Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 171–178

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Bioactivity of 2,4-Diacyl Analogues of Paclitaxel

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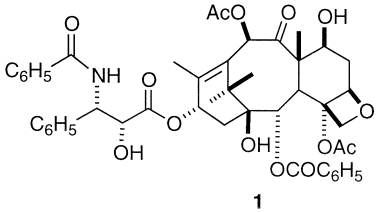
Received 2 June 2000; accepted 18 August 2000

Abstract—The 2,4-diacyl paclitaxel analogues **8a**–**8r** were prepared from paclitaxel by acylation of 4-deacetyl-2-debenzoylpaclitaxel 1,2-carbonate (**3**) followed either by hydrolysis of the carbonate and acylation or by direct treatment of the carbonate with an aryllithium. Some of the resulting derivatives showed significantly improved tubulin assembly activity and cytotoxicity as compared with paclitaxel; in some cases this improvement was especially significant for paclitaxel-resistant cell lines.

Introduction

Paclitaxel (Taxol®, **1**) has been recognized as one of the most important chemotherapeutic agents for clinical treatment of ovarian and breast cancer for the past decade. Its potential against non small-cell lung cancer, head and neck cancer, and other types of cancer is also under various stages of clinical investigation. Tremendous chemical research efforts have been made in the past years, which have established the fundamental structure–activity relationships of the paclitaxel molecule, and have provided analogues for biochemical studies to elucidate the precise mechanism of action for the development of second-generation agents.[1] These studies have shown that both the C-4 acetate group[2,3] and the C-2 benzoyl group[4] are important contributors to paclitaxel's activity. Paclitaxel analogues with other C-2[4,5] and C-4[3] acyl groups have also been made, and many of these are as active or even more active than paclitaxel; very recently the carbonate derivative **2** was disclosed by Bristol-Myers Squibb as a clinical candidate.[6] These results suggest that further exploration of the effect of different acyl groups at the C-2 and C-4 positions could be fruitful for the discovery of further candidates for drug development.

Up to this point most of the structure–activity studies of paclitaxel have focused on changing only one functional group, although recently some studies that involve the manipulation of more than one group have appeared.[5,7] We thus elected to investigate the effect of modifying both the C-2 and C-4 acyl groups, in the expectation that some of these might prove to have superior activity to paclitaxel. We also elected to investigate the synthesis of two C-4 analogues which would be expected to have improved water-solubility as compared with paclitaxel. A number of paclitaxel derivatives[8] and prodrugs[9] have been prepared by attaching functional groups that could increase the molecule's water solubility at C-2′, C-7, or C-10 positions, but the C-4 position has not yet been investigated for this purpose.



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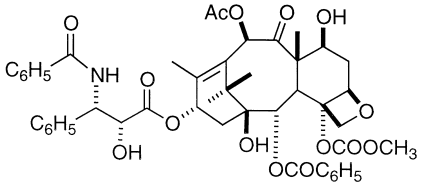

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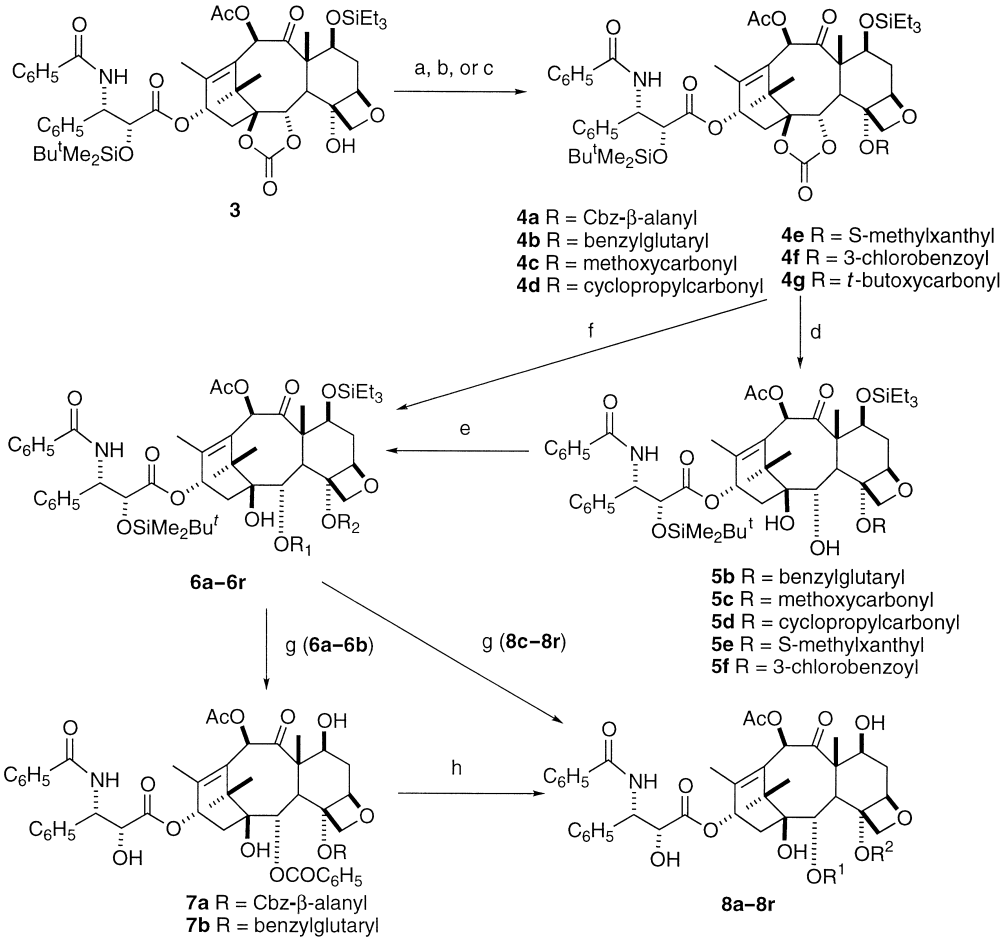
0968-0896/01/$ - see front matter
PII: S0968-0896(00)00233-9

