Synthesis and Structure–Activity Relationships of New Antitumor Taxoids. Effects of Cyclohexyl Substitution at the C-3′ and/or C-2 of Taxotere (Docetaxel)

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Synthesis and cytotoxicity of the new analogs (**11**–**13**) of docetaxel possessing cyclohexyl groups instead of phenyl groups at the C-3′ and/or C-2 benzoate positions are described. The C-2 cyclohexanecarboxylate analog of paclitaxel (**15**) is also synthesized for comparison. The potency of these new taxoids were examined for their inhibitory activity for microtubule disassembly and also for their cytotoxicity against murine P388 leukemia cell line as well as doxorubicin-resistant P388 leukemia cell line (P388/Dox). It is found that 3′-dephenyl-3′-cyclohexyldocetaxel (**11**) (0.72*T*) and 2-(hexahydro)docetaxel (**12**) (0.85*T*) possess strong inhibitory activity for microtubule disassembly equivalent to docetaxel (0.7*T*), which is more potent than paclitaxel (1.0*T*). The results clearly indicate that phenyl or an aromatic group at C-3′ or C-2 is not a requisite for strong binding to the microtubules. This finding has opened an avenue for development of new nonaromatic analogs of docetaxel and paclitaxel. 3′-Dephenyl-3′-cyclohexyl-2-(hexahydro)docetaxel (**13**) (2*T*) turns out to be a substantially weaker inhibitor. The cytotoxicities of **11**–**13** against P388 are, however, in the same range that is 8–12 times weaker than docetaxel and 4–6 times weaker than paclitaxel, i.e., **13** shows equivalent cytotoxicity to that of **11** or **12** in spite of much lower microtubule disassembly inhibitory activity. The cytotoxicities of these new taxoids against the P388/Dox cell line are only 2–2.5 times lower than that of docetaxel. The potency of 2-(hexahydro)paclitaxel (**15**) for these assays is much lower than the docetaxel counterpart **12**. The significant loss of activity *in vivo* against B16 melanoma is observed for **11**–**13**, i.e., **11** is only marginal (T/C = 38% at 20 mg/kg/day), and **12** and **13** are inactive (T/C = 76% and 79%, respectively). This could be ascribed to faster metabolism, faster excretion or other bioavailability problems.

Introduction

Taxol (paclitaxel), a complex diterpene isolated from the bark of *Taxus brevifolia*, is currently considered one of the most exciting leads in cancer chemotherapy.[2–4] In the late 1992, paclitaxel was approved by FDA in the United States for the treatment of advanced ovarian cancer. Moreover, the results of ongoing clinical trials for the treatment of breast and lung cancers are also very encouraging.[5,6] Taxotere (docetaxel), a promising anticancer agent, is the first semisynthetic taxoid prepared so far that exhibits a stronger antitumor activity than paclitaxel for *in vitro* and *in vivo* experimental models.[7–10] Docetaxel is currently in phase II and III clinical trials for different types of cancers in the United States, Europe and Japan.[11,12] Paclitaxel and docetaxel belong to a spindle poison family and both act as potent mitosis inhibitors with a unique mechanism of action, i.e., by promoting the assembly of tubulin to form stable microtubules and by inhibiting the disassembly of microtubules to tubulins.[13,14]

Studies on the structure–activity relationships (SAR) of paclitaxel and docetaxel have disclosed the essential role of the *N*-acylphenylisoserine moiety at C-13 and the benzoate at C-2 as well as the oxetane ring for their strong antitumor activity.[8,15–17] However, little has been reported on the modification at the C-3′ phenyl and the C-2 benzoate,[18] which would be of significance for the SAR study of antitumor taxoids. We will describe here the synthesis and cytotoxicity of the new analogs of docetaxel possessing cyclohexyl groups instead of phenyl groups at the C-3′ and/or C-2 benzoate positions. The C-2 cyclohexanecarboxylate analog of paclitaxel is also synthesized and assayed for comparison.

Syntheses of Cyclohexyl Analogs of Docetaxel and Paclitaxel

The β-lactam synthon method[19] has proven to be a powerful protocol for the semisynthesis of paclitaxel,[20a,b,23] docetaxel,[21,22] and their analogs[20c,22] through an efficient coupling with baccatin III derivatives. For these syntheses, (2*R*,3*S*)-*N*-acyl-β-lactam (**1a** or **1b**) serves as the direct precursor of the C-13 side chain.[20–23] The β-lactam **1** is readily obtained from enantiomerically pure (3*R*,4*S*)-3-hydroxy-4-phenylazetidin-2-one (**2**), which is prepared through an efficient and highly stereoselective lithium chiral ester enolate–imine cyclocondensation, *via* protection of 3-hydroxyl followed by *N*-*tert*-butoxycarbonylation or *N*-benzoylation.[20a,c,21]

To introduce a cyclohexyl in place of the phenyl group at the C-3′ of docetaxel, the β-lactam **2** was hydrogenated on 5% Rh on carbon at 80 °C and 800 psi of hydrogen to give enantiomerically pure (3*R*,4*S*)-3-hydroxy-4-cyclohexyl-β-lactam (**3**) in 85% yield (Scheme **1**). Protection of the 3-hydroxyl of **3** as ethoxyethyl (EE) ether (100% yield) followed by *N*-acylation with di-*tert*-butyl dicarbonate in dichloromethane in the presence

