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New Natural Epothilones from *Sorangium cellulosum*, Strains So ce90/B2 and So ce90/D13: Isolation, Structure Elucidation, and SAR Studies

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In addition to epothilones A (**1**) and B (**2**), 37 natural epothilone variants and epothilone-related compounds were isolated from the culture broth of a 700 L fermentation of *Sorangium cellulosum*, strain So ce90/B2. Of these, only the 12,13-desoxyepothilones, epothilone C (**14**) and D (**15**), were produced in significant amounts (3−6 mg/L); the 21-hydroxy derivatives and epothilones E (**3**) and F (**4**), in low and variable amounts due to further degradation by the producing organism. Most of the other epothilone variants were produced only in 1−100 μg/L amounts. The new compounds are very similar in structure to the parent compounds **1**, **2** and **14**, **15** and are presumably the result of the imperfect selectivity of the biosynthetic enzymes for acetate and propionate. Further, epothilones containing an oxazole moiety (**10**−**13**) in the side chain instead of a thiazole as well as ring-expanded 18-membered macrolides, epothilones I (**30**−**35**), and a ring contracted 14-membered macrolide, epothilone K (**36**), were found as very minor metabolites. The mutant strain, So ce90/D13, instead of macrolactones, produced short-chain carboxylic acids **40**, **41**, and **42** bearing the characteristic thiazole side chain. The structures of the new epothilones were elucidated on the basis of comprehensive NMR and MS data. The new epothilone variants were tested in a cytotoxicity assay with mouse fibroblasts (cell line L929), and structure−activity relationships were established. Several new natural epothilones showed activity comparable to **1** and **2**, but in no case exceeded that of **2**.

The epothilones A (**1**) and B (**2**) were first discovered as antifungal and cytotoxic metabolites in the myxobacterium *Sorangium cellulosum*; they were isolated and their structure was elucidated.[1] Later they were rediscovered as inhibitors of the tubulin system in a target-oriented screening designed for the detection of Taxol mimetics.[2] The epothilones like Taxol and other taxoids stabilize microtubules, leading to arrest of the cell cycle and eventually to apoptosis.[2] Although their chemical structures are quite diverse, both groups of compounds appear to bind at the same or closely related binding sites on β-tubulin. Among several beneficial properties, most interestingly, the epothilones are highly active against cell lines resistant to Taxol and other anticancer agents. This resistance is commonly based on the induction of the P-glycoprotein drug-transport system and in case of taxoids also on mutations in the β-tubulin gene. Fortunately the epothilones are not sensitive to both mechanisms,[2,3] raising high hopes that a new drug can be developed that overcomes the acquired resistance in cancer chemotherapy. This observation stimulated intense synthetic activities leading to several total syntheses of epothilones A−F and en route to a great variety of structural variants and stereoisomers.[4] From these, detailed structure−activity relationships (SAR) could be derived by in vitro studies.[4,5] Synthetic **2** and in particular **15** (desoxyepothilone B) demonstrated efficacy against human tumor xenografts that are refractory to Taxol.[6] Epothilone B (**2**) and its lactam analogue[7] have been in phase I clinical trials since early last year. Also, the biosynthetic gene cluster for epothilone has been cloned, sequenced,[8,9] and expressed in a *Streptomyces* host.[9] This

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approach could lead to structural modifications through combinatorial biosynthesis with possibly improved therapeutic properties. We concentrated our efforts with the same goal on the chemical modification of epothilones[10] produced by fermentation and on the exploitation of the natural variability of the biosynthesis. The latter strategy also generated a considerable number of epothilone variants on a small scale[11] for in vitro SAR studies, especially after sufficient material became available as a byproduct from large-scale production of **1** and **2**.

Results and Discussion

The large-scale production and isolation of epothilone A (**1**) and B (**2**) proceeded according to the published method[1] with the exception that the mutant strain So ce90/B2 was used and the LH-20 chromatography replaced by a filtration over Florisil. The crude extract from a 700 L fermentation batch according to HPLC analysis contained the abundant epothilones **1** and **2**, small amounts of two further epothilones later designated C (**14**) and D (**15**), and many trace compounds with the characteristic epothilone UV chromophore. By passing the extract through a column of Florisil the majority of very lipophilic and polar components of the matrix were eliminated and the weight was reduced significantly. Clearly, epothilone variants with extreme polarities, if present, were also lost this way; however, the most polar epothilones E (**3**) and F (**4**) and the liphophilic epothilones I_1-I_6 (**30**−**35**) observed in the crude extract by HPLC were recovered. Further separation of the fractions obtained above on RP-18 silica gel focused on the isolation of the four major epothilones **1**, **2**, **14**, and **15**. Side fractions were pooled to give polar and lipophilic subfractions, which were further separated by sequential reversed-phase and normal phase chromatography. In most cases pure compounds were obtained; otherwise a third chromatographic step was employed. Obviously, the yields given in the Experimental Section do not indicate the amounts produced by the organism, but rather reflect the difficulties of separation. Particularly the late fractions showed additional peaks with considerably higher masses compared to epothilones due to overlap with a different kind of metabolites belonging to the group of spirangiens.[12]

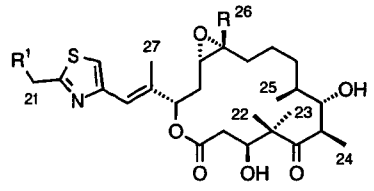
Contrary to strain So ce90/B2, which is an overproducer of **1** and **2**, mutant So ce90/D13 is a nonproducer. However, when the crude extract from the adsorber resin was re-extracted with *n*-butanol, HPLC analysis indicated the presence of small amounts of several very polar compounds exhibiting the typical epothilone UV chromophore. Three compounds, **40**, **41**, and **42**, were isolated by preparative RP-18 chromatography.

Epothilones C (**14**) and D (**15**) showed molecular masses reduced by 16 mu and were considerably more lipophilic than **1** and **2** (Figure 1). The NMR spectra indicated that the epoxide is replaced by a *cis* double bond in these compounds, and epothilones **1** and **2** were obtained on reaction with dimethyl dioxirane or *m*CPBA; thus both groups of compounds had the same absolute configuration. These samples of epothilones C (**14**) and D (**15**) served to identify the intermediates of the first total syntheses of epothilones in late 1996.[13]

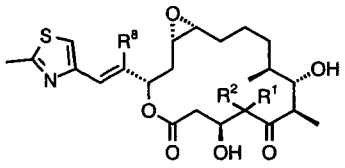
The most polar epothilone derivatives, designated E (**3**) and F (**4**), showed masses that were 16 mu higher than **1** and **2**, respectively, indicating oxygenation. The yields of these compounds increased with the duration of the fermentation and with lower amounts of added adsorber resin. This suggests that these compounds are degradation products made by the producing organism, a process that can almost entirely be suppressed by the addition of more resin to scavenge the excreted primary fermentation products. Examination of the NMR spectra of **3** and **4** clearly showed oxidation of the C-21 methyl. The signal at δ 2.7 had disappeared, and a new signal at δ 4.9 corresponding to two protons confirmed that **3** and **4** were the 21-hydroxy derivatives of **1** and **2**. Particularly epothilone E (**3**) can also be obtained by biotransformation of various strains of *S. cellulosum*,[14] whereas **4** is preferably synthesized via a Polonovsky type rearrangement of epothilone B *N*-oxide.[10e,f] Several total syntheses of epothilone E[18,19] and F[19,20] have been published.

Judging from the elemental composition, epothilone A_9 (**8**) was an isomer of epothilone E. The hydroxylation occurred at the allylic C-27 methyl, shifting the carbon NMR signal to δ 57.2 and the protons to an AB system at δ 4.11 and 4.49. A group of three compounds related to **1** showed a lower molecular mass by 14 mu. Two of them, epothilone A_1 (**5**) and A_2 (**6**), showed in the NMR spectrum a signal for a methyl group coupled to a C-4 methine instead of a gem-dimethyl group. The methine was further coupled to H-3 with 7.7 Hz in **5** and 4.8 Hz in **6**, which, however, did not allow assignment of relative configuration at C-4. Epothilone A_8 (**7**) lacks the C-27 methyl group and shows proton signals for a *trans* C-16/C-17 double bond at δ 6.52 and 6.64 with $J = 15.6$ Hz. In epothilone B_{10} (**9**), with a molecular weight of 14 mu higher than epothilone B, the C-21 methyl group is replaced by ethyl, which in the proton NMR gives rise to triplet/quartet signals at δ 1.39 and 3.01. Another set of four epothilone variants showed a conspicous hypsochromic shift by 12 nm of both characteristic UV bands, which indicated a change in the thiazole moiety. Indeed, proton and carbon NMR signals were shifted significantly, in particular, C-18 upfield by 15 ppm and C-19 downfield by 19 ppm. This and the molecular weight reduced by 16 mu compared to their thiazole counterparts proved that epothilones G_1 (**10**), G_2 (**11**), H_1 (**12**), and H_2 (**13**) were oxazoles. A large group of analogues related to epothilones C and D showed consistently a molecular weight of 14 mu lower than their C/D counterparts. The structures were easily deduced from the proton NMR spectra as des-methyl analogues at C-4 (**16**, **17**, **18**, **19**), C-6 (**20**), C-8 (**21**), and C-16 (**26**). Two further analogues of epothilone C showed in addition significant shifts of proton signals for H-12 and H-13 with $J = 15.1$ Hz coupling indicating *trans*-epothilone C_1/C_2 structures **28** and **29**. Additional double bonds were observed in epothilones C_5 (**22**), D_5 (**23**), and C_6 (**24**) having a molecular weight of 2 mu less than their C and D conterparts. In epothilone C_5 and D_5 the additional double bond is located in C-8/C-9 position and in epothilone C_6 in C-10/C-11 position. According to the NOE between H-7 and H-9 in **22** the 8*E* configuration was deduced, whereas vicinal coupling constants of 15.5 and 11 Hz in **24** indicate a 10*E*,12*Z* configuration.