Results and Discussion

Synthesis of 2,4-diacyl analogues of paclitaxel

Since we wished to modify both the C-2 and C-4 positions, we elected to use a modification of Holton's carbonate protection method[10] for this purpose. Protection of paclitaxel as its 2′-(*tert*-butyldimethylsilyl)-7-(triethylsilyl) derivative, followed by selective hydrolysis with Triton B and reaction with carbonyldiimidazole gave the cyclic carbonate **3** in approximately 50% overall yield from paclitaxel.[11]

Compound **3** was acylated at the C-4 position by one of three different acylation methods. In one method, designated method A and exemplified by the synthesis of the water-soluble precursors **4a** and **4b**, compound **3** was treated with 10 equivalents of monobenzyl glutaric acid or *N*-carbobenzyloxy-β-alanine, 12 equivalents of dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-pyrrolidinopyridine for 24 h at room temperature to afford compounds **4a** and **4b** in 72–87% yield (Scheme 1). In a second method, designated method B and exemplified by the synthesis of the compounds **4c**, **4d**, **4f** and **4g**, compound **3** was treated with LHMDS in THF at −78 °C, followed by treatment with the appropriate acid chloride or with di-*t*-butyldicarbonate. Method C was used to prepare the xanthate **4e**; in this method compound **3** was treated with sodium hydride



6a, 8a R^1 = benzoyl, R^2 = β-alanyl
6b, 8b R^1 = benzoyl, R^2 = glutaryl
6c, 8c R^1 = 3-methoxybenzoyl, R^2 = methoxycarbonyl
6d, 8d R^1 = 3-methylbenzoyl, R^2 = methoxycarbonyl
6e, 8e R^1 = 3-chlorobenzoyl, R^2 = methoxycarbonyl
6f, 8f R^1 = 3-azidobenzoyl, R^2 = methoxycarbonyl
6g, 8g R^1 = 3,3-dimethylacryloyl, R^2 = methoxycarbonyl
6h, 8h R^1 = 3-methoxybenzoyl, R^2 = cyclopropylcarbonyl
6i, 8i R^1 = 3-azidobenzoyl, R^2 = cyclopropylcarbonyl
6j, 8j R^1 = 3-chlorobenzoyl, R^2 = cyclopropylcarbonyl
6k, 8k R^1 = 2,4-difluorobenzoyl, R^2 = cyclopropylcarbonyl
6l, 8l R^1 = 2,5-dimethoxybenzoyl, R^2 = cyclopropylcarbonyl
6m, 8m R^1 = 2,4-dichlorobenzoyl, R^2 = cyclopropylcarbonyl
6n, 8n R^1 = 3-azidobenzoyl, R^2 = S-methylxanthyl
6o, 8o R^1 = 3-chlorobenzoyl, R^2 = S-methylxanthyl
6p, 8p R^1 = 3-methoxybenzoyl, R^2 = S-methylxanthyl
6q , 8q R^1 = 3-chlorobenzoyl, R^2 = 3-chlorobenzoyl
6r, 8r R^1 = 3-methoxybenzoyl, R^2 = t-butoxycarbonyl

(a) DCC, RCOOH, cat. 4-PP, toluene, 12–24 h; (b) LHMDS/THF, -78°C, RCOCl; (c) NaH/THF, CS_2, MeI; (d) LiOH, THF/H_2O; (e) RCOOH, DCC/DMAP or DCC/4-PP, toluene; (f) ArLi, THF, -78°C; (g) HF-py, THF; (h) H_2, Pd-C, EtOAc.

Scheme 1.

at room temperature, followed by treatment with carbon disulfide and methyl iodide.[12]

To complete the preparation of the analogues, it was necessary to install the C-2 benzoyl, substituted benzoyl, or acyl group on the C-2 position. This was accomplished in two different ways. In method D, used for the synthesis of compounds **6b**, **f**, **g**, **i**–**q**, the 1,2-carbonate was hydrolyzed with LiOH to give the diols **5b**–**5f**. These diols were then acylated with the appropriate carboxylic acids in the presence of DCC and DMAP to give the C-2 derivatives **6b**, **f**, **g**, **i**–**q**. As an example, hydrolysis of the 1,2-carbonate **4b** with lithium hydroxide afforded the 1,2-diol **5b** in 85% yield. Benzoylation at C-2 provided the C-4 acid **6b** in 61% yield from **5b** (Scheme 1).

In method E the carbonate **4** was treated directly with the desired aryllithium compound, as first reported by Holton.[10] Thus treatment of the β-alanyl derivative **4a** with phenyllithium at −78 °C for 15 min gave the protected 4-deacetyl-4-β-alanyl-paclitaxel derivative **6a** in 80% yield (Scheme 1).

Conversion to the final products **8a**–**8r** was accomplished by deprotection of the silyl groups with HF/pyridine. In the case of the β-alanyl and glutaryl derivatives **6a** and **6b** the 2′-*O* and 7-*O* silyl protecting groups were first removed with HF/pyridine or HCl/MeOH to give the derivatives **7a** and **7b**, and the benzyl or carbobenzyloxy protecting groups were then removed by hydrogenation to give the final products **8**a and **8b**

Bioactivity of 2,4-diacyl analogues of paclitaxel

The bioactivities of the 2,4-diacylpaclitaxels were evaluated in two different assay systems. The first assay was for their ability to assemble microtubular protein (tubulin unresolved from microtubule-associated proteins) at 37 °C. Cytotoxicity assays were obtained against colon carcinoma using both normal and paclitaxel-resistant HCT 116 cells. These data are summarized in the Table 1, together with the data for paclitaxel itself for comparison.

