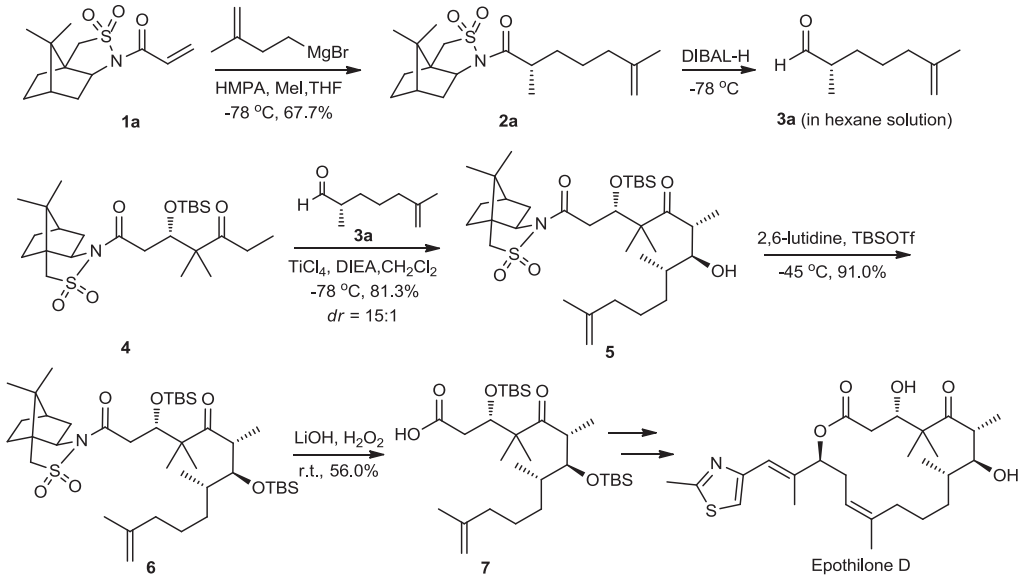
With the successful synthesis of Epo D, similar strategy was applied to prepare 9-Me analogues, while with different C7–C12 aldehydes. The various conditions to supply conjugation adducts **2b**/**2c** were listed in Table 1.

The conjugate addition of Grignard reagent followed by direct methylation of the generated enolate provided sultam **2c** in 75% (entry 1, Table 1) [21], and the conjugate addition of the same Grignard reagent to alpha substituted amide [13b,21], followed by protonation also provided compound **2c** as the main product (entry 2, Table 1). The replacement of Grignard reagent with copper lithium reagent failed to provide any product (entry 3, Table 1) [22]. The addition of methyl magnesium bromide to conjugate amide provided methyl ketone as the main product (entry 4, Table 1); however, with catalytic amount of cuprous iodide, compound **2c** was obtained in 62% yield (entry 5, Table 1). Fortunately, with catalytic amount of cuprous chloride, the conjugate addition followed by direct methylation provided sultam **2b** and **2c** in 70% yield and 1:1 ratio (entry 6, Table 1) [22,23]. Both compound **2b** and **2c** were analyzed with X-ray crystallographic techniques to confirm their absolute stereochemistry (Fig. 2).

Aldehydes **3b** and **3c** were then obtained by reduction with DIBAL-H and precipitation of the by-product sultam (Scheme 4). Aldehyde **3b** in hexane was used directly in the next aldol reaction, and the aldol adduct **14** was isolated in 75% yield and over 20:1 diastereoselectivity. The TES protection of **14** supplied compound



Scheme 1. Retrosynthetic analysis of Epo D and its C9-modified analogues.



Scheme 2. Synthesis of Epo D through approach **1**.

15, and the following RCM reaction with Zhan catalyst-1B provided **16** and **17** as Z/E isomer in about 1:1.7 ratio (Scheme 5). Deprotection of two protection groups with TFA/CH_2Cl_2 provided final products **18** and **19** respectively.

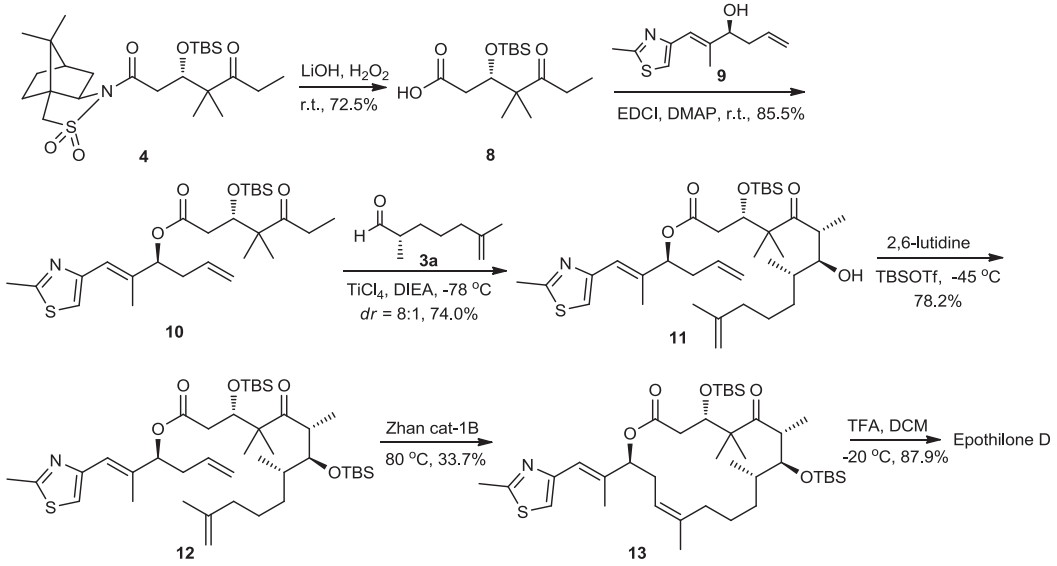
The 9-(S)-Me epo D **24** along with the E isomer **25** were prepared using the similar sequence to prepare compound **18** (Scheme 6), and the synthetic sequence starting from **10** and **3c** was also only 4 steps.

2.2. Biological activity

With Epo D analogues **18**, **19**, **24**, **25** in hand, we conducted a bioassay against sensitive breast cancer cell line MCF and ADR resistant breast cancer cell line MCF-7/ADR (Table 2). Although all analogues were less potent than the mother molecule Epo D, the 9-(R)-Me epo D **18** demonstrated significant higher activity (about 35 times) than the 9-(S)-Me epo D **24**. In addition, the *Z* isomers of 9-Me epo D (compounds **18** and **24**) were more potent than their corresponding *E* isomers (compounds **19** and **25**). Furthermore, we evaluated tubulin polymerization of Epo D, compounds 9-(R)-Me epo D **18** and 9-(S)-Me epo D **24** for their tubulin polymerization inhibitory activity using literature method [24], tubulin polymerization for Epo D, compounds **18** and **24** at 5 μM were 43.3%, 15.7% and 8.5% respectively. This result is consistent to their inhibitory activities against cancer cells growth.

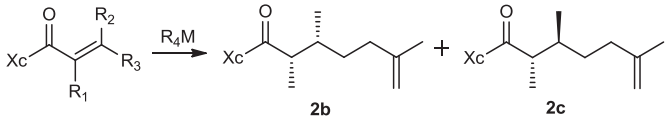
2.3. Computational analysis

The conformations of epothilones were investigated with electron-crystallography [25], NMR [3,26–34] and computational analysis [33–39]. The optimized conformations of Epo D, **18**, and **24** were subjected to geometry optimization with DFT at the B3LYP/6-31G (d) level using Gaussian 03 [40]. Fig. 3 shows the superposition of conformations of Epo D and **18**, Epo D and **24**. From Fig. 3, the



Scheme 3. Synthesis of Epo D through approach **2**.

Table 1
Synthesis of the C7–C12 segment.



Entry	Xc	R_1	R_2	R_3	R_4M	**2b**/**2c**[g]	% yield[h]
1[a]	L-camphorsultam	H	H	Me	CH_2=C(CH_3)CH_2CH_2MgBr/HMPA/MeI	**2c**	75
2[b]	L-camphorsultam	Me	H	Me	CH_2=C(CH_3)CH_2CH_2MgBr	**2c**	67
3[c]	D-camphorsultam	Me	H	Me	[CH_2=C(CH_3)CH_2CH_2]$_2$CuLi	NR[i]	–
4[d]	L-camphorsultam	H	H	CH_2=C(CH_3)CH_2CH_2	CH_3MgBr/HPMA/MeI	Methyl ketone	–
5[e]	L-camphorsultam	H	H	CH_2=C(CH_3)CH_2CH_2	CuI/CH_3MgBr/HPMA/MeI	**2c**	62
6[f]	L-camphorsultam	H	H	Me	CH_2=C(CH_3)CH_2CH_2MgBr/CuCl/HMPA/MeI	**2b**/**2c** (1:1)	70[j]

[a] THF, −78 °C to r.t., 7 h.
[b] THF, −78 °C, 3 h.
[c] THF, −40 °C, 24 h.
[d] THF, −78 °C to −40 °C, 3 h.
[e] THF, −78 °C to r.t., 10 h.
[f] THF, −78 °C to r.t., 10 h.
[g] The major product after purification.
[h] Yield of the major diastereomer after purification.
[i] No reaction.
[j] The total yield of the **2b** and **2c**.

ring of **18** matches better with Epo D than **24**. The RMSD between the ring atoms of Epo D and **18** is 0.09 Å, whereas the RMSD between the ring atoms of Epo D and **24** is 0.14 Å. The (S)-methyl modification changes the shape of the ring of Epo D more than (R)-methyl. The best docking poses of Epo D, **18** and **24** to the structure of α, β-tubulin (PDB ID: 1TVK) using AutoDock v.4.2 (http://autodock.scripps.edu) were further used to calculate the binding free energy using MM-PBSA from AMBER 11 [41]. The binding energy of **18** and **24** to the structure of α, β-tubulin are 5.50 kcal/mol and 8.14 kcal/mol higher than Epo D respectively. This information is helpful to explain that the biological activities of compounds Epo D, **18**, **24** against MCF-7 cancer cell lines are in the descending order (IC_{50} of Epo D, **18**, **24** against MCF-7 is 0.014, 0.99, 35.4 μM respectively). Our results indicate that the C9 site is probably not well tolerated to modification to maintain the bioactivities.

