Synthesis of Biologically Active Taxol Analogues with Modified Phenylisoserine Side Chains†

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Taxol (1) is a highly potent antitumor agent, exerting its mechanism of action by promoting the assembly of stable microtubules in cells. We are reporting on the first synthesis and biological evaluation of taxol derivatives with substituted phenyl rings at the C-13 *N*-benzoyl-(2′*R*,3′*S*)-3′-phenylisoserine side chain of taxol (1). Two taxol derivatives were synthesized, one possessing a *N*-(*p*-chlorobenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserine side chain (2) and the other one a *N*-benzoyl-(2′*R*,3′*S*)-3′-(*p*-chlorophenyl)isoserine side chain (3). The synthesis of the novel phenylisoserine side chains was achieved through the asymmetric synthesis of 3-hydroxy-4-aryl-2-azetidinone derivatives via the ester enolate–imine cyclocondensation reaction. The 2-azetidinones 14 and 15 were acylated with *p*-chlorobenzoyl chloride and benzoyl chloride, respectively, to form the *N*-acyl β-lactams 16 and 17. Subsequent coupling of 16 and 17 to 7-(triethylsilyl)baccatin III (6) in the presence of pyridine and DMAP afforded, after removal of the protecting groups, the desired taxol analogues 2 and 3 in excellent yields. The newly synthesized derivatives 2 and 3 were tested in the tubulin assembly assay and also evaluated for their cytotoxicity against B16 melanoma cells. It was found that the taxol derivatives 2 and 3 had activity comparable to taxol (1).

Introduction

Taxol (1), a complex diterpene, isolated[1] in small quantities from the bark of *Taxus brevifolia*[2,3] is currently considered a most exciting lead in cancer chemotherapy.[4] Since taxol (1) displayed high cytotoxicity against a variety of cancer cell lines[5] and significant activity against xenografts of human tumors in mice, it was selected for clinical development in 1977.[6] Taxol (1) is currently in phase II clinical trials in the United States.[6] Activity against advanced cisplatin refractory ovarian cancer has been established.[6,7]

In vitro studies on taxol (1) have revealed a new and unique mechanism of blocking cell replication in HeLa cells and fibroblast cells.[8] It has been shown that taxol (1) promotes the assembly of stable microtubules, which cannot be depolymerized by calcium ion, cold, or microtubule disassembling drugs.[9] Taxol (1) exerts its effect in the absence of GTP, normally required for tubulin assembly, or microtubule associated proteins.[10] At saturation, taxol (1) binds reversibly to polymerized tubulin with an approximate stoichiometry of 1 mol of taxol (1) per polymerized dimer.[9]

Despite the encouraging biological results, it seemed until recently that it would not be possible to develop taxol (1) for clinical use because only small amounts[11] of taxol (1) can be isolated from the bark of *Taxus brevifolia*. However, a recent report has shown that a more readily available taxol precursor, 10-deacetylbaccatin III (5), can be isolated from the needles of *Taxus baccata*, a yew abundant in Europe.[12] Of note is that the needles are a regenerable source and that harvest does not threaten the survival of the species.

Two very efficient approaches have been reported for the conversion of 10-deacetylbaccatin III (5) and baccatin III (4) to taxol (1) via coupling to *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine[12] or an appropriately protected 3-hydroxy-4-phenyl-2-azetidinone.[13,14] Results by us[15,16] and

† This paper is dedicated to Professor Lester A. Mitscher on the occasion of his 60th birthday.

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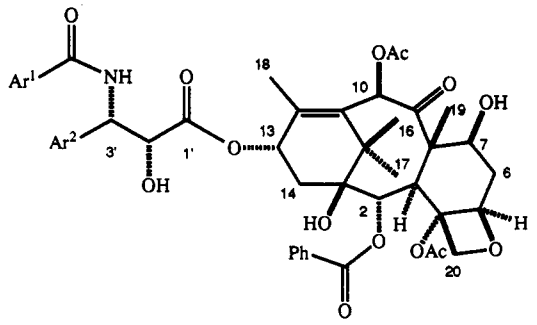
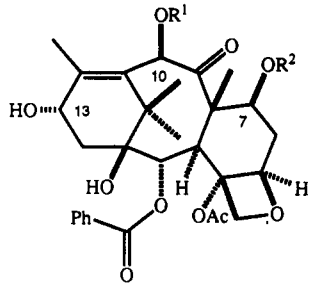


(1) Ar^1, Ar^2 = phenyl = taxol

(2) Ar^1 = *p*-chlorophenyl, Ar^2 = phenyl

(3) Ar^1 = phenyl, Ar^2 = *p*-chlorophenyl



(4) baccatin III R^1 = Ac, R^2 = H

(5) 10-deacetyl baccatin III R^1, R^2 = H

(6) 7-triethylsilyl baccatin III R^1 = Ac, R^2 = $SiEt_3$

Figure 1. Structures of taxol (1), taxol analogues (2, 3), baccatin III (4), and baccatin III derivatives (5, 6).

others[12,17-20] have recently provided practical approaches toward the synthesis of *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine and optically active 3-hydroxy-4-phenyl-2-azetidinones, thus facilitating the semisynthesis of taxol (1) and its analogues (Scheme I).

A variety of structure–activity studies concerning analogues of taxol and 10-deacetyltaxol have been published. Since our studies are concerned with modifications at the C-13 side chain of taxol (1), we will provide a short overview on this topic. Two excellent reviews on other aspects of structure–activity relationships of taxol derivatives have been published recently.[21] It has been definitely shown that the side chain at C-13 and the diterpene moiety of taxol are both essential for biological activity. Baccatin III (4), 10-deacetylbaccatin III (5), and *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine are devoid of significant antitumor activity.[22]

Taxol derivatives, possessing an intact diterpene moiety, but carrying simplified side chains at C-13, derived from acetic acid, hydroxyacetic acid, cinnamic acid, crotonic acid, and 3-phenylpropionic acid were found to be inactive.[23-26] All C-13 phenylserine derivatives of taxol investigated thus far displayed significantly lower biological activities than taxol (1).[26]

C-13 (*R*)- and (*S*)-lactic acid, 13-((*R*)-*N*-benzoylisoserine), and 13-((*S*)-*N*-benzoylisoserine) derivatives of taxol had greatly reduced biological activity.[27] C-13 (*R*)- and (*S*)-3′-mandelic acid as well as C-13 (*R*)- and (*S*)-3′-(*N*-*t*-BOC-amino)-3′-phenylpropionic acid taxol derivatives had significant activity (ID_{50}/ID_{50} (taxol) = 2.3–4.5) but were not as active as taxol (1).[26,27]

The results described above underscore the necessity for the presence of the 3′-*N*-acylamino and to a lesser degree for the 2′-hydroxy group at the C-13 taxol side chain for optimal activity. Interesting in this context, however, is the observation that the taxol analogue possessing a 2′,3′-dihydroxy-3′-phenylpropionic acid side chain (mixture of diastereoisomers) is almost equiactive to taxol (1), whereas a 3′-amino-2′-hydroxy-3′-phenylpropionic acid derivative displayed greatly reduced activity.[26]

Derivatives which carry a substituent at the 2′-hydroxyl group are inactive, unless the group is susceptible to enzymatic hydrolysis in vivo to release the parent compound taxol (1) (prodrug approach).[28-30]

The relationship between the stereochemistry at the C-13 side chain and tubulin assembly properties was investigated.[26] It was typically found that derivatives with 2′*R*,3′*S* side chain configuration (taxol stereochemistry) were more active than the corresponding 2′*S*,3′*R* side chain isomers. However, one exception was observed. The two taxol derivatives with the 2′*R*,3′*R* and 2′*S*,3′*S* configuration (anti) at the *N*-benzoyl-3′-phenylisoserine side chain were almost as active (ID_{50}/ID_{50}(taxol) = 1.3) as taxol (1).[26]

Only a few taxol derivatives have been reported in which the *N*-benzoyl group of the C-13 side chain has been replaced.[26] However, such a derivative, taxotere, 13-(*N*-*t*-BOC-(2′*R*,3′*S*)-3′-phenylisoseryl)-10-deacetylbaccatin III was found to be even more active than taxol (1) and is currently in clinical trials in France.[4,26] The corresponding