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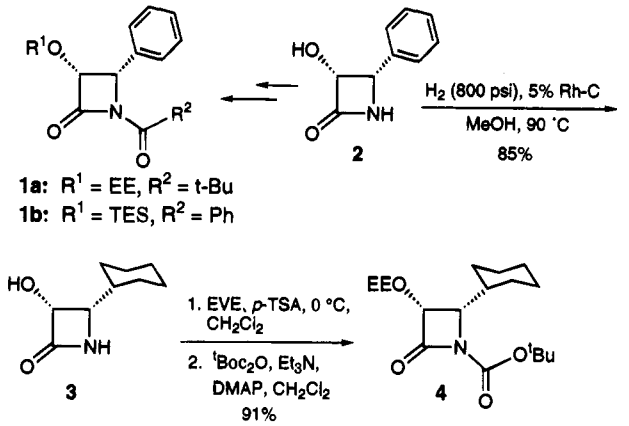
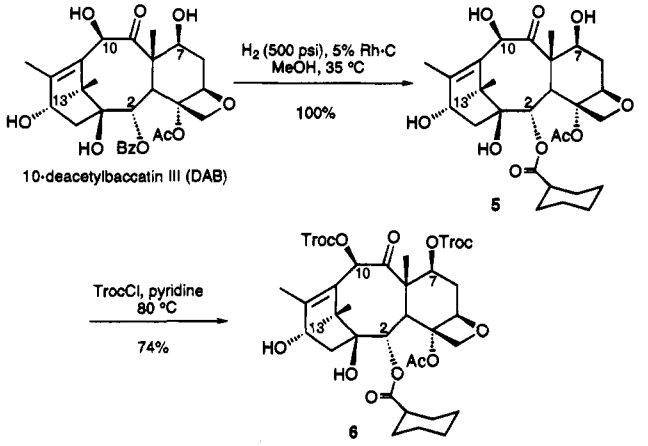
Scheme 1



Scheme 2



of triethylamine and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) gave enantiomerically pure *N*-(*tert*-butoxycarbonyl)-β-lactam (**4**) in 91% yield (Scheme 1).

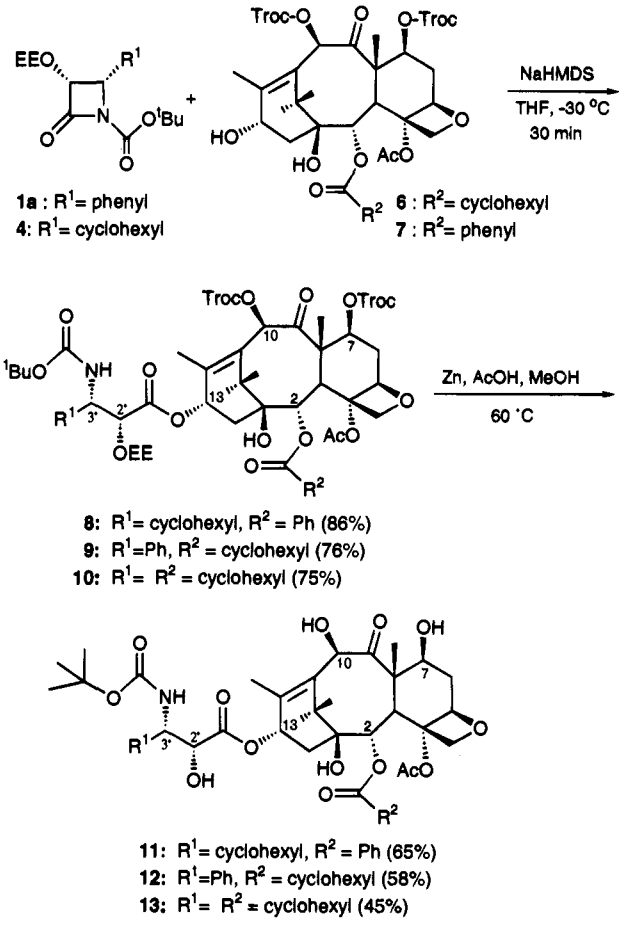
For the substitution of the 2-benzoate of docetaxel with 2-cyclohexanecarboxylate, we prepared the 2-cyclohexyl derivative (**5**) of 10-deacetylbaccatin III (DAB) through hydrogenation of DAB over 5% Rh on carbon at 35 °C and 500 psi of hydrogen, which gave **5** in quantitative yield.[24] It is noteworthy that the Δ^{11} double bond remains intact under these conditions. Selective protection of the hydroxyl groups of **5** at C-7 and C-10 as 2,2,2-trichloroethoxycarbonate (Troc) afforded 7,10-Ditroc-2-hexahydro-DAB (**6**) in 74% yield (Scheme 2).

3′-Dephenyl-3′-cyclohexyldocetaxel (**11**) was synthesized through an efficient coupling of **4a** with 7,10-Ditroc-DAB[25] based on our protocol using NaHMDS as the base,[20] giving **8** in 86% yield, followed by deprotection under the Commerçon conditions,[26] i.e., Zn, AcOH, MeOH at 60 °C (65% yield) (Scheme 3).

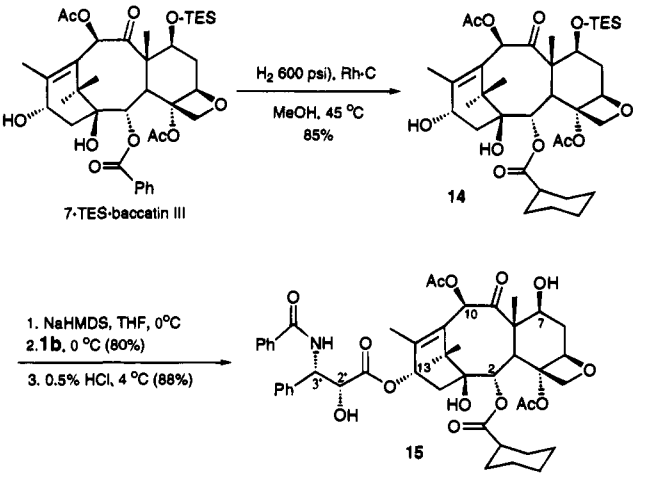
In a similar manner, 2-(hexahydro)docetaxel (**12**) and 3′-dephenyl-3′-cyclohexyl-2-(hexahydro)docetaxel (**13**) were synthesized through couplings of **1a** with **6** and **4a** with **6**, respectively, in moderate to fairly good yields (Scheme 3). These yields are not optimized.

2-(Hexahydro)paclitaxel (**15**) was also synthesized through coupling of **1b** with 7-TES-2-(hexahydro)baccatin III (**14**), which was obtained by hydrogenation of 7-TES-baccatin III over 5% Rh on carbon at 45 °C and 600 psi of hydrogen in methanol (85%), followed by

Scheme 3



Scheme 4



deprotection under the modified Grenoble–Gif conditions,[27] i.e, 0.5% HCl in ethanol at 4 °C for 48 h (Scheme 4).

