Synthesis and Evaluation of Some 10-Mono- and 2′,10-Diesters of 10-Deacetylpaclitaxel

Koppaka V. Rao,* Rajendra S. Bhakuni, James Johnson, and Ravi S. Oruganti

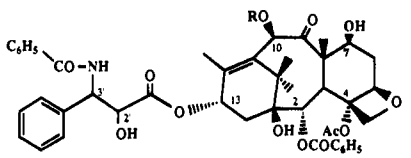
Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, Florida 32610

Received May 16, 1995®

10-Deacetylpaclitaxel, isolated from the bark of *Taxus brevifolia*, was converted into paclitaxel in one composite step (trimethylsilylation, acetylation, and desilylation) and in an overall yield of 80–85%. A series of 10-monoesters of 10-deacetylpaclitaxel are prepared by protection of the 2′- and 7-hydroxyls with a chloroacetyl group, acylation, and deprotection. Depending on the reaction conditions, the 10-monoesters, either exclusively or accompanied by the 2′,10-diesters, are formed. The mono- and diesters were evaluated using the L-1210 cell culture assay. The 10-monoesters were comparable to paclitaxel and more active than the corresponding 2′,10-diesters. The 10-[(4-methoxyphenyl)acetyl], 10-(2-nitrobenzoyl), and 10-(phenylacetyl) esters were found to be somewhat more active than paclitaxel.

Introduction

Paclitaxel (**1**), isolated from the bark of *Taxus brevifolia*,[1] is currently viewed as an important lead compound on the basis of its unique mechanism of action[2] and promising anticancer activity.[3–5] 10-Deacetylpaclitaxel (**2**) is an important natural analogue of **1**[6–8] with a similar level of activity.



1: R = $COCH_3$ PACLITAXEL

2: R = H 10-DEACETYL PACLITAXEL

Although the preparation and activity of variously substituted paclitaxel esters (e.g., 2′ and/ or 7) have been reported,[9–15] those at the C-10-position have not. In view of the currently better availability of 10-deacetylpaclitaxel,[6,16] we describe here the preparation and evaluation of a number of its 10-monoesters and 2′,10-diesters.

Chemistry

The basic method for preparing the 10-esters consisted of protecting the 2′- and 7-positions by chloroacetylation, acylation of the 10-hydroxyl with the desired acyl group, and deprotection.

The initial protection step gave the desired 2′,7- bis(monochloroacetate) as the major product **3** (77%), along with the 2′,7,10-tris(monochloroacetate) **4** (11%). Acylation of the 10-position of the 2′,7-bis(monochloroacetate) was carried out using the acid chloride (or the anhydride) in pyridine or the acid/DCC. Following the 10-acylation, the product was subjected to deprotection by treatment with thiourea in ethanol.[18] It was found, however, that, if the residual acylating agent was not decomposed before the deprotection, 10–30% of the 2′,-10-diester was also formed as a byproduct. On the basis of this observation, methods for making one or the other as the major product were developed. The primary esters such as the 10-(2-nitrobenzoate) and the 10-(2-nitrophenylacetate) were also converted to the corresponding 2-amino derivatives for a comparison of their activities.

A special case of a 10-ester of 10-deacetylpaclitaxel is paclitaxel (**1**) itself, and a convenient method for the conversion of **2** to **1** would be desirable. Using the above sequence based on chloroacetylation, an overall yield of 60% was obtained. With the use of trimethylsilyl protecting group, all three steps (protection, acetylation, and deprotection) could be carried out as a "one-pot reaction", with an overall yield of 85%.

All the esters were characterized by ^1H NMR, ^1H COSY, and mass spectra. The compounds are listed in Table 1, with their yield and key spectral data. The various esters listed in Table 1 were tested for their cytotoxic activity in the L-1210 cell culture sytem, and the activities are listed in Table 3.

