Notes

Synthesis of a Photoaffinity Taxol Analogue and Its Use in Labeling Tubulin

Debjani Dasgupta,† Haeil Park,‡ Geraldine C. B. Harriman,‡ Gunda I. Georg,‡ and Richard H. Himes*,†

Departments of Biochemistry and Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

Received April 25, 1994®

A photoaffinity analogue of taxol, *N*-([3,5-^{3}H]-4-azidobenzoyl)-*N*-debenzoyltaxol (**7**), was synthesized and used to photolabel microtubules. Approximately 20% of the noncovalently bound analogue becomes covalently bound upon irradiation at 300 nm. Incorporated label was stable to a 50% ethanol solution and sodium dodecyl sulfate. About 80% of the incorporated label was found in the β-subunit and 20% in the α-subunit. Incorporation did not occur into unpolymerized tubulin, consistent with the fact that taxol binds only to polymerized tubulin, and was decreased by the presence of taxol. Little or no nonspecific labeling occurs. This analogue is currently being used to identify taxol binding site(s) on tubulin.

Introduction

Taxol is a diterpenoid isolated from the Western Yew, *Taxus brevifolia*,[1] which has antimitotic and antineoplastic activities (for recent reviews, see ref 2 and 3). Special interest in taxol has developed due to its exciting antitumor activity, limited availability, and unique mechanism of action. Microtubules are the cellular targets of taxol, but unlike other known antimitotic drugs which inhibit microtubule assembly, taxol promotes assembly and stabilizes microtubules.[4] Thus, taxol alters the normal equilibrium between the tubulin dimer and microtubules, shifting the equilibrium in favor of microtubules. An interesting property of this antimitotic agent is that it binds to tubulin only when the protein is polymerized with a stoichiometry of about 1 mol of taxol bound/mol of tubulin.[5] The detailed mechanism of action by which taxol exerts its effect on microtubules is not known. In trying to understand the interaction of taxol with microtubules, it would be helpful to know the binding site(s) on the tubulin molecule. To accomplish this aim, we have synthesized an azido analogue of taxol, *N*-(4-azidobenzoyl)-*N*-debenzoyltaxol (**6**)[6] (Figure 1), and are using it as a probe for the taxol binding site(s). Previously we have demonstrated that this compound is a good analogue of taxol, with about 50% of the activity of taxol in promoting tubulin assembly.[6] This communication reports on some initial studies of the interaction of this analogue with microtubules which indicate that it will be a useful probe of the taxol site.

Results

Synthesis of AzB-taxol (6). *N*-(4-Azidobenzoyl)-*N*-debenzoyltaxol (**6**)[6] was synthesized as detailed in Scheme 1.[7] Azetidinone **1** was prepared via the ester enolate–imine cyclocondensation reaction as previously described by us.[8–10] Deprotonation of **1** followed by acylation with benzyl chloroformate provided *N*-acyl β-lactam **2** in 90% yield. Reaction between 7-(triethylsilyl)baccatin III (7-TES-baccatin III)[11] and β-lactam **2** in the presence of NaH yielded *N*-CBZ taxol analogue **3** in 93% yield.[9] Removal of the silyl and the *N*-CBZ protecting groups was achieved in 92% and 94% yield,

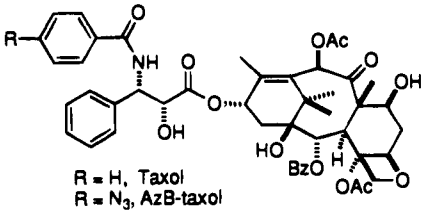
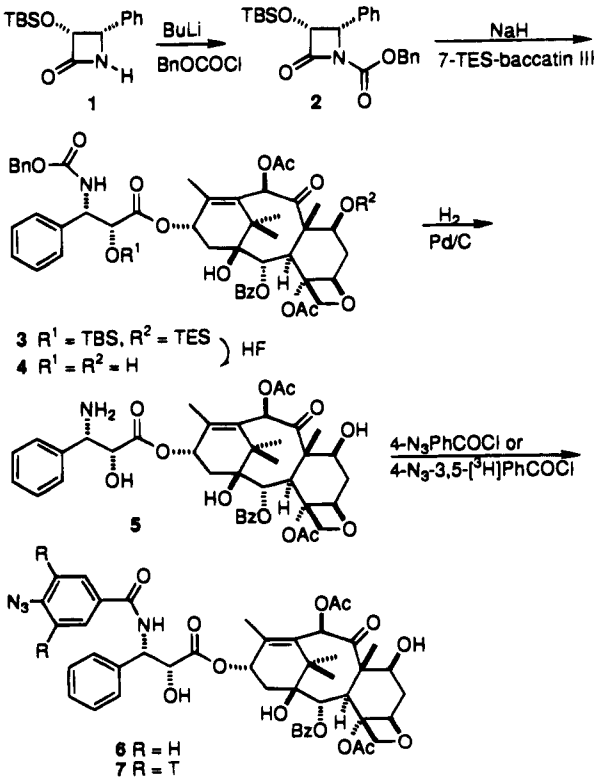