Microtubule Disassembly Inhibitory Activity and Cytotoxicity of the Cyclohexyl Analogs

Biological activities of the cyclohexyl analogs of docetaxel **11**–**13** and 2-(hexahydro)paclitaxel (**15**) were evaluated in three assay systems, i.e., inhibition of microtubule disassembly[28] and cytotoxicity against murine P388 leukemia cell lines as well as doxorubicin resistant P388 leukemia (P388/Dox) cell line[29] (see the Experimental Section). The results are summarized in Table 1.

Table 1. Microtubule Disassembly Inhibitory Activity and *in Vitro* Cytotoxicity of the Cyclohexyl Analogs

taxoid	microtubule disassembly inhibitory activity[a] IC_{50}/IC_{50}(paclitaxel)	cytotoxicity against P388 cell line[b] (μg/mL)	cytotoxicity against P388/Dox[c] (μg/mL)
docetaxel	0.70*T*	0.008	1.5
11	0.72*T*	0.063	3.1
12	0.85*T*	0.090	6.3
13	2.0*T*	0.076	3.6
15	1.7*T*	0.45	7.5

[a] IC_{50} represents the concentration of an agent leading to 50% inhibition of the rate of microtubule disassembly. IC_{50}(paclitaxel) is the IC_{50} value of paclitaxel in the same assay. In the same assay, the IC_{50} of paclitaxel is 0.015 μM. [b] IC_{50} represents the concentration that inhibits 50% of cell proliferation. [c] P388/Dox = doxorubicin-resistant murine leukemia P388 cell line.

As Table 1 shows, the introduction of one cyclohexyl moiety to docetaxel virtually does not affect the tubulin binding ability, i.e., **11** (1.0Tt) and **12** (1.17Tt) (Tt = IC_{50} of docetaxel). Cytotoxicities of these analogs, however, appear to be more sensitive to the modification, i.e., **11** and **12** show ca. 8-fold and 12-fold weaker activities than docetaxel against P388 cell line, respectively. It is worthy of note that **11** is only 2 times less active than docetaxel against P388/Dox cell line. On the other hand, the substitution of two phenyl groups at the C-3′ and the C-2 benzoate positions with two cyclohexyl groups (**13**) results in a substantial loss of activity in the microtubule disassembly inhibition (2.86Tt), but cytotoxicity (9.5Tt, P388; 2.4Tt, P388/Dox) is at the same level as those of **11** and **12**. 2-(Hexahydro)paclitaxel (**15**) shows much weaker activity than 2-(hexahydro)docetaxel (**12**) in the same assays.

Although extensive conformational analyses of paclitaxel and docetaxel have been performed by NMR spectroscopy and molecular modeling studies,[30] the binding conformations of these drugs are still to be determined. Nevertheless, it has been believed that phenyl groups at the C-3′ and C-2 positions play an important role in the microtubules-binding process through their hydrophobic interactions with microtubules.[30] In this context, it is worthy of note that **11**–**13** and **15** are the first active paclitaxel/docetaxel analogs reported to date without such a phenyl moiety at C-3′ and/or C-2.[31] *The results reported here clearly indicate that phenyl or an aromatic group at C-3′ or C-2 is not a requisite for strong binding to the tubulin receptor.*

As mentioned above, the bis-cyclohexyl analog **13** exhibits unexpectedly weaker inhibitory activity for tubulin disassembly than mono-cyclohexyl analogs **11** and **12** in that the observed activity is much lower than that anticipated based on additivity of substituent effect. This fact may imply that the two cyclohexyl moieties of **13** are spatially in close proximity to render an undesired change in the binding conformation, although *in vitro* cytotoxicity of **13** does not appear to reflect this change. Our molecular modeling study on these cyclohexyl analogs using the SYBYL 6.0 program on the basis of the "hydrophobic collaps" conformation in aqueous media proposed by Vander Velde et al.[30c] strongly supports this implication. Figure 1 shows the Chem 3D representations of hypothetical comformations to be recognized by tubulin receptor for **11**–**13** together with that of docetaxel.

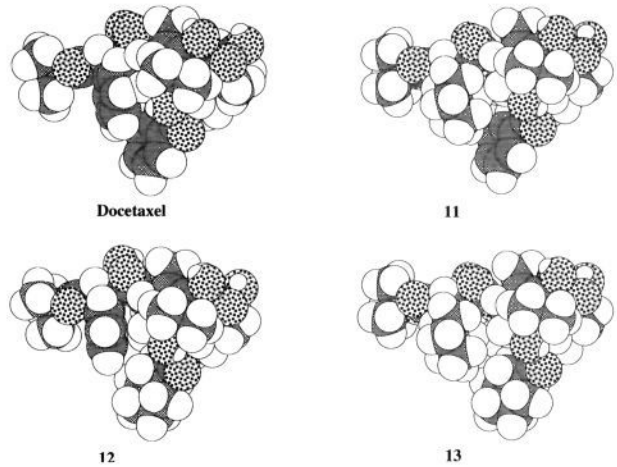


Figure 1. Chem 3D representations of docetaxel and new taxoids **11**–**13**.

Antitumor Activity of the Cyclohexyl Analogs *in Vivo*

The *in vivo* antitumor activity of **11**, **12**, and **13** were evaluated against B16 melanoma in $B6D2F_1$ mice (see the Experimental Section).[32] The antitumor activity is expressed by the T/C (%) value in which *T* is the median tumor weight of tolerated mice and *C* is the median tumor weight of control mice; Measurements were made when *C* is approximately equal to 1 g. Results are as follows: **11**, T/C = 38% (20 mg/kg/day); **12**, T/C = 76% (32.2 mg/kg/day); **13**, T/C = 79% (12.4 mg/kg/day). The results clearly indicate that **11** is only marginally active at the maximal *tested* dose, but **12** (at the maximal *tested* dose) and **13** (at the maximal *tolerated* dose) are inactive *in vivo*. Under the same conditions, docetaxel, showed excellent activity, i.e., T/C = 0% at 20 mg/kg/day. The observed significant loss of activity *in vivo* could be ascribed to faster metabolism, e.g., oxidations by P450, faster excretion, or other bioavailability problems, but it is apparent that further investigation is necessary to deduce any conclusion.