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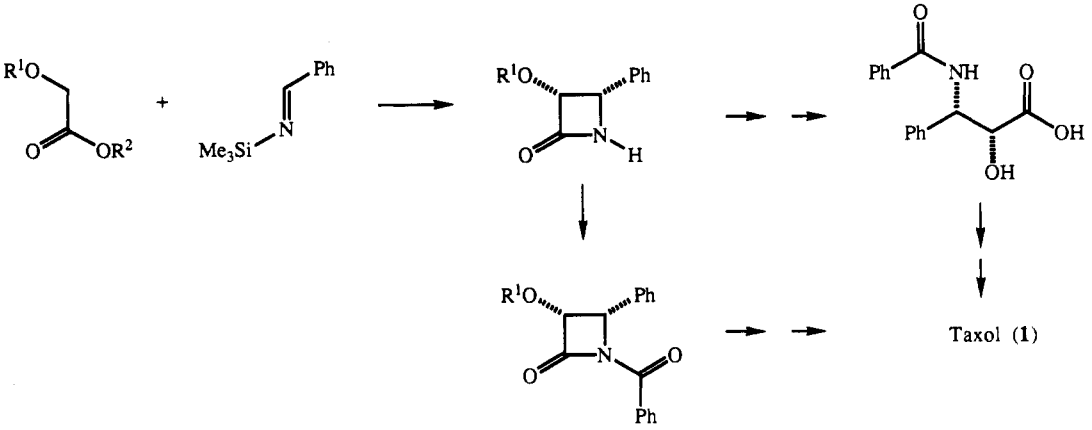
Scheme I



N-tiglyl (cephalomannine), *N*-tosyl, and *N*-hexanoyl analogues of taxol (1) were also found to have significant cytotoxicity as well as the *N*-glutaryl derivative, which should provide access to taxol derivatives with enhanced solubility.[26]

In summary, the structure–activity studies have demonstrated that the presence of the phenylisoserine side chain with correct absolute stereochemistry is significant for strong cytotoxicity. Structural alterations at the side chain are not very well tolerated with the exception of the replacement of the *N*-benzoyl group such as in taxotere and related compounds. Since the side chain does not tolerate major changes without significant loss of activity, we decided to investigate more subtle structural modifications in an effort to evaluate the influence of substituent effects at the two aromatic rings of the C-13 side chain on taxol potency.

We would now like to report on the synthesis and biological evaluation of the first derivatives of taxol, possessing substituents at the two phenyl rings of the C-13 taxol side chain.[31]

We synthesized baccatin III 13-[*N*-(*p*-chlorobenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserinate] (2) and baccatin III 13-[*N*-benzoyl-(2′*R*,3′*S*)-3′-(*p*-chlorophenyl)isoserinate] (3). This choice was made in accordance with the Topliss operational scheme for analogue synthesis in drug design.[32] In the context of drug design, it is also of interest that hydroxylation at the para position of the phenyl group at C-3′ of the C-13 side chain of taxol is a major pathway for taxol metabolism (rat bile).[33] It was found that this metabolite was 10 times less cytotoxic than taxol. Thus, the introduction of a substituent at the para position of the phenyl group at C-3′ may have a favorable effect on the pharmacokinetic properties of taxol (1).

Chemistry

We recently reported[15] that the taxol C-13 side chain, *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine can be synthesized (Scheme I) in high enantiomeric purity via the highly efficient ester enolate–imine condensation[34-36] of optically active glycolates and *N*-(trimethylsilyl)imines.

Since *N*-trimethylsilyl aldimines are readily available[37] from aldehydes and lithium bis(trimethylsilyl)amide, the ester enolate cyclocondensation allows for the facile introduction of different substituents at the C-4 position of the β-lactam ring system. Thus, the methodology is very well suited for the synthesis of 3-phenylisoserine analogues with modified C-3 phenyl groups. Subsequent N-acylation of the β-lactam nitrogen also provides access to phenylisoserine derivatives with different substituents at the C-3 amino group.

Our synthetic sequence starts with the reaction between glycolate 7, utilizing (−)-*trans*-2-phenylcyclohexanol[38] as the chiral auxiliary and (trimethylsilyl)imines[37] 8 (Ar^2 = phenyl) and 9 (Ar^2 = *p*-chlorophenyl) (Scheme II). The desired β-lactams 10 (Ar^2 = phenyl)[15] and 11 (Ar^2 = *p*-chlorophenyl) were synthesized in 85% and 70% yield, respectively.

The enantiomeric purity of these β-lactams was found to be 96% ee for β-lactam 10 and 91% ee for β-lactam 11, as determined by HPLC on a chiral column.[15,39] The absolute stereochemistry of β-lactam 10 had been previously determined by us through chemical conversion to *N*-benzoyl-(2*R*,3*S*)-phenylisoserine.[12,15] The absolute stereochemistry of β-lactam 11 was therefore inferred from β-lactam 10.

It has recently been shown[13,14] that *N*-benzoyl β-lactams of type 16 and 17 are excellent acylating agents in the coupling step with 7-(triethylsilyl)baccatin III (6) for the formation of taxol (1). Therefore, β-lactams 10 and 11

(31) For a preliminary account of this work, see: Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R. Novel Biologically Active Taxol Analogues: Baccatin III 13-(*N*-(*p*-chlorobenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserinate) and Baccatin III 13-(*N*-benzoyl-(2′*R*,3′*S*)-3′-(*p*-chlorophenyl)isoserinate). *Bioorg. Med. Chem. Lett.* **1992**, *2*, 295–298.

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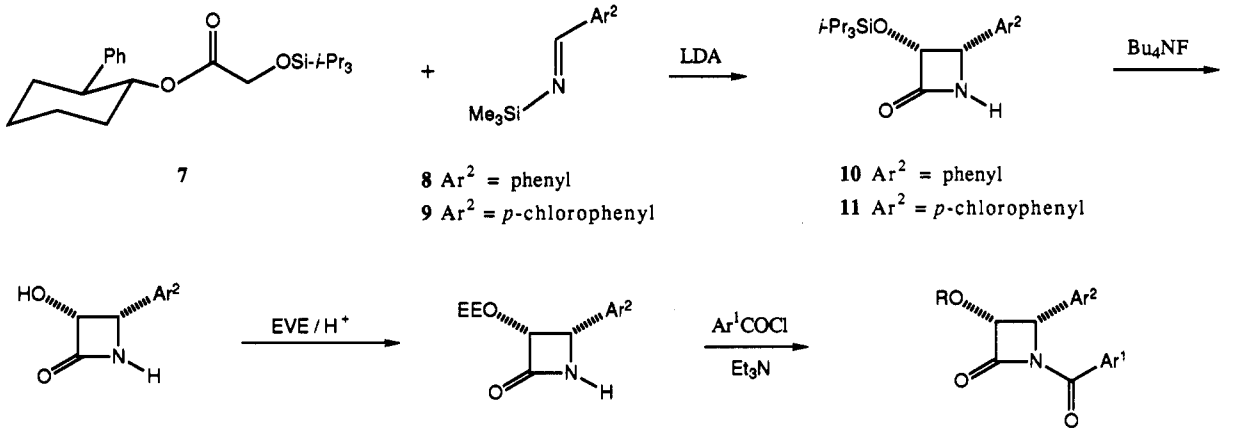
(39) For β-lactam 10, see ref 15. β-Lactam 11 was synthesized in optically active form and also as racemic mixture. The enantiomeric excess was determined by HPLC analysis on a chiral column: CHIRACEL OD from Daicel Chemical Industries. Solvent: hexanes/2-propanol (13:1).

