Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 267–273

TETRAHEDRON

Studies on taxol biosynthesis. Preparation of 5α-acetoxytaxa-4(20),11-dien-2α,10β-diol derivatives by deoxygenation of a taxadiene tetra-acetate obtained from Japanese yew

Tohru Horiguchi,[a] Christopher D. Rithner,[a] Rodney Croteau[b] and Robert M. Williams[a,*]

[a]*Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA*
[b]*Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA*

Received 31 July 2002; accepted 4 September 2002

Abstract—The putative metabolite, 5α-acetoxytaxa-4(20),11-dien-2α,10β-diol (**7**), which is a promising candidate as a biosynthetic pathway triol in taxol biosynthesis, has been prepared by Barton deoxygenation of the C-14-hydroxyl group of a differentially protected derivative of natural 2α,5α,10β-triacetoxy-14β-(2-methyl)-butyryloxytaxa-4(20),11-diene (**8**), a major taxoid metabolite isolated from Japanese 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

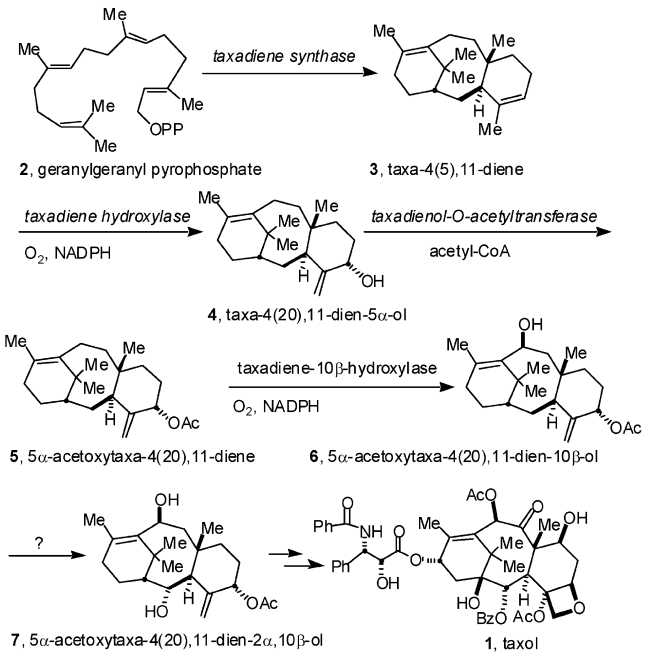
The approval of taxol (Paclitaxel, **1**) as a therapeutic agent against ovarian and other types of cancer has continued to elicit interest in both the chemical synthesis and biosynthesis of this agent.[1,2] A diverse retinue of approaches for the synthesis of the taxane framework[3] and for taxol itself[4] have evolved but the complexity of the fascinating structure of taxol mandates lengthy syntheses, that result in low overall yields rendering totally synthetic approaches to this potent antitumor agent impractical for large-scale manufacturing of clinical-grade material. The Pacific yew, *Taxus brevifolia* Nutt., the initial source for commercial scale production of taxol, 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 semisynthesis from 10-deacetylbaccatin III, that can be isolated from the needles of the European yew *Taxus baccata*, a renewable resource, have been adopted as the current commercial method for taxol production.[5] However, it is expected that taxol will become utilized for other types of cancer and as a consequence, pressure on the yew population worldwide would increase significantly. Alternative biological methods for taxol production has 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. To this end, we have sought to elucidate a detailed understanding of the steps of taxol biosynthesis and the identification of the associated genes.

We have previously reported a combination of in vivo feeding studies and investigations with cell-free enzyme systems, using yew stem tissue or suspension cultured *Taxus* sp. cells as bioconversion vectors. These investigations have revealed 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, to the first oxygenated metabolite, taxa-4(20),11-diene-5α-ol (**4**).[8] The third step appears to involve taxadienol-*O*-acetyltransferase, that subsequently acylates **4** to provide the acetate **5**, which has proven to be a superior substrate for downstream hydroxylation reactions.[9]

After the formation of **5**, we have observed that the downstream hydroxylation reactions enter a very complex matrix and the elucidation of a single linear path to taxol has proven extremely challenging. This appears to be due to the softening of substrate specificity by several of the remaining hydroxylating enzymes that can accept **5** as a suitable substrate in vitro or presumably, in vivo. We have deployed two major approaches to identify the genes and associated intermediates from **5** to taxol. In the first approach, we have obtained a set of related full-length cytochrome P-450 clones by the method of differential display of

Keywords: deoxygenation; taxadiene; taxol mandates.
* Corresponding author. Tel.: +1-9704916747; fax: +1-9704913944; e-mail: rmw@chem.colostate.edu

0040–4020/03/$ - see front matter
PII: S0040-4020(02)01450-3



Scheme 1. Early steps in taxol biosynthesis.

mRNA-reverse transcription-PCR, followed by traditional library screening. Clones are selected based on sequence homology to other plant cytochrome P-450s and used to individually transform *Saccharomyces cerevisiae* and the transformed yeast clones are then screened for hydroxylase activity with several synthetic, labeled taxoids as substrates. In one instance, we identified a clone that produced 5α-acetoxytaxa-4(20),11-dien-10β-ol (**6**) employing taxa-4(20),11-dien-5α-acetate (**5**) as a substrate.[10] This material has been characterized by ^{1}H NMR and mass spectroscopy and has been shown to incorporate into taxol in vivo.