Conclusion

It is found that phenyl or an aromatic group at C-3′ or C-2 is not a requisite for strong binding to the microtubules. This significant finding has opened an avenue for development of new nonaromatic analogs of docetaxel and paclitaxel, which may have better activity than the parent drugs. In fact, some 3′-alkyl and 3′-alkenyl analogs of docetaxel have been found to be more cytotoxic than docetaxel and paclitaxel, which possess equivalent *in vivo* antitumor activity to docetaxel, i.e., better than paclitaxel. Results will be reported elsewhere.

Further SAR studies on new taxoids bearing nonaromatic substituents at the C-3′ position as well as at the C-2 benzoate position are actively underway in these laboratories.

Experimental Section

General Methods. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 1600 series spectrophotometer. ^1H, ^{13}C, and 2D NMR spectra were measured with a Bruker AC 250 or a General Electric QE-300 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).

Materials. 10-Deacetylbaccatin III was obtained from Rhône-Poulenc Rorer. 7-(Triethylsilyl)baccatin III and 7,10-bis(trichloroacetyl)-10-deacetylbaccatin III were either prepared by literature methods[20a,33] or obtained from Rhône-Poulenc Rorer. (3*R*,4*S*)-4-Phenyl-3-hydroxy-2-azetidinone was prepared by the method developed in these laboratories.[20a] Phosphocellulose P11 was obtained from Whatman Co. Paclitaxel and docetaxel were obtained from the National Cancer Institute and Rhône-Poulenc Rorer, respectively.

Cell Lines and Culture Conditions. Murine leukemia P388 cell line and a doxorubicin-resistant P388 cell line (P388/Dox) were obtained from the tumor bank of the National Cancer Institute. These cell lines were grown in RPMI 1640 medium containing 2-mercaptoethanol (10 μM), L-glutamine (2 mM), penicillin (200 units/mL), and streptomycin (200 μg/mL) and supplemented with 10% (v/v) foetal calf serum. Doxorubicin (1 μg/mL) was added to the medium of P388/Dox.

Mice and Tumor Model. The murine tumor used for *in vivo* evaluation, B16 melanoma is currently passaged in our laboratory and is maintained in the mouse strain of origin C57BL/6. It was transplanted into B6D2F$_1$ mice for *in vivo* assay. Mice were bred at IFFA CREDO (L'Abresle, Lyon, France) from strains obtained from The Jackson Laboratory, Bar Harbor, ME. Mice were over 18 g at the start of the *in vivo* assay. They were supplied food and water *ad libitum*.

Preparation of Pure Tubulin. Porcine brain tubulin was prepared by three cycles of polymerization–depolymerization[34] followed by chromatography on phosphocellulose P11.[35] The eluted tubulin, depleted of microtubule associated proteins (Maps), was concentrated by ultrafiltration, adjusted to 0.05 M 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.8, 0.25 mM $MgCl_2$, 0.5 mM ethylenebis(oxyethylenenitrilo)-*N,N,N',N'*-tetraacetic acid (EGTA), 3.4 M glycerol, and 0.2 mM guanosine 5'-triphosphate (GTP). The tubulin, thus prepared, was stored at −80 °C at a concentration of 5–10 mg/mL.

(3*R*,4*S*)-4-Cyclohexyl-3-hydroxy-2-azetidinone (3). A solution of (3*R*,4*S*)-4-phenyl-3-hydroxy-2-azetidinone (**2**, 500 mg, 3.06 mmol) in methanol (10 mL) was stirred under an atmosphere of hydrogen (800 psi) at 90 °C in the presence of 5% rhodium on charcoal (15 mg). After 5 days, the hydrogen pressure was released, and the suspension was filtered through a pad of Celite to remove the catalyst. The filtrate was concentrated under reduced pressure, and the resulting solid was recrystallized in ethyl acetate to give 440 mg (85%) of **3** as a white solid: mp 140–140.5 °C; $[\alpha]^{20}_D$ +65.1° (*c* 0.66, CH_3OH); ^{1}H NMR (250 MHz, CD_3OD) δ 0.75–1.10 (m, 2 H), 1.12–1.35 (m, 3 H), 1.40–2.00 (m, 6 H), 3.28 (dd, *J* = 9.7, 4.6 Hz, 1 H), 4.81 (d, *J* = 4.6 Hz, 1 H); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.75–1.00 (m, 2 H), 1.10–1.35 (m, 3 H), 1.37–1.55 (m, 1 H), 1.58–1.85 (m, 5 H), 3.10 (dd, *J* = 9.6, 4.7 Hz, 1 H), 4.67 (m, 1 H), 5.87 (d, *J* = 7.8 Hz, 1 H), 8.21 (bs, 1 H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 25.1, 25.4, 26.1, 28.8, 29.2, 37.5, 59.0, 76.4, 170.2; IR (KBr) 3312, 3219, 2928, 1726 cm^{-1}. Anal. Calcd for $C_9H_{15}NO_2$: C, 63.88; H, 8.93; N, 8.28. Found: C, 63.70; H, 9.00; N, 8.06.

(3*R*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-cyclohexyl-3-(1-ethoxyethoxy)-2-azetidinone (4). To a solution of 3-hydroxy-4-cyclohexyl-2-azetidinone **3** (320 mg, 1.9 mmol) and *p*-toluenesulfonic acid (TsOH, 10 mg) in 20 mL of THF at 0 °C was added 375 μL (3.9 mmol) of ethyl vinyl ether. After 2 h at 0 °C, the reaction mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to yield 460 mg (100%) of 3-(1-ethoxyethoxy)-4-cyclohexyl-2-azetidinone as a white solid: mp 87-89 °C; $[\alpha]^{20}_D$ + 83° (*c* 0.76, $CHCl_3$); ^{1}H NMR δ (250 MHz, $CDCl_3$) 0.84 (m, 2 H), 1.07–1.34 (m, 9 H), 1.66 (m, 6 H), 3.32 (m, 1 H), [3.42 (q, *J* = 7.7 Hz), 3.54 (q, *J* = 7.7 Hz), 3.65 (q, *J* = 7.7 Hz), 3.74 (q, *J* = 7.7 Hz), 2 H], 4.81 (m, 1H), [4.80 (m), 4.90 (q, *J* = 5.2 Hz), 1 H], 6.92 (bs, 1 H); IR ($CHCl_3$) 3412, 2989, 2931, 1760, 1443, 1155, 1114 cm^{-1}. Anal. Calcd for $C_{13}H_{27}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.82; H, 9.66; N, 5.64.