Scheme II



7

8 Ar^2 = phenyl
9 Ar^2 = *p*-chlorophenyl

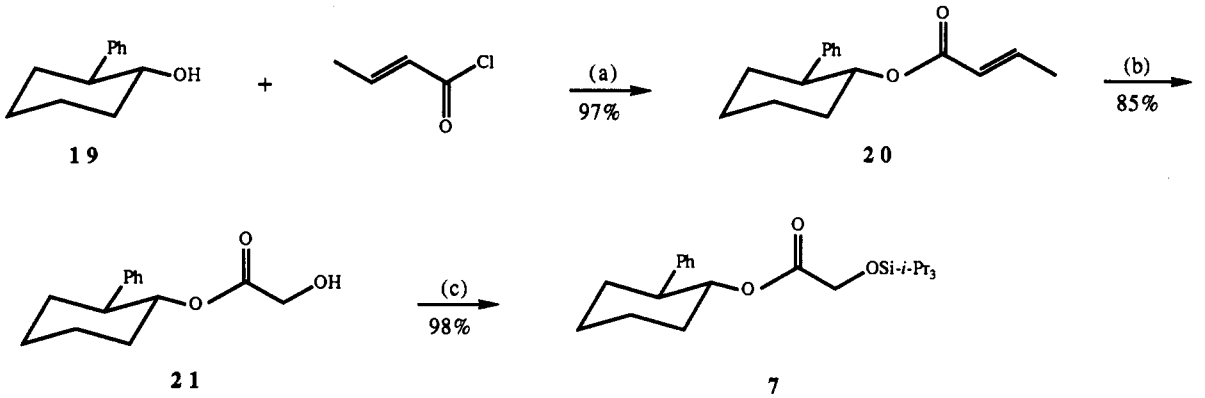
10 Ar^2 = phenyl
11 Ar^2 = *p*-chlorophenyl

12 Ar^2 = phenyl
13 Ar^2 = *p*-chlorophenyl

14 Ar^2 = phenyl
15 Ar^2 = *p*-chlorophenyl

16 Ar^2 = phenyl, Ar^1 = *p*-chlorophenyl, R = EE
17 Ar^2 = *p*-chlorophenyl, Ar^1 = phenyl, R = EE
18 Ar^2 = phenyl, Ar^1 = *p*-chlorophenyl, R = Si-*i*-Pr_3

Scheme III[a]



19 **20** **21** **7**

[a] (a) AgCN (3 equiv), toluene, 7 h reflux; (b) O_3, $NaBH_4$; (c) *i*-Pr_3SiCl, imidazole, DMF.

were first desilylated with tetra-*n*-butylammonium fluoride in tetrahydrofuran, followed by protection[40] of the resulting 3-hydroxy-4-aryl-2-azetidinones **12** and **13** with ethyl vinyl ether (EVE) under acid catalysis as their ethoxyethyl (EE) derivatives **14** and **15**. Both were obtained as a 1:1 mixture of diastereoisomers in an overall yield of 93% and 87%, respectively. This conversion was necessary because β-lactam **18** (R = triisopropylsilyl, Ar^1 = *p*-ClPh, Ar^2 = Ph), possessing a triisopropylsilyl protecting group at the 3-hydroxy group of the β-lactam ring produced only a 5% yield of the product, when coupled to 7-(triethylsilyl)baccatin III (**6**). Apparently, the triisopropylsilyl protecting group causes steric hindrance in the coupling reaction with **6**. The diastereomeric ethoxyethyl derivatives **14** and **15** were converted to their *N*-*p*-chlorobenzoyl and *N*-benzoyl derivatives **16** and **17** via reaction with *p*-chlorobenzoyl chloride and benzoyl chloride in dichloromethane in the presence of triethylamine and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in 95% and 90% yield, respectively.

As illustrated in Scheme III, glycolate **7** was synthesized in three steps and in good overall yield from readily available[38] (−)-*trans*-2-phenylcyclohexanol (**19**) by reaction with crotonyl chloride, followed by ozonolysis of **20** with reductive ($NaBH_4$) workup, and protection of **21** with triisopropylsilyl chloride.

7-(Triethylsilyl)baccatin III (**6**) was prepared in quantitative yield by cleavage[41] of the C-13 side chain of a mixture of taxol (**1**) and cephalomannine with tetrabutylammonium borohydride in dichloromethane, followed by protection of the C-7 hydroxyl group with triethylsilyl chloride.[12]

Coupling between β-lactams **16** and **17** and 7-(triethylsilyl)baccatin III (**6**) in the presence of pyridine and DMAP (Scheme IV) proceeded in excellent yield to form taxol derivatives **22** and **23** (91% and 89% yield, respectively). Due to the presence of an additional chiral center at the ethoxyethyl protecting group of the C-13 side chain, derivatives **22** and **23** were obtained as diastereomeric mixtures (**22a**:**22b** in a 1.9:1 ratio and **23a**:**23b** in a 1.3:1 ratio). Subsequent hydrolysis under mild acid conditions[12] removed the ethoxyethyl as well as the triethylsilyl protecting group to generate the desired novel taxol derivatives **2** and **3** in excellent yields (90% and 92%, respectively).

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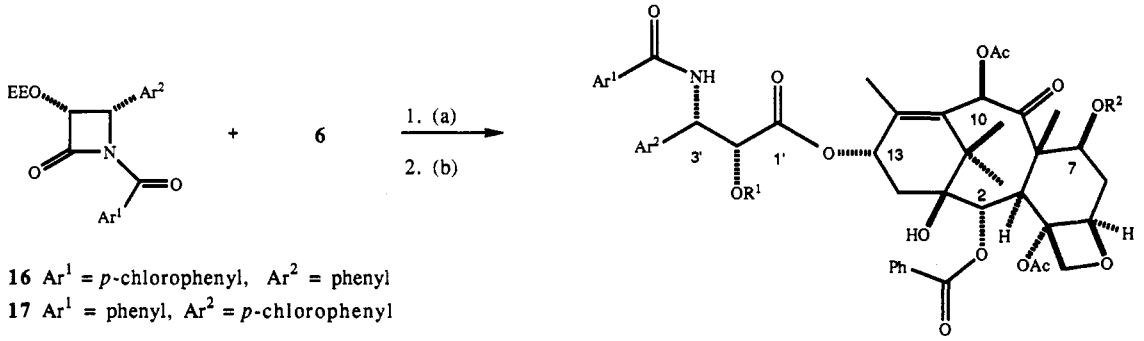
Scheme IV



16 Ar^1 = *p*-chlorophenyl, Ar^2 = phenyl
17 Ar^1 = phenyl, Ar^2 = *p*-chlorophenyl

(a) ß-lactam (5 equiv), pyridine, 4-dimethylaminopyridine (1 equiv.), 25 °C, 24 h; (b) EtOH / 0.5% HCl (1:1), 0 °C, 4 days

22 Ar^1 = *p*-chlorophenyl, Ar^2 = phenyl, R^1 = ethoxyethyl, R^2 = triethylsilyl
23 Ar^1 = phenyl, Ar^2 = *p*-chlorophenyl, R^1 = ethoxyethyl, R^2 = triethylsilyl
2 Ar^1 = *p*-chlorophenyl, Ar^2 = phenyl, R^1, R^2 = H
3 Ar^1 = phenyl, Ar^2 = *p*-chlorophenyl, R^1, R^2 = H