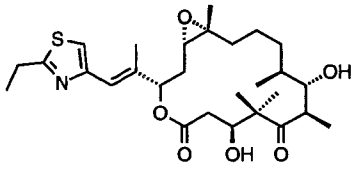
Epothilones C_7 (**25**) and C_9 (**27**), with a molecular weight of 16 mu higher than epothilone C, are hydroxylated analogues. The position of the hydroxyl at C-14 and C-27, respectively, follows from the existence of only one H-14 at δ 4.62 in **25** and an AB system at δ 4.47 and 4.20 for H-27 in **27**. The most lipophilic group of compounds, designated epothilones I_1-I_6 (**30**−**35**), can be assigned the basic structure of epothilone C and D (Figure 2). These compounds show molecular weights of 14, 28, and 42 mu higher than their counterparts and consistently have a macrolide ring enlarged by two aliphatic carbon atoms, C-9′ and C-10′. Epothilones I_1 and I_2 (**30** and **31**) are type C



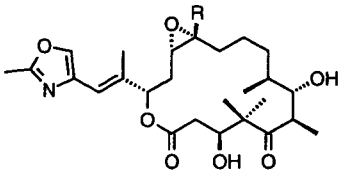
Epothilone A (**1**) R^1 = H; R = H [493]
Epothilone B (**2**) R^1 = H; R = Me [507]
Epothilone E (**3**) R^1 = OH; R = H [509]
Epothilone F (**4**) R^1 = OH; R = Me [523]



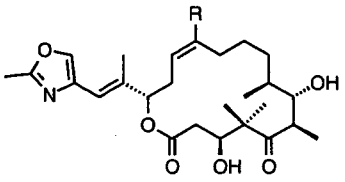
Epothilone A_1 (**5**) R^1 = H; R^2, R^8 = Me [479]
Epothilone A_2 (**6**) R^2 = H; R^1, R^8 = Me [479]
Epothilone A_8 (**7**) R^8 = H; R^1, R^2 = Me [479]
Epothilone A_9 (**8**) R^1, R^2 = Me; R^8 = CH_2OH [509]



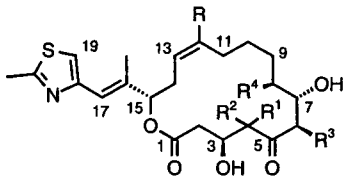
Epothilone B_{10} (**9**) [521]



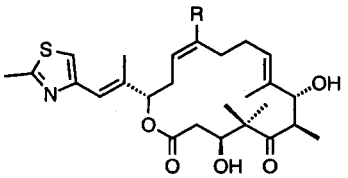
Epothilone G_1 (**10**) R = H [477]
Epothilone G_2 (**11**) R = Me [491]



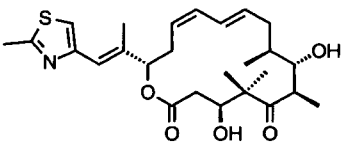
Epothilone H_1 (**12**) R = H [461]
Epothilone H_2 (**13**) R = Me [475]



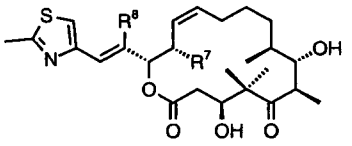
Epothilone C (**14**) R^1, R^2, R^3, R^4 = Me; R = H [477]
Epothilone D (**15**) R^1, R^2, R^3, R^4, R = Me [491]
Epothilone C_1 (**16**) R^1 = H; R^2, R^3, R^4 = Me; R = H [463]
Epothilone D_1 (**17**) R^1 = H; R^2, R^3, R^4 = Me; R = Me [477]
Epothilone C_2 (**18**) R^2 = H; R^1, R^3, R^4 = Me; R = H [463]
Epothilone D_2 (**19**) R^2 = H; R^1, R^3, R^4 = Me; R = Me [477]
Epothilone C_3 (**20**) R^3 = H; R^1, R^2, R^4 = Me; R = H [463]
Epothilone C_4 (**21**) R^4 = H; R^1, R^2, R^3 = Me; R = H [463]



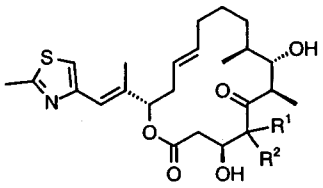
Epothilone C_5 (**22**) R = H [475]
Epothilone D_5 (**23**) R = Me [489]



Epothilone C_6 (**24**) [475]



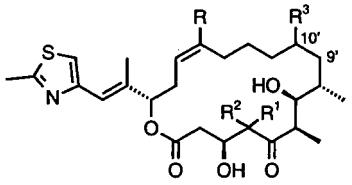
Epothilone C_7 (**25**) R^7 = OH; R^8 = Me [493]
Epothilone C_8 (**26**) R^8, R^7 = H [463]
Epothilone C_9 (**27**) R^8 = CH_2OH; R^7 = H [493]



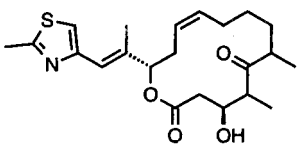
trans-Epothilone C_1 (**28**) R^1 = H; R^2 = Me [463]
trans-Epothilone C_2 (**29**) R^1 = Me; R^2 = H [463]

Figure 1. Epothilone A−H variants (16-membered lactones, molecular weights are given in brackets).

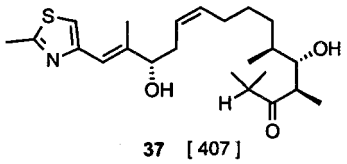
epothilones with an extra acetate (+28 mu) or propionate (+42 mu) unit introduced during polyketide biosynthesis. This gives rise to two extra methylene signals in the NMR for **30** and a distinct extra methyl doublet at δ 0.91 in addition to multiplets for a methine and methylene group for **31**. Epothilone I_3 (**32**) is the counterpart of I_2 (**31**), belonging to the epothilone D series. Epothilones I_4−I_6 (**33**, **34**, **35**) have in common a methyl group at C-10′ and a molecular weight of 14 mu less than their closest relatives, I_2 and I_3. The NMR spectra show that they have only one methyl group on C-4, similar to epothilones C_1 and D_1. Epothilone K (**36**) lacks, according to the elemental composition and NMR data, the C-7/C-8 ring segment. It is still a lactone, though with the ring size reduced to 14 atoms. The remaining six compounds (**37**−**42**) have in common the characteristic UV chromophore of epothilones, and instead of the lactone a free hydroxy or methoxy group at C-15 shifting H-15 upfield by 0.6−0.9 ppm. From the NMR spectra can be seen that **37** and **38** belong to the epothilone C, and **39** to the D series. They have shorter chain lengths, and the terminating groups are an isopropyl ketone (**37**) or ethyl ketone (**38**, **39**). In **40** and **41** the remaining chain is even shorter, terminating with C-11 as a carboxyl group. With R representing a proton or methyl group these



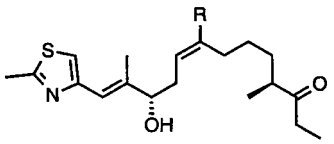
Epothilone I_1 (**30**) R, R^3 = H; R^1, R^2 = Me [505]
Epothilone I_2 (**31**) R = H; R^1, R^2, R^3 = Me [519]
Epothilone I_3 (**32**) R, R^1, R^2, R^3 = Me [533]
Epothilone I_4 (**33**) R, R^2 = H; R^1, R^3 = Me [505]
Epothilone I_5 (**34**) R, R^1, R^3 = Me; R^2 = H [519]
Epothilone I_6 (**35**) R, R^2, R^3 = Me; R^1 = H [519]



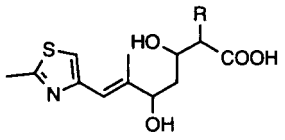
Epothilone K (**36**) [405]



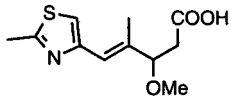
37 [407]



38 R = H [335]
39 R = Me [349]



40 R = H [271]
41 R = Me [285]



42 [241]

Figure 2. Epothilone I−K variants (18- and 14-membered lactones) and linear fragments **37**−**42** (molecular weights are given in brackets).

compounds can be assigned to the epothilone C and D series. The smallest fragment reminiscent of epothilone was carboxylic acid **42**, which, in addition to the signals of the chromophore, exhibited only signals of an ABX system at δ 2.62, 2.55, and 4.18 and a methoxy at δ 3.30.

Although the structures of the epothilone variants described above were derived in a straightforward manner from the molecular mass, elemental composition, and proton NMR spectra, in most cases also carbon spectra (Table 1 and Experimental Section) and occasionally ^{1}H−^{1}H COSY spectra were measured and found in perfect agreement with the structures proposed. The absolute configuration of epothilones C, D, E, and F followed from semisynthetic interconversions.[10] Homologous centers in other compounds were assigned analogously, whereas newly created centers remained unassigned as C-4 in epothilones A_1 (**5**), A_2 (**6**), C_1 (**16**), D_1 (**17**), C_2 (**18**), D_2 (**19**), *trans*-C_1 (**28**), and *trans*-C_2 (**29**) and C-4 and C-10 in epothilones I_4, I_5, and I_6 (**33**−**35**). The same applies for C-4 and C-6 in epothilone K (**36**), C-13 in **40**, and C-12 and C-13 in **41**. Interestingly, compounds **40**, **41**, and **42** obtained from the mutant strain So ce90/D13 seem to have an inverted configuration at C-15, as indicated by the optical rotation.[14] Since their first isolation, a number of epothilone variants described above have been independently obtained by semisynthesis and biotransformation as epothilones B_{10} (**9**),[10c] E (**3**), and F (**4**)[10d,16] or by total synthesis, as epothilones C_4 (**21**),[17] E (**3**),[18,19] F (**4**),[19,20] G_1 (**10**),[17,21] G_2 (**11**),[17,22] H_1 (**12**),[17,21] H_2 (**13**),[17,22] and I_1 (**30**).[23]

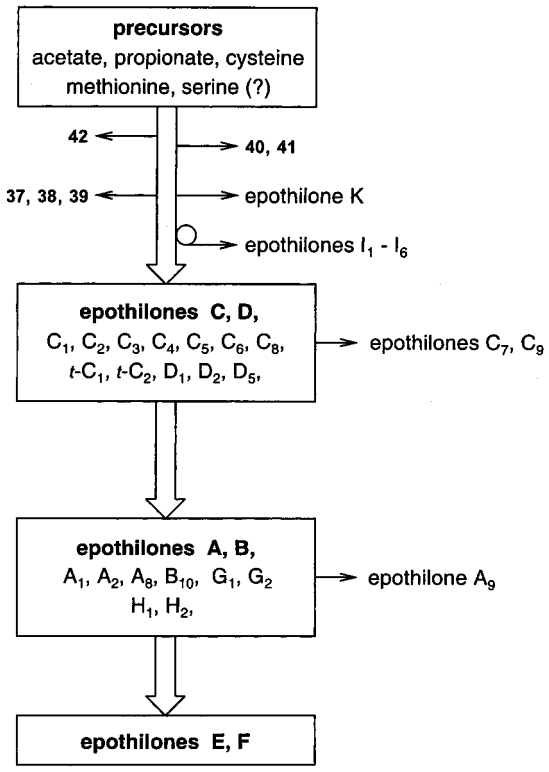
From a biosynthetic point of view the epothilone variants can be arranged in three basic groups (Scheme 1). Clearly, the epothilones C and D are the primary products of polyketide synthesis which are processed by decorating enzymes first to the 12,13-epoxides, epothilones A and B.[24] Subsequently hydroxylation of the terminal methyl C-21 gives epothilones E and F,[16] and that of methyl C-27 gives epothilone A_9. Similarly hydroxylation of epothilone C at C-14 and C-27 leads to epothilones C_7 and C_9. The second group of epothilones, I_1−I_6, arise from failure of the polyketide synthase in that, after introduction of C-9/C-10, an additional cycle of chain elongation with either malonyl-CoA or methylmalonyl-CoA is performed. After that, the regular sequence of synthetic steps is completed including release from the enzyme by lactone formation. The opposite seems to occur in the formation of epothilone K, where chain elongation by C-7/C-8 and methylation of C-4 are skipped,[25] and the synthesis is completed to give a 14-membered lactone. The linear ketones **37**, **38**, and **39** seem to be formed by premature release from the enyzme as β-keto acids which undergo decarboxylation. In the blocked mutant So ce90/D13 polyketide synthesis appears to be severely disturbed. Intermediates of early stages are released as carboxylic acids **40**, **41**, and **42** possibly because they have the wrong configuration at C-15.[14] The majority of side products from the wild strain are homologues or isomers of the epothilones A−D with changed methylation pattern. Their formation apparently results from the incorrect selection of malonyl-CoA or methylmalonyl-CoA by the acyl carrier protein. However, from the relative yields of these side products the reliability of this process is still 99.9% or more. Only in C-12/C-13 position is the selectivity low and variable, leading to a ratio of epothilones A and C compared with B and D of 8:2 to 1:1 depending on the culture conditions. From mutation experiments[26] and the sequences of the biosynthesis gene cluster[8,9] it can be concluded that the same enzyme is responsible for the formation of both series of compounds. As the sequence homology of the acyltransferase indicates malonyl selectivity for introduction of the C-12/C-13 building block, it must be assumed that at least in this case additional factors are involved in the selection process. Interestingly a similar situation is observed for C-9′/C-10′ in epothilones I_1−I_6 (**30**−**35**). Selection of serine instead of cysteine by the adenylating enzyme appears to be responsible for the formation of the oxazole analogues, epothilones G and H. However, due to the low yield of these compounds, this cannot be probed by a labeling experiment. A failure of