3. Conclusion

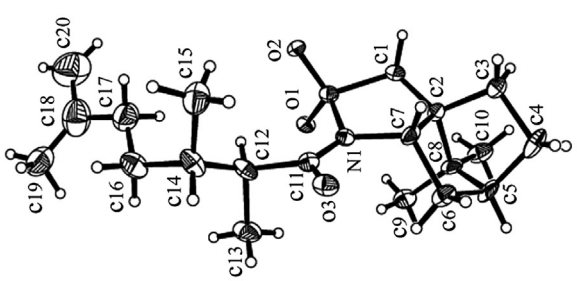
In summary, we completed the total synthesis of epothilone D and its 9-Me analogues via a combinatorial strategy. This strategy involved the common precursor E and varies of aldehydes (compounds **3a**, **3b** and **3c**). The synthesis of each aldehyde was only 3 steps, and the syntheses shared the one-pot strategy of conjugate addition followed by alkylation. The next reduction with DIBAL-H provides the volatile and unstable aldehydes in hexane, which were used directly in the next aldol reactions. The linear steps and total steps for the syntheses of common precursor E were 6 steps and 11 steps respectively, and additional 4 linear steps may supply epothilone D or one of its 9-Me analogues. Therefore, this combinatorial approach is highly efficient for the preparation of epothilone analogues with modification at C9 position.

On the other hand, the biological activities of 9-Me analogues indicated that conformation adopted by **18** (Fig. 3, purple) is probably closer to the bioactive confirmation than the conformation adopted by **24** (Fig. 3, yellow), but modification at C9 position still reduces the biological activity; this phenomena may be explained by the computer modeling.

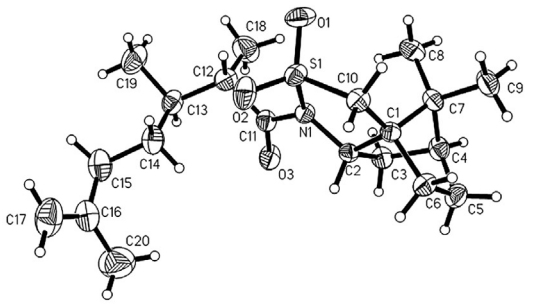
4. Experimental section

4.1. Chemistry

All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. The used solvents were purified and dried according to common procedures. Other chemicals and solvents were commercially available. The phosphate buffer solution (pH 7.2, 0.1 M) was prepared by dissolving disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$, 25.79 g) and sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$, 4.37 g) in distilled, deionized water (1000 mL).

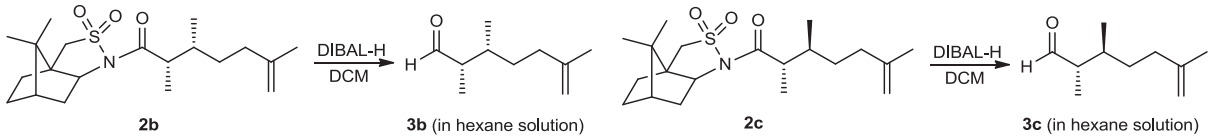


X-ray of sultam-2c



X-ray of sultam-2b

Fig. 2. X-ray of sultam **2c** and sultam **2b**.



Scheme 4. Preparation of fragment B.

High-resolution mass spectra (HRMS) were obtained with an FTICR-MS (Ion spec 7.0T) spectrometer. 1H NMR spectra were obtained by using a Bruker AC-P 300, AV 400, AV 600. Chemical shifts are reported in parts per million (ppm) relative to either a tetramethylsilane internal standard or solvent signals. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants and integration. ^{13}C NMR spectra were recorded using a Bruker AC-P 300 (75 MHz) or AV 400 spectrometer (100 MHz) using $CDCl_3$ or CH_3CD as the solvent. Chemical shifts (δ) are reported in parts per million measured relative to the solvent peak. IR spectra were recorded with a Bio-Rad FTS 6000 Fourier infrared spectrometer.

4.2. Synthesis of compounds

4.2.1. Synthesis of compound ***2a***

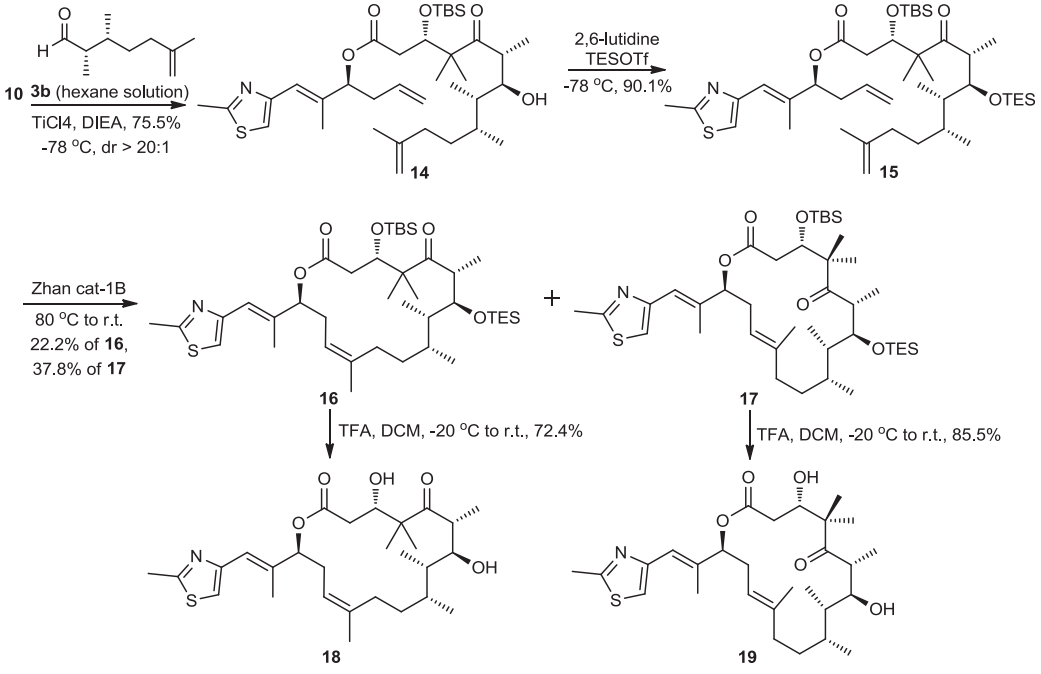
To a solution of compound **1** (1.98 g, 7.0 mmol) in THF (30 mL) was added Grignard reagents (18.9 mmol) under argon atmosphere at −78 °C, and the resulting solution was stirred at same temperature for 2 h. Then HMPA (7.4 mL, 49.0 mmol) and CH_3I (4.6 mL, 70.0 mmol) was added at −78 °C and the reaction mixture was warmed up slowly to 25 °C in 5 h. The reaction was quenched by addition of sat. NH_4Cl (15 mL), and was stirred for 10 min. After the solvents were removed under reduced pressure, the residue was diluted with water (20 mL) and EtOAc (50 mL), and the aqueous phase was extracted with EtOAc (2 × 30 mL) again. Then the combined organic phase was washed with H_2O (10 mL) and brine (10 mL), dried over $MgSO_4$. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (5% EtOAc in hexane) to obtain compound **2a** as a white solid (1.63 g, 67.7%). $R_f = 0.75$ (hexane/EtOAc = 4:1); ^1H NMR (400 MHz, $CDCl_3$) δ 4.66 (m, 2H), 3.89 (t, J = 8.4 Hz, 1H), 3.46 (m, 2H), 3.06 (m, 1H), 1.97–2.05 (m, 4H), 1.74–1.91 (m, 4H), 1.68 (s, 3H), 1.34–1.45 (m, 5H), 1.19 (m, 3H), 1.15 (s, 3H), 0.96 (s, 3H).

