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Studies on Taxol biosynthesis: preparation of taxadiene-diol and triol derivatives by deoxygenation of taxusin

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ABSTRACT

The putative Taxol biosynthesis metabolites, taxa-4(20),11(12)-diene-5α,13α-diol (**7**), taxa-4(20),11(12)-diene-5α,9α,13α-triol (**9**), and taxa-4(20),11(12)-diene-5α,10β,13α-triol (**10**), have been prepared by Barton deoxygenation of the *C*-9 and *C*10-hydroxyl groups of protected derivatives of taxusin, a major taxoid metabolite isolated from Yew heart wood. The synthetic protocol devised is amenable for the preparation of isotopically labeled congeners that will be useful to probe further intermediate steps in the biosynthesis of Taxol.

1. Introduction

The approval of Taxol (Paclitaxel, **1**) as a clinically useful therapeutic agent against ovarian and other types of cancer continues to stimulate interest in both the chemical synthesis and the biogenesis of this agent.[1,2] Numerous approaches for the synthesis of the taxane framework[3] and for Taxol itself[4] have been reported but the architectural complexity of the Taxol core mandates lengthy syntheses, with low overall yields rendering chemical synthesis approaches to this drug impractical for large-scale manufacturing of clinical-grade material. The bark of *Taxus brevifolia* Nutt., the Pacific yew tree, served as the initial source for commercial-scale production of Taxol.[1] However, this species grows in environmentally sensitive areas of the Pacific Northwest and has become an untenable source for large-scale Taxol production. Alternative approaches for Taxol production such as semi-synthesis from 10-deacetylbaccatin III, which can be isolated from the needles of the European yew *Taxus baccata*, a renewable resource, were subsequently adopted as the current commercial method for Taxol production.[5] Additionally, it is reasonable to anticipate that Taxol will become utilized for other types of cancer and as a consequence, pressure on the population of yew species worldwide will increase significantly. Alternative sources for Taxol production based on biological systems have become an important goal and our laboratories have sought to employ emerging technologies based on the genetic manipulation of *Taxus* sp. cell cultures to address this problem. In order to meet these objectives, we have sought to elucidate a detailed understanding of the steps of Taxol biosynthesis and the identification of the associated genes.

Previously, we have reported a combination of in vivo feeding studies and investigations with cell-free enzyme systems, using *Taxus* sp. stem tissue or suspension cultured *Taxus* sp. cells as bioconversion vectors. The experimental results of these investigations have led us to surmise that the early steps of Taxol biosynthesis proceed in sequence from the initial conversion of geranylgeranyl pyrophosphate (**2**) to the parent diene **3** catalyzed by taxadiene synthase (Scheme 1).[6,7] Following this first committed step in Taxol biosynthesis, taxadiene hydroxylase, a cytochrome *P*-450-dependent enzyme, regioselectively hydroxylates **3** with allylic transposition of the 4,5-double bond, to the first oxygenated metabolite, taxa-4(20),11(12)-diene-5α-ol (**4**).[8] The resulting intermediate taxadienol **4** can be subsequently acetylated through the action of taxadienol-*O*-acetyltransferase to provide the corresponding acetate **5**.[9] Studies have shown that taxa-4(20),11(12)-diene-5α-acetate (**5**) was converted to taxa-4(20),11(12)-diene-5α,10β-diol-5-acetate (**6**) by taxoid 10β-hydroxylase.[10–12] Experiments also demonstrated that taxa-4(20),11(12)-diene-5α-ol (**4**) was converted by a 13α-hydroxylase to taxa-4(20),11(12)-diene-5α,13α-diol (**7**, Scheme 1),[12b] thereby introducing uncertainties as to the precise order of the early hydroxylation and C5-acetylation diverging from the confirmed intermediate taxadienol (**4**). The complementary but opposite substrate selectivities of the 13α-hydroxylase and the 10β-hydroxylase, which favors taxadien-5α-ol and taxadien-5α-acetate as substrates, respectively, as well as feeding studies of *Taxus* sp. cells with the relevant intermediates,[13] suggest a bifurcation in the early stages of Taxol biosynthesis.[14] Based on the relative abundance of the several hundreds defined taxoids bearing oxy-functional groups at various positions, the

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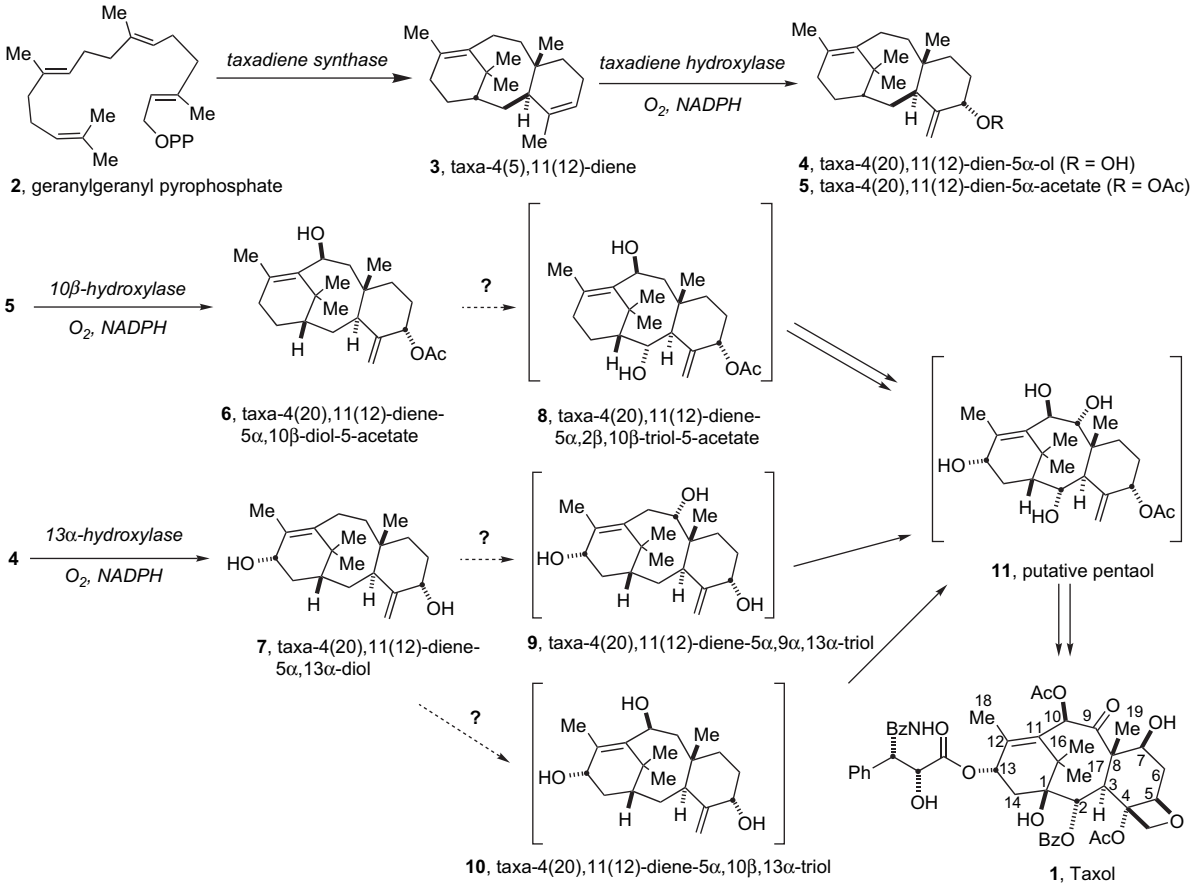