Looking first at the tubulin-assembly data, it can be seen that several of the analogues have improved assembly ability as compared with paclitaxel. This is true in particular for the methoxy carbonyl analogues **8c**–**8g** and the cyclopropanecarbonyl analogues **8h**–**8m**. These results are consistent with previous work which indicated that these two C-4 acyl groups have a beneficial effect on the activity of paclitaxel.[3c] It can also be noted that the effect of making changes at both the C-2 and C-4 positions appears to be additive, at least to some extent. Thus 2-debenzoyl-2-(3-azidobenzoyl)paclitaxel has an activity in the tubulin assembly assay that is about three times as great as paclitaxel (analogue/paclitaxel 0.37),[13] while 2-debenzoyl-4-deacetyl-2-(3-azidobenzoyl)-4-(cyclopropylcarbonyl) paclitaxel (**8i**) has an analogue/paclitaxel ratio of 0.08; this is approximately the product of the analogue/paclitaxel ratios of the two singly modified compounds.

Turning next to the cytotoxicity measurements, it can be noted that improved tubulin-assembly activity does not necessarily correlate with improved cytotoxicity against a colon carcinoma line HCT116. As an example, the compounds **8i** and **8j** both have excellent tubulin-assembly activity (12–16 times that of paclitaxel), but they are only modestly more cytotoxic than paclitaxel. The most interesting compound from a cytotoxicity point of view turns out to be **8k**, which is about as cytotoxic as paclitaxel against parental HCT116 tumor cells, but is

Table 1. Synthetic methods for and bioactivity of 2-aroyl-4-acylpaclitaxel analogues

	Method 3→4	Method 4→6	C-2 Substituent	C-4 Substituent	Tubulin data $EC_{0.01}$ (μM)	Anal/PT	HCT116 (nM)	116/VM46 (nM)	Anal/PT	*R*/*S* ratio
Taxol			Benzoyl	Acetyl	6.9±1.1	1	2.0	203	1	101
8a	A	E	Benzoyl	β-Alanyl	NT[a]	—	8.12	> 113	3.85	> 14
8b	A	D	Benzoyl	Glutaryl	NT	—	> 108	> 108	> 54	NM[b]
	NA	NA	Benzoyl[c]	Methoxycarbonyl	2.8	0.41	2	NT	1.0	—
8c	B	E	3-Methoxybenzoyl	Methoxycarbonyl	6.2	0.9	6.7	867	3.3	129
8d	B	E	3-Methylbenzoyl	Methoxycarbonyl	5.5	0.8	2.1	44	1	21
8e	B	E	3-Chlorobenzoyl	Methoxycarbonyl	1.7±0.2	0.4	2.2	82	1.1	37
8f	B	D	3-Azidobenzoyl	Methoxycarbonyl	NT	—	1.3	92	0.7	71
8g	B	D	3,3-Dimethylacryloyl	Methoxycarbonyl	NT	—	4.0	> 118	2.0	> 30
	NA	NA	Benzoyl[c]	Cyclopropylcarbonyl	1.6	0.24	1.0	NT	0.5	—
8h	B	E	3-Methoxybenzoyl	Cyclopropylcarbonyl	9.0	1.3	2	40	1	20
8i	B	D	3-Azidobenzoyl	Cyclopropylcarbonyl	0.55	0.08	1.7	14.4	0.9	8.5
8j	B	D	3-Chlorobenzoyl	Cyclopropylcarbonyl	0.41	0.06	1.0	16.2	0.5	16.2
8k	B	D	2,4-Difluorobenzoyl	Cyclopropylcarbonyl	1.3±0.1	0.2	2.1	8.2	1.0	3.9
8l	B	D	2,5-Dimethoxybenzoyl	Cyclopropylcarbonyl	3.4±0.8	0.5	1.8	45.1	0.9	25.1
8m	B	D	2,4-Dichlorobenzoyl	Cyclopropylcarbonyl	5.4±2.9	0.7	2.5	76.6	1.3	30.6
8n	C	D	3-Azidobenzoyl	*S*-Methylxanthyl	250±0	42	16.8	134.8	8.4	8.03
8o	C	D	3-Chlorobenzoyl	*S*-Methylxanthyl	21±5.9	3.5	5.7	58.6	2.9	10.3
8p	C	D	3-Methoxybenzoyl	*S*-Methylxanthyl	53±41	8.9	4.7	53.2	2.4	11.3
8q	A	D	3-Chlorobenzoyl	3-Chlorobenzoyl	420±140	144	2430	2460	1210	NM[b]
8r	B	E	3-Methoxybenzoyl	*t*-Butoxycarbonyl	NT	—	> 106	> 106	> 50	NM[b]

[a]NT (the sample was not tested in this assay system).
[b]NM (the data is not meaningful).
[c]Data for this compound from ref 3c.

significantly more cytotoxic than paclitaxel to a paclitaxel resistant variant HCT116/VM46. This multidrug resistant cell line exhibits overexpression of P-glycoprotein, a cell surface drug efflux pump, which limits the intracellular accumulation of paclitaxel and other lipophilic anticancer agents. It is possible that this derivative, and others which demonstrate a more modest decrease in resistance, are pumped out less well by P-glycoprotein, contributing to the diminished resistance. Thus, compounds of this type may be of interest in the future as candidates for drug development.

Experimental

General experimental methods

Unless otherwise noted, all materials were used as received from a commercial supplier without further purification. All anhydrous reactions were performed in oven-dried glassware under argon. Anhydrous tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone. Anhydrous toluene was distilled from sodium. Dichloromethane was distilled from calcium hydride. All reactions were monitored by E. Merck analytical thin-layer chromatography (TLC) plates (silica gel 60 GF, aluminum back) and analyzed with 254 nm UV light and/or vanillin/sulfuric acid spray. Silica gel for column chromatography was purchased from E. Merck (230–400 mesh). Preparative thin-layer chromatography (PTLC) plates (silica gel 60 GF) were purchased from Analtech. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ on a Varian Unity 400 spectrometer (operating at 399.951 MHz for ^{1}H and 100.578 MHz for ^{13}C) or a Bruker WP 360 spectrometer (operating at 360.140 MHz for ^{1}H and 90.562 MHz for ^{13}C), and were assigned by comparison of chemical shifts and coupling constants with those of related compounds and by appropriate 2D-NMR techniques. All 2D-NMR spectra were obtained on the Varian Unity 400 spectrometer. Chemical shifts were reported as δ-values using residual $CHCl_3$ as internal reference, and coupling constants were reported in Hertz. Mass spectra (LRFABMS/HRFABMS) were obtained at the Nebraska Center for Mass Spectrometry, University of Nebraska.

