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Syntheses and Biological Activity of C-3′-Difluoromethyl-Taxoids

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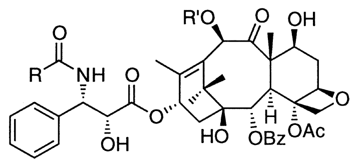
Abstract—A series of new taxoids bearing difluoromethyl group at the C-3′ position and modifications at the C-10 and C-14 positions has been synthesized and their biological activities studied. The in vitro cytotoxicity assay results indicate that these newly developed taxoids exhibit comparable to several times better activity against drug-sensitive cell line LCC6-WT, and 40–70 times better activity against the corresponding drug-resistant cancer cell line LCC6-MDR as compared to that of paclitaxel. Apoptosis analysis has revealed the exceptional activity of SB-T-12843 (**1e**) in inducing apoptosis in both MDR-bearing and MDR-negative cancer cells. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Over the past several years, Taxol® (paclitaxel)[1] and its semisynthetic analogue, Taxotère® (docetaxel),[2] have been at the forefront of the development of potent anticancer agents.[3–12] These drugs possess the same unique and effective mechanism of action[13,14] and exhibit strong activity against several cancers that are not effectively treated with other chemotherapeutic drugs. Paclitaxel received FDA approval for the treatment of advanced ovarian cancer (December, 1992) and breast cancer (April, 1994), while docetaxel was approved for the treatment of breast cancer (May, 1996). Clinical trials for other cancers, as well as combination therapies, are also currently in progress worldwide.[7,15] While these two drugs have made significant progress in the treatment of various forms of cancer, reports have also indicated that their use results in an assortment of undesirable side-effects.[16] In addition to these side-effects, the onset of multi-drug resistance (MDR)[17] clearly illustrates the necessity for the development of the "second-generation taxoids" that exhibit fewer undesirable side-effects and improved activity against drug-resistant tumors.



Paclitaxel: R = Ph, R′ = Ac
Docetaxel: R = ^{t}BuO, R′ = H

In the course of our structure–activity relationship (SAR) study of taxoids, we set out to incorporate fluorine into taxoids to study the effects of fluorine-incorporation on the cytotoxicity of the resulting analogues,[12,18] blocking of known metabolic pathways of taxoids,[19–21] and the use of fluorine(s) as probe(s) for the conformational analysis of these antitumor agents.[22–24] The synthesis and biological activities of other fluorinated taxoids have also been reported by Bristol-Myers Squibb laboratories.[25–29]

As a part of our continuing SAR study of taxoids,[11,12] we have become interested in developing new taxoids bearing a difluoromethyl group at C-3′ position (**1a–e** and **2a–f**, Fig. 1). We have also studied the ability of a new CF_2H-taxoid, which possesses the strongest cytotoxicity in this series against drug-resistant cancer cells, in inducing apoptosis in drug-sensitive and drug-resistant cancer cells, since the aim of chemotherapy is the induction of apoptosis in cancer cells. We describe here the synthesis and biological activities of a series of C-3′-dephenyl-3′-difluoromethyldocetaxel analogues derived

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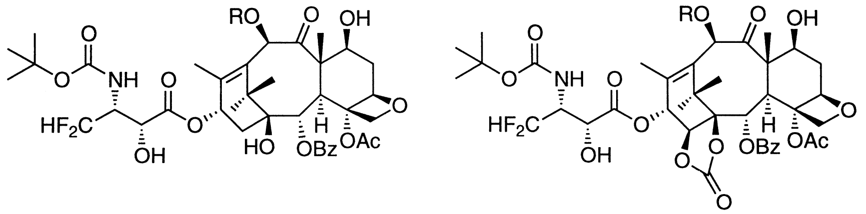


1a (SB-T-1283): R = H
1b (SB-T-1284): R = Ac
1c (SB-T-12841): R = Me_2N-CO
1d (SB-T-12842): R = Et-CO
1e (SB-T-12843): R = Me_3CCH_2-CO

2a (SB-T-101100): R = H
2b (SB-T-101101): R = Ac
2c (SB-T-101104): R = Me_2N-CO
2d (SB-T-101103): R = Et-CO
2e (SB-T-101105): R = Me_3CCH_2-CO
2f (SB-T-101102): R = *c*-Pr-CO

Figure 1. 3′-CF_2-taxoids **1** and **2**.

from 10-deacetylbaccatin III (DAB) and 14β-hydroxy-10-deacetylbaccatin III (14β-OH-DAB).

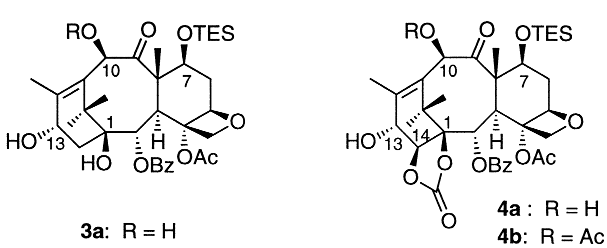
Results and Discussion

Synthesis

A series of novel C-3′-difluoromethyl-taxoids was synthesized from DAB and 14β-OH-DAB using the coupling protocols developed in these laboratories.[9,11,12,30] 7-TES-baccatin III and its 10-modified analogues **3a–e** (TES = triethylsilyl) were prepared in two steps from DAB, employing previously published methods.[31,32] The C-7 hydroxyl group of DAB was protected as a triethylsilyl ether, followed by modification of the C-10 hydroxyl group with chlorotriethylsilane, acyl chlorides, or *N*,*N*-dimethylcarbamoyl chloride using LiHMDS as the base to afford baccatins **3a–e** in high to excellent yields (Fig. 2).[33] 7-TES-10-modified-baccatin-1,14-carbonates **4a–f** were also prepared in good to excellent yields from 14β-OH-DAB,[34] through (a) protection of the C-7 hydroxyl group of 14β-OH-DAB as a triethylsilyl ether, (b) protection of 1,14-*cis*-diol as cyclic 1,14-carbonate with triphosgene and pyridine, and (c) modification of C-10 hydroxyl group with chlorotriethylsilane, acyl chlorides, or *N*,*N*-dimethylcarbamoyl chloride (Fig. 2).

(3*R*,4*S*)-1-PMP-3-TIPSO-4-(2-methylprop-1-enyl)-β-lactam (**5**, PMP = *p*-methoxyphenyl, TIPSO = triisopropylsiloxy) with extremely high enantiomeric purity (>98% ee) was prepared in excellent yield through a highly efficient chiral enolate-imine cyclocondensation reaction developed in these laboratories.[30,34–37] Ozonolysis of the 4-alkenyl-β-lactam at low temperature afforded the corresponding 4-formyl-β-lactam **6** in quantitative yield. Treatment of 4-formyl-β-lactam **6** with 3.0 equiv of dialkylaminosulfur trifluorides (DAST)[38] in dichloromethane for 5 h at room temperature afforded 1-PMP-3-OTIPS-4-CF_2H-β-lactam **7** in good yield. There was no detectable epimerization of the β-lactam in the fluorination step (Scheme 1). Subsequent treatment of β-lactam **7** with ceric ammonium nitrate (CAN) removing the *N*-PMP group, followed by *t*-Boc protection of the resulting *N*-H-β-lactam, afforded 1-*t*-Boc-3-OTIPS-4-CF_2H-β-lactam **8** in good yield (Scheme 1).

