Synthesis and Structure−Activity Relationships of Nonaromatic Taxoids: Effects of Alkyl and Alkenyl Ester Groups on Cytotoxicity

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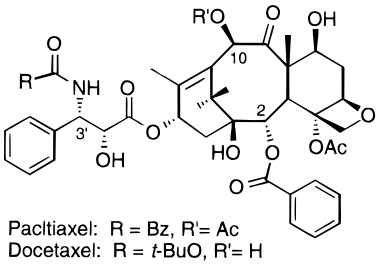
Several new nonaromatic taxoids are synthesized by means of the β-lactam synthon method. These include taxoids modified with 3-methylbut-2-enoate, 3-methylbutanoate, and cyclohexanecarboxylate groups in place of the benzoate at the C-2 position. In addition, taxoids with 2-methylprop-1-enyl, 2-methylpropyl, *(E)*-prop-1-enyl, and cyclohexyl groups at the C-3′ position are also prepared in combination with the modifications at C-2. The alkyl and alkenyl ester groups at C-2 displayed pronounced effects on the *in vitro* cytotoxicity. Two of the fully aliphatic taxoids possess similar or stronger activity than paclitaxel and docetaxel. It is clear that the 2-benzoate does not play a unique role, and replacement with the appropriate alkyl and alkenyl groups provides taxoids with equivalent or superior activity.

Introduction

The naturally occurring antitumor agent paclitaxel[1] has shown exceptional efficacy in cancer chemotherapy and has been approved by the FDA for the treatment of advanced ovarian cancer and breast cancer as of December 1992 and April 1994, respectively. Docetaxel,[2] a semisynthetic analog, has also exhibited significant clinical distinction and was approved for the treatment of breast cancer in May 1996 by the FDA. These compounds possess a unique mechanism of action as promoters of tubulin assembly and inhibitors of microtubule disassembly.[3−5] However, despite their potent antitumor activity, these drugs often result in a number of undesired side effects and are subject to multidrug resistance (MDR).[6] Thus, it is essential to develop new anticancer agents with fewer side effects, improved pharmacological properties, and activity against cancers not effectively treated by existing anticancer drugs.



Paclitaxel: R = Bz, R'= Ac
Docetaxel: R = *t*-BuO, R'= H

Several structure−activity relationship studies have looked at the effects of the 3′-phenyl and the 2-benzoate moieties[7] on the biological activity of paclitaxel and its analogs. Multiple reports concerning substituted phenyl rings for both C-2 and C-3′ have appeared.[8] With few exceptions many of these showed substantially decreased activity. Heteroaromatic groups have also been included at these positions. The Kansas group has reported the preparation of several 2-*O*-heteroaromatic replacements[9] and found that they had detrimental effects on the activities compared to paclitaxel, with the exception that certain groups, such as thienyl, retained comparable microtubule assembly properties. Notable is the work of Nicolaou[10] who prepared a series of aryl and heteroaryl analogs as the C-2 position using a ring-opening reaction of a protected 1,2-carbonate with organolithium reagents. Once again moieties such as furyl and thienyl exhibited similar activities to paclitaxel, while larger groups showed substantially decreased activities. Clearly the size of the aryl or heteroaryl group at C-3′ and C-2 has prominent effects on the biological activity of these taxoids.

Saturation of the phenyl rings at the C-3′ position and the 2-benzoate has been investigated by us[11] and others.[12,13] In general it has been shown that a cyclohexyl group at the C-3′ position or cyclohexanecarbonyl group at C-2 exhibited weaker cytotoxicity *in vitro* while little effect was observed on the tubulin binding ability. We[14,15] and others[16,17] have reported novel taxoids containing alkyl and alkenyl groups at the C-3′ position such as 2-methylprop-1-enyl, 2-methylpropyl, *(E)*-prop-1-enyl, and *tert*-butyl. These analogs have in most cases shown superior activity compared to paclitaxel. More importantly, we have recently reported C-10-modified 3′-alkyl and 3′-alkenyl taxoids which exhibit exceptional potency against multidrug resistant cell lines expressing the MDR phenotype.[18] However, the replacement of the 2-benzoate with simple alkyl and alkenyl esters in conjunction with modifications at the C-3′ position has not been studied. We report here the synthesis of new taxoids replacing all phenyl groups of paclitaxel and docetaxel by alkyl and alkenyl groups and their effects on cytotoxicity.

Taxoid Synthesis

Alkyl and alkenyl groups such as 2-methylprop-1-enyl, 2-methylpropyl, *(E)*-prop-1-enyl, and *tert*-butyl were chosen for investigation in place of the phenyl at the C-2 benzoate position. Several approaches reported in the literature were attempted,[7,10,13] but each approach had its own drawback or was not compatible with our goal of modifying both C-2 and C-3′ positions. After these unsuccessful attempts, we decided to use a modified version of Chen's method[13] choosing to oxidize the 13-OH to give 13-oxo-7-TES-baccatin III (**1**)[10] as shown

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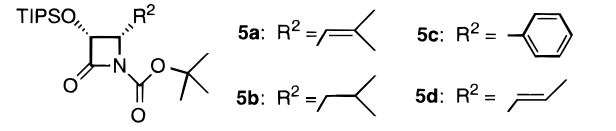
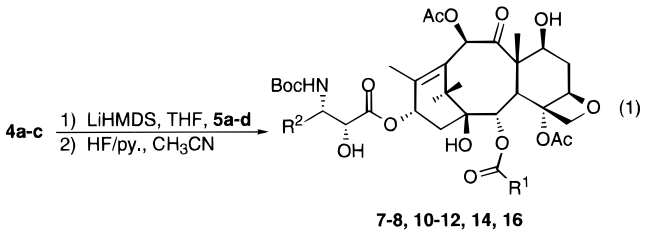
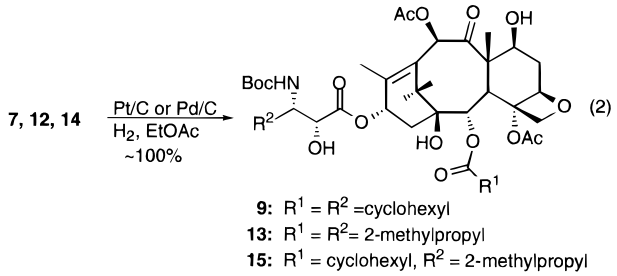
in Scheme 1. The use of **1** serves as protection for the C-13 position and also allows the esterification to proceed under milder conditions as the 2-OH is more exposed due to a conformational change in the baccatin core resulting from oxidation of the C-13 position. Acetylation of 10-deacetyl-13-oxo-7-TES-baccatin III followed by reduction using Red-Al afforded a 75% yield of **2**. DCC coupling using 3 equiv of dimethylacrylic acid furnished **3** in 92% yield, and subsequent $NaBH_4$ reduction of the 13-ketone afforded **4a**. Esterification with other acids such as tiglic acid or pivalic acid did not afford any reaction. Prolonged reaction times and heating to 65 °C still gave no reaction. This is attributed to the α-methyl substitution on these acids showing the sensitive steric requirements at this position. Hydrogenation of the olefin was carried out to yield **4b** in quantitative fashion using Pd/C in EtOAc under 1 atm of H_2. The 2-(cyclohexylcarbonyl)baccatin **4c** was obtained via hydrogenation of 7-TES-baccatin III using the literature procedure.[12]

Coupling reactions were then carried out using methodology developed independently by our group[19−21] and Holton et al.[22] Thus treatment of protected baccatins **4a**−**c** and enantiomerically pure (3*R*,4*S*)-1-*t*-Boc-3-TIPSO-4-modified-azetidin-2-ones[23,24] (**5a**−**d**) using LiHMDS in THF at −40 °C afforded 7-TES-2′-TIPS-protected taxoids (eq 1). Deprotection using HF/pyridine produced taxoids **7**, **8**, **10**−**12**, **14**, and **16** in fair to good yields. Results are summarized in Table 1.





