Isolation and Characterization of New Epothilone Analogues from Recombinant *Myxococcus xanthus* Fermentations

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Nine new epothilone analogues (**6**−**8**, **10**, **11a**, **11b**, **12**, **13**, and **15**) were isolated from fermentations of *Myxococcus xanthus* strains engineered with modified polyketide synthase genes. The epothilone structures were elucidated primarily through interpretation of 1D and 2D NMR data. 4-Desmethyl-10,11-didehydroepothilone D (**6**) displayed activity against several tumor cell lines, including a multi-drug-resistant cell line.

Epothilones are cytotoxic polyketide natural products originally isolated from the bacterium *Sorangium cellulosum*[1] and found to kill dividing cells by stabilizing microtubules.[2] While epothilones appear to share a common tubulin binding site with the cancer drug paclitaxel,[3] they are active against several paclitaxel-resistant cell lines,[4] making them attractive candidates for development as new cancer drugs. The syntheses and semisyntheses of several epothilone analogues have been achieved, allowing a general structure−activity relationship to be established.[5−7] In addition to the synthetically derived analogues, many naturally occurring epothilone biosynthetic side products have been isolated from large-scale fermentations of the producing organism.[8]

The polyketide synthase gene (PKS) cluster responsible for the biosynthesis of epothilone B (**2**) has been cloned[9] and expressed in *Myxococcus xanthus*.[10,11] Deactivation of the EpoK gene[9] encoding a cytochrome P_{450} in this strain resulted in the production of epothilone D (**4**), an analogue that lacks the epoxide moiety and displays a higher therapeutic index.[12] To produce additional epothilone analogues, three other mutants were generated. To create the first two mutants, the enoyl reductase domain ER5 was deactivated in the **2**- and the **4**-producing *M. xanthus* strains to create strains K165-79.7 and K165-76.2, respectively; each of these strains was designed to produce the 10,11-didehydro analogue of the parent compound.[13] The third mutant was generated by deactivating the ketoreductase domain KR6 of the **4**-producing strain to generate strain K39-164, which produces 9-keto-epothilone D (**9**).[13] Like the wild-type *S. cellulosum* strain, these mutants each produced several analogues in addition to the major epothilone product. Here we describe the isolation, structure determination, and cytotoxicity of several of these side products.

Results and Discussion

In addition to 10,11-didehydroepothilone D (**5**), three new compounds were isolated from a 1000 L fermentation of *M. xanthus* K165-76.2. Epothilone-containing sidestreams from the purification of **5** from this fermentation were pooled and further separated by reversed-phase MPLC and HPLC to yield 4-desmethyl-10,11-didehydroepothilone D (**6**), 6-desmethyl-10,11-didehydroepothilone D (**7**), and 9-keto-10,11-didehydroepothilone D (**8**), all as yellow oils.

Compound **6** has a molecular formula of $C_{26}H_{37}NO_5S$, consistent with a structure having one fewer methyl group than **5**. The ^{1}H NMR spectrum contained six methyl signals, three of which appeared as well-resolved doublets (J = 6−7 Hz). This loss of a methyl group combined with the introduction of splitting in one of the remaining methyl signals suggested that compound **6** lacks the *gem*-dimethyl characteristic of the epothilones. Examination of the 2D NMR spectra confirmed this hypothesis; only one methyl group displayed HMBC coupling to C-4, and the protons of the remaining methyl (H-22 or H-23) were coupled to H-4 (δ 3.19). The 4-desmethyl analogues of several epothilones have been identified previously.[8]

Compound **7** is isomeric with **6**. In the ^{1}H NMR spectrum of **7**, a doublet methyl signal and the quartet of doublets generally observed for H-6 in epothilones were both absent, suggesting that there is no methyl group at position 6. 1D and 2D NMR spectra measured in $CDCl_3$ were consistent with the rest of the structure being identical to **5**, but overlapping signals prevented unambiguous attachment of the remaining doublet methyl to C-8. To alleviate this problem, the ^{1}H, ^{13}C, HSQC, and COSY NMR data were again collected, this time using CD_3CN as the NMR solvent. In CD_3CN, one of the H-9 protons (δ 2.28) was well-resolved from the other signals, and a COSY correlation between this proton and H-10 (δ 5.73) confirmed its identity. Additional COSY correlations between H-9 and H-8 and between H-8 and the H-25 methyl confirmed that the doublet methyl is attached to C-8.

A molecular formula of $C_{27}H_{37}NO_6S$ for compound **8** was established by the ^{13}C NMR spectrum and HRMS data. In addition to the characteristic epothilone UV chromophore (λ_{max} 247 nm), **8** exhibited an absorption peak at 286 nm, indicative of extended conjugation. The ^{13}C NMR spectrum displayed a ketone function (δ 206.5) not present in **5**. HMBC correlations between this additional ketone carbon and H-10, H-11, and H-25 located it at position 9. Additional HSQC, HMBC, and COSY correlations were consistent with the rest of the structure being identical to **5**.