Table 1. ^{13}C Chemical Shifts for Selected Epothilone Variants ($CDCl_3$, 75 or 100 MHz)

carbon	12	14	16	18	20	21	22	24	26	27	28	29	30
1	170.3	170.4	169.9	170.2	170.2	170.3	170.5	170.2	170.4	170.0	169.7	169.9	171.1
2	39.2	39.3	40.7	38.9	39.3	39.7	38.7	39.2	39.2	40.9	39.9	40.1	38.6
3	72.6	72.4	67.8	68.2	72.9	72.5	74.4	72.0	72.3	70.6	67.0	70.2	74.7
4	53.2	53.4	52.2	50.2	53.5	53.9	51.9	53.1	53.1	54.2	51.4	50.0	51.6
5	220.4	220.6	217.5	216.0	216.7	221.7	221.6	220.2	220.4	221.6	217.2	217.0	221.3
6	42.0	41.8	45.8	48.7	37.9	42.7	46.8	41.5	41.9	40.3	48.9	49.8	43.7
7	74.2	74.2	76.8	75.0	71.0	70.9	77.3	71.4	74.3	74.1	77.7	76.8	74.9
8	38.6	38.6	37.5	36.4	37.5	32.3	136.4	37.0	38.6	38.9	35.2[a]	36.0	34.3
9	32.5	31.8	30.0	30.6	33.5	24.9	128.0	35.5	32.5	32.6	28.1	28.0	29.3
9′													24.8
10	27.61	27.5	27.3	28.1	27.7	28.4	27.0	133.6	27.7	27.4	25.1	27.1	28.2
10′													28.6
11	27.59	27.6	27.4	28.1	28.0	26.8	26.5	127.5	27.5	27.8	31.3	32.3	26.6
12	133.6	133.5	133.0	133.6	132.8	133.9	132.6	132.2	133.6	133.6	133.4	134.5	133.8
13	124.9	125.0	125.3	124.0	125.2	125.3	125.1	124.2	124.6	124.6	125.2	125.5	124.6
14	31.7	31.5	31.5	31.4	31.7	31.8	31.4	32.2	32.9	31.4	36.4[a]	36.3	31.6
15	78.3	78.5	78.1	78.6	78.2	78.4	77.6	78.2	74.5	78.3	79.5	77.8	78.8
16	138.0[b]	138.7	137.6	138.1	139.0	139.1	137.8	137.7	129.6	139.9	136.6	137.1	137.4
17	115.9	119.5	119.6	119.1	119.0	119.0	118.9	119.7	125.4	124.8	119.6	120.0	120.0
18	137.5[b]	152.1	152.1	152.1	152.0	151.9	152.3	152.2	152.5	151.0	152.0	152.2	152.4
19	135.7	115.8	116.2	115.7	115.6	115.6	115.7	116.2	116.0	118.9	115.6	116.1	116.2
20	160.9	165.0	164.9	164.8	165.1	165.1	164.8	165.0	166.4	167.3	165.5	165.0	64.8
21	13.8	19.1	19.1	19.0	19.1	19.0	19.1	19.2	19.3	19.2	18.9	19.1	19.3
22	19.2	18.7		11.1	16.4	17.1	21.7	19.6	19.1	15.2	9.0		23.6
23	22.6	22.7	10.5		22.7	23.3	22.0	21.4	22.5	23.8		13.5	19.3
24	13.6	13.5	14.0	14.0		11.1	14.4	11.5	13.7	13.2	14.8	14.7	13.7
25	15.6	15.5	16.4	17.2	14.0		12.1	16.7	15.6	15.2	17.7	17.4	16.5
27	16.1	15.9	15.7	16.1	16.1	16.2	16.2	15.8		57.3	16.1	15.8	15.5

[a,b] Assignments may be interchanged.

Scheme 1. Proposed Biosynthetic Relationships of Epothilone Variants



individual enoyl reductases may lead to the extra double bonds in epothilone C_5 (**22**), D_5 (**23**), and C_6 (**24**).

The biological activities of the epothilones A−K (**1**−**36**) and the epothilone fragments **37**−**39** were determined by growth inhibition of the mouse fibroblast cell line L929. The data summarized in Table 2 confirm results from total synthesis of analogues and semisynthetic derivatives.[4,5] Epothilones with a C-12 methyl group are consistently more active by a factor of 4−20 than the unsubstituted ones. Similarly the C-6, C-8, and C-16 methyl substitutents are essential for good activity, whereas C-4 may be monomethylated. In the epothilone D series a C-4 monomethyl analogue of unknown configuration, epothilone D_2 (**19**), is even significantly more active than epothilone D (**15**). An additional hydroxy group on C-27 reduces activity significantly and on C-21 slightly, whereas on C-14 an improvement by a factor of 2 compared to epothilone C is observed. The change from C-20 methyl to ethyl and from thiazole to oxazole has no influence on the activity. In conclusion, it appears that during evolution some of the most active epothilone variants have been optimized in their production by *Sorangium cellulosum*. Remarkably, 39 other epothilone-producing strains of *S. cellulosum* collected from all over the world exhibit a very similar product spectrum with the exception of two strains that produce exclusively epothilone A.[26]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 241 instrument. UV spectra were recorded on a Shimadzu UV-2102 PC scanning spectrometer. IR spectra were measured with a Nicolet 20DXB FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ on Bruker DMX-600, ARX-400, and DPX-300 spectrometers and referenced to residual solvent at 7.25 and 77.0 ppm for ^1H and ^{13}C, respectively. For the majority of compounds only a selection of significant NMR data are given. For complete data see the Supporting Information. EI and DCI (reactant gas isobutane) mass spectra were obtained on a Finnigan MAT 95 spectrometer, and high-resolution data were acquired using peak matching (M/DM = 10 000 and M/DM = 8000 for EI and DCI, respectively). Pure compounds were characterized by analytical HPLC on Nucleosil C18 (column 125 × 2 mm, 5 μm, flow 0.3 mL/min, acetonitrile/10 mM ammonium acetate buffer, pH 6.5, gradient 50:50 → 60:40 in 15 min then isocratic for 15 min, diode array detection).

Organisms and Isolation of Epothilones A−K (1−36) and Compounds 37−39. *Sorangium cellulosum*, strain So ce90/B2, was cultivated on a 700 L scale in the presence of 14 L Amberlite XAD 16 adsorber resin and harvested as described

Table 2. Cytotoxic Activity of Epothilones **1**−**39** for the Mouse Fibroblast Cell Line L929 (IC_{50} in ng/mL)

	epothilone				
structural type	A_Y	B_Y	C_Y	D_Y	*trans* C_Y
parent	(**1**) 4	(**2**) 1	(**14**) 60	(**15**) 20	
21-hydroxy (E&F)	(**3**) 10	(**4**) 1.5			
oxazoles (G&H)	(**10**) 6	(**11**) 1	(**12**) 120	(**13**) 11	
4-desmethyl (X_1)	(**5**) 20		(**16**) 900	(**17**) 35	(**28**) 400
4-desmethyl (X_2)	(**6**) 7		(**18**) 800	(**19**) 7	(**29**) 80
6-desmethyl (X_3)			(**20**) 1500		
8-desmethyl (X_4)			(**21**) 800		
8,9-didehydro (X_5)			(**22**) 1500	(**23**) 200	
10,11-didehydro (X_6)			(**24**) 120		
14-hydroxy (X_7)			(**25**) 25		
16-desmethyl (X_8)	(**7**) 20		(**26**) 250		
27-hydroxy (X_9)	(**8**) 100		(**27**) 200		
20-ethyl (X_{10})		(**9**) 1.5			
ring expanded			(**30**) 800		
ring expanded			(**31**) 400	(**32**) 600	
ring expanded			(**33**) 300	(**34**) 100	
ring expanded			(**35**) 600		
ring contracted			(**36**) 300		
miscellaneous			(**37**) 800		
miscellaneous			(**38**) >4000	(**39**) >4000	

elsewhere.[1] Concentration of the aqueous/methanolic extract and re-extraction of the water phase with ethyl acetate yielded 271 g of a dark viscous oil. This was disolved in 6:4 hexane/ethyl acetate and applied to an open column with Florisil (Fluka, 60−100 mesh, 1.5 L, column diameter 10 cm). Elution with 11 L of 6:4 hexane/ethyl acetate gave several fractions containing 76 g of refined material. These were further separated by RP-18 chromatographly on a Merck Prep-bar system to give 29.8 g of epothilone A (**1**), 10.3 g of B (**2**), 3.8 g of C (**14**), and 2.2 g of D (**15**). Polar and lipophilic side fractions were combined to give fractions of 4.2 and 0.4 g. The next separation step consisted of RP-HPLC (column Macherey & Nagel SP 250/21 Nucleosil 120-7 C18) using MeCN/H_2O mixtures (ranging from 35% to 65% MeCN) as mobile phase. In most cases, final purification was achieved using silica HPLC (column Knauer Vertex 250/16 Nucleosil 100-7) with dichloromethane/2-propanol mixtures (ranging from 1.5% to 4% 2-propanol). Otherwise a third chromatography step was performed using an RP column with MeOH/H_2O mixtures.

Isolation of Compounds 40−42. *S. cellulosum*, strain So ce90/D13, was cultivated in a 10 L bioreactor in the presence of 0.2 L Amberlite XAD 16 adsorber resin and harvested as described elsewhere.[1] Re-extraction of the H_2O phase with ethyl acetate followed by *n*-butanol gave a combined crude extract of 90 mg. Separation in three batches on a Nucleosil C18 column (250 × 20 mm, 7 μm, solvent system 46:52:2 methanol/water/acetic acid, detection at 254 nm) furnished **40** (5.2 mg), **41** (2.5 mg), and **42** (6.3 mg).