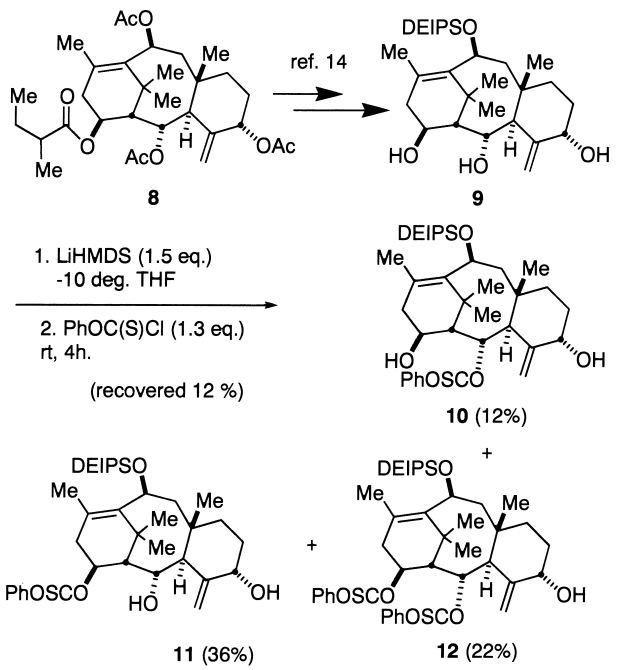
In a second approach, we have examined microsomal bioconversion of synthetic substrate **5** with induced *Taxus cuspidata*. microsomes to more polar products. This approach however, yielded several diol mono-acetates including 5α-acetoxytaxa-4(20),11-dien-2α-ol[11] and 5α-acetoxytaxa-4(20),11-dien-13α-ol along with other polyols tentatively identified as triols, tetraols, and pentaols by GC–MS.[12] Comparable preliminary bioconversion studies with microsomes from uninduced *Taxus canadensis* cells, have also shown the conversion of **5**, primarily to a more polar metabolite tentatively identified by LC–MS as a pentaol monoacetate.[6a] Based on the existing map of naturally co-occurring taxoids, the subsequent order of hydroxylation downstream from **6** appears likely to occur at C2 and C9, then C13.[13] Based on this supposition, a very likely intermediate downstream from **6** is 5α-acetoxy taxadien-2α,10β-diol (**7**) or 5α-acetoxy taxadien-9α (or β),10β-diol.

Due to the very low yield of the intermediate metabolites that may be obtained from natural sources, we have relied heavily on synthetic, tritium-labeled taxadienes **3**,[15] **4**,[8] **5**[8] and **6**[14] as substrates from which in vivo and in vitro bioconversion strategies have been utilized to identify lightly oxygenated taxoids downstream of these substances. It must be stressed that, bioconversion of synthetic **6** is presently only capable of conveniently generating sub-milligram quantities of a given metabolite, which has proven insufficient for the substrate requirements of down-stream bioconversion experiments. We have thus devoted considerable effort to devising totally synthetic and semi-synthetic methods to prepare these lightly oxygenated taxoids, such as compound **7**. Herein, we report a method to semi-synthetically prepare **7** and its derivatives from 2α,5α,10β-triacetoxy-14β-(2-methyl)-butyryloxytaxa-4(20),11-diene (**8**), a component of Japanese Yew heart wood.[16] This method is easily applicable for the preparation of multi-milligram quantities of stable- and/or radio-isotopomers of **7**.

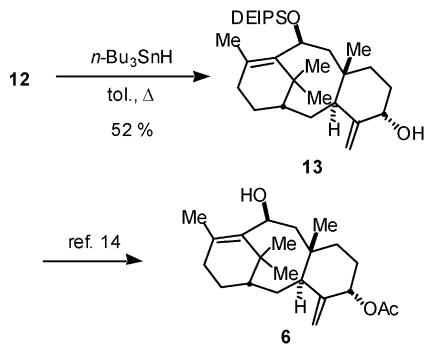
2. Results and discussion

The starting substrate, 10β-diethylisopropylsilyloxy-4(20),11-taxadiene-2α,5α,14β-triol (**9**), was prepared from 2α,5α,10β-triacetoxy-14β-(2-methyl)-butyryloxytaxa-4(20),11-diene (**8**), following the method we have previously reported in good yield.[14] Although the reaction of triol **9** with carbon disulfide and methyl iodide resulted in the selective formation of the 2α-methyldithiocarboxyl ester,[14] the reaction of triol **9** with phenylchlorothiono carbonate under similar conditions gave the 2α-phenoxy-thiocarbonyl ester **10** as a minor product (12%) along with the desired 14β-phenoxythiocarbonyl ester **11** (36%) and the diacylated material **12** (22%) (Scheme 2).