Tubulin-assembly assay

Twice cycled microtubule protein was prepared following the procedure of Williams and Lee[14] and stored in liquid nitrogen before use. Quantification of tubulin polymerization potency was accomplished following a modified procedure of Swindell et al.[15] These modifications, in part, result in the expression of tubulin polymerization potency as an effective concentration for any given compound. For this method, different compound concentrations in polymerization buffer (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, pH 6.6) were added to microtubule protein in polymerization buffer at 37 °C in microcuvette wells of a Beckman (Beckman Instruments) Model DU 7400 UV spectrophotometer. A final microtubule protein concentration of 1.0 mg/mL and compound concentrations of 2.5, 5.0, and 10 μM were used. Initial slopes of absorbance change measured every 10 s were calculated by the program accompanying the instrument after initial and final times of the linear region encompassing at least 3 time points were manually defined. Under these conditions linear variances were generally $< 10^{-6}$, slopes ranged from 0.03 to 0.002 A unit/min, and maximum absorbance was 0.15 A unit. Effective concentration ($EC_{0.01}$) is defined as the interpolated concentration capable of inducing an initial slope of 0.01 A unit/min and is calculated using the formula: $EC_{0.01}$ = concentration/slope.

Cytotoxicity assay

Human colon carcinoma lines HCT116 and HCT116/VM46 were maintained in RPMI 1640 media/10% fetal bovine serum at 37 °C under 5% CO_2. For the assay, cells were seeded in 96-well microtiter plates and 24 h later test compounds were added. After a 72 h drug exposure, live cells were quantified by tetrazolium dye conversion using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2*H*-tetrazolium, inner salt) and measuring the difference in optical density (OD). The results were expressed as IC_{50} values; the drug concentration required to inhibit the OD by 50% relative to no drug control. The average IC_{50} value for paclitaxel in the experiments presented here was 2.0 nM.

Synthetic procedures

In order to conserve space, representative examples only of the various synthetic procedures used are given below. All isolated final products **8a**–**8r** were characterized by HRMS and by ^{1}H NMR spectroscopy, and had spectroscopic data consistent with their assigned structures. All final compounds tested for bioactivity had a purity >95%, as determined from their ^{1}H NMR spectra.

Acylation at C-4 by method A. 2′-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(*O*-benzylglutaryl)-paclitaxel (4b). To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-paclitaxel[11] (**3**, 57 mg, 0.059 mmol) in dry toluene (3.0 mL) was added DCC (310 mg, 1.50 mmol), 4-PP (7 mg, catalytic amount), and glutaric acid monobenzyl ester (133 μL, 0.72 mmol). The resulting suspension was stirred at room temperature for 24 h and was then filtered through a pad of silica gel and rinsed with EtOAc. The filtrate was concentrated and purified by preparative TLC (silica gel, 1000 μM; EtOAc: hexanes, 4:6) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(*O*-benzylglutaryl)-paclitaxel (**4b**, 60 mg, 87%). ^{1}H NMR ($CDCl_3$, 399.951 MHz) δ 7.77 (m, 2H), 7.55–7.27 (m, 13H), 7.01 (d, J = 10.4 Hz, 1H), 6.42 (s, 1H), 6.12 (t, J = 9.6 Hz, 1H), 5.62 (dd, J = 10.4, 2.4 Hz, 1H), 5.08 (AB q, 2H), 4.91 (d, J = 9.6 Hz, 1H), 4.64 (d, J = 9.6 Hz, 1H), 4.63 (d, J = 2.4 Hz, 1H), 4.50 (d, J = 6.0 Hz, 1H), 4.49 (d, J = 9.6 Hz, 1H), 4.41 (dd, J = 10.8 Hz, 8.0, 1H), 3.45 (d, J = 6.4 Hz, 1H), 2.76 (m, 1H), 2.61–1.92 (m, 9H), 2.15 (s, 3H), 2.00 (s, 3H), 1.75 (s, 3H), 1.32 (s, 3H), 1.22 (s, 3H), 0.91 (t, J = 8.8 Hz, 9H), 0.83 (s, 9H), 0.57 (q, J = 8.8 Hz, 6H), −0.02 (s, 3H), −0.20 (s, 3H); ^{13}C NMR ($CDCl_3$,

90.562 MHz) δ 201.9, 172.31, 172.27, 171.8, 169.0, 167.0, 152.6, 143.7, 137.9, 131.8, 131.2, 128.8, 128.7, 128.6, 128.4, 128.24, 128.18, 127.0, 126.8, 89.7, 84.2, 81.3, 80.1, 77.2, 76.3, 75.6, 74.6, 71.5, 70.6, 66.5, 60.0, 55.7, 43.4, 41.5, 37.9, 35.1, 32.9, 32.3, 25.5, 25.4, 21.0, 20.7, 18.2, 14.8, 10.1, 6.7, 5.2, −5.1, −5.5.

Acylation at C-4 by method B. 2′-*tert*-Butyldimethylsilyl-7-triethylsilyl-4-acylpaclitaxel 1,2-carbonate derivatives (4c, 4d). To a solution of compound **3** (32.0 mg, 0.033 mmol) at −78 °C in freshly distilled anhydrous THF (0.5 mL) was added lithium hexamethyldisilamide (0.125 mmol) via syringe under argon. The mixture was stirred at −78 °C for 15 min. The desired electrophile (cyclopropyl carbonyl chloride or methyl chloroformate, 0.250 mmol) was added via syringe and the mixture was further stirred for 15 min at −78 °C. The reaction mixture was then allowed to warm to 0 °C, diluted with EtOAc (5 mL), and quenched with water. The organic layer was washed with dil. HCl (1 N), dil. $NaHCO_3$, water, and finally brine. The organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude product. The crude product thus obtained was purified using PTLC (silica gel, 500 μM; ethyl acetate:hexanes, 1:3) to yield compounds **4c** and **4d** (80–90%).