Epothilone A (1): colorless crystals; mp 95 °C (from ethyl acetate/hexanes); 29.8 g; t_R 4.6 min; $[\alpha]^{22}_D$ −47.1 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (17800), 249 (12500); IR (KBr) ν_{max} 3476, 2974, 2938, 2882, 1738, 1692 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (1H, s, H-19), 6.60 (1H, bs, H-17), 5.44 (1H, dd, J = 8.8, 2.8 Hz, H-15), 4.20 (1H, bdd, J = 10.5, 3.2 Hz, H-3), 4.03 (1H, bs, 3-OH), 3.80 (1H, dd, J = 4.7, 4.1 Hz, H-7), 3.25 (1H, dq, J = 4.7, 6.9 Hz, H-6), 3.03 (1H, ddd, J = 8.2, 4.2, 4.0 Hz, H-13), 2.92 (1H, ddd, J = 7.5, 4.0, 3.9 Hz, H-12), 2.70 (3H, s, H-21), 2.65 (1H, bs, 7-OH), 2.54 (1H, dd, J = 14.6, 10.5 Hz, H-2a), 2.40 (1H, dd, J = 14.6, 3.2 Hz, H-2b), 2.14 (1H, ddd, J = 15.1, 4.2, 2.8 Hz, H-14a), 2.09 (3H, d, J = 1.3 Hz, H-27), 1.88 (1H, ddd, J = 15.1, 8.8, 8.2 Hz, H-14b), 1.76 (1H, m, H-8), 1.75 (1H, m, H-11a), 1.56 (1H, m, H-10a), 1.45 (3H, m, H-9 and H-10b), 1.44 (1H, m, H-11b), 1.37 (3H, s, H-23), 1.18 (3H, d, J = 6.9 Hz, H-24), 1.10 (3H, s, H-22), 1.01 (3H, d, J = 7.0 Hz, H-25); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.1 (s, C-5), 170.6 (s, C-1), 165.1 (s, C-20), 151.8 (s, C-18), 137.5 (s, C-16), 119.8 (d, C-17), 116.2 (d, C-19), 76.6 (d, C-15), 74.5 (d, C-7), 73.1 (d, C-3), 57.5 (d, C-12), 54.7 (d, C-13), 53.0 (s, C-4), 43.3 (d, C-6), 39.0 (t, C-2), 36.3 (d, C-8), 31.5 (t, C-14), 30.5 (t, C-9), 27.2 (t, C-11), 23.5 (t, C-10), 21.7 (q, C-23), 20.2 (q, C-22), 19.1 (q, C-21), 17.1 (q, C-25), 15.7 (q, C-27), 14.1 (q, C-24); EIMS m/z 493 [M]$^+$ (22), 421 (20), 306 (70), 166 (50), 165 (84), 164 (100).

Epothilone B (2): colorless crystals; mp 93−94 °C (from ethyl acetate); 10.3 g; t_R 5.3 min; $[\alpha]^{22}_D$ −35.0 (*c* 0.7, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (18 600), 249 (14 100); IR (KBr) ν_{max} 3487, 2966, 2935, 2880, 1738, 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.96 (1H, s, H-19), 6.58 (1H, bs, H-17), 5.41 (1H, dd, J = 7.8, 3.0 Hz, H-15), 4.21 (1H, m, H-3), 4.19 (1H, bs, 3-OH), 3.76 (1H, ddd, J = 4.2, 3.5, 2.7 Hz, H-7), 3.29 (1H, dq, J = 4.2, 6.9 Hz, H-6), 2.80 (1H, dd, J = 7.7, 4.6 Hz, H-13), 2.68 (3H, s, H-21), 2.64 (1H, bd, J = 2.7 Hz, 7-OH), 2.54 (1H, dd, J = 14.1, 9.6 Hz, H-2a), 2.35 (1H, dd, J = 14.1, 3.0 Hz, H-2b), 2.10 (1H, ddd, J = 15.1, 4.7, 3.1 Hz, H-14a), 2.08 (3H, d, J = 1.3 Hz, H-27), 1.91 (1H, ddd, J = 15.1, 7.8, 7.7 Hz, H-14b), 1.73 (1H, m, H-8), 1.70 (1H, m, H-11a), 1.49 (1H, m, H-10a), 1.42 (4H, m, H-9, H-10b, and H-11b), 1.36 (3H, s, H-23), 1.27 (3H, s, H-26), 1.16 (3H, d, J = 6.9 Hz, H-24), 1.07 (3H, s, H-22), 0.99 (3H, d, J = 7.0 Hz, H-25); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.7 (s, C-5), 170.6 (s, C-1), 165.2 (s, C-20), 151.9 (s, C-18), 137.6 (s, C-16), 119.7 (d, C-17), 116.1 (d, C-19), 77.3 (d, C-15), 74.1 (d, C-7), 72.9 (d, C-3), 61.7 (d, C-12), 61.4 (s, C-13), 53.2 (s, C-4), 42.9 (d, C-6), 39.3 (t, C-2), 36.5 (d, C-8), 32.4 (t, C-11), 32.1 (t, C-14), 30.8 (t, C-9), 22.8 (q, C-26), 22.4 (t, C-10), 21.5 (q, C-23), 19.7 (q, C-22), 19.2 (q, C-21), 17.1 (q, C-25), 15.9 (q, C-27), 13.6 (q, C-24); EIMS m/z 507 [M]$^+$ (15), 421 (20), 306 (70), 166 (50), 165 (84), 164 (100); HREIMS m/z 507.2568 (calcd for $C_{26}H_{39}NO_6S$, 507.2577).

Epothilone E (3): colorless amorphous solid; 140 mg; t_R 2.5 min; $[\alpha]^{22}_D$ −37.1 (*c* 0.56, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (17800), 247 (13000); IR (KBr) ν_{max} 3489, 2964, 2933, 2876, 1735, 1690, 1466, 1261, 1058, 979 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.10 (1H, s, H-19), 4.87 (2H, s, 21-H_2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3 (s, C-20), 152.3 (s, C-18), 117.1 (d, C-19), 62.2 (t, C-21); EIMS m/z 509 [M]$^+$ (2), 338 (9), 322 (30), 180 (62), 156 (60), 138 (33), 100 (100), 71 (66); HRDCIMS m/z 510.2558 (M + H$^+$) (calcd for $C_{26}H_{40}NO_7S$, 510.2526).

Epothilone F (4): colorless amorphous solid; 45 mg; t_R 2.8 min; $[\alpha]^{22}_D$ −27.4 (*c* 0.46, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (19 100), 249 (13 800); IR (KBr) ν_{max} 3437, 2959, 2930, 2874, 1735, 1690, 1467, 1264, 1060, 979 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (1H, s, H-19), 4.92 (2H, s, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0 (s, C-20), 152.3 (s, C-18), 117.0 (d, C-19), 62.2 (t, C-21); EIMS m/z 523 [M]$^+$ (19, 353 (21), 336 (59), 198 (45), 181 (99), 180 (100), 156 (35), 129 (25); HREIMS m/z 523.2635 (calcd for $C_{27}H_{41}NO_7S$, 523.2604).

Epothilone A_1 (5): colorless amorphous solid; 3.1 mg; t_R 3.5 min; $[\alpha]^{22}_D$ −69 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (ϵ) 208 (19 600), 247 (13 600); IR (KBr) ν_{max} 3437, 2959, 2931, 2876, 1732, 1710, 1455, 1259, 978 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz)

δ 4.12 (1H, m, H-3), 3.37 (1H, bd, J = 7.5 Hz, 3-OH), 3.21 (1H, dq, J = 7.7, 7.0 Hz, H-4), 2.78 (1H, dd, J = 16.8, 4.3 Hz, H-2a), 2.66 (1H, dq, J = 3.9, 7.0 Hz, H-6), 2.65 (1H, dd, J = 16.8, 5.2 Hz, H-2b), 1.21 (3H, d, J = 7.0 Hz, H-23), 1.22 (3H, d, J = 7.0 Hz, H-24); EIMS m/z 479 $[M]^+$ (21), 322 (31), 306 (65), 304 (47), 168 (45), 166 (73), 164 (100), 151 (30), 140 (35); HREIMS m/z 479.2317 (calcd for $C_{27}H_{41}NO_5S$, 479.2342).

Epothilone A_2 (6): colorless amorphous solid; 14.5 mg; t_R 3.4 min; $[\alpha]^{22}_D$ −12.0 (c 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (15100), 248 (15500); IR (KBr) ν_{max} 3438, 2963, 2929, 2875, 1734, 1706, 1458, 1262, 981 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.26 (1H, ddd, J = 8.5, 4.8, 4.7 Hz, H-3), 3.54 (1H, bs, 3-OH), 3.09 (1H, dq, J = 4.8, 7.0 Hz, H-4), 2.98 (1H, dq, J = 7.9, 7.0 Hz, H-6), 2.60 (1H, dd, J = 15.1, 8.5 Hz, H-2a), 2.50 (1H, dd, J = 15.1, 4.7 Hz, H-2b), 1.22 (3H, d, J = 7.0 Hz, H-24), 1.15 (3H, d, J = 7.0 Hz, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 216.2 (s, C-5), 69.1 (d, C-3), 50.3 (d, C-4), 49.6 (d, C-6), 39.4 (t, C-2), 13.9 (q, C-24), 12.4 (q, C-22); EIMS m/z 479 $[M]^+$ (18), 322 (38), 306 (78), 304 (59), 168 (48), 166 (96), 164 (100), 151 (33), 140 (38); HREIMS m/z 479.2318 (calcd for $C_{27}H_{41}NO_5S$, 479.2342).

Epothilone A_8 (7): colorless amorphous solid; 38.7 mg; t_R 3.4 min; $[\alpha]^{22}_D$ −76.2 (c 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (15 300), 248 (15 500); IR (KBr) ν_{max} 3440, 2967, 2932, 2876, 1736, 1691, 1467, 1252, 979 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.95 (1H, s, H-19), 6.64 (1H, dd, J = 15.6, 0.9 Hz, H-17), 6.52 (1H, dd, J = 15.6, 6.6 Hz, H-16), 5.68 (1H, dddd, J = 7.8, 6.6, 3.2, 0.9 Hz, H-15), 2.10 (1H, ddd, J = 15.0, 5.5, 3.2 Hz, H-14a), 1.90 (1H, ddd, J = 15.0, 7.8, 7.3 Hz, H-14b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.2 (s, C-18), 128.4 (d, C-16), 125.9 (d, C-17), 116.4 (d, C-19), 72.7 (d, C-15), 32.5 (t, C-14); EIMS m/z 479 $[M]^+$ 10, 308 (27), 292 (57), 164 (51), 152 (100), 126 (92); HRDCIMS m/z 480.2401 (calcd for $C_{25}H_{38}NO_6S$, 480.2401).

Epothilone A_9 (8): colorless amorphous solid; 4.4 mg; t_R 3.4 min; $[\alpha]^{22}_D$ −38 (c 0.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (15500), 253 (14 100); IR (KBr) ν_{max} 3423, 2965, 2932, 2877, 1736, 1690, 1463, 1249, 1014, 979 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.10 (1H, s, H-19), 6.72 (1H, dd, J = 10.7, 4.3 Hz, 27-OH), 6.60 (1H, bs, H-17), 5.69 (1H, dd, J = 11.6, 2.0 Hz, H-15), 4.49 (1H, ddd, J = 12.9, 4.3, 1.2 Hz, H-27a), 4.11 (1H, ddd, J = 12.9, 10.7, 1.0 Hz, H-27b), 2.03 (1H, ddd, J = 14.7, 2.2, 2.0 Hz, H-14a), 1.85 (1H, m, H-14b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.7 (s, C-18), 138.9 (s, C-16), 125.2 (d, C-17), 119.5 (d, C-19), 76.7 (d, C-15), 57.2 (t, C-27), 31.8 (t, C-14); EIMS m/z 509 $[M]^+$ (9), 491 (4), 322 (28), 321 (25), 180 (45), 167 (40), 166 (100), 165 (49), 154 (47), 138 (33); HREIMS m/z 509.2467 (calcd for $C_{26}H_{39}NO_7S$, 509.2447).

Epothilone B_{10} (9): colorless amorphous solid; 1.1 mg; t_R 7.4 min; $[\alpha]^{22}_D$ −27 (c 0.15, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (15800), 247 (12500); IR (KBr) ν_{max} 3434, 2962, 2930, 2876, 2858, 1733, 1692, 1461, 1259, 1052, 981 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.99 (1H, s, H-19), 3.01 (2H, q, J = 7.6 Hz, H-21), 1.39 (3H, t, J = 7.6 Hz, H-28); EIMS m/z 521 $[M]^+$ (22), 449 (7), 350 (18), 334 (57), 248 (16), 234 (27), 196 (41), 182 (59), 180 (96), 178 (100), 166 (44), 154 (44); HREIMS m/z 521.2808 (calcd for $C_{28}H_{43}NO_6S$, 521.2811).