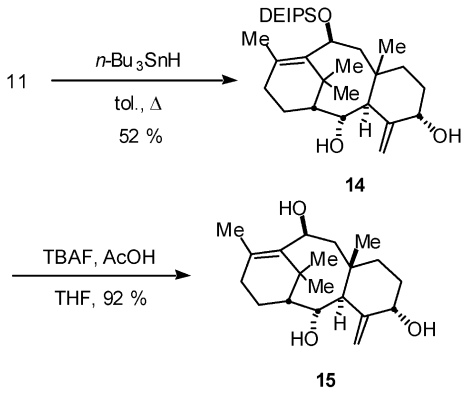
All three compounds proved useful and as described in detail below, served as substrates from which the known biogenetic intermediate **6**, putative metabolite **7**, the free 2α,5α,10β-triol **15** and 5α,10β,14β-triol **19** were prepared. These four substances are valuable as authentic standards to facilitate the identification of metabolites bioconverted from **4**, **5** and **6**.[6a,10,12]



Scheme 2.



Scheme 3.



Scheme 4.

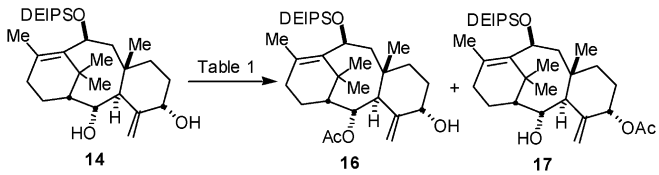
presence of imidazole in DMF resulted in quantitative conversion to regioisomer **10**. This presumably occurred by transposition of the 14-phenoxythiocarbonyl group to the 2-position catalyzed by imidazole. Based on the failure of this approach, direct acetylation of diol **14** was examined. (Table 1).

Acetylation of **14** with AcCl in THF containing DMAP (Table 1, entry 1) or with acetic acid in the presence of DCC (Table 1, entry 2) gave an inseparable mixture of the C-2-monoacetate **16** and the C-5-monoacetate **17**. Alternatively, acetylation of **14** with AcCl or AcBr utilizing LHMDS in THF at −18°C, provided the desired C5-monoacetate **17** as the sole product in low yields (Table 1, entries 3 and 4). Although the net conversion of **14** to **17** was poor, recovery of the starting material was reasonably high and the reaction was clean enough to render this the most practical approach.

Although removal of the *O*-DEIPS group from compound **14** or from 5α-acetoxy-10β-diethylisopropylsilyloxytaxa-4(20),11-diene with TBAF in THF proceeded efficiently in 24 h, subjecting the structurally related mixture of **16** and **17** to these conditions required 14 days, and the diol monoacetate products proved inseparable. We found that the DEIPS group of **17** was more easily removed by treatment with HF–pyridine complex for 4 h at room temperature to provide **7** in 94% isolated yield (Scheme 5).

Treatment of 2α-phenoxythiocarbonylester **10** under similar conditions gave the desired C2-deoxygention product **18** in 52% yield. (Scheme 6). Removal of

Table 1.



Entry	Conditions	Product (%)	Recovered 14 (%)
1	(1.2 equiv.) AcCl, (1.3 equiv.) DMAP, THF, 0°C to rt	**16**+**17** (17%; 1:1 mixture)	83
2	(1.2 equiv.) AcOH, (2 equiv.) DMAP, (2 equiv.) DCC, (2 equiv.) HOBt, THF, rt	**16**+**17** (22%; 3:1 mixture)	68
3	(1.05 equiv.) AcCl, (1.1 equiv.) LHMDS, THF, −18°C to rt	**17** (8%)	65
4	(1.05 equiv.) AcBr, (1.1 equiv.) LHMDS, THF, −18°C to rt	**17** (12%)	74

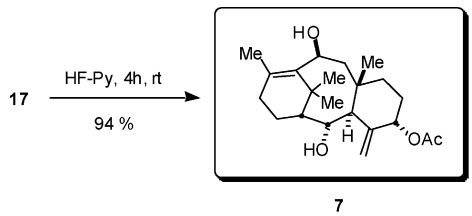
Treatment of 2α,14β-di-phenoxythiocarbonyl ester **12** with tri-*n*-butyl tin hydride in the presence of AIBN in toluene at 100–120°C, provided **13** in 52% yield (Scheme 3). We have previously described the conversion of **13** into 5α-acetoxy taxa-4(20),11-dien-10β-ol **6**, which has been identified as an early stage intermediate.[14]

Treatment of 14-phenoxythiocarbonylester **11** under similar conditions gave the desired C14-deoxygention product **14** in 52% yield. (Scheme 4). Removal of the *O*-DEIPS group from **14** was accomplished with TBAF in acetic acid-THF, furnishing triol **15** in 92% yield. (Scheme 4).

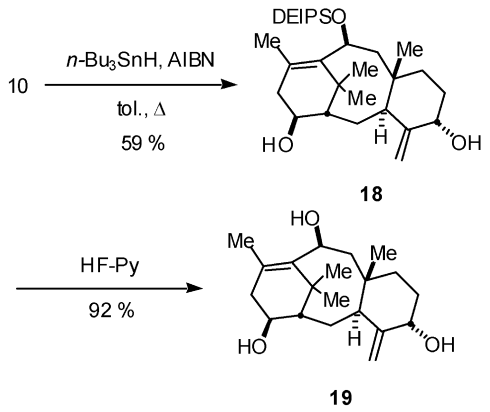
In order to regioselectively introduce the acetyl moiety onto the C5-hydroxy group, selective and differential protection of diol **11** was examined. Treatment of **11** with TESCl in the

the *O*-DEIPS group from **18** was accomplished with HF–pyridine complex for 4 h at room temperature, furnishing triol **19** in 92% yield.