Compound **15** was isolated from a 5 L fermentation of *M. xanthus* strain K165-79.7 that had been grown with dissolved oxygen maintained at 50% of saturation.[14] XAD-16 resin harvested from the fermentation was eluted with methanol, and successive MPLC and HPLC steps yielded **15**. This compound has a molecular formula of $C_{27}H_{40}NO_6S$, consistent with the incorporation of one oxygen atom into **5**. The ^{13}C NMR spectrum indicated one double bond fewer than in **5**, suggesting that **15** contains an additional ring. HSQC, HMBC, COSY, and TOCSY data allowed unam-

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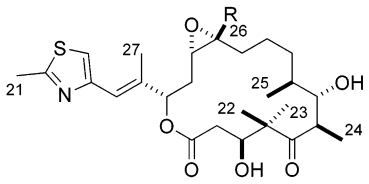
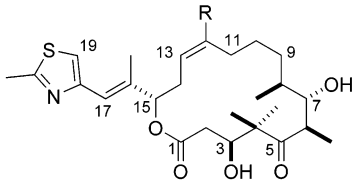
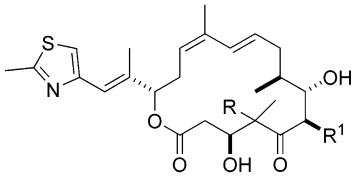
Chart 1



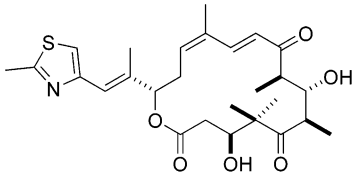
epothilone A (**1**) R=H
epothilone B (**2**) R=CH_3



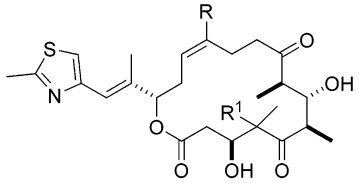
epothilone C (**3**) R=H
epothilone D (**4**) R=CH_3



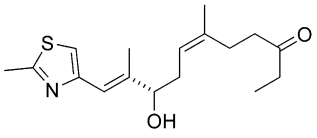
10,11-didehydroepothilone D (**5**) R=R^1=CH_3
4-desmethyl-10,11-didehydroepothilone D (**6**) R=H, R^1=CH_3
6-desmethyl-10,11-didehydroepothilone D (**7**) R=CH_3, R^1=H



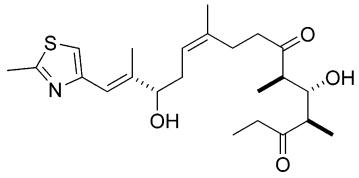
9-keto-10,11-didehydroepothilone D (**8**)



9-keto-epothilone D (**9**) R=R^1=CH_3
4-desmethyl-9-keto-epothilone C (**10**) R=H, R^1=H
4-desmethyl-9-keto-epothilone D (**11a**, **11b**) R=CH_3, R^1=H

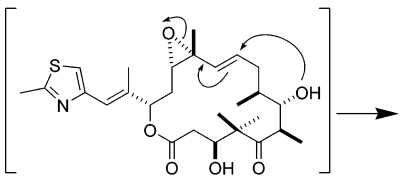


12

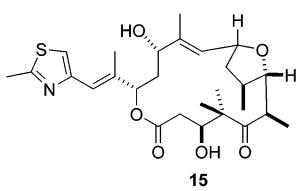


13

biguous assignment of the carbon connectivity. The chemical shifts of C-3, C-7, C-10, and C-13 indicated that all four are bonded to oxygen atoms; to account for the molecular formula, two of these oxygenated carbons must be part of a cyclic ether. The signals of the hydroxyl protons at positions 3 and 13 were visible in the ^{1}H NMR spectrum, and their HMBC and COSY correlations to neighboring atoms confirmed their assignments. The cyclic ether, therefore, must include carbons 7 and 10, indicating a tetrahydrofuran structure. Significant Na^+ and K^+ adducts in the mass spectrum further supported the postulated molecular formula by suggesting that the ion observed at *m*/*z* 506.2571 is the $[M + H]^+$ ion and not a fragment generated upon ionization of a hypothetical parent diol. Unfortunately, no HMBC correlation linking H-7 and H-10 across the ether bridge was observed. It is chemically reasonable, however, that this fermentation initially generated its expected product, **14**, which then underwent a vinylogous Payne rearrangement to yield **15** (Figure 1). On the basis of a strong NOESY signal between H-26 and H-10, the newly generated 11,12 double bond was determined to be of the *E*-configuration. The stereochemistry at positions 7 and 13 is presumed to be the same as in **2**.