Discussion of Results

Hydroxyl protection by the chloroacetyl group[18] is evaluated in the paclitaxel series and found to be quite satisfactory. The esters are readily formed, are stable during the subsequent reactions, and are removable under relatively mild conditions, by alcohol and thiourea. For the bischloroacetylation of **2**, use of 3 or more mol equiv (instead of 2–2.2) of the anhydride gave a much faster reaction (15–20 min) to give the diester in a 77% yield. As has been noted before (e.g., Deutsch[10]) the 2′-hydroxyl is readily acylated, followed by the 7-hydroxyl, giving the desired diester. The triester (11%) can be recycled to **2**.

During the deprotection step (EtOH/thiourea at 65 °C), immediately following the esterification at the 10-position (and without workup), a substantial degree of acylation of the newly freed 2′-OH takes place, by the still present acylating agent, forming varying amounts of the 2′,10-diester. This can be avoided by stirring with methanol (30 min) to destroy the acylating agent. Interestingly, the diesters are actually made from the same 2′,7-protected compound, only by using more reagent and not removing it before deprotection. The diesters were formed almost exclusively: e.g., **23** (47%), **25** (59%), and **26** (76%). The formation of the diesters

* To whom enquiries should be addressed.

® Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0022-2623/95/1838-3411$09.00/0

Table 1. Yield and Spectral Data on 10-Deacetylpaclitaxel Esters

compd	10-deacetylpaclitaxel	yield (%)	mass peaks	NMR signals 2′	3′	10
3	2′,7-bis(chloroacetyl)-	77	964,[b] 946, 870, 846, 603, 585	5.58 d (3)	6.01 dd (3.3, 9.3)	5.31 br s
4	2′,7,10-tris(chloroacetyl)-	11	1040[b]	5.59 d (3)	6.02 dd (3.3, 9.3)	6.25 s
5	2′,7-bis(chloroacetyl)-10-acetyl-	71				
6	10-butyryl-	74	882,[b] 597, 537, 509, 286, 268	4.77 br s	5.77 dd (2.4, 8.7)	6.18 s
7	10-hexanoyl-	72	910,[b] 625, 509, 286, 268, 240	4.79 br s	5.78 dd (2.4, 8.6)	6.26 s
8	10-octanoyl	62	938,[b] 653, 509, 286, 268, 240	4.79 d (1.9)	5.79 dd (2.4, 8.9)	6.26 s
9	10-cinnamoyl	81	942,[b] 657, 509, 286, 268, 240	4.78 d (2.6)	5.77 dd (3, 9)	6.41 s
10	10-hydrocinnamoyl-	80	966,[b] 944, 659, 509, 286, 268	4.78 br s	5.79 dd	6.26 s
11	10-benzoyl-	83	916,[b] 631, 509, 286, 268, 240	4.79 br s	5.79 dd	6.54 s
12	10-(2-nitrobenzoyl)	89	961,[b] 676, 509, 286, 268, 240	4.79 d (2.6)	5.78 dd (2.4, 9)	6.54 s
13	10-(2-aminobenzoyl)-	75	931,[b] 646, 509, 286, 268, 240	4.79 br s	5.78 dd (2.4, 8.7)	6.48 s
14	10-(phenylacetyl)-	84	930,[b] 645, 509, 286, 268, 240	4.76 br s	5.77 dd	6.27 s
15	10-[(2-nitrophenyl)acetyl]-	90	975,[b] 690, 509, 286, 268, 240	4.79 d (2.4)	5.78 dd (2.1, 9)	6.26 s
16	10-[(2-aminophenyl)acetyl]-	63	945,[b] 660, 509, 286, 268, 240	4.80 br s	5.7 dd (2.7, 8.7)	6.27 s
17	10-[(4-methoxyphenyl)acetyl]-	76	960,[b] 675, 509, 286, 268, 240	4.79 br s	5.78 dd	6.27 s
18	10-(phenoxymethyl)-	83	946,[b] 794, 643, 509, 286, 240	4.79 br s	5.78 dd	6.27 s
19	2′,10-dibutyryl-[a]	25[a]	952,[b] 597, 509, 356, 338, 286	5.51 d (3.3)	5.95 dd	6.30 s
20	2′,10-dihexanoyl-[a]	19[a]	1008[b], 625, 509, 384, 366, 286	5.50 d (3)	5.95 dd (3, 9)	6.29 s
21	2′,10-dioctanoyl-	62	1064,[b] 653, 509, 412, 394, 286	5.5 d (3)	5.95 dd (3, 9)	6.29 s
22	2′,10-dicinnamoyl-	47	1072,[b] 657, 509, 416, 398, 286	5.64 d (2.8)	6.0 dd (3, 9)	6.45 s
23	2′,10-bis(2-nitrobenzoyl)-	47	1110,[b] 676, 658, 509, 435, 417	5.75 d (3)	6.07 dd (3, 9)	6.56 s
24	2′,10-bis(phenylacetyl)[a]	15[a]	1048,[b] 645, 509, 404, 386, 286	5.41 d (3)	5.88 dd (3, 9)	6.29 s
25	2′,10-bis[(2-nitrophenyl)acetyl]-	59	1138,[b] 690, 509, 449, 431, 286	5.49 d (3)	5.93 dd (3, 9)	6.27 s
26	2′,10-bis[(4-methoxyphenyl)acetyl]-	63	1108,[b] 675, 509, 434, 416, 286	5.42 d (3)	5.88 dd (3, 9)	6.29 s