4.2.2. Synthesis of compound ***3a***

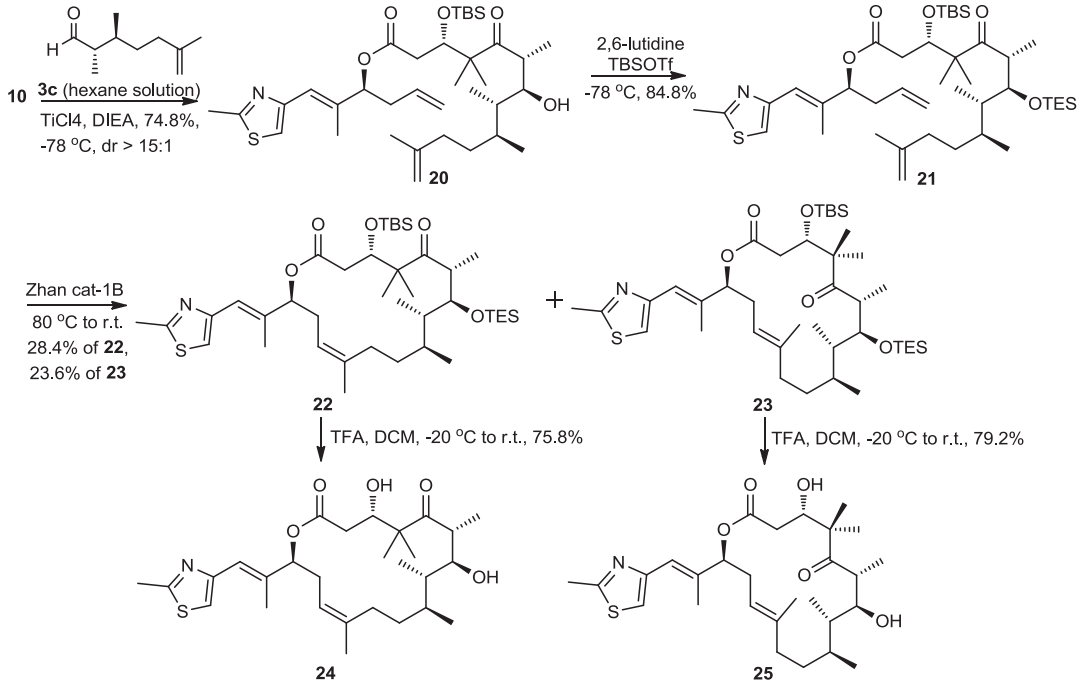
To a solution of compound **2a** (1.06 g, 3.0 mmol) in CH_2Cl_2 (6 mL) was added DIBAL-H (4.8 mL, 4.8 mmol, 1 M in CH_2Cl_2) under argon atmosphere at −78 °C. After stirred at −78 °C for 4 h, the reaction was quenched by addition of $NaHSO_4$ (1.15 g) in water (20 mL), and was diluted with hexane (4 mL). The organic phase was removed by needle to a 25 mL flask which contained 10 g of 4 Å molecular sieves, and the aqueous phase was extracted with hexane (2 × 3 mL). The organic solution was concentrated under reduced pressure carefully until a white crystal appeared. The clear hexane layer (about 10 mL) was store in −20 °C overnight, and the upper clear solution was used for the next step directly.

4.2.3. Synthesis of compound ***5***

To a solution of compound **4** (775 mg, 1.55 mmol) in CH_2Cl_2 (5 mL) was added $TiCl_4$ (1.7 mL, 1.7 mmol, 1 M in DCM) under argon atmosphere at −78 °C, followed by addition of DIEA (0.29 mL, 1.7 mmol) to obtain a dark red solution. The reaction mixture was stirred at −78 °C for 1 h. Then compound **3a** in hexane (obtained from above reaction) was added, and the resulting solution was warmed up slowly to −12 °C in 10 h. The reaction was quenched by addition of aqueous of phosphate solution (8 mL, pH = 7.2).



Scheme 5. Preparation of **18** from **10** and **3b**.



Scheme 6. Preparation of **24** from **10** and **3c**.

The resulting solution was stirred for 10 min and was diluted with Et_2O (20 mL). The aqueous phase was extracted with Et_2O (2 × 10 mL) again, and the combined organic phase was dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The crude product was purified by column chromatography (5% EtOAc in hexane) to obtain alcohol **5** as a colorless oil (404 mg, 81.3%). R_f = 0.50 (hexane/EtOAc = 4:1); $[\alpha]_D^{20}$ = −75.0 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3507, 3073, 2957, 2953, 2886, 2857.6, 1691, 1650, 1461, 1388, 1331, 1168, 1078, 991, 843, 775; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 1H), 4.64 (s, 1H), 4.56 (t, J = 4.0 Hz, 1H), 3.85 (m, 1H), 3.47 (m, 1H), 3.43 (m, 2H), 3.27 (d, J = 9.2 Hz, 1H), 3.20 (m, 1H), 2.93 (dd, J = 17.6, 5.2 Hz, 1H), 2.60 (dd, J = 17.6, 3.6 Hz, 1H), 2.19–2.23 (m, 1H), 1.96–2.10 (m, 3H), 1.85–1.90 (m, 3H), 1.69 (s, 3H), 1.24–1.54 (m, 6H), 1.19 (s, 3H), 1.15 (s, 3H), 1.14 (s, 3H), 1.01 (d, J = 6.8 Hz, 3H), 0.94 (s, 3H), 0.86 (s, 9H), 0.82 (d, J = 6.8 Hz, 4H), 0.10 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 146.4, 109.8, 75.1, 72.1, 65.4, 54.6, 53.2, 48.6, 47.9, 44.8, 41.8, 41.0, 38.5, 38.4, 35.5, 33.0, 32.7, 26.6, 26.1, 24.9, 22.7, 22.6, 21.0, 20.1, 18.6, 18.2, 15.6, 9.8, −4.1, −4.9; HRMS (ESI) (M + H$^+$) calculated for $C_{34}H_{61}NO_6SSi$: 640.4062, found 640.4063.

4.2.4. Synthesis of compound 6

To a solution of alcohol **5** (1.32 g, 2.06 mmol) in CH_2Cl_2 (15 mL) was added 2,6-lutidine (0.72 mL, 6.20 mmol) at −45 °C, followed by addition of TBSOTf (0.95 mL, 4.13 mmol). After stirred at −45 °C for 6 h, the reaction mixture was warmed to 20 °C. Then the reaction was quenched by addition of H_2O (20 mL), and was diluted with CH_2Cl_2 (40 mL) and H_2O (20 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic phase was dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The resulting residue was purified by column chromatography (3% EtOAc in hexane) to obtain compound **6** as a colorless oil (1.41 g, 91.0%). R_f = 0.70 (hexane/EtOAc = 4:1); $[\alpha]_D^{20}$ = −51.0 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3384, 3374, 2955, 2931, 2886, 2857, 1695, 1464, 1388, 1332, 1252, 1136, 1082, 987, 939, 833, 773; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 1H), 4.65 (s, 1H), 4.57 (t, J = 4.4 Hz), 3.86 (m, 1H), 3.73 (m, 1H), 3.42 (m, 2H), 3.10 (m, 1H), 2.90 (dd, J = 17.6, 4.8 Hz, 1H), 2.60 (dd, J = 17.2, 3.6 Hz, 1H), 2.25–2.29 (m, 1H), 1.95–2.12 (m, 3H), 1.88–1.90 (m, 3H), 1.69 (s, 3H), 1.26–1.58 (m, 7H), 1.22 (s, 3H), 1.16 (s, 3H), 1.08 (s, 3H), 1.03 (d, J = 6.8 Hz, 3H), 0.96 (s, 3H), 0.89 (s, 9H), 0.87 (s, 9H), 0.85 (m, 4H), 0.11 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.1, 170.2, 146.0, 110.0, 78.3, 72.2, 65.3, 54.3, 53.1, 48.5, 47.9, 45.3, 44.9, 42.2, 38.5, 38.4, 33.0, 30.2, 26.6, 26.4, 26.1, 25.7, 23.4, 22.5, 21.0, 20.1, 18.7, 18.3, 18.2, 16.2, −3.4, −3.5, −4.1, −4.8; HRMS (ESI) (M + H$^+$) calculated for $C_{40}H_{75}NO_6SSi_2$: 754.4926, found 754.4935.

Table 2
Cytotoxicity and tubulin polymerization inhibitory activity of Epo D and its new analogues.

Compound	MCF-7 (IC_{50}, μM)[a]	MCF-7/ADR (IC_{50}, μM)[a]	% tubulin polymerization at 5 μM
Epo D	0.014	0.010	43.3
18	0.99	0.51	15.7
19	7.7	7.3	ND[b]
24	35.4	ND[b]	8.5
25	93.4	>100	ND[b]

[a] Cells were exposed to compounds for 72 h.
[b] Not determined.