10,11-didehydroepothilone B (**14**)



15

Figure 1. Hypothetical cyclization of **14** to **15**.

Compounds **10**−**13** were purified from the combined products of a 1000 L and a 500 L fermentation of *M. xanthus* strain K39-164, the major products of which were **3** and its epimer at the 8-position (3.4 g total). Purification sidestreams were pooled and then further separated by reversed-phase MPLC and HPLC to yield 4-desmethyl-9-keto-epothilone C (**10**), two diastereomers of 4-desmethyl-9-keto-epothilone D (**11a** and **11b**), and two epothilone fragments (**12** and **13**).

Compound **10** has a molecular formula of $C_{25}H_{35}NO_6S$. Its ^{1}H NMR spectrum displays five methyl groups, including three doublets, indicative of a single methyl group attached to C-4. The C-26 methyl group observed at δ 1.65 in **4** is absent, and an additional olefinic signal is present at δ 5.50, suggeting that **10** is an analogue of epothilone C (**3**).[8] The additional 1D and 2D NMR data were consistent with this structure.

Diastereomers **11a** and **11b** each have a molecular formula of $C_{26}H_{37}NO_6S$. Their ^{1}H NMR spectra each contained signals for six methyl groups, including a doublet methyl attached to C-4. The 1D and 2D NMR data confirmed the assignment of the C-4 doublet methyl group and were consistent with the rest of the structure being identical with **9**. It has been observed previously that naturally occurring 4-desmethyl epothilones often exist as pairs of diastereomers epimeric at position 4,[8] and we presume that **11a** and **11b** differ only in the stereochemistry of the methyl group attached to C-4.

Compound **12** has a molecular formula of $C_{17}H_{25}NO_2S$. Despite its relatively small size, it displayed the characteristic UV spectrum of epothilones, suggesting that it is an epothilone fragment. Its ^{1}H NMR spectrum displayed four methyl groups, including a triplet signal (δ 1.02)

Table 1. Cytotoxicity of Epothilones against Human Cancer-Derived Cell Lines (IC_{50}, nM)[a,b]

	cell line			
compound	MCF-7	NCI/ADR	SF-268	NCI-H460
3	97 ± 5	310 ± 7	340 ± 40	280 ± 20
4	16 ± 1	44 ± 2	18 ± 8	30 ± 1
5	47 ± 3	250 ± 30	92 ± 6	47 ± 1
6	250 ± 14	395 ± 35	390 ± 1	345 ± 7
9	500−1000	>1000	>1000	>1000

[a] For details of cell lines used, see Experimental Section. [b] Compounds **7**, **8, 10**, **11a**, **11b**, **12**, **13**, and **15** exhibited IC_{50} values of >1000 nM for all cell lines in the panel.

indicative of an ethyl group; its ^{13}C NMR spectrum displayed one ketone function, but no ester carbonyl. HSQC, COSY, and HMBC correlations placed the carbon at δ 35.9 in the ethyl group, and H-8 and H-9 showed correlations to C-9. These data were consistent with a truncated epothilone terminating at the C-8 methyl group. Chemical shifts and 2D correlations for the rest of the molecule were consistent with this structure.

Compound **13** has a molecular formula of $C_{23}H_{35}NO_4S$. Like **12**, **13** is an epothilone fragment terminating in an ethyl group. Its ^1H and ^{13}C spectra indicated six methyl groups and two ketone carbonyls, but no ester carbonyl. The ethyl protons H-22 and H-4 showed HMBC correlations to the C-5 ketone, indicating chain termination at the methyl attached to C-4. Chemical shifts and 2D correlations were similar to those in **12**.

The cytotoxicity of each of the new epothilones was measured against several human tumor-derived cell lines, including a multi-drug-resistant breast tumor line (NCI/ADR); results of these assays are listed in Table 1. In each case, oxidation of the C-9 methylene to a ketone eliminated cytotoxicity. The most active of the new analogues was compound **6**; this finding is consistent with the findings of Hardt and co-workers that cell growth inhibition activity of epothilones is relatively insensitive to removal of C-4 methyl groups.[8] However, aside from this moderate residual activity of **6**, none of the analogues presented here displayed significant cytotoxicity.