[a] Isolated as a byproduct. [b] (M + H) + peak.

Table 3. Activities of the 10-Mono- and 2′,10-Diesters

no.	compound	IC_{50} (mM)	r[a]	rel poten
1	paclitaxel	0.024	0.98	1
3	2′,7-bis(monochloroacetyl)-10-deacetylpaclitaxel	0.039	0.99	0.62
4	2′,7,10-tris(monochloroacetyl)-10-deacetylpaclitaxel	0.033	0.98	0.73
5	2′,7-bis(monochloroacetyl)paclitaxel	0.037	0.99	0.65
6	10-butyryl-10-deacetylpaclitaxel	0.023	0.96	1.02
7	10-hexanoyl-10-deacetylpaclitaxel	0.036	0.96	0.67
8	10-octanoyl-10-deacetylpaclitaxel	0.1	0.96	0.24
9	10-cinnamoyl-10-deacetylpaclitaxel	0.052	0.99	0.46
10	10-hydrocinnamoyl-10-deacetylpaclitaxel	0.022	0.96	1.1
11	10-benzoyl-10-deacetylpaclitaxel	0.028	0.99	0.88
12	10-(2-nitrobenzoyl)-10-deacetylpaclitaxel	0.02	0.94	1.2
13	10-(2-aminobenzoyl)-10-deacetylpaclitaxel	0.042	0.98	0.57
14	10-(phenylacetyl)-10-deacetylpaclitaxel	0.024	0.95	1
15	10-[(2-nitrophenyl)acetyl]-10-deacetylpaclitaxel	0.028	0.95	0.86
16	10-[2-aminophenyl)acetyl]-10-deacetylpaclitaxel	0.061	0.99	0.39
17	10-[(4-methoxyphenyl)acetyl]-10-deacetylpaclitaxel	0.019	0.92	1.26
18	10-(phenoxyacetyl)-10-deacetylpaclitaxel	0.044	0.96	0.55
19	2′,10-dibutyryl-10-deacetylpaclitaxel	0.21	0.99	0.11
20	2′,10-dihexanoyl-10-deacetylpaclitaxel	>0.6		
21	2′,10-dioctanoyl-10-deacetylpaclitaxel	>0.5		
22	2′,10-dicinnamoyl-10-deacetylpaclitaxel	>0.6		
23	2′,10-bis(2-nitrobenzoyl)-10-deacetylpaclitaxel	0.059	0.99	0.41
24	2′,10-bis(phenylacetyl)-10-deacetylpaclitaxel	0.136	0.98	0.24
25	2′,10-bis[(2-nitrophenyl)acetyl]-10-deacetylpaclitaxel	0.066	0.98	0.36
26	2′,10-bis[(4-methoxyphenyl)acetyl]-10-deacetylpaclitaxel	0.117	0.99	0.21

[a] r = correlation coefficient.

is more common when the acylating agents are somewhat stable to water or methanol (e.g., octanoic acid/DCC).