Table I. ^1H NMR Data (ppm) for 1–3 and 22a–23b[a]

protons at	1	2	3	22a	22b	23a	23b
C-2	5.67 (d, 7.0)	5.67 (d, 7.0)	5.66 (d, 7.0)	5.69 (m)	5.69 (m)	5.67 (m)	5.67 (m)
C-2′	4.78 (d, 2.6)	4.78 (bs)	4.76 (bs)	4.63 (d, 2.6)	4.70 (d, 2.6)	4.60 (d, 2.6)	4.70 (d, 2.6)
C-3	3.77 (d, 7.0)	3.79 (d, 7.0)	3.79 (d, 7.0)	3.82 (d, 7.2)	3.81 (d, 7.2)	3.82 (d, 6.4)	3.81 (d, 6.4)
C-3′	5.76 (dd, 2.0, 9.0)	5.76 (dd, 2.4, 8.8)	5.76 (dd, 2.4, 8.8)	5.69 (m)	5.69 (m)	5.67 (m)	5.67 (m)
C-4 OAc	2.36 (s)	2.37 (s)	2.37 (s)	2.53 (s)	2.38 (s)	2.52 (s)	2.38 (s)
C-5	4.92 (d, 8.0)	4.94 (d, 9.0)	4.93 (d, 7.9)	4.96 (d, 7.9)	4.93 (d, 7.9)	4.96 (d, 7.2)	4.93 (d, 7.2)
C-6a	1.85 (m)	1.81 (m)	1.90 (m)	1.90 (m)	1.90 (m)	1.90 (m)	1.90 (m)
C-6b	2.49 (m)	2.49 (m)	2.51 (m)	2.08 (m)	2.08 (m)	2.08 (m)	2.08 (m)
C-7	4.36 (m)	4.39 (m)	4.39 (m)	4.45 (m)	4.45 (m)	4.46 (m)	4.46 (m)
C-10	6.26 (s)	6.26 (s)	6.27 (s)	6.43 (s)	6.43 (s)	6.43 (s)	6.43 (s)
C-10 OAc	2.22 (s)	2.24 (s)	2.22 (s)	2.17 (s)	2.17 (s)	2.17 (s)	2.17 (s)
C-13	6.20 (bt, 9)	6.22 (bt, 8.8)	6.22 (bt, 8.8)	6.23 (m)	6.23 (m)	6.25 (m)	6.25 (m)
C-14	2.28 (m)	2.31 (m)	2.30 (m)	2.28 (m)	2.28 (m)	2.30 (m)	2.30 (m)
C-16	1.21 (s)	1.23 (s)	1.21 (s)	1.23 (s)	1.22 (s)	1.23 (s)	1.22 (s)
C-17	1.13 (s)	1.14 (s)	1.13 (s)	1.19 (s)	1.18 (s)	1.17 (s)	1.18 (s)
C-18	1.77 (s)	1.78 (s)	1.80 (s)	2.00 (s)	2.00 (s)	2.01 (s)	2.02 (s)
C-19	1.67 (s)	1.68 (s)	1.67 (s)	1.70 (s)	1.70 (s)	1.70 (s)	1.70 (s)
C-20	4.17, 4.27 (AB q, 8)	4.18, 4.30 (AB q, 8.3)	4.18, 4.30 (AB q, 8.3)	4.18, 4.31 (m)	4.18, 4.31 (m)	4.20, 4.32 (m)	4.20, 4.32 (m)
NH	7.22 (d, 9)	6.99 (d, 8.8)	7.12 (d, 8.8)	7.06 (d, 8.8)	7.28 (d, 8.8)	7.14 (d, 8.8)	7.25 (d, 8.8)
CH_2CH_3				0.98 (t, 7.0)	1.05 (t, 7.0)	1.02 (t, 7.0)	1.08 (t, 7.0)
$CHCH_3$				1.25 (d, 5.4)	1.28 (d, 5.4)	1.31 (d, 5.3)	1.31 (d, 5.3)
CH_2CH_3				3.25 (m)	3.25 (m)	3.29 (m)	3.29 (m)
$CHCH_3$				4.53 (q, 5.4, 10.7)	4.79 (q, 5.4, 10.7)	4.57 (q, 5.3, 10.7)	4.82 (q, 5.3, 10.7)

[a] Multiplicities and coupling constants in hertz are given in parentheses. The spectrum of 1 was recorded at 500 MHz; all other data were obtained at 300 MHz.

Table II. ^1H NMR Data (ppm) for Aromatic Protons of 1–3[a]

no.	*o*-$PhCO_2$	*m*-$PhCO_2$	*p*-$PhCO_2$	*o*-ArCONH	*m*-ArCONH	*p*-ArCONH	*o*-Ar	*m*-Ar	*p*-Ar
1	8.11 (d, 7.2)	7.49 (t, 7.2)	7.59 (t, 7.2)	7.72 (d, 7.2)	7.36 (t, 7.2)	7.46 (t, 7.2)	7.45 (d, 7.2)	7.39 (t, 7.2)	7.32 (t, 7.2)
2	8.13 (dd, 1.4, 7.2)	7.51 (t, 7.2)	7.62 (t, 7.2)	7.68 (d, 7.2)	7.37 (d, 7.2)		7.48 (d, 7.2)	7.42 (t, 7.2)	7.37 (t, 7.2)
3	8.12 (dd, 1.4, 7.2)	7.51 (t, 7.2)	7.62 (t, 7.2)	7.72 (dd, 1.4, 7.2)	7.38 (t, 7.2)	7.50 (t, 7.2)	7.42 (d, 7.2)	7.37 (d, 7.2)	

[a] Multiplicities and coupling constants in hertz are given in parentheses. The spectrum of 1 was recorded at 500 MHz; all other data were obtained at 300 MHz.

All newly synthesized compounds displayed spectral data in agreement with the assigned structures. They were also confirmed by MS or FABMS. The ^1H NMR assignments of taxol derivatives 2 and 3 were made by comparison of their ^1H NMR spectra with those of taxol (1)[42] and related analogues.[26,27] All of the spectral characteristics of 2 and 3 were similar to taxol (1) (Table I) with exception of the aromatic region.

As expected, derivative 2 displayed ^1H NMR data in the aromatic region, consistent with a *p*-chloro-substituted *N*-benzoyl derivative, and analogue 3 with a *p*-chloro-substituted 3′-phenyl derivative (Table II). The assignment of the ^1H NMR data of the aromatic protons of taxol (1), and the taxol derivatives 2 and 3 was made by inspection of their COSY spectra (500 MHz).

Biological Testing

The activity of taxol analogues 2 and 3 was compared to taxol in two assays. The first measures the ability of taxol to stimulate the assembly of tubulin into microtubules. Under the conditions of our assay, tubulin (free of microtubule-associated proteins) does not assemble in the absence of taxol. Thus, the assay is diagnostic of taxol-like activity. Figure 2 presents the results of an experiment comparing the ability of a 5 μM concentration of 2 and 3 in comparison to taxol, baccatin III and *N*-benzoyl-(2*R**,3*S**)-3-phenylisoserine to promote the assembly reaction.

Another experiment was done to determine the extent of assembly at different concentrations of the compounds

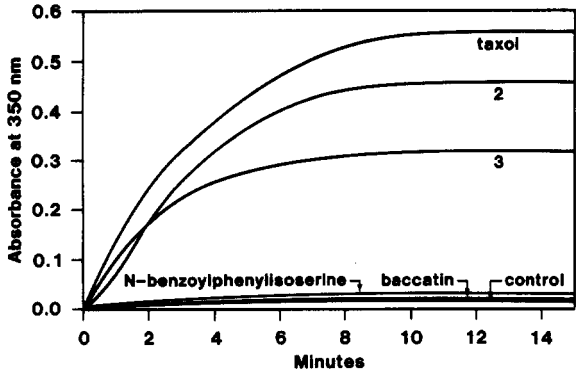


Figure 2. Tubulin at 10 μM was polymerized at 37 °C in the presence of 5 μM taxol or its derivatives and 0.5 mM GTP in PEM buffer. The increase in turbidity was monitored by the apparent absorbance at 350 nm.

Table III. Activity of Taxol Analogues in the Tubulin Assembly Assay[a]

compound	ED_{50},[b] μM	ED_{50}/ED_{50} (taxol)
taxol (1)	0.7	1.0
2	1.7	2.4
3	1.3	1.9
baccatin III (4)	>10[c]	>14
N-benzoyl-(2*R**,3*S**)-3-phenylisoserine	>10[c]	>14

[a] The concentration of tubulin was 10 μM. [b] ED_{50} = the concentration which produces 50% of the maximum absorbance increase at 350 nm. [c] No assembly was found at 10 μM, the highest concentration used.