Epothilone G_1 (10): colorless amorphous solid; 52.3 mg; t_R 3.4 min; $[\alpha]^{22}_D$ −39.7 (c 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 203 (15 200), 236 (15 100); IR (KBr) ν_{max} 3456, 2962, 2933, 2876, 1736, 1691, 1585, 1466, 1262, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.47 (1H, s, H-19), 6.33 (1H, bs, H-17), 2.43 (3H, s, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0 (s, C-20), 137.4 (s, C-18), 136.7 (s, C-16), 135.9 (d, C-19), 116.4 (d, C-17), 13.8 (9, C-21); EIMS m/z 477 $[M]^+$ (4), 405 (7), 290 (40), 152 (39), 150 (100), 148 (23), 124 (23); HREIMS m/z 477.2684 (calcd for $C_{26}H_{39}NO_7$, 477.2727).

Epothilone G_2 (11): colorless amorphous solid; 9.4 mg; t_R 3.8 min; $[\alpha]^{22}_D$ −22.6 (c 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 202 (21 500), 236 (14 800); IR (KBr) ν_{max} 3456, 2965, 2934, 2877, 1737, 1690, 1586, 1464, 1250, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.48 (1H, s, H-19), 6.33 (1H, bs, H-17), 5.43 (1H, dd, J = 7.1, 3.6 Hz, H-15), 2.44 (3H, s, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0 (s, C-20), 137.4 (s, C-18), 136.5 (s, C-16), 135.9 (d, C-19), 116.3 (d, C-17), 13.8 (q, C-21); EIMS m/z 491-$[M]^+$ (21), 419 (6), 320 (18), 304 (39), 166 (42), 152 (57), 150 (100), 149 (44), 148 (58), 124 (35), 109 (33); HREIMS m/z 491.2878 (calcd for $C_{27}H_{41}NO_7$, 491.2883).

Epothilone H_1 (12): colorless amorphous solid; 3.0 mg; t_R 8.6 min; $[\alpha]^{22}_D$ −84 (c 0.2, MeOH); UV (MeOH) λ_{max} nm (ϵ) 203 (19 600), 237 (12 000); IR (KBr) ν_{max} 3436, 2933, 2880, 2860, 1734, 1688, 1585, 1251, 1007 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.47 (1H, s, H-19), 6.31 (1H, bs, H-17), 2.43 (3H, s, H-21); ^{13}C NMR, see Table 1; EIMS m/z 461 $[M]^+$ (6), 310 (5), 274 (10), 273 (7), 171 (63), 152 (100), 148 (18), 111 (15); HREIMS m/z 461.2743 (calcd for $C_{26}H_{39}NO_6$, 461.2777).

Epothilone H_2 (13): colorless amorphous solid; 1.5 mg; t_R 10.6 min; $[\alpha]^{22}_D$ −44 (c 0.25, MeOH); UV (MeOH) λ_{max} nm (ϵ) 203 (14 500), 236 (12 200); IR (KBr) ν_{max} 3436, 2967, 2935, 2880, 1734, 1690, 1586, 1251, 1007 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.46 (1H, s, H-19), 6.30 (1H, bs, H-17), 2.43 (3H, s, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0 (s, C-20), 138.4 (s, C-16), 137.5 (s, C-18), 135.6 (d, C-19), 115.8 (d, C-17), 13.8 (q, C-21); EIMS m/z 475 $[M]^+$ (11), 288 (9), 287 (5), 188 (7), 171 (32), 152 (100), 111 (10); HREIMS m/z 475.2913 (calcd for $C_{27}H_{41}NO_6$, 475.2934).

Epothilone C (14): colorless amorphous solid; 3.8 g; t_R 11.8 min; $[\alpha]^{22}_D$ −74.4 (c 2.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 213 (16 200), 248 (12 500); IR (KBr) ν_{max} 3446, 2931, 2878, 2857, 1734, 1688, 1508, 1250, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.97 (1H, s, H-19), 6.61 (1H, bs, H-17), 5.45 (1H, m, H-12), 5.38 (1H, m, H-13), 5.29 (1H, dd, J = 9.7, 2.1 Hz, H-15), 4.25 (1H, bd, J = 11.3 Hz, H-3), 3.72 (1H, bdd, J = 3.6, 2.1 Hz, H-7), 3.62 (1H, bs, 3-OH), 3.14 (1H, dq, J = 2.1, 6.8 Hz, H-6), 3.11 (1H, bs, 7-OH), 2.69 (3H, s, H-21), 2.68 (1H, m, H-14a), 2.50 (1H, dd, J = 15.1, 11.3 Hz, H-2a), 2.33 (1H, dd, J = 15.1, 2.7 Hz, H-2b), 2.27 (1H, m, H-14b), 2.20 (1H, m, H-11a), 2.08 (3H, bs, H-27), 2.01 (1H, m, H-11b), 1.74 (1H, m, H-8), 1.66 (1H, m, H-10a),1.36 (1H, m, H-9a), 1.33 (3H, s, H-23), 1.21 (2H, m, H-9b and H-10b), 1.18 (3H, d, J = 6.8 Hz, H-24), 1.07 (3H, s, H-22), 0.99 (3H, d, J = 7.1 Hz, H-25); ^{13}C NMR, see Table 1; EIMS m/z 477 $[M]^+$ (15), 459 (6), 405 (5), 306 (7), 290 (23), 171 (66), 168 (100), 164 (26), 151 (19), 111 (11); HREIMS m/z 477.2547 (calcd for $C_{26}H_{39}NO_5S$, 477.2549).

Epothilone D (15): colorless amorphous solid; 2.2 g; t_R 14.4 min; $[\alpha]^{22}_D$ −61.3 (c 2.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (18 400), 248 (13 200); IR (KBr) ν_{max} 3445, 2966, 2935, 2878, 1734, 1688, 1508, 1251, 978 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, s, H-19), 6.57 (1H, bs, H-17), 5.21 (1H, dd, J = 9.9, 2.0 Hz, H-15), 5.13 (1H, ddd, J = 10.1, 4.8, 0.5 Hz, H-13), 4.27 (1H, dd, J = 11.0, 2.6 Hz, H-3), 3.71 (1H, dd, J = 4.2, 2.4 Hz, H-7), 3.41 (1H, bs, 3-OH), 3.15 (1H, dq, J = 2.4, 6.9 Hz, H-6), 3.01 (1H, bs, 7-OH), 2.68 (3H, s, H-21), 2.63 (1H, ddd, J = 15.0, 10.1, 9.9 Hz, H-14a), 2.45 (1H, dd, J = 14.7, 11.0 Hz, H-2a), 2.30 (1H, m, H-11a), 2.28 (1H, dd, J = 14.7, 2.6 Hz, H-2b), 2.22 (1H, bd, J = 15.0 Hz, H-14b), 2.06 (3H, d, J = 1.3 Hz, H-27), 1.87 (1H, m, H-11b), 1.74 (1H, m, H-8), 1.67 (1H, m, H-10a), 1.65 (3H, bs, H-26), 1.33 (3H, s, H-23), 1.27 (3H, m, H-9 and H-10b), 1.18 (3H, d, J = 6.9 Hz, H-24), 1.06 (3H, s, H-22), 1.01 (3H, d, J = 7.0 Hz, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 220.7 (s, C-5), 170.4 (s, C-1), 165.0 (s, C-20), 152.1 (s, C-18), 139.3 (s, C-12), 138.5 (s, C-16), 120.9 (d, C-13), 119.3 (d, C-17), 115.7 (d, C-19), 79.0 (d, C-15), 74.2 (d, C-7), 72.3 (d, C-3), 53.6 (s, C-4), 41.7 (d, C-6), 39.7 (t, C-2), 38.5 (d, C-8), 32.6 (t, C-14), 31.8 (t, C-9), 31.6 (t, C-11), 25.4 (t, C-10), 22.9 (q, C-23), 22.9 (q, C-26), 19.1 (q, C-21), 18.1 (q, C-22), 15.9 (q, C-27), 15.8 (q, C-25), 13.4 (q, C-24); EIMS m/z 491 $[M]^+$ (18), 476 (2), 304 (15), 171 (27), 168 (100), 164 (20), 111 (11); HREIMS m/z 491.2703 (calcd for $C_{27}H_{41}NO_5S$, 491.2705).

Epothilone C_1 (16): colorless amorphous solid; 32.4 mg; t_R 7.2 min; $[\alpha]^{22}_D$ −114.0 (c 10.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (16 500), 248 (12 500); IR (KBr) ν_{max} 3440, 2933, 2877, 2858, 1730, 1708, 1457, 1244, 981 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.40 (1H, ddd, J = 6.2, 6.1, 6.1 Hz, H-3), 3.01 (1H, dq, J = 5.7, 6.9 Hz, H-6), 3.01 (1H, bs, 3-OH), 2.84 (1H, dq, J = 5.2, 7.0 Hz, H-4), 2.64 (1H, dd, J = 15.9, 7.1 Hz, H-2a), 2.54 (1H, dd, J = 15.9, 6.1 Hz, H-2b), 1.19 (3H, d, J = 6.9 Hz, H-24), 1.14 (3H, d, J = 6.9 Hz, H-23); ^{13}C NMR, see Table 1; EIMS m/z 463 $[M]^+$ (5), 324 (8), 290 (8), 204 (7), 168 (100), 164 (15), 139 (36); HREIMS m/z 463.2381 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

Epothilone D_1 (17): colorless amorphous solid; 5.3 mg; t_R 8.7 min; $[\alpha]^{22}_D$ −119 (*c* 0.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 208 (18 300), 249 (11 900); IR (KBr) ν_{max} 3439, 2965, 2934, 2877, 1729, 1707, 1456, 1250, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.42 (1H, ddd, J = 7.1, 6.3, 5.5 Hz, H-3), 3.07 (1H, dq, J = 6.5, 6.9 Hz, H-6), 2.95 (1H, dq, J = 4.7, 7.0 Hz, H-4), 2.69 (1H, dd, J = 16.0, 6.3 Hz, H-2a), 2.59 (1H, dd, J = 16.0, 7.1 Hz, H-2b), 2.46 (1H, bs, 3-OH), 1.24 (3H, d, J = 6.9 Hz, H-24), 1.14 (3H, d. J = 7.0, H-23); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 217.0 (s, C-5), 67.7 (d, C-3), 52.1 (d, C-4), 23.1 (q, C-26), 14.5 (q, C-24), 9.7 (q, C-23); EIMS *m/z* 477 $[M]^+$ (13), 304 (19), 303 (31), 218 (40), 204 (41), 168 (100), 164 (45), 157 (25), 139 (18); HREIMS *m/z* 477.2544 (calcd for $C_{26}H_{39}NO_5S$, 477.2549).