For the conversion of **2** into paclitaxel, a reaction with important commercial potential, a trimethylsilyl protecting group is better than the chloroacetyl, although the conditions used are critical. With hexamethyldisilazane, protection of the 2′- and 7-hydroxyls was complete in 15−20 min, with little or no trisether. The subsequent acetylation (Ac_2O) at C-10, likewise, was complete in 20 min. Stirring with water for 20 min followed by HCl leads to deprotection giving **1** (76%) and ca. 13% of the paclitaxel 2′-acetate. Brief reaction of this mixture with MeOH/Me_2NH converts the latter into paclitaxel in an overall yield of 85%.

The ^1H NMR spectra of the 10-mono- and 2′,10-diesters were consistent with those of the other mono- or diesters of **2**. The signal due to 10-H moves from 5.3 to 6.2 ppm, that due to the 2′-H from 4.8 to 5.6 ppm, and even that due to the 3′-H from 5.78 to 6.0 ppm, as a result of the acylation at the 2′-position.

In general, all of the 10-monoesters and most of the 2′,10-diesters studied showed significant activity in the L-1210 cell culture assay, comparable to that of paclitaxel (Table 3). Several of the monoesters were as active as **1**. Three of the diesters, **20** (dihexanoyl), **21** (dioctanoyl), and **22** (dicinnamoyl), were inactive even at 10 × IC_{50} of the corresponding monoesters. The diesters that showed activity had higher IC_{50}'s than the monoesters (i.e., 2−5 times less potent).

In the homologous series (acetate, butyrate, hexanoate, and octanoate), the potency decreased as the chain length increased. Even in the diesters, the dihexanoate and dioctanoate were inactive, compared to the dibutyrate. In the series benzoate, phenylacetate, and phenylpropionate (hydrocinnamate), the activity was essentially the same. The 10-cinnamate was less active than the 10-hydrocinnamate, and the dicinnamate was inactive. In the two sets of examples the 10-(2-nitrobenzoate) vs 10-(2-aminobenzoate) and the 2-nitro- vs 2-aminophenylacetate, the nitro compounds were more active than the amino compounds. The slightly greater degree of activity exhibited by the 10-(2-nitrobenzoate) and the 10-(4-methoxyphenylacetate) over that of paclitaxel suggests that further study of substituted benzoates and phenylacetates will be justified.

Experimental Section

10-Deacetylpaclitaxel used in this study was isolated from the bark of *T. brevifolia* as described earlier.[6,16]

2′,7-Bis(monochloracetyl)-10-deacetylpaclitaxel 3. To **2** (1 g, 1.2 mM) in DMF (5 mL) and CH_2Cl_2 (100 mL) were added DMAP (47 mg) and chloroacetic anhydride (0.67 g, 3.9 mM) with stirring. Standard workup was performed after 15 min. The product from three such batches was combined and chromatographed (SiO_2/$CHCl_3$/ligroin, 1:1). Compound **4** was eluted first: yield, 435 mg (ether/ligroin, 1:1); mp 154−156 °C. Anal. C, H, N.

Elution with $CHCl_3$/ligroin (3:1) gave **3**: yield, 2.67 g (ether); mp 166−168 °C. Anal. C, H, N.

2′,7-Bis(monochloracetyl)paclitaxel (5). Ac_2O (0.05 mL) was added to 3 (16 mg, 0.016 mM) in pyridine (0.1 mL). After 4 h, the product was recovered and purified by preparative TLC ($CHCl_3$−MeOH, 95:5) to yield **5** (14 mg): mp 164−166 °C. It was identical with paclitaxel 2′,7-bis(chloroacetate) prepared as under **3**. Anal. C, H, N.