Each of the minor products of epothilone fermentations described here was isolated in an amount ranging from 1% to 12% of the amount of the major epothilone isolated (except for compound **15**, which was isolated as the major fermentation product). This large proportion of "unintended" products in our engineered *M. xanthus* strains can be compared with that measured for the naturally occurring producer *S. cellulosum*, which produces epothilone A (**1**), **2**, **4**, and **9**; extracts from this strain have been shown to contain at least 35 additional epothilone products, each isolated in amounts from 0.02% to 0.3% of that of the major product.[8] This apparent difference in biosynthetic fidelity may suggest that the engineered strains have a diminished substrate or reaction specificity as compared with wild-type strains. It is also possible that the unnatural epothilone intermediates are bound in less productive conformations by PKS modules including acyltransferases, ketoreductases, and methyltransferases, thereby resulting in truncated polyketide products. While continuing research should address the factors affecting the specificity of engineered PKSs, these side products can further the continuing search for novel polyketide "natural" products with beneficial biological activities.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded in $CDCl_3$ solution (unless otherwise noted) at 300 K with a Bruker DRX 400 spectrometer equipped with a QNP *z*-axis gradient probehead. Chemical shifts were referenced to δ 7.26 and 77.0 for ^1H and ^{13}C spectra, respectively. Chemical shifts for samples in CD_3CN were referenced to δ 1.94 and 1.24 for ^1H and ^{13}C spectra, respectively. For each compound, ^1H, ^{13}C, COSY, HMBC, and multiplicity-edited HSQC experiments were carried out. HRESIMS were obtained by manual peak matching versus internal standards by high-resolution mass spectrometry using an Applied Biosystems Mariner TOF spectrometer configured with a Turbo-Ionspray source in positive-ion mode. Preparative, isocratic, HPLC was carried out on a Polaris C_{18} column (MetaChem, 10 μm, 21.2 × 250 mm).

Isolation of 6−8. Compound **5** was isolated from a 1000 L fermentation of *M. xanthus* K165-76.2 using the method described for **4**.[15] During that isolation, sidestreams from the defatting and C_{18} columns were observed by diode-array HPLC and LC-MS to contain epothilone derivatives. Sidestreams from the defatting column were chromatographed on C_{18} sorbent (Bakerbond, 40 μm) with a H_2O−MeOH gradient to yield two epothilone-containing pools. Each pool was further purified by preparative HPLC in 50% MeCN to yield **6** (18 mg) and **8** (32 mg). A C_{18} sidestream fraction from the purification of **5** was further fractionated by two rounds of preparative HPLC, first in 50% MeCN and then in 67% MeOH, to yield **7** (11 mg).

Isolation of 15. The XAD resin harvested from a 5 L fermentation of *M. xanthus* K165-79.7 was eluted with MeOH, and the extract was chromatographed on Diaion HP-20SS (Supelco) sorbent using a H_2O−MeOH gradient. The fraction eluting in 75% MeOH was chromatographed on C_{18} in 65% MeOH and then further purified by preparative HPLC in 30% MeCN to yield **15** (4 mg).

Isolation of 10−13. Sidestreams from the purification of **9** from a 1000 L and a 500 L fermentation of *M. xanthus* K39-164 were observed by diode-array HPLC and LC-MS to contain additional epothilones and were pooled together. The resulting pool was chromatographed on HP-20SS sorbent with a H_2O−MeOH gradient; the epothilone-containing fractions were pooled and further separated into five fractions by C_{18} chromatography in 55% MeOH. Additional C_{18} chromatography, followed by preparative HPLC, of each of the pools yielded compounds **10** (104 mg), **11a** (406 mg), **11b** (240 mg), **12** (62 mg), and **13** (32 mg).

Cytotoxicity Measurements. For cytotoxicity measurements, stock epothilone solutions were prepared at 10 mM in DMSO and stored at −20 °C. Human breast carcinoma cell line MCF-7, multi-drug-resistant breast carcinoma cell line NCI/ADR, non-small-cell lung carcinoma cell line NCI-H460, and glioma cell line SF-268 were obtained from the National Cancer Institute. All cell lines were maintained in RPMI-1640 medium (Gibco/BRL, Rockville, MD) supplemented with 2 mM L-glutamine, 25 mM HEPES, and 10% FBS (Hyclone, Logan, UT). Cells were maintained in a humidified incubator at 37 °C in 5% CO_2. Tumor cells were seeded in 100 μL at 5000 (MCF-7), 7500 (NCI/ADR), 5000 (NCI-H460), and 7500 (SF-268) cells per well in 96-well plates. Cells were allowed to adhere for 24 h. Each compound ranging from 0.001 to 1000 nM in 100 μL was added to cells in triplicate wells. After 3 days, cells were fixed at 4 °C for 1 h with 10% trichloroacetic acid and then stained with 0.2% sulforhodamine B (SRB)/1% acetic acid for 20 min at room temperature. The cells were washed with 1% acetic acid to remove unbound dye, and the bound SRB was then extracted with 200 μL of 10 mM Tris base. The UV absorbance was measured at 515 nm using a 96-well microtiter plate reader (Spectra Max 250, Molecular Devices).