4.2.5. Synthesis of compound 7

To a solution of compound **6** (613 mg, 0.81 mmol) in THF (35 mL) and H_2O (8.7 mL) was added LiOH·H_2O (509 mg, 12.1 mmol) and H_2O_2 (10.8 mL, 30%, 131.2 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 30 min, and was stirred at 25 °C for 24 h. The reaction mixture was quenched by addition of saturated $NaHSO_4$ (50 mL) at 0 °C, and was stirred for 10 min. The solvent was removed under reduced pressure, and $NaHSO_4$ solid was added to adjust the pH = 4–5. The resulting solution was extracted with EtOAc (3 × 30 mL), and the combined organic phase was dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The resulting residue was purified by column chromatography (0.5% AcOH and 6% EtOAc in hexane) to obtain acid

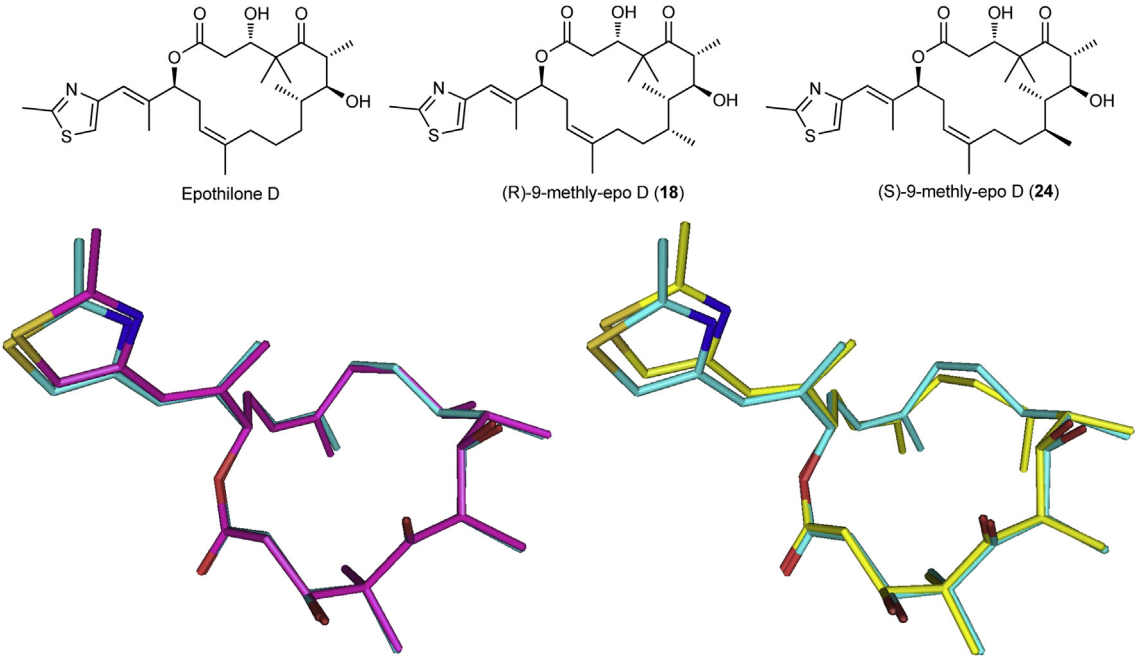


Fig. 3. Left: Superposition of geometry optimized conformations of Epo D (cyan) and **18** (purple). Right: Superposition of geometry optimized conformations of Epo D (cyan) and **24** (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

7 as a colorless oil (252 g, 56.0%). $R_f = 0.50$ (1% AcOH and 20% EtOAc in hexane); IR (KBr/cm^{-1}): 3074, 2954, 2930, 2857, 1711, 1650, 1469, 1253, 1086, 987, 941, 876, 832, 773; ^{1}H NMR (100 MHz, $CDCl_3$) δ 4.69 (s, 1H), 4.66 (s, 1H), 4.39 (dd, $J = 6.8$, 3.2 Hz, 1H), 3.78 (d, $J = 6.4$ Hz, 1H), 3.14 (dq, $J = 7.2$, 7.2 Hz, 1H), 2.49 (dd, $J = 16.4$, 3.2 Hz, 1H), 2.30 (dd, $J = 16.4$, 6.4 Hz, 1H), 2.04–1.96 (m, 2H), 1.70 (s, 3H), 1.63–1.36 (m, 5H), 1.24 (s, 3H), 1.16 (s, 3H), 1.09 (s, 3H), 1.05 (d, $J = 6.8$ Hz, 3H), 0.92 (d, $J = 7.2$ Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.10 (s, 3H), 0.06 (3s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 218.5, 177.6, 146.2, 110.0, 77.9, 73.6, 53.7, 45.4, 40.3, 38.8, 38.5, 31.2, 30.5, 29.9, 26.4, 26.2, 25.8, 25.7, 23.8, 22.6, 19.1, 18.7, 18.4, 18.0, 16.0, −3.4, −3.5, −4.1, −4.4.

*4.2.6. Synthesis of compound **8***

To a solution of compound **4** (7.5 g, 15.0 mmol) in THF (620 mL) and H_2O (156 mL) was added $LiOH{\cdot}H_2O$ (9.45 g, 22.5 mmol) and H_2O_2 (200 mL, 30%) at 0 °C. The reaction mixture was stirred at the same temperature for 30 min, and was stirred at 25 °C for 24 h. Then the reaction was quenched by addition of $NaHSO_3$ (66.8 mg) in water (800 mL) at 0 °C, and was stirred for 10 min. The solution was concentrated under reduced pressure, and $NaHSO_4$ was added to adjust the pH = 4–5. The solution was extracted with EtOAc (3 × 100 mL), and the organic phase was dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The resulting residue was purified by column chromatography (0.5% AcOH and 7% EtOAc in hexane) to obtain acid **8** as a colorless oil (3.29 g, 72.5%). $R_f = 0.30$ (1% AcOH and 20% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.47 (m, 1H), 2.51 (m, 3H), 2.31 (dd, $J = 16.4$, 6.8 Hz, 1H), 1.13 (s, 3H), 1.07 (s, 3H), 0.99 (t, $J = 7.2$ Hz, 3H), 0.83 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.4, 178.3, 73.7, 52.8, 39.5, 32.0, 26.1, 21.2, 20.8, 18.3, 7.9, −4.2, −4.7.

*4.2.7. Synthesis of compound **10***

To a solution of compound **8** (1.1 g, 3.62 mmol) and alcohol **9** (1.51 g, 7.22 mmol) in CH_2Cl_2 (20 mL) was added DMAP (0.885 g, 7.22 mmol) and EDCI (2.77 g, 14.44 mmol) at room temperature, and the reaction mixture was stirred at 20 °C for 2 h. Then the reaction was diluted with water (60 mL), and was extracted with Et_2O (2 × 120 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was concentrated under reduced pressure. The crude product was purified by column chromatograph (10% EtOAc in hexane) to obtain ester **10** as a colorless oil (1.60 g, 85.5%). $R_f = 0.65$ (0.5% AcOH and 7% EtOAc in hexane); $[\alpha]_D^{20} = -28.2$ (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 2954, 2930, 2856, 1737, 1705, 1643, 1505, 1471, 1374, 1293, 1248, 1177, 1088, 1047, 825, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.48 (s, 1H), 5.71 (m, 1H), 5.28 (t, $J = 6.8$ Hz, 1H), 5.11–5.03 (m, 2H), 4.46 (dd, $J = 6.4$, 4.0 Hz, 1H), 2.69 (s, 3H), 2.60–2.42 (band, 5H), 2.31 (dd, $J = 16.8$, 6.4 Hz, 1H), 2.06 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H), 0.97 (t, $J = 7.2$ Hz, 3H), 0.84 (s, 9H), 0.06 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.2, 171.3, 164.8, 152.6, 136.9, 133.5, 121.4, 118.1, 116.6, 79.0, 73.7, 52.8, 39.7, 37.7, 31.9, 26.1, 21.1, 20.9, 19.4, 18.3, 14.8, 17.9, −4.0, −4.8; HRMS (ESI) (M + H^+) calculated for $C_{26}H_{43}NO_4SSi$: 494.2755, found 494.2759.

*4.2.8. Synthesis of compound **11***

To a solution of ester **10** (253 mg, 0.51 mmol) in CH_2Cl_2 (2 mL) was added $TiCl_4$ (0.56 mL, 1 M in CH_2Cl_2) at −78 °C, followed by addition of DIEA (0.09 mL, 0.56 mmol) to obtain a dark red solution. After stirred at −78 °C for 1 h, compound **3a** (1.5 mmol) was added to the reaction solution. The reaction mixture was warmed up slowly to −12 °C, and was stirred for 15 h. Then the reaction was quenched by addition of aqueous of phosphate solution (3 mL, pH = 7.2), and was stirred for 10 min. The resulting mixture was diluted with Et_2O (20 mL), the aqueous layer was extracted with Et_2O (20 mL) again. The combined organic phase was dried over $MgSO_4$, and the solvent was concentrated under reduced pressure. The crude product was purified by column chromatograph (5% EtOAc in hexane) to obtain alcohol **11** as a colorless oil (239 mg, 74.0%). $R_f = 0.35$ (hexane/EtOAc = 9:1); $[\alpha]_D^{20} = -51.4$ (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 2929, 2856, 1735, 1684, 1506, 1471, 1376, 1292, 1252, 1178, 1079, 976, 283, 832, 776; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.48 (s, 1H), 5.70 (m, 1H), 5.28 (t, $J = 6.8$ Hz, 1H), 5.07 (d, $J = 17.2$ Hz, 1H), 5.04 (d, $J = 10.0$ Hz, 1H), 4.66 (s, 1H), 4.64 (s, 1H), 4.40 (m, 1H), 3.44 (s, 1H), 3.26 (m, 2H), 2.69 (s, 3H),

2.48–2.43 (band, 3H), 2.31 (dd, J = 17.2, 6.0 Hz, 1H), 2.05 (s, 3H), 1.96 (m, 2H), 1.69 (s, 3H), 1.69–1.31 (band, 4H), 1.18 (s, 3H), 1.09 (s, 3H), 1.02 (d, J = 6.8 Hz, 3H), 0.87 (s, 9H), 0.82 (d, J = 6.8 Hz, 3H), 0.10 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 164.8, 152.6, 146.4, 136.8, 133.5, 121.3, 118.0, 116.6, 109.8, 79.0, 74.8, 73.7, 54.1, 41.5, 40.4, 38.4, 37.7, 35.7, 32.8, 26.2, 25.0, 22.6, 22.2, 20.2, 19.4, 18.3, 15.5, 14.8, 9.9, −4.1, −4.6; HRMS (ESI) (M + H^+) calculated for $C_{35}H_{59}NO_5SSi$: 634.3956, found 634.3954.