Table IV. Cytotoxic Activity of the Taxol Analogues against B16 Melanoma Cells[a]

compound	ED_{50},[b] nM	ED_{50}/ED_{50} (taxol)
taxol (1)	28	1.0
2	43	1.5
3	61	2.2
baccatin III (4)	>1000[c]	>36
N-benzoyl-(2*R**,3*S**)-3-phenylisoserine	>10000[d]	>360

[a] Conditions are described under the Experimental Section. [b] ED_{50} = the concentration which produces 50% inhibition of proliferation after a 40-h incubation. At the end of this time, the control contained about 7×10^5 cells/well. [c] At 1 μM, 40% inhibition occurred. [d] At 42 μM, 64% inhibition occurred.

and the concentration which produced a 50% effect (ED_{50}) was determined (Table III). The analogues **2** and **3** displayed an ED_{50}/ED_{50}(taxol) of 2.4 and 1.9, respectively, in this assay. Baccatin III and *N*-benzoyl-(2*R**,3*S**)-3-phenylisoserine had no activity at the concentrations tested.

The cytotoxicity of the analogues was also tested against B16 melanoma cells in culture. The concentrations of the compounds which produced 50% inhibition of proliferation after 40 h (ED_{50}) are listed in Table IV. This assay also showed the chloro derivatives to have good taxol-like activity. The ED_{50}/ED_{50}(taxol) were found to be 1.5 and 2.2, respectively. Baccatin III (**4**) and *N*-benzoyl-(2*R**,3*S**)-3-phenylisoserine had very little activity in this assay at the concentrations tested.

Conclusions

Taxol analogues **2** and **3**, possessing a *N*-(*p*-chlorobenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserine side chains and *N*-benzoyl-(2′*R*,3′*S*)-3′-[(*p*-chlorophenyl)isoserine] side chain, respectively, were prepared in good overall yields from baccatin III and 3-hydroxy-4-aryl-2-azetidinones. The synthetic methodology utilized in these studies allows for the synthesis of a variety of C-13 side chain modified taxol analogues. The taxol derivatives **2** and **3** displayed significant activity in the microtubule assembly assay and against B16 melanoma cell proliferation. Thus, the introduction of the *p*-chloro substituents at the two phenyl groups of the C-13 taxol side chain did not significantly alter the activity of taxol.

More extensive structure–activity studies with respect to the influence of substituents at the aromatic ring systems of the C-13 taxol side chain on biological activity as well as a more indepth evaluation of the cytotoxicity of analogues **2** and **3** are underway in our laboratory.

Experimental Procedures

Chemistry. General. ^1H NMR spectra were recorded with a General Electric QE-300, a Varian VXR-300 spectrometer, or a Brucker AM-500 in parts per million (ppm) using tetramethylsilane as the internal standard. IR spectra were recorded with a Perkin-Elmer 1420 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Melting points were measured with a Thomas Hoover capillary melting point apparatus and are uncorrected. HPLC analysis for the determination of enantiomeric excess was carried out with a Waters Model 590 programmable solvent delivery module and a Waters Model 481 LC spectrometer detector (at 254 nm), equipped with a Waters 740 Data Module integrator using a chiral column J. T. Baker DIACEL-CHIRAL OD employing hexanes/2-propanol (13/1) as the solvent system with a flow rate of 0.2 mL/min. Mass spectra were obtained from a ZAB HS mass spectrometer (VG Analytical Ltd, Manchester, U.K.) equipped with a 11/250 data system by Dr. Todd Williams and Robert Drake. Fast-atom-bombardment mass spectrometry experiments were performed using a xenon gun operated at 8-keV energy and 0.8-mA emission. Sample in CH_2Cl_2 was added to thioglycerol as the matrix. Exact mass FAB experiments were carried out at 1:10 000 resolution using linear voltage scans under data system control and collecting continuum data in multichannel analyser (MCA) mode. Polyethylene glycol peaks served as bracketing calibrant ions. All reactions requiring anhydrous conditions were performed using a positive atmosphere of nitrogen in oven-dried glassware. Tetrahydrofuran (THF) was distilled from benzophenone ketyl prior to use. Diisopropylamine was refluxed and distilled from calcium hydride prior to use. *n*-Butyllithium in hexanes was titrated prior to use. Thin-layer chromatography was carried out on Merck silica gel 60 F-254 plates of 0.25-mm thickness. Column chromatography was carried out by flash chromatography on Aldrich silica gel (70–230 mesh). Intermediates **7**, **15**, **22**, and **23** were judged to be pure by ^1H NMR and ^{13}C NMR spectroscopy (see supplementary material available) and thus subjected to the next step without further analysis of purity. Both taxol analogues **2** and **3** were found to be pure compounds by ^1H NMR and ^{13}C NMR spectroscopy (see supplementary material available). The high purity of **2** and **3** was additionally established by HPLC analysis.[43]

(1*R*,2*S*)-2-Phenyl-1-cyclohexyl Crotonate (20). To a stirred solution of (−)-(1*R*,2*S*)-2-phenyl-1-cyclohexanol (**19**) (2.0 g, 11.34 mmol) in anhydrous toluene (25 mL) were added silver cyanide (4.05 g, 34.02 mmol) and crotonyl chloride (2.77 mL, 28.93 mmol) sequentially, and the mixture was refluxed for 7 h under nitrogen. The reaction mixture was diluted with ether (50 mL) and passed through a pad of Celite. The organic layer was washed with 10% sodium bicarbonate and brine and dried over anhydrous sodium sulfate. The crude product was submitted to column chromatography on silica gel using ethyl acetate/hexanes (1:9) as the eluent to give 2.67 g (97% yield) of **20** as a colorless oil: $[\alpha]_D$ −57.9° (*c* 3.08, $CHCl_3$); IR (neat) 2930, 2850, 1710, 1650, 1180 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25–1.53 (bm, 4 H, H_4 and H_5), 1.72 (d, *J* = 6 Hz, 3 H, CH_3), 1.80–1.95 (m, 3 H, H_3 and H_5), 2.10–2.22 (m, 1 H, H_6), 2.42 (ddd, $J_{2,3eq}$ = 5.4 Hz, $J_{1,2}$ = 10.8 Hz, $J_{2,3ax}$ = 10.8 Hz, 1 H, H_2), 5.0 (ddd, $J_{1,6eq}$ = 5.4 Hz, $J_{1,2}$ = 10.8 Hz, $J_{1,6ax}$ = 10.8 Hz, 1 H, H_1), 5.55 (d, *J* = 16 Hz, 1 H, $H_{2'}$), 6.72 (sextet, $J_{2',3'}$ = 16 Hz, $J_{3',4'}$ = 6 Hz, 1 H, $H_{3'}$), 7.17–7.35 (m, 5 H, ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 17.7, 24.7, 25.8, 32.3, 34.0, 49.6, 75.5,

122.8, 126.2, 127.4, 128.1, 143.1, 143.2, 165.7; MS-CI m/z 262 ($M^+ + NH_4^+$), 245 (MH^+), 176, 159, 91, 69, 41. Anal. ($C_{16}H_{20}O_2$) C, H, N.

(1*R*,2*S*)-2-Phenyl-1-cyclohexyl Glycolate (21). Ozone was bubbled through a stirred solution of **20** (2.65 g, 10.84 mmol) in methylene chloride (25 mL) at −78 °C until a blue color persisted. The reaction mixture was stirred for 5 min at −78 °C, and the excess ozone was removed by a stream of nitrogen. The reaction was quenched by treating with dimethyl sulfide (4.78 mL, 65.09 mmol) at −78 °C, and the mixture was allowed to warm to ambient temperature and stirred for 2 h. To the reaction mixture at 0 °C was added 25 mL of ethanol followed by the addition of sodium borohydride (615 mg, 16.26 mmol). After 30 min the reaction was quenched with saturated ammonium chloride and the solvent was removed in vacuo. The residue was dissolved in water and extracted with ethyl acetate (3 × 75 mL). The organic layer was washed with saturated sodium chloride and dried over anhydrous sodium sulfate. Purification of the crude product by flash column chromatography (1:4 ethyl acetate/hexanes) provided 2.15 g (85%) of **21** as a colorless oil: $[\alpha]_D$ −2.08° (*c* 0.95, CH_3OH); IR (CH_2Cl_2) 3460 (broad), 2920, 2850, 1730, 1220, 1210 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.2–1.65 (bm, 4 H, H_4 and H_5), 1.7–1.95 (m, 3 H, H_3 and H_6), 2.10–2.15 (m, 1 H, H_6), 2.64 (ddd, $J_{2,3eq}$ = 3.7 Hz, $J_{2,3ax}$ = 11.5 Hz, $J_{1,2}$ = 10.5 Hz, 1 H, H_2), 2.77 (bs, 1 H, OH), 3.63 (d, J = 16.8 Hz, 1 H, $H_{2'}$), 3.84 (d, J = 16.8 Hz, 1 H, $H_{2'}$), 5.03 (ddd, $J_{1,6eq}$ = 4.5 Hz, $J_{1,2}$ = 10.5 Hz, $J_{1,6ax}$ = 10.5 Hz, 1 H, H_1), 7.10–7.30 (m, 5 H, ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.2, 25.2, 31.7, 33.2, 49.1, 59.7, 76.4, 126.0, 126.9, 127.8, 142.2, 172.0; MS-EI m/z 234 (M^+), 158, 130, 91. Anal. ($C_{14}H_{18}O_3$) C, H, N.