4-Desmethyl-10,11-didehydroepothilone D (6): yellow oil; $[\alpha]_D$ −36.3° (*c* 0.003, MeOH); UV (MeOH) λ_{max} (ϵ) 213 (22300), 242 (27600) nm; IR (film) ν_{max} 3468, 2932, 1706, 1507, 1456, 1376, 1251, 1182, 980 cm^{-1}; ^1H NMR δ 7.00 (1H, s, H-19), 6.70 (1H, br s, H-17), 6.42 (1H, br d, J = 15.5 Hz, H-11), 5.72 (1H, ddd, J = 15.5, 9.5, 5.0 Hz, H-10), 5.24 (2H, m, H-13, H-15), 4.36 (1H, dt, J = 8.0, 5.0 Hz, H-3), 3.92 (1H, m, H-7), 3.19

Table 2. ^{13}C NMR Chemical Shifts of Epothilone Analogues ($CDCl_3$, 100 MHz)

carbon	6	7	8	10	11a	11b	12	13	15
1	169.9	169.8	170.6	170.3	170.2	170.4			170.8
2	38.7	37.7	38.1	39.1	39.1	39.2			38.4
3	68.5	71.7	73.3	69.9	68.9	70.8			70.5
4	49.7	53.5	52.1	48.8	48.2	48.5		35.2	53.0
5	216.6	216.8	219.2	216.8	216.5	217.4		215.6	216.7
6	47.6	37.7	46.7	49.1	49.2	49.8		48.4	43.1
7	72.6	69.2	75.5	70.0	70.5	70.1		71.1	84.4
8	37.1	37.0	46.5	51.4	50.4	51.5	35.9	47.9	36.1
9	35.8	38.6	206.5	212.2	213.3	212.5	211.4	214.5	41.5
10	129.7	130.7	126.4	38.4	38.8	37.1	40.2	39.8	75.1
11	129.1	127.5	135.2	20.9	25.6	25.6	25.9	25.7	124.1
12	135.9	135.6	139.6	131.1	136.4	137.6	136.7	136.7	141.5
13	122.4	123.3	134.2	125.2	120.1	120.1	121.7	122.0	74.8
14	32.0	31.9	32.0	30.4	30.4	31.5	34.0	34.1	36.6
15	78.7	77.2	77.4	78.7	77.1	79.8	77.0	77.2	77.2
16	138.3	142.0	138.0	136.6	136.6	136.2	141.8	141.8	138.2
17	119.0	115.4	119.0	120.1	119.1	121.3	118.7	118.8	118.9
18	151.4	150.0	150.5	151.9	151.9	152.1	152.7	152.8	152.4
19	116.0	115.4	116.2	116.3	115.8	116.6	115.3	115.5	116.4
20	165.4	166.0	165.0	164.8	164.7	164.9	164.4	164.6	164.7
21	18.7	17.9	18.7	19.0	18.8	19.2	19.0	19.2	19.2
22	11.8	16.8	21.7	13.0	11.5	13.8		7.6	19.6
23		21.9	22.5						20.9
24	11.3		15.9	8.2	8.9	8.3		11.8	11.6
25	16.9	15.6	14.9	14.0	13.3	14.1	25.9	12.4	17.0
26	21.3	20.8	20.1		22.7	23.7	21.1	23.3	16.6
27	15.7	16.6	15.6	15.1	15.6	14.9	14.4	14.5	16.2

(1H, qd, J = 7.0, 5.5 Hz, H-4), 2.95 (1H, qd, J = 7.0, 4.5 Hz, H-6), 2.73 (3H, s, H-21), 2.65 (1H, m, H-14a), 2.54 (1H, dd, J = 15.5, 8.0 Hz, H-2a), 2.44 (1H, m, H-9b), 2.40 (1H, m, H-2b), 2.35 (1H, m, H-14b), 2.12 (1H, m, H-9a), 2.08 (3H, br s, H-27), 1.83 (1H, m, H-8), 1.76 (3H, s, H-26), 1.20 (3H, d, J = 7.0 Hz, H-24), 1.10 (3H, d, J = 7.0 Hz, H-22), 1.01 (3H, d, J = 6.5 Hz, H-25); ^{13}C NMR, see Table 2; HRESIMS m/z 476.2464 [M + H]$^+$ (calcd for $C_{26}H_{38}NO_5S$, 476.2465).