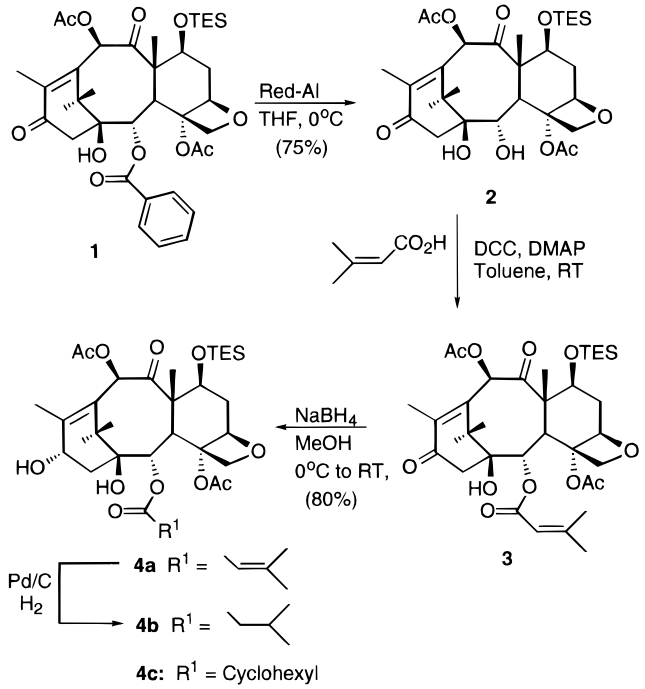
Taxoid **9** was prepared in quantitative yield by reduction of the 3′-phenyl ring of **7** with Pt/C in EtOAc under 1 atm of H_2 in EtOAc for 36 h. Similarly, taxoids **13** and **15** were prepared from **12** and **14** via hydrogenation over Pd/C under 1 atm of H_2 in 24 h. Results are summarized in eq 2.



Biological Evaluation of New Taxoids

The *in vitro* cytotoxicity of the new taxoids was evaluated for potency against several human tumor cell lines: A121 (ovarian carcinoma), A549 (non-small-cell lung carcinoma), HT-29 (colon carcinoma), MCF7 (mammary carcinoma), and the drug resistant cell line MCF7-R (mammarian carcinoma 180-fold resistant to doxorubicin). Results are shown in Table 2 with the values for paclitaxel, docetaxel, and SB-T-1212[14] shown for comparison.

Scheme 1. Preparation of Baccatin **4**



As Table 2 shows, several of the new taxoids exhibit similar or enhanced activity compared to paclitaxel and docetaxel. As previously reported, taxoids **7** and **9** bearing cyclohexanecarbonyl groups at the C-2 position show diminished activity compared to paclitaxel and docetaxel, particularly for **9**. SB-T-1212 exhibits very potent cytotoxicity, better than docetaxel, particularly against MCF7 and MCF7-R cell lines. Taxoid **10** bearing 2-methylprop-1-enyl groups at both C-3′ and C-2 is more active than both paclitaxel and docetaxel showing that a benzoate group at C-2 is not necessary for strong cytotoxicity in lieu of the appropriate alkyl, alkenyl, or cyclohexyl group. However, taxoids **11**−**13** bearing a combination of 2-methylprop-1-enyl and 2-methylpropyl groups at C-3′ and C-2 were all less active than **10**, showing the considerable sensitivity for the modification at these positions. It is apparent, however, from **14**−**16** that in the case of the 2-cyclohexanecarbonyl taxoids, the C-3′ position exerts marked effects on the activity. In fact, taxoid **14** shows better activity than docetaxel, especially against the MCF7 and MCF7-R cell lines. The 3′-(2-methylprop-1-enyl) taxoid **14** is clearly the most potent of all analogs bearing a 2-cyclohexanecarbonyl group, with a significant increase in activity as compared to 3′-((*E*)-prop-1-enyl) taxoid **16** as well as **7** and **9**.

While the size of the group at C-3′ exerts marked effects on the cytotoxicity of the analog, the C-2 substituent has prominent effects on the biological activity as well. Whereas a cyclohexanecarbonyl group at C-2 diminishes the activity, the placement of the proper alkyl or alkenyl group at the C-3′ position brings about an increase in activity comparable to that of docetaxel. This is clearly seen from the results for compounds **14**−

Table 1. Syntheses of Taxoids through Coupling and Deprotection

Baccatin	R^1	β-Lactam	R^2	Taxoid	Yield(%)[a]
4c		**5c**		**7**	93[b]
4a		**5c**		**8**	55
4a		**5a**		**10**	51
4b		**5a**		**11**	53
4a		**5b**		**12**	68
4c		**5a**		**14**	72
4c		**5d**		**16**	71

[a] Isolated two-step yield for coupling and deprotection. [b] Deprotection was carried out using TBAF/HOAc in THF.

Table 2. *In Vitro* Cytotoxicity (IC_{50}, nM)[a] of Modified Taxoids **7**−**16**

Taxoid	C-2 (R^1)	C-3' (R^2)	A121 (ovarian)	A549 (NSCL)	HT-29 (colon)	MCF7 (breast)	MCF7-R (breast)
Paclitaxel			6.1	3.6	3.2	1.7	299
Docetaxel			1.2	1.0	1.2	1.0	235
SB-T-1212			0.5	0.3	0.6	0.6	12
7			4.8	5.7	5.6	1.8	412
8			2.7	2.9	3.5	1.1	140
9			43	49	59	10	591
10			**0.9**	**0.9**	**1.4**	**0.5**	**38**
11			17	14	16	11	589
12			54	30	89	84	>1000
13			55	47	52	43	1081
14			**1.5**	**1.3**	**1.7**	**0.5**	**26**
15			3.2	7.7	5.6	1.8	57
16			6.6	12	8.5	3.2	446

[a] The concentration of compound which inhibits 50% of the growth of human tumor cell line: A121 (ovarian carcinoma), A549 (non-small-cell lung carcinoma), HT-29 (colon carcinoma), MCF7 (mammary carcinoma), and MCF7-R (mammarian carcinoma 180-fold resistant to doxorubicin), after 72 h drug exposure.[30]

16. A Chem 3D representation of taxoid **10** based on the hydrophobic cluster model,[25−27] i.e., the dihedral angle of $H^{2'}-C^{2'}-C^{3'}-H^{3'}$ is set to be 180° and energy minimized, is shown in Figure 1. This conformation indicates a very strong clustering of the 2-methylprop-1-enyl groups at C-2 and C-3′ with the C-4 acetyl methyl. It appears that a rigid moiety, such as a ring or olefin, is required at the C-3′ and C-2 positions for strong activity. When alkyl groups that can freely rotate such as 2-methylpropyl are introduced at these positions (taxoids **11**−**13**), the activity is discernibly reduced. The conformational analysis of these taxoids based on NMR and molecular modeling including restrained molecular dynamics is actively underway and will be reported elsewhere.