Figure 1. Structures of taxol and AzB-taxol.

Scheme 1



* To whom correspondence should be addressed.
† Department of Biochemistry.
‡ Department of Medicinal Chemistry.
® Abstract published in *Advance ACS Abstracts*, August 1, 1994.

0022-2623/94/1837-2976$04.50/0

Table 1. Photoincorporation of Label Under Different Conditions[a]

condition	incorporation, mol/mol of tubulin
polymerized tubulin	0.15 ± 0.03 (n = 6)[b]
incubation in the dark	0.008 ± 0.002 (n = 4)
preirradiation of AzB-taxol[c]	0.016 ± 0.002 (n = 4)
unpolymerized tubulin (on ice)	0.03 ± 0.004 (n = 3)
tubulin in 8 M urea	0.015 ± 0.005 (n = 3)

[a] [^{3}H]AzB-taxol was incubated with the protein at a molar ratio of 1:1 for 15 min before irradiation at 300 nm or dark incubation for an additional 20 min. [b] n = number of experiments. [c] Preirradiated [^{3}H]AzB-taxol was added to microtubules preformed in 10% dimethyl sulfoxide.

respectively, to supply key intermediate *N*-debenzoyltaxol (**5**). Reaction of **5** with 4-azidobenzoyl chloride gave the desired taxol photoaffinity label **6** in 72% yield, and treatment of **5** with 4-azido-[3,5-^{3}H]benzoyl chloride provided the tritiated analogue **7**. 4-Azido-[3,5-^{3}H]-benzoyl chloride was synthesized from succinimidyl 4-azido[3,5-^{3}H]benzoate via hydrolysis with NaOH followed by treatment with thionyl chloride. This conversion was necessary because the direct reaction between succinimidyl 4-amido-[3,5-^{3}H]benzoate and **5** in the presence of base was slow and low yielding.

Photolabeling of Tubulin. The wavelength dependency of incorporation of [^{3}H]AzB-taxol into tubulin was first measured. Irradiation of the taxol analogue–microtubule complex was done for 20 min using 254, 300, and 350 nm lamps. Using an initial molar ratio of tubulin/AzB-taxol of 2:1, radiation at 254 nm led to the incorporation of 0.13 mol of [^{3}H]AzB-taxol/mol of tubulin, at 300 nm the value was 0.12 mol of [^{3}H]AzB-taxol/mol of tubulin, and at 350 nm the incorporation fell to 0.025 mol of [^{3}H]AzB-taxol/mol of tubulin. Because of the greater potential of producing radiation-induced damage to the taxol derivative and tubulin at 254 nm, we conducted further studies using the 300 nm lamp.

A number of control experiments were performed (Table 1). For example, incubation in the dark did not lead to covalent incorporation. To rule out the possibility that irradiation of the analogue produced a fairly stable product which could react with polymerized tubulin, perhaps in a nonspecific manner, the azido derivative was irradiated for 20 min prior to its addition to microtubules which had been preformed in the presence of 10% dimethyl sulfoxide.[12] This mixture was incubated for another 20 min in the dark before analysis for incorporation. A negligible amount of label was incorporated into tubulin under these conditions. Taxol binds to microtubules but not to unpolymerized tubulin.[5] To demonstrate that incorporation only occurred into polymerized tubulin, irradiation of tubulin and [^{3}H]AzB-taxol was performed at 0 °C. This resulted in little incorporation of label. Incorporation of [^{3}H]AzB-taxol into tubulin denatured in 8 M urea was also negligible (Table 1).

