Supplementary Material (ESI) for Chemical Communications

Novel Epothilone Lactones by an Unusual Diversion of the Grubbs' Metathesis Reaction

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Electronic Supplementary Information

Materials and Methods.

Genaral synthetic procedures. NMR spectra were obtained on Bruker Avance 600, JEOL Eclipse 500, Varian Unity 400, or Varian Inova 400 spectrometers in $CDCl_3$ or CD_3CN. The chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. High-resolution FAB mass spectra were obtained on a JEOL HX110 Dual Focusing Mass Spectrometer. THF was distilled from sodium-benzophenone and dichloromethane was distilled from calcium hydride. Other reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using flash silica gel (32-63μ). Preparative thin-layer chromatography (PTLC) separations were carried out on 500 or 1000 μ Uniplate thin layer chromatography plates. All reactions were carried out under a nitrogen atmosphere unless otherwise noted.

12α,13α-Epoxide 8. To a solution of allylic alcohol **7**[1] (26 mg, 0.049 mmol) and 4 Å molecular sieves (60 mg) in CH_2Cl_2 (0.5 mL) at -20 ^oC was added diethyl-D-tartrate (12 mg, 0.0588 mmol, 1.2 eq) in CH_2Cl_2 (0.1 mL) and titanium isopropoxide (14 mg, 0.049 mmol, 1.0 eq) in CH_2Cl_2 (0.1 mL). After stirring at that temperature for 1 h, *t*-butyl hydroperoxide (30 μL, 5 M in decane, 0.0147 mmol, 3.0 eq) was added and the resulting reaction mixture was stirred at -20 ^oC for an additional 2 h. The reaction mixture was then filtered through Celite® into saturated aqueous Na_2SO_4 solution (10 mL), rinsing with EtOAc (10 mL). The subsequent biphasic mixture was stirred for 1 h and two layers were separated. The aqueous phase was re-extracted with EtOAc (10 mL × 3), and the combined organic extracts were

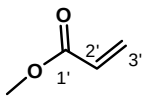
dried over anhydrous Na_2SO_4, and the solvents were removed *in vacuo* to give a crude oil. Purification of this oil by preparation thin layer chromatography over silica gel using 80% EtOAc in hexanes as eluent furnished a mixture of α-epoxide triol **8** and β-epoxide triol **10** (22.6 mg, 84%, containing around 20% β-epoxide diastereoisomer, which cannot be separated *via* chromatography over silica gel). This mixture was used directly for the next step without further purification.

26-Acryloyloxy-α-epoxide 9. To a solution of the mixture of triols **8** and **10** (22.6 mg, 0.041 mmol) in dichloromethane (1.5 mL) was added sequentially Et_3N (29 μL, 0.205 mmol, 5 eq), acryloyl chloride (6 μL, 0.0738 mmol, 1.8 eq) and DMAP (1 mg) at 0 °C. The resulting reaction mixture was allowed to stir for 30 min prior to being quenched by the addition of saturated $NaHCO_3$. The mixture was extracted with ethyl acetate (10 mL × 3), the combined organic extracts were dried over anhydrous Na_2SO_4, and the solvents were removed *in vacuo*. The crude product obtained was subjected to preparative thin layer chromatography over silica gel, eluting with 30% EtOAc in hexances for three times, to provide pure 26-acryloyloxy-α-epoxide **9** (15.7 mg, 79%) as a colorless syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.58 (s, 1H), 6.39 (d, *J* = 17.6 Hz, 1H), 6.08 (dd, *J* = 17.6, 10.4 Hz, 1H), 5.84 (d, *J* = 10.8 Hz, 1H), 5.66 (m, 1H), 5.50 (d, *J* = 10.8 Hz, 1H), 5.12 (d, *J* = 16.4 Hz, 1H), 5.09 (m, 1H), 4.50 (m, 1H), 4.18 (d, *J* = 12.8 Hz, 1H), 4.06 (d, *J* = 12.4 Hz, 1H), 3.91 (m, 1H), 3.28 (m, 1H), 3.12 (dd, *J* = 7.6, 5.2 Hz, 1H), 2.66 (s, 3H), 2.68-2.61 (m, 3H), 2.56-2.44 (m, 2H), 2.12-2.01 (m, 2H), 2.07 (s, 3H), 1.88-1.71 (m, 3H), 1.49-1.27 (m, 4H), 1.08-0.99 (m, 1H), 1.03 (s, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.5, 170.2, 165.7, 164.9, 152.0, 137.6, 133.8, 131.7, 127.7, 119.4, 118.5, 116.3, 76.8, 72.2, 71.0, 66.9, 59.6, 59.1, 57.2, 43.2, 40.2, 39.6, 35.5, 31.3, 30.4, 25.8, 19.1, 19.0, 15.7, 15.2, 10.1. HRFABMS: calcd for $C_{32}H_{46}NO_8S$ (M+H) 604.2944, found 604.2981.

Internal lactone Epothilone 12. To a solution of diene **9** (12 mg, 0.0199 mmol) in dichloromethane (6 mL) was added second-generation Grubbs catalyst (6 mg, 0.006965 mmol, 0.35 eq) in dichloromethane (3.5 mL) for a period of 3 h *via* syringe pump under N_2. The resulting reaction mixture was allowed to stir for 20 h at 25 °C, and then the dichloromethane was removed under reduced pressure. The residue obtained was subjected to preparative thin layer chromatography over silica gel, eluting with 50% EtOAc in hexanes, to furnish **12** (2.84 mg, 23.7%) and starting material **9** (8.18 mg, 68.2%). Compound **12** was obtained as a colorless oil. IR (film) cm^{-1}: 3506 (OH), 1786 (5-membered lactone), 1733, 1729 (CO, COO), 1697(unsaturated ester). ^{1}H NMR (400 MHz, CD_3CN) and ^{13}C NMR (100 MHz, CD_3CN) see Table 1. HRFABMS: calcd for $C_{31}H_{42}NO_9S$ (M+H) 604.2580, found 604.2538.