Scheme 1. Possible divergence of taxadiene to lightly oxygenated taxoids and convergence to a putative pentaol.

subsequent order of hydroxylation reactions downstream appears likely to occur at C10, followed by C13 and C9, and later C7 and C2, the precise timing of acetylation at C5 is, however, uncertain.[14] Considering numerous possible parallel sequences of oxygenation, we have decided to investigate a series of plausible intermediates downstream from **4** by way of the *C*13-oxygenated species[7] that can be converted into taxa-4(20),11(12)-diene-5α,9α,13α-triol (**9**) or taxa-4(20),11(12)-diene-5α,10β,13α-triol (**10**). In order to evaluate the possible intermediacy of such species, we required access to authentic specimens of **7**, **9**, and **10**, since we have found that sufficiently good substrates for subsequent hydroxylation reactions are often produced in trace amounts and whose structures are very difficult to confirm. We report herein, the semi-synthesis of **7**, **9**, and **10** by radical deoxygenation of taxusin.

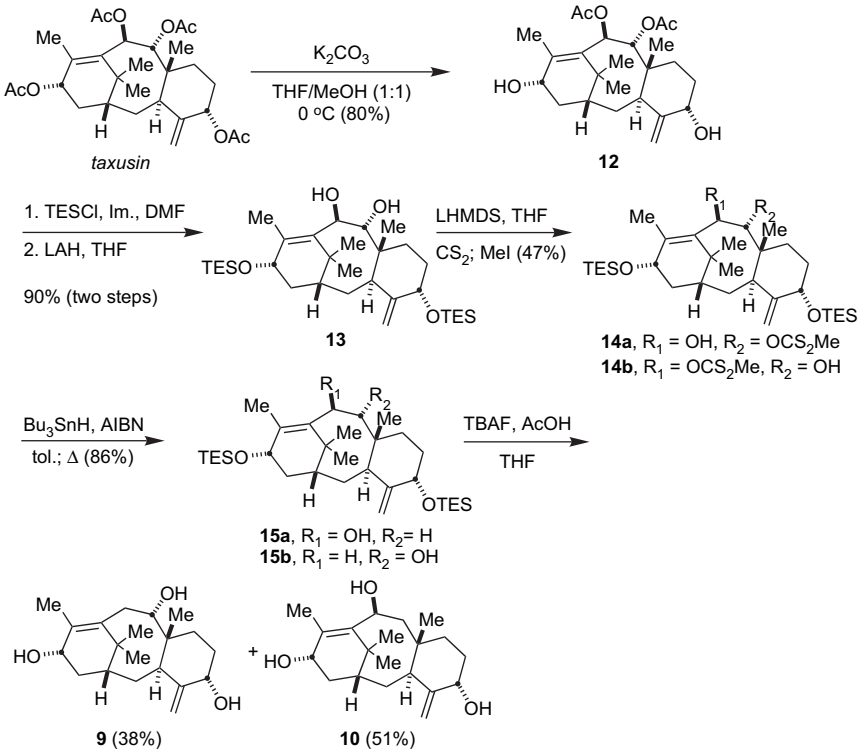
2. Results and discussion

Taxusin was selected as a starting substrate due to its structural relatedness to a variety of putative pathway metabolites and its high natural abundance in European and Japanese yew heart woods. Previous studies from our laboratory have revealed that taxusin can be deacetylated efficiently to the corresponding tetraol and re-protected with modest efficiency to form a series of differentially protected polyols that can be selectively deoxygenated by classical radical deoxygenation methods with the exception of the *C*13 alcohol.[15] All attempts to remove the *C*13 alcohol result in rearrangement of the *C*11(12)-bridgehead alkene to the *C*12(13) alkene. Despite our failure to deoxygenate taxusin at C-13, this substrate proved to be useful for making lightly oxygenated taxoids with oxygenation at C13.