Acylation at C-4 by method C. 4-Deacetyl-4-(*S*-methylxanthyl)-2′-*tert*-butyl-dimethylsilyl-7-triethylsilylpaclitaxel-1,2-carbonate (4e). 4-Deacetyl-2′-*tert*-butyldimethyl-silyl-7-triethylsilylpaclitaxel 1,2-carbonate (**3**) (80.0 mg, 0.0825 mmol) was dissolved in freshly distilled dry THF (0.5 mL). Sodium hydride (6.0 mg, 0.250 mmol) was added at room temperature, followed by carbon disulfide (0.2 mL, excess). After stirring for 5 min at room temperature methyl iodide (0.1 mL, excess) was added and the mixture stirred at room temperature. Reaction was completed in 1 h. The mixture was diluted with EtOAc and washed with dil HCl, water and brine. The organic layer was separated, dried over Na_2SO_4, and evaporated. The crude product thus obtained was purified by preparative TLC (1000 μM; silica gel; EtOAc:hexanes, 1:3) to yield amorphous solid **4e** (77.4 mg, 90%). FABMS *m/z* (rel. int.) $[M+H]^+$ 1052 (7), 1022 (15), 514 (4), 400 (20), 354 (75), 105 (100); HRFABMS *m/z* $[M+H]^+$ 1052.4120 ($C_{53}H_{73}NO_{13}S_2Si_2$ requires 1052.4062).

Acylation at C-2 by method D. 2′-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel. To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(*O*-benzylglutaryl)-paclitaxel (**4b**, 30 mg, 0.025 mmol) in THF (1.5 mL) was added 7 drops of water and lithium hydroxide monohydrate ($LiOH{\cdot}H_2O$, 26 mg, excess). The mixture was stirred at room temperature for 2 h and was then diluted with EtOAc, washed successively with dilute HCl (1 N), saturated $NaHCO_3$, water, and brine. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ; EtOAc:hexanes, 6:4) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**5b**, 25 mg, 85%), which was subjected directly to the next reaction. To a solution of **5b** (30 mg, 0.026 mmol) in dry toluene (3.0 mL) was added DCC (64 mg, 0.31 mmol), DMAP (2 mg, catalytic amount), and benzoic acid (30 mg, 0.25 mmol). The resulting suspension was stirred at 80 °C for 48 h and was then purified by preparative TLC (silica gel, 1000 μM; EtOAc:hexanes, 4:6) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel **6b** (20 mg, 61%). ^1H NMR ($CDCl_3$, 399.951 MHz) δ 8.12 (m, 2H), 7.72 (m, 2H), 7.56–7.27 (m, 16H), 7.07 (d, $J=9.2$ Hz, 1H), 6.44 (s, 1H), 6.21 (t, $J=8.8$ Hz, 1H), 6.15 (br d, $J=7.6$ Hz, 1H), 5.68 (d, $J=8.0$ Hz, 1H), 5.09 (s, 2H), 4.88 (br d, $J=8.8$ Hz, 1H), 4.67 (d, $J=2.4$ Hz, 1H), 4.47 (dd, $J=10.4$ Hz, 6.8, 1H), 4.29 (d, $J=8.4$ Hz, 1H), 4.20 (d, $J=8.4$ Hz, 1H), 3.81 (d, $J=6.8$ Hz, 1H), 3.01 (m, 1H), 2.74 (m, 1H), 2.52–1.70 (m, 8H), 2.16 (s, 3H), 2.03 (s, 3H), 1.72 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.92 (t, 9H), 0.79 (s, 9H), 0.58 (q, 6H), −0.02 (s, 3H), −0.27 (s, 3H).

Acylation at C-2 by method E. 2′-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel. To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-1, 2-carbonato-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel (**4a**, 7.0 mg, 0.0060 mmol) in dry THF (1.0 mL) at −78 °C was added PhLi (1.8 M in hexanes, 20 μL, 0.036 mmol). The solution was stirred at −78 °C for 15 min, and was then quenched with dilute HCl (1 N) and extracted with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μM; EtOAc:hexanes, 4:6) to give 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel **6a** (6.0 mg, 80%) which was directly subjected to the next reaction.

4-Deacetyl-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel. A solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel (**6a**, 15 mg, 0.012 mmol) in freshly prepared 5% HCl/MeOH (1 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μM; EtOAc:hexanes, 7:3) to afford 4-deacetyl-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel **7a** (9.0 mg, 74%). ^1H NMR ($CDCl_3$, 399.951 MHz) δ 8.06 (d, $J=7.2$ Hz, 2H), 7.81 (d, $J=7.6$ Hz, 2H), 7.62–7.26 (m, 17H), 6.21 (s, 1H), 6.20 (t, $J=8.8$ Hz, 1H), 5.69 (dd, $J=8.4$, 4.0 Hz, 1H), 5.65 (d, $J=7.2$ Hz, 1H), 5.43 (m, 1H), 4.99 (br s, 2H), 4.89 (d, $J=8.0$ Hz, 1H), 4.72 (dd, $J=6.8$, 4.4 Hz, 1H), 4.38 (m, 1H), 4.27 (d, $J=8.8$ Hz, 1H), 4.17 (d, $J=8.8$ Hz, 1H), 4.15 (m, 1H), 3.70 (d, $J=7.2$ Hz, 1H), 3.57 (d, $J=6.8$ Hz, 1H), 3.56 (m, 1H), 2.86 (t, $J=6.8$ Hz, 2H), 2.52 (m, 1H), 2.48 (d, $J=4.0$ Hz, 1H), 2.23 (s, 3H), 2.12 (m, 1H), 2.05 (m, 1H), 1.87 (m, 1H), 1.75 (br s, 3H), 1.66 (s, 3H), 1.24 (s, 3H), 1.12 (s, 3H).