12β,13β-Epoxide 10. β-Epoxide triol **10** was prepared as a pure compound using diethyl-L-tartrate instead of diethyl-D-tartrate following the procedure described previously for the synthesis of α-epoxide triol **8**. Compound **10** was obtained as a colorless oil in 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.59 (s, 1H), 5.66 (m, 1H), 5.42 (d, *J* = 7.2 Hz, 1H), 5.13 (d, *J* = 16.4 Hz, 1H), 5.10 (d, *J* = 10.8 Hz, 1H), 4.37 (d, *J* = 11.2 Hz, 1H), 4.29 (s, 1H), 3.73 (d, *J* = 12.8 Hz, 1H), 3.69 (dd, *J* = 6.0, 2.0 Hz, 1H), 3.59 (d, *J* = 12.0 Hz, 1H), 3.41 (m, 1H), 3.12 (dd, *J* = 8.0, 3.6 Hz, 1H), 2.85 (br.s, 1H), 2.69 (s, 3H), 2.64-2.53 (m, 4H), 2.34 (m, 1H), 2.18 (m, 1H), 2.06 (s, 3H), 2.11-1.88 (m, 3H), 1.78-1.63 (m, 2H), 1.55-1.23 (m, 3H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.04 (s, 3H), 0.98 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.7, 170.8, 165.8, 152.6, 138.3, 133.6, 119.2, 119.1, 116.2, 77.1, 72.9, 72.89, 71.6, 64.4, 64.0, 57.9, 42.3, 39.8, 39.7, 35.9, 31.6, 31.3, 29.0, 20.7, 16.7, 16.1, 15.5, 12.0. HRFABMS: calcd for $C_{29}H_{44}NO_7S$ (M+H) 550.2838, found 550.2836.

Table S1. ^{1}H-(400 Mz, CD_3CN)and ^{13}C-NMR Data (100 Mz, CD_3CN) of **12**, δ in ppm, *J* = Hz

	δ(C)(HSQC)	δ(H)	^{1}H-^{1}H COSY	HMBC(H→C)
C(1)	167.7(CO)	—	—	—
CH_2(2)	35.3(CH_2)	2.92 (d, *J*=7.2)	H-3, H-2(2.62)	N/A
		2.62 (d, *J*=7.2)	H-3, H-2(2.92)	C-1, C-3, C-4
CH (3)	77.9(CH)	5.13 (t, *J*=7.6)	H_2-2	C-1, C-2, C-5, C-23 (4-CH_3)
C(4)	55.0(C)	—	—	—
C(5)	212.1(CO)	—	—	—
CH(6)	44.7(CH)	3.37 (m)	H-7, H_3-24(6-CH_3)	C-5, C-7, C-24(6-CH_3)
CH(7)	76.3(CH)	3.70 (m)	H-6	C-6, C-9
CH(8)	35.7(C)	1.38 (m)	H_3-25(8-CH_3)	C-25 (8-CH_3)
CH_2(9)	30.0(CH_2)	1.58 (m)	H_2-10	C-12
CH_2(10)	22.1(CH_2)	1.61 (m)	H_2-9	C-9, C-12
CH_2 (11)	29.0 (CH_2)	2.04, 1.55 (m)	—	C-12, C-13
C (12)	61.9 (C)	—	—	—
CH(13)	57.1(CH)	3.04(dd, *J*=7.6, 5.2)	H_2-14	C-11, C-14
CH_2 (14)	29.2 (CH_2)	2.10 (m)	H-13, H-15	C-12, C-13, C-15
		1.74 (m)	H-13, H-15	C-12, C-13
CH(15)	76.5 (CH)	5.39 (m)	H-14	N/A
C (16)	135.9(C)	—	—	—
CH(17)	118.4 (CH)	6.51 (s)	—	C-15, C-16, C-18, C-19, C-27
C (18)	158.0 (C)	—	—	—
CH(19)	118.1(CH)	7.25 (s)	—	C-18, C-20
C (20)	165.2(C)	—	—	—
CH_3(21)	18.6 (CH_3)	2.64 (s)	H_2-21	C-18, C-19, C-20
CH_2 (22)	41.0 (CH_2)	3.52 (d, *J*=16.4)	H-22(2.53)	C-1", C-4, C-5,C-23(4-CH_3)
		2.53 (d, *J*=16.4)	H-22(3.52)	C-1", C-3, C-4, C-23(4-CH_3)
CH_3(23)	16.7 (4-CH_3)	1.15 (s)	—	C-3, C-4, C-5, C-22
CH_3(24)	15.0 (6-CH_3)	1.10 (d, *J*=7.2)	H-6	C-5, C-6, C-7
CH_3(25)	17.3 (8-CH_3)	0.94 (d, *J*=6.4)	H-8	C-7, C-8, C-9
CH_2 (26)	65.4 (CH_2)	4.39 (d, *J*=12.8)	H-26 (4.12)	C-12, C-13, C-1'
		4.12 (d, *J*=12.4)	H-26 (4.39)	C-12, C-13, C-1'
CH_3 (27)	15.3 (16-CH_3)	2.17 (s)	—	C-15, C-16, C-17, C-18
C(1")	174.4 (C)	—	—	—
	165.9 (C1', COO)	—	—	—
	127.9 (C2', CH)	6.05(dd,*J*=17.6, 0.4)	H_2-3'	C-1', C-3'
	131.6 (C3', CH_2)	6.27 (d, *J*=17.6)	H-2'	C-1', C-2'
		5.76 (d, *J*=10.8)	H-2'	C-1', C-2'