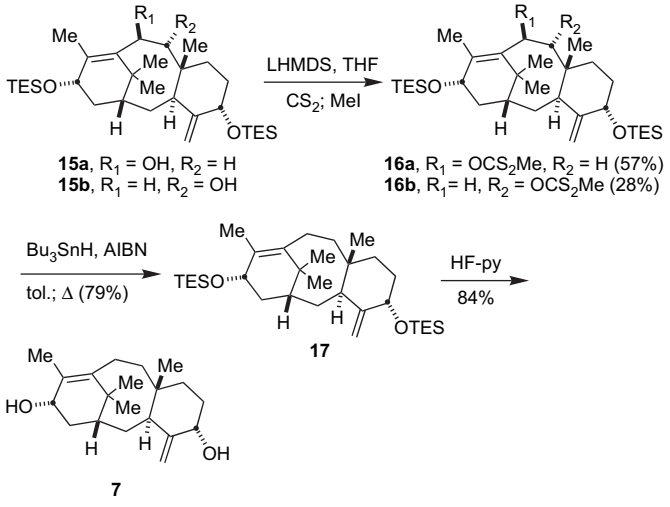
Taxusin was partially deacylated by treatment with K_2CO_3 in THF/MeOH at 0 °C for 3 days, which gave the 5,13-diol **12** predominantly with some 9,10-diol. A small amount of the more polar taxusin tetraol was produced but easily separated (Scheme 2). The diol **12** was treated with TESCl in the presence of imidazole in DMF followed by deacetylation of the 9,10-diacetyl groups using lithium aluminum hydride (LAH) in THF, which provided diol **13** in high yield. Exposure of **13** to LHMDS followed by addition of carbon disulfide and methyl iodide gave an inseparable mixture of the xanthates **14a** and **14b** in approximately 1:1 ratio. The *C*-9 and *C*-10-hydroxyl residues were successfully removed by Barton deoxygenation, respectively, to give an inseparable mixture of **15a** and **15b**. Finally, removal of the *O*-TES ethers by treatment with TBAF in the presence of acetic acid in THF gave the desired triols taxa-4(20),11(12)-diene-5α,10β,13α-triol (**10**) and taxa-4(20),11(12)-diene-5α,9α,13α-triol (**9**), which were separable by silica gel chromatography (Scheme 2).

The mixture of **15a**/**15b** also proved useful for the synthesis of the corresponding taxa-4(20),11(12)-diene-5α,13α-diol. As shown in Scheme 3, **15a**/**15b** was subjected to treatment with LHMDS in THF followed by the addition of carbon disulfide and methyl iodide to give a mixture of the xanthates **16a**/**16b**, which could be separated on column but was used as a mixture in the following step. Barton deoxygenation of **16a**/**16b** yielded the protected diol **17** in 79% yield. Final treatment of **17** with HF/pyridine complex afforded taxa-4(20),11(12)-diene-5α,13α-diol (**7**) (Scheme 3).

While this route provided the first authentic specimens of **9** and **10**, it proved to be capricious due to the lability of the *O*-TES ethers. As such, a more robust, second-generation synthesis was devised as shown in Scheme 4 where *O*-diethyl-*iso*-propylsilyl ethers (ODEIPS) were employed.



Scheme 2. Synthesis of taxa-4(20),11(12)-diene-5α,10β,13α-triol (**10**) and taxa-4(20),11(12)-diene-5α,9α,13α-triol (**9**).



Scheme 3. Synthesis of taxa-4(20),11(12)-diene-5α,13α-diol.

In addition, we found that conversion of taxusin into the corresponding tetraol followed by protection of the 9,10-diol unit as its cyclic carbonate proved advantageous and reproducible.

Taxusin was converted into tetraol **18** by global reductive removal of the acetates with LAH. Cyclic carbonate formation followed by protection of the *C*5 and *C*13 hydroxyl groups as *O*-DEIPS ethers proceeded in 62% overall yield. Reductive removal of the cyclic carbonate followed by xanthate formation gave the inseparable xanthates **22a**/**22b**, which were directly subjected to Barton deoxygenation conditions. Final removal of the silyl ethers with tetra-*n*-butylammonium fluoride (TBAF) produced **9** and **10** (77% and 17% yields, respectively). Similarly, **23a** and **23b** were converted to **7** in good overall yield as shown in Scheme 5.

The synthesis of these substrates with deuterium labeling is enabled through the use of tri-*n*-butyltin deuteride in the radical deoxygenation step.

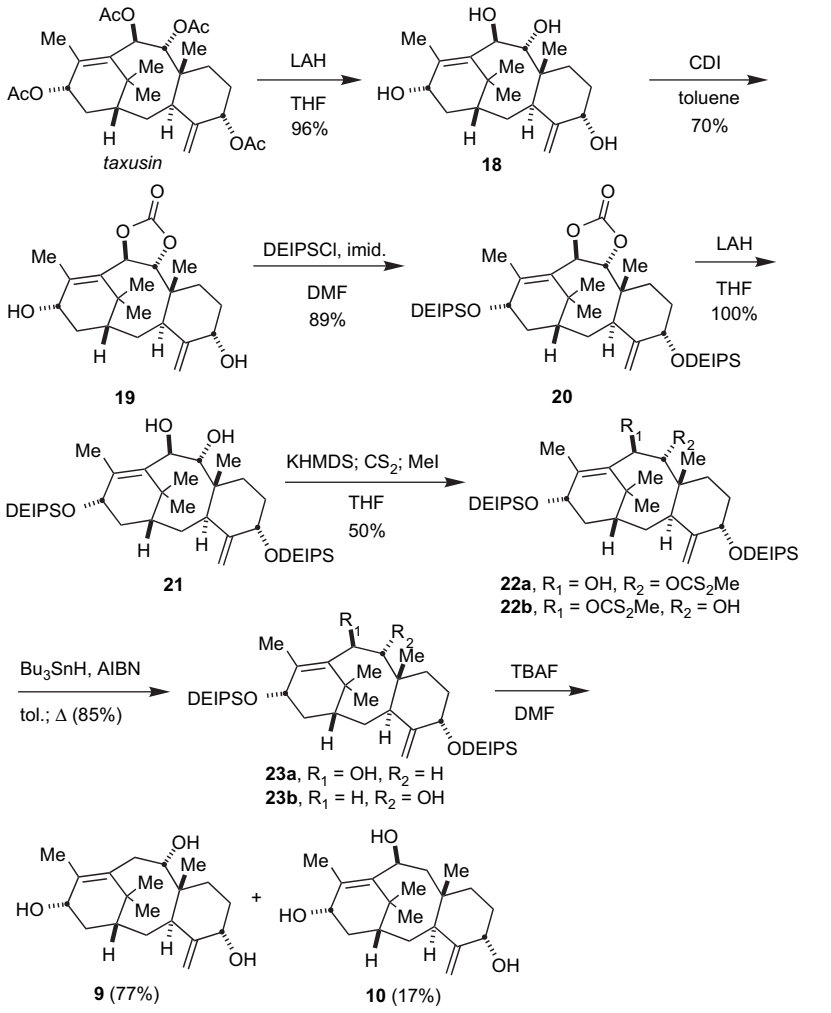
3. Experimental

3.1. 5,13-Dideacetyltaxusin (12)