In summary, a series of taxoids with alkyl and alkenyl modifications at both the C-2 and C-3′ positions have been prepared and evaluated. It has been shown that the phenyl of the C-2 benzoate can be replaced by certain alkyl or alkenyl groups, such as 2-methylpropenyl in **10**, with the taxoid exhibiting equivalent or improved activity compared to paclitaxel.

Experimental Section

General Methods. Melting points were measured with a Thomas Hoover capillary melting point apparatus and are

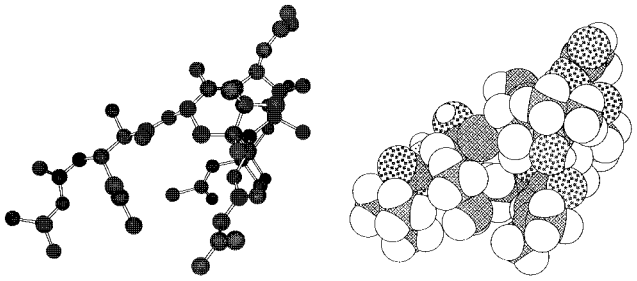


Figure 1. Chem 3D representation of taxoid **10**.

uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 1600 FT-IR spectrophotometer with neat samples. ^{1}H, ^{13}C, and 2D nuclear magnetic resonance (NMR) spectra were measured using a General Electric QE-300 or a Bruker AC-250 spectrometer using tetramethylsilane as the internal standard. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Thin layer chromatography was performed on Merck DC-alufolien with Kieselgel 60F-254. Column chromatography was carried out on silica gel 60 (230−400 mesh ASTM, Merck). FAB-HRMS were performed at the UCR Mass Spectrometry Facility, Riverside, CA.

Materials. The chemicals were purchased from Aldrich Co. and Sigma and purified before use by standard methods. THF was freshly distilled over sodium and benzophenone. 7-(Triethylsilyl)baccatin III (7-TES) was prepared by the literature method.[28] (3*R*,4*S*)-3-[(Triisopropylsilyl)oxy]-4-(2-methylprop-1-enyl)-1-(*tert*-butoxycarbonyl)azetidin-2-ones **5** were prepared by literature procedures.[14] 13-Oxo-7-TES-baccatin III (**1**) was prepared by the literature method.[10]

2-Debenzoyl-13-oxo-7-TES-baccatin III (2). To a mixture of 530 mg (0.76 mmol) of **1** in 14 mL of THF at 0 °C was added dropwise 0.5 mL of Red-Al (Aldrich; 70% wt solution in toluene). The reaction was monitored by TLC, and Red-Al was added dropwise until all starting material was consumed, usually within 1 h. The reaction was quenched by addition of 10 mL of a saturated solution of potassium sodium tartrate (Rochelle salt) and the aqueous layer extracted with 2 × 50 mL of Et_2O. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated. The crude oil was subjected to chromatography on silica gel (hexane:EtOAc = 1:1) to afford 337 mg (75%) of **2** as a clear film: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.55 (q, J = 7.8 Hz, 6 H), 0.89 (t, J = 7.8 Hz, 9 H), 1.13 (s, 3 H), 1.16 (s, 3 H), 1.61 (s, 3 H), 2.01 (s, 3 H), 2.10 (s, 3 H), 2.17 (s, 3 H), 2.43 (m, 1 H), 2.54 (d, J = 19.9 Hz, 1 H), 2.80 (d, J = 19.9 Hz, 1 H), 2.92 (bd, J = 5.3 Hz, 1 H), 3.53 (d, J = 6.2 Hz, 1 H), 3.93 (d, J = 6.2 Hz, 1 H), 3.93 (bt, 1 H), 4.40 (dd, J = 9.9, 6.8 Hz, 1 H), 4.53 (d, J = 9.0 Hz, 1 H), 4.61 (d, J = 9.0 Hz, 1 H), 4.88 (d, J = 9.2 Hz, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 5.2, 6.7, 9.7, 13.5, 18.2, 20.8, 21.6, 32.7, 37.3, 42.7, 43.1, 46.1, 59.5, 72.3, 72.4, 76.1, 77.5, 81.3, 83.8, 140.0, 153.1, 168.9, 170.0, 198.9, 201.2; FAB-HRMS (NBA) m/e 595.296 (MH^+, $C_{30}H_{47}NO_{10}Si$ requires 595.293).

2-Debenzoyl-2-(3-methylbut-2-enoyl)-13-oxo-7-TES-baccatin III (3). Compound **2** (135 mg, 0.23 mmol), DMAP (83 mg, 0.68 mmol), DCC (140 mg, 0.68 mmol), and dimethylacrylic acid (68 mg, 0.68 mmol) were dissolved in 3 mL of toluene and allowed to stir overnight. The reaction was quenched with 10 mL of saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with 2 × 20 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Silica gel chromatography (hexane:EtOAc = 1:1) afforded 141 mg of **3** (92%) as a white film: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, J = 7.8 Hz, 6 H), 0.90 (t, J = 7.8 Hz, 9 H), 1.15 (s, 3 H), 1.22 (s, 3 H), 1.81−1.87 (m, 1 H), 2.06 (s, 3 H), 2.11 (s, 3 H), 2.20 (s, 3 H), 2.27−2.54 (m, 1 H), 2.58 (d, J = 20.1 Hz, 1 H), 2.78 (d, J = 20.1 Hz, 1 H), 3.78 (d, J = 6.3 Hz, 1 H), 4.13 (d, J = 8.4 Hz, 1 H), 4.40−4.46 (m, 2 H), 4.90 (d, J = 7.8 Hz, 1 H), 5.44 (d, J = 6.3 Hz, 1 H), 5.63 (bs, 1 H), 6.54 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.4, 8.3, 12.9, 15.5, 15.6, 16.4, 19.5, 21.3, 22.6, 25.5, 27.8, 32.0, 37.2, 38.3, 41.1, 54.2, 66.1, 67.0, 70.8, 72.9, 75.1, 78.8, 109.5, 135.0, 147.8, 156.2, 161.9, 163.8, 165.1, 193.5, 195.2; HRMS (DCI) m/e 677.3341 (MH^+, $C_{30}H_{47}NO_{10}Si$ requires 677.3357).