4.2.9. Synthesis of compound **12**

To a solution of alcohol **11** (200 mg, 0.32 mmol) in CH_2Cl_2 (5 mL) was added 2,6-lutidine (0.11 mL, 0.95 mmol) at −45 °C, followed by addition of TBSOTf (0.14 mL, 0.63 mmol). The reaction mixture was warmed up to 20 °C after stirred at −45 °C for 5 h. Then the reaction was quenched by addition of H_2O (5 mL), and was diluted with CH_2Cl_2 (20 mL) and H_2O (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic phase was dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The crude product was purified by column chromatograph (3% EtOAc in hexane) to obtain compound **12** as a colorless oil (184 mg, 78.2%). R_f = 0.75 (hexane/EtOAc = 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.49 (s, 1H), 5.70 (m, 1H), 5.29 (t, J = 6.8 Hz, 1H), 5.05 (m, 2H), 4.68 (s, 1H), 4.65 (s, 1H), 4.34 (dd, J = 6.0, 3.6 Hz, 1H), 3.72 (d, J = 4.8 Hz, 1H), 3.15 (m, 1H), 2.69 (s, 3H), 2.54–2.44 (band, 3H), 2.28 (dd, J = 16.8, 6.0 Hz, 1H), 2.06 (s, 3H), 1.97 (m, 2H), 1.69 (s, 3H), 1.50 (m, 1H), 1.33–1.29 (band, 3H), 1.25–1.21 (band, 4H), 1.04–1.03 (band, 6H), 0.91–0.88 (band, 21H), 0.10 (s, 3H), 0.06 (s, 3H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.9, 171.4, 164.7, 152.7, 146.2, 137.0, 133.6, 121.3, 118.0, 116.6, 110.0, 78.9, 77.8, 74.2, 53.5, 45.4, 40.5, 38.9, 38.5, 37.7, 31.1, 30.7, 26.4, 26.2, 25.9, 23.4, 22.6, 20.5, 19.4, 18.7, 18.4, 17.9, 15.6, 14.8, 1.2, −3.4, −3.6, −4.1, −4.5.

4.2.10. Synthesis of compound **13**

To a solution of Zhan Catalyst-1B (20 mg, 0.027 mmol) in 1,2-dichoroethane (80 mL) was added compound **12** (55 mg, 0.074 mmol) in 1,2-dichoroethane (3 mL) at 80 °C. After stirred for 4 h, the reaction mixture was cooled to 20 °C, and was stirred at 20 °C for overnight. The reaction solution was concentrated under reduced pressure. The crude product was purified by column chromatograph (2% EtOAc in hexane) to obtain compound **13** and its isomer as a colorless oil (18 mg, 33.7%). R_f = 0.70 (hexane/EtOAc = 9:1).

4.2.11. Synthesis of epothilone D

To a solution of **13** (5 mg, 0.0069 mmol) was added a freshly prepared 20% CF_3COOH solution in CH_2Cl_2 (0.5 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 1 h, and warmed to 0 °C in 2 h. Sodium Bicarbonate was added to adjust the pH to 7. The resulting solution was extracted with EtOAc (3 × 2 mL), and the combined organic phase was concentrated under reduced pressure. The crude product was purified by column chromatograph (10% EtOAc in hexane) to obtain Epo D as a colorless oil (3 mg, 87.9%). R_f = 0.75 (hexane/EtOAc = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.58 (s, 1H), 5.21 (d, J = 8.8 Hz, 1H), 5.14 (dd, J = 10.0, 4.8 Hz, 1H), 4.29 (d, J = 7.2 Hz, 1H), 3.72 (m, 1H), 3.54 (br s, 1H), 3.16 (m, 1H), 3.05 (br s, 1H), 2.69 (s, 3H), 2.65–2.59 (band, 1H), 2.46 (dd, J = 14.8, 11.2 Hz, 1H), 2.35–2.21 (band, 3H), 2.06 (d, J = 0.8 Hz, 3H), 1.91–1.85 (m, 1H), 1.76–1.68 (band, 2H), 1.66 (s, 3H), 1.34 (s, 3H), 1.31–1.25 (band, 4H), 1.19 (d, J = 6.8 Hz, 3H), 1.07 (s, 3H), 1.01 (d, J = 6.8 Hz, 3H).

4.2.12. Synthesis of compound **2c**

To the solution of Grignard reagents (63.0 mmol) was added **1b** (8.9 g, 31.5 mmol, Entry 1, Table 1) in THF (130 mL) at −78 °C, and the resulting mixture was stirred at −78 °C for 2 h. Then HMPA (31.7 mL, 220.5 mmol) and CH_3I (19.7 mL, 315 mmol) was added by syringe at −78 °C, and the reaction mixture was warmed up slowly to 20 °C in 5 h. The reaction was quenched by addition of sat NH_4Cl (50 mL), and was stirred for 10 min. The organic solvent was removed by reduced pressure. The residue diluted with water (100 mL), and was extracted with EtOAc (3 × 150 mL). The combined organic phase was washed with H_2O (50 mL) and brine (50 mL), dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The crude product purified by column chromatograph (5% EtOAc in hexane) to obtain compound **2c** as a white solid (8.65 g, 75.0%). R_f = 0.70 (hexane/EtOAc = 4:1); $[\alpha]_D^{20}$ = +82.0 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3073, 2965, 1683, 1649, 1453, 1411, 1391, 1323, 1271, 1219, 1162, 1135, 1064, 1038, 879, 773, 723, 640, 620, 501, 447; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 2H), 3.89 (t, J = 6.4 Hz, 1H), 3.46 (q, J = 26.8, 14.0 Hz, 2H), 2.88 (m, 1H), 2.10–2.04 (band, 2H), 1.97–1.81 (band, 5H), 1.70 (s, 3H), 1.68 (m, 1H), 1.42–1.30 (band, 2H), 1.18 (d, J = 7.2 Hz, 3H), 1.14 (s, 3H), 0.95 (s, 3H), 0.93 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 145.9, 109.7, 65.0, 53.2, 48.1, 47.6, 45.7, 44.5, 38.4, 34.6, 34.4, 32.7,30.6 26.4, 22.4, 20.8, 19.8, 17.6, 16.5; HRMS (ESI) (M + H^+) calculated for $C_{20}H_{34}NO_3S$: 368.2254, found 368.2251.

4.2.13. Synthesis of compound **2b**

To a solution of Grignard reagents (41.0 mmol) and CuCl (1 g, cat) was added compound **1b** (4.40 g, 15.55 mmol, entry 6, Table 1) in THF (200 mL) at −78 °C, and the resulting mixture was stirred at −78 °C for 2 h. Then HMPA (14.8 mL, 108.9 mmol) and CH_3I (9.2 mL, 155.5 mmol) was added by syringe at −78 °C, and the reaction mixture was warmed up slowly to 20 °C in 5 h. The reaction was quenched by addition of sat NH_4Cl (30 mL), and was stirred for 10 min. The organic solvent was removed under reduced pressure. The residue was diluted with water (40 mL), and was extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with H_2O (30 mL) and brine (30 mL), dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The crude product was purified by column chromatograph (5% EtOAc in hexane) to obtain compound **2b** as a white solid (2.02 g, 35.2%). R_f = 0.70 (hexane/EtOAc = 4:1); $[\alpha]_D^{20}$ = +46.8 (c = 10 mg/mL, $CHCl_3$). IR (KBr/cm^{-1}): 3339, 3080, 2979, 1774, 1678, 1649, 1456, 1418, 1383, 1336, 1270, 1218, 1194, 1136, 1063, 1039, 878, 684, 588; ^{1}H NMR (100 MHz, $CDCl_3$) δ 4.66 (s, 2H), 3.90 (t, J = 6.2 Hz, 1H), 3.46 (q, J = 25.2, 13.6 Hz, 2H), 2.88 (m, 1H), 2.11–2.04 (band, 3H), 1.99–1.84 (band, 5H), 1.70 (s, 3H), 1.52 (m, 1H), 1.40–1.23 (band, 2H), 1.17 (d, J = 7.2 Hz, 3H), 1.15 (s, 3H), 0.96 (s, 3H), 0.92 (d, J = 6.8 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.1, 146.0, 109.7, 65.0, 53.2, 48.1, 47.7, 46.0, 44.5, 38.4, 35.3, 33.9, 33.2,32.7, 26.4, 22.4, 20.8, 19.8, 16.3, 15.5; HRMS (ESI) (M + H^+) calculated for $C_{20}H_{34}NO_3S$: 368.2254, found 368.2256.