Epothilone C_2 (18): colorless amorphous solid; 58.4 mg; t_R 8.0 min; $[\alpha]^{22}_D$ −11.6 (*c* 10.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (15 500), 249 (12 100); IR (KBr) ν_{max} 3428, 2962, 2929, 2877, 2859, 1734, 1705, 1460, 1251, 982 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.42 (1H, dddd, J = 9.4, 5.6, 4.2, 4.1 Hz, H-3), 3.93 (1H, d, J = 5.6 Hz, 3-OH), 3.12 (1H, dq, J = 4.2, 7.0 Hz, H-4), 3.00 (1H, dq, J = 6.9, 7.0 Hz, H-6), 1.26 (3H, d, J = 7.0 Hz, H-24), 1.15 (3H, d, J = 7.0 Hz, H-23); ^{13}C NMR, see Table 1; EIMS *m/z* 463 $[M]^+$ (7), 324 (7), 306 (8), 290 (17), 168 (100), 164 (14), 139 (27); HREIMS *m/z* 463.2392 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

Epothilone D_2 (19): colorless amorphous solid; 13.1 mg; t_R 8.0; $[\alpha]^{22}_D$ −12.5 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (15 400), 248 (11 200); IR (KBr) ν_{max} 3436, 2965, 2930, 2877, 1732, 1705, 1458, 1253, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.27 (1H, m, H-3), 3.19 (1H, bs, 3-OH), 3.07 (1H, dq, J = 4.3, 7.0 Hz, H-4), 2.95 (1H, dq, J = 5.6, 7.0 Hz, H-6), 1.19 (3H, d, J = 6.9 Hz, H-24), 1.17 (3H, d. J = 7.0, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 216.8 (s, C-5), 69.7 (d, C-3), 48.6 (d, C-4), 48.4 (d, C-6), 39.9 (t, C-2), 12.4 (q, C-23), 12.7 (q, C-24); EIMS *m/z* 477 $[M]^+$ (22), 304 (19), 303 (17), 218 (22), 204 (25), 168 (100), 164 (28), 157 (31), 139 (21); HREIMS *m/z* 477.2545 (calcd for $C_{26}H_{39}NO_5S$, 477.2549).

Epothilone C_3 (20): colorless amorphous solid; 32.5 mg; t_R 9.4 min; $[\alpha]^{22}_D$ −62.1 (*c* 5.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (16 200), 248 (12 300); IR (KBr) ν_{max} 3432, 2928, 2878, 2858, 1736, 1698, 1252, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.93 (1H, ddd, J = 9.5, 2.3, 1.4 Hz, H-7), 3.56 (1H, bd, J = 2.3 Hz, 7-OH), 2.70 (1H, dd, J = 18.0, 1.4 Hz, H-6a), 2.36 (1H, dd, J = 18.0, 9.5 Hz, H-6b), ^{13}C NMR, see Table 1; EIMS *m/z* 463 $[M]^+$ (28), 290 (14), 168 (100), 164 (36), 157 (44), 151 (25); HREIMS *m/z* 463.2379 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

Epothilone C_4 (21): colorless amorphous solid; 6.5 mg; t_R 9.7 min; $[\alpha]^{22}_D$ −75.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (17 200), 248 (12 500); IR (KBr) ν_{max} 3434, 2974, 2932, 2859, 1735, 1686, 1252, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.78 (1H, m, H-7), 3.46 (1H, d, J = 0.9 Hz, 7-OH), 3.01 (1H, dq, J = 0.5, 7.0 Hz, H-6), 1.51 (2H, m, H-8), 1.37 (2H, m, H-9); ^{13}C NMR, see Table 1; EIMS *m/z* 463 $[M]^+$ (7), 276 (15), 171 (33), 168 (100), 164 (23), 151 (22), 111 (13); HREIMS *m/z* 463.2373 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

Epothilone C_5 (22): colorless amorphous solid; 7.3 mg; t_R 8.7 min; $[\alpha]^{22}_D$ −158 (*c* 0.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 205 (19500), 247 (12700); IR (KBr) ν_{max} 3447, 2972, 2927, 1737, 1690, 1450, 1252, 1181, 986 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.37 (1H, m, H-9), 4.29 (1H, dd, J = 6.0, 2.6 Hz, H-7), 3.17 (1H, dq, J = 6.0, 6.9 Hz, H-6), 2.44 (1H, bs, 7-OH), 2.20 (1H, m, H-10a), 2.12 (1H, m, H-10b), 1.67 (3H, bs, H-25), 1.27 (3H, s, H-23), 1.21 (3H, d, J = 6.9 Hz, H-24), 1.15 (3H, s, H-22); ^{13}C NMR, see Table 1; EIMS *m/z* 475 $[M]^+$ (6), 392 (7), 304 (6), 288 (33), 204 (76), 171 (19), 168 (100), 164 (12); HREIMS *m/z* 475.2380 (calcd for $C_{26}H_{37}NO_5S$, 475.2392).

Epothilone D_5 (23): colorless amorphous solid; 0.9 mg; t_R 11.0 min; $[\alpha]^{22}_D$ −150 (*c* 0.2, MeOH); UV (MeOH) λ_{max} nm (ϵ) 205 (23 300), 248 (13 600); IR (KBr) ν_{max} 3439, 2967, 2927, 1736, 1690, 1451, 1254, 1181, 987 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.34 (1H, bs, H-9), 4.30 (1H, bd, J = 4.9 Hz, H-7), 3.17 (1H, dq, J = 4.9, 7.0 Hz, H-6), 2.65 (1H, d, J = 2.1 Hz, 7-OH), 2.25 (2H, m, H-10a and H-11a), 2.20 (1H, m, H-10b); ^{13}C NMR, see Table 1; EIMS *m/z* 489 $[M]^+$ (4), 406 (4), 338 (7), 302 (13), 218 (35), 171 (10), 168 (100), 153 (20), 125 (10); HREIMS *m/z* 489.2536 (calcd for $C_{27}H_{39}NO_5S$, 489.2549).

Epothilone C_6 (24): colorless amorphous solid; 2.9 mg; t_R 9.8 min; $[\alpha]^{22}_D$ −205.2 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 218 (24 600), 237 (28 800); IR (KBr) ν_{max} 3435, 2967, 2927, 2882, 1732, 1688, 1465, 1258, 988 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.43 (1H, dd, 15.5, 10.8 Hz, H-11), 6.11 (1H, dd, J = 10.8, 10.6 Hz, H-12), 5.75 (1H, ddd, J = 15.5, 8.3, 5.6 Hz, H-10), 5.34 (1H, m, H-13), 2.48 (2H, m, H-9); ^{13}C NMR, see Table 1; EIMS *m/z* 475 $[M]^+$ (13), 387 (2), 316 (4), 288 (15), 230 (16), 204 (9), 171 (18), 168 (100), 164 (14), 151 (17); HREIMS *m/z* 475.2361 (calcd for $C_{26}H_{37}NO_5S$, 475.2392).

Epothilone C_7 (25): colorless amorphous solid; 0.9 mg; t_R 3.4 min; $[\alpha]^{22}_D$ −11 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 203 (18 300), 247 (12 500); IR (KBr) ν_{max} 3437, 2958, 2926, 2856, 1732, 1691, 1464, 1382, 1260 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.59 (1H, ddd, J = 11.1, 11.1, 3.8 Hz, H-12), 5.40 (1H, dd, J = 11.1, 9.2, H-13), 5.03 (1H, d, J = 9.3 Hz, H-15), 4.62 (1H, dd, J = 9.3, 9.2 Hz, H-14); EIMS *m/z* 493 $[M]^+$ 493 $[M]^+$(4), 306 (7), 290 (6), 192 (9), 168 (100), 100 (11); HREIMS *m/z* 493.25002 (calcd for $C_{26}H_{39}NO_6S$, 493.2498).

Epothilone C_8 (26): colorless amorphous solid; 26.3 mg; t_R 9.0 min; $[\alpha]^{22}_D$ −75.2 (*c* 2.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (16 800), 248 (17 800); IR (KBr) ν_{max} 3443, 2932, 2881, 1734, 1689, 1465, 1255, 1183, 976 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.93 (1H, s, H-19), 6.62 (1H, dd, J = 15.6, 0.6 Hz, H-17), 6.49 (1H, dd, J = 15.6, 6.6 Hz, H-16), 5.52 (1H, dddd, J = 9.5, 6.6, 2.8, 0.6 Hz, H-15), 2.67 (1H, ddd, J = 14.9, 9.5, 8.4 Hz, H-14a), 2.30 (1H, bd, J = 14.9 Hz, H-14b); ^{13}C NMR, see Table 1; EIMS *m/z* 463 $[M]^+$ (21), 310 (10), 276 (21), 171 (83), 154 (100), 150 (27), 111 (18); HREIMS *m/z* 463.2382 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

Epothilone C_9 (27): colorless amorphous solid; 3.0 mg; t_R 10.2 min; $[\alpha]^{22}_D$ −93.4 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 209 (15 200), 254 (15 700); IR (KBr) ν_{max} 3416, 2966, 2932, 1736, 1689, 1463, 1249, 1011 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, s, H-19), 6.65 (1H, bs, H-17), 6.56 (1H, dd, J = 10.6, 4.4 Hz, 27-OH), 5.52 (1H, dd, J = 11.6, 2.0 Hz, H-15), 4.47 (1H, ddd, J = 12.5, 4.4, 1.3 Hz, H-27a), 4.20 (1H, ddd, J = 12.5, 10.6, 0.9 Hz, H-27b), 2.80 (1H, ddd, J = 14.8, 11.6, 11.0 Hz, H-14a), 2.01 (1H, ddd, J = 14.8, 3.9, 2.0 Hz, H-14b); ^{13}C NMR, see Table 1; EIMS *m/z* 493 $[M]^+$ (17), 306 (64), 184 (50), 171 (30), 167 (38), 166 (100), 138 (12); HREIMS *m/z* 493.2502 (calcd for $C_{26}H_{39}NO_6S$, 493.2498).

***trans*-Epothilone C_1 (28):** colorless amorphous solid; 1.4 mg; t_R 8.7 min; $[\alpha]^{22}_D$ −84 (*c* 0.2, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (17 400), 248 (12 900); IR (KBr) ν_{max} 3433, 2961, 2933, 2879, 1730, 1708, 1457, 1251, 975 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.00 (1H, s, H-19), 6.64 (1H, bs, H-17), 5.45 (1H, ddd, J = 15.2, 6.5, 6.5 Hz, H-12), 5.42 (1H, dd, J = 6.4, 3.7 Hz, H-15), 5.35 (1H, dt, J = 15.2, 7.1 Hz, H-13), 4.42 (1H, m, H-3), 3.58 (1H, ddd, J = 8.1, 7.9, 2.8 Hz, H-7), 3.24 (1H, m, H-6), 3.14 (1H, dq, J = 4.0, 6.9 Hz, H-6), 2.92 (1H, d, J = 7.9 Hz, 7-OH), 2.71 (3H, s, H-21), 2.71 (2H, m, H-2), 2.53 (2H, m, H-14), 2.17 (1H, d, J = 2.17 Hz, 3-OH), 2.11 (1H, m, H-11a), 2.06 (3H, bs, H-27), 1.93 (1H, m, H-11b), 1.68 (1H, m, H-9a), 1.65 (1H, m, H-10a), 1.33 (1H, m, H-8), 1.26 (3H, d, J = 6.8 Hz, H-24), 1.16 (1H, m, H-10b), 1.12 (3H, d, J = 6.9 Hz, H-22), 1.07 (1H, m, H-9b), 1.00 (3H, d, J = 6.8 Hz, H-25); ^{13}C NMR, see Table 1; EIMS *m/z* 463 $[M]^+$ (6), 290 (21), 289 (20), 204 (23), 194 (19), 190 (22), 168 (100), 164 (48), 157 (14), 152 (19), 151 (17), 139 (15), 111 (18); HREIMS *m/z* 463.2371 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

***trans*-Epothilone C_2 (29):** colorless amorphous solid; 4.5 mg; t_R 8.5 min; $[\alpha]^{22}_D$ −3 (*c* 1.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (15 800), 248 (11 900); IR (KBr) ν_{max} 3435, 2963, 2931, 2878, 1731, 1706, 1457, 1273, 979 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 4.13 (1H, dddd, J = 6.7, 6.2, 5.6, 5.1 Hz, H-3), 3.18 (1H, d, J = 5.6 Hz, 3-OH), 3.06 (1H, dq, J = 8.2, 7.1 Hz, H-6), 2.98 (1H, dq, J = 6.2, 7.0 Hz, H-4), 2.64 (1H, dd, J = 15.1, 6.7 Hz, H-2a), 2.54 (1H, dd, J = 15.1, 5.1 Hz, H-2b), 1.15 (3H, d, J = 7.0 Hz, H-23); ^{13}C NMR, see Table 1; EIMS *m/z* 463 $[M]^+$ (13), 290 (11), 190 (10), 168 (100), 164 (20), 157 (26), 139 (17); HREIMS *m/z* 463.2383 (calcd for $C_{25}H_{37}NO_5S$, 463.2392).