(4*R*)-4-Allyl-4-demethyl-26-(acryloyloxy)epothilone B 11. 26-Acryloyloxy-β-epoxide **11** was synthesized from β-epoxide triol **10** employing the same procedure as that described for 26-acryloyloxy-α-epoxide **9**. Compound **11** was obtained as a colorless syrup in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.55 (s, 1H), 6.37 (d, *J* = 16.4 Hz, 1H), 6.08 (dd, *J* = 16.4, 10.8 Hz, 1H), 5.83 (d, *J* = 10.8 Hz, 1H), 5.40 (dd, *J* = 8.8, 1.6 Hz, 1H), 5.12 (d, *J* = 16.8 Hz, 1H), 5.08 (m, 1H), 4.38 (m, 1H), 4.28 (d, *J* = 12.4 Hz, 1H), 4.03 (d, *J* = 12.4 Hz, 1H), 3.67 (m, 1H), 3.36 (m, 1H), 2.95 (dd, *J* = 8.4, 2.8 Hz, 1H), 2.78 (br.s, 1H), 2.67-2.64 (m, 1H), 2.65 (s, 3H), 2.59 (d, *J* = 7.6 Hz, 2H), 2.56 (dd, *J* = 14.0, 2.8 Hz, 1H), 2.33 (d, *J* = 14.0, 1.6 Hz, 1H), 2.17 (dt, *J* = 18.8, 3.2 Hz, 1H), 2.09-2.02 (m, 1H), 2.04 (s, 3H), 1.98-1.87 (m, 2H), 1.78-1.70 (m, 2H), 1.58-1.48 (m, 2H), 1.43-1.36 (m, 2H), 1.30-1.24 (m, 1H), 1.06 (d, *J* = 6.8 Hz, 3H), 1.02 (s, 3H), 0.95 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.6, 170.8, 165.9, 165.6, 151.8, 138.1, 133.6, 131.8, 128.0, 119.8, 119.1, 116.3, 77.1, 72.6, 71.7, 66.4, 62.2, 58.9, 57.9, 42.1, 39.8, 35.8, 31.6, 31.5, 29.3, 20.6, 19.2, 16.7, 16.2, 16.0, 15.4, 11.7. HRFABMS: calcd for $C_{32}H_{46}NO_8S$ (M+H) 604.2944, found 604.2976.

Internal lactone 13. The epothilone analog **13** was prepared from diene **11** according to a similar procedure to that described for the conversion of **9** to **12**. Compound **13** was obtained as a colorless oil in 24.8% yield (69.7% starting material was recovered). IR(film) cm^{-1}: 3506 (OH), 1786 (5-membered lactone), 1733, 1730 (CO, COO), 1697(unsaturated ester).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 6.62 (s, 1H), 6.37 (d, *J* = 17.6 Hz, 1H), 6.05 (dd, *J* = 17.6, 10.4 Hz, 1H), 5.84 (d, *J* = 10.8 Hz, 1H), 5.37 (m, 1H), 4.98 (dd, *J* = 10.8, 2.8 Hz, 1H), 4.17 (d, *J* = 12.8 Hz, 1H), 4.07 (d, *J* = 12.4 Hz, 1H), 3.78 (m, 1H), 3.27 (d, *J* = 16.4 Hz, 1H), 3.21 (m, 1H), 3.01 (dd, *J* = 7.6, 5.2 Hz, 1H), 2.67 (s, 3H), 2.51 (d, *J* = 16.4 Hz, 1H), 2.73-2.50 (m, 3H), 2.20-2.14 (m, 1H), 2.16 (s, 3H), 1.92-1.83 (m, 2H), 1.74-1.64 (m, 3H), 1.47-1.38 (m, 3H), 1.33-1.13 (m, 2H), 1.19 (d, *J* = 7.2 Hz, 3H), 1.18 (s, 3H), 1.00 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3CN) δ 211.9, 174.1, 168.0, 165.6, 165.3, 152.9, 137.2, 131.3, 128.2, 119.8, 118.1, 77.7, 77.6, 76.9, 66.5, 61.6, 60.1, 55.0, 44.8, 41.2, 35.4, 34.9, 32.5, 28.8, 28.1, 21.9, 18.6, 17.8, 16.7, 15.4, 14.2. HRFABMS: calcd for $C_{31}H_{42}NO_9S$ (M+H) 604.2580, found 604.2574.