6-Desmethyl-10,11-didehydroepothilone D (7): yellow oil; $[\alpha]_D$ −53.2° (c 0.0079, MeOH); UV (MeOH) λ_{max} (ϵ) 215 (24100), 240 (34000) nm; IR (film) ν_{max} 3400, 2923, 1732, 1506, 1378, 1251, 1185, 1036, 966, 852 cm^{-1}; ^1H NMR δ 7.02 (1H, s, H-19), 6.70 (1H, br s, H-17), 6.51 (1H, dd, J = 15.5, 1.5 Hz, H-11), 5.69 (1H, ddd, J = 15.5, 10.5, 3.5 Hz, H-10), 5.25 (2H, m, H-13, H-15), 4.31 (1H, dd, J = 11.0, 2.5 Hz, H-3), 4.00 (1H, ddd, J = 11.0, 2.5, 1.5 Hz, H-7), 2.77 (3H, s, H-21), 2.75 (1H, m, H-14a), 2.50 (1H, dd, J = 17.5, 1.5 Hz, H-6a), 2.35 (3H, m, H-2a, H-9a, H-6b), 2.25 (1H, dd, J = 14.0, 6.0 Hz, H-14b), 2.13 (1H, m, H-2b), 2.08 (3H, br s, H-27), 2.05 (2H, m, H-8, H-9b), 1.81 (3H, br s, H-26), 1.26 (3H, s, H-23), 1.00 (3H, s, H-22), 0.99 (3H, d, J = 7.0 Hz, H-25); ^1H NMR (CD_3CN) δ 7.13 (1H, br s, H-19), 6.63 (1H, dd, J = 15.5, 2.0 Hz, H-11), 6.55 (1H, br s, H-17), 5.73 (1H, ddd, J = 15.5, 10.5, 3.5 Hz, H-10), 5.29 (dd, J = 11.0, 5.5 Hz, H-13), 5.17 (1H, dd, J = 11.0, 1.0 Hz, H-15), 4.33 (1H, br d, J = 10.5 Hz, H-3), 3.92 (1H, dt, J = 10.5, 2.5 Hz, H-7), 2.89 (1H, dt, J = 14.0, 11.0 Hz, H-14a), 2.66 (3H, s, H-21), 2.64 (1H, m, H-6a), 2.53 (1H, dd, J = 17.5, 2.5 Hz, H-6b), 2.28 (1H, br d, J = 15.0 Hz, H-9), 2.18 (3H, m, H-2, H-9, H-14), 2.14 (3H, d, J = 1.5 Hz, H-27), 2.00 (1H, d, J = 2.5 Hz, H-2), 1.95 (1H, m, H-8), 1.80 (3H, br s, H-26), 1.18 (3H, s, H-23), 0.95 (3H, d, J = 7.0 Hz, H-25), 0.91 (3H, s, H-22); ^{13}C NMR, see Table 2; ^{13}C NMR (CD_3CN) δ 216.9 (C-5), 170.6 (C-1), 165.7 (C-20), 153.6 (C-18), 139.1 (C-16), 136.1 (C-12), 131.5 (C-10), 128.6 (C-11), 124.5 (C-13), 119.9 (C-17), 117.8 (C-19), 79.0 (C-15), 72.4 (C-3), 70.1 (C-7), 54.2 (C-4), 39.4 (C-2), 38.8 (C-6), 38.1 (C-8), 38.1 (C-9), 32.7 (C-14), 21.4 (C-23), 20.9 (C-26), 19.3 (C-21), 17.3 (C-23), 16.2 (C-25), 15.3 (C-27); HRESIMS m/z 476.2453 [M + H]$^+$ (calcd for $C_{26}H_{38}NO_5S$, 476.2465).

9-Keto-10,11-didehydroepothilone D (8): yellow oil; $[\alpha]_D$ −84.2° (c 0.0052, MeOH); UV (MeOH) λ_{max} (ϵ) 210 (17500), 247 (14900), 286 (10600) nm; IR (film) ν_{max} 3467, 2975, 2934, 1734, 1687, 1624, 1589, 1508, 1452, 1377, 1254, 1181, 1152, 1047, 978 cm^{-1}; ^1H NMR δ 7.65 (1H, d, J = 16.0 Hz, H-11), 7.00 (1H, s, H-21), 6.56 (1H, br s, H-17), 6.22 (1H, d, J = 16.0 Hz, H-10), 5.87 (1H, t, J = 9.0 Hz, H-13), 5.26 (1H, d, J = 9.0 Hz, H-15), 3.88 (1H, m, H-3), 3.85 (1H, m, H-7), 3.11 (1H, qd, J = 7.0, 7.0 Hz, H-6), 3.03 (1H, qd, J = 7.5, 2.5 Hz, H-8), 2.87 (1H, m, H-14a), 2.73 (3H, s, H-21), 2.58 (1H, dd, J = 16.5, 1.5 Hz, H-2a), 2.44 (1H, dd, J = 14.0, 8.0 Hz, H-14b), 2.33 (1H, dd, J = 16.5, 11.5 Hz, H-2b), 2.11 (3H, s, H-27), 1.86 (3H, s, H-26), 1.36 (3H, d, J = 7.5 Hz, H-24), 1.24 (3H, s, H-23), 1.23 (3H, d, J = 7.0 Hz, H-24), 1.11 (3H, s, H-22); ^{13}C NMR, see Table 2; HRESIMS m/z 504.2408 [M + H]$^+$ (calcd for $C_{27}H_{38}NO_6S$, 504.2414).