(1*R*,2*S*)-2-Phenyl-1-cyclohexyl [(Triisopropylsilyl)oxy]-acetate (7). To a stirred solution of **21** (1.05 g, 4.52 mmol) in dimethylformamide (10 mL) were added imidazole (923 mg, 13.56 mmol) and triisopropylsilyl chloride (1.30 g, 6.78 mmol) sequentially at ambient temperature under nitrogen. The reaction mixture was stirred for 36 h, diluted with diethyl ether (50 mL), and washed with water. The combined organic layers were dried over anhydrous sodium sulfate. Purification of the crude product by flash column chromatography (1:20 ethyl acetate/hexanes) provided 1.72 g (98%) of **7** as a colorless oil: $[\alpha]_D$ −19.2° (*c* 1.17, $CHCl_3$); IR (CH_2Cl_2) 2940, 2860, 1760, 1740, 1150 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.93–0.99 (m, 21 H, SiC_9H_{21}), 1.30–1.62 (m, 4 H, H_4 and H_5), 1.70–2.0 (m, 3 H, H_3 and H_6), 2.10–2.19 (m, 1 H, H_6), 2.66 (ddd, $J_{2,3eq}$ = 4.0 Hz, $J_{1,2}$ = 10.6 Hz, $J_{2,3ax}$ = 11.5 Hz, 1 H, H_2), 3.90 (d, J = 16.6 Hz, 1 H, $H_{2'}$), 4.07 (d, J = 16.6 Hz, 1 H, $H_{2'}$), 5.07 (ddd, $J_{1,6eq}$ = 4.0 Hz, $J_{1,6ax}$ = 10.6 Hz, $J_{1,2}$ = 1 H, H_1), 7.16–7.30 (m, 5 H, ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.7, 17.6, 24.6, 25.7, 32.2, 34.0, 49.6, 61.6, 75.0, 126.3, 127.3, 128.2, 142.8, 170.8; MS-CI m/z 408 ($M^+ + NH_4^+$), 391 (MH^+), 159, 91.

(3*R*,4*S*)-3-[(Triisopropylsilyl)oxy]-4-phenyl-2-azetidinone (10). To a stirred solution of diisopropylamine (0.30 mL, 2.20 mmol) in THF (2.0 mL) at −78 °C was added 2.5 M solution of *n*-butyllithium (2.20 mL) in THF. This mixture was warmed to 0 °C, stirred for 30 min, and then cooled back to −78 °C. To the mixture was added a solution of **7** (781 mg, 2.0 mmol) in THF (2.0 mL). The solution was stirred for 2 h at −78 °C, followed by the addition of *N*-(trimethylsilyl)benzaldimine (**8**) (2.0 mmol) in THF (2.0 mL). The mixture was stirred at −78 °C for 4 h and then slowly allowed to warm to room temperature and further stirred overnight. The reaction was quenched with a saturated solution of ammonium chloride (50 mL), and the reaction mixture was extracted with diethyl ether (2 × 50 mL). The combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Purification of the crude product by flash column chromatography (1:6 ethyl acetate/hexanes) gave 542 mg (85%) of β-lactam **10** as a colorless solid: mp 78–79 °C; $[\alpha]_D$ +55.7° (*c* 1.59, $CHCl_3$); 96% ee; IR (CH_2Cl_2) 3260, 2930, 2850, 1760, 1190, 1170 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.86–0.91 (m, 21 H, SiC_9H_{21}), 4.81 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H_4), 5.17 (dd, $J_{1,3}$ = 2.6 Hz, $J_{3,4}$ = 4.7 Hz, 1 H, H_3), 7.06 (bs, 1 H, NH), 7.30–7.40 (m, 5 H, ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.6, 17.3, 17.4, 59.6, 79.6, 127.8, 127.9, 128.1, 136.3, 170.4; MS-EI m/z 320 (MH^+), 319 (M^+), 233, 161, 75, 59. Anal. ($C_{18}H_{29}NO_2Si$) C, H, N.

(3*R*,4*S*)-3-[(Triisopropylsilyl)oxy]-4-(*p*-chlorophenyl)-2-azetidinone (11). The same procedure was used as for the formation of β-lactam **10** utilizing *p*-chlorobenzaldimine (**9**): 70% yield as a colorless oil; $[\alpha]_D$ +44.95° (*c* 1.90, CH_2Cl_2); 91% ee; IR (CH_2Cl_2) 3260, 2940, 2860, 1760, 1260, 1190 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.8–0.96 (m, 21 H, SiC_9H_{21}), 4.76 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H_4), 5.13 (dd, $J_{1,3}$ = 2.4 Hz, $J_{3,4}$ = 4.7 Hz, 1 H, H_3), 6.70 (bs, 1 H, NH), 7.27 (q, J = 8.3 Hz, 16.9 Hz, 4 H, ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.6, 17.4, 17.5, 59.1, 79.7, 128.1, 129.5, 133.8, 135.0, 170.5; MS-EI m/z 353 (MH^+), 310, 157, 75, 59; HRMS calcd for $C_{18}H_{28}ClNO_2Si$ 353.1578, found 353.1585. Anal. ($C_{18}H_{28}ClNO_2Si$) C, H, N.

(3*R*,4*S*)-3-Hydroxy-4-phenyl-2-azetidinone (12). A solution of **10** (200 mg, 0.62 mmol) in THF (5 mL) was treated with a 1 M solution of tetra-*n*-butylammonium fluoride (0.93 mL) at room temperature for 1 h under nitrogen. The reaction mixture was poured into water and extracted with ethyl acetate (3 × 25 mL). The combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Purification of the crude product by passing through a short silica gel column using ethyl acetate as the eluent gave 98 mg of **12** (98% yield) as a colorless solid: mp 185–186 °C; $[\alpha]_D$ +193° (*c* 1.63, MeOH); IR (KBr) 3360, 3250, 1740, 1735 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 4.83 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H_4), 5.04 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H_3), 7.25–7.45 (m, 5 H, ArH); ^{13}C NMR (300 MHz, CD_3OD) δ 61.7, 81.3, 130.0, 130.3, 130.8, 139.7, 174.6; MS-EI m/z 163 (M^+), 149, 120, 106, 91, 77, 51. Anal. ($C_9H_9NO_2$) C, H, N.

(3*R*,4*S*)-3-Hydroxy-4-(*p*-chlorophenyl)-2-azetidinone (13). The same procedure was used as for the formation of β-lactam **12**: 95% yield, colorless solid; $[\alpha]_D$ +150° (*c* 0.26, MeOH); IR (CH_2Cl_2) 3300, 1760, 1735 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 5.21 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H_4), 5.43 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H_3), 7.67 (d, J = 8.1 Hz, 2 H, ArH), 7.75 (d, J = 8.1 Hz, 2 H, ArH); ^{13}C NMR (300 MHz, CD_3OD) δ 58.5, 78.7, 128.3, 129.1, 133.5, 136.0, 171.9; MS-EI m/z 197 (M^+), 154, 140, 125, 91, 77, 51; HRMS calcd for $C_9H_8ClNO_2$ 197.0244, found 197.0248. Anal. ($C_9H_8ClNO_2$) C, H, N.