To a solution of 540 mg (2.24 mmol) of 3-(1-ethoxyethoxy)-4-cyclohexyl-2-azetidinone, 14 mg (0.11 mmol) of DMAP, and 624 μL (4.48 mmol) of triethylamine in 20 mL of dichloromethane at 0 °C was added a solution of 733 mg (3.36 mmol) of di-*tert*-butyl dicarbonate dissolved in 5 mL of dichloromethane. The reaction mixture was stirred 4 h at room temperature, and then water was added. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (hexane:EtOAc, 5:1) to yield 696 mg (91%) of **4** as a colorless oil: $[\alpha]^{20}_D$ +62.5° (*c* 1.12, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.10–1.28 (m, 6 H), 1.15 (t, *J* = 7.0 Hz, 3 H), [1.27 (d, *J* = 5.4 Hz), 1.31 (d, *J* = 5.4 Hz), 3 H], [1.45 (s), 1.46 (s), 9 H], 1.63–1.70 (m, 5 H), [3.43 (dq, *J* = 9.2, 7.0 Hz), 3.62 (m), 3.75 (d, *J* = 7.0 Hz), 3.78 (d, *J* = 7.0 Hz), 2 H], 3.85 (t, *J* = 6.1 Hz, 1 H), [4.78 (q, *J* = 5.4 Hz), 4.88 (m), 1H], [4.85 (d, *J* = 6.1 Hz), 4.86 (d, *J* = 6.1 Hz), 1 H]; ^{13}C NMR (63 MHz, $CDCl_3$) δ 15.1, (20.2, 20.4), (26.0, 26.1), 26.3, (27.3, 27.9), (29.0, 29.2), (30.0, 30.2), (37.5, 37.6), (61.2, 62.5), (62.1, 62.3), (75.4, 75.8), 83.1, 100.1, 148.7, (166.7, 166.8); IR (neat) 2980, 2931, 2854, 1807, 1725, 1450, 1370, 1329, 1212, 1118 cm^{-1}. Anal. Calcd for $C_{18}H_{31}NO_5$: C, 63.32; H, 9.15; N, 4.10. Found: C, 63.15; H, 8.97; N, 3.96.

10-Deacetyl-2-(hexahydro)baccatin III (5). A solution of 10-deacetylbaccatin III (480 mg, 0.88 mmol) in methanol (15 mL) was stirred under an atmosphere of hydrogen (500 psi) at 35 °C in the presence of 5% rhodium on charcoal (48 mg). After 24 h the hydrogenation reaction was judged complete by TLC (EtOAc), and the suspension was filtered through a pad of Celite to remove the catalyst. The filtrate was concentrated under reduced pressure to give **5** (485 mg) in quantitative yield as a white solid: mp 173–175 °C; $[\alpha]^{20}_D$ −36.2° (*c* 1.00, CH_3OH); ^{1}H NMR (250 MHz, CD_3OD) δ 0.97 (s, 3 H), 1.01 (s, 3 H), 1.20–2.20 (m, 13 H), 1.60 (s, 3 H), 1.96 (s, 3 H), 2.12 (s, 3 H), 2.26 (m, 1H), 2.41 (m, 1 H),3.80 (d, *J* = 7.3 Hz, 1 H), 4.11 (d, *J* = 8.0 Hz, 1H), 4.19 (dd, *J* = 6.6, 11.0 Hz, 1 H), 4.38 (d, *J* = 8.0 Hz, 1 H), 4.73 (bt, *J* = 8.1 Hz, 1 H), 4.97 (bd, *J* = 8.4 Hz, 1 H), 5.23 (s, 1 H), 5.33 (d, *J* = 7.3 Hz, 1 H); ^{13}C NMR (63 MHz, CD_3OD) δ 10.5, 15.4, 20.9, 22.8, 26.6, 26.8, 27.1, 27.3, 30.0, 30.5, 37.6, 40.6, 44.0, 45.0, 48.3, 59.0, 68.2, 72.8, 76.0, 76.2, 77.9, 79.4, 81.9, 86.1, 135.6, 144.7, 172.2, 177.8, 212.0. Anal. Calcd for $C_{29}H_{42}O_{10}$: C, 63.26; H, 7.69. Found: C, 63.22; H, 7.74.

10-Deacetyl-7,10-bis[(2,2,2-trichloroethoxy)carbonyl]-2-(hexahydro)baccatin III (6). To a stirred solution of **5** (210 mg, 0.038 mmol) in pyridine (4.1 mL) was added 2,2,2-trichloroethyl chloroformate (108 μL, 0.078 mmol). The reaction mixture was stirred at 80 °C for 30 min, and then water was added to it. The mixture was extracted with ethyl acetate, and the organic layer was dried over $MgSO_4$. After evaporation of solvent, the resulting crude oil was purified by column chromatography on silica gel (hexane:EtOAc, 1.5:1) to give 253 mg (74%) of protected baccatin **6** as a white solid: mp 160–162 °C; $[\alpha]^{20}_D$ −51° (*c* 1.00, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.08 (s, 3 H), 1.09 (s, 3 H), 1.20–2.35 (m, 14 H), 1.79 (s, 3 H), 2.13 (s, 3 H), 2.21 (s, 3 H), 2.64 (m, 1 H), 3.85 (d, *J* = 6.9 Hz, 1 H), 4.14 (d, *J* = 8.1 Hz, 1H), 4.50 (d, *J* = 8.1 Hz, 1 H), 4.59 (*J* = 11.8 Hz, 1H), 4.74 (d, *J* = 11.9 Hz, 1H), 4.80 (d, *J* = 11.9 Hz, 1 H), 4.91 (d, *J* = 11.8 Hz, 1 H), 4.85–4.95 (m, 1 H), 4.99 (bd, *J* = 8.4 Hz, 1 H), 5.41 (d, *J* = 6.9 Hz, 1 H), 5.57 (dd, *J* = 7.2, 10.7 Hz, 1 H), 6.23 (s, 1H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 105, 15.3, 20.0, 22.4, 25.0, 25.5, 26.5, 28.3, 29.3, 33.1, 37.9, 42.5, 43.5, 47.1, 56.1, 67.6, 73.5, 76.3, 76.6, 76.9, 77.3, 78.6, 79.6, 80.0, 83.8, 94.1, 94.2, 130.5, 146.7, 153.1, 153.15, 170.8, 176.9, 201.2. Anal. Calcd for $C_{35}H_{44}O_{14}Cl_6$: C, 46.63; H, 4.92. Found: C, 46.50; H, 5.00.