4.2.14. Synthesis of compound **3c**

To a solution of compound **2c** (682 mg, 1.86 mmol) in CH_2Cl_2 (5 mL) was added DIBAL-H (3.2 mL, 3.2 mmol, 1 M in CH_2Cl_2) at −78 °C. The reaction mixture was stirred at −78 °C for 4 h. Then the reaction was quenched by addition of $NaHSO_4$ (800 mg) in water (8 mL), and was diluted with hexane (4 mL). The organic phase was removed by syringe to a 25 mL flask which contained 8 g 4 Ao molecular sieve. The aqueous phase was extracted with hexane (3 mL). The combined organic phase was concentrated under reduced pressure carefully until a white crystal appeared. The clear hexane layer (about 7 mL) was store in −20 °C overnight, and the upper clear solution was used for the next step directly.

4.2.15. Synthesis of compound **3b**

To a solution of compound **2b** (1.39 g, 3.94 mmol) in CH_2Cl_2 (8 mL) was added DIBAL-H (6 mL, 6 mmol, 1 M in CH_2Cl_2) at −78 °C.

The reaction mixture was stirred at −78 °C for 4 h. Then the reaction was quenched by addition of $NaHSO_4$ (1.44 mg) in water (11 mL), and was diluted with hexane (8 mL). The organic phase was removed by syringe to a 50 mL flask which contained 15 g 4 Ao molecular sieve. And the aqueous phase extracted with hexane (5 mL). The combined organic phase was concentrated under reduced pressure carefully until a white crystal appeared. The clear hexane layer (about 13 mL) was store in −20 °C overnight, and the upper clear solution was used for the next step directly.

*4.2.16. Synthesis of compound **14***

To a solution of ester **10** (705 mg, 1.43 mmol) in CH_2Cl_2 (7 mL) was added $TiCl_4$ (1.6 mL, 1 M in CH_2Cl_2) at −78 °C, followed by addition of DIEA (0.27 mL, 1.6 mmol). After stirred at −78 °C for 1 h, a solution of compound **3b** (1.7 mmol) in hexane (6 mL) was added to the reaction solution. The resulting reaction mixture was warmed up slowly to −12 °C in 10 h. Then the reaction was quenched by addition of aqueous of phosphate solution (3 mL, pH = 7.2). The resulting solution was stirred for 10 min and was diluted with Et_2O (30 mL). The aqueous layer was extracted with Et_2O (2 × 10 mL), and the combined organic phase was dried over $MgSO_4$. The solvent was concentrated under reduced pressure. The crude product was purified by column chromatography (5% EtOAc in hexane) to obtain alcohol **14** as a colorless oil (701 mg, 75.5%). R_f = 0.35 (hexane/EtOAc = 9:1); $[\alpha]_D^{20}$ = −37.8 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3519, 3069, 2953, 2856, 1730, 1681, 1645, 1506, 1464, 1379, 1290, 1253, 1183, 1095, 997, 968, 875, 835, 777; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.48 (s, 1H), 5.71 (m, 1H), 5.29 (t, J = 6.8 Hz, 1H), 5.07 (m, 2H), 4.66 (br s, 2H), 4.43 (t, J = 4.8 Hz, 1H), 3.46 (s, 1H), 3.39 (d, J = 9.6 Hz, 1H), 3.23 (m, 1H), 2.69 (s, 3H), 2.50–2.42 (band, 3H), 2.33 (dd, J = 17.2, 6.0 Hz, 1H), 2.06 (s, 3H), 2.03–1.96 (band, 3H), 1.71 (s, 3H), 1.60 (m, 1H), 1.35–1.25 (band, 2H), 1.17 (s, 3H), 1.12 (s, 3H), 1.04–1.03 (band, 3H), 0.88 (s, 9H), 0.69–0.67 (band, 6H), 0.10 (s, 3H), 0.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 164.5, 152.4, 146.6, 136.6, 133.3, 121.2, 117.8, 116.4, 109.3, 78.9, 73.3, 71.7, 54.0, 41.1, 40.1, 38.4, 37.5, 36.0, 34.1, 31.1, 26.0, 22.5, 21.9, 19.9, 19.2, 18.1, 14.6, 12.6, 9.4, 9.3, −4.3, −4.9; HRMS (ESI) (M + H$^+$) calculated for $C_{36}H_{62}NO_5SSi$: 648.4112, found 648.4112.

*4.2.17. Synthesis of compound **15***

To a solution of alcohol **14** (183 mg, 0.283 mmol) in CH_2Cl_2 (5 mL) was added 2, 6-lutidine (0.14 mL, 1.13 mmol) at −78 °C, followed by addition of TESOTf (0.20 mL, 0.849 mmol). After stirred 1.5 h at −78 °C, the reaction was quenched by addition of sat $NaHCO_3$ (7 mL), diluted with CH_2Cl_2 (20 mL) and H_2O (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was concentrated under reduced pressure. The crude product was purified by column chromatograph (3% EtOAc in hexane) to obtain compound **15** as a colorless oil (195 mg, 90.1%). R_f = 0.75 (hexane/EtOAc = 9:1); $[\alpha]_D^{20}$ = −48.8 (c = 10 mg/mL, $CHCl_3$). IR (KBr/cm^{-1}): 3077, 2957, 2878, 2858, 1737, 1699, 1646, 1506, 1471, 1382, 1293, 1253, 1181, 1081, 988, 885, 817, 812; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.49 (s, 1H), 5.71 (m, 1H), 5.28 (t, J = 6.8 Hz, 1H), 5.06 (m, 2H), 4.67 (d, J = 4.4 Hz, 2H), 4.22 (m, 1H), 3.69 (dd, J = 5.7, 2.0 Hz, 1H), 3.22 (m, 1H), 2.69 (s, 3H), 2.62 (dd, J = 17.2, 3.2 Hz, 1H), 2.47 (m, 2H), 2.21 (m, 1H), 2.06 (s, 3H), 1.99 (m, 1H), 1.71 (s, 3H), 1.48 (m, 1H), 1.36–1.25 (band, 7H), 1.04 (s, 3H), 0.99 (d, J = 6.4 Hz, 3H), 0.94 (t, J = 8.0 Hz, 9H), 0.87 (s, 9H), 0.75 (t, J = 6.6 Hz, 6H), 0.59 (m, 6H), 0.11 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 164.5, 152.5, 146.4, 136.7, 133.4, 121.2, 117.8, 116.4, 109.5, 78.8, 75.4, 74.4, 52.9, 44.7, 41.7, 39.9, 37.5, 36.0, 34.6, 31.4, 26.0, 22.7, 22.5, 21.4, 19.2, 18.1, 14.5, 13.8, 11.1, 11.0, 7.2, 5.5, −4.5, −4.6; HRMS (ESI) (M + H$^+$) calculated for $C_{42}H_{76}NO_5SSi_2$: 762.4977, found 762.4976.

*4.2.18. Synthesis of compounds **16** and **17***

To a solution of Zhan Catalyst-1B (25 mg, 0.034 mmol) in 1,2-dichoroethane (125 mL) was added compound **15** (95 mg, 0.125 mmol) in 1,2-dichoroethane (5 mL) slowly at 80 °C. After stirred for 4 h, the reaction mixture was cooled to 20 °C, and was stirred for overnight. Then the reaction solution was concentrated under reduced pressure. The crude product was purified by column chromatograph (2% EtOAc in hexane) to obtain compound **16** and compound **17** as a colorless oil (55 mg, 60.0%). R_f = 0.70 (hexane/EtOAc = 9:1); Compound **16**: $[\alpha]_D^{20}$ = −7.2 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3151, 2972, 2902, 2841, 2723, 1739, 1696, 1505, 1461, 1410, 1379, 1299, 1249, 1200, 1161, 983, 939, 775; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.56 (s, 1H), 5.13 (m, 1H), 5.04 (dd, J = 9.2, 2.4 Hz, 1H), 4.10–4.08 (band, 2H), 3.07 (m, 1H), 2.71 (s, 3H), 2.76–2.67 (band, 1H), 2.59 (dd, J = 16.0, 8.4 Hz, 1H), 2.34 (m, 1H), 2.11 (s, 3H), 1.81–1.71 (band, 2H), 1.65 (s, 3H), 1.49–1.39 (band, 2H), 1.25 (s, 3H), 1.19 (s, 3H), 1.15 (s, 3H), 1.10 (d, J = 6.8 Hz, 3H), 0.99 (t, J = 8.0 Hz, 9H), 0.93–0.91 (band, 6H), 0.85 (s, 9H), 0.67 (q, J = 16.0, 8.0 Hz, 6H), 0.10 (s, 3H), −0.08 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.8, 169.9, 163.5, 151.7, 139.5, 137.4, 118.9, 118.1, 115.1, 78.9, 74.7, 52.4, 46.5, 40.5, 38.8, 34.0, 31.9, 31.2, 28.8, 28.7, 25.1, 25.0, 23.5, 23.3, 18.2, 17.5, 16.3, 14.1, 13.9, 13.2, 6.2, 4.5, −4.7, −6.5; HRMS (ESI) (M + H$^+$) calculated for $C_{40}H_{72}NO_5SSi_2$: 734.4664, found 734.4664.