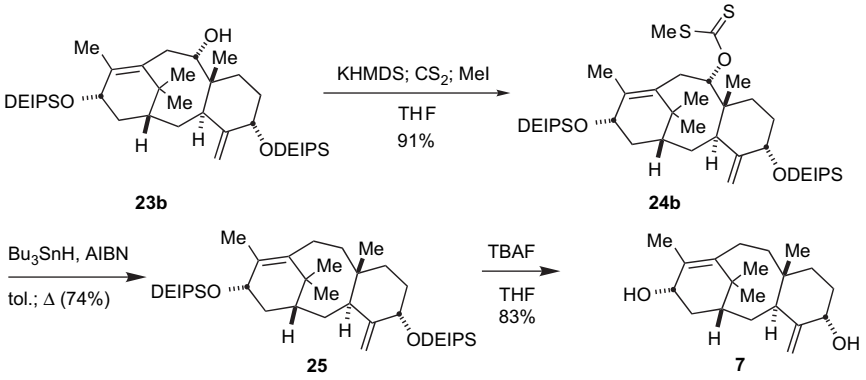
To a solution of taxusin (120 mg, 0.24 mmol) in THF/MeOH (4 mL, 1:1) was added K_2CO_3 (49 mg, 0.36 mmol) at 0 °C. The mixture was stirred for 3 days at 0 °C, after which time EtOAc (20 mL) and satd NH_4Cl (aq) (5 mL) were added to the mixture. The organic layer was separated and washed with brine. The organic layer was concentrated and purified by chromatography (silica gel, hexanes/EtOAc 1:1), yielding **12** (80 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (d, J=10.2 Hz, 1H), 5.69 (d, J=10.2 Hz, 1H), 5.04 (s, 1H), 4.69 (s, 1H), 4.40–4.24 (br s, 1H), 4.28 (s, 1H), 3.32 (d, J=8.4 Hz, 1H), 3.24 (d, J=4.5 Hz, 1H), 2.90–2.70 (comp, 2H), 2.20 (d, J=1.2 Hz, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.94–1.54 (comp, 7H), 1.49 (s, 3H), 1.24–1.16 (m, 1H), 0.88 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.1, 152.3, 141.7, 134.8, 110.7, 77.7, 74.3, 73.6, 68.5, 43.4, 39.5, 38.6, 36.3, 35.8, 32.8, 30.5, 27.2, 26.6, 25.9, 21.2, 20.9, 17.1, 16.7; IR (NaCl) 3450 (br), 2944, 1724, 1242 cm^{-1}; HRMS calcd for $C_{24}H_{35}O_6$ (M^+−H) 419.2434, found 419.2422.

3.2. 5α,13α-Bis(triethylsilyloxy)-taxa-4(20),11(12)-diene-9α,10β-diol (13)

To the solution of **12** (1.32 g, 3.13 mmol) and imidazole (534 mg, 7.85 mmol) in DMF (5 mL), TESCl (991 mg, 6.57 mmol) was added at 0 °C. The mixture was stirred for 24 h at rt. The mixture was diluted by diethyl ether (40 mL), washed with water, NH_4Cl (aq), and brine, and dried over Na_2SO_4, yielding a crude mixture, which was used in the next reaction without further purification. Thus, a solution of the crude diacetate prepared above and LAH (238 mg,



Scheme 4. Cyclic carbonate route to **9** and **10**.



Scheme 5. Conversion of **23b** to **7**.

6.26 mmol) in THF (10 mL) were stirred at rt for 15 h. The mixture was diluted by THF/H_2O (3:1, 10 mL) at 0 °C. The mixture was filtered and the filtrate was diluted with EtOAc. The mixture was washed by NH_4Cl (aq) and brine, dried over Na_2SO_4, and condensed in vacuo, yielding diol **13** (1.60 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 (s, 1H), 4.80 (d, J=9.8 Hz, 1H), 4.82–4.75 (m, 1H), 4.58 (s, 1H), 4.15 (t, J=2.6 Hz, 1H), 4.12 (d, J=9.8 Hz, 1H), 2.98 (d, J=5.6 Hz, 1H), 2.36 (td, J=9.2, 14.0 Hz, 1H), 2.48–2.22 (br s, 2H), 1.99 (s, 3H), 1.76–1.50 (comp, 7H), 1.50 (s, 3H), 1.17 (s, 3H), 1.18–1.10 (m, 1H), 1.2–0.88 (comp, 21H), 0.70–0.55 (comp, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 140.0, 135.5, 109.4, 79.6, 74.7, 73.0, 69.4, 43.1, 42.0, 39.7, 36.7, 35.9, 32.0, 31.6, 29.0, 28.5, 25.6, 18.4, 14.9, 7.2, 5.1, 4.7.

3.3. Taxa-4(20),11(12)-diene-5α,10β,13α-triol (10)

3.3.1. Procedure A

To the solution of **13** (137 mg, 0.24 mmol) in THF (3 mL), LHMDS (0.25 mmol, 1 M THF solution) was added at 0 °C. After stirring for 5 min, CS_2 (0.1 mL) was added to the mixture. The mixture was stirred for 30 min at rt and then MeI (16 μL, 36 mg, 0.25 mmol) was

added. The mixture was stirred for another 14 h at rt. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC (hexanes/EtOAc 30:1), yielding an inseparable mixture of **14a** and **14b** (75 mg, 47%).

To a solution of **14a** and **14b** (75 mg, 0.11 mmol) obtained above in toluene (2 mL), AIBN (cat.) and *n*-Bu_3SnH (63 μL, 0.23 mmol) were added. The mixture was stirred at reflux for 3 h. The mixture was concentrated and purified by flash chromatography (silica gel, hexanes/EtOAc 11:1), yielding an inseparable mixture of **15a** and **15b** (54 mg, 86%).