2-Debenzoyl-2-(3-methylbut-2-enoyl)-7-TES-baccatin III (4a). Compound **3** (70 mg, 0.10 mmol) was dissolved in 4 mL of MeOH, and 1 mL of THF and $NaBH_4$ (100 mg, 2.0 mmol) were added in small portions at 0 °C. The reaction was kept at 0 °C for 5 h and quenched by addition of 5 mL of saturated aqueous NH_4Cl; the mixture was allowed to stir for 5 min. The aqueous layer was extracted with 2 × 20 mL of CH_2Cl_2, dried over $MgSO_4$, filtered, and concentrated. Silica gel chromatography (hexane:EtOAc = 2:1) afforded 17 mg (25%) of recovered **3** and 42 mg of **4a** (60%, 80% based on 75% conversion) as a white film: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.48−0.51 (m, 6 H), 0.84 (t, J = 7.8 Hz, 9 H), 0.96 (s, 3 H), 1.08 (s, 3 H), 1.55 (s, 3 H), 1.87 (s, 3 H), 1.57−1.60 (m, 1 H), 2.08−2.12 (m, 1 H), 2.41−2.52 (m, 1 H), 3.68 (d, J = 6.3 Hz, 1 H), 4.07 (d, J = 8.4 Hz, 1 H), 4.36 (d, J = 8.4 Hz, 1 H), 4.73 (bt, 1 H), 4.87 (d, J = 9.3 Hz, 1 H), 5.30 (d, J = 6.6 Hz, 1 H), 5.60 (bs, 1 H), 6.3 (bs, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 5.3, 6.7, 10.0, 14.9, 20.0, 22.7, 37.3, 38.2, 42.9, 47.4, 58.7, 68.0, 72.3, 73.1, 75.9, 78.3, 80.7, 84.3, 87.4, 103.0, 115.4, 132.7, 144.0, 160.0, 167.4, 169.4, 171.0, 202.4; FAB-HRMS (NBA-NaCl) m/e 701.3329 (M^+ + Na, $C_{35}H_{54}NO_{11}Si$ requires 701.3327).

2-Debenzoyl-2-(3-methylbutanoyl)-7-TES-baccatin III (4b). Compound **4a** (21 mg, 0.031 mmol) was hydrogenated under 1 atm of H_2 in the presence of 20 mg of 10% Pd/C in 3 mL of EtOAc. After 22 h the reaction mixture was filtered through a pipet column of silica gel (EtOAc) and concentrated to afford 21 mg (100%) of **4b** as a white film: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.52−0.61 (m, 6 H), 0.91 (t, J = 7.9 Hz, 19 H), 0.94 (s, 3 H), 1.00 (d, J = 3.5 Hz, 6 H), 1.12 (s, 3 H), 1.61 (s, 3 H), 1.78−1.91 (m, 1 H), 2.02−2.27 (m, 4 H), 2.15 (s, 3 H), 2.16 (s, 3 H), 2.45−2.57 (m, 1 H), 3.75 (d, J = 6.7 Hz, 1 H), 4.15 (d, J = 7.9 Hz, 1 H), 4.44 (d, J = 7.9 Hz, 1 H), 4.79 (bt, J = 8.2 Hz, 1 H), 4.95 (d, J = 8.9 Hz, 1 H), 5.46 (d, J = 6.5 Hz, 1 H), 6.41 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 5.3, 6.7, 9.9, 14.9, 20.0, 20.9, 22.5, 22.6, 25.4, 26.9, 37.2, 37.9, 42.8, 43.9, 47.2, 58.6, 67.9, 72.3, 74.0, 75.8, 76.7, 78.6, 80.6, 84.4, 132.6, 144.0, 169.3, 170.9, 174.3, 202.2; FAB-HRMS (NBA-NaCl) m/e 681.3661 (MH^+, $C_{35}H_{57}O_{11}Si$ requires 681.3670).

10-Acetyl-2-(cyclohexylcarbonyl)-2-debenzoyldocetaxel (7). To a mixture of 16 mg (0.023 mmol) of **4c** and 14 mg (0.035 mmol) of **5c** in 2 mL of THF was added 0.032 mL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition of 10 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 15 mL portions of Et_2O, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford 90 mg (95%) of a white solid. This material was dissolved in 3 mL of THF, and 2 drops of HOAc and 0.26 mL of TBAF (1.0 M in THF) were added dropwise. After 36 h, the solvent was removed, and silica gel chromatography (hexane:EtOAc = 1:1 to 1:2) afforded 66 mg (98%) of **7** as a white solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.99 (s, 3 H), 1.14 (s, 3 H), 1.20−2.43 (m, 14 H), 1.31 (s, 9 H), 1.61 (s, 3 H), 1.73 (s, 3 H), 2.16 (bs, 6 H), 2.40−2.58 (s, 1 H), 3.58 (d, J = 6.9 Hz, 1 H), 4.08 (d, J = 8.2 Hz, 1 H), 4.40 (d, J = 8.2 Hz, 1 H), 4.34 (dd, J = 10.0, 6.6 Hz, 1 H), 4.52 (bs, 1 H), 4.88 (d, J = 10.0 Hz, 1 H), 5.11 (d, J = 9.0 Hz, 1 H), 5.28 (bd, J = 9.0 Hz, 1 H), 5.35 (d, J = 6.9 Hz, 1 H), 6.09 (t, J = 8.7 Hz, 1 H), 6.15 (s, 1 H), 7.20−7.33 (m, 5 H). All data are in agreement with reported literature values.[12]

10-Acetyl-2-debenzoyl-2-(3-methylbut-2-enoyl)docetaxel (8). To a mixture of 30 mg (0.044 mmol) of **4a** and 28 mg (0.066 mmol) of **5c** in 3 mL of THF was added 0.061 mL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition of 10 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 20 mL portions of EtOAc, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford 33 mg (70%). A solution of this material (31 mg, 0.028 mmol) was dissolved in 2 mL of pyridine and 2 mL of CH_3CN, treated with 0.5 mL of HF/pyridine (70% wt solution) at 0 °C, and allowed to warm to room temperature for 3 h. The reaction was quenched with 10 mL of 1 N HCl, and the mixture was extracted with 2 × 15 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (hexane:EtOAc = 1:2) afforded

18 mg (78% and 55%, two-step yield) of **8** as a white film: $[\alpha]^{20}_D$ −45.0° (*c* 0.1, $CHCl_3$); IR ($CDCl_3$, cm^{-1}) 3424, 3036, 2966, 1742, 1736, 1731, 1725, 1701, 1660, 1631, 1554, 1378, 1243, 1161; ^1H NMR (250 MHz, $CDCl_3$) δ 1.10 (s, 3 H), 1.24 (s, 3 H), 1.40 (s, 9 H), 1.62 (s, 3 H), 1.80 (s, 3 H), 1.96 (s, 3 H), 1.97 (m, 1 H), 2.19 (s, 3 H), 2.22 (s, 3 H), 2.24 (s, 3 H), 2.23 (m, 2 H), 2.43 (m, 1 H), 3.31 (bd, *J* = 5.1 Hz, 1 H), 3.68 (bd, *J* = 6.5 Hz, 1 H), 4.17 (d, *J* = 8.4 Hz, 1 H), 4.44 (d, *J* = 8.4 Hz, 1 H), 4.37 (m, 1 H), 4.56 (bd, 1 H), 4.95 (m, 2 H), 5.18 (d, *J* = 9.5 Hz, 1 H), 5.36 (d, *J* = 9.8 Hz, 1 H), 5.42 (d, *J* = 6.8 Hz, 1 H), 5.68 (bs, 1 H), 6.09 (bt, 1 H), 6.15 (s, 3 H), 7.30 (m, 5 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.6, 14.9, 20.6, 20.8, 21.7, 22.6, 26.7, 27.7, 28.2, 35.4, 43.3, 45.8, 56.3, 58.6, 72.1, 72.8, 73.3, 75.7, 76.9, 78.7, 84.5, 115.2, 126.8, 128.1, 133.0, 133.2, 138.4, 142.2, 155.4, 160.6, 167.1, 170.2, 171.3, 172.2, 173.1, 203.8; FAB-HRMS (NBA) *m/e* 828.3827 (MH^+, $C_{43}H_{58}NO_{15}$ requires 828.3806).