General Procedure for the Coupling of a β-Lactam Intermediate with a 7,10-DiTroc-baccatin III. To a solution of 1.5 equiv of β-lactam (0.05 mol) and 1.0 equiv of 7,10-diTroc-10-deacetylbaccatin III or 7,10-diTroc-10-deacetyl-2-hexahydrobaccatin III (0.033 mol) in THF cooled at −30 °C was added dropwise 1.5 equiv of NaHMDS (0.95 M in hexane). The reaction mixture was stirred for 30 min at −30 °C, and then the reaction was quenched by addition of saturated aqueous NH_4Cl. The aqueous layer was extracted with ethyl ether, and the combined organic layers were washed with

brine, dried over $MgSO_4$, and concentrated. The crude solid was purified by column chromatography on silica gel (hexanes: EtOAc, 3:1 then 1:1) to afford the coupling product as a diastereoisomeric mixture and the unreacted 7,10-diTroc-baccatin.

3'-Cyclohexyl-3'-dephenyl-2'-(1-ethoxyethyl)docetaxel (8): a mixture of two diastereomers; 86% isolated yield with 12% recovery of 7,10-diTroc-baccatin III (**7**) (98% conversion yield). Anal. Calcd for $C_{53}H_{69}NO_{19}Cl_6$: C, 51.47; H, 5.62; N, 1.13. Found: C, 51.43; H, 5.75; N, 1.18.

2'-(1-Ethoxyethyl)-2-(hexahydro)docetaxel (9): a mixture of two diastereomers; 76% isolated yield with 16% recovery of baccatin **7** (90% conversion yield). Anal. Calcd for $C_{53}H_{69}NO_{19}Cl_6$: C, 51.47; H, 5.62; N, 1.13. Found: C, 51.45; H, 5.83; N, 1.20.

3'-Cyclohexyl-3'-dephenyl-2'-(1-ethoxyethyl)-2-(hexahydro)docetaxel (10): a mixture of two diastereomers; 75% isolated yield with 18% recovery of baccatin **7** (91% conversion yield). Anal. Calcd for $C_{53}H_{75}NO_{19}Cl_6$: C, 51.22; H, 6.08; N, 1.13. Found: C, 51.07; H, 6.06; N, 1.09.

General Procedure for the Final Deprotection. To a solution of a 2-EE-7,10-diTroc-docetaxel analog in a 1:1 acetic acid/methanol mixture was added Zn dust (200% in weight). The resulting suspension was vigorously stirred at 60 °C for a few hours, and then the solid materials were filtered off. After removal of solvent under reduced pressure, the resulting crude residue was dissolved in ethyl acetate and washed with aqueous $NaHCO_3$ and brine. The organic phase was concentrated and the crude oil was purified by flash column chromatography on silica gel (hexane:EtOAc, 1:2) to afford the corresponding docetaxel analog.

3'-Cyclohexyl-3'-dephenyldocetaxel (11): 65% yield; white solid; $[\alpha]^{20}_D$ −41° (*c* 0.49, CH_3OH); IR (KBr) 3450, 2970, 2930, 2835, 1715 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.00–1.30 (m, 11 H), 2.24–2.40 (m, 2 H), 2.43 (s, 3 H), 2.60 (m, 1 H), 3.38 (bs, 1 H, OH), 3.70 (bt, J = 9.0 Hz, 1 H), 3.90 (d, J = 7.0 Hz, 1 H), 3.90 (d, J = 7.5 Hz, 1 H), 4.20 (m, 2 H), 4.34 (d, J = 7.5 Hz, 1 H), 4.49 (bs, 1 H), 4.70 (d, J = 9.0 Hz, 1 H), 4.98 (bd, J = 9.0 Hz, 1 H), 5.70 (d, J = 7.0 Hz, 1 H), 6.20 (bt, J = 9.0 Hz, 1 H), 7.50 (m, 2 H), 7.63 (m, 1 H), 8.12 (m, 2 H). Anal. Calcd for $C_{43}H_{59}NO_{14}$: C, 63.45; H, 7.31; N, 1.72. Found: C, 63.26; H, 7.44; N, 1.59.

2-(Hexahydro)docetaxel (12): 58% yield; white solid; mp 179–182 °C; $[\alpha]^{20}_D$ −41.3° (*c* 0.945, $CHCl_3$); IR ($CHCl_3$) 3426, 2980, 3016, 2931, 2856, 1714 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3 H), 1.21 (s, 3 H),1.25–2.35 (m, 14 H), 1.42 (s, 9 H), 1.71 (s, 3 H), 1.81 (s, 3 H), 2.25 (s, 3 H), 2.59 (m, 1 H), 3.34 (d, J = 5.3 Hz, 1 H, OH), 3.78 (d, 1 H, J = 6.9 Hz, 1 H), 4.18 (d, J = 8.2 Hz, 1H), 4.18 (m, 1 H), 4.48 (d, J = 8.2 Hz, 1 H), 4.58 (bs, 1 H), 4.95 (d, J = 8.7 Hz, 1 H), 5.17–5.20 (m, 2 H), 5.44 (m, 2 H), 6.15 (bt, J = 8.8 Hz, 1 H), 7.30–7.50 (m, 5 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.8, 14.3, 20.4, 22.4, 25.1, 25.5, 25.6, 26.4, 28.2, 28.4, 29.3, 35.2, 36.8, 42.9, 43.5, 46.3, 56.3, 57.6, 71.9, 72.3, 73.7, 74.2, 74.5, 76.7, 78.8, 80.2, 80.8, 84.3, 126.8, 128.0, 128.7, 135.8, 138.4, 155.4, 170.3, 170.9, 177.0, 211.3. Anal. Calcd for $C_{43}H_{59}NO_{14}$: C, 63.45; H, 7.31; N, 1.72. Found: C, 63.29; H, 7.26; N, 1.62.