To a solution of **15a** and **15b** (67 mg, 0.12 mmol) in THF were added TBAF (0.26 mmol, 1 M THF solution) and AcOH (1 drop). After 9 h, additional TBAF (0.52 mmol) was added and the mixture was stirred for 72 h. The mixture was diluted with EtOAc and washed with NH_4Cl (aq) and brine. The organic layer was concentrated and purified by preparative TLC (silica gel, hexanes/EtOAc 1:1, then $CHCl_3$/MeOH 20:1), yielding triol **10** (20 mg, 51%) and triol **9** (15 mg, 38%).

3.3.2. Procedure B

A solution of **23a** and **23b** (280 mg, 0.485 mmol) and TBAF (651 mg, 2.50 mmol) in DMF (30 mL) was stirred at 55 °C for 12 h. The mixture was diluted with EtOAc (75 mL), and washed with H_2O (3×50 mL) and brine (50 mL). The organic layer was concentrated and purified by flash chromatography (silica gel, eluted with hexanes/EtOAc 3:2 then 1:1), yielding triol **9** (120 mg, 77%) and triol **10** (27 mg, 17%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (dd, J=5.7, 11.7 Hz, 1H), 4.97 (s, 1H), 4.64 (s, 1H), 4.38–4.22 (comp, 2H), 3.22 (s, 1H), 2.88–2.60 (comp, 3H), 2.18 (dd, J=11.7, 14.4 Hz, 1H), 2.12–2.00 (m, 1H), 2.01 (d, J=1.2 Hz, 3H), 1.72–1.52 (comp, 7H), 1.48 (s, 3H), 1.38–1.10 (comp, 2H), 0.93 (s, 3H), 0.66 (s, 3H); ^{13}C NMR (100 MHz, CD_3CN) 156.2, 142.5, 136.9, 109.6, 75.2, 69.1, 68.7, 48.4, 41.5, 39.8, 39.7, 37.6, 35.9, 34.1, 34.0, 32.3, 25.9, 22.5, 21.5, 17.2; IR (KBr) 3349 (br), 2927, 1652, 1456, 1004, 894 cm^{-1}; HRMS (M^++Na) calcd for $C_{20}H_{32}O_3Na$ 343.2244, found 343.2240.

3.4. Taxa-4(20),11(12)-diene-5α,9α,13α-triol (9)

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.03 (s, 1H), 4.69 (s, 1H), 4.40–4.30 (br s, 1H), 4.32 (s, 1H), 4.14–4.02 (m, 1H), 3.55 (s, 1H), 2.96–2.70 (comp, 3H), 2.54 (dt, J=3.8, 13.2 Hz, 1H), 2.30–2.15 (br s, 1H), 2.00 (s, 3H), 1.93 (dt, J=5.1, 13.2 Hz, 1H), 1.80–1.60 (comp, 5H), 1.55–1.45 (m, 1H), 1.38–1.32 (m, 1H), 1.33 (s, 3H), 1.20 (dd, J=3.9, 15.3 Hz, 1H), 0.92 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 156.4, 138.2, 136.3, 109.6, 76.8, 75.9, 69.6, 46.7, 40.9, 40.6, 38.1, 37.6, 37.1, 32.8, 32.5, 28.4, 26.6, 26.3, 19.0, 17.6; IR (NaCl) 3350 (br), 2941, 1448, 1101 cm^{-1}; HRMS calcd for $C_{20}H_{32}O_3Na$ (M^++Na) 343.2244, found 343.2240.

3.5. 5α,13α-Bis(triethylsilyloxy)-10β-*S*-methyl dithiocarboxytaxa-4(20),11(12)-diene (16a) and 5α,13α-di(triethylsilyloxy)-9α-*S*-methyl dithiocarboxytaxa-4(20),11(12)-diene-10β-ol (16b)

To the solution of the mixture of **15a** and **15b** (62 mg, 0.11 mmol) in THF (2 mL), LHMDS (0.12 mL, 1 M THF solution) was added at 0 °C. After stirring for 10 min, CS_2 (0.1 mL) was added to the mixture. The mixture was stirred for 2 h at rt and then MeI (7 μL, 0.12 mmol) was added. The mixture was stirred for 3 h at rt. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by PTLC (hexanes/EtOAc 100:1), yielding **16a** (41 mg, 57%) and **16b** (20 mg, 28%). These were used for the next step without further purification.

3.6. 5α,13α-Bis(triethylsilyloxy)-taxa-4(20),11(12)-diene (17)

To a solution of the mixture of **16a** and **16b** (61 mg, 0.095 mmol) obtained above in toluene (2 mL), AIBN (cat.) and *n*-Bu_3SnH (52 μL, 0.19 mmol) were added. The mixture was stirred at reflux for 3 h. The mixture was concentrated and purified by chromatography (silica gel, heptane), yielding **17** (40 mg, 79%). ^{1}H NMR (400 MHz, benzne-d_6) δ 4.96–4.78 (m, 1H), 4.64 (s, 1H), 4.184 (t, J=3.0 Hz, 1H), 3.33 (m, 1H), 2.90 (dt, J=3.6, 12.8 Hz, 1H), 2.44 (td, J=9.2, 13.6 Hz, 1H), 2.41 (dt, J=4.8, 13.2 Hz, 1H), 2.14 (s, 3H), 2.15–2.00 (comp, 2H), 1.80–1.60 (comp, 5H), 1.38 (s, 3H), 1.44–1.34 (m, 1H), 1.30–1.20 (m, 1H), 1.16 (s, 3H), 1.14–1.00 (comp, 18H), 1.00–0.90 (comp, 2H), 0.80–0.62 (comp, 15H); ^{13}C NMR (100 MHz, benzne-d_6) 156.8, 137.0, 133.8, 108.7, 75.0, 70.1, 42.6, 41.7, 40.8, 40.5, 37.0, 36.5, 35.0, 32.9, 31.1, 30.1, 28.2, 26.3, 23.3, 15.3, 7.8, 7.7, 5.8, 5.4; IR (NaCl): 2951, 2361, 1457, 1239, 1118, 1062, 1007, 886, 838, 739 cm^{-1}.