10-Acetyl-2-(cyclohexylcarbonyl)-3′-cyclohexyl-2-debenzoyl-3′-dephenyldocetaxel (9). Compound **7** (15 mg, 0.017 mmol) was hydrogenated under 1 atm of H_2 in the presence of 20 mg of 5% Pt/C in 3 mL of EtOAc. After 24 h the reaction mixture was filtered through a pipet column of silica gel (EtOAc) and concentrated to afford 15 mg (100%) of **9** as a white film: $[\alpha]^{20}_D$ −62.9° (*c* 0.7, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.92−2.28 (m, 25 H), 1.05 (s, 3 H), 1.20 (s, 3 H), 1.38 (s, 9 H), 1.66 (s, 3 H), 1.83 (s, 3 H), 2.20 (s, 3 H), 2.28 (s, 3 H), 2.45 (d, *J* = 3.7 Hz, 1 H), 2.46−2.62 (m, 1 H), 3.26 (d, *J* = 5.6 Hz, 1 H), 3.62−3.69 (m, 2 H), 4.16 (d, *J* = 8.0 Hz, 1 H), 4.35−4.48 (m, 3 H), 4.67 (d, *J* = 10.0 Hz, 1 H), 4.96 (d, *J* = 8.3 Hz, 1 H), 5.42 (d, *J* = 6.9 Hz, 1 H), 6.13 (t, *J* = 8.7 Hz, 1 H), 6.24 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.8, 20.8, 21.8, 22.7, 25.2, 25.5, 25.7, 26.0, 26.2, 26.7, 28.2, 28.5, 29.4, 29.9, 30.1, 35.0, 35.6, 39.1, 43.2, 43.5, 45.4, 57.9, 58.6, 70.2, 72.1, 72.6, 74.4, 75.6, 76.6, 77.2, 79.0, 79.6, 80.8, 132.8, 142.6, 155.8, 170.1, 170.9, 171.3, 174.8, 177.2, 203.8; FAB-HRMS (NBA) *m/e* 862.4612 (MH^+, $C_{45}H_{68}NO_{15}$ requires 862.4589).

10-Acetyl-3′-dephenyl-2-debenzoyl-2-(3-methylbut-2-enoyl)-3′-(2-methylprop-1-enyl)docetaxel (10). To a mixture of 30 mg (0.044 mmol) of **4a** and 28 mg (0.066 mmol) of **5a** in 3 mL of THF was added 0.066 mL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition of 10 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 20 mL portions of EtOAc, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford 33 mg. A solution of this material (25 mg, 0.023 mmol) was dissolved in 1 mL of pyridine and 1 mL of CH_3CN, treated with 0.5 mL of HF/pyridine (70% wt solution) at 0 °C, and allowed to warm to room temperature for 3 h. The reaction was quenched with water, and the mixture was extracted with 2 × 25 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (hexane:EtOAc = 1:2) afforded 15 mg (50%, two-step yield) of **10** as a white film: $[\alpha]^{20}_D$ −33.3° (*c* 0.3, $CHCl_3$); IR ($CDCl_3$, cm^{-1}) 3426, 3018, 2975, 1636, 1336, 1214; ^1H NMR (250 MHz, $CDCl_3$) δ 1.11 (s, 3 H), 1.24 (s, 3 H), 1.40 (s, 9 H), 1.62 (s, 3 H), 1.73 (s, 3 H), 1.76 (s, 3 H), 1.86 (s, 3 H), 1.92 (m, 1 H), 1.96 (s, 3 H), 2.19 (s, 3 H), 2.22 (s, 3 H), 2.24 (s, 3 H), 2.33 (m, 2 H), 2.54 (m, 1 H), 3.69 (d, *J* = 6.7 Hz,1 H), 4.38 (dd, *J* = 10.5, 7.7 Hz, 1 H), 4.17 (d, *J* = 8.4 Hz, 1 H), 4.44 (d, *J* = 8.4 Hz, 1 H), 4.74 (m, 1H), 4.96 (d, *J* = 9.5 Hz, 1 H), 5.30 (d, *J* = 8.2 Hz, 1 H), 5.44 (d, *J* = 6.6 Hz, 1 H), 5.68 (bs, 1 H), 6.12 (t, *J* = 8.5 Hz, 1 H), 6.27 (bs, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.9, 18.6, 20.6, 20.8, 21.7, 22.4, 25.7, 26.6, 27.7, 28.2, 35.4, 35.6, 43.2, 45.7, 51.5, 58.6, 72.1, 72.5, 73.3, 73.5, 75.7, 76.9, 78.6, 79.9, 80.8, 84.5, 115.3, 120.5, 133.0, 137.1, 142.5, 155.4, 160.4, 167.1, 170.2, 171.2, 173.2, 203.9; FAB-HRMS (NBA-NaCl) *m/e* 828.380 (M^+ + Na, $C_{41}H_{59}NO_{15}Na$ requires 828.387).

10-Acetyl-2-debenzoyl-3′-dephenyl-3′-(2-methylprop-1-enyl)-2-(3-methylbutanoyl)docetaxel (11). To a mixture of 16 mg (0.023 mmol) of **4b** and 14 mg (0.035 mmol) of **5a** in 2 mL of THF was added 0.032 mL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition of 10 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 15 mL portions of Et_2O, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford a white solid. A solution of this material was dissolved in 1.5 mL of pyridine and 1.5 mL of CH_3CN, treated with 0.5 mL of HF/pyridine (70% wt solution) at 0 °C, and allowed to warm to room temperature for 3 h. The reaction was quenched with 10 mL of saturated $NaHCO_3$, and the mixture was extracted with 2 × 20 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (hexane:EtOAc = 1:2) afforded 10 mg (53%, two-step yield) of **11** as a white film: $[\alpha]^{20}_D$ −43.4° (*c* 0.01, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.94 (d, 6 H), 1.09 (s, 3 H), 1.24 (s, 3 H), 1.42 (s, 9 H), 1.63 (s, 3 H), 1.74 (s, 3 H), 1.77 (s, 3 H), 1.86−1.92 (m, 2 H), 2.04−2.34 (m, 2 H), 2.23 (s, 3 H), 2.24 (s, 3 H), 2.45 (bd, 1 H), 2.50−2.62 (m, 1 H), 3.31 (d, *J* = 7.0 Hz, 1 H), 3.69 (d, *J* = 6.6 Hz, 1 H), 4.17−4.21 (m, 2 H), 4.39−4.31 (m, 1 H), 4.48 (d, *J* = 8.2 Hz, 1 H), 4.70−4.75 (m, 2 H), 4.97 (d, *J* = 8.0 Hz, 1 H), 5.30 (bs, 1 H), 6.13 (t, *J* = 8.2 Hz, 1 H), 6.27 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.9, 18.6, 20.8, 21.6, 22.4, 22.5, 25.3, 25.7, 26.7, 28.3, 35.1, 35.5, 43.2, 43.8, 45.6, 51.6, 58.6, 72.3, 73.7, 74.3, 75.6, 77.2, 79.0, 80.0, 80.7, 120.5, 132.8, 138.0, 142.7, 155.3, 170.2, 170.8, 171.3, 173.6, 174.2, 203.8; FAB-HRMS (NBA) *m/e* 808.4088 (MH^+, $C_{41}H_{62}NO_{15}$ requires 808.4119).