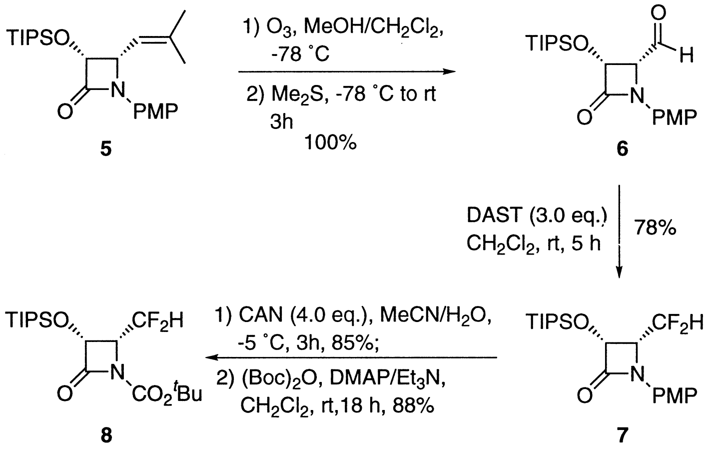
Novel taxoids bearing a difluoromethyl group at the C-3′ position and modification at the C-10 position were synthesized by applying the ring-opening coupling protocol developed in these laboratories.[9,11,12,30,36,37,39] The coupling of 4-CF_2H-β-lactam **8** with 7-TES-10-modified-baccatins **3** or 7-TES-10-modified-baccatin-1,14-carbonates **4** was carried out under the standard conditions of our protocol. Thus, treatment of a mixture of baccatin (**3** or **4**) and β-lactam **8** in THF with LiHMDS or NaHMDS at −40 °C for 30 min afforded the corresponding coupling products, which were treated with HF/pyridine to give desired 3′-CF_2H-taxoids, **1** and **2**, in good to excellent yields (Scheme 2 and Table 1).



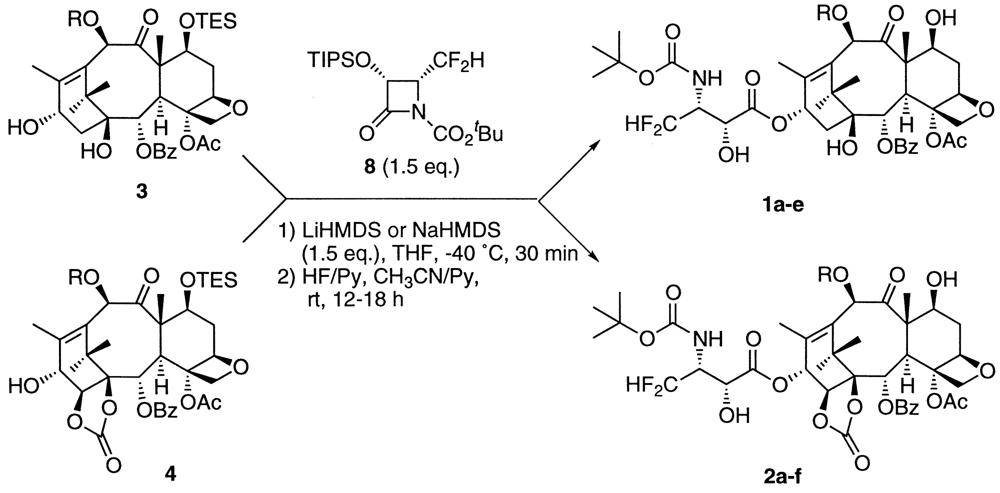
3a: R = H
3b: R = Ac
3c: R = Me_2N-CO
3d: R = Et-CO
3e: R = Me_3CCH_2-CO

4a : R = H
4b: R = Ac
4c: R = Me_2N-CO
4d: R = Et-CO
4e: R = Me_3CCH_2-CO
4f: R = *c*-Pr-CO

Figure 2. C-10-modified baccatins **3** and **4**.



Scheme 1. Synthesis of optically pure 4-CF_2H-β-lactam **8**.



Scheme 2. Coupling of baccatins **3** and **4** with β-lactam **8**.

Table 1. Synthesis of 3′-CF_2-taxoids

Baccatin	β-Lactam	R	Taxoid	Yield (%, 2 steps)
3a	**8**	H	**1a**	71
3b	**8**	Ac	**1b**	54
3c	**8**	Me_2N-CO	**1c**	51
3d	**8**	Et-CO	**1d**	62
3e	**8**	Me_3CCH_2-CO	**1e**	57
4a	**8**	H	**2a**	59
4b	**8**	Ac	**2b**	86
4c	**8**	Me_2N-CO	**2c**	79
4d	**8**	Et-CO	**2d**	60
4e	**8**	Me_3CCH_2-CO	**2e**	81
4f	**8**	*c*-Pr-CO	**2f**	73

Cytotoxicity assay

Cytotoxicities of these new 3′-CF_2H-C-10-modified-taxoids, **1** and **2**, were evaluated in vitro against a human breast cancer cell line, LCC6-WT, and the corresponding drug-resistant cell line, LCC6-MDR.[40] The results are shown in Table 2. The assay results for paclitaxel, docetaxel, and doxorubicin are also shown for comparison.

As Table 2 shows, all of the 3′-CF_2H-taxoid-1,14-carbonates (**2**) exhibit activity comparable to those of paclitaxel and docetaxel against drug-sensitive cell line LCC6-WT, and 3–7 times better activity than those of paclitaxel against drug-resistant cancer cell line LCC6-MDR. Most 3′-CF_2H taxoids (**1**) in this series exhibit 3–6 times better activity against drug-sensitive cell line LCC6-WT, and 40–70 times better activity against drug-resistant cancer cell line LCC6-MDR than that of paclitaxel. All these new taxoids show 2–3 orders of magnitude higher potency than that of doxorubicin against both drug-sensitive and drug-resistant cell lines.

There is no correlation between the activities against the drug-sensitive and drug-resistant cell lines for both series of taxoids. For example, taxoid **1e** (R = *tert*-butylacetyl), the least active against the drug-sensitive cell line LCC6-WT, exhibits strongest cytotoxicity against the drug-resistant cell line LCC6-MDR. In the same manner, taxoid **2f** (R = cyclopropanecarbonyl), that is the least active against LCC6-WT, shows the highest potency against LCC6-MDR in this series.

It should be noted that the C-10 modification substantially improves the cytotoxicity of taxoids (**1a** versus

Table 2. In vitro cytotoxicity (IC_{50}, nM)[a] of 3′-CF_2H-taxoids **1** and **2**

Taxoid	R	LCC6-WT[b]	LCC6-MDR[c]
Paclitaxel	—	3.1	346
Docetaxel	—	1.0	120
Doxorubicin	—	180	2900
1a	H	0.96	43.5
1b	Ac	0.50	8.79
1c	Me_3N-CO	0.48	8.20
1d	Et-CO	1.0	5.69
1e	Me_2CCH_2-CO	1.34	5.04
2a	H	3.2	> 100[d]
2b	Ac	1.9	64
2c	Me_2N-CO	2.8	53
2d	Et-CO	3.2	63
2e	Me_3CCH_2-CO	3.2	133
2f	*c*-Pr-CO	5.6	49

[a] The concentration of compound which inhibits 50% of the growth of the human tumor cell line after 72 h drug exposure.
[b] LCC6-WT: human breast carcinoma.
[c] LCC6-MDR: MDR1 transduced human breast carcinoma.
[d] 20% inhibition at 100 nM.

Table 3. DNA fragmentation rate (%)[a] of MDA-MB 231,[b] MCF-7 ADRr,[c] CEM,[d] and CEM VBLr[e] cells after treatment with taxoids

Cancer cell	C (nM)	SB-T-12843 (**1e**)	Paclitaxel	Docetaxel	Control
MDA-MB 231	0	—	—	—	1.55
	0.01	2.8	5.5	3.5	—
	0.1	4.6	2.8	5	—
	0.5	6.2	4.2	13	—
	2	39.3	3.3	52	—
MCF-7 ADRr	0	—	—	—	4.1
	20	14.4	7.1	7.5	—
	50	52.8	5.6	5	—
	200	76.2	8.1	9.4	—
	500	89.5	7.8	7.7	—
CEM	0	—	—	—	8.1
	10	22.7	13.6	9.8	—
	50	19.3	14.4	13.2	—
	100	33.7	16.6	12	—
	500	30.8	14.9	13.4	—
	1000	46.9	15.8	14	—
	5000	52.2	13.9	15	—
	10000	25.1	14.8	15	—
CEM VBLr	0	—	—	—	1.55
	10	22.7	9.8	13.6	—
	50	19.3	13.2	14.4	—
	100	33.7	12	16.6	—
	500	30.8	13.4	14.9	—
	1000	46.9	14	15.8	—
	5000	52.2	15	13.9	—
	10000	25.1	15	14.8	—

[a] Data obtained after 72 h of cell exposure to the taxoids.
[b] MDA-MB 231: human breast cancer cell line.
[c] MCF-7 ADRr: human breast adriamycin (ADR)-resistant cancer cell line.
[d] CEM VBLr: human leukemia CEM vinblastine (VBL)-resistant cell line.
[e] CEM: human leukemia CEM cell line.