The structures of compounds **7**, **15**, and **19** were fully characterized by 1D (^{1}H and ^{13}C) and 2D (HH, HSQC, and HMBC) NMR analysis.



Scheme 5.



Scheme 6.

In summary, 5α-acetoxytaxadien-2α,10β-diol (**7**), the corresponding triol **15**, and 5α,10β,14β-triol **19** have been conveniently prepared from the readily available 2α,5α,10β-triacetoxy-14β-(2-methyl)-butyryloxytaxa-4(20),11-diene **8**, one of the main taxoid components in Japanese yew heart wood. The synthetic methodology described here is readily amenable for the preparation of isotopically labeled congeners that are required for our ongoing biosynthetic studies. Utilization of these new lightly oxygenated taxoids is presently being explored to probe downstream steps in the biosynthesis of taxol and will be reported on in due course.

3. Experimental

3.1. Preparation of phenoxythiocarbonylesters 10–12

To a solution of **9** (18.8 mg, 0.040 mmol) in THF (2 mL), LHMDS (60 μL as 1 M THF solution) was added at −10°C. After being stirred for 5 min, PhOCSCl (7 μL, 0.052 mmol) was added and the mixture was stirred for 2 h at rt. The reaction mixture was diluted with EtOAc (15 mL) and washed with brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography (silica gel, eluted with $CHCl_3$/MeOH 20:1), yielding **10** (3.0 mg, 12%) and recovered **9** 12%. The more polar fraction was additionally purified by chromatography (silica gel, eluted with hexane/EtOAc 6:1) to give **11** (8.6 mg, 36%), and **12** (6.5 mg, 22%).

3.1.1. Compound 10

$[\alpha]_D^{20}$=+98 (*c* 0.13, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (m, 2H, Arom.), 7.30 (m, 1H, Arom.), 7.12 (dd, *J*=0.3, 8.3 Hz, 1H, Arom.), 5.92 (dd, *J*=2.1, 6.4 Hz, 1H, H-2), 5.18 (s, 1H, H-20), 5.07 (dd, *J*=5.8, 11.3 Hz, 1H, H-10), 4.82 (s, 1H, H-20), 4.22 (dd, *J*=2.6, 2.7 Hz, 1H, H-5), 4.15 (m, 1H, H-14), 3.39 (d, *J*=6.2 Hz, 1H, H-3), 2.64 (dd, *J*=9.2, 18.3 Hz, 1H, H-13), 2.47 (dd, *J*=5.1, 18.3 Hz, 1H, H-13), 2.36 (dd, *J*=7.0, 14.9 Hz, 1H, H-9), 2.06 (dt, *J*=6.8, 12.6 Hz, 1H, H-7), 1.98 (s, 3H, Me-18), 1.94 (d, *J*=1.9 Hz, 1H, H-1), 1.70 (m, 2H, H-6), 1.74 (s, 3H, Me-16), 1.55 (dd, *J*=5.5, 15.1 Hz, 1H, H-9), 1.25 (s, 3H, Me-17), 1.10 (ddd, *J*=2.1, 4.3, 13.4 Hz, 1H, H-7), 1.03–0.90 (m, 13H, $[CH_3CH_2]_2SiCH[CH_3]_2$), 0.88 (s, 3H, Me-19), 0.67–0.58 (m, 4H, $[CH_3CH_2]_2SiCH[CH_3]_2$); ^{13}C NMR (100 MHz, $CDCl_3$) 193.6, 153.6, 148.0, 138.7, 131.6, 129.7, 126.8, 122.3,113.8, 84.3, 76.8, 68.2, 67.7, 63.0, 48.7, 41.9, 41.2, 40.3, 38.0, 33.3, 31.8, 31.3, 26.2, 23.0, 21.2, 17.6, 17.6, 13.2, 7.4, 7.3, 4.1; IR (NaCl) 3420, 2952, 1490, 1457, 1293, 1200, 1047, 1013, 688 cm^{-1}; HR-FABMS calcd for $C_{34}H_{52}O_5SiSNa$ (M^+Na) 623.3202, found 623.3226.