(3*R*,4*S*)-3-[((*R)-1′-Ethoxyethyl)oxy]-4-phenyl-2-azetidinone (14).** To a stirred solution of **12** (100 mg, 0.61 mmol) in THF (5 mL) at 0 °C was added freshly distilled ethyl vinyl ether (0.211 mL, 3.05 mmol), followed by the addition of a catalytic amount of *p*-toluenesulfonic acid. After 0.5 h the reaction mixture was diluted with diethyl ether (50 mL), washed with saturated sodium bicarbonate and brine, and dried over anhydrous sodium sulfate. Purification of the crude product by flash column chromatography (1:2 ethyl acetate/hexanes) provided **14** (136 mg, 95% yield) as a viscous colorless oil in a 1:1 ratio of diastereoisomers: IR (neat) 3250, 2980, 2920, 1755, 1370, 1270, 1250 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (d, J = 5.4 Hz, 1.5 H, $CHCH_3$), 1.03 (d, J = 5.4 Hz, 1.5 H, $CHCH_3$), 1.09 (t, J = 7.0 Hz, 1.5 H, OCH_2CH_3), 1.11 (t, J = 7.0 Hz, 1.5 H, OCH_2CH_3), 3.14–3.25 (m, 0.5 H, OCH_2CH_3), 3.30–3.42 (m, 1 H, OCH_2CH_3), 3.56–3.68 (m, 0.5 H, OCH_2CH_3), 4.47 (q, J = 5.4 Hz, 10.7 Hz, 0.5 H, $CHCH_3$), 4.66 (q, J = 5.4 Hz, 10.7 Hz, 0.5 H, $CHCH_3$), 4.80 (d, $J_{3,4}$ = 4.7 Hz, 0.5 H, H_4), 4.84 (d, $J_{3,4}$ = 4.7 Hz, 0.5 H, H_4), 5.12–5.19 (m, 1 H, H_3), 6.70 (bs, 0.5 H, NH), 6.75 (bs, 0.5 H, NH), 7.35 (m, 5 H, ArH); MS-EI m/z 236 (MH^+), 207, 181, 106, 73, 61, 44; HRMS-FAB calcd for $C_{13}H_{18}NO_3$ 236.1287, found 236.1266. Anal. ($C_{13}H_{18}NO_3$) C, H, N.

(3*R*,4*S*)-3-[((*R)-1′-Ethoxyethyl)oxy]-4-(*p*-chlorophenyl)-2-azetidinone (15).** The same procedure was used as for the formation of β-lactam **14**: 90% yield of a colorless oil in a 1:1 ratio of diastereoisomers; IR (neat) 3270, 3040, 2970, 1760, 1260 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (d, J = 5.4 Hz, 1.5 H, $CHCH_3$), 1.07 (d, J = 5.4 Hz, 1.5 H, $CHCH_3$), 1.09 (t, J = 7.2 Hz, 1.5 H, OCH_2CH_3), 1.15 (t, J = 7.2 Hz, 1.5 H, OCH_2CH_3), 3.17–3.29 (m, 0.5 H, OCH_2CH_3), 3.29–3.44 (m, 1 H, OCH_2CH_3), 3.57–3.69 (m, 0.5 H, OCH_2CH_3), 4.47 (q, J = 5.4 Hz, 10.7 Hz, 0.5 H, $CHCH_3$), 4.67 (q, J = 5.4 Hz, 10.7 Hz, 0.5 H, $CHCH_3$), 4.78 (d, $J_{3,4}$ = 4.7 Hz, 0.5 H, H_4), 4.82 (d, $J_{3,4}$ = 4.7 Hz, 0.5 H, H_4), 5.12–5.18 (m, 1 H, H_3), 7.02 (bs, 0.5 H, NH), 7.05 (bs, 0.5 H, NH), 7.24–7.34 (m, 4 H, ArH); MS-EI m/z 270 (MH^+), 240, 224, 180, 125, 73, 45; HRMS calcd (MH^+) for $C_{13}H_{16}ClNO_3$ 270.0897, found 270.0885.

(3*R*,4*S*)-1-(*p*-Chlorobenzoyl)-3-[((*R)-1′-ethoxyethyl)oxy]-4-phenyl-2-azetidinone (16).** To a stirred solution of **14** (250 mg, 1.06 mmol) in methylene chloride (5 mL) at 0 °C were added

triethylamine (0.29 mL, 2.12 mmol), a catalytic amount of 4-(dimethylamino)pyridine, and *p*-chlorobenzoyl chloride (0.20 mL, 1.59 mmol) sequentially. After 45 min the reaction mixture was diluted with diethyl ether (50 mL) and washed with 2% HCl, saturated solution of sodium bicarbonate, and brine and dried over anhydrous sodium sulfate. Purification of the crude product by flash column chromatography (1:4 ethyl acetate/hexanes) gave **16** (371 mg, 95%) as a viscous colorless oil in a 1:1 ratio of diastereoisomers: IR (CH_2Cl_2) 2920, 2850, 1790, 1680, 1290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.03 (d, J = 5.3 Hz, 1.5 H, $CHCH_3$), 1.05–1.15 (m, 4.5 H, $CHCH_3$ and OCH_2CH_3), 3.20–3.30 (m, 0.5 H, OCH_2CH_3), 3.30–3.45 (m, 1 H, OCH_2CH_3), 3.60–3.75 (m, 0.5 H, OCH_2CH_3), 4.56 (q, J = 5.4 Hz, 10.8 Hz, 0.5 H, $CHCH_3$), 4.75 (q, J = 5.4 Hz, 10.8 Hz, 0.5 H, $CHCH_3$), 5.28 (d, $J_{3,4}$ = 6.2 Hz, 1 H, H_4), 5.40 (d, $J_{3,4}$ = 6.2 Hz, 0.5 H, H_3), 5.43 (d, $J_{3,4}$ = 6.2 Hz, 0.5 H, H_3), 7.2–7.98 (m, 7 H, ArH), 8.0 (d, J = 2.2 Hz, 2 H, ArH); MS-EI m/z 374 (MH^+), 312, 173, 73, 52; HRMS-FAB calcd for $C_{20}H_{20}ClNO_4$ 374.1159, found 374.1135. Anal. ($C_{20}H_{20}ClNO_4$) C, H, N.

(3*R*,4*S*)-1-Benzoyl-3-[((*R)-1′-ethoxyethyl)oxy]-4-(*p*-chlorophenyl)-2-azetidinone (17).** The same procedure was used as for the synthesis of β-lactam **16**; yield 90% as a colorless oil in a 1:1 ratio of diastereoisomers; IR (CH_2Cl_2) 2970, 2920, 1795, 1670, 1490, 1290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, J = 5.4 Hz, 1.5 H, $CHCH_3$), 1.10–1.20 (m, 4.5 H, $CHCH_3$ and OCH_2CH_3), 3.26–3.32 (m, 0.5 H, OCH_2CH_3), 3.32–3.42 (m, 1 H, OCH_2CH_3), 3.64–3.76 (m, 0.5 H, OCH_2CH_3), 4.58 (q, J = 5.4 Hz, 10.8 Hz, 0.5 H, $CHCH_3$), 4.76 (q, J = 5.4 Hz, 10.8 Hz, 0.5 H, $CHCH_3$), 5.26 (d, $J_{3,4}$ = 6.2 Hz, 1 H, H_4), 5.38 (d, $J_{3,4}$ = 6.2 Hz, 0.5 H, H_3), 5.41 (d, $J_{3,4}$ = 6.2 Hz, 0.5 H, H_3), 7.33–7.60 (m, 7 H, ArH), 8.0–8.11 (m, 2 H, ArH); MS-EI m/z 374 (MH^+), 328, 316, 105, 77, 73, 45; HRMS calcd for $C_{20}H_{20}ClNO_4$ 373.1081, found 373.1085. Anal. ($C_{20}H_{20}ClNO_4$) C, H, N.