4-Deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (7b). To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**6b**, 6.0 mg,

0.0048 mmol) in dry THF (1 mL) was added HF-pyridine (70%, 150 μL, excess) and the solution was stirred at room temperature for 4 h. The reaction mixture was diluted with EtOAc and washed with saturated Na HCO_3 and dilute HCl (1 N), the organic layers were combined and washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 500 μM; EtOAc:hexanes, 7:3) to afford 4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel **7b** (3.5 mg, 71%). ^{1}H NMR ($CDCl_3$, 399.951 MHz) δ 8.11 (m, 2H), 7.75 (m, 2H), 7.60–7.27 (m, 16H), 7.09 (d, J = 8.4 Hz, 1H), 6.24 (s, 1H), 6.19 (t, J = 8.8 Hz, 1H), 5.73 (dd, J = 8.8, 3.2 Hz, 1H), 5.66 (d, J = 7.2 Hz, 1H), 5.07 (s, 2H), 4.87 (br d, J = 8.0 Hz, 1H), 4.77 (dd, J = 6.0, 3.2 Hz, 1H), 4.41 (m, 1H), 4.28 (d, J = 8.4 Hz, 1H), 4.18 (d, J = 8.4 Hz, 1H), 3.77 (d, J = 6.8 Hz, 1H), 3.65 (d, J = 6.0 Hz, 1H), 2.75–2.50 (m, 3H), 2.46 (d, J = 4.0 Hz, 1H), 2.44–1.84 (m, 7H), 2.24 (s, 3H), 1.76 (br s, 3H), 1.68 (s, 3H), 1.25 (s, 3H), 1.13 (s, 3H).

4-Deacetyl-4-glutaryl-paclitaxel (8b). A catalytic amount of palladium on activated carbon (5%) was added to a solution of 4-deacetyl-4-(*O*-benzylglutaryl)-paclitaxel (**7b**, 3.5 mg, 0.0035 mmol) in EtOAc (1 mL). This suspension was stirred in an atmosphere of hydrogen gas for 1 h. TLC analysis indicated complete conversion of the starting material to a very polar compound. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford 4-deacetyl-4-glutaryl-paclitaxel (**8b**, 3.0 mg, 94%). ^{1}H NMR ($CDCl_3$, 399.951 MHz) δ 8.10 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 7.6 Hz, 2H), 7.62–7.28 (m, 12H), 6.24 (s, 1H), 6.15 (t, J = 8.4 Hz, 1H), 5.70 (dd, J = 8.8, 4.8 Hz, 1H), 5.66 (d, J = 7.2 Hz, 1H), 4.88 (br d, J = 8.0 Hz, 1H), 4.77 (d, J = 4.0 Hz, 1H), 4.41 (m, 1H), 4.28 (d, J = 8.8 Hz, 1H), 4.19 (d, J = 8.8 Hz, 1H), 3.75 (d, J = 6.8 Hz, 1H), 3.42 (m, 1H), 2.75–2.40 (m, 5H), 2.23 (s, 3H), 2.20–1.89 (m, 5H), 1.75 (br s, 3H), 1.67 (s, 3H), 1.23 (s, 3H), 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 100.578 MHz) δ 203.8, 175.5, 173.8, 172.8, 172.3, 171.3, 167.2, 142.5, 137.4, 133.7, 132.9, 132.0, 130.1, 129.3, 128.8, 128.65, 128.61, 128.3, 127.3, 127.1, 84.6, 81.2, 77.8, 76.4, 75.5, 75.0, 73.2, 71.8, 71.7, 58.4, 55.9, 45.5, 43.8, 35.4, 34.6, 32.9, 29.9, 26.8, 22.1, 20.8, 20.7, 14.6, 9.6. HRFABMS calculated for $C_{50}H_{55}NO_{16}$ $(M + Li)^+$ 932.2931, found 932.2955, error 2.6 ppm.

4-Deacetyl-4-β-alanyl-paclitaxel (8a). A catalytic amount of palladium on activated carbon (5%) was added to a solution of 4-deacetyl-4-(*N*-carbobenzyloxy-β-alanyl)-paclitaxel (**7a**, 9.0 mg, 0.0089 mmol) in EtOAc (2 mL). This suspension was stirred in an atmosphere of hydrogen gas for 2 h. TLC analysis indicated complete conversion of the starting material to a very polar compound. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford 4-deacetyl-4-β-alanyl-paclitaxel (**8a**, 7.5 mg, 94%). ^{1}H NMR ($CDCl_3$, 399.951 MHz) δ 8.04 (m, 2H), 7.82 (m, 2H), 7.62–7.28 (m, 12H), 6.22 (s, 1H), 6.21 (t, J = 8.8 Hz, 1H), 5.73 (dd, J = 8.8, 4.0 Hz, 1H), 5.67 (d, J = 7.2 Hz, 1H), 4.93 (d, J = 8.0 Hz, 1H), 4.73 (d, J = 4.0 Hz, 1H), 4.41 (m, 1H), 4.29 (d, J = 8.4 Hz, 1H), 4.16 (d, J = 8.8 Hz, 1H), 3.70 (d, J = 7.6 Hz, 1H), 3.19 (m, 1H), 2.92–2.77 (m, 3H), 2.53 (m, 2H), 2.35–2.15 (m, 6H), 2.24 (s, 3H), 1.87 (m, 1H), 1.78 (br s, 3H), 1.66 (s, 3H), 1.26 (s, 3H), 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 90.562 MHz) δ 203.5, 172.8, 172.7, 171.4, 167.0, 166.4, 142.6, 139.0, 134.0, 133.8, 132.9, 131.7, 130.0, 129.1, 128.7, 128.64, 128.58, 128.0, 127.6, 127.0, 84.4, 81.5, 79.7, 77.2, 76.2, 75.4, 75.1, 73.5, 72.0, 71.1, 58.3, 55.6, 45.7, 43.2, 36.8, 36.3, 35.3, 34.8, 26.7, 22.4, 20.9, 14.7, 9.7. HRFABMS calculated for $C_{48}H_{54}N_2O_{14}$ $(M + H)^+$ 883.3653, found 883.3647, error 0.7 ppm.