3.1.2. Compound 11

$[\alpha]_D^{20}$=+39 (*c* 0.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J*=7.9, 8.1 Hz, 2H, Arom.), 7.29 (m, 1H, Arom.), 7.12 (m, 2H, Arom.), 5.49 (dd, *J*=5.1, 9.4 Hz, 1H, H-14), 5.46 (s, 1H, H-20), 5.17 (s, 1H, H-20), 5.05 (dd, 1H, *J*=5.8, 11.1 Hz, H-10), 4.13 (m, 1H, H-2), 4.21 (br.s, 1H, H-5), 3.04 (d, *J*=5.8 Hz, 1H, H-3), 2.85 (d, *J*=10.2 Hz, 1H, OH-2), 2.78 (dd, *J*=9.4, 18.5 Hz, 1H, H-13), 2.64 (dd, *J*=4.9, 18.1 Hz, 1H, H-13), 2.22 (dd, *J*=4.9, 18.1 Hz, 1H, H-9), 2.12 (br.s, 1H, H-1), 2.00 (m, 1H, H-7), 1.99 (s, 3H, Me-18), 1.73 (m, 2H, H-6), 1.66 (s, 3H, Me-16), 1.49 (dd, *J*=5.8, 15.1 Hz, 1H, H-9), 1.19 (s, 3H, Me-17), 1.08 (m, 1H, H-7), 1.04–0.88 (m, 13H, $[CH_3CH_2]_2SiCH[CH_3]_2$), 0.85 (s, 3H, Me-19), 0.68 –0.58 (m, 4H, $[CH_3CH_2]_2SiCH[CH_3]_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.5, 153.4, 149.3, 139.4, 129.8, 129.7, 126.8, 122.1, 114.8, 82.6, 76.7, 69.8, 67.7, 63.0, 60.6, 48.8, 42.1, 40.4, 37.9, 33.6, 31.9, 31.5, 25.8, 22.6, 21.1, 17.6, 17.6, 14.4, 13.2, 7.4, 7.3, 4.1; IR (NaCl) 3404, 2951, 1592, 1490, 1456, 1343, 1279, 1192, 1056, 688 cm^{-1}; HR-FABMS calcd for $C_{34}H_{51}O_4SiS$ (M^+-H_2O+1) 583.3277, found 583.3267.

3.1.3. Compound 12

$[\alpha]_D^{20}$=+36 (*c* 0.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (m, 4H, Arom.), 7.22 (m, 2H, Arom.), 7.04 (d, 4H, Arom.), 5.91 (dd, *J*=1.6, 6.4 Hz, 1H, H-2), 5.53 (dd, *J*=4.7, 9.4 Hz, 1H, H-14), 5.14 (s, 1H, H-20), 5.06 (dd, *J*=5.8, 11.3 Hz, 1H, H-10), 4.82 (s, 1H, H-20), 4.19 (br.s, 1H, H-5), 3.40 (d, *J*=6.4 Hz, 1H, 1H, H-3), 3.01 (dd, *J*=9.2, 18.8 Hz, 1H, H-13), 2.51 (m, 1H, H-13), 2.33 (dd, *J*=11.3, 15.1 Hz, 1H, H-9), 2.27 (br.s, 1H, H-1), 2.05 (m, 1H, H-7), 1.98 (s, 3H, Me-18), 1.74 (s, 3H, Me-16), 1.70 (m, 2H, H-6), 1.54 (m, 1H, H-9), 1.18 (s, 3H, Me-17), 1.08 (m, 1H, H-7), 1.00–0.88 (m, 13H, $[CH_3CH_2]_2SiCH[CH_3]_2$), 0.86 (s, 3H, Me-19), 0.66–0.56 (m, 4H, $[CH_3CH_2]_2SiCH[CH_3]_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.0, 193.7, 153.6, 147.2, 139.2, 130.7, 129.7, 129.7, 126.7, 122.2, 122.2, 114.3, 83.1. 81.5, 76.6, 67.7, 58.3, 48.7, 41.2, 40.3, 38.5, 37.8, 33.4, 32.1, 30.9, 25.9, 23.0, 21.1, 17.6, 17.6, 13.2, 7.4, 7.3, 4.1; IR (NaCl): 3447, 2953, 1593, 1490, 1457, 1437, 1419, 1288, 1098, 1012, 954, 926, 882, 869, 823, 759, 726, 689, 668 cm^{-1}; HR-FABMS calcd for $C_{41}H_{56}O_6SiS_2Na$ (M^+Na) 759.3185, found 759.3178.

3.1.4. 10β-Diethylisopropylsilyloxytaxa-4(20),11-dien-5α-ol (13)

To a solution of **12** (4.4 mg, 0.0056 mmol) in toluene (2 mL), AIBN (cat.) and nBu$_3$SnH (16 μL, 0.056 mmol) were added. The mixture was stirred at reflux temperature for 3 h. The mixture was condensed and purified by chromatography (silica gel, eluted with hexane/EtOAc

9:1), yielding crude **13** (1.4 mg, 53%). All pertinent spectroscopic and analytical data is reported in Ref. 14.