3.7. Taxa-4(20),11(12)-diene-5α,13α-diol (7)

3.7.1. Procedure A

To a solution of **17** (25 mg, 0.047 mmol) in pyridine (2 mL) was added HF/pyridine (0.2 mL, 70% solution in pyridine) at 0 °C. The mixture was stirred for 3 h at rt, diluted with EtOAc (30 mL), and washed with saturated $CuSO_4$, water, NH_4Cl (aq), and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography (silica gel, $CHCl_3$/MeOH 9:1), yielding **7** (12 mg, 84%).

3.7.2. Procedure B

To a solution of **25** (60 mg, 0.107 mmol) in THF (10 mL) were added TBAF (140 mg, 0.535 mmol). The mixture was stirred at 55 °C overnight and concentrated and purified by flash chromatography (silica gel, hexanes/EtOAc 3:2), yielding diol **7** (27 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.96 (s, 1H), 4.63 (s, 1H), 4.38–4.24 (br s, 1H), 4.27 (t, J=2.6 Hz, 1H), 3.51 (br s, 1H), 2.85 (dd, J=5.4, 13.2 Hz, 1H), 2.83–2.70 (comp, 2H), 2.23 (dt, J=5.7, 12.9 Hz, 1H), 2.18–2.09 (br s, 2H), 1.95 (s, 3H), 2.00–1.90 (m, 1H), 1.82–1.60 (comp, 5H), 1.32 (s, 3H), 1.28–1.16 (comp, 2H), 1.00 (ddd, J=2.4, 4.5, 13.2 Hz, 1H), 0.88 (s, 3H), 0.60 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7, 139.5, 132.5, 109.5, 74.7, 68.7, 40.4, 39.6, 39.24, 39.18, 36.2, 35.8, 32.4, 32.1, 31.4, 27.4, 25.4, 24.9, 22.2, 16.3; IR (NaCl): 3359 (br), 2921, 1439, 1008 cm^{-1}; HRMS calcd for $C_{20}H_{32}O_2Na$ (M^++Na) 327.2294, found 327.2292.

3.8. Cyclic carbonate (19)

A solution of tetraol **18** (6.41 g, 19.1 mmol) and CDI (5.0 g, 30.8 mmol) in toluene (300 mL) was heated at 70 °C overnight. After cooling to rt, the mixture was concentrated and the residue was purified by flash chromatography (silica, 1:1 EtOAc/hexanes) to give **19** as a white powder (4.80 g, 70%). ^{1}H NMR (300 MHz, acetone-d_6) δ 5.79 (d, J=12.0 Hz, 1H), 5.09 (s, 1H), 4.95 (d, J=12.0 Hz, 1H), 4.72 (s, 1H), 4.45 (app dt, J=4.5, 9.8 Hz, 1H), 4.31 (d, J=2.4 Hz, 1H), 4.17 (d, J=2.1 Hz, 1H), 3.76 (d, J=9.6 Hz, 1H), 3.16–3.10 (br s, 1H), 2.84 (ddd, J=9.0, 10.2, 15.3 Hz, 1H), 2.18 (d, J=1.2 Hz, 3H), 1.99 (dd, J=4.5, 13.5 Hz, 1H), 1.86–1.80 (comp, 5H), 1.46 (s, 3H), 1.48–1.40 (m, 1H), 1.18 (d, J=4.8, 15.3 Hz, 1H), 1.03 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 155.7, 154.0, 150.5, 133.7, 110.8, 88.2, 81.1, 75.1, 69.3, 41.5, 41.4, 40.2, 37.6, 36.8, 33.9, 31.7, 28.8, 27.1, 26.3, 18.3, 17.8; IR (NaCl): 3407 (br), 2944, 2877, 1801, 1190, 1016 cm^{-1}; HRMS calcd for $C_{21}H_{30}O_5Na$ (M^++Na) 385.1991, found 385.1982.

3.9. Cyclic carbonate (20)

A solution of diol **19** (475 mg, 1.31 mmol), imidazole (700 mg, 10.2 mmol), and DEIPSCl (0.63 mL, 0.34 mmol) in DMF (7 mL) was

stirred at rt for 40 h. The mixture was diluted with EtOAc (25 mL), and washed with H_2O (3×25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by flash chromatography (silica, 1:20 then 1:10 EtOAc/hexanes) to give **20** as a clear liquid (726 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.58 (d, J=12.0 Hz, 1H), 5.06 (d, J=12.0 Hz, 1H), 4.96 (s, 1H), 4.84 (app t, J=9.0 Hz, 1H), 4.60 (s, 1H), 4.18 (t, J=2.3 Hz, 1H), 2.70–2.63 (s, 1H), 2.41 (td, J=9.3, 14.1 Hz, 1H), 2.03 (d, J=0.6 Hz, 3H), 1.88–1.73 (comp, 2H), 1.67–1.55 (comp, 4H), 1.50 (s, 3H), 1.53–1.44 (m, 1H), 1.18 (s, 3H), 1.10–0.83 (comp, 30H), 0.70–0.54 (comp, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 152.2, 149.2, 129.2, 110.1, 87.1, 79.6, 77.0, 74.1, 69.4, 42.0, 39.6, 39.4, 35.7, 35.4, 32.4, 30.9, 28.2, 28.0, 25.3, 17.6, 17.5, 17.4, 15.4, 13.0, 12.8, 7.20, 7.18, 7.15, 7.10, 3.7, 3.5, 3.4, 3.1; IR (NaCl): 2944, 2877, 1809, 1463, 1184 cm^{-1}; HRMS calcd for $C_{35}H_{62}O_5Si_2Na$ (M^++Na) 641.4034, found 641.4050.