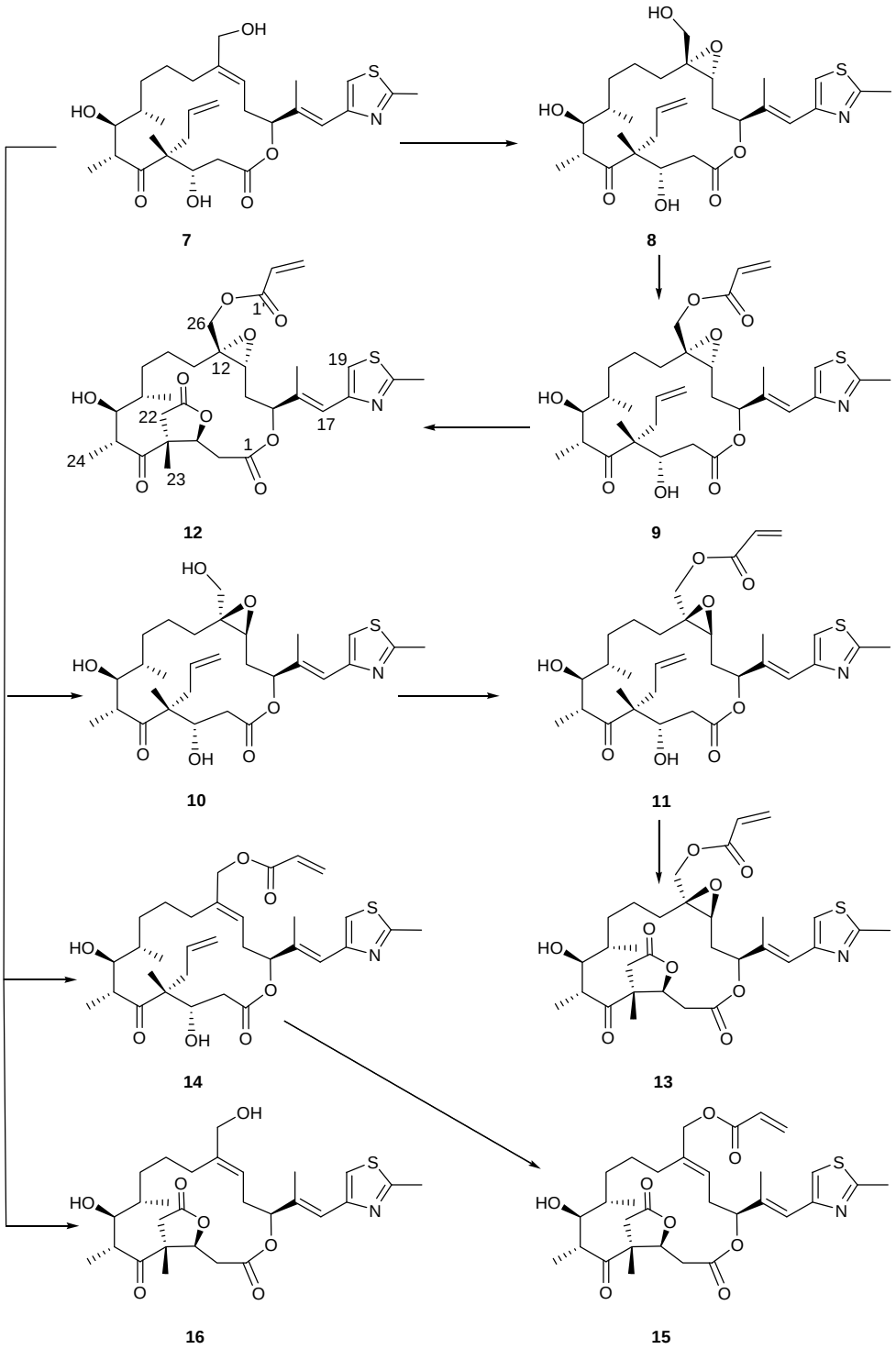
(4*R*)-4-Allyl-4-demethyl-26-(acryloyloxy)epothilone D 14. 26-Acryloyloxy-macrolactone **14** was prepared directly from triol **7** following the procedure described previously for the preparation of **9** from **8**. Compound **14** was obtained as a colorless oil in 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (s, 1H), 6.55 (s, 1H), 6.36 (dd, *J* = 17.2, 1.6 Hz, 1H), 6.09 (dd, *J* = 17.2, 10.4 Hz, 1H), 5.84 (dd, *J* = 10.6, 1.2 Hz, 1H), 5.60 (m, 1H), 5.45 (dd, *J* = 10.2, 4.8 Hz, 1H), 5.27 (d, *J* = 8.0 Hz, 1H), 5.11 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.07 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.58 (d, *J* = 12.8 Hz, 1H), 4.49 (d, *J* = 12.8 Hz, 1H), 4.41

(dd, *J* = 11.0, 3.2 Hz, 1H), 3.64 (d, *J* = 5.6 Hz, 1H), 3.49 (bs, 1H), 3.19 (qd, *J* = 5.6, 1.2 Hz, 1H), 3.08 (d, *J* = 1.6 Hz, 1H), 2.66 (m, 1H), 2.65 (s, 3H), 2.60-2.42 (m, 3H), 2.38-2.26 (m, 3H), 2.04 (s, 3H), 2.03 (m, 1H), 1.77 (m, 2H), 1.63 (m, 1H), 1.40 (m, 1H), 1.28 (m, 1H), 1.11 (d, *J* = 7.2 Hz, 3H), 1.0 (s, 3H), 0.99 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 219.6, 170.2, 165.9, 151.7, 138.9, 137.0, 133.4, 131.0, 128.4, 125.3, 119.2, 118.9, 115.8, 78.2, 73.6, 73.3, 71.6, 67.6, 57.7, 41.4, 40.1, 39.6, 37.6, 32.4, 31.5, 28.3, 24.6, 19.0, 16.0, 15.5, 14.6, 12.1. HRFABMS: calcd for $C_{32}H_{46}NO_7S$ (M+H) 588.2995, found 588.3007.

Internal lactone Epothilone 15. The epothilone analog **15** was prepared from diene **14**, using a similar procedure to that described for the conversion of **9** to **12**, as a colorless oil in approximately 40% yield (55% starting material was recovered). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (s, 1H), 6.50 (s, 1H), 6.27 (dd, *J* = 17.6, 1.2 Hz, 1H), 6.01 (dd, *J* = 17.6, 10.4 Hz, 1H), 5.61 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.35 (m, 1H), 5.32 (t, *J* = 4.4, 1H), 5.00 (dd, *J* = 12.0, 2.8 Hz, 1H), 4.86 (d, *J* = 14.8 Hz, 1H), 4.23 (d, *J* = 14.0 Hz, 1H), 3.80 (m, 1H), 3.66 (d, *J* = 16.4 Hz, 1H), 3.56 (m, 1H), 2.68 (s, 3H), 2.60-2.54 (m, 2H), 2.46 (d, *J* = 16.0 Hz, 1H), 2.42-2.32 (m, 3H), 2.21 (s, 3H), 1.78-1.64 (m, 4H), 1.34-1.22 (m, 3H), 1.20 (d, *J* = 6.8 Hz, 3H), 1.17 (s, 3H), 1.03 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.8, 174.2, 167.4, 166.1, 164.7, 153.2, 136.3, 134.8, 131.5, 128.2, 118.7, 118.5, 67.2, (there are three carbon's signals were hidden among solvent signal), 55.3, 43.8, 41.6, 36.7, 35.4, 29.5, 28.8, 27.4, 19.6, 17.9, 16.7, 16.4, 15.9. HRFABMS: calcd for $C_{31}H_{42}NO_8S$ (M+H) 588.2626, found 588.2611.