2′-*O*-*tert*-Butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (5c). To a solution of 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-4-(methoxycarbonyl)-paclitaxel 1,2-carbonate (**4c**, 50 mg, 0.49 mmol) in THF (1 mL) and water (0.1 mL) was added LiOH (20 mg, 0.64 mmol) and stirred at room temperature for 1.5 h. The mixture was taken up in EtOAc (10 mL) and washed with water, brine and dried over sodium sulfate. The residue obtained after concentration was purified by PTLC (silica gel, 1000 μM; EtOAc:hexane, 2:3) to furnish 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**5c**, 20 mg, 59% yield based on the recovery of 15 mg unreacted starting compound) and 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-paclitaxel (6 mg). Compound **5c**: ^{1}H NMR: δ, −0.27 (s, 3H), −0.06 (s, 3H), 0.54–0.60 (m, 6H), 0.79 (s, 9H), 0.89–0.96 (m,9H), 1.03 (s, 3H), 1.09 (s, 3H), 1.22–1.27 (m, 1H), 1.85 (s, 3H), 1.86–1.95 (m, 1H), 2.04 (s, 3H), 2.08–2.14 (m, 1H), 2.12 (s, 3H), 2.25–2.29 (m, 1H), 2.45–2.53 (m, 1H), 3.24 (d, J = 4.3 Hz, 1H), 3.30 (s, 1H), 3.52 (d, J = 6.9 Hz, 1H), 3.86 (s, 3H), 3.92–3.95 (m, 1H), 4.37–4.41 (m, 1H), 4.52 (d, J = 1.4 Hz, 1H), 4.65 (d, J = 9.5 Hz, 1H), 4.70 (d, J = 9.6 Hz, 1H), 4.98 (d,J = 7.8 Hz, 1H), 5.70 (d, J = 9.3 Hz, m 1H), 6.18 (dd, J = 8.7, 8.2 Hz, 1H), 6.35 (s, 1H), 7.09 (d, J = 9.5 Hz, 1H), 7.29–7.52 (m, 8H), 7.72 (d, J = 7.2 Hz, 2H); ^{13}C NMR δ −5.78, −5.25, 5.23, 6.71, 10.32, 14.21, 18.14, 20.81, 21.12, 25.42, 25.48, 26.26, 35.41, 37.24, 42.92, 46.73, 55.41, 55.87, 58.09, 71.46, 72.05, 73.75, 74.96, 75.14, 77.78, 78.03, 83.66, 84.26, 126.22, 126.62, 126.91, 126.99, 127.93, 128.49, 128.59, 128.83, 131.86, 134.17, 143.20, 138.41, 139.72, 152.91, 167.56, 169.24, 171.51, 202.27; HRFABMS: m/z $[M + H]^+$ 994.4822 (C_{52} $H_{76}NO_{14}Si_2$ requires 994.4802).

2′-*O*-*tert*-Butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-2-(3-azidobenzoyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (6f). Toluene (0.8 mL) was added to a mixture of 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-4-(methoxycarbonyl)-paclitaxel **5c** (10 mg, 0.01 mmol), *m*-azidobenzoic acid (16 mg, 0.09 mmol), DCC (20 mg), and pyrrolidinopyridine (1.0 mg), and the reaction mixture was stirred at room temperature for 24 h and then purified by PTLC (silica gel, 1000 μM; EtOAc:hexane, 2:3) to yield 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-2-(3-azidobenzoyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**6f**, 9.0 mg, 79%). ^{1}H NMR: δ −0.30 (s, 3H), −0.03 (s, 3H), 0.54–0.61 (m, 6H), 0.78 (s, 9H), 0.91–0.94 (m,9H), 1.15 (s, 3H), 1.22 (s, 3H), 1.66–1.68 (m, 1H), 1.70 (s, 3H), 1.81 (s, 3H), 1.89–1.96 (m, 1H), 2.06 (s, 3H), 2.12–2.17 (m, 1H), 2.17 (s, 3H), 2.38–2.44 (m, 1H), 2.52–2.55 (m, 1H), 3.94 (d,

J = 6.87 Hz, 1H), 4.03 (s, 3H), 4.21 (d, J = 8.85 Hz, 1H), 4.37 (d, J = 8.24 Hz, 1H), 4.43–4.48 (dd, J = 6.87, 6.56 Hz, 1H), 4.67 (d, J = 1.68 Hz, 1H), 5.10 (d, J = 7.63 Hz, 1H), 5.71 (d, J = 7.02 Hz, 1H), 5.77 (d, J = 9.76 Hz, 1H), 6.20–6.24 (dd, J = 7.94, 9.16 Hz, 1H), 6.46 (s, 1H), 7.09 (d, J = 9.31 Hz, 1H), 7.21–7.52 (m, 10H), 7.73–7.78 (d, J = 7.17 Hz, 3H), 7.92 (d, J = 7.78 Hz, 1H); ^{13}C NMR δ −5.21, −5.25, 6.73, 10.09, 14.40, 18.11,20.84, 21.09, 25.48, 26.50, 35.58, 32.12, 43.23, 46.71, 55.44, 56.47, 58.25, 70.86, 72.03, 75.01, 75.10, 75.25, 78.53, 83.17, 83.97, 120.45, 124.00, 126.49, 126.56, 126.93, 127.79, 128.57, 128.73, 130.25, 131.70, 133.65, 134.30, 140.43, 140.89, 152.56, 166.10, 166.91, 169.25, 171.51, 201.47. HRFABMS: m/z $[M+H]^+$ 1139.5061 ($C_{59}H_{79}N_4O_{15}Si_2$ requires 1139.5080).