3'-Cyclohexyl-3'-dephenyl-2-(hexahydro)docetaxel (13): 45% yield; white solid; mp 174–177 °C; $[\alpha]^{20}_D$ −40° (*c* 1.00, CH_3OH); IR ($CHCl_3$) 3437, 3017, 2983, 2935, 2856, 1739, 1731, 1713, 1695 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.90–2.35 (m, 25 H), 1.04 (s, 3 H), 1.19 (s, 3 H), 1.40 (s, 9 H), 1.69 (s, 3 H), 1.87 (s, 3 H), 2.30 (s, 3 H), 2.56 (m, 1 H), 3.57 (d, J = 5.0 Hz, 1 H), 3.67 (bt, J = 10.0 Hz, 1 H), 3.75 (d, J = 6.8 Hz, 1 H), 4.17 (d, J = 8.0 Hz, 1H), 4.15–4.25 (m, 1 H), 4.27 (bs, 1 H, OH), 4.46 (bd, J = 5.0 Hz, 1 H), 4.48 (d, J = 8.0 Hz, 1 H), 4.76 (d, J = 10.0 Hz, 1 H), 4.97 (d, J = 8.5 Hz, 1 H), 5.20 (s, 1 H), 5.43 (d, J = 6.8 Hz, 1 H), 6.12 (bt, J = 8.4 Hz, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.8, 14.3, 20.6, 22.6, 25.2, 25.5, 25.6, 26.0, 26.1, 26.3, 28.2, 28.4, 29.3, 29.8, 30.1, 35.1, 36.8, 38.9, 43.0, 43.5, 46.2, 57.6, 57.8, 70.3, 71.9, 72.5, 74.3, 74.5, 76.7, 78.7, 79.5, 80.7, 84.3, 135.5, 138.9, 155.8, 170.2, 174.6, 177.1, 211.4. Anal. Calcd for $C_{43}H_{65}NO_{14}$: C, 62.99; H, 7.99; N, 1.71. Found: C, 63.27; H, 7.85; N, 1.74.

2-Hexahydro-7-(triethylsilyl)baccatin III (14). A solution of 7-TES-baccatin III (40 mg, 0.59 mmol) in methanol (10 mL) was stirred under an atmosphere of hydrogen (600 psi) at 45 °C in the presence of 5% rhodium on charcoal (40 mg). After 48 h, the hydrogenation reaction was judged complete by TLC (EtOAc), and the suspension was filtered through a pad of Celite to remove the catalyst. The filtrate was concentrated under reduced pressure and purified by flash column chromatography on silica gel (hexane:EtOAc, 1.5:1) to give **14** (355 mg) in 85% yield as a white solid: mp 137–140 °C; $[\alpha]^{20}_D$ −57.4° (*c* 1.76, $CHCl_3$); ^1H NMR (250 MHz, CD_3OD) δ 0.56 (m, 6 H), 0.90 (t, 9 H), 0.99 (s, 3 H), 1.04 (s, 3 H), 1.20-2.30 (m, 14 H), 1.57 (s, 3 H), 2.05 (s, 3 H), 2.09 (s, 3 H), 2.12 (s, 3 H), 3.73 (d, J = 7.0 Hz, 1 H), 4.09 (d, J = 7.9 Hz, 1H), 4.39 (d, J = 7.9 Hz, 1 H), 4.48 (dd, J = 6.8, 10.3 Hz, 1 H), 4.70 (bt, J = 8.0 Hz, 1 H), 4.95 (bd, J = 9.1 Hz, 1 H), 5.34 (d, J = 7.0 Hz, 1 H), 6.41 (s, 1 H); ^{13}C NMR (63 MHz, CD_3OD) δ 6.4, 7.3, 10.7, 15.6, 21.0, 21.4, 22.8, 26.6, 26.9, 27.1, 27.3, 30.0, 30.6, 38.5, 40.4, 44.2, 45.1, 48.5, 59.9, 68.3, 74.0, 75.8, 77.5, 77.8, 79.2, 81.7, 85.7, 133.2, 147.2, 170.9, 172.2, 177.8, 205.4. Anal. Calcd for $C_{37}H_{58}O_{11}Si$: C, 62.86; H, 8.27. Found: C, 62.83; H, 7.92.

2-(Hexahydro)paclitaxel (15). To a solution of *N*-benzoyl-β-lactam **1a** (65 mg, 0.17 mmol) and 2-hexahydro-7-(triethylsilyl)baccatin III (**14**) (80 mg, 0.11 mmol) in THF (3.4 mL) at 0 °C was added dropwise NaHMDS (170 μL , 0.17 mmol). The reaction mixture was stirred for 45 min at 0 °C, and then saturated aqueous NH_4Cl was added for quenching. The aqueous layer was extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The crude residue was purified by column chromatography on silica gel (hexane:EtOAc, 3:1 then 2:1) to give 97 mg (80%) of coupling product 2-hexahydro-7-(triethylsilyl)paclitaxel as a white solid and 13.5 mg (17%) of the unreacted baccatin (**14**).

2-Hexahydro-2',7-bis(triethylsilyl)paclitaxel: mp 138–141 °C; $[\alpha]^{20}_D$ −46.5° (*c* 1.35, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.44 (m, 6 H), 0.58 (m, 6 H), 0.82 (t, 9 H), 0.92 (t, 9 H), 1.16 (bs, 6 H), 1.20–2.40 (m, 14 H), 1.65 (s, 3 H), 1.98 (s, 3 H), 2.16 (s, 3 H), 2.43 (s, 3 H), 2.53 (m, 1 H), 3.70 (d, J = 6.9 Hz, 1 H), 4.18 (d, J = 8.0 Hz, 1H), 4.44 (dd, J = 6.7, 10.4 Hz, 1 H), 4.50 (d, J = 8.0 Hz, 1 H), 4.66 (d, J = 1.6 Hz, 1 H), 4.96 (bd, J = 8.6 Hz, 1 H), 5.46 (d, J = 6.9 Hz, 1 H), 5.61 (bd, J = 8.5 Hz, 1 H), 6.20 (bt, J = 9.0 Hz, 1 H), 6.42 (s, 1 H), 7.14 (d, J = 8.5 Hz, 1 H, NH), 7.30-7.60 (m, 8 H), 7.80 (d, J = 7.7 Hz, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 4.3, 4.8, 5.3, 6.5, 6.7, 10.0, 14.1, 20.8, 21.3, 23.0, 25.1, 25.5, 25.7, 26.6, 28.5, 29.4, 35.0, 37.1, 43.2, 43.6, 46.5, 55.8, 58.4, 71.5, 72.1, 74.2, 74.8, 74.9, 76.6, 78.9, 80.9, 84.3, 126.5, 127.0, 127.9, 128.6, 128.7, 131.7, 133.6, 134.1, 138.5, 140.2, 166.9, 169.2, 170.0, 177.2, 201.7. Anal. Calcd for $C_{59}H_{85}NO_{14}Si_2$: C, 65.10; H, 7.87; N, 1.29. Found: C, 64.87; H, 7.99; N, 1.31.