Internal lactone Epothilone 16. The epothilone analog **16** was prepared directly from triol **7** by the procedure described previously for the synthesis of **12** as a colorless oil in 17.7% yield (71.7% starting material was recovered). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (s, 1H), 6.62 (s, 1H), 5.48 (dd, *J* = 8.2, 7.4 Hz, 1H), 5.31 (dd, *J* = 6.0, 3.6 Hz, 1H), 5.05 (dd, *J* = 10.4, 2.7 Hz, 1H), 4.08 (d, *J* = 12.4 Hz, 1H), 4.01 (d, *J* = 12.9 Hz, 1H), 3.76 (m, 1H), 3.40 (br.d, 1H), 3.31 (d, *J* = 17.0 Hz, 1H), 3.28 (m, 1H), 2.70 (s, 3H), 2.70-2.68 (m, 3H), 2.46 (d, *J* = 17.0 Hz, 1H), 2.51-2.32 (m, 3H), 2.11 (s, 3H), 1.78-1.64 (m, 3H), 1.34-1.22 (m, 3H), 1.22 (d, *J* = 6.6 Hz, 3H), 1.19 (s, 3H), 1.03 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, CD_3CN) δ 212.2, 174.1, 168.2, 164.7, 152.1, 143.5, 134.7, 119.9, 119.1, 118.7, 77.9, 77.0, 70.3, 65.4, 55.7, 44.1, 41.3, 36.7, 35.8, 29.9, 28.3, 27.8, 26.6, 17.8, 17.7, 16.1, 15.9, 15.0. HRFABMS: calcd for $C_{28}H_{40}NO_7S$ (M+H) 534.2525, found 534.2513.

Scheme S1. Synthesis of compounds **8** – **16**



(4*R*)-4-Allyl-4-demethyl-epothilone B (19). To a stirred solution of 12,13-β-epoxy-triol **10** (14.1 mg, 0.02568 mmol) in dichloromethane (0.5 mL) at 0 ^{o}C was added Et_3N (11 μL, 0.077 mmol, 3 eq) followed by tosyl chloride (7.3 mg, 0.0385 mmol, 1.5 eq) and 4-DMAP (3.1 mg, 0.02568 mmol, 1.0 eq). The resulting reaction mixture was warmed to 25 ^{o}C and stirred for 1 h before saturated aqueous NH_4Cl

solution (10 mL) was added. The mixture was extracted with EtOAc (10 mL × 3), the combined organic extracts were dried (Na_2SO_4), and the solvents were removed *in vacuo*. The residue obtained was then dissolved in acetone (1 mL) and treated with NaI (12 mg, 0.07704 mmol, 3 eq). After stirring at 25 ^oC for 30 h, the solvent was removed under reduced pressure and the residue was purified by preparative thin layer chromatography (40% EtOAc in hexanes) to provide iodide (12.5 mg, 74% for two steps) as a colorless syrup. ^1H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.58 (s, 1H), 5.67 (m, 1H), 5.41 (d, *J* = 7.6 Hz, 1H), 5.14 (d, *J* = 16.8 Hz, 1H), 5.11 (d, *J* = 10.0 Hz, 1H), 4.41 (m, 1H), 4.09 (br.s, 1H), 3.69 (m, 1H), 3.38 (m, 1H), 3.31 (d, *J* = 10.4 Hz, 1H), 3.09 (d, *J* = 10.4 Hz, 1H), 3.01 (dd, *J* = 8.0, 3.2 Hz, 1H), 2.86 (br.s, 1H), 2.69 (s, 3H), 2.72-2.53 (m, 3H), 2.42-2.34 (m, 2H), 2.07 (s, 3H), 2.18-1.92 (m, 2H), 1.86-1.69 (m, 3H), 1.56-1.46 (m, 3H), 1.38-1.30 (m, 1H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.05 (s, 3H), 0.99 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.9, 170.8, 165.6, 151.7, 138.0, 133.5, 119.9, 119.2, 116.4, 77.0, 73.0, 71.8, 64.7, 63.1, 57.8, 42.3, 39.8, 39.7, 35.9, 32.8, 31.0, 30.5, 21.1, 19.2, 16.7, 16.0, 15.5, 12.6, 12.0. HRFABMS: calcd for $C_{29}H_{43}NO_6SI$ (M+H) 660.1856, found 660.1819.

The iodide prepared in the preceding step (12.5 mg, 0.018968 mmol) and sodium cyanoborohydride (12 mg, 0.18968 mmol, 10 eq) were dissolved in HMPA and the resulting mixture was heated at 45 ^oC for 43 h. After cooling to 25 ^oC, brine (10 mL) was added and the mixture was extracted with EtOAc (10 mL × 5). The combined organic extracts were dried (Na_2SO_4), the solvents were removed under reduced pressure, and the residue was passed through a short plug of silica gel to remove traces of HMPA (40% EtOAc in hexanes). The solvents was evaporated, and the residue was purified by preparative thin layer chromatography using 20% EtOAc in hexanes as eluent to provide pure epothilone B analog **19** (5 mg, 49.5%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.58 (s, 1H), 5.67 (m, 1H), 5.41 (d, *J* = 8.8 Hz, 1H), 5.14 (d, *J* = 17.2 Hz, 1H), 5.11 (d, *J* = 9.6 Hz, 1H), 4.41 (br.d, *J* = 9.2 Hz, 1H), 4.25 (br.s, 1H), 3.69 (br.s, 1H), 3.38 (m, 1H), 2.88 (br.s, 1H), 2.79 (dd, *J* = 6.8, 5.6 Hz, 1H), 2.69 (s, 3H), 2.66-2.55 (m, 3H), 2.42-2.33 (m, 1H), 2.18-2.142 (m, 1H), 2.07 (s, 3H), 1.94-1.86 (m, 1H), 1.82-1.69 (m, 2H), 1.69-1.46 (m, 2H), 1.42-1.34 (m, 3H), 1.28 (s, 3H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.06 (s, 3H), 0.99 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.9, 170.9, 165.6, 151.8, 138.5, 133.6, 119.57, 119.51, 116.2, 77.4, 72.9, 71.7, 63.1, 62.1, 57.8, 42.1, 39.8, 39.7, 36.0, 33.4, 32.3, 31.7, 22.8, 21.1, 19.2, 16.7, 16.0, 15.4, 11.9. HRFABMS: calcd for $C_{29}H_{44}NO_6S$ (M+H) 534.2889, found 534.2853.