3.10. 5α,13α-Bis(diethylisopropylsilyloxy)-taxa-4(20),11(12)-diene-9α,10β-diol (21)

A mixture of the cyclic carbonate **20** (1.5 g, 2.42 mmol) and $LiAlH_4$ (184 mg, 4.85 mmol) in THF (150 mL) was stirred at rt for 6 h whereupon EtOAc (10 mL) was added slowly to quench excess $LiAlH_4$. The mixture was partitioned between EtOAc (80 mL), H_2O (40 mL), and brine (40 mL). The aqueous layer was extracted with EtOAc (2×80 mL) and the combined organic layers were washed with brine (150 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by flash chromatography (silica gel, 1:7 then 1:3 EtOAc/hexanes) to give **21** as a white solid (1.44 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.89 (s, 1H), 4.80 (t, J=1.2 Hz, 1H), 4.76 (d, J=9.9 Hz, 1H), 4.57 (s, 1H), 4.15 (s, 1H), 4.10 (d, J=9.9 Hz, 1H), 2.90 (d, J=5.4 Hz, 1H), 2.50–2.20 (br s, 2H), 2.33 (td, J=9.3, 13.2 Hz, 1H), 1.97 (d, J=1.2 Hz, 3H), 1.72–1.52 (comp, 7H), 1.47 (s, 3H), 1.15 (s, 3H), 1.04–0.84 (comp, 27H), 0.84 (s, 3H), 0.70–0.54 (comp, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 139.7, 135.3, 109.6, 79.4, 74.7, 72.7, 69.3, 42.9, 42.0, 39.4, 36.6, 35.5, 31.9, 31.4, 29.0, 28.5, 25.5, 18.3, 17.6, 17.54, 17.52, 17.49, 14.5, 13.1, 12.9, 7.24, 7.21, 7.17, 3.8, 3.6, 3.5, 3.2; IR (NaCl): 3402 (br), 2944, 1463, 1072, 1014 cm^{-1}; HRMS calcd for $C_{34}H_{64}O_4Si_2Na$ (M^++Na) 615.4241, found 615.4238.

3.11. 5α,13α-Bis(diethylisopropylsilyloxy)-9α-*S*-methyl dithiocarboxytaxa-4(20),11(12)-diene (24b)

A solution of KHMDS (1.18 mL, 0.5 M in toluene, 0.59 mmol) was added to **23b** (200 mg, 0.347 mmol) in THF (20 mL) at −10 °C. The mixture was stirred at 0 °C for 10 min and then at rt for 20 min, whereupon carbon disulfide (36 μL, 0.58 mmol) was added and the solution was stirred for another 30 min. MeI (36 μL, 0.54 mmol) was then added and the mixture was allowed to stir overnight. The mixture was then partitioned between EtOAc (25 mL) and 1% AcOH in water (25 mL), and the organic layer separated. The organic layer was washed with brine (25 mL) and dried over Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by flash chromatography (silica gel, hexanes then 1:100 EtOAc/hexanes) to give **24b** as a clear oil (210 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.20 (dd, J=5.7, 11.7 Hz, 1H), 4.92 (s, 1H), 4.80 (app t, J=9.0 Hz, 1H), 4.61 (s, 1H), 4.18 (s, 1H), 3.19 (d, J=5.1 Hz, 1H), 2.89 (t, J=12.9 Hz, 1H), 2.56 (s, 3H), 2.55–2.48 (br s, 1H), 2.32 (td, J=9.0, 14.1 Hz, 1H), 2.20–2.05 (m, 1H), 1.98 (s, 3H), 1.80–1.55 (comp, 6H), 1.63 (s, 3H), 1.17–1.08 (m, 1H), 1.11 (s, 3H), 1.05–0.90 (comp, 26H), 0.71 (s, 3H), 0.70–0.60 (comp, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.6, 154.2, 136.3, 132.1, 109.9, 90.7, 74.4, 60.4, 45.2, 41.3, 39.6, 36.8, 35.2, 31.2, 30.7, 30.5, 28.9, 28.5, 26.7, 19.0, 18.3, 17.7, 17.6, 17.55, 17.53, 14.7, 13.1, 12.9, 7.29, 7.25, 7.2, 3.8, 3.6, 3.5, 3.2; IR (NaCl): 2945, 2872, 1645, 1466, 1240, 1078 cm^{-1}; HRMS calcd for $C_{36}H_{65}O_3S_2Si_2$ (M^+−H) 665.3914, found 665.3905.

3.12. 5α,13α-Bis(diethylisopropylsilyloxy)-4(20),11(12)-diene (25)

A solution of **24b** (160 mg, 0.24 mmol), AIBN (20 mg, 0.12 mmol), and Bu_3SnH (140 mg, 127 μL, 0.48 mmol) in toluene (15 mL) was heated at 115 °C for 3 h. The mixture was concentrated and the residue purified by flash chromatography (silica gel, hexanes then 1:100 EtOAc/hexanes) to give **25** as clear oil (100 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.81 (s, 1H), 4.75 (t, J=8.9 Hz, 1H), 4.55 (s, 1H), 4.14 (t, J=3.5 Hz, 1H), 3.06 (d, J=6.3 Hz, 1H), 2.76 (ddd, J=5.4, 12.6, 14.1 Hz, 1H), 2.33 (td, J=9.3, 13.8 Hz, 1H), 2.16 (dt, J=4.1, 13.2 Hz, 1H), 2.10–1.90 (comp, 2H), 1.89 (s, 3H), 1.80–1.40 (comp, 6H), 1.37 (s, 3H), 1.33–1.12 (comp, 3H), 1.05 (s, 3H), 1.02–0.84 (comp, 25H), 0.65 (s, 3H), 0.74–0.62 (comp, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.4, 136.4, 133.3, 107.8, 73.9, 69.3, 42.0, 41.2, 39.9, 39.7, 36.8, 35.7, 34.8, 32.1, 30.7, 30.1, 27.8, 25.4, 22.7, 17.7, 17.6, 17.5, 14.6, 13.1, 12.9, 7.33, 7.30, 7.27, 7.2, 3.9, 3.7, 3.5, 3.3; IR (NaCl): 2941, 1458, 1016 cm^{-1}; HRMS calcd for $C_{34}H_{63}O_2Si_2$ (M^+−H) 559.4365, found 559.4367.