1b–e; **2a** versus **2b–d, f**). However, in contrast to the C-3′-alkyl and alkenyl series of taxoids previously reported,[33,34] the effect of the size of the C-10 substitution on cytotoxicity is very small except for the case of **2e**.

Apoptosis assay

Besides blocking cell replication by inhibiting the depolymerization of microtubules, paclitaxel, like vincristine, was also found to induce apoptosis.[41] This appears to suggest that perturbation of the microtubule assembly could trigger programmed cell death. Since the 3′-CF_2H-taxoids exhibit exceptional potency in the cytotoxicity assay, it would be of great interest to study the activity of these taxoids in inducing apoptosis. Thus, we selected compound SB-T-12843 (**1e**) to perform the apoptosis analysis against four cell lines, i.e., leukemia cells, CEM and CEM VBLr; breast cancer cells, MDA-MB 231 and MCF-7 ADRr. The CEM VBLr and MCF-7 ADRr cell lines overexpress P-gp protein. MDA-MB 231 cells were preferred over the MDR-negative MCF-7 wt, because this cell line does not overexpress P-gp protein under the selective pressure of xenobiotics. The data for paclitaxel and docetaxel were also obtained for comparison.

We scored the occurrence of apoptosis by evaluating DNA fragmentation by flow cytometry, a marker of apoptosis in a number of experimental models,[42] including cells tested for taxoids.[43] Table 3 shows the quantitative data of DNA fragmentation rate obtained from analysis of SB-T-12843 (**1e**) against the four cell lines assayed. The rates for paclitaxel and docetaxel in the same assay are also shown for comparison purposes. Figure 3 summarizes the DNA fragmentation data for SB-T-12843 (**1e**), paclitaxel, and docetaxel by normalizing the results as percentage of the control. As Figure 3 illustrates, the three drugs exhibit similar ability in inducing apoptosis in CEM cells, while only SB-T-12843 induces apoptosis in CEM VBLr cells. This finding is consistent with the cytotoxicity assay results showing the exceptional activity of SB-T-12843 (**1e**) against P-gp overexpressing cells. The same phenomena are observed in breast cancer cells where SB-T-12843 starting from 50 nM induces a dose-dependent increase in the DNA fragmentation of MCF-7 ADRr cells. SB-T-12843 (**1e**) exhibits a similar potency to that of docetaxel and a higher potency than that of paclitaxel in MDA-MB231 cells.

These findings indicate that SB-T-12843 (**1e**) can induce apoptosis in the P-gp negative cells at a level similar to that of docetaxel and higher than that of paclitaxel. On the other hand, in the P-gp positive cells SB-T-12843 (**1e**) induces a remarkably higher level of apoptosis at lower concentrations as compared to those of paclitaxel and docetaxel. Further studies are currently in progress in these laboratories to ascertain if the enhanced apoptotic ability of the taxoids against P-gp positive MDR cancer cells is closely linked to their ability to induce the disruption of microtubules and the cell division cycle arrest.

Conclusions

Novel taxoids bearing a difluoromethyl group at the C-3′ position and modifications at the C-10 and C-14

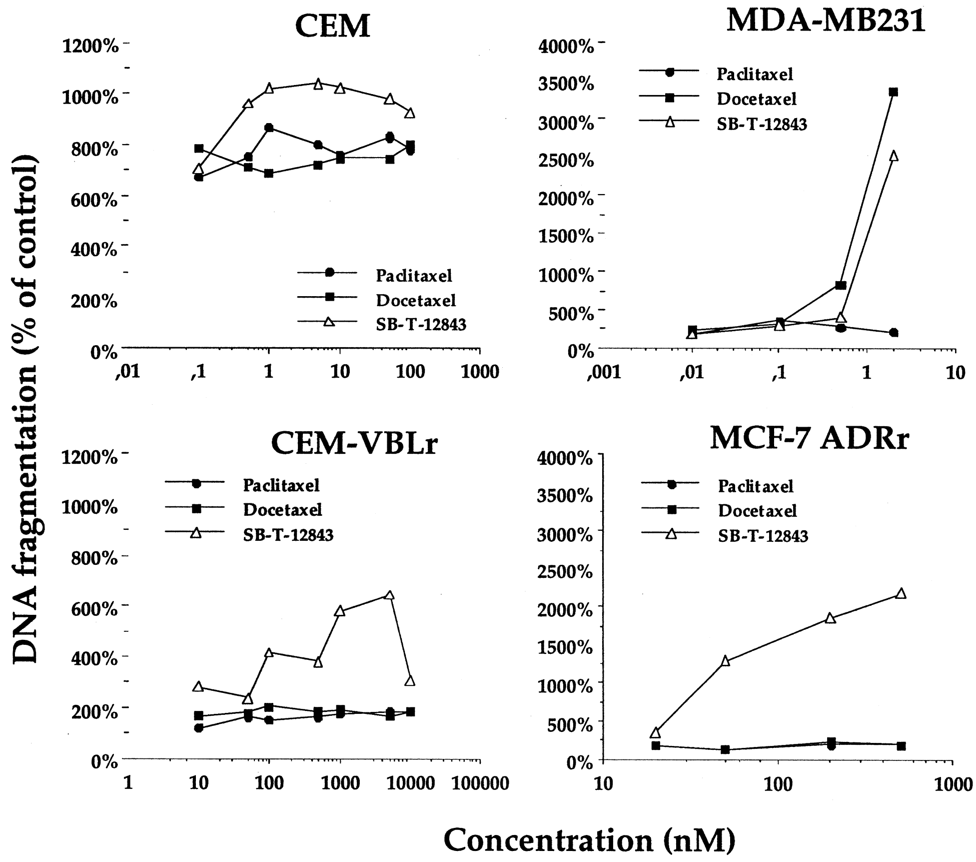


Figure 3. Apoptosis assay of paclitaxel, docetaxel, and SB-T-12843 (**1e**) on MDA-MB 231, MCF-7 ADRr, CEM and CEM VBLr cells.

positions have been synthesized and their biological activities examined. The newly developed taxoids exhibit higher potency against drug-sensitive cell line LCC6-WT, and 40–70 times higher potency against drug-resistant cancer cell line LCC6-MDR as compared to that of paclitaxel. One of these novel CF_2-taxoids, SB-T-12843 (**1e**), is found to possess exceptional activity in inducing apoptosis in both MDR-bearing and MDR-negative cancer cells.

Experimental

Chemical synthesis

General methods. ^1H and ^{13}C NMR spectra were measured on a Bruker AC-250 NMR spectrometer or a Varian 300 NMR spectrometer using tetramethylsilane as the internal standard. Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter. IR spectra were recorded on a Perkin–Elmer Model 1600 FT-IR spectrophotometer. TLC was performed on Merck DC-alufolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60 (Merck; 230–400 mesh ASTM). Elemental analyses were performed at M-H-W Laboratory, Phoenix, AZ. High resolution mass spectra were obtained from the University of California, Riverside Mass Spectrometry Facility, Riverside, CA; or Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL.

Materials. The chemicals were purchased from Aldrich Co. and Sigma, and purified before use by standard methods. Tetrahydrofuran was freshly distilled under sodium metal and benzophenone. Dichloromethane was also distilled immediately prior to use under nitrogen from calcium hydride. 7-TES-10-modified baccatins **3a–e** and 7-TES-10-modified-baccatin-1,14-carbonates **4a–f** were prepared using our published procedures.[33,34] (3*R*,4*S*)-1-PMP-3-TIPSO-4-(2-methylprop-1-enyl)-β-lactam (**5**) was prepared through a highly efficient chiral ester enolate-imine cyclocondensation reaction previously reported from our laboratories.[30,34,36,37]

(3*R*,4*S*)-1-(4-Methoxylphenyl)-3-triisopropylsiloxy-4-formylazetidin-2-one (6). Nitrogen gas was bubbled into a solution of 4-(2-methyl-2-propenyl)-β-lactam **5** (500 mg, 1.239 mmol) in MeOH:CH_2Cl_2 (1:2, 27 mL) at −78 °C for 3 min. Then, O_3 gas was bubbled into the solution till the color of the solution turned blue (2 min), and N_2 was bubbled into the solution for another 3 min. Dimethyl sulfide (0.458 mL, 6.194 mmol) was added to the solution and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred for 3 h at room temperature. The solvents were removed in vacuo and the residue was further purified on a neutral alumina column using hexane:ethyl acetate (4:1 followed by 2:1) as the eluant to afford **6** as a white solid (467.5 mg, quantity): mp 78–80 °C; $[\alpha]_D^{20}$ +158.0 (*c* 0.50, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 1.06 (m, 21H), 3.74 (s, 3H), 4.43 (dd, *J* = 5.1, 3.8 Hz, 1H), 5.25 (d, *J* = 5.4 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 9.72 (d, *J* = 3.8 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 11.6, 17.4, 55.3, 64.2, 78.6, 114.4, 117.8, 130.7, 156.7, 164.2, 199.6. Anal. calcd for $C_{20}H_{31}NO_4Si$: C, 63.63; H, 8.28; N, 3.71. Found: C, 63.80; H, 8.05; N, 3.72.