The time course of photoincorporation of labeled AzB-taxol into tubulin is shown in Figure 2. Maximum incorporation of the analogue was observed within 15 min of irradiation. When the ^{3}H-labeled azido derivative was used at varying concentrations to form microtubules and then irradiated at 300 nm for 20 min, the extent of incorporation increased to a plateau value of about 0.22 mol of AzB-taxol/mol of tubulin (Figure 3). In other experiments, the amount of derivative noncovalently bound before irradiation was compared to the amount covalently bound after subsequent irradiation (Table 2). These data indicate that approximately 20% of the noncovalently bound AzB-taxol became covalently incorporated.

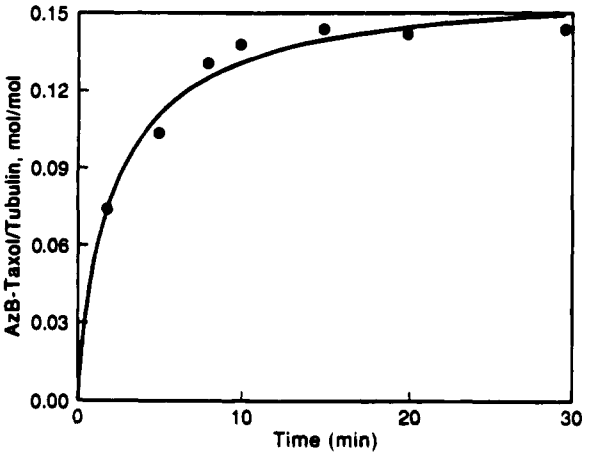


Figure 2. Time dependence of covalent incorporation of labeled AzB-taxol into polymerized tubulin. [^{3}H]AzB-taxol and tubulin, both at 10 μM, were incubated before irradiation at 300 nm for the time periods shown. Details are given in the Experimental Section.

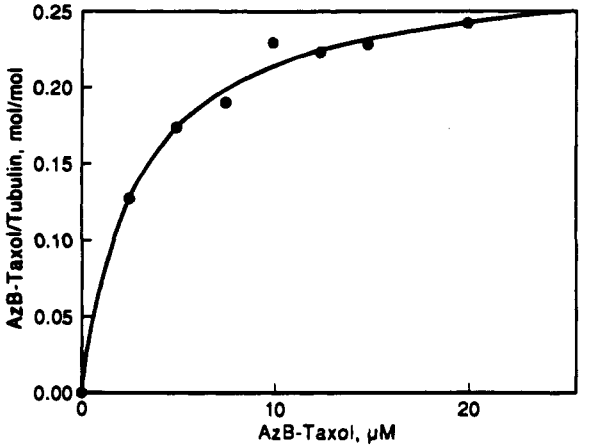


Figure 3. Concentration dependence of label incorporation. [^{3}H]AzB-taxol was incubated with 5 μM tubulin at different molar ratios before irradiation for 20 min. Details are given in the Experimental Section.

Table 2. Comparison of Noncovalent Binding and Photoincorporation[a]

initial ratio of AzB-taxol/tubulin	AzB-taxol bound/tubulin, mol/mol	
	noncovalent	covalent
0.5	0.50	0.13
1.0	0.85	0.17
2.0	1.22	0.23
3.0	1.20	0.23
4.0	1.19	0.24

[a] Samples containing [^{3}H]AzB-taxol at different concentrations and 5 μM tubulin were incubated for 20 min at 37 °C. One set (covalent) was irradiated for 20 min and treated as described in the Experimental Section. The other set was centrifuged at 40000g, and the pellets were washed with buffer, centrifuged, and dissolved in 0.1 M NaOH for determination of protein and radioactivity.