2-Debenzoyl-2-(3-azidobenzoyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (8f). To a solution of 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-2-(3-azidobenzoyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel **6f** (8.0 mg, 0.007 mmol) in dry THF was added HF-pyridine (0.2 mL) in a Teflon vial. The reaction mixture was stirred at room temperature for 2 h. The mixture was then diluted with EtOAc (10 mL) and washed thoroughly with dilute sodium bicarbonate, dilute HCl, water and finally brine. The organic layer was dried over Na_2SO_4 and evaporated to yield crude product, which was purified by PTLC (500 μM; EtOAc:hexane, 55:45) to give 2-debenzoyl-2-(3-azidobenzoyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**8f**, 6.3 mg, 98%). ^{1}H NMR δ 1.14 (s, 3H), 1.24 (s, 3H), 1.67 (s, 3H), 1.85 (s, 3H), 1.88 (s, 1H), 1.89–1.93 (m, 1H), 2.24 (s, 3H), 2.35–2.57 (m, 4H), 3.57 (d, J = 4.89 Hz, 1H), 3.77 (s, 3H), 3.84 (d, J = 6.86 Hz, 1H), 4.20 (d, J = 8.39 Hz, 1H), 4.34–4.41 (m, 2H), 4.47–4.79 (m, 1H), 4.99 (d, J = 9.00 Hz, 1H), 5.69 (d, J = 7.17 Hz, 1H), 5.80 (d, J = 8.85 Hz, 1H), 6.18–6.22 (dd, J = 7.78, 9.00 Hz, 1H), 6.27 (s, 1H), 6.87 (d, J = 9.01 Hz, 1H), 7.24–7.51 (m, 10H), 7.57 (d, J = 7.63 Hz, 2H), 7.70 (d, J = 7.02 Hz, 2H), 7.82–7.83 (m, 1H), 7.94 (d, J = 7.79 Hz, 1H). HRFABMS: m/z $[M+H]^+$ 911.3343 ($C_{47}H_{51}N_4O_{15}$ requires 911.3350).

2′-*O*-*tert*-Butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-2-(3,3-dimethylacryloyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (6g). A mixture of the 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**5c**, 10 mg, 0.01 mmol), 3,3-dimethylacrylic acid (10 mg, 0.1 mmol), DCC (20 mg) and pyrrolidinopyridine (1.0 mg) in toluene (0.8 mL) was stirred at room temperature for 24 h and purified by PTLC (silica gel, 1000 μM; EtOAc:hexane, 3:7) to yield 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-2-(3,3-dimethylacryloyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**6g**, 7.0 mg, 95% based on the recovery of 2 mg unreacted starting compound). ^{1}H NMR δ −0.35 (s, 3H), −0.04 (s, 3H), 0.52–0.62 (m, 6H), 0.78 (s, 9H), 0.87–0.93 (m,9H), 1.14 (s, 3H), 1.18 (s, 3H), 1.25 (s, 3H), 1.67 (s, 3H), 1.89 (s, 3H), 1.97 (d, J = 0.9 Hz, 3H), 2.02 (d, J = 0.9 Hz, 3H), 1.91–2.22 (m, 3H), 2.16 (s, 3H), 2.19 (s, 3H), 2.52–2.55 (m, 1H), 3.78 (d, J = 6.7 Hz, 1H), 4.00 (s, 3H), 4.22 (d, J = 8.8 Hz, 1H), 4.39–4.43 (dd, J = 6.9, 6.7 Hz, 1H), 4.52 (d, J = 8.4 Hz, 1H), 4.65 (d, J = 1.7 Hz, 1H), 5.03 (d, J = 7.6 Hz, 1H), 5.48 (d, J = 6.7 Hz, 1H), 5.67–5.70 (m, 2H), 6.20–6.25 (dd, J = 9.5, 9.8 Hz, 1H), 6.43 (s, 1H), 7.13 (d, J = 8.7 Hz, 1H), 7.27–7.55 (m, 8H), 7.78 (d, J = 6.9 Hz, 1H). HRFABMS: m/z $[M+H]^+$ 1076.5206 ($C_{57}H_{82}NO_{15}Si_2$ requires 1076.5223).

2-Debenzoyl-2-(3,3-dimethyl-acryloyl)-4-deacetyl-4-(methyl carbonate)-paclitaxel (8g). To a solution of 2′-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-2-debenzoyl-2-(3,3-dimethylacryloyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**6g**, 6.0 mg, 0.005 mmol) in dry THF (1 mL) was added HF-pyridine (0.2 mL) in a Teflon vial. The reaction mixture was stirred at room temperature for 2 h. After usual work up the residue obtained was purified by PTLC (500 μM, 55% EtOAc/hexane) to give 2-debenzoyl-2-(3,3-dimethylacryloyl)-4-deacetyl-4-(methoxycarbonyl)-paclitaxel (**8g**, 3.9 mg, 98%). ^{1}H NMR δ 1.10 (s, 3H), 1.21 (s, 3H), 1.63 (s, 3H), 1.79 (d, J = 1.2 Hz, 3H), 1.83 (s, 3H), 1.86–1.89 (m, 1H), 1.95 (d, J = 1.1 Hz, 3H), 2.19 (s, 3H), 2.23 (s, 3H), 2.26–2.34 (m, 2H), 2.44 (d, J = 4.3 Hz, 1H), 2.50–2.58 (m, 1H), 3.58 (d, J = 4.2 Hz, 1H), 3.70 (d, J = 6.7 Hz, 1H), 3.78 (s, 3H), 4.20 (d,J = 8.2 Hz, 1H), 4.34 (m, 1H), 4.51 (d, J = 8.8 Hz, 1H), 4.74–4.76 (dd, J = 2.3, 2.1 Hz, 1H), 4.99 (d, J = 7.8 Hz, 1H), 5.46 (d, J = 6.7 Hz, 1H), 5.71 (s, 1H), 5.78 (d, J = 8.8 Hz, 1H), 6.15 (m, 1H), 6.23 (s, 1H), 6.95 (d, J = 9.8 Hz, 1H), 7.30–7.55 (m, 8H), 7.77 (d, J = 7.0 Hz, 1H). HRFABMS: m/z $[M+H]^+$ 848.3501 ($C_{45}H_{54}NO_{15}$ requires 848.3493).

Acknowledgements

Financial support by the National Cancer Institute, NIH (Grant Number CA-69571) and by Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, is gratefully acknowledged. High resolution mass spectra were obtained at the Nebraska Center for Mass Spectrometry.

References and Notes

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