(3*R*,4*S*)-1-(4-Methoxyphenyl)-3-triisopropylsiloxy-4-difluoromethylazetidin-2-one (7). To a solution of 800 mg (2.12 mmol) of 4-formyl-β-lactam **6** in 13 mL of methylene chloride was added dropwise 0.84 mL (6.37 mmol) of diethylaminosulfur trifluoride at room temperature. The reaction was monitored by TLC, quenched after 5 h with water, and extracted with ethyl acetate. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel (hexane:EtOAc = 6:1) to afford 665 mg (78%) of the product **7** as a light yellow oil: $[\alpha]_D^{20}$ +55.9 (*c* 0.34, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.12–1.26 (m, 21H), 3.78 (s, 3H), 4.31–4.40 (m, 1H), 5.17 (d, *J* = 5.1 Hz, 1H), 6.07 (ddd, *J* = 55.7, 53.5, 5.3 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 11.89, 17.65, 55.39, 58.41(m), 75.75, 75.84, 114.21, 118.93, 130.65, 156.73, 165.10; ^{19}F NMR ($CDCl_3$) δ −119.3 (ddd, *J* = 298.7, 53.5, 7.1 Hz, 1F), −123.7 (ddd, *J* = 298.7, 56.2, 7.1 Hz, 1F). Anal. calcd for $C_{20}H_{31}NO_3SiF_2$: C, 60.12; H, 7.82; N, 3.51. Found: C, 60.81; H, 7.84; N, 3.60.

(3*R*,4*S*)-1-*tert*-Butoxycarbonyl-3-triisopropylsiloxy-4-difluoromethylazetidin-2-one (8). To a solution of 638 mg (1.6 mmol) of 4-difluoromethyl-β-lactam **7** in 40 mL of CH_3CN at −10 °C was added 3.5 g (6.4 mmol) of ceric(IV) ammonium nitrate (CAN) dropwise over a period of 1 h at −10 °C. The reaction mixture was stirred at −5 °C for an additional 2 h and quenched with the addition of 75 mL of water. The reaction mixture was extracted with ethyl acetate and the combined organic layers were washed with 5% $NaHSO_3$, 10% Na_2CO_3, 5% $NaHSO_3$, and brine. The organic layer was then dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel (hexane:EtOAc = 4:1 to 2:1) afforded 397 mg (85%) of the *N*-H-β-lactam as a light yellow oil: $[\alpha]_D^{20}$ +33.3 (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.09–1.21 (m, 21H), 3.79–3.91 (m, 1H), 5.07 (br s, 1H), 5.86 (ddd, *J* = 54.2, 54.1, 6.5 Hz, 1H), 6.79 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.74, 17.44, 55.24 (m), 77.85, 77.97, 169.02; ^{19}F NMR ($CDCl_3$) δ −121.3 (ddd, *J* = 299.4, 53.7, 7.3 Hz, 1F), −122.6 (ddd, *J* = 299.4, 54.2, 7.0 Hz, 1F). Anal. calcd for $C_{13}H_{25}F_2NO_2Si$: C, 53.21; H, 8.59; N, 4.77. Found: C, 53.08; H, 8.72; N, 4.87.

To a solution of *N*-H-β-lactam thus obtained (384 mg, 1.31 mmol), 25 mg of DMAP, and 0.37 mL (2.62 mmol) of triethylamine in 7 mL of dry methylene chloride was added, at 0 °C, 315 mg (1.44 mmol) of di-*tert*-butyl dicarbonate in 2 mL of methylene chloride. The reaction mixture was stirred at room temperature for 3 h, quenched with saturated aqueous NH_4Cl, and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous NH_4Cl and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel (hexane:EtOAc = 5:1) to afford product **8** as a colorless oil (452 mg, 88%): ^1H NMR ($CDCl_3$) δ 1.06–1.21 (m, 21 H), 1.48 (s, 9H), 4.20–4.31 (m, 1H), 5.11 (d, *J* = 5.8 Hz, 1H), 5.99 (ddd, *J* = 54.2, 54.2, 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.74, 17.44, 17.50, 27.83, 57.32 (m), 76.02, 84.18, 147.51, 164.73; ^{19}F NMR ($CDCl_3$) δ −122.9 (ddd, *J* = 297.9, 53.8, 8.9 Hz, 1F), −127.4 (ddd, *J* = 297.9, 55.0, 8.5 Hz, 1F). Anal. calcd for $C_{18}H_{33}F_2NO_4Si$: C, 54.94; H, 8.45; N, 3.56. Found: C, 55.02; H, 8.33; N, 3.68.

General procedure for synthesis of 3′-dephenyl-3′-difluoro-taxoids 1 and 2. A general procedure is described for the synthesis of 3′-dephenyl-3′-difluoromethyl-docetaxel **1a**: To a solution of 7,10-di-TES baccatin III **3a** (54 mg, 0.07 mmol) and 4-difluoromethyl-β-lactam **8** (52 mg, 0.13 mmol) in 2 mL dry THF was added 1.0 M NaHMDS in THF (0.11 mL, 0.11 mmol) dropwise at −40 °C, and the solution was stirred at −40 °C for 30 min. The reaction was quenched with aqueous saturated ammonium chloride solution (5 mL), and the aqueous layer was extracted with dichloromethane (25 mL × 3). The combined extracts were then dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified on a silica gel column using hexane:ethyl acetate (6:1) as the eluant to afford the coupling product 2′-triisopropylsilyl-3′-difluoromethyl-7,10-di-triethylsilyldocetaxel as a white solid (66 mg, 82.5%): ^1H NMR ($CDCl_3$) δ 0.52–0.73 (m 12H), 0.92–1.21 (m, 45H), 1.30 (s, 9H), 1.61–1.65 (m, 4H), 1.82–1.89 (m, 4 H), 2.20–2.37 (m, 4H), 2.43–2.58 (m, 1H), 3.83 (d, *J* = 6.9 Hz, 1H), 4.15 (d, *J* = 8.3 Hz, 1H), 4.25–4.48 (m, 3H), 4.83 (br s, 1 H), 4.89 (d, *J* = 8.6 Hz, 1H), 5.00 (d, *J* = 10.2 Hz, 1H), 5.15 (s, 1H), 5.65 (d, *J* = 6.9 Hz, 1H), 5.77 (ddd, *J* = 56.1, 55.8, 6.3 Hz, 1H), 6.10 (t, *J* = 8.8 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 2H).