10-Acetyl-2-debenzoyl-3′-dephenyl-2-(3-methylbut-2-enoyl)-3′-(2-methylpropyl)docetaxel (12). To a mixture of 37 mg (0.055 mmol) of **4a** and 33 mg (0.082 mmol) of **5b** in 2.5 mL of THF was added 0.07 mL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition of 10 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 20 mL portions of EtOAc, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford a white solid. A solution of this material was dissolved in 1.5 mL of pyridine and 1.5 mL of CH_3CN, treated with 0.5 mL of HF/pyridine (70% wt solution) at 0 °C, and allowed to warm to room temperature for 6.5 h. The reaction was quenched with 15 mL of water, and the mixture was extracted with 2 × 15 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (hexane:EtOAc = 1:2) afforded 30 mg (68%, two-step yield) of **12** as a white film: $[\alpha]^{20}_D$ −44.0° (*c* 0.75, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.92−0.96 (m, 6 H), 1.10 (s, 3 H), 1.22 (s, 3 H), 1.38 (s, 9 H), 1.62 (s, 3 H), 1.65−1.69 (m, 1 H), 1.80−1.86 (m, 1 H), 1.84 (s, 3 H), 1.96 (s, 3 H), 2.18 (s, 3 H), 2.22 (s, 3 H), 2.25 (s, 3 H), 2.32−2.60 (m, 3 H), 3.25 (bs, 1 H), 3.67 (d, *J* = 6.5 Hz, 1 H), 4.05−4.19 (m, 3 H), 4.36 (dd, *J* = 10.6, 6.6 Hz, 1 H), 4.43 (d, *J* = 8.4 Hz, 1 H), 4.64 (d, *J* = 9.6 Hz, 1 H), 4.95 (d, *J* = 8.0 Hz, 1 H), 5.43 (d, *J* = 6.6 Hz, 1 H), 5.70 (bs, 1 H), 6.11 (t, *J* = 8.6 Hz, 1 H), 6.26 (bs, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.8, 20.6, 20.8, 21.7, 22.0, 22.5, 23.2, 24.6, 26.6, 27.7, 28.2, 35.3, 35.6, 41.2, 43.2, 45.7, 51.4, 58.6, 72.1, 72.7, 73.3, 75.7, 76.8, 79.7, 80.9, 84.5, 115.4, 133.0, 142.5, 155.6, 160.3, 167.1, 170.0, 171.2, 174.0, 203.9; FAB-HRMS (NBA) *m/e* 808.4088 (MH^+, $C_{41}H_{62}NO_{15}$ requires 808.4119).

10-Acetyl-2-debenzoyl-3′-dephenyl-2-(3-methylbutanoyl)-3′-(2-methylpropyl)docetaxel (13). Compound **12** (17 mg, 0.021 mmol) was hydrogenated under 1 atm of H_2 in the presence of 20 mg of 10% Pd/C in 3 mL of EtOAc. After 24 h the reaction mixture was filtered through a pipet column of silica gel (EtOAc) and concentrated to afford 17 mg (100%) of **13** as a white film: $[\alpha]^{20}_D$ −46.0° (*c* 0.2, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.92−1.01 (m, 12 H), 1.08 (s, 3 H), 1.22 (s, 3 H), 1.39 (s, 9 H), 1.60−1.69 (m, 6 H), 1.86 (s, 3 H), 1.78−1.92 (m, 1 H), 2.14−2.36 (m, 4 H), 2.22 (s, 3 H), 2.25 (s, 3 H), 2.45−2.56 (m, 2 H), 3.67 (d, *J* = 6.7 Hz, 1 H), 4.07−4.21 (m, 4 H), 4.38 (bt, 1 H), 4.48 (d, *J* = 8.0 Hz, 1 H), 4.59 (d, *J* = 9.6 Hz, 1 H), 4.97 (d, *J* = 8.2 Hz, 1 H), 5.41 (d, *J* = 6.8 Hz, 1 H), 6.10 (t, *J* = 9.2 Hz, 1 H), 6.26 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.2, 14.9, 20.8, 21.6, 21.9, 22.5, 23.3, 24.7, 25.2, 26.7, 28.2, 34.9, 35.5, 41.2, 43.2, 43.8, 45.5, 51.4, 58.6, 72.1, 72.6, 73.0, 74.3, 75.6, 76.6, 78.9, 79.7, 80.8, 84.5, 132.8, 142.6, 155.6, 170.0, 171.3, 174.1, 203.8; FAB-HRMS (NBA) *m/e* 810.4322 (MH^+, $C_{41}H_{64}NO_{15}$ requires 810.4276).

10-Acetyl-2-(cyclohexylcarbonyl)-2-debenzoyl-3′-dephenyl-3′-(2-methylprop-1-enyl)docetaxel (14). To a mixture of 80 mg (0.114 mmol) of **4c** and 70 mg (1.71 mmol) of **5a** in 7 mL of THF was added 0.16 mL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition

of 15 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 25 mL portions of EtOAc, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford 97 mg (92%). A solution of this material (95 mg, 0.085 mmol) was dissolved in 3 mL of pyridine and 3 mL of CH_3CN, treated with 0.5 mL of HF/pyridine (70% wt solution) at 0 °C, and allowed to warm to room temperature for 3 h. The reaction was quenched with water, and the mixture was extracted with 2 × 25 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (hexane:EtOAc = 1:2) afforded 54 mg (78% and 72%, two-step yield) of **14** as a white film: $[\alpha]^{20}_D$ −40.0° (*c* 0.75, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3 H), 1.21 (s, 3 H), 1.25 (m, 2 H), 1.40 (s, 9 H), 1.62 (s, 3 H), 1.57−1.80 (m, 4 H), 1.62 (s, 3 H), 1.71 (s, 3 H), 1.85 (s, 3 H), 2.18−2.28 (m, 1 H), 2.21 (s, 3 H), 2.23 (s, 3 H), 2.53 (m, 2 H), 3.43 (d, *J* = 6.8 Hz, 1 H), 3.65 (d, *J* = 7.0 Hz, 1 H), 4.15 (d, *J* = 8.0 Hz, 1 H), 4.45 (d, *J* = 8.0 Hz, 1 H), 4.36 (m, 1 H), 4.70 (m, 1 H), 4.81 (d, *J* = 8.5 Hz, 1 H), 4.95 (d, *J* = 8.2 Hz, 1 H), 5.27 (d, *J* = 8.5 Hz, 1 H), 5.40 (d, *J* = 7.0 Hz, 1 H), 6.10 (t, *J* = 8.6 Hz, 1 H), 6.24 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.4, 14.9, 18.5, 20.8, 21.7, 22.3, 25.1, 25.5, 25.7, 26.6, 28.2, 28.4, 29.3, 35.1, 35.5, 43.1, 43.5, 45.6, 51.6, 58.5, 72.1, 73.7, 74.4, 75.6, 79.1, 79.9, 80.7, 84.5, 120.5, 132.7, 137.9, 142.6, 155.4, 170.1, 171.2, 173.0, 177.0, 203.8; FAB-HRMS (NBA) *m*/*e* 834.4275 (MH^+, $C_{44}H_{63}NO_{15}$ requires 834.4265).