Internal lactone 18. The epothilone B analog **18** was obtained from (4*R*)-4-allyl-4-demethyl-epothilone B (**19**) according to the procedure described previously for the preparation of **12**, as a colorless oil in 28% yield (56% starting material was recovered). ^1H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 6.63 (s,

1H), 5.41 (br.d, *J* = 5.6 Hz, 1H), 4.96 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.81 (m, 1H), 3.29 (d, *J* = 17.2 Hz, 1H), 3.25 (m, 1H), 2.83 (dd, *J* = 9.2, 4.4 Hz, 1H), 2.75 (dd, *J* = 16.8, 11.2 Hz, 1H), 2.70 (s, 3H), 2.56 (dd, *J* = 16.8, 3.2 Hz, 1H), 2.53 (d, *J* = 16.4 Hz, 1H), 2.26-2.21 (m, 1H), 2.17 (s, 3H), 1.86 (m, 1H), 1.76 (m, 1H), 1.68-1.52 (m, 4H), 1.48-1.21 (m, 3H), 1.27 (s, 3H), 1.23 (d, *J* = 6.4 Hz, 3H), 1.21 (s, 3H), 1.05 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.3, 172.5, 167.6, 165.0, 152.5, 136.1, 120.4, 117.7, 78.1, 77.7, 77.3, 62.2, 61.1, 54.9, 44.2, 41.3, 35.7, 35.5, 32.7, 32.5, 29.6, 23.1, 22.6, 19.4, 18.1, 17.2, 15.5, 15.0. HRFABMS: calcd for $C_{28}H_{40}NO_7S$ (M+H) 534.2525, found 534.2499.

Iodide 21. To a solution of primary alcohol **20**[1] (19 mg, 0.02497 mmol) in THF (2.5 mL) was added PPh_3 (13 mg, 0.0499 mmol, 2 eq), followed by imidazole (5.1 mg, 0.07491 mmol, 3 eq) and iodine (25 mg, 0.09988 mmol, 4 eq). The resulting reaction mixture was allowed to stir at 25 °C for 30 min prior to being quenched by the addition of saturated $Na_2S_2O_3$ solution (10 mL). The subsequent mixture was extracted with ether (10 mL × 3), the combined organic extracts were dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure. The residue obtained was purified by preparative thin layer chromatography eluting with 5% EtOAc in hexanes to furnish iodide **21** (19.3 mg, 89%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.56 (s, 1H), 5.61 (m, 1H), 5.48 (m, 1H), 5.04-5.01 (m, 3H), 4.12 (m, 1H), 4.00 (d, *J* = 9.0 Hz, 1H), 3.89 (d, *J* = 8.6 Hz, 2H), 3.05 (m, 1H), 2.81 (br.s 1H), 2.71 (s, 3H), 2.65 (m, 2H), 2.44-2.22 (m, 3H), 2.10 (s, 3H), 1.72-1.47 (m, 4H), 1.26-1.07 (m, 2H), 1.11 (d, *J* = 7.2 Hz, 3H), 1.10 (s, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.92 (s, 9 H), 0.82 (s, 9H), 0.11 (s, 3H), 0.07 (s, 3H), -0.009 (s, 3H), -0.17 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 214.3, 170.7, 164.8, 152.4, 143.9, 138.3, 132.9, 124.0, 119.7, 118.9, 116.3, 80.7, 74.5, 60.5, 56.8, 48.2, 40.9, 33.2, 31.2, 28.6, 27.0, 26.4, 26.2, 19.3, 18.7, 18.6, 15.4, 14.3, 13.1, -3.1, -3.35, -3.6, -5.3. HRFABMS: calcd for $C_{41}H_{71}NO_5SSi_2I$ (M+H) 872.3636, found 872.3594.