3.1.5. Barton deoxygenation of 11: 10β-diethyliso-propylsilyloxy taxa-4(20),11-dien-2α, 5α-diol (14)

To a solution of **11** (17.3 mg, 0.029 mmol) in toluene (2 mL), AIBN (cat.) and nBu$_3$SnH (78 μL, 0.29 mmol) were added. The mixture was stirred at reflux temperature for 3 h. The mixture was condensed and purified by chromatography (silica gel, eluted with hexane/EtOAc 6:1–5:1 hexane/EtOAc and fianlly EtoAc only), yielding crude **14** (6.8 mg, 52%): $[\alpha]_D^{20}$=+32 (c 0.68, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 (dd, J=1.5, 1.7 Hz, 1H, H-20), 5.13 (d, J=1.7 Hz, 1H, H-20), 4.98 (dd, J=5.5, 11.3 Hz, 1H, H-10), 4.17 (d, J=2.1 Hz, 1H, H-5), 4.02 (t, J=6.4 Hz, 1H, H-2), 3.20 (d, J=6.2 Hz, 1H, H-3), 2.32 (ddd, J=4.7, 11.3, 17.9 Hz, 1H, H-13), 2.19 (dd, J=11.5, 14.9 Hz, 1H, H-9), 2.06–1.86 (m, 3H, H-7, H-13, and H-14), 1.86 (s, 3H, Me-18), 1.78–1.56 (m, 4H, H-1, H-6, and OH-2), 1.54 (s, 3H, Me-16), 1.52–1.36 (m, 3H, H-9, H-14, and OH-5), 1.08 (s, 3H, Me-17), 1.02 (dt, 1H, J=14.3, 4.1 Hz, H-7), 1.00–0.82 (m, 13H, [CH_3CH$_2$]$_2$SiCH[CH_3]$_2$), 0.81 (s, 3H, Me-19), 0.64–0.44 (m, 4H, [CH$_3$$CH_2$]$_2$-SiCH[CH$_3$]$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) 150.5, 137.5, 132.2, 114.1, 76.8, 70.6, 68.0, 55.2, 48.7, 41.4, 40.4, 37.2, 33.6, 32.4, 32.0, 30.0, 25.5, 22.9, 21.3, 18.2, 17.7, 17.6, 13.2, 7.4, 7.4, 4.1; IR (KBr):3369, 2930, 2361, 1635, 1457, 1386, 1281, 1232, 1053, 1011, 915, 827, 723 cm^{-1}; HR-FABMS calcd for $C_{27}H_{47}O_3Si$ (M$^+$−H) 447.3294, found 447.3288.

3.1.6. Deprotection of DEIPS group of compound 14 to compound 15. Taxa-4(20),11-dien-2α, 5α,10β-triol

To a solution of **14** (5.4 mg, 0.012 mmol) in THF were added TBAF (0.06 mmol, 60 μL as 1 M THF solution) and AcOH (one drop). The mixture was stirred for 24 h. The mixture was diluted with EtOAc and washed with aqueous NH_4Cl and brine. The organic layer was condensed and purified by chromatography (silica gel, eluted with $CHCl_3$/MeOH 20:1), yielding crude **15** (3.5 mg, 92%): $[\alpha]_D^{20}$=+19 (c 0.17, EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.32 (s, 1H, H-20), 5.19 (s, 1H, H-20), 5.12 (dd, J=5.5, 11.7 Hz, 1H, H-10), 4.23 (t, J=2.8 Hz, 1H, H-5), 4.06 (br.s, 1H, H-2), 3.22 (d, J=6.2 Hz, 1H, H-3), 2.50–2.30 (m, 2H, H-13 and OH), 2.23 (dd, J=11.9, 14.5 Hz, 1H, H-9), 2.11–1.95 (m, 4H, H-1, H-7, H-13, and H-14), 1.92 (s, 3H, Me-18), 1.78–1.68 (m, 2H, H-6), 1.60 (s, 3H, Me-16), 1.52 (m, 1H, H-9), 1.50 (m, 1H, H-14), 1.15 (s, 3H, Me-17), 1.10 (ddd, J=2.1, 4.7, 13.4 Hz, 1H, H-7), 0.87 (s, 3H, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.2 (C4), 136.6 (C12), 135.5 (C11), 114.3 (C20), 76.7 (C5), 70.6 (C2), 68.1 (C10), 55.3 (C1), 47.2 (C9), 41.3 (C3), 40.5 (C8), 37.2 (C15), 33.6 (C7), 32.7 (C17), 32.0 (C6), 30.2 (C13), 25.7 (C16), 22.8 (C19), 21.5 (C18), 18.2 (C14); IR (KBr) 3406, 2925, 1653, 1631, 1445, 1380, 1261, 1097, 1026, 925, 802, 681, 468 cm^{-1}: HR-EIMS (M$^+$Na) calcd for $C_{20}H_{32}O_3Na$ 343.2249, found 343.2239.

3.1.7. 5α-Acetoxy-10β-diethylisopropylsilyloxy taxa-4(20),11-dien-2α-ol (17)