*4.2.19. Synthesis of compound **18***

To a solution of **16** (30 mg, 0.041 mmol) was added a freshly prepared 20% CF_3COOH solution in CH_2Cl_2 (0.7 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 1 h, and warmed to 0 °C in 30 min. Sodium bicarbonate was added to adjust the pH to 7. The resulting solution was extracted with EtOAc (3 × 3 mL), and the combined organic mixture was concentrated under reduced pressure. The crude product was purified by column chromatograph (10% EtOAc in hexane) to obtain target molecule **18** as a colorless oil (15 mg, 72.4%). R_f = 0.25 (hexane/EtOAc = 3:1); $[\alpha]_D^{20}$ = −58.6 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3496, 2958, 2931, 1737, 1685, 1507, 1376, 1249, 1155, 1083, 1002, 977, 858, 849, 765, 704; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.57 (s, 1H), 5.22 (d, J = 10.2 Hz, 1H), 5.07 (dd, J = 9.2, 2.4 Hz 1H), 4.30 (dd, J = 10.8, 2.0 Hz, 1H), 3.81 (d, J = 6.4 Hz, 1H), 3.60 (br s, 1H), 3.23 (q, J = 6.8 Hz, 1H), 3.18 (s, 1H), 2.68 (s, 3H), 2.60 (m, 1H), 2.45 (m, 1H), 2.33–2.19 (band, 3H), 2.06 (s, 3H), 1.78 (m, 1H), 1.68 (s, 3H), 1.66–1.58 (band, 1H), 1.44–1.36 (band, 1H), 1.33 (s, 3H), 1.28–1.21 (band, 2H), 1.12 (t, J = 8.0 Hz, 6H), 1.06 (s, 3H), 1.00 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.0, 170.6, 165.1, 152.0, 139.5, 139.4, 119.2, 119.2, 115.7, 79.1, 72.2, 72.1, 53.8, 41.8, 41.8, 39.8, 38.0, 33.0, 32.8, 30.9, 23.9, 22.2, 19.7, 19.1, 17.7, 16.2, 15.7, 11.6; HRMS (ESI) (M + H$^+$) calculated for $C_{28}H_{44}NO_5S$: 506.2935, found 506.2934.

*4.2.20. Synthesis of compound **19***

To a solution of **17** (35 mg, 0.047 mmol) was added a freshly prepared 20% CF_3COOH solution in CH_2Cl_2 (0.7 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 1.5 h, and warmed to 0 °C in 30 min. Sodium Bicarbonate was added to adjust the pH to 7. The resulting solution extracted with EtOAc (3 × 3 mL), and the combined organic mixture was concentrated under reduced pressure. The crude product was purified by column chromatograph (10% EtOAc in hexane) to obtain target molecule **19** as a colorless oil (21 mg, 85.5%). R_f = 0.25 (hexane/EtOAc = 3:1); $[\alpha]_D^{20}$ = −85.2 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3493, 2969, 2930, 1776, 1736, 1689, 1508, 1376, 1181, 1026, 960, 852, 713; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.59 (s, 1H), 5.26 (t, J = 5.2 Hz, 1H), 5.10 (t, J = 7.2 Hz, 1H), 4.32 (d, J = 10.0 Hz, 1H), 4.07 (br s, 1H), 3.71 (d, J = 4.8 Hz, 1H), 3.48–3.43 (band, 2H), 2.69 (s, 3H), 2.56 (m, 1H), 2.43–2.29 (band, 3H), 2.12 (m, 1H), 2.04 (s, 3H), 1.91 (m, 1H),

1.68–1.61 (band, 1H), 1.59 (s, 3H), 1.53–1.45 (band, 1H), 1.32 (s, 3H), 1.27–1.21 (band, 2H), 1.16 (d, J = 7.2 Hz, 3H), 1.03 (s, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.80 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.5, 170.3, 163.5, 151.2, 139.1, 135.6, 118.1, 117.2, 114.3, 77.0, 73.8, 70.3, 52.1, 43.4, 41.2, 37.7, 36.1, 32.2, 31.7, 29.6, 19.4, 18.1, 17.7, 15.3, 15.0, 14.4, 14.0, 11.2; HRMS (ESI) (M + H^+) calculated for $C_{28}H_{44}NO_5S$: 506.2935, found 506.2937.

*4.2.21. Synthesis of compound **20***

To a solution of compound **10** (710 mg, 1.44 mmol) in 7 mL CH_2Cl_2 was added $TiCl_4$ (1.6 mL, 1.6 mmol, 1 M in CH_2Cl_2) at −78 °C, followed by addition of DIEA (0.27 mL, 1.6 mmol). After being stirred at −78 °C for 1 h, a solution of compound **3c** (1.5 mmol) in hexane (6 mL) was added to the reaction solution. The resulting reaction mixture was warmed up slowly to −12 °C in 10 h. The reaction was quenched by addition of aqueous of phosphate solution (3 mL, pH = 7.2). Then the resulting solution was stirred for 10 min, and was diluted with ether (30 mL). The aqueous layer was extracted with Et_2O (2 × 10 mL), and the combined organic phase was dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by column chromatography (5% EtOAc in hexane) to obtain alcohol **20** as a colorless oil (700 mg, 74.8%). R_f = 0.35 (hexane/EtOAc = 9:1); $[\alpha]_D^{20}$ = −33.2 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3509, 2960, 2858, 1737, 1684, 1646, 1506, 1471, 1378, 1295, 1254, 1181, 1094, 995, 975, 883, 866, 778; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.49 (s, 1H), 5.70 (m, 1H), 5.29 (t, J = 6.6 Hz, 1H), 5.06 (m, 2H), 4.65 (br s, 2H), 4.41 (t, J = 4.8 Hz, 1H), 3.47 (d, J = 10 Hz, 1H), 3.44 (s, 1H), 3.21 (m, 1H), 2.68 (s, 3H), 2.50–2.45 (band, 3H), 2.31 (dd, J = 17.2, 5.6 Hz, 1H), 2.06 (s, 3H), 1.99–1.85 (band, 3H), 1.68 (s, 3H), 1.52 (band, 4H), 1.22–1.17 (band, 5H), 1.11 (s, 3H), 1.03–1.01 (band, 3H), 0.87 (s, 9H), 0.71–0.69 (band, 3H), 0.10 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 222.1, 171.0, 164.6, 152.4, 146.6, 136.6, 133.3, 121.2, 117.9, 116.5, 109.5, 78.9, 73.6, 71.7, 53.8, 41.2, 40.4, 40.2, 37.5, 36.1, 31.2, 27.9, 26.0, 22.4, 21.7, 20.4, 19.2, 18.1, 18.1, 14.6, 10.3, 9.4, −4.3, −4.8; HRMS (ESI) (M + H^+) calculated for $C_{36}H_{62}NO_5SSi$: 648.4112, found 648.4111.

*4.2.22. Synthesis of compound **21***

To a solution of alcohol **20** (380 mg, 0.586 mmol) in CH_2Cl_2 (13 mL) was added 2,6-lutidine (0.28 mL, 2.35 mmol) at −78 °C, followed by addition of TESOTf (0.40 mL, 1.76 mmol). After stirred at −78 °C for 1.5 h, the reaction mixture was quenched by addition of sat $NaHCO_3$ (15 mL), diluted with CH_2Cl_2 (50 mL) and H_2O (30 mL). The aqueous layer was separated and was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by column chromatograph (3% EtOAc in hexane) to obtain compound **21** as a colorless oil (379 mg, 84.8%). R_f = 0.75 (hexane/EtOAc = 9:1); $[\alpha]_D^{20}$ = −77.2 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3076, 2958, 2879, 2858, 1738, 1697, 1646, 1506, 1471, 1378, 1293, 1263, 1180, 1087, 988, 917, 885, 813; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.48 (s, 1H), 5.70 (m, 1H), 5.27 (t, J = 6.8 Hz, 1H), 5.05 (m, 2H), 4.66 (br s, 2H), 4.25 (m, 1H), 3.84 (t, J = 4.8 Hz, 1H), 3.18 (m, 1H), 2.68 (s, 3H), 2.59 (dd, J = 17.2, 3.2 Hz, 1H), 2.46 (m, 2H), 2.23 (dd, J = 17.2, 6.0 Hz, 1H), 2.10 (m, 1H), 2.06 (s, 3H), 1.87 (m, 1H), 1.70 (s, 3H), 1.74–1.63 (band, 2H), 1.34 (band, 1H), 1.26 (s, 3H), 1.05–0.97 (band, 7H), 0.94 (t, J = 8.0 Hz, 9H), 0.86 (s, 9H), 0.82–0.72 (band, 6H), 0.60 (m, 6H), 0.10 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.0, 171.3, 164.5, 152.6, 146.6, 136.8, 133.4, 121.1, 117.8, 116.4, 109.6, 78.7, 75.5, 75.1, 53.0, 44.9, 43.8, 40.1, 37.5, 35.9, 31.9, 29.5, 26.0, 23.2, 22.5, 21.1, 19.3, 18.9, 18.2, 14.6, 12.8, 12.1, 7.2, 5.5, −4.4, −4.6; HRMS (ESI) (M + H^+) calculated for $C_{42}H_{76}NO_5SSi_2$: 762.4977, found 762.4969.