4-Desmethyl-9-keto-epothilone C (10): yellow oil; $[\alpha]_D$ −9.2° (c 0.0493, MeOH); UV (MeOH) λ_{max} (ϵ) 212 (25140), 248 (20600) nm; IR (film) ν_{max} 3435, 2974, 2937, 1704, 1506, 1455, 1374, 1265, 1182, 1053, 1001 cm^{-1}; ^1H NMR δ 6.95 (1H, s, H-19), 6.54 (1H, s, H-17), 5.50 (1H, m, H-12), 5.41 (1H, m, H-13), 5.33 (1H, dd, J = 6.5, 5.5 Hz, H-15), 4.17 (1H, q, J = 5.5 Hz, H-3), 4.12 (1H, d, J = 10.5 Hz, H-7), 2.92 (1H, qd, J = 6.5, 6.5 Hz, H-4), 2.66 (3H, s, H-21), 2.60 (1H, m, H-8), 2.50 (2H, m, H-2), 2.45 (1H, m, H-6), 2.35 (1H, m, H-11a), 2.26 (1H, m, H-11b), 2.04 (3H, d, J = 1.0 Hz, H-27), 1.23 (3H, d, J = 7.0 Hz, H-25), 1.08 (3H, d, J = 7.0 Hz, H-24), 1.04 (3H, d, J = 7.0 Hz, H-22); ^{13}C NMR, see Table 2; HRESIMS m/z 478.2278 [M + H]$^+$ (calcd for $C_{25}H_{36}NO_6S$, 478.2258).

4-Desmethyl-9-keto-epothilone D, diastereomer 1 (11a): yellow oil; $[\alpha]_D$ −79.8° (c 0.0151, MeOH); UV (MeOH) λ_{max} (ϵ) 210 (30200), 248 (22500) nm; IR (film) ν_{max} 3488, 2973, 2939, 1706, 1507, 1454, 1376, 1250, 1181, 1040, 1004, 980 cm^{-1}; ^1H NMR δ 6.91 (1H, s, H-19), 6.47 (1H, s, H-17), 5.38 (1H, t, J = 5.0 Hz, H-15), 5.08 (1H, t, J = 6.0 Hz, H-13), 4.08 (1H, m, H-3), 4.06 (1H, m, H-7), 2.95 (1H, dq, J = 6.5, 5.5 Hz, H-4), 2.66 (2H, m, H-10), 2.64 (1H, m, H-8), 2.62 (3H, s, H-21), 2.57 (1H, m, H-14a), 2.53 (1H, m, H-2a), 2.50 (1H, m, H-6), 2.48 (1H, m, H-11a), 2.45 (2H, m, H-2b, H-14b), 2.00 (1H, m, H-11b), 1.95 (3H, s, H-27), 1.59 (3H, s, H-26), 1.14 (3H, d, J = 7.0 Hz, H-25), 1.07 (3H, d, J = 6.5 Hz, H-22), 1.00 (3H, d, J = 6.5 Hz, H-24); ^{13}C NMR, see Table 2; HRESIMS m/z 492.2406 [M + H]$^+$ (calcd for $C_{26}H_{38}NO_6S$, 492.2414).

4-Desmethyl-9-keto-epothilone D, diastereomer 2 (11b): yellow oil; $[\alpha]_D$ +24.8° (c 0.0527, MeOH); UV (MeOH) λ_{max} (ϵ) 212 (25580), 248 (19600) nm; IR (film) ν_{max} 3444, 1968, 1448, 1373, 1250, 1162, 974, 841 cm^{-1}; ^1H NMR δ 6.98 (1H, s, H-19), 6.54 (1H, s, H-17), 5.26 (1H, t, J = 6.0 Hz, H-15), 5.09 (1H, t, J = 2.5 Hz, H-13), 4.19 (1H, d, J = 10.0 Hz, H-7), 4.15 (1H, m, H-3), 2.95 (1H, qd, J = 7.0, 7.0 Hz, H-4), 2.69 (3H, s, H-21), 2.66 (1H, m, H-8), 2.55 (2H, m, H-2), 2.48 (1H, m, H-6), 2.45 (3H, m, H-10b, H-14), 2.40 (1H, m, H-11a), 2.12 (1H, m, H-11b), 2.07 (3H, d, J = 1.0 Hz, H-27), 1.68 (3H, s, H-26), 1.27 (3H, d, J = 7.0 Hz, H-25), 1.13 (3H, d, J = 7.0 Hz, H-24), 1.10 (3H, d, J = 7.0 Hz, H-22); ^{13}C NMR, see Table 2; HRESIMS m/z 492.2428 [M + H]$^+$ (calcd for $C_{26}H_{38}NO_6S$, 492.2414).