To a solution of coupling product thus obtained (66.0 mg, 0.057 mmol) in 4 mL of pyridine:acetonitrile (1:1) was added dropwise HF:pyridine (70:30, 0.6 mL) at 0 °C, and the mixture was stirred at room temperature for 12 h. The reaction was quenched with aqueous saturated sodium carbonate solution (5.0 mL). The mixture was then diluted with ethyl acetate (70 mL), washed with aqueous saturated copper sulfate solution (10 mL × 3) and water (10 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude product was purified on a silica gel column using hexane:ethyl acetate (1:1) as the eluant to afford new taxoid **1a** as a white solid (38.0 mg, 86% yield): mp 158.0–160.0 °C; $[\alpha]_D^{20}$ −88.5 (*c* 0.26, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.11 (s, 3H), 1.22 (s, 3H), 1.32 (s, 9H), 1.72–1.77 (m, 5H), 1.88 (s, 3H), 2.17–2.33 (m, 1H), 2.38 (s, 3H), 2.51–2.62 (m, 1H), 3.87 (d, *J* = 6.9 Hz, 1H), 4.00 (br s, 1H), 4.14–4.49 (m, 5H), 4.64 (br s, 1H), 4.94 (d, *J* = 8.7 Hz, 1H), 5.19 (d, *J* = 10.1 Hz, 1H), 5.27 (s, 1H), 5.65 (d, *J* = 6.9 Hz, 1H), 5.66–6.10 (m, 2H), 6.22 (t, *J* = 8.5 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 8.09 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (CDCl$_3$) δ 9.64, 14.46, 20.75, 22.44, 26.30, 28.02, 35.45, 36.86, 43.16, 46.56, 54.58 (m), 57.75, 68.53, 71.94, 72.87, 74.56, 74.86, 78.62, 80.78, 81.05, 84.21, 114.13, 118.03, 128.77, 129.08, 130.22, 133.71, 136.04, 138.31, 155.15, 167.13, 170.34, 172.64; ^{19}F NMR (CDCl$_3$) δ −128.6 (ddd, *J* = 287, 56.1, 8.3 Hz, 1F), −126.1 (ddd, *J* = 287, 55.5, 11.3 Hz, 1F). Anal. calcd for $C_{38}H_{49}F_2NO_{14}$: C, 58.38; H, 6.32. Found: C, 58.19; H, 6.51.

3′-Dephenyl-3′-difluoromethyl-10-acetyldocetaxel (1b). White solid; 54% yield (two steps); mp 158.0–160.0 °C; $[\alpha]_D^{20}$ −113 (*c* 0.16, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 1.15 (s, 3H), 1.25 (s, 3H), 1.31 (s, 9H), 1.68 (s, 3H), 1.89 (br s, 4 H), 2.24 (br s, 4H), 2.40 (s, 3H), 2.49–2.61 (m, 1H), 3.41 (br s, 1H), 3.81 (d, *J* = 6.9 Hz, 1H), 4.17 (d, *J* = 8.2 Hz, 1H), 4.31 (d, *J* = 8.2 Hz, 1H), 4.36–4.47 (m, 2H), 4.63 (s, 1H), 4.95 (d, *J* = 9.2 Hz, 1H), 5.04 (d, *J* = 10.1 Hz, 1H), 5.64–6.10 (m, 2H), 6.20–6.30 (m, 2H), 7.50 (t, *J* = 7.4 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 8.12 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (CDCl$_3$) δ 9.56, 14.83, 20.84, 21.90, 22.50, 26.72, 35.39, 35.59, 43.25, 45.65, 54.56 (m), 58.61, 68.42, 72.17, 72.94, 74.98, 75.51, 79.04, 81.07, 84.43, 114.09, 128.76, 129.06, 130.22, 133.26, 133.71, 141.67, 155.05, 167.18, 170.34, 171.25, 172.38, 203.61; ^{19}F NMR (CDCl$_3$) δ −126.24 (ddd, *J* = 287.2, 55.5, 11.7 Hz, 1F), −128.56 (ddd, *J* = 287.2, 56.2, 8.6 Hz, 1F). Anal. calcd for $C_{40}H_{51}F_2NO_{15}$: C, 58.32; H, 6.24. Found: C, 58.07; H, 6.01.

3′-Dephenyl-3′-difluoromethyl-10-(*N*,*N*-dimethyl-carbamoyl)docetaxel (1c). White solid; 51% yield (two steps); mp 168.0–170.0 °C; $[\alpha]_D^{20}$ −52.0 (*c* 0.25, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 1.15 (s, 3H), 1.24 (s, 3H), 1.30 (s, 9H), 1.66 (s, 3H), 1.74–1.82 (m, 1H), 1.90 (br s, 4H), 2.21–2.37 (m, 1H), 2.39 (s, 3H), 2.44–2.58 (m, 1H), 2.95 (s, 3H), 3.04 (s, 3H), 3.58 (br s, 1H), 3.80 (d, *J* = 6.9 Hz, 1H), 4.16 (d, *J* = 8.3 Hz, 1H), 4.30 (d, *J* = 8.3 Hz, 1H), 4.38–4.49 (m, 2H), 4.63 (br s, 1H), 4.96 (d, *J* = 8.6 Hz, 1 H), 5.13 (d, *J* = 10.1 Hz, 1H), 5.63–6.10 (m, 2H), 6.18–6.29 (m, 2H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (CDCl$_3$) δ 9.38, 14.90, 22.32, 22.51, 26.70, 28.00, 35.40, 36.06, 36.66, 43.25, 45.63, 54.56 (m), 58.51, 68.45, 68.64, 72.46, 72.90, 75.11, 76.12, 79.13, 80.85, 81.10, 84.67, 110.24, 114.14, 116.04, 128.75, 129.06, 130.24, 133.54, 133.68, 142.26, 155.09, 156.12, 167.15, 170.29, 172.34, 205.59; ^{19}F NMR (CDCl$_3$) δ −126.2 (ddd, *J* = 286.7, 55.7, 10.6 Hz, 1F), −128.2 (ddd, *J* = 286.7, 56.0, 9.2 Hz, 1F). Anal. calcd for $C_{41}H_{54}F_2NO_{15}$: C, 57.75; H, 6.38. Found: C, 57.53; H, 6.38.

3′-Dephenyl-3′-difluoromethyl-10-propanoyldocetaxel (1d). White solid; 62% yield (two steps); mp 134.0–136.0 °C; $[\alpha]_D^{20}$ −108 (*c* 0.26, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 0.94 (t, *J* = 7.2 Hz, 3H), 1.14 (s, 3H), 1.21–1.52 (m, 14H), 1.69 (br s, 4H), 1.90 (br s, 4H), 2.21–2.59 (m, 7H), 3.51 (br s, 1H), 3.81 (d, *J* = 6.8 Hz, 1H), 4.16 (d, *J* = 8.3 Hz, 1H), 4.30 (d, *J* = 8.3 Hz, 1H), 4.39–4.47 (m, 2H), 4.62 (br s, 1H), 4.94 (d, *J* = 9.4 Hz, 1H), 5.11 (d, *J* = 10.0 Hz, 1H), 5.63–6.10 (m, 2H), 6.20–6.29 (m, 2H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (CDCl$_3$) δ 9.56, 13.71, 14.80, 21.93, 22.20, 22.50, 26.72, 26.88, 28.00, 33.90, 35.41, 35.56, 43.24, 45.68, 54.58 (m), 58.59, 68.43, 72.18, 72.91, 75.00, 75.27, 79.03, 80.93, 81.09, 84.46, 114.11, 128.75, 129.04, 130.22, 133.38, 133.70, 141.73, 155.06, 167.16, 170.36, 172.35, 174.01, 203.51; ^{19}F NMR (CDCl$_3$) δ −126.2 (ddd, *J* = 286.9, 55.5, 11.4 Hz, 1F), −128.6 (ddd, *J* = 286.9, 56.0, 9.4 Hz, 1F). Anal. calcd for $C_{41}H_{53}F_2NO_{15}$: C, 58.77; H, 6.38. Found: C, 58.51; H, 6.45.