Paclitaxel (1). Method A: A solution of **5** (50 mg, 0.049 mM) in EtOH (5 mL) was heated with thiourea (150 mg) at 65 °C for 5 h. After standard workup, the product was purified by preparative TLC (CH_2Cl_2/MeOH/Me_2CO, 13:0.5:1.5) to yield **1**: mp 220−222 °C, identical with an authentic sample.

Method B: After the acetylation of **3** (51 mg, 0.053 mM), the reaction mixture was stirred with EtOH (5 mL) for 30 min and then heated with thiourea (150 mg) at 65 °C for 5 h. Standard workup and preparative TLC gave **1**: yield, 39 mg (86%).

Method C: To **2** (101 mg, 0.0124 mM) in MeCN (2 mL) were added DMAP (10 mg) and hexamethyldisilazane (**1** mL, 4.75 mM). After 15 min Ac_2O (1 mL) was added followed (after 20 min) by water (4 mL) and after another 20 min with 0.5 N HCl (4 mL). Standard workup and preparative TLC gave **1** (yield, 81 mg (76%)), along with paclitaxel 2′-acetate[20] (14 mg, 13%). In subsequent experiments, the crude reaction product in MeOH (2 mL) was treated with Me_2NH (to make 0.2 M). Following the disappearance of paclitaxel 2′-acetate (TLC), the solution was neutralized (0.2 M HCl) and concentrated, the solid filtered, and the paclitaxel recrystallized from acetone/ligroin: yield, 85%.

10-Esters of 10-Deacetylpaclitaxel. Method A: 10-Butyroyl-10-deacetylpaclitaxel (6). To a solution of **3** (100 mg, 0.104 mM) and DMAP (14 mg) in CH_2Cl_2 (10 mL) and $N(Et)_3$ (1 mL) was added butyryl chloride (0.8 mL). After 1 h and standard workup, the product was stirred with EtOH (5 mL) for 30 min and then heated (65 °C, 3.5 h) with thiourea (262 mg). Recovery and chromatography (SiO_2/$CHCl_3$/ligroin) gave **6**. Anal. C, H, N. The same procedure was used for **7**, **11**, **12**, and **18**.

10-Hexanoyl-10-deacetylpaclitaxel (7). Anal. C, H, N.

10-Benzoyl-10-deacetylpaclitaxel (11). Anal. C, H, N.

10-(2-Nitrobenzoyl)-10-deacetylpaclitaxel (12). Anal. C, H, N.

10-(Phenoxyacetyl)-10-deacetylpaclitaxel (18). Anal. C, H, N.

10-Esters of 10-Deacetylpaclitaxel. Method B: 10-Cinnamoyl-10-deacetylpaclitaxel (9). A mixture of **3** (40 mg, 0.042 mM), DMAP (4 mg), DCC (46 mg, 0.223 mM), and cinnamic acid (40 mg, 0.27 mM) was stirred in toluene (5 mL) for 6 h. After 2 h the cooled, filtered mixture was stirred (20 min) with MeOH (4 mL) and then heated with EtOH (5 mL) and thiourea (122 mg) at 65 °C for 4 h. The standard workup and preparative TLC gave **9**: 32 mg. Anal. C, H, N. The same procedure was used for **8**, **10**, **14**, **15**, and **17**.

10-Octanoyl-10-deacetylpaclitaxel (8). Anal. C, H, N.

10-Hydrocinnamoyl-10-deacetylpaclitaxel (10). Anal. C, H, N.

10-(Phenylacetyl)-10-deacetylpaclitaxel (14). Anal. C, H, N.

10-[(2-Nitrophenyl)acetyl]-10-deacetylpaclitaxel (15). Anal. C, H, N.