Hydroxyketone 12: yellow oil; $[\alpha]_D$ +1.8° (c 0.0274, MeOH); UV (MeOH) λ_{max} (ϵ) 211 (17600), 247 (12800) nm; IR (film) ν_{max} 3416, 2973, 2935, 1711, 1507, 1448, 1376, 1184, 1114, 1028 cm^{-1}; ^1H NMR δ 6.90 (1H, s, H-19), 6.52 (1H, br s, H-17), 5.17 (1H, t, J = 7.0 Hz, H-13), 4.11 (1H, t, J = 6.5 Hz, H-15), 2.66 (3H, s, H-21), 2.45 (2H, m, H-10), 2.38 (2H, q, J = 7.5 Hz, H-8), 2.31 (2H, m, H-14), 2.30 (1H, m, H-11a), 2.26 (1H, m, H-11b), 1.98 (3H, d, J = 1.0 Hz, H-27), 1.64 (3H, d, J = 1.0 Hz, H-26), 1.00 (3H, t, J = 7.5 Hz, H-25); ^{13}C NMR, see Table 2; HRESIMS m/z 308.1663 [M + H]$^+$ (calcd for $C_{17}H_{26}NO_2S$, 308.1679).

Dihydroxydione 13: yellow oil; $[\alpha]_D$ −6.8° (c 0.0256, MeOH); UV (MeOH) λ_{max} (ϵ) 212 (26700), 246 (20000) nm; IR (film) ν_{max} 3404, 2935, 1703, 1450, 975 cm^{-1}; ^1H NMR δ 6.94 (1H, s, H-19), 6.55 (1H, s, H-17), 5.21 (1H, t, J = 7.0 Hz, H-13), 4.15 (1H, t, J = 6.0 Hz, H-15), 4.10 (1H, t, J = 6.0 Hz, H-7), 2.69 (3H, s, H-21), 2.67 (1H, m, H-8), 2.63 (2H, m, H-6, H-10a), 2.54 (1H, m, H-4a), 2.52 (1H, m, H-10b), 2.45 (1H, m, H-4b), 2.35 (2H, m, H-14), 2.30 (1H, m, H-11a), 2.27 (1H, m, H-11b), 2.02 (3H, d, J = 1.0 Hz, H-27), 1.68 (3H, d, J = 1.0 Hz, H-26), 1.15 (3H, d, J = 2.5 Hz, H-24 or H-25), 1.13 (3H, d, J = 2.5 Hz, H-24 or H-25), 1.02 (3H, t, J = 7.0 Hz, H-22); ^{13}C NMR, see Table 2; HRESIMS m/z 422.2354 [M + H]$^+$ (calcd for $C_{23}H_{36}NO_4S$, 422.2360).

Epothilone tetrahydrofuran 15: pale yellow oil; UV (MeOH) λ_{max} (ϵ) 210 (19800), 247 (13500) nm; IR (film) ν_{max}

3400, 2939, 1725, 1695, 1507, 1455, 1381, 1262, 1189, 1151, 1044, 1010, 933, 880 cm^{-1}; ^1H NMR δ 6.92 (1H, s, H-19), 6.63 (1H, br s, H-17), 5.46 (1H, dt, J = 9.0, 1.0 Hz, H-11), 5.19 (1H, dd, J = 6.5, 2.0 Hz, H-15), 4.57 (1H, ddd, J = 10.0, 10.0, 5.5 Hz, H-10), 4.24 (1H, ddd, J = 11.0, 5.5, 2.5 Hz, H-3), 4.12 (1H, td, J = 8.0, 2.0 Hz, H-13), 3.87 (1H, dd, J = 9.0, 4.5 Hz, H-7), 3.62 (1H, d, J = 5.5 Hz, OH-3), 3.59 (1H, qd, J = 7.0, 4.5 Hz, H-6), 2.69 (3H, s, H-21), 2.65 (1H, m, H-8), 2.54 (1H, dd, J = 15.5, 2.5 Hz, H-2a), 2.46 (1H, m, H-2b), 2.41 (1H, m, H-14a), 2.18 (2H, m, H-9a, H-14b), 2.17 (3H, br s, H-27), 2.09 (1H, d, J = 8.0 Hz, OH-13), 1.75 (3H, br s, H-26), 1.38 (3H, s, H-23), 1.36 (1H, m, H-9b), 1.11 (3H, d, J = 6.5 Hz, H-25), 1.03 (3H, d, J = 7.0 Hz, H-24), 1.02 (3H, s, H-22); ^{13}C NMR, see Table 2; HRESIMS m/z 506.2589 $[M + H]^+$ (calcd for $C_{27}H_{40}NO_6S$, 506.2571).

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