To demonstrate that the azido analogue interacted with the taxol site on tubulin, a competition experiment was performed. Increasing concentrations of unlabeled

Table 3. Distribution of AzB-taxol Incorporation into the α- and β-subunits[a]

initial ratio of AzB-taxol/tubulin	photoincorporation of AzB-taxol, %	
	α-subunit	β-subunit
0.5 ($n = 2$)	20 ± 10	80 ± 10
1.0 ($n = 3$)	14 ± 2	86 ± 2
2.0 ($n = 3$)	19 ± 10	81 ± 10

[a] Tubulin (5 μM) was incubated with different concentrations of [^{3}H]AzB-taxol at 37 °C for 20 min and then irradiated at 300 nm for 20 min. Tubulin subunits were separated by SDS–PAGE and the stained bands cut out and treated as detailed in the Experimental Section. n = number of experiments.

taxol were added to 5 μM tubulin and 5 μM [^{3}H]AzB-taxol before polymerization and irradiation. Taxol decreased the extent of incorporation of the analogue with almost complete inhibition occurring at 7.5 μM taxol (data not shown).

To determine whether labeling was localized to one of the tubulin subunits, the labeled protein was subjected to SDS–PAGE and the bands corresponding to the α- and β-subunits were cut out and counted. The results of several experiments performed at different ratios of [^{3}H]AzB-taxol to tubulin showed that about 80% of the label was found in the β-subunit (Table 3).

Discussion

To help characterize the interaction sites between taxol and tubulin dimers in a microtubule, we and others have synthesized several azido analogues of the drug.[6,13–16] Substitutions at the 7-position have led to derivatives with various degrees of taxol-like activity, e.g., 7-(azidobenzoyl)taxol,[13] 7-(azidotetrafluorobenzoyl)-taxol,[6] 7-(azidonitrobenzoyl)taxol,[14] and 7-[[3-[3-(trifluoromethyl)-[^{3}H]diazirin-3-yl]phenoxy]acetyl]taxol.[15] By substituting an azidobenzoyl group for the *N*-benzoyl ring on the phenylisoserine side chain of taxol, we, and others,[16] have produced an analogue with good taxol-like properties. This derivative is about 50% as active as taxol in a tubulin assembly assay and about 20% as active in a cell culture assay.[6] The reasonable activity of this analogue is consistent with the known fact that the *N*-benzoyl group can be substituted with a variety of substituents with little loss of biological activity.[8,17–21] The only other analogue which has been used for photolabeling studies is 7-[[3-[3-(trifluoromethyl)-[^{3}H]-diazirin-3-yl]phenoxy]acetyl]taxol.[15] This analogue appears to label tubulin in a nonspecific manner and is a poor analogue of taxol.[15] Photolabeling of tubulin by taxol itself, using the direct photolabeling method, has been studied, but no information on the efficiency of labeling was presented.[22] We have also used this approach but found that the labeling is quite inefficient. The *N*-(azidobenzoyl)taxol derivative that we have synthesized specifically labels the protein with good efficiency (20%). That the labeling is covalent is indicated by its stability to 50% ethanol and SDS–PAGE. Specificity of labeling was shown by the fact that the extent of labeling reached a saturating level at concentrations of the derivative which would saturate the binding site (Figure 3) and by the fact that taxol decreased the extent of labeling.

Covalent incorporation of AzB-taxol occurred primarily in the β-subunit, but about 20% of the incorporated label was found in the α-subunit. This may indicate that the *N*-benzoyl group of taxol occupies a site such that it can interact with both subunits. Effective photoaffinity analogues with reactive groups at other positions of the molecule would be useful to explore this possibility. Rao et al.,[16] who found that labeling of the β-subunit by AzB-taxol occurs in the N-terminal region of the protein, reported no labeling of the α-subunit. This difference in labeling pattern could be due to the fact that Rao et al. photolabeled tubulin in the presence of microtubule-associated proteins and/or used autoradiography to detect labeling. The analogue which we have synthesized appears to be useful for defining at least part of the taxol-binding domain, and our studies are now directed at identifying the peptide(s) which are labeled by AzB-taxol.

Experimental Section

Materials. Taxol was a gift from Hauser Chemical Research, Inc., Boulder, CO. Succinimidyl 4-azido-[3,5-^{3}H]benzoate was purchased from DuPont. Bovine brain tubulin was prepared by a temperature-dependent assembly–disassembly procedure[23] followed by phosphocellulose–biogel P-10 chromatography.[24]

Synthetic Procedures. For general experimental information, see ref 8.