3′-Dephenyl-3′-difluoromethyl-10-(3,3-dimethylbutanoyl)-docetaxel (1e). White solid; 57% yield (two steps); mp 141.0–143.0 °C; $[\alpha]_D^{20}$ −86.3 (*c* 0.51, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 1.10 (s, 9H), 1.14 (s, 3H), 1.23 (s, 3H), 1.30 (s, 9H), 1.67–1.73 (m, 4H), 1.87 (br s, 4H), 2.19–2.46 (m, 6H), 2.49–2.68 (m, 1H), 3.46 (br s, 1H), 3.81 (d, *J* = 6.9 Hz, 1H), 4.17 (d, *J* = 8.3 Hz, 1H), 4.30 (d, *J* = 8.3 Hz, 1H), 4.38–4.52 (m, 2 H), 4.63 (br s, 1H), 4.95 (d, *J* = 9.3 Hz, 1H), 5.06 (d, *J* = 10.0 Hz, 1H), 5.64–6.10 (m, 2H), 6.20–6.27 (m, 2H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (CDCl$_3$) δ 9.58, 144.20, 14.80, 22.00, 22.51, 26.78, 28.00, 29.64, 31.03, 35.48, 43.22, 45.64, 47.93, 54.58 (m), 58.62, 68.53, 72.24, 72.93, 75.02, 75.18, 76.51, 79.07, 80.94, 81.18, 84.49, 114.11, 128.76, 129.05, 130.22, 133.57, 133.70, 141.69, 155.05, 167.16, 170.35, 172.34, 172.47, 203.52; ^{19}F NMR (CDCl$_3$) δ −126.2 (ddd, *J* = 287.2, 55.5, 11.4 Hz, 1F), −128.5 (ddd, *J* = 287.2, 56.3, 8.5 Hz, 1F). Anal. calcd for $C_{44}H_{59}F_2NO_{15}$: C, 60.06; H, 6.76. Found: C, 60.12; H, 6.99.

3′-Dephenyl-3′-difluoromethyldocetaxel-1,14-carbonate (2a). White solid; 59% yield (two steps); mp 184–187 °C; $[\alpha]_D^{20}$ −25.0 (*c* 0.32, CHCl$_3$); ^{1}H NMR (CD$_3$OD) δ 1.25 (s, 3H), 1.27 (s, 3H), 1.37 (s, 9H), 1.43 (m, 1H), 1.73 (s, 3H), 1.79 (m, 1H), 1.95 (s, 3H), 2.44 (m, 1H), 2.52 (s, 3H), 3.84 (d, *J* = 7.5 Hz, 1H), 4.24 (m, 3H), 4.52 (m, 1H), 4.71 (br s, 1H), 4.91 (d, *J* = 6.8 Hz, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 5.29 (s, 1H), 5.69–6.17 (dt, *J* = 56.4, 6.5 Hz, 1H), 6.07 (d, *J* = 7.5 Hz, 1H), 6.41 (d, *J* = 6.5 Hz, 1H), 6.80 (d, *J* = 10.0 Hz, 1H), 7.55 (t, *J* = 7.4 Hz, 2H), 7.67 (t, *J* = 7.1 Hz, 1H), 8.10 (d, *J* = 7.7 Hz, 2H); ^{13}C NMR (CD$_3$OD) δ 10.5, 14.6, 22.5, 23.0, 26.2, 28.7, 37.3, 43.0, 49.4, 59.3, 70.2, 70.8, 72.3, 75.3, 75.6, 77.0, 81.3, 81.8, 85.7, 87.4, 89.7, 103.2, 116.4, 129.6, 130.2, 131.2, 135.4, 136.8, 138.2, 165.8, 166.4, 167.4, 169.0, 172.4, 209.3. Anal. calcd for $C_{39}H_{47}O_{16}N_1F_2$: C, 56.86; H, 5.75; N, 1.70. Found: C, 57.09; H, 5.73; N, 1.68.

3′-Dephenyl-3′-difluoromethyl-10-acetyldocetaxel-1,14-carbonate (2b). White solid; 86% yield (two steps); mp 188–189 °C; $[\alpha]_D^{20}$ −40.0 (*c* 0.15, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 1.27 (s, 3H), 1.32 (s, 12H), 1.71 (s, 3H), 1.85 (m, 1H), 1.89 (s, 3H), 2.24 (s, 3H), 2.49 (s, 3H), 2.54 (m, 1H), 3.71 (d, *J* = 7.3 Hz, 1H), 4.21 (d, *J* = 8.4 Hz, 1H), 4.28 (d, *J* = 8.4 Hz, 1H), 4.39 (dd, *J* = 10.5, 6.8 Hz, 1H), 4.50 (m, 1H), 4.72 (s, 1H), 4.84 (d, *J* = 6.6 Hz, 1H), 4.91 (d, *J* = 8.9 Hz, 1H), 5.27 (d, *J* = 10.0 Hz, 1H), 5.90 (td, *J* = 56.1, 5.5 Hz, 1H), 6.09 (d, *J* = 7.5 Hz, 1H), 6.27 (s, 1H), 6.44 (d, *J* = 6.2 Hz, 1H), 7.49 (t, *J* = 7.3 Hz, 2H), 7.60 (t, *J* = 7.2 Hz, 1H), 8.08 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.7, 14.8, 20.7, 22.5, 23.0, 25.8, 28.0, 35.5, 41.8, 45.2, 54.3 (m), 58.7, 68.9, 69.2, 71.7, 74.7, 75.0, 79.5, 80.5, 81.2, 84.2, 88.1, 110.3, 114.2, 118.1, 127.8, 129.0, 130.0, 133.7, 134.1, 139.0, 151.8, 155.3, 164.7, 170.9, 171.4, 202.0. Anal. calcd for $C_{41}H_{49}F_2NO_{17}$: C, 56.88; H, 5.70; N, 1.62. Found: C, 56.66; H, 5.88; N, 1.74.

3′-Dephenyl-3′-difluoromethyl-10-(*N*,*N*-dimethylcarbamoyl)docetaxel-1,14-carbonate (2c). White solid; 79% yield (two steps); mp 174–176 °C; $[\alpha]_D^{20}$ −45.0 (*c* 0.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 15H), 1.70 (s, 3H), 1.83 (m, 1H), 1.91 (s, 3H), 2.48 (s, 3H), 2.53 (m, 1H), 2.96 (s, 3H), 3.04 (s, 3H), 3.71 (d, *J* = 7.4 Hz, 1H), 4.21 (d, *J* = 8.4 Hz, 1H), 4.28 (d, *J* = 8.4 Hz, 1H), 4.44 (m, 2H), 4.70 (s, 1H), 4.84 (d, *J* = 6.6 Hz, 1H), 4.92 (d, *J* = 8.5 Hz, 1H), 5.44 (d, *J* = 10.0 Hz, 1H), 5.90 (td, *J* = 56.3, 5.1 Hz, 1H), 6.08 (d, *J* = 7.5 Hz, 1H), 6.22 (s, 1H), 6.46 (d, *J* = 5.9 Hz, 1H), 7.49 (t, *J* = 7.3 Hz, 2H), 7.60 (t, *J* = 7.2 Hz, 1H), 8.08 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.5, 14.9, 22.5, 23.4, 25.9, 28.0, 35.3, 36.1, 36.7, 41.8, 45.2, 54.3 (m), 58.6, 68.9, 69.3, 72.0, 74.9, 75.4, 75.9, 79.7, 80.6, 81.0, 84.5, 87.4, 88.3, 110.4, 114.3, 118.2, 127.9, 129.0, 130.0, 133.9, 134.1, 139.6, 152.0, 155.3, 155.8, 164.7, 170.9, 171.4, 203.9. Anal. calcd for $C_{42}H_{52}F_2NO_{17}$: C, 56.37; H, 5.86; N, 3.13. Found: C, 56.31; H, 5.91; N, 3.04.

3′-Dephenyl-3′-difluoromethyl-10-propanoyldocetaxel-1,14-carbonate (2d). White solid; 60% yield (two steps); mp 177–180 °C; $[\alpha]_D^{20}$ −62.5 (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.20–1.40 (m, 20H), 1.71 (s, 3H), 1.89 (s, 3H), 2.39 (m, 7H), 3.73 (m, 2H), 3.85 (s, 3H), 4.22 (dd, *J* = 17.2, 8.5 Hz, 2H), 4.39 (m, 1H), 4.50 (m, 1H), 4.71 (br s, 1H), 4.84 (d, *J* = 6.6 Hz, 1H), 4.90 (d, *J* = 8.6 Hz, 1H), 5.15 (d, *J* = 9.9 Hz, 1H), 5.67–6.07 (dt, *J* = 56.4, 6.5 Hz, 1H), 6.08 (d, *J* = 7.6 Hz, 1H), 6.27 (s, 1H), 6.44 (d, *J* = 6.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 7.1 Hz, 1H), 8.04 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.0, 9.7, 14.9, 22.5, 23.1, 25.9, 27.5, 28.1, 35.5, 41.8, 45.2, 58.7, 68.8, 69.3, 71.8, 74.5, 75.2, 76.0, 79.5, 80.6, 81.4, 84.3, 87.3, 88.1, 127.9, 129.0, 130.0, 134.1, 138.9, 151.7, 155.3, 164.7, 170.8, 171.5, 174.3, 202.1. Anal. calcd for $C_{42}H_{51}O_{17}N_1F_2$: C, 57.33; H, 5.84; N, 1.59. Found: C, 56.97; H, 5.87; N, 1.61.