To a solution of **14** (40 mg, 0.089 mmol) in THF (4 mL), LHMDS (0.10 mmol, 100 μL as 1 M THF solution) was added at −18°C. After being stirred for 5 min, acetyl bromide (7 μL, 0.098 mmol) was added and the mixture was stirred for 2 h at rt. The mixture was diluted with EtOAc (15 mL) and washed with aqueous $NaHCO_3$, aqueous NH_4Cl, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography (silica gel, eluted with hexane/EtOAc 4:1–3:1), yielding **17** (5.1 mg, 12%) and recovered **14** (29.5 mg, 74%). Employing the same procedure using AcCl and diol **14** (28.4 mg, 0.063 mmol) gave **17** (2.4 mg, 8%) and recovered **14** (65%). Data for **17**: $[\alpha]_D^{20}$=+47 (c 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (dd, J=1.2, 1.7 Hz, 1H, H-20), 5.32 (d, J=1.3 Hz, 1H, H-20), 5.23 (dd, J=2.6, 3.0 Hz, 1H, H-5), 4.99 (dd, J=5.8, 11.3 Hz, 1H, H-10), 4.06 (d, J=4.7 Hz, 1H, H-2), 2.96 (d, J=6.4 Hz, 1H, H-3), 2.43 (m, 1H, H-13), 2.28 (dd, J=11.3, 14.9 Hz, 1H, H-9), 2.12 (s, 3H, MeCO),1.96–1.80 (m, 4H, H-1, H-7, H-13, and H-14) 1.91 (s, 3H, Me-18), 1.75 (m, 2H, H-6), 1.60 (m, 1H, OH), 1.59 (s, 3H, Me-16), 1.50 (dd, J=5.3, 14.7 Hz, 1H, H-7), 1.50 (m, 1H, H-14), 1.15 (ddd, J=3.2, 3.6, 13.0 Hz, 1H, H-7), 1.12 (s, 3H, Me-17), 1.02–0.92 (m, 13H, [CH_3CH$_2$]$_2$SiCH[CH_3]$_2$), 0.87(s, 3H, Me-19), 0.66–0.56 (m, 4H, [CH$_3$$CH_2$]$_2$SiCH[CH$_3$]$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.19 (Ac), 145.4, 138.3, 131.3, 117.4 (C20), 79.0 (C5), 70.6 (C2), 67.9 (C10), 55.2 (C1), 48.7 (C9), 43.4 (C3), 40.0, 37.2, 34.3 (C7), 32.3 (Me), 30.2 (C13), 29.6 (C6), 25.5 (Me), 23.1 (Me), 22.2 (Ac), 21.3 (Me), 18.2 (C14), 17.7 (DEIPS), 13.3 (DEIPS), 7.0 (DEIPS), 4.2 (DEIPS); IR (NaCl): 3502, 2951, 1739, 1635, 1464, 1368, 1239, 1198, 1105, 1049, 1015, 957, 881, 827, 726, 668, 417 cm^{-1}; HR-FABMS (M$^+$−H) calcd for $C_{29}H_{49}O_4Si$ 489.3400, found 489.3385.

3.1.8. 5α-Acetoxytaxa-4(20),11-dien-2α,10β-diol (7)

To a solution of **17** (4.3 mg, 0.088 mmol) in pyridine (2 mL) was added HF-Py (0.2 mL, 70% solution in pyridine) at 0°C. The mixture was stirred for 4 h at room temperature and was diluted with EtOAc (30 mL) and washed with saturated $CuSO_4$, water, aqueous NH_4Cl, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography (silica gel, eluted with hexane/EtOAc 2:1), yielding **7** (3.0 mg, 94%): $[\alpha]_D^{20}$=+42 (c 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ, 5.43 (dd, J=1.5, 1.7 Hz, 1H, H-20), 5.31 (d, J=1.7 Hz, 1H, H-20), 5.21 (dd, J=2.8, 3.0 Hz, 1H, H-5), 5.06 (dd, J=5.5, 11.7 Hz, 1H, H-10), 4.03 (br.s, 1H, H-2), 2.91 (d, J=6.2 Hz, 1H, H-3), 2.47 (m, 1H, H-13), 2.26 (dd, J=11.7, 14.7 Hz, 1H, H-9), 2.08 (s, 3H, Ac), 2.00 (m, 2H, H-13 and H-14), 1.98 (m, 1H, H-1), 1.94 (m, 1H, H-7), 1.91 (s, 3H, Me-18), 1.80 (m, 2H, H-6), 1.63 (m, 1H, H-9), 1.60 (m, 1H, OH), 1.57 (s, 3H, Me-16), 1.50 (m, 1H, H-14), 1.19 (s, 3H, Me-17), 1.16 (ddd, J=3.2, 3.4, 13.0 Hz, 1H, H-7), 0.86 (s, 3H, Me-19); IR (NaCl) 3420, 2985, 2931, 1734, 1456, 1447, 1369, 1242, 1198, 1074, 1019, 958, 943, 755, 667 cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1(Ac), 145.1, 137.4, 134.4, 117.5 (C20), 78.9 (C5), 70.5 (C2), 67.9 (C10), 55.3 (C1), 47.2 (C9), 43.3 (C3), 40.1 (C8), 37.2 (C15), 34.3 (C17), 32.55 (C17), 30.4 (C13), 29.7 (C6), 25.7 (C16), 23.0 (C19), 22.1 (Ac), 21.5 (C18), 18.2 (C14); HR-FABMS (M$^+$Na) calcd for $C_{22}H_{34}O_4Na$ 385.2355, found 385.2365.