Epothilone I_1 (30): colorless amorphous solid; 7.1 mg; t_R 18.6 min; $[\alpha]^{22}_D$ −32.8 (*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 204 (14 600), 249 (8800); IR (KBr) ν_{max} 3437, 2960, 2927, 2855,

1733, 1695, 1459, 1367, 1261, 1180, 1039, 978 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.96 (1H, s, H-19), 6.54 (1H, bs, H-17), 5.49 (1H, ddd, J = 10.3, 7.3, 7.3 Hz, H-12), 5.33 (1H, dd, J = 8.3, 4.4 Hz, H-15), 5.31 (1H, m, H-13), 4.15 (1H, ddd, J = 8.0, 5.0, 4.6 Hz, H-3), 3.80 (1H, m, H-7), 3.21 (1H, dq, J = 6.0, 6.9 Hz, H-6), 2.89 (1H, d, J = 5.0 Hz, 3-OH); 2.70 (3H, s, H-21), 2.65 (1H, ddd, J = 15.8, 8.5, 8.3 Hz, H-14a), 2.42 (2H, m, H-2), 2.35 (1H, m, H-14b), 2.27 (1H, bd, J = 3.3 Hz, 7-OH), 2.13 (1H, m, H-11a), 2.09 (3H, d, J = 1.2 Hz, H-27), 2.00 (1H, m, H-11b), 1.72 (1H, m, H-8), 1.40 (2H, m, H-10′), 1.37 (1H, m, H-9′a), 1.36 (2H, m, H-9), 1.32 (3H, s, H-23), 1.27 (3H, m, H-9′ b and H-10), 1.13 (3H, d, J = 6.9 Hz, H-24), 1.09 (3H, s, H-22), 0.94 (3H, d, J = 6.9 Hz, H-25); ^{13}C NMR, see Table 1; EIMS m/z (%) 505 $[M]^+$ (10), 417 (2), 318 (25), 260 (5), 190 (15), 171 (25), 168 (100), 97 (15); HREIMS m/z 505.2869 (calcd for $C_{28}H_{43}NO_5S$, 505.2862).

Epothilone I_2 (31): colorless amorphous solid; 4.0 mg; t_R 20.3 min; $[\alpha]^{22}_D$ −68.5 (c 1.0, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (12 600), 249 (9200); IR (KBr) ν_{max} 3437, 2963, 2928, 2855, 1735, 1696, 1461, 1376, 1292, 1177, 977 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.95 (1H, s, H-19), 6.53 (1H, bs, H-17), 5.40 (1H, m, H-12), 5.38 (1H, dd, J = 9.8, 3.3 Hz, H-15), 5.37 (1H, m, H-13), 4.21 (1H, ddd, J = 8.6, 3.8, 3.6 Hz, H-3), 3.85 (1H, ddd, J = 8.5, 5.8, 2.2 Hz, H-7), 3.18 (1H, dq, J = 8.5, 7.0 Hz, H-6), 2.70 (3H, s, H-21), 2.65 (1H, ddd, J = 15.2, 9.8, 9.0 Hz, H-14a), 2.51 (1H, d, J = 3.6 Hz, 3-OH), 2.37 (2H, m, H-2), 2.32 (1H, bd, J = 15.2 Hz, H-14b), 2.09 (3H, d, J = 1.3 Hz, H-27), 2.07 (2H, m, H-11), 1.78 (1H, m, H-8), 1.65 (1H, d, J = 5.8 Hz, 7-OH), 1.57 (1H, m, H-10′ a), 1.44 (1H, m, H-10a), 1.42 (1H, m, H-9′), 1.32 (3H, s, H-23), 1.21 (1H, m, H-10′ b), 1.17 (3H, d, J = 7.0 Hz, H-24), 1.13 (2H, m, H-9), 1.06 (3H, s, H-22), 0.95 (3H, d, J = 7.0 Hz,H-25), 0.91 (3H, d, J = 6.5 Hz, H-25′), 0.68 (1H, m, H-10b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 220.4 (s, C-5), 171.3 (s, C-1), 164.7 (s, C-20), 152.4 (s, C-18), 137.6 (s, C-16), 134.5 (d, C-12), 125.3 (d, C-13), 119.6 (d, C-17), 116.2 (d, C-19), 78.6 (d, C-15), 77.2 (d, C-7), 75.0 (d, C-3), 51.0 (s, C-4), 44.6 (d, C-6), 38.2 (t, C-2), 36.9 (t, C-9), 34.5 (t, C-10), 32.6 (d, C-8), 32.0 (t, C-14), 30.0 (d, C-9′), 27.4 (t, C-11), 26.6 (t, C-10′), 25.0 (q, C-22), 21.5 (q, C-25′), 19.3 (q, C-21), 17.9 (q, C-25), 17.7 (q, C-23), 15.8 (q, C-24), 15.6 (q, C-27); EIMS m/z 519 (%) $[M]^+$ (11), 431 (4), 348 (6), 332 (24), 274 (15), 168 (100), 84 (25); HREIMS m/z 519.3009 (calcd for $C_{29}H_{45}NO_5S$, 519.3018).

Epothilone I_3 (32): colorless amorphous solid; 0.9 mg; t_R 19.7 min; $[\alpha]^{22}_D$ −64 (c 0.17, MeOH); UV (MeOH) λ_{max} nm (ϵ) 203 (15 800), 249 (9000); IR (KBr) ν_{max} 3436, 2958, 2925, 2854, 1734, 1697, 1457, 1376, 1261, 1180, 1014, 978 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.67 (3H, s, H-26), 1.31 (3H, s, H-23), 1.27 (1H, m, H-10a), 1.16 (3H, d, J = 7.0 Hz, H-24), 1.07 (3H, s, H-22), 0.95 (3H, d, J = 7.0 Hz,H-25), 0.92 (3H, d, J = 6.5 Hz, H-25′), 0.75 (1H, m, H-10b); EIMS m/z (%) 533 $[M]^+$ (12), 445 (2), 346 (19), 288 (6), 204 (12), 168 (100), 97 (8); HREIMS m/z 533.3169 (calcd for $C_{30}H_{47}NO_5S$, 533.3175).

Epothilone I_4 (33): colorless amorphous solid; 3.3 mg; t_R 24.3 min; $[\alpha]^{22}_D$ −51 (c 0.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (13 400), 250 (9800); IR (KBr) ν_{max} 3435, 2958, 2927, 2876, 2856, 1733, 1711, 1458, 1376, 1275, 1173, 983 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.09 (1H, dddd, J = 9.6, 8.1, 4.5, 3.3 Hz, H-3), 3.57 (1H, bs, 3-OH), 2.83 (1H, dq, J = 8.1, 7.1 Hz, H-4), 1.17 (3H, d, J = 7.1 Hz, H-23), 0.79 (1H, m, H-10b); EIMS m/z (%) 505 $[M]^+$ (2), 448 (2), 332 (8), 274 (4), 204 (5), 168 (100), 97 (5); HREIMS m/z 505.2867 (calcd for $C_{28}H_{43}NO_5S$, 505.2862).

Epothilone I_5 (34): colorless amorphous solid; 2.2 mg; t_R 24.2 min; $[\alpha]^{22}_D$ −36 (c 0.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 208 (14 400), 249 (9700); IR (KBr) ν_{max} 3438, 2960, 2927, 2874, 2856, 1733, 1711, 1457, 1377, 1275, 1179, 981 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.05 (1H, dddd, J = 7.5, 7.2, 5.9, 4.6 Hz, H-3), 3.17 (1H, d, J = 5.9 Hz, 3-OH), 2.94 (1H, dq, J = 7.2, 7.1 Hz, H-4), 2.87 (1H, dq, J = 6.5, 6.9 Hz, H-6), 1.11 (3H, d, J = 6.9 Hz, H-24); EIMS m/z 519 $[M]^+$ (4), 362 (2), 346 (16), 288 (8), 232 (4), 218 (26), 204 (30), 168 (100), 139 (18), 97 (9); HREIMS m/z 519.3015 (calcd for $C_{29}H_{45}NO_5S$, 519.3018).

Epothilone I_6 (35): colorless amorphous solid; 1.7 mg; t_R 28.0 min; $[\alpha]^{22}_D$ −40 (c 0.35, MeOH); UV (MeOH) λ_{max} nm (ϵ) 204 (14 600), 250 (9000); IR (KBr) ν_{max} 3437, 2962, 2926, 2872, 2855, 1727, 1708, 1457, 1378, 1261, 1177, 983 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.22 (1H, tdd, J = 6.1, 5.6, 4.8 Hz, H-3), 3.13 (1H, d, J = 5.6 Hz, 3-OH), 3.05 (1H, dq, J = 4.8, 7.0 Hz, H-4), 2.79 (1H, dq, J = 5.6, 6.9 Hz, H-6), 1.21 (3H, d, J = 7.0 Hz, H-22), 1.16 (3H, d, J = 6.9 Hz, H-24); EIMS m/z 519 $[M]^+$ (4), 462 (2), 346 (17), 288 (6), 232 (4), 204 (24), 168 (100), 139 (24), 97 (10); HREIMS m/z 519.3009 (calcd for $C_{29}H_{45}NO_5S$, 519.3018).

Epothilone K (36): colorless amorphous solid; 0.4 mg; t_R 10.6 min; $[\alpha]^{22}_D$ −7 (c 0.08, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (16 700), 248 (12 500); IR (KBr) ν_{max} 3431, 2963, 2927, 2856, 1731, 1712, 1262, 1093, 1021, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.95 (1H, s, H-19), 6.51 (1H, bs, H-17), 5.49 (3H, m, H-15, H-13, and H-12), 4.04 (1H, dddd, J = 7.9, 7.6, 6.9, 3.3 Hz, H-3), 3.36 (1H, dq, J = 6.9, 6.8 Hz, H-6), 2.83 (1H, d, J = 7.6 Hz, 3-OH), 2.75 (1H, ddd, J = 16.1, 6.6, 3.4 Hz, H-14a), 2.74 (1H, dd, J = 15.3, 3.3 Hz, H-2a), 2.71 (3H, s, H-21), 2.58 (2H, m, H-14b and H-8), 2.50 (1H, dd, J = 15.3, 7.9 Hz, H-2b), 2.29 (1H, m, H-11a), 2.10 (1H, m, H-11b), 2.09 (3H, d, J = 0.7 Hz, H-27), 1.78 (1H, m, H-9a), 1.65 (1H, m, H-10a), 1.48 (1H, m, H-10b), 1.18 (1H, m, H-9b), 1.15 (3H, d, J = 6.8 Hz, H-22), 1.03 (3H, d, J = 6.5 Hz, H-25); EIMS m/z 405 $[M]^+$ (38), 317 (12), 260 (9), 232 (10), 204 (14), 190 (16), 168 (100), 164 (30), 151 (28); HREIMS m/z 405.1976 (calcd for $C_{26}H_{39}NO_5S$, 405.1974).