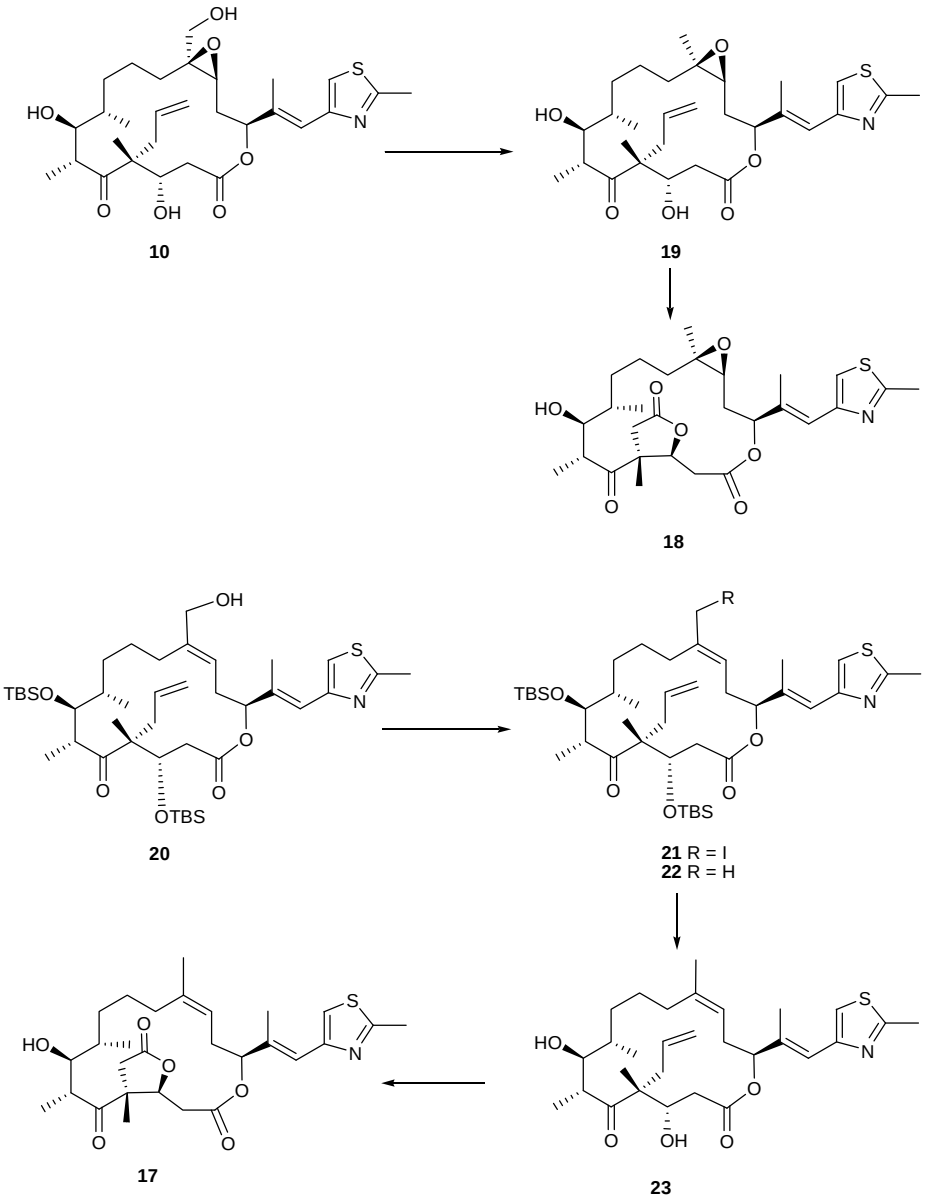
Protected epothilone D analog 22. Iodide **21** (19.3 mg, 0.022 mmol) and sodium cyanoborohydride (14 mg, 0.22 mmol, 10 eq) were dissolved in HMPA (0.2 mL) and the resulting mixture was heated at 45 °C for 22 h. After cooling to 25 °C, the reaction mixture was diluted with 0.2 mL of EtOAc and then directly loaded onto preparative thin layer chromatography, eluting with 15% EtOAc in hexanes, to yield pure epothilone D analog **22** (10 mg, 60.6%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.54 (s, 1H), 5.61 (m, 1H), 5.16 (t, *J* = 7.6 Hz, 1H), 5.05-4.97 (m, 3H), 4.12 (m, 1H), 3.90 (d, *J* = 8.6 Hz, 1H), 3.07 (m, 1H), 2.83 (br.s 1H), 2.70 (s, 3H), 2.68 (m, 2H), 2.45-2.33 (m, 3H), 2.10 (s, 3H), 1.73 (m, 1H), 1.66 (s, 3H), 1.12-0.82 (m, 2H), 1.11 (d, *J* = 7.2 Hz, 3H), 1.10 (s, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.94 (s, 9 H), 0.83 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H), -0.007 (s, 3H), -0.13 (s, 3H). ^{13}C NMR (125

MHz, $CDCl_3$) δ 214.4, 171.0, 164.6, 152.6, 147.0, 138.9, 133.0, 119.4, 119.1, 118.8, 115.9, 79.8, 74.7, 60.5, 56.8, 40.9, 32.7, 31.9, 31.4, 26.5, 26.3, 23.1, 21.1, 19.3, 18.8, 18.7, 15.5, 14.3, -3.1, -3.5, -3.6, -5.3. HRFABMS: calcd for $C_{41}H_{72}NO_5SSi_2$ (M+H) 746.4670, found 746.4688.

(4*R*)-4-Allyl-4-demethyl-epothilone D (23). To a solution of **22** (13.3 mg, 0.0134 mmol) in THF (6 mL) was added HF.Py (6 mL) at 0 °C. The resulting reaction mixture was allowed to warm up to 25 °C and the reaction was allowed to proceed for 24 h at 25 °C. The reaction was quenched by careful, portionwise addition into saturated aqueous $NaHCO_3$ solution (10 mL) with further addition of sufficient solid $NaHCO_3$ to ensure complete neutralization. The mixture was then extracted with EtOAc (10 mL × 3), the combined organic extracts were dried (Na_2SO_4), and the solvents were removed under reduced pressure to afford crude oil. Preparative thin layer chromatography of this oil over silica gel, eluting with 40% ethyl acetate in hexanes, gave **23** (7 mg, 76%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.54 (s, 1H), 5.60 (m, 1H), 5.23 (d, *J* = 8.8 Hz, 1H), 5.11-5.06 (m, 3H), 4.39 (br.d, *J* = 11.6 Hz, 1H), 3.66 (d, *J* = 5.2 Hz, 1H), 3.47 (d, *J* = 5.2 Hz, 1H), 3.17 (m, 2H), 2.66 (s, 3H), 2.65-2.44 (m, 4H), 2.31-2.20 (m, 3H), 2.03 (s, 3H), 1.87-1.74 (m, 3H), 1.62 (s, 3H), 1.32-1.20 (m, 2H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.00 (s, 3H), 0.99 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.4, 170.3, 165.0, 151.9, 139.3, 138.2, 133.2, 121.1, 119.0, 118.9, 115.5, 78.7, 73.7, 71.6, 57.5, 41.2, 39.8, 39.5, 38.1, 32.6, 31.5, 31.4, 24.6, 22.9, 19.0, 15.9, 15.4, 14.6, 12.4. HRFABMS: calcd for $C_{29}H_{44}NO_5S$ (M+H) 518.2940, found 518.2897.

Internal lactone epothilone D 17. The epothilone D analog **17** was prepared from (4*R*)-4-allyl-4-demethyl-epothilone D (**23**), according to the procedure described previously for the synthesis of **12** except that 1.2 equivalents of Grubbs' catalyst were used. The product was obtained as a colorless oil in 88% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (s, 1H), 6.60 (s, 1H), 5.27 (d, *J* = 8.8 Hz, 1H), 5.16 (dd, *J* = 8.8, 5.2 Hz, 1H), 5.05 (dd, *J* = 10.4, 3.3 Hz, 1H), 3.75 (m, 1H), 3.30 (d, *J* = 17.0 Hz, 1H), 3.27 (m, 1H), 2.73-2.62 (m, 2H), 2.70 (s, 3H), 2.51 (dd, *J* = 10.1, 3.5 Hz, 1H), 2.46 (d, *J* = 17.0 Hz, 1H), 2.18 (s, 3H), 1.90-1.84 (m, 1H), 1.72-1.63(m, 1H), 1.67 (s, 3H), 1.51-1.49 (m, 1H), 1.39-1.30 (m, 1H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.17 (s, 3H), 1.03 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.1, 172.9, 167.5, 164.8, 152.7, 138.5, 137.1, 120.8, 119.6, 117.3, 80.6, 78.1, 76.3, 55.3, 43.1, 41.0, 37.1, 35.9, 32.3, 32.0, 30.7, 26.8, 23.3, 19.4, 17.2, 16.8,15.4, 15.3,12.4. HRFABMS: calcd for $C_{29}H_{40}NO_6S$ (M+H) 518.2576, found 518.2549.