(3*R*,4*S*)-1-Benzoyl-3-[(triisopropylsilyl)oxy]-4-(*p*-chlorophenyl)-2-azetidinone (18). The same procedure was used as for the synthesis of β-lactam **16**: 96% yield, colorless oil; $[\alpha]_D$ +100° (*c* 1.15, $CHCl_3$); IR (CH_2Cl_2) 2940, 2860, 1795, 1720, 1670, 1490, 1290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91–0.98 (m, 21 H, SiC_9H_{21}), 5.24 (d, $J_{3,4}$ = 6.1 Hz, 1 H, H_4), 5.39 (d, $J_{3,4}$ = 6.1 Hz, 1 H, H_3), 7.34–7.61 (m, 7 H, ArH), 8.01–8.03 (m, 2 H, ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.6, 17.3, 17.4, 60.5, 76.5, 128.2, 128.6, 129.6, 129.8, 131.8, 132.5, 133.5, 134.3, 165.1, 166.2; MS-EI m/z 457 (M^+), 414, 310, 267, 226, 105, 77, 51; HRMS calcd for $C_{25}H_{32}ClNO_2Si$ 457.1840, found 457.1840. Anal. ($C_{25}H_{32}ClNO_2Si$) C, H, N.

7-(Triethylsilyl)baccatin III 13-[*N*-(*p*-Chlorobenzoyl)-*O*-((*R)-1″-ethoxyethyl)-(2′*R*,3′*S*)-3′-phenylisoserinate] (22).** 7-(Triethylsilyl)baccatin III (50 mg, 0.071 mmol) was reacted with 5 equiv of β-lactam **16** (132 mg, 0.355 mmol) and 4-(dimethylamino)pyridine (8 mg, 0.071 mmol) in pyridine (2 M in 7-(triethylsilyl)baccatin III) at 25 °C for 24 h. The reaction mixture was submitted to flash column chromatography using ethyl acetate/hexanes (1:4) as the eluant to give 0.069 g (91%) of **22** as colorless crystals and as a 1:1.9 mixture of diastereoisomers with 5% recovery of 7-(triethylsilyl)baccatin III: mp 187 °C (CH_2Cl_2/pentane); IR (CH_2Cl_2) 3420, 2940, 2860, 1730, 1720, 1660, 1230 cm^{-1}; MS-FAB m/z 1074 (MH^+), 1096 ($M + Na^+$), 1002, 307.

7-(Triethylsilyl)baccatin III 13-[*N*-Benzoyl-*O*-((*R)-1″-ethoxyethyl)-(2′*R*,3′*S*)-3′-(*p*-chlorophenyl)isoserinate] (23).** The same procedure was used as for the synthesis of compound **22**: 89% yield of **23** as colorless crystals and as a 1:1.3 mixture of diastereoisomers; mp 174 °C (CH_2Cl_2/pentane); IR (CH_2Cl_2) 3420, 2940, 2860, 1730, 1720, 1660, 1230 cm^{-1}; MS-FAB 1074 (MH^+), 1096 ($M + Na^+$), 1002, 320.

Baccatin III 13-[*N*-(*p*-Chlorobenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserinate] (2). Compound **22** was treated with 1:1 0.5% HCl/EtOH solution at 0 °C for 4 days. The reaction mixture was washed with 10% sodium bicarbonate solution and brine and dried over anhydrous sodium sulfate. Purification of the crude product by flash column chromatography (1:1 ethyl acetate/hexanes) gave **2** (90%) as colorless crystals: mp 182–183 °C dec (CH_2Cl_2/pentane); the purity of **2** was verified by HPLC analysis;[43] $[\alpha]_D$ −46° (*c* 0.52, MeOH); IR (CH_2Cl_2) 3400, 3040, 2980, 2940, 1730, 1710, 1660 cm^{-1}; ^{13}C NMR (500 MHz, $CDCl_3$) δ 9.6, 14.8, 20.9, 21.8, 22.6, 26.9, 29.7, 35.6, 43.2, 45.6, 55.0, 58.6, 72.2, 72.4, 73.0, 74.9, 75.5, 76.5, 79.0, 81.1, 84.4, 127.0, 128.4, 128.5, 128.7, 129.0, 129.1, 129.1, 130.2, 132.0, 133.2, 133.7, 137.8, 138.3, 141.9, 166.0, 167.0, 170.4, 171.3, 172.7, 203.6; MS-FAB 910 ($M + Na^+$), 888 (MH^+), 509, 429, 215, 139; HRMS-FAB calcd for $C_{47}H_{50}ClNO_{14}$ 888.2977, found 888.2998.

Baccatin III 13-[*N*-Benzoyl-(2′*R*,3′*S*)-3′-(*p*-chlorophenyl)-isoserinate] (3). The same procedure was used as for the synthesis of compound **2**: yield 92% of **3** as colorless crystals; mp 179–180 °C dec (CH_2Cl_2/pentane); the purity of **3** was verified by HPLC analysis;[43,44] $[\alpha]_D$ −37° (*c* 0.75, MeOH); IR (CH_2Cl_2) 3400, 3040, 2980, 2920, 1730, 1710, 1660 cm^{-1}; ^{13}C NMR (500 MHz, $CDCl_3$) δ 9.5, 14.8, 20.8, 21.7, 22.6, 26.8, 29.6, 35.6, 43.1, 45.7, 54.3, 58.6, 72.1, 72.3, 72.9, 74.9, 75.5, 76.5, 78.9, 81.2, 84.3, 127.0, 128.5, 128.7, 128.7, 129.0, 129.1, 130.2, 132.0, 133.3, 133.4, 133.7, 134.1, 136.7, 141.7, 166.9, 167.0, 170.4, 171.2, 172.4, 203.6; MS-FAB 888 (MH^+), 429, 185; HRMS-FAB calcd for $C_{47}H_{50}ClNO_{14}$ 888.2977; found 888.2998.

Biological Testing.[29] B16 Melanoma Cell Proliferation. Cells were seeded in 24-well plates at 7.5 × 10^4 cells/well and grown in Dulbecco's modified minimal essential medium (MEM) containing 10% bovine calf serum at 37 °C for 24 h in a 97% humidified atmosphere of 5.5% CO_2. The medium was then replaced with fresh medium containing taxol or its derivatives and dissolved in DMSO in concentrations ranging from 7.5 × 10^{-9} M to 1 × 10^{-7} M for taxol and the chloro derivatives **2** and **3** and up to 1 μM for baccatin III (**4**) and 42 μM for *N*-benzoyl-(2*R**,3*S**)-3-phenylisoserine. The final concentration of DMSO in the cell medium was 0.5% or less. This amount of DMSO did not have any effect on cell proliferation as determined from control experiments. After 40 h, the cells were released by trypsinization and counted in a Coulter counter.

Tubulin Preparation and Assembly. Tubulin free of microtubule-associated proteins was purified from bovine brain as previously described.[45] The assembly reaction was done at 37 °C in PEM buffer (0.1 M Pipes, pH 6.9, 1 mM EGTA, and 1 mM $MgSO_4$) at a protein concentration of 1 mg/mL (10 μM) in the presence of taxol or taxol analogues and 0.5 mM GTP. The reaction was monitored by the increase in the apparent absorbance at 350 nm.

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Supplementary Material Available: ^1H NMR and ^{13}C NMR spectra of compounds **2**, **3**, **7**, **15**, **22**, and **23** (12 pages). Ordering information is given on any current masthead page.

(43) Ringel, I.; Horwitz, S. B. Taxol is Converted to 7-Epitaxol, a Biologically Active Isomer, in Cell Culture Medium. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 692–698.

(44) HPLC analysis demonstrated the purity of the sample. In addition to the major peak, we observed the presence a small peak eluting close (prior) to the major peak. We believe that this additional peak is due to the presence of the (2′*S*,3′*R*)-**3** derivative. The formation of this diastereoisomer is expected since the enantiomeric excess of the β-lactam utilized in the acylation of **6** is 91% ee. The presence of the 7-epi derivative was ruled out on the basis of the elution order of taxol (**1**) and 7-epitaxol. Under the same HPLC conditions as utilized by us, Ringel and Horwitz found that taxol elutes before 7-epitaxol.[43]

(45) Algaier, J.; Himes, R. H. The Effect of Dimethyl Sulfoxide on the Kinetics of Tubulin Assembly. *Biochim. Biophys. Acta* **1988**, *954*, 235–243.