10-[(4-Methoxyphenyl)acetyl]-10-deacetylpaclitaxel (17). Anal. C, H, N.

2′,10-Diesters of 10-Deacetylpaclitaxel. Method A: 2′,-10-Dibutyryl-10-deacetylpaclitaxel (19). The same procedure as given under **6** was used. After the acylation step, the mixture was directly heated with ethanol (8 mL) and thiourea (170 mg) at 65 °C for 7 h. After the standard workup and preparative TLC, **19** was obtained (23 mg, 25%) along with **6** (52 mg, 62%). Anal. C, H, N. The same procedure was used for **20** and **23**.

2′,10-Dihexanoyl-10-deacetylpaclitaxel (20). Anal. C, H, N.

2′,10-Bis(2-nitrobenzoyl)-10-deacetylpaclitaxel (23). Anal. C, H, N.

2′,10-Diesters of 10-Deacetylpaclitaxel. Method B: 2′,-10-Dioctanoyl-10-deacetyl paclitaxel (21). The same procedure used for **9** was used, except that deprotection with thiourea was carried out without removal of the acylating agent. Anal. C, H, N.

The same procedure was used for **22** and **24**−**26**.

2′,10-Dicinnamoyl-10-deacetylpaclitaxel (22). Anal. C, H, N.

2′,10-Bis(phenylacetyl)-10-deacetylpaclitaxel (24). Anal. C, H, N.

2′,10-Bis[(2-nitrophenyl)acetyl]-10-deacetylpaclitaxel (25). Anal. C, H, N.

2′,10-Bis[(4-methoxyphenyl)acetyl]-10-deacetylpaclitaxel (26). Anal. C, H, N.

10-(2-Aminobenzoyl)-10-deacetylpaclitaxel (13). A solution of **12** (80 mg, 0.083 mM) in EtOH (6 mL) and AcOH (0.3 mL) was subjected to hydrogenation using 5% Pd−C for 8 h. After filtration and concentration to dryness, the product was purified by preparative TLC: yield, 58 mg. Anal. C, H, N.

10-[(2-Aminophenyl)acetyl]-10-deacetylpaclitaxel (16). The above procedure was used with **15** (60 mg, 0.06 mM): yield, 36 mg. Anal. C, H, N.

L-1210 Cytotoxicity Assay. The method described by Thayer et al. was used.[19] The compounds in DMSO were added (0.01 mL) to the wells containing the L-1210 cells (150 000/mL). After 48 h, 1 mL of the culture was mixed with 1 mL of trypan blue, 0.1 mL of this mixture was placed on a hemocytometer (Fisher), and the unstained, viable cells present in the five gridded areas were counted. This number × 4 000 gives the number of cells in 1 mL of the culture in the well.

Controls with and without DMSO and an active positive control, which, in this case, was paclitaxel, were also run. The IC_{50} was determined from the plot of log(conc) vs percent inhibition, and the latter was obtained by the equation:

$$\text{percent inhibition } (\%) = 1 - (T_d - T_0/T_c - T_0) \times 100$$

where T_d is the number of cells/mL of the treated samples, T_0 is the initial number of cells, and T_c is the average number of cells/mL of the controls.

The values given in Table 3 are the averages from four different tests, each being tested in quadruplicate. The term r is the correlation coefficient from the linear regression.

Supporting Information Available: Table 2 that lists complete ^{1}H NMR data on compounds listed in Table 1 (6 pages). Ordering information is given on any current masthead page.

References

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. Plant antitumor agents VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.

(2) Schiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of microtubule assembly *in vitro* by taxol. *Nature* **1979**, *277*, 665–667.

(3) Rowinsky, E. K.; Cazenave, L. A.; Donhower, R. C. Taxol: a novel investigational antimicrotubule agent. *J. Natl. Cancer Inst.* **1990**, *82*, 1247–1258.