Scheme S2. Synthesis of compounds **17** – **23**



Antiproliferative measurements. Antiproliferative activity against A2780 ovarian cancer cells was determined using the Alamar Blue assay as previously described.[2]

In vitro microtubule assays. Promotion of tubulin assembly was measured in 96-well plates and ED_{50} values were calculated as described.[3] Inhibition constants for epothilone analogs binding to GMPcPP-stabilized microtubules were assessed by competition between the molecules and a fluorescent taxane (3'-*N*-*m*-aminobenzamido-3'-*N*-debenzamidopaclitaxel) as described.[4]

Molecular Modeling. The 3-D structures of epoD, **16**, **17**, and **18** were constructed to closely fit the electron crystallographic (EC) pose of epoA bound to tubulin.[5] The resulting structures were then fully

optimized with the MMFF/GBSA/H_2O force field[6] to provide the nearest local minima. The latter were separately subjected to 150,000 steps of Monte Carlo conformational searching with the MMFF/GBSA/H_2O force field within an energy window of 10 kcal/mol. The procedure delivers 29928, 6535 and 3452 converged conformers with global minima found 2, 16 and 13 times, respectively. ROCS[7] was subsequently applied to search for the corresponding EC-like conformers using the EC epoA conformer as target template. The top ROCS matches were flexibly Glide-docked[8,9] into the electron crystallographic structure of tubulin.[5] The best docking pose was chosen on the basis of the Emodel scoring function together with visualization to ensure a reasonable binding mode. Finally, 10 ps of molecular dynamics at 273K for each structure in the binding pocket was performed to remove short contacts between ligands and protein residues in the respective complexes. Relative binding free energies were estimated by MMGBSA energy calculations[10,11] for the refined docking complexes of **16**, **17**, and **18**. To investigate the physical properties of the ligands, Qikprop calculations were used to assess MDCK and Caco-2 permeabilities for the final docked ligands.

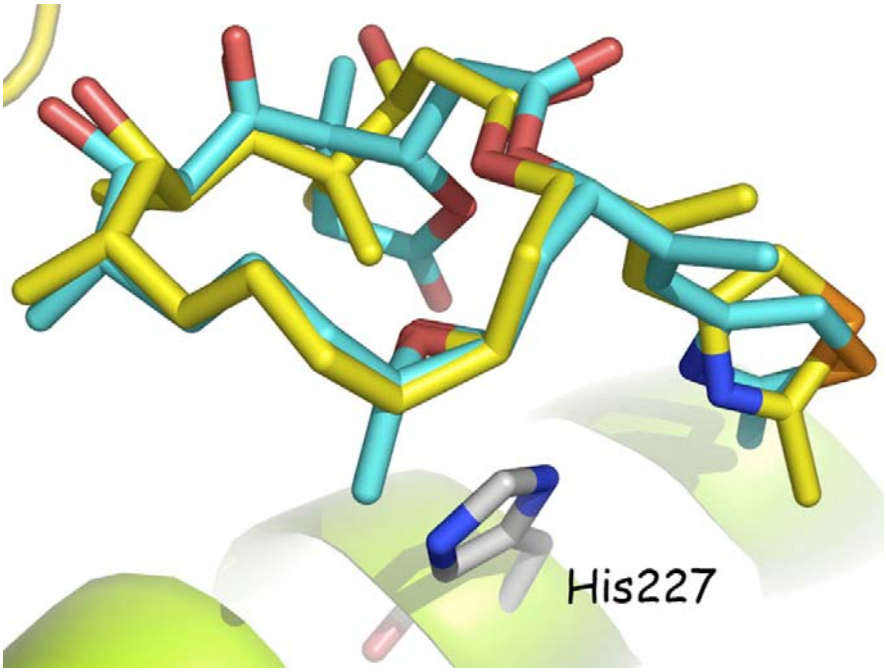
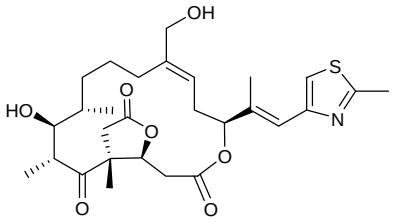
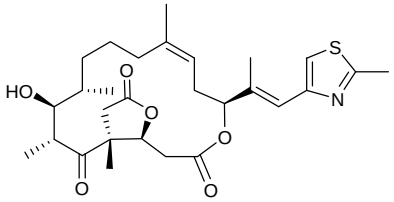
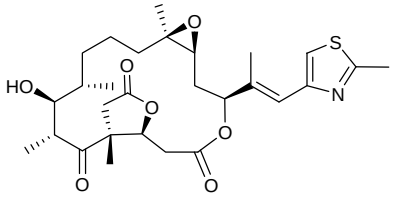


Figure S1. MMFF-energy minimized structure of lactone **18** (cyan) superimposed on the EC-determined conformation of epoA (yellow) in tubulin.

Permeability Calculations for Epothilones with QikProp.[8]

Table 2. Permeability and Binding Energy Calculations (MMGBSA) (3.4) for **16**, **17**, **18**, and EpoD.

	Caco-2 permeability[a] (nm/sec)	MDCK permeability[a] (nm/sec)	ΔG from MMGBSA (Kcal/mol)
 **16**	188	150	-18
 **17**	434	370	-21
 **18**	392	332	-23
EpoD	535	464	-22

[a] Caco-2 and MDCK <25 poor, >500 great.

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