10-Acetyl-2-(cyclohexylcarbonyl)-2-debenzoyl-3′-dephenyl-3′-(2-methylpropyl)docetaxel (15). Compound **14** (16 mg, 0.019 mmol) was hydrogenated under 1 atm of H_2 in the presence of 20 mg of 10% Pd/C in 2 mL of EtOAc. After 24 h the reaction mixture was filtered through a pipet column of silica gel (EtOAc) and concentrated to afford 16 mg (100%) of **15** as a white film: $[\alpha]^{20}_D$ −43.2° (*c* 0.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3 H), 1.19 (s, 3 H), 1.37 (s, 9 H), 1.24−1.26 (m, 2 H), 1.61 (s, 3 H), 1.65 (s, 3 H), 1.52−1.86 (m, 10 H), 2.03−2.24 (m, 4 H), 2.21 (s, 3 H), 2.24 (s, 3 H), 2.49−2.53 (m, 2 H), 3.24 (d, *J* = 6.1 Hz, 1 H), 3.65 (d, *J* = 6.9 Hz, 1 H), 4.12−4.17 (m, 4 H), 4.36 (m, 1 H), 4.46 (d, *J* = 8.2 Hz, 1 H), 5.41 (d, *J* = 6.9 Hz, 1 H), 6.11 (t, *J* = 8.6 Hz, 1 H), 6.24 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.9, 20.8, 21.8, 22.0, 22.4, 23.0, 23.4, 24.7, 24.9, 25.2, 25.5, 25.7, 28.0, 29.3, 35.1, 35.5, 41.3, 43.1, 43.6, 45.5, 51.4, 58.6, 72.1, 72.5, 73.1, 74.5, 75.6, 79.2, 79.7, 80.9, 84.5, 132.8, 142.5, 155.5, 170.0, 171.2, 173.9, 177.0, 203.8; FAB-HRMS (NBA) *m*/*e* 836.4432 (MH^+, $C_{43}H_{66}NO_{15}$ requires 836.4472).

10-Acetyl-2-(cyclohexylcarbonyl)-2-debenzoyl-3′-dephenyl-3′-((*E*)-prop-1-enyl)docetaxel (16). To a mixture of 20 mg (0.030 mmol) of **4c** and 18 mg (0.045 mmol) of **5d** in 3 mL of THF was added 45 μL of LiHMDS (1.0 M in THF) at −40 °C. After 20 min the reaction was quenched by addition of 10 mL of aqueous NH_4Cl. The aqueous layer was extracted with 2 × 20 mL portions of EtOAc, dried over $MgSO_4$, filtered, and concentrated. The crude residue was subjected to silica gel chromatography (hexane:EtOAc = 1:1) to afford a white solid. A solution of this material was dissolved in 2 mL of pyridine and 3 mL of CH_3CN, treated with 0.5 mL of HF/pyridine (70% wt solution) at 0 °C, and allowed to warm to room temperature for 3 h. The reaction was quenched with 10 mL of 1 N HCl, and the mixture was extracted with 2 × 15 mL of EtOAc, dried over $MgSO_4$, filtered, and concentrated. Chromatography on silica gel (hexane:EtOAc = 1:2) afforded 17 mg (71%, two-step yield) of **16** as a white film: $[\alpha]^{20}_D$ −48.2° (*c* 0.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3 H), 1.20 (s, 3 H), 1.26 (m, 2 H), 1.40 (s, 9 H), 1.61 (s, 3 H), 1.56−1.80 (m, 4 H), 1.71 (s, 3 H), 1.74 (s, 3 H), 1.84 (s, 3 H), 2.17−2.23 (m, 1 H), 2.21 (s, 3 H), 2.26 (s, 3 H), 2.53 (m, 2 H), 3.65 (d, *J* = 7.0 Hz, 1 H), 4.15 (d, *J* = 7.9 Hz, 1 H), 4.46 (d, *J* = 7.9 Hz, 1 H), 4.36 (dd, *J* = 10.6, 6.8 Hz, 1 H), 4.53 (m, 1 H), 4.92 (m, 2 H), 5.27 (d, *J* = 8.5 Hz, 1 H), 5.42 (d, *J* = 6.8 Hz, 1 H), 5.51 (dd, *J* = 17.5, 4.9 Hz, 1 H), 5.71 (m, 1 H), 6.15 (t, *J* = 8.4 Hz, 1 H), 6.25 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.9, 17.8, 20.8, 21.6, 22.5, 25.1, 25.5, 25.6, 26.7, 28.0, 28.2, 28.4, 29.3, 34.9, 35.5, 43.1, 43.5, 45.5, 54.7, 58.6, 72.1, 73.1, 74.3, 75.6, 79.0, 80.0, 80.8, 84.5, 122.0, 127.3, 128.9, 132.9, 142.4, 155.3, 170.2, 171.3, 173.0, 177.2, 203.8; FAB-HRMS (NBA-NaCl) *m*/*e* 820.4120 (M^+ + Na, $C_{42}H_{62}NO_{15}$ requires 820.4154).

Cytotoxicity Assay *in Vitro*.[29] Tumor cell growth inhibition was dertermined according to the method established by Skehan et al.[30] Human tumor cells (A121 ovarian carcinoma, HT-29 colon carcinoma, A549 non-small-cell lung carcinoma, and MCF7 breast carcinoma) were plated at a density of 400 cells/well in 96-well plates and allowed to attach overnight. These cell lines were maintained in RPMI-1640 medium (Roswell Park Memmorial Institute growth medium) supplemented with 5% fetal bovine serum and 5% Nu serum (Collaborative Biomedical Product, MA). Taxanes were solubilized in DMSO and further diluted with RPMI-1640 medium. Triplicate wells were exposed to various treatments. After 72 h incubation, 100 μL of ice-cold 50% trichloroacetic acid (TCA) was added to each well, and the samples were incubated for 1 h at 4 °C. Plates were then washed five times with water to remove TCA and serum proteins, and 50 μL of 0.4% sulforhodamine B (SRB) was added to each well. Following a 5 min incubation, plates were rinsed five times with 0.1% acetic acid and air-dried. The dye was then solubilized with 10 mM Tris base (pH 10.5) for 5 min on a gyratory shaker. Optical density was measured at 570 nm. The IC_{50} values were then calculated by fitting the concentration−effect curve data with the sigmoid-E_{max} model using nonlinear regression, weighted by the reciprocal of the square of the predicted effect.[31]

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References

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. Plant Antitumor Agents. VI. The Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor Agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* **1971**, *93*, 2325−2327.

(2) Guéritte-Voegelein, F.; Mangatal, L.; Guénard, D.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. Structure of a Synthetic Taxol Precursor: *N-tert*-Butoxycarbonyl-10-deacetyl-*N*-debenzoyltaxol. *Acta Crystallogr.* **1990**, *C46*, 781−784.

(3) Shiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of Microtubule Assembly *in vitro* by Taxol. *Nature* **1979**, *277*, 665−667.

(4) Manfredi, J. J.; Horwitz, S. B. Taxol: an Antimitotic Agent with a New Mechanism of Action. *Pharmacol. Ther.* **1984**, *25*, 83−125.

(5) Ringel, I.; Horwitz, S. B. Studies with RP 56976 (Taxotère): A Semisynthetic Analogue of Taxol. *J. Natl. Cancer Inst.* **1991**, *83*, 288−291.

(6) Verweij, J.; Clavel, M.; Chevalier, B. Paclitaxel (Taxol) and Docetaxel (Taxotere): Not Simply Two of a Kind. *Ann. Oncol.* **1994**, *5*, 495−505.