3′-Dephenyl-3′-difluoromethyl-10-(3,3-dimethylbutanoyl)-docetaxel-1,14-carbonate (2e). White solid; 81% yield (two steps); mp 165–167 °C; $[\alpha]_D^{20}$ −51.4 (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 9H), 1.20 (s, 6H), 1.25 (s, 9H), 1.61 (s, 3H), 1.75 (m, 1H), 1.81 (s, 3H), 2.27 (s, 3H), 2.40 (s, 2H), 2.45 (m, 1H), 3.63 (d, *J* = 7.5 Hz, 1H), 4.11 (d, *J* = 8.5 Hz, 1H), 4.19 (d, *J* = 8.4 Hz, 1H), 4.25 (dd, *J* = 10.9, 6.7 Hz, 1H), 4.39 (m, 1H), 4.61 (s, 1H), 4.76 (d, *J* = 6.7 Hz, 1H), 4.85 (d, *J* = 8.9 Hz, 1H), 5.79 (td, *J* = 56.0, 5.8 Hz, 1H), 5.99 (d, *J* = 7.3 Hz, 1H), 6.23 (s, 1H), 6.31 (d, *J* = 6.1 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.52 (t, *J* = 7.3 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.5, 14.3, 22.0, 22.6, 25.5, 27.8, 29.3, 30.8, 35.5, 41.5, 45.5, 47.6, 54.3 (m), 58.3, 68.5, 69.2, 70.9, 74.2, 75.8, 79.6, 80.4, 80.6, 84.3, 88.1, 110.4, 114.3, 118.2, 127.6, 128.8, 129.7, 133.7, 134.0, 138.6, 151.9, 156.9, 164.8, 166.6, 170.9, 171.1, 171.5, 201.6. Anal. calcd for $C_{45}H_{57}F_2NO_{17}$: C, 58.63; H, 6.23; N, 1.52. Found: C, 58.48; H, 6.18; N, 1.51.

3′-Dephenyl-3′-difluoromethyl-10-cyclopropanylcarbonyl-docetaxel-1,14-carbonate (2f). White solid; 73% yield (two steps); mp 186–188 °C; $[\alpha]_D^{20}$ −40.4 (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (m, 1H), 1.03–1.44 (m, 21H), 1.70 (s, 3H), 1.79 (m, 1H), 1.89 (s, 3H), 2.44 (m, 4H), 3.72 (d, *J* = 7.5 Hz, 1H), 4.22 (dd, *J* = 17.2, 8.5 Hz, 2H), 4.39 (m, 1H), 4.50 (m, 1H), 4.71 (br s, 1H), 4.84 (d, *J* = 6.6 Hz, 1H), 4.90 (d, *J* = 8.6 Hz, 1H), 5.17 (d, *J* = 10.0 Hz, 1H), 5.67–6.07 (dt, *J* = 56.4, 6.5 Hz, 1H), 6.08 (d, *J* = 7.6 Hz, 1H), 6.25 (s, 1H), 6.44 (d, *J* = 6.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 7.1 Hz, 1H), 8.04 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 9.4, 9.7, 12.9, 14.9, 22.5, 23.2, 26.0, 28.1, 35.4, 41.8, 45.1, 58.7, 68.9, 69.3, 71.8, 74.5, 75.3, 76.0, 79.5, 84.3, 87.3, 88.1, 127.9, 129.0, 130.0, 134.1, 139.1, 152.0, 155.3, 164.5, 170.8, 171.5, 174.9, 202.2. Anal. calcd for $C_{43}H_{51}O_{17}N_1F_2$: C, 57.91; H, 5.76; N, 1.57. Found: C, 57.70; H, 5.89; N, 1.63.

Cytotoxicity assay

Materials and methods. Human tumor cell lines MDA435/LCC6-WT and MDR1 were provided by Dr. R. Clarke, Lombardi Cancer Center, Georgetown University School of Medicine. Cell lines were propagated as monolayers in RPMI-1640 containing 5% FCS, 5% NuSerum IV, 20 mM HEPES, 2 mM L-glutamine at 37 °C in a 5% CO_2 humidified atmosphere. The doubling times for the cell lines range between 20 and 24 h.

Growth inhibition assay. In 96 well microtiter plates assessment of cell growth inhibition was determined according to the methods of Skehan et al.[40] Briefly, cells were plated at 2000 cells/well in 96 well plates and incubated at 37 °C for 15–18 h prior to drug addition to allow cell attachment. Compounds were dissolved in 100% DMSO and further diluted in RPMI-1640 containing 10 mM HEPES. Each cell line was treated with 10 concentrations of compound (5 log range). After a 72 h incubation, 100 μL of ice-cold 50% TCA was added to each well and incubated for 1 h at 4 °C. Plates were then washed 5 times with tap water to remove TCA, low-molecular-weight metabolites and serum proteins. Then, 50 μL of 0.4% sulforhodamine B (SRB) was added to each well. Following a 5 min incubation at room temperature, plates were rinsed 5 times with 0.1% acetic acid and air dried. Bound dye was dissolved with 10 mM Tris base (pH 10.5) for 5 min on a gyratory shaker. Optical density was measured at 570 nm.

Data analysis. Data were fit with the Sigmoid-Emax concentration–effect model[44] with nonlinear regression, weighted by the reciprocal of the square of the predicted response. The fitting software was developed at RPCI with MicroSoft FORTRAN, and uses Marquardt[45] algorithm as adapted by Nash[46] for the nonlinear regression. The concentration of drug which resulted in 50% growth inhibition (IC_{50}) was calculated.

Apoptosis assay

Cell culture. Two breast cancer cell lines (MDA-MB 231 and MCF-7 ADRr) and one human leukemia cell line (CEM VBLr) were used. The MDA-MB 231 cells were purchased from the ATCC (Rockville, MD); the MDR line MCF-7 ADRr, selected for adriamycin (ADR) resistance, was kindly provided by Dr. K. H. Cowan (National Cancer Institute, NIH, Bethesda, MD).[47] MDA-MB 231 cells were grown in MEM and MCF-7 ADRr cells in RPMI 1640. Media were supplemented with 10% FCS and 200 U/mL penicillin. MCF-7 ADRr cells were grown in the presence of 10 μM ADR. Cells, propagated as monolayer culture in 75 cm^2 tissue-culture flasks, were trypsinized weekly and plated at a density of 8×10^4 cell/mL.

The human leukemia CEM cells and their P-gp counterpart, vinblastine-resistant subline CEM VBLr cells, were a gift of Dr. M. Cianfriglia (Istituto Superiore di Sanità, Rome, Italy). Cells were grown in RPMI 1640 medium supplemented with 10% FCS and 200 U/mL penicillin. To maintain the presence of P-gp, CEM VBLr cells were cultured in the presence of 100 ng/mL of vinblastine in RPMI 1640 supplemented with 10% FCS and 200 U/mL penicillin.

Cells were seeded at $2–3\times10^5$ cells/mL and split at a ratio of 1:3 everyday. All cells were incubated at 37 °C under 5% CO_2, 95% air in a high humidity atmosphere.

DNA fragmentation analysis. A typical procedure is described for CEM VBLr cells. CEM VBLr cells were plated at a concentration of 1×10^5 cells/mL in the specific medium supplemented as above, containing compounds to be tested or vehicle alone. After 72 h, cells were harvested and nuclei isolated and stained using a solution containing 0.1% sodium citrate, 0.1% NP40, 4 mM EDTA and 50 μg/mL propidium iodide (PI) as DNA dye. Incubation of cells with staining solution lasted a minimum of 12 h at 4 °C. DNA ploidy analysis was performed and a minimum of 20,000 nuclei acquired with an Epics-XL flow cytometer (Coulter, Hialeah, FL). DNA fluorescence was collected in linear mode, and pulse signal processing was used to set a doublet discrimination gate (data not shown). Cell cycle analysis was performed using the Multicycle software package (Phoenix, San Diego, CA).