2-Hexahydro-7-(triethylsilyl)paclitaxel (20 mg) was treated with 0.5% aqueous HCl/EtOH solution (1/2, 2.2 mL) at 4 °C for 48 h. The reaction mixture was washed with 5% aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Purification of the crude product by flash column chromatography on silica gel (hexane:EtOAc, 1:2) gave **15** (88%) as a white solid: mp 200–205 °C dec; $[\alpha]^{20}_D$ −45.1° (*c* 0.975, $CHCl_3$); IR ($CHCl_3$) 3499, 3435, 3017, 2933, 2856, 1731, 1660, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.07 (s, 3 H), 1.20–2.35 (m, 14 H), 1.21 (s, 3 H), 1.63 (s, 3 H), 1.75 (s, 3 H), 2.22 (s, 3 H), 2.26 (s, 3 H), 2.53 (m, 1 H), 2.53 (d, J = 3.7 Hz, 1 H, OH), 3.65 (d, J = 6.8 Hz, 1 H), 3.74 (d, J = 5.2 Hz, 1 H, OH), 4.17 (d, J = 8.0 Hz, 1 H), 4.35 (m, 1 H), 4.47 (d, J = 8.0 Hz, 1 H), 4.75 (dd, J = 2.5, 5.2 Hz, 1 H), 4.95 (bd, J = 8.5 Hz, 1 H), 5.43 (d, J = 6.8 Hz, 1 H), 5.72 (dd, J = 2.5, 8.8 Hz, 1 H), 6.17 (bt, J = 8.7 Hz, 1 H), 6.23 (s, 1 H), 7.07 (d, J = 8.8 Hz, 1 H, NH), 7.30–7.60 (m, 8 H), 7.78 (d, J = 6.9 Hz, 2 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 14.8, 20.8, 21.6, 22.5, 25.1, 25.5, 25.7, 26.8, 28.4, 29.3, 35.2, 35.5, 43.1, 43.5, 45.5, 55.1, 58.6, 72.1, 72.3, 73.2, 74.2, 75.5, 76.6, 79.0, 80.6, 80.9, 84.5, 127.0, 128.3, 128.7, 128.9, 132.0, 133.0, 133.7, 138.0, 141.9, 167.1, 170.4, 171.2, 172.6, 177.0, 203.6. Anal. Calcd for $C_{59}H_{57}NO_{14}$: C, 65.64; H, 6.68; N, 1.63. Found: C, 65.49; H, 6.55; N, 1.58.

Microtubule Disassembly Assay. A stock solution of tubulin (*vide supra*) was supplemented with 6 mM $MgCl_2$ and 1 mM GTP at 0–2 °C and used at a concentration of 9 μM

(0.9 mg/mL). A taxoid in ethanol (0.1–5 μM) was added to the tubulin solution. The polymerization was initiated by a temperature shift from 6 °C to 37 °C in a cell with a 1-cm light path and monitored turbidimetrically at 350 nM[34a] with a UVIKON 931 spectrophotometer (KONTRON) equipped with a thermostatically controlled cell holder. After 30 min, cold-reversibility was evaluated by shifting the temperature at 6 °C until the drop in turbidity was completed. The depolymerization rate was calculated from the slope of the decrease in turbidity as a function of time. The IC_{50} value indicates the concentration of an inhibitor, i.e., a taxoid, for which the rate is decreased by 50%. Paclitaxel was used as the standard and IC_{50}(taxoid)/IC_{50}(paclitaxel) was calculated.

Cell Line Assay *in Vitro*.[29] The concentration of a taxoid giving 50% of growth inhibition (IC_{50}) was determined on the basis of three separate experiments in a 96-well microculture plates.[29] Cell lines seeded at 1×10^5 cells/mL (0.2 mL/well) were grown for 96 h in the presence of various concentrations of a taxoid (each point in quadruplicate). Cells were then incubated for 16 h with 0.02% neutral red. The cells were washed and lyzed with 1% sodium dodecyl sulfate (SDS). The incorporation of the dye reflecting cellular growth and viability was evaluated by the measurement of the optical density for each well at 540 and 346 nm, using a Titertek multiwell spectrophotometer. Experiments were carried out in comparison with docetaxel. Stock solutions of taxoids were prepared in ethanol at 10 mg/mL and stored at −20 °C. Further dilutions were made by adding water.

Antitumor Activity Assay *in Vivo*.[10,32] Tumor fragments of B16 melanoma (1 mm^3, 30–60 mg) were grafted subcutaneously (sc) on day 0 in $B6D2F_1$ mice (5 mice/group). Taxoids were first dissolved in ethanol, polysorbate 80 was added, and the final dilution was made with 5% glucose in water (5/5/90; v/v/v). The pH of the final solution was 5. Taxoids (0.4 mL/mouse) were administered intraveneously (iv) on days of 5, 7, and 9. Animals were observed for toxicity and tumor growth. Tumors were measured with a caliper. Tumor weights were calculated from 2-dimensional measurements: tumor weight (mg) = $(lw^2)/2$, where l and w are the tumor length and width, respectively. Antitumor activity was expressed by the T/C value obtained at the maximal tolerated dose (no lethality, body weight loss <20%) wherein T is the median tumor weight of treated animals and C is the median tumor weight of control animals (measurements were performed when C was approximately equal to 1 g). A T/C value of 42% or less is considered significant antitumor activity by the National Cancer Institute.

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