Dihydroxyketone 37: colorless amorphous solid; 2.9 mg; t_R 9.5 min; $[\alpha]^{22}_D$ −28 (c 0.4, MeOH); UV (MeOH) λ_{max} nm (ϵ) 211 (16 100), 247 (12 100); IR (KBr) ν_{max} 3431, 2967, 2929, 2875, 1704, 1462, 1381, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, s, H-19), 6.55 (1H, bs, H-17), 5.56 (1H, dtt, J = 10.8, 7.3, 1.4 Hz, H-12), 5.39 (1H, dtt, J = 10.8, 7.3, 1.4 Hz, H-13), 4.17 (1H, t, J = 6.6 Hz, H-15), 3.50 (1H, ddd, J = 8.7, 2.6, 2.6 Hz, H-7), 3.10 (1H, d, J = 2.6, 7-OH), 2.90 (1H, dq, J = 2.6, 7.2 Hz, H-6), 2.77 (1H, sep, J = 6.9 Hz, H-4), 2.70 (3H, s, H-21), 2.40 (2H, m, H-14), 2.07 (2H, m, H-11), 2.04 (3H, d, J = 1.1 Hz, H-27), 1.78 (1H, bs, 15-OH), 1.74 (1H, m, H-9a), 1.50 (1H, m, H-8), 1.46 (1H, m, H-10a), 1.27 (1H, m, H-10b), 1.11 (1H, m, H-9b), 1.094 (3H, d, J = 6.9 Hz, H-23), 1.089 (3H, d, J = 6.9 Hz, H-22), 1.08 (3H, d, J = 7.2 Hz, H-24), 0.82 (3H, d, J = 6.7 Hz, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 220.5 (s, C-5), 164.6 (s, C-20), 152.9 (s, C-18), 141.5 (s, C-16), 133.4 (d, C-12), 125.0 (d, C-13), 119.2 (d, C-17), 115.6 (d, C-19), 77.2 (d, C-15), 74.9 (d, C-7), 44.9 (d, C-6), 40.0 (d, C-4), 35.5 (d, C-8), 33.5 (t, C-14), 32.3 (t, C-9), 27.9 (t, C-11), 26.9 (t, C-10), 19.2 (q, C-21), 18.6 (q, C-23), 18.1 (q, C-22), 15.6 (q, C-25), 14.4 (q, C-27), 9.3 (q, C-24); EIMS m/z 407 $[M]^+$ (0.1), 204 (0.8), 168 (100), 140 (3.4); HREIMS m/z 407.2488 (calcd for $C_{23}H_{37}NO_3S$, 407.2494).

Hydroxyketone 38: colorless amorphous solid; 6.5 mg; t_R 8.7 min; $[\alpha]^{22}_D$ −25 (c 0.5, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (17 700), 247 (13 400); IR (KBr) ν_{max} 3427, 2971, 2933, 2878, 2858, 1709, 1457, 1377, 1186, 1023 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.95 (1H, s, H-19), 6.55 (1H, bs, H-17), 5.52 (1H, dtt, J = 10.9, 7.2, 1.4 Hz, H-12), 5.39 (1H, dtt, J = 10.9, 7.1, 1.2 Hz, H-13), 4.18 (1H, ddt, J = 3.4, 0.4, 6.7 Hz, H-15), 2.71 (3H, s, H-21), 2.51 (1H, bq, J = 6.8 Hz, H-8), 2.48 (1H, dq, J = 17.7, 7.4 Hz, H-6a), 2.41 (1H, dq, J = 17.7, 7.2 Hz, H-6b), 2.39 (2H, ddd, J = 7.1, 6.7, 1.4 Hz, H-14), 2.06 (2H, ddt, 7.2, 1.2, 7.0 Hz, H-11), 2.05 (3H, d, J = 1.4 Hz, H-27), 1.81 (1H, d, J = 3.4 Hz, 15-OH), 1.66 (1H, m, H-9a), 1.32 (1H, m, H-9b), 1.31 (2H, m, H-10), 1.06 (3H, d, J = 6.9 Hz, H-25), 1.04 (3H, dd, J = 7.4, 7.2 Hz, H-24); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 215.3 (s, C-7), 164.6 (s, C-20), 152.9 (s, C-18), 141.5 (s, C-16), 132.7 (d, C-12), 125.3 (d, C-13), 119.2 (d, C-17), 115.6 (d, C-19), 77.2 (d, C-15), 46.0 (d, C-8), 34.3 (t, C-14), 33.5 (t, C-6), 32.7 (t, C-9), 27.5 (t, C-11), 27.3 (t, C-10), 19.2 (q, C-21), 16.5 (q, C-25), 14.4 (q, C-27), 7.8 (q, C-24); EIMS m/z 335 $[M]^+$ (2), 317 (4), 170 (27), 169 (67), 168 (100), 140 (20); HREIMS m/z 335.1912 (calcd for $C_{19}H_{29}NO_2S$, 335.1919).

Hydroxyketone 39: colorless amorphous solid; 0.8 mg; t_R 10.4 min; $[\alpha]^{22}_D$ −9 (0.02, MeOH); UV (MeOH) λ_{max} nm (ϵ) 203 (19 100), 244 (12 500); IR (KBr) ν_{max} 3430, 2970, 2934, 2877, 1710, 1458, 1377, 1184 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, s, H-19), 6.55 (1H, bs, H-17), 5.17 (1H, t, J = 7.3 Hz, H-13), 4.13 (1H, m, H-15), 2.70 (3H, s, H-21), 2.51 (1H, bq, J = 6.8 Hz, H-8), 2.47 (1H, dq, J = 17.7, 7.2 Hz, H-6a), 2.41

(1H, dq, J = 17.7, 7.2 Hz, H-6b), 2.33 (2H, bdd, J = 7.3, 6.8 Hz, H-14), 2.05 (3H, d, J = 1.2 Hz, H-27), 2.03 (2H, m, H-11), 1.71 (1H, d, J = 3.2 Hz, 15-OH), 1.69 (3H, d, J = 1.3 Hz, H-26), 1.62 (1H, m, H-9a), 1.32 (3H, m, H-10 and H-9b), 1.06 (3H, d, J = 6.9 Hz, H-25), 1.03 (3H, t, J = 7.2 Hz, H-24); EIMS m/z 349 $[M]^+$ (0.7), 331 (1.7), 168 (100), 140 (5.1); HRDCIMS m/z 350.2145 (calcd for $C_{20}H_{31}NO_2S$, 350.2154).

Dihydroxycarboxylic acid 40: colorless, amorphous solid; 5.2 mg; $[\alpha]^{22}_D$ +9 (*c* 0.3, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (15 900), 248 (12 200); IR (KBr) ν_{max} 3365, 2926, 2843, 1715, 1583, 1435, ^{1}H NMR (CD_3OD), 400 MHz) δ 7.20 (1H, s, H-19), 6.59 (1H, s, H-17), 4.6 (1H, m, H-15), 4.42 (1H, m, H-13), 2.71 (3H, s, H-21), 2.45 (1H, m, H-12b), 2.38 (1H, m, H-12a), 1.95 (3H, s, H-27), 1.78 (1H, m, H-14b), 1.73 (1H, m, H-14a); EIMS m/z 271 $[M]^+$ (4), 253 (24), 164 (100), 151 (57), 140 (79), 97 (53), HREIMS m/z 271.3333 (calcd For $C_{12}H_{17}NO_4S$ 271.3341).

Dihydroxycarboxylic acid 41: colorless amorphous solid; 2.5 mg; $[\alpha]^{22}_D$ +2 (*c* 0.6, MeOH); UV (MeOH) λ_{max} nm (ϵ) 212 (16 000), 248 (12 100); IR (KBr) ν_{max} 3430, 2965, 2932, 1710, 1460, 1370 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.99 (1H, s, H-19), 6.56 (1H, s, H-17), 4.72 (1H, dd, J = 9.1, 6.0 Mz, H-15), 3.86 (1H, ddd, J = 10.0, 7.0, 4.1 Hz, H-13), 2.70 (3H, s, H-21), 2.41 (1H, dq, J = 10.0, 7.0 Hz, H-12), 2.29 (1H, ddd, J = 10.0, 7.0 Hz, H-14b), 2.10 (3H, s, H-27), 1.93 (1H, ddd, J = 10.0, 7.0, 3.2 Hz, H-14a), 1.43 (3H, d, J = 7,1 Hz, H-26); ^{13}C NMR (CD_3OD, 100 MHz). 174.3 (s, C-11), 167.0 (s, C-20), 154.0 (s, C-18), 144.8 (s, C-16) 122.5 (d, C-17), 118.8 (d, C-19), 74.5 (d, C-15), 67.0 (d, C-13), 52.0 (d, C-12), 43.6 (t, C-14), 18.7 (q, C-21), 14.9 (q, C-27), 13.7 (q, C-26); EIMS m/z 285 $[M]^+$ (5), 207 (38), 149 (96), 97 (65), 57 (85); HREIMS m/z 285.1019 (calcd For $C_{13}H_{19}NO_4S$, 285.1035).

Methoxycarboxylic acid 42: colorless amorphous solid; 6.3 mg; $[\alpha]^{22}_D$ +11 (*c* 1.2, MeOH); UV (MeOH) λ_{max} nm (ϵ) 210 (15 900), 249 (11 800); IR (KBr) ν_{max} 3427, 2960, 1700, 1444 cm^{-1}; ^{1}H NMR (CD_3OD), 300 MHz) δ 7.28 (1H, s, H-19), 6.59 (1H, s, H-17), 4.18 (1H, dd, J = 8.6 Hz, H-15), 3.30 (3H, s, 15-OMe), 2.72 (3H, s, H-21), 2.62 (1H, dd, J = 15.3, 9.2, 8.6 Hz, H-14b), 2.55 (1H, ddd, J = 15.3, 5.1, 4.6 Hz, H-14a), 2.01 (3H, s, H-27); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.6 (s, C-13), 167.0 (s, C-20), 153.3 (s, C-18), 139.5 (s, C-16), 122.4 (d, C-17), 85.0 (d, C-15), 56.8 (q, 15-OMe), 40.9 (t, C-14), 18.7 (q, C-21), 13.6 (q, C-27); EIMS m/z 241 $[M]^+$ (37), 226 (37), 208 (28), 182 (87), 164 (100), 151 (32), 128 (20), 97 (21); HREIMS m/z 241.0775 (calcd For $C_{11}H_{15}NO_3S$, 241.0773).

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Supporting Information Available: HPLC elution profile of a *S. cellulosum* crude extract, scheme of separation of compounds **1**−**39**, and ^{1}H and ^{13}C NMR data of epothilones **3**−**27**, **29**, **32**−**35**. This material is available free of charge via the Internet at http://pubs.acs.org.

Noted Added after ASAP: Figures 1 and 2 and Scheme 1 are in different positions than in the ASAP version posted on June 8, 2001. The current version was posted on June 18, 2001.

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(14) Compound **42** and a close analogue of it from the total synthesis of epothilone show specific optical rotations of $[\alpha]^{22}_D$ +11 and −11, respectively (ref 15, compound **23**). A proof of the absolute configuration of **37**−**42** by chemical degradation of epothilones A and B is under way.

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(25) Alternatively, however less probably, acetate is incorporated instead of propionate followed by C-methylation.

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