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References

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2. Guéritte-Voegelein, F.; Mangatal, L.; Guénard, D.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. *Acta Crystall.* **1990**, *C46*, 781.
3. Rowinsky, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. *Seminars in Oncology* **1992**, *19*, 646.
4. Guénard, D.; Guéritte-Vogelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160.
5. Suffness, M. In *Annual Reports in Medicinal Chemistry*; J. A. Bristol, Ed.; Academic: San Diego, 1993; Vol. 28; Chapter 32, pp. 305–314.
6. Suffness, M. *Taxol: Science and Applications*. CRC Press: New York, 1995.
7. Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. *Taxane Anticancer Agents: Basic Science and Current Status*. American Chemical Society: Washington, DC, 1995.
8. Kingston, D. G. I. In *Taxane Anticancer Agents: Basic Science and Current Status; ACS Symp. Ser. 583*; G. I. Georg; T. T. Chen; I. Ojima and D. M. Vyas, Eds.; American Chemical Society: Washington, DC, 1995; pp 203–216.
9. Holton, R. A.; Biediger, R. J.; Boatman, P. D. In *Taxol®: Science and Applications*; M. Suffness, Ed.; CRC Press: New York, 1995; pp 97–121.
10. Boge, T. C.; Georg, G. I. In *Enantioselective Synthesis of β-Amino Acids*; E. Juaristi, Ed.; Wiley-VCH: New York, 1997; pp 1–43.
11. Ojima, I.; Kuduk, S. D.; Chakravarty, S. In *Adv. Med. Chem.*; B. E. Maryanoff and A. B. Reitz, Eds.; JAI Press: Greenwich, CT, 1998; Vol. 4, pp 69–124.
12. Ojima, I.; Lin, S.; Wang, T. *Curr. Med. Chem.* **1999**, *6*, 927.
13. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
14. Horwitz, S. B. In *Stony Brook Symposium on Taxol and Taxotère*; Stony Brook: NY, 14–15 May 1993; Abstracts 23–24.
15. Arbuck, S. G.; Blaylock, B. A. In *Taxol®: Science and Applications*; M. Suffness, Ed.; CRC Press: Boca Raton, 1995; pp 379–415.
16. Verweij, J.; Clavel, M.; Chevalier, B. *Ann. Oncol.* **1994**, *5*, 495–505.
17. Seidman, A. D. In *Stony Brook Symposium on Taxol and Taxotère*; Stony Brook, NY, 14–15 May 1993; Abstracts pp 14–16.
18. Ojima, I.; Kuduk, S. D.; Slater, J. C.; Gimi, R. H.; Sun, C. M. *Tetrahedron* **1996**, *52*, 209.
19. Cresteil, T.; Monsarrat, B.; Alvinerie, P.; Tréluyer, J. M.; Vieira, I.; Wright, M. *Cancer Res.* **1994**, *54*, 386.
20. Vuilhorgne, M.; Gaillard, C.; Sanderlink, G. J.; Royer, I.; Monsarrat, B.; Dubois, J.; Wright, M. In *Taxane Anticancer Agents: Basic Science and Current Status*. American Chemical Society: Washington, DC, 1995; pp 98–110.
21. Ojima, I.; Inoue, T.; Chakravarty, S. *J. Fluorine Chem.* **1999**, *97*, 3.
22. Ojima, I.; Kuduk, S. D.; Slater, J. C.; Gimi, R. H.; Sun, C. M.; Chakravarty, S.; Ourevich, M.; Abouabdellah, A.; Bonnet-Delpon, D.; Bégué, J.-P.; Veith, J. M.; Pera, P.; Bernacki, R. J. In *Biomedical Frontiers of Fluorine Chemistry ACS Symp. Ser. 639*; I. Ojima; J. R. McCarthy and J. T. Welch, Eds.; American Chemical Society: Washington, DC, 1996; pp 228–243.

23. Ojima, I.; Kuduk, S. D.; Chakravarty, S.; Ourevitch, M.; Bégué, J.-P. *J. Am. Chem. Soc.* **1997**, *119*, 5519.

24. Ojima, I.; Inoue, T.; Slater, J. C.; Lin, S.; Kuduk, S. C.; Chakravarty, S.; Walsh, J. J.; Gilchrist, L.; McDermott, A. E.; Cresteil, T.; Monsarrat, B.; Pera, P.; Bernacki, R. J. In *Asymmetric Fluoroorganic Chemistry: Synthesis, Application, and Future Directions; ACS Symp. Ser. 746*; Ramachandran, Ed.; American Chemical Society: Washington, DC, 1999; Chapter 12.

25. Kant, J.; Huang, S.; Wong, H.; Fairchild, C.; Vyas, D.; Farina, V. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2471.

26. Chen, S.-H.; Huang, S.; Farina, V. *Tetrahedron Lett.* **1994**, *35*, 41.

27. Chen, S.-H.; Kadow, J. F.; Farina, V.; Fairchild, C. R.; Johnston, K. A. *J. Org. Chem.* **1994**, *59*, 6156.

28. Chen, S. H.; Kant, J.; Mamber, S. W.; Roth, G. P.; Wei, J.; Marshall, D.; Vyas, D.; Farina, V. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2223.

29. Chen, S.-H.; Wei, J.-M.; Long, B. H.; Fairchild, C. A.; Carboni, J.; Mamber, S. W.; Rose, W. C.; Johnston, K.; Casazza, A. M.; Kadow, J. F.; Farina, V.; Vyas, D. M.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2741.

30. Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C.-M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985.

31. Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 5917.

32. Kant, J.; O'Keeffe, W. S.; Chen, S.-H.; Farina, V.; Fairchild, C.; Johnston, K.; Kadow, J. F.; Long, B. H.; Vyas, D. *Tetrahedron Lett.* **1994**, *35*, 5543.

33. 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. *J. Med. Chem.* **1996**, *39*, 3889.

34. Ojima, I.; Slater, J. S.; Kuduk, S. D.; Takeuchi, C. S.; Gimi, R. H.; Sun, C.-M.; Park, Y. H.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Med. Chem.* **1997**, *40*, 267.

35. Ojima, I.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Slater, J. C.; Lavelle, F.; Veith, J. M.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2631.

36. Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224.

37. Ojima, I.; Lin, S.; Chakravarty, S.; Fenoglio, I.; Park, Y. H.; Sun, C.; Appendino, G.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Org. Chem.* **1998**, *63*, 1637.

38. Puech, F.; Gosselin, G.; Imbach, J.-L. *J. Chem. Soc., Chem. Commun.* **1989**, 955.

39. Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol®: Science and Applications*; M. Suffness, Ed.; CRC Press: New York, 1995; pp 317–375.

40. Skehan, P.; Streng, R.; Scudierok, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107.

41. Ireland, C. M.; Pittman, S. M. *Biochem. Pharmacol.* **1995**, *49*, 1491.

42. Darzynkiewicz, Z.; Bruno, S.; Del Bino, G.; Gorczyca, W.; Hotz, M. A.; Lassota, P.; Traganos, F. *Cytometry* **1992**, *13*, 795.

43. Distefano, M.; Scambia, G.; Ferlini, C.; Gaggini, C.; Vincenzo, R. D.; Riva, A.; Bombardelli, E.; Ojima, I.; Fattorossi, A.; Panici, P. B.; Mancuso, S. *Int. J. Cancer* **1997**, *72*, 844–850.

44. Holford, N. H. G.; Scheiner, L. B. *Clin. Pharmaco.* **1981**, *6*, 429.

45. Marquardt, D. W. *J. Soc. Ind. Appl. Math.* **1963**, *11*, 431.

46. Nash, J. C. *Compact Numerical Method for Computers: Linear Algebra and Function Minimisation*. John Wiley & Sons: New York, 1979.

47. Scambia, G.; Ranelletti, F.O.; Benedetti Panici, P.; De Vincenzo, R.; Bonanno, G.; Ferranadina, G.; Piantelli, M.; Bussa, S.; Rumi, C.; Cianfriglia, P.; Mancuso, S. *Cancer Chemother. Pharmacol.* **1994**, 459.