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References and notes

1. (a) Suffness, M. *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; American Chemical Society: Washington, DC, 1995; pp 1–17; (b) Suffness, M.; Wall, M. E. *Taxol: Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995; pp 3–25; (c) Holmes, F. A.; Kudelka, A. P.; Kavanagh, J. J.; Huber, M. H.; Ajani, J. A.; Valero, V. *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; American Chemical Society: Washington, DC, 1995; pp 31–57; (d) Golspiel, B. R. *Pharmacotherapy* **1997**, *17*, 110S–125S.
2. Paclitaxel is the generic name for Taxol®, a registered trademark of Bristol–Myers Squibb; because of its greater familiarity, the term "Taxol" is used throughout.
3. For a review, see: Swindell, C. S. *Org. Prep. Proced. Int.* **1991**, *23*, 465–543.
4. (a) Holton, R. A.; Somoza, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. S.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1597–1598; (b) Holton, R. A.; Somoza, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. S.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1599–1600; (c) Nicolaou, K. C.; Nantermet, P. G.; Ueno, H.; Guy, R. K.; Coulandouros, E. A.; Sorensen, E. J. *J. Am. Chem. Soc.* **1995**, *117*, 624–633; (d) Nicolaou, K. C.; Liu, J. J.; Yangh, H.; Claiborne, C. F.; Hwang, C. K.; Nakada, M.; Nantermet, P. G.; Ueno, H.; Guy, R. K.; Sorensen, E. J. *J. Am. Chem. Soc.* **1995**, *117*, 634–644; (e) Nicolaou, K. C.; Liu, J. J.; Yangh, H.; Claiborne, C. F.; Renaud, J.; Nantermet, P. G.; Guy, R. K.; Shibayama, K. *J. Am. Chem. Soc.* **1995**, *117*, 645–652; (f) Nicolaou, K. C.; Ueno, H.; Liu, J. J.; Yangh, H.; Renaud, J.; Nantermet, P. G.; Paulvannan, K.; Chadha, R. *J. Am. Chem. Soc.* **1995**, *117*, 653–659; (g) Danishefsky, S. J.; Masters, J. J.; Young, W. B.; Link, J. T.; Snyder, L. B.; Magee, T. B.; Jung, D. K.; Isaacs, R. C. A.; Bornman, W. G.; Alaimo, C. A.; Coburn, C. A.; DiGrandi, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2843–2859; (h) Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Granicher, C.; Houze, J. B.; Janichen, J.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Mucciaro, T. P.; Muhlebach, M.; Natchus, M. G.; Paulsen, H.; Rawlins, D. B.; Satkofsky, J.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E.; Tomooka, K. I. *J. Am. Chem. Soc.* **1997**, *119*, 2755–2756; (i) Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Houze, J. B.; Krauss, N. E.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Natchus, M. G.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E. *J. Am. Chem. Soc.* **1997**, *119*, 2757–2758; (j) Mukaiyama, T.; Shiina, I.; Iwadare, H.; Saitoh, M.; Nishimura, T.; Ohkawa, N.; Sakoh, H.; Nishimura, K.; Tani, Y.; Hasegawa, M.; Yamada, K.; Saitoh, K. *Chem.—Eur. J.* **1999**, *5*, 121–161; (k) Kusama, H.; Hara, R.; Kawahara, S.; Nishimori, T.; Kashima, H.; Nakamura, N.; Morihira, K.; Kuwajima, I. *J. Am. Chem. Soc.* **2000**, *122*, 3811–3820.
5. Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15–44.

6. (a) Hezari, M.; Croteau, R. *Planta Med.* **1997**, *63*, 291–295; (b) Walker, K.; Croteau, R. *Phytochemistry* **2001**, *58*, 1–7.
7. (a) Koepp, A. E.; Hezari, M.; Zajicek, J.; Vogel, B. S.; LaFever, R. E.; Lewis, N. G.; Croteau, R. *J. Biol. Chem.* **1995**, *270*, 8686–8690; (b) Hezari, M.; Lewis, N. G.; Croteau, R. *Arch. Biochem. Biophys.* **1995**, *322*, 437–444; (c) Lin, X.; Hezari, M.; Koepp, A. E.; Floss, H. G.; Croteau, R. *Biochemistry* **1996**, *35*, 2968–2977.
8. Hefner, J.; Rubenstein, S. M.; Ketchum, R. E. B.; Gibson, D. M.; Williams, R. M.; Croteau, R. *Chem. Biol.* **1996**, *3*, 479–489.
9. (a) Walker, K.; Ketchum, R. E. B.; Hezari, M.; Gat, J.; wfnerfield, D.; Goleniowski, M.; Barthol, A.; Croteau, R. *Arch. Biochem. Biophys.* **1999**, *364*, 273–279; (b) Walker, K.; Schoendorf, A.; Croteau, R. *Arch. Biochem. Biophys.* **2000**, *374*, 371–380.
10. Schoendorf, A.; Rithner, C. D.; Williams, R. M.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1501–1506.
11. Vazquez, A.; Williams, R. M. *J. Org. Chem.* **2000**, *65*, 7865–7869.
12. (a) Wheeler, A. L.; Long, R. M.; Ketchum, R. E. B.; Rithner, C. D.; Williams, R. M.; Croteau, R. *Arch. Biochem. Biophys.* **2001**, *390*, 265–278; (b) Jennewein, S.; Rithner, C. D.; Williams, R. M.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13595–13600.
13. Ketchum, R. E. B.; Croteau, R. B. The Taxoid Metabolome and the Elucidation of the Paclitaxel Biosynthetic Pathway in Cell Suspension Cultures of Taxus. In *Plant Metabolomics, Biotechnology in Agriculture and Forestry*; Saito, K., Dixon, R., Willmitzer, L., Eds.; Springer: Heidelberg, 2006; Vol. 57, pp 291–309.
14. Kaspera, R.; Croteau, R. *Phytochem. Rev.* **2006**, *5*, 433–444.
15. (a) Horiguchi, T.; Rithner, C. D.; Croteau, R.; Williams, R. M. *J. Org. Chem.* **2002**, *67*, 4901–4903; (b) Horiguchi, T.; Rithner, C. D.; Croteau, R.; Williams, R. M. *Tetrahedron* **2003**, *59*, 267–273; (c) Ketchum, R. E. B.; Horiguchi, T.; Qiu, D.; Williams, R. M.; Croteau, R. B. *Phytochemistry* **2007**, *68*, 335–341.