3.1.9. Barton deoxygenation of 10:10β-diethyliso-propylsilyloxy-taxa-4(20),11-dien-5α,14β -diol (18)

To a solution of **10** (99.1 mg, 0.0165 mmol) in toluene (4 mL), AIBN (cat.) and nBu$_3$SnH (0.44 mL, 1.65 mmol) were added. The mixture was stirred at reflux temperature for 3 h. The mixture was condensed and purified by chromatography (silica gel, eluted with $CHCl_3$/MeOH 20:1), yielding crude **18** (44 mg, 59%): $[\alpha]_D^{20}=+88$ (c 0.57, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.08 (dd, J=5.5, 11.5 Hz, 1H, H-10), 4.97 (s, 1H, H-20), 4.67 (s, 1H, H-20), 4.24 (dd, J=2.8, 3.0 Hz, 1H, H-5), 3.75 (dd, J=5.3, 9.2 Hz, 1H, H-14), 2.86 (d, J=4.3 Hz, 1H, H-3), 2.52 (dd, J=9.2, 18.3 Hz, 1H, H-13), 2.40 (ddd, J=1.1, 4.3, 18.5 Hz, 1H, H-9), 2.26 (dd, J=11.5, 14.7 Hz, 1H, H-9), 2.07 (dt, J=6.2, 12.8 Hz, 1H, H-7), 1.91 (d, J=1.1 Hz, 3H, Me-18), 1.75–1.63 (5H, m, H-1, H-2, and H-6), 1.56 (s, 3H, Me-27), 1.47 (dd, J=5.5, 14.9 Hz, 1H, H-9), 1.18 (s, 3H, Me-16), 1.08 (ddd, J=2.1, 4.5, 13.0 Hz, 1H, H-7), 1.02–0.9 (m, 13H, [CH_3CH$_2$]$_2$SiCH[CH_3]$_2$), 0.68–0.58 (m, 4H, [CH$_3$$CH_2$]$_2$-SiCH[CH$_3$]$_2$), 0.65 (s, 3H, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 140.7, 130.8, 109.7, 74.5, 72.0, 68.0, 55.9, 49.3, 42.6, 39.7, 38.8, 35.4, 33.5, 31.7, 30.2, 27.0, 26.2, 21.9, 21.2, 17.7, 17.6, 13.3, 7.4, 7.4, 4.1; IR (NaCl) 3374, 2954, 1457, 1380, 1232, 1057, 926, 826, 729, 668; HR-FABMS (M$^+$Na) calcd for $C_{27}H_{48}O_3SiNa$ 471.3270, found 471.3275.

3.1.10. Deprotection of DEIPS group of compound 18 to compound 19: taxa-4(20),11-dien-5α 10β,14β-triol

To a solution of **18** (9.4 mg, 0.021 mmol) in pyridine (2 mL) was added HF-Py (0.2 mL, 70% solution in pyridine) at 0°C. The mixture was stirred for 4 h at room temperature and was diluted with EtOAc (30 mL) and washed with saturated $CuSO_4$, water, aqueous NH_4Cl, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography (silica gel, eluted with $CHCl_3$/MeOH 9:1), yielding crude **19** (6.3 mg, 94%): $[\alpha]_D^{20}=+105$ (c 0.24, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96 (dd, J=5.5, 11.7 Hz, 1H, H-10), 4.79 (s, 1H, H-20), 4.56 (t, J=1.3 Hz, 1H, H-20), 3.88 (dd, J=3.2, 3.6 Hz, 1H, H-5), 3.61 (dd, J=6.8, 7.0 Hz, 1H, H-14), 2.81 (m, 1H, H-3), 2.41 (d, J=7.7 Hz, 2H, H-13), 2.22 (dd, J=11.7, 14.5 Hz, 1H, H-9), 2.03 (ddd, J=5.8, 12.8, 13.0 Hz, 1H, H-7), 1.85 (s, 3H, Me-18), 1.65 (s, 3H, Me-16), 1.61–1.49 (m, 5H, H-1, H-2, and H-6), 1.46 (dd, J=5.5, 14.5 Hz, 1H, H-9), 1.34 (s, 3H, Me-17), 0.96–0.80 (m, 3H, 10-OH, 14-OH, and H-7), 0.61 (s, 3H, Me-19), 0.58 (m, 1H, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 140.7, 133.2, 108.9, 74.5, 71.7, 68.0, 56.5, 48.5, 43.1, 40.1, 39.2, 35.6, 33.9, 32.6, 30.9, 27.4, 26.8, 22.2, 21.5; IR (KBr) 3362, 2924, 1646, 1445, 1377, 1262, 995, 948, 896, 757 cm^{-1}; HR-FABMS (M$^+$Na) calcd for $C_{20}H_{32}O_3Na$ 434.2249, found 434.2248.

Acknowledgements

We thank the National Institutes of Health (Grant CA 70375 to R. M. W., and Grant CA 55254 to R. C) for financial support. We thank Dr John Greaves (Department of Chemistry, University of California, Urvine) for exact mass measurement of compound **15**. We thank Professor Takayuki Oritani (Laboratory of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, Japan) and Hida Ichii Ittoubori Kyoudou Kumiai of The Engraving Craftsman Association, Hida, Japan for the generous gift of the Japanese yew heart wood. We thank the Yamada Science Foundation for fellowship support (to T. H.).

References

1. (a) Suffness, M. In *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. In *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. In *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. USA* **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. USA* **2001**, *98*, 13595–13600.
13. (a) Floss, H. G.; Mocek, U. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995; pp 3–25. (b) Croteau, R.; Hefner, J.; Hezari, M.; Lewis, N. G. *Curr. Top. Plant Physiol.* **1995**, *15*, 94–104.
14. Horiguchi, T.; Rithner, C. D.; Croteau, R.; Williams, R. M. *J. Org. Chem.* **2002**, *67*, 4901–4903.
15. (a) Rubenstein, S. M.; Williams, R. M. *J. Org. Chem.* **1995**, *60*, 7215–7223. (b) Rubenstein, S. M.; Vazquez, A.; Williams, R. M. *J. Labeled Cmpd Radiopharm.* **2000**, *43*, 481–491.
16. Sugiyama, T.; Oritani, T. *Biosci. Biotech. Biochem.* **1994**, *58*, 1923–1924.