(4) Holmes, F. A.; Walters, R. S.; Thriault, R. L.; Forman, A. D.; Newton, L. K.; Raber, M. N.; Buzdar, A. U.; Frye, D. K.; Hortobagyi, G. N. *J. Natl. Cancer Inst.* **1991**, *83*, 1797–1805.

(5) Borman, S. Scientists mobilize to increase supply of anticancer drug taxol. *Chem. Eng. News* **1991**, *69*, 11–18.

(6) Rao, K. V. Taxol and related taxanes I. Taxanes of *Taxus brevifolia* bark. *Pharm. Res.* **1993**, *10*, 521–524.

(7) McLaughlin, J. L.; Miller, R. W.; Powell, R. G.; Smith, C. R., Jr. 19-Hydroxy baccatin III, 10-deacetyl cephalomannine and 10-deacetyl taxol. *J. Natl. Prod.* **1981**, *44*, 312–319.

(8) Senilh, V.; Blechert, S.; Colin, M.; Fuenard, D.; Picot, F.; Potier, P.; Varenne, P. Mise en evidence de noveaux analogues du taxol extraits de *Taxus baccata*. *J. Nat. Prod.* **1984**, *47*, 131–137.

(9) Gueritte-Voegelein, F.; Guenard, D.; Lavelle, F.; Le Goff, M. T.; Mangatal, L.; Potier, P. Relationship between the structure of taxol analogues and their antimitotic activity. *J. Med. Chem.* **1991**, *34*, 992–998.

(10) Deutsch, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M. M.; Zalkow, L. H. Synthesis of congeners and prodrugs 3. Water-soluble prodrugs of taxol with potential antitumor activity. *J. Med. Chem.* **1989**, *32*, 788–792.

(11) Magri, N. F.; Kingston, D. G. I. Modified taxols 4. Synthesis and biological activity of taxols modified in the side chain. *J. Nat. Prod.* **1988**, *51*, 298–306.

(12) Kingston, D. G. I.; Samaranayake, G.; Ivey, C. A. The chemistry of taxol, a clinically useful anticancer agent. *J. Nat. Prod.* **1990**, *53*, 1–12.

(13) Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Leslie Gunatilaka, A. A.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. Unexpectedly facile hydrolysis of the 2-benzoate group of taxol and synthesis of analogues with increased activities. *J. Am. Chem. Soc.* **1994**, *116*, 4097–4098.

(14) Georg, G. I.; Cheruvallath, Z.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. Synthesis of biologically active taxol analogues with modified phenyl isoserine side chains. *J. Med. Chem.* **1992**, *35*, 4230–4237.

(15) Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. Biologically active taxol analogues with deleted A ring side chain substituents and variable C-2′ configurations. *J. Med. Chem.* **1991**, *34*, 1176–1184.

(16) Rao, K. V.; Hanuman, J. B.; Alvarez, C.; Stoy, M.; Juchum, J.; Davies, R. M.; Baxley, R. A new large scale process for taxol and related taxanes from *Taxus brevifolia*. *Pharm. Res.* **1995**, *12*, 1003–1010.

(17) Blay, P. K. S.; Thibault, P.; Thiberge, N.; Kiecken, B.; Lebrun, A.; Mercure, C. Analysis of taxol and related taxanes from *Taxus canadensis* using liquid chromatography combined with mass spectrometry or tandem mass spectrometry. *Rapid Comm. Mass Spectrometry* **1993**, *7*, 626–634.

(18) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley and Sons, Inc.: New York, 1991; p 92.

(19) Thayer, P. S.; Himmelfarb, P.; Watts, G. L. Cytotoxicity assays with L-1210 cells *in vitro*. Comparison with L-1210 *in vivo* and KB cells *in vitro*. *Cancer Chemother. Rep.* **1971**, 1–10.

(20) Mellado, W.; Magri, N. F.; Kingston, D. G. I.; Garcia-Arenas, R.; Orr, G. A.; Horwitz, S. B. Preparation and biological activity of taxol acetates. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 329–336.

JM950222L