(7) Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. Unexpectedly Facile Hydrolysis of the 2-Benzoate Group of Taxol and Syntheses of Analogs with Increased Activities. *J. Am. Chem. Soc.* **1994**, *116*, 4097−4098.

(8) Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. The Medicinal Chemistry of Taxol. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC Press: New York, 1995; pp 317−375.

(9) Georg, G. I.; Harriman, G. C. B.; Ali, S. M.; Datta, A.; Hepperle, M.; Himes, R. H. Synthesis of 2-*O*-Heteroaroyl Taxanes: Evaluation of Microtubule Assembly Promotion and Cytotoxicity. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 115−118.

(10) Nicolaou, K. C.; Renaud, J.; Nantermet, P. G.; Couladouros, E. A.; Guy, R. K.; Wrasidlo, W. Chemical Synthesis and Biological Evaluation of C-2 Taxoids. *J. Am. Chem. Soc.* **1995**, *117*, 2409−2420.

(11) Ojima, I.; Duclos, O.; Zucco, M.; Bissery, M.-C.; Combeau, C.; Vrignaud, P.; Riou, J. F.; Lavelle, F. Synthesis and Structure-Activity Relationships of New Antitumor Taxoids. Effects of Cyclohexyl Substitution at the C-3′ and/or C-2 of Taxotère (Docetaxel). *J. Med. Chem.* **1994**, *37*, 2602−2608.

(12) Boge, T. C.; Himes, R. H.; Vander Velde, D. G.; Georg, G. I. The Effect of the Aromatic Rings of Taxol on Biological Activity and Solution Conformation: Synthesis and Evaluation of Saturated Taxol and Taxotère Analogues. *J. Med. Chem.* **1994**, *37*, 3337−3343.

(13) Chen, S.-H.; Farina, V.; Wei, J.-M.; Long, B.; Fairchild, C.; Mamber, S. W.; Kadow, J. F.; Vyas, D.; Doyle, T. W. Structure-Activity Relationships of Taxol®: Synthesis and Biological Evaluation of C2 Taxol Analogs. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 479−482.
(14) Ojima, I.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Slater, J. C.; Lavelle, F.; Veith, J. M.; Bernacki, R. J. Synthesis and Biological Activity of 3′-Alkyl- and 3′-Alkenyl-3′-Dephenyldocetaxels. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2631−2634.
(15) Ojima, I.; Park, Y. H.; Fenoglio, I.; Duclos, O.; Sun, C.-M.; Kuduk, S. D.; Zucco, M.; Appendino, G.; Pera, P.; Veith, J. M.; Bernacki, R. J.; Bissery, M.-C.; Combeau, C.; Vrignaud, P.; Riou, J. F.; Lavelle, F. Syntheses and Structure-Activity Relationships of New Taxoids. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series 583; American Chemical Society: Washington, DC, 1995; pp 262−275.
(16) Ali, S. M.; Hoemann, M. Z.; Aubé, J.; Mitscher, L. A.; Georg, G. I.; McCall, R.; Jayasinghe, L. R. Novel Cytotoxic 3′-(*tert*-Butyl) 3′-Dephenyl Analogs of Paclitaxel and Docetaxel. *J. Med. Chem.* **1995**, *38*, 3821−3828.
(17) Li, L.; Thomas, S. A.; Klein, L. L.; Yeung, C. M.; Maring, C. J.; Grampovnik, D. J.; Lartey, P. A.; Plattner, J. J. Synthesis and Biological Evaluation of C-3′-Modified Analogs of 9(R)-Dihydrotaxol. *J. Med. Chem.* **1994**, *37*, 2655−2663.
(18) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J.; Pera, P.; Bernacki, R. J. Syntheses and Structure-Activity Relationships of the Second Generation Antitumor Taxoids. Exceptional Activity against Drug-Resistant Cancer Cells. *J. Med. Chem.* **1996**, *39*, 3889−3896.
(19) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C.-M.; Brigaud, T. New and Efficient Approaches to the Semisynthesis of Taxol and Its C-13 Side Chain Analogs by Means of β-Lactam Synthon Method. *Tetrahedron* **1992**, *48*, 6985−7012.
(20) Ojima, I.; Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. D. A Highly Efficient Route to Taxotère by the β-Lactam Synthon Method. *Tetrahedron Lett.* **1993**, *34*, 4149−4152.
(21) Ojima, I.; Zucco, M.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Park, Y. H. *N*-Acyl-3-hydroxy-β-lactams as Key Intermediates for Taxotère and Its Analogs. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2479−2482.
(22) Holton, R. A.; Biediger, R. J.; Boatman, P. D. Semisynthesis of Taxol and Taxotere. In *Taxol: Science and Applications*; Suffness, M., Ed.; CRC Press: New York, 1995; pp 97−121.
(23) Ojima, I. β-Lactam Synthon Method - Enantiomerically Pure β-Lactams as Synthetic Intermediates. In *The Organic Chemistry of β-Lactam Antibiotics*; Georg, G. I., Ed.; VCH Publishers: New York, 1992; pp 197−255.
(24) Ojima, I. Recent Advances in β-Lactam Synthon Method. *Acc. Chem. Res.* **1995**, *28*, 383−389.
(25) Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. "Hydrophobic Collapse" of Taxol and Taxotere Solution Conformations in Mixtures of Water and Organic Solvent. *J. Am. Chem. Soc.* **1993**, *115*, 11650−11651.
(26) Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heerding, J. M.; Krauss, N. E. NMR and Molecular Modeling Study of the Conformations of Taxol and of its Side Chain Methylester in Aqueous and Non-Aqueous Solution. *Tetrahedron* **1993**, *49*, 6545−6560.
(27) (a) Paloma, L. G.; Guy, R. K.; Wrasidlo, W.; Nicolaou, K. C. Conformation of a Water-Soluble Derivative of Taxol in Water by 2D-NMR Spectroscopy. *Chem. Biol.* **1994**, *2*, 107−112. (b) Ojima, I.; Kuduk, S. D.; Slater, J. C.; Gimi, R. H.; Sun, C. M.; Chakravarty, S.; Ourevitch, M.; Abouabdellah, A.; Bonnet-Delpon, D.; Bégué, J.-P.; Veith, J. M.; Pera, P.; Bernacki, R. J. Synthesis, Biological Activity, and Conformational Analysis of Fluorine-Containing Taxoids. In *Biomedical Frontiers of Fluorine Chemistry*; Ojima, I., McCarthy, J. R., Welch, J. T., Eds.; ACS Symposium Series 639; American Chemical Society: Washington, DC, 1996; Chapter 17, pp 228−243.
(28) Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. A Highly Efficient, Practical Approach to Natural Taxol. *J. Am. Chem. Soc.* **1988**, *110*, 5917−5919.
(29) Sharma, A.; Straubinger, R. M.; Ojima, I.; Bernacki, R. J. Antitumor Efficacy of Taxane Liposomes on a Human Ovarian Tumor Xenograft in Nude Athymic Mice. *J. Pharm. Sci.* **1995**, *84*, 1400−1404.
(30) Skehan, P.; Streng, R.; Scudierok, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(31) Motulsky, H. J.; Ransnas, L. A. Fitting Curves to Data Using Nonlinear Regression: A Practical and Nonmathematical Review. *FASEB J.* **1987**, *1*, 365−374.

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