*4.2.23. Synthesis of compounds **22** and **23***

To a solution of Zhan Catalyst-1B (40 mg, 0.055 mmol) in 1,2-dichoroethane (180 mL) was added compound **21** (180 mg, 0.236 mmol) in 1,2-dichoroethane (10 mL) slowly at 80 °C. After stirred at the same temperature for 4 h, the reaction mixture was cooled to 20 °C, and was stirred for overnight. Then the reaction solution was concentrated under reduced pressure. The crude product was purified by column chromatograph (2% EtOAc in hexane) to obtain compound **22** and compound **23** as a colorless oil (90 mg, 52.0%). R_f = 0.70 (hexane/EtOAc = 9:1); Compound **22**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.56 (s, 1H), 5.31 (d, J = 9.2 Hz, 1H), 5.15 (d, J = 4.4 Hz, 1H), 4.81 (m, 1H), 4.08 (d, J = 7.2 Hz, 1H), 3.17 (m, 1H), 2.81–2.74 (band, 1H), 2.71 (s, 3H), 2.62–2.54 (band, 2H), 2.36 (d, J = 16.0 Hz, 1H), 2.15 (s, 3H), 2.12–2.01 (band, 3H), 1.65 (s, 3H), 1.62 (m, 1H), 1.25 (m, 1H), 1.18 (d, J = 6.8 Hz, 1H), 1.07 (s, 3H), 1.07–0.97 (band, 12H), 0.92 (d, J = 6.8 Hz, 3H), 0.88 (m, 1H), 0.85 (s, 9H), 0.74 (d, J = 6.4 Hz, 3H), 0.67 (m, 6H), 0.11 (s, 3H), 0.07 (s, 3H); HRMS (ESI) (M + H^+) calculated for $C_{40}H_{72}NO_5SSi_2$: 734.4664, found 734.4654.

*4.2.24. Synthesis of **24***

To a solution of **22** (35 mg, 0.047 mmol) was added a freshly prepared 20% CF_3COOH solution in CH_2Cl_2 (0.7 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 30 min, and warmed to 0 °C in 30 min. Sodium bicarbonate was added to adjust the pH to 7. The resulting solution extracted with EtOAc (3 × 3 mL), and the combined organic mixture was concentrated under reduced pressure. The crude product was purified by column chromatograph (10% EtOAc in hexane) to obtain target molecule **24** as a colorless oil (18 mg, 75.8%). R_f = 0.25 (hexane/EtOAc = 3:1); $[\alpha]_D^{20}$ = −68.4 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3482, 2959, 1775, 1733, 1689, 1507, 1455, 1378, 1253, 1159,1035, 1006, 983, 819, 775, 715; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.50 (s, 1H), 5.26 (dd, J = 8.8, 3.2 Hz, 1H), 5.14 (t, J = 6.4 Hz, 1H), 4.08 (m, 1H), 3.83 (d, J = 6.8 Hz, 1H), 3.44 (br s, 1H), 3.24 (q, J = 6.8 Hz, 1H), 2.85 (br s, 1H), 2.70 (s, 3H), 2.59–2.51 (band, 2H), 2.41–2.37 (band, 1H), 2.16–2.11 (band, 1H), 2.08 (s, 3H), 1.93 (m, 1H), 1.74 (m, 1H), 1.63 (s, 3H), 1.55–1.48 (band, 2H), 1.33 (s, 3H), 1.25 (m, 1H), 1.19–1.11 (band, 1H), 1.09 (s, 3H), 1.07 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.81 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.1, 170.8, 164.7, 152.3, 140.0, 137.6, 119.5, 118.7, 116.1, 79.2, 73.3, 72.0, 52.2, 42.7, 42.7, 38.3, 37.2, 32.6, 31.7, 30.4, 21.3, 20.5, 19.2, 18.7, 16.9, 15.5, 10.8, 10.0; HRMS (ESI) (M + H^+) calculated for $C_{28}H_{44}NO_5S$: 506.2935, found 506.2936.

*4.2.25. Synthesis of **25***

To a solution of **23** (18 mg, 0.025 mmol) was added a freshly prepared 20% CF_3COOH solution in CH_2Cl_2 (0.6 mL) at −20 °C. The reaction mixture was stirred at −20 °C for 50 min, and warmed to 0 °C in 1.5 h. Sodium bicarbonate was added to adjust the pH to 7. The resulting solution was extracted with EtOAc (3 × 3 mL), and the combined organic mixture was concentrated under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexane) to obtain target molecule **25** as a colorless oil (10 mg, 79.2%). R_f = 0.25 (hexane/EtOAc = 3:1); $[\alpha]_D^{20}$ = −10.4 (c = 10 mg/mL, $CHCl_3$); IR (KBr/cm^{-1}): 3498, 2962, 2921, 1777, 1731, 1684, 1507, 1456, 1375, 1176, 1016, 975, 867, 758; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.53 (s, 1H), 5.40 (dd, J = 11.2, 3.2 Hz, 1H), 5.11 (t, J = 11.2 Hz, 1H), 4.31 (m, 1H), 3.34–3.22 (band, 2H), 2.70 (s, 3H), 2.50 (m, 1H), 2.40–2.26 (band, 2H), 2.13 (m, 1H), 2.07 (s, 3H), 2.03 (m, 1H), 1.80 (m, 1H), 1.66 (s, 3H), 1.57–1.49 (band, 2H), 1.35 (s, 3H), 1.25 (s, 3H), 1.22–1.11 (band, 1H), 1.06–1.03 (band, 6H), 0.94 (d, J = 10.8 Hz, 3H), 0.68 (d, J = 11.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.1, 172.2, 164.8, 152.3, 141.5, 137.8, 120.1, 118.7, 116.4, 79.7, 71.1, 69.7, 54.0, 41.1, 40.2, 38.3, 38.0, 32.9, 31.9, 31.7, 29.7, 22.0, 19.1, 17.0,

15.4, 14.8, 10.0, 8.9; HRMS (ESI) ($M + H^+$) calculated for $C_{28}H_{44}NO_5S$: 506.2935, found 506.2938.

4.3. Procedure for testing tubulin inhibitory activity of Epo D, compounds **18** and **24**

Fresh animal brain tissue was isolated, and the meninges and large blood vessels were removed. The cut tissue was washed with cold MES buffer (1 mL per gram) for 2 times. Then the mixture was mashed until become homogenate. The mixture was centrifuged at 105,000 g, 4 °C for 1 h. The supernatant was separated, and the microtubule polymerization buffer was added. And after incubated at 37 °C or 30 min, the mixture was centrifuged at 105,000 g, 26 °C for 1 h. Cold MES buffer with 1/10 volume of homogenate was added into the supernatant, the mixture was gently vortex until the precipitate was completely dissolved. The solution was stored in ice for 0.5 h, and the low and high temperature centrifugals were repeated for two times. The precipitate was suspended in MES buffer, and the tubulin was diluted with MES buffer to 2 mg/mL. 5 μL $ATPNa_2$ (40 mmol/L) and 195 μL tubulin were added into a cold 96-well plates, and then a certain concentration of compound was added into each well of the cold 96-well plates. The values of OD350 were detected every 3 min with Ultraviolet spectrophotometer at 37 °C for 30 min.

4.4. Computational methods

The structure of Epo D, 9-(R)-Me epo D and 9-(S)-Me epo D were built in Maestro [42] and cleaned up by performing a short force-field minimization. Epo D, 9-(R)-Me epo D and 9-(S)-Me epo D were subjected to full geometry optimization with DFT at the B3LYP/6-31G (d) level using Gaussian 03.

Autodock 4.2 was used to perform the docking simulation of fully optimized Epo D, 9-(R)-Me epo D and 9-(S)-Me epo D. Structure of α, β-tubulin (PDB ID: 1TVK) was used as our initial protein model for docking (Fig. 4).

The binding energy [43] of 9-(R)-Me epo D and 9-(S)-Me epo D to the structure of α, β-tubulin are 5.50 kcal/mol and 8.14 kcal/mol higher than Epo D respectively. ΔG binding = ΔE (ligand in protein) + ΔE (ligand in water).

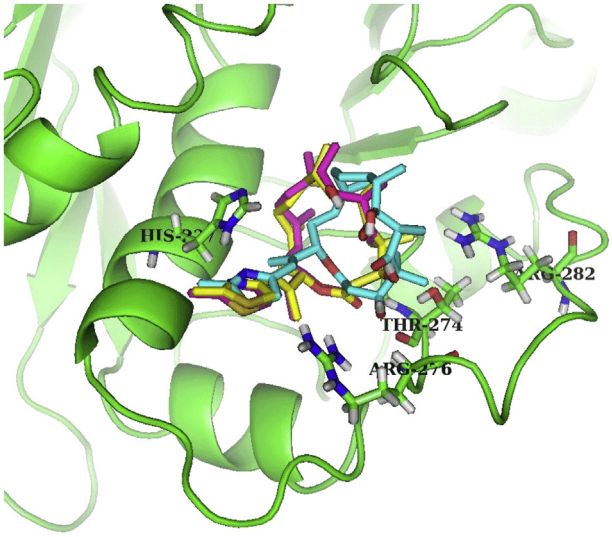


Fig. 4. Superposition of best docking poses of Epo D (cyan), 9-(R)-Me epo D (purple) and 9-(S)-Me epo D (yellow) to structure of α, β-tubulin (PDB ID: 1TVK). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.08.003.

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