(3*R*,4*S*)-1-(Benzyloxycarbonyl)-3-[(*tert*-butyldimethylsilyl)oxy]-4-phenyl-2-azetidinone (2). To a stirred solution of β-lactam 1 (180 mg, 0.65 mmol) in THF (2 mL) was added *n*-butyllithium (2.5 M in hexane, 0.31 mL, 0.78 mmol) followed by benzyl chloroformate (0.11 mL, 0.78 mmol) at −78 °C. The reaction mixture was stirred for 10 min at −78 °C, and the reaction was quenched with brine at 0 °C. The reaction mixture was extracted with diethyl ether (2 × 50 mL), washed with brine, and dried over anhydrous $MgSO_4$. After the solvent was evaporated *in vacuo*, purification of the crude residue was accomplished by flash column chromatography (ethyl acetate/hexane = 1:20) and gave **2** (233 mg, 90%) as a viscous oil: HRMS *m/e* calcd for $(M + 1)^+$ $C_{23}H_{30}NO_4Si$, 412.1944; found, 412.1930; $[\alpha]_D$ 38.7° (c = 2.0, chloroform).

7-(Triethylsilyl)baccatin III 13-[*N*-(Benzyloxycarbonyl)-2′-[*O*-(*tert*-butyldimethylsilyl)oxy]-(2′*R*,3′*S*)-3′-phenylisoserinate] (3). To a solution of 7-(triethylsilyl)baccatin III (75 mg, 0.11 mmol) in THF (1 mL) at 0 °C was added a NaH suspension (60% in mineral oil, 171 mg, 4.3 mmol) in THF (1 mL) by syringe. To the reaction mixture was added a solution of **2** (67 mg, 0.17 mmol) in THF (1 mL) at 0 °C. After the reaction mixture had stirred at 35 °C for 1 h, another portion of **2** (50 mg, 0.11 mmol) in THF (1 mL) was added to the reaction mixture. The reaction mixture was stirred at 35 °C for 1 h. Then the reaction mixture was cooled to 0 °C, the reaction quenched with cold brine, and the mixture extracted with diethyl ether (2 × 25 mL) and dried with anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave the crude product, which was purified by flash column chromatography (ethyl acetate/hexane = 1:10) to yield **3** (108 mg, 93%) as a white solid: HRMS *m/e* calcd for $(M + 1)^+$ $C_{60}H_{82}NO_{15}Si_2$, 1112.5223; found, 1112.5253; $[\alpha]_D$-36.7° (c = 2.15, chloroform).

Baccatin III 13-[*N*-(Benzyloxycarbonyl)-(2*R*,3*S*)-3-phenylisoserinate] (4). To a solution of **3** (72 mg, 0.066 mmol) in pyridine (1 mL) was added pyridinium HF (0.5 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and allowed to warm up to room temperature. After 2 h, the reaction mixture was recooled to 0 °C and another portion of pyridinium HF (0.5 mL) was added. The reaction mixture stirred for 30 min at 0 °C and was then allowed to warm up to room temperature. After 3 h, the reaction mixture was diluted with diethyl ether, washed with 1% HCl (aq) and water, and dried over anhydrous Na_2SO_4. Removal of the solvent *in vacuo* and purification of the crude material by flash column chromatography (chloroform/MeOH = 50:1) gave **4** (53 mg, 92%) as a white solid: HRMS *m/e* calcd for (M + 1) $C_{48}H_{54}NO_{15}$, 884.3493; found, 884.3493; $[\alpha]_D$ −24° (c = 1.0, chloroform).

***N*-Debenzoyltaxol (5).** A solution of **4** (105 mg, 0.12 mmol) in MeOH (2.5 mL) containing 10% Pd/C (20 mg) was shaken

under H_2 at 50 psi in a Parr apparatus. After 2 h, additional 10% Pd/C (10 mg) was added and shaking was continued for 3 h. The catalyst was removed by filtration through Celite, and the solvent was evaporated *in vacuo* to give **5**. Precipitation of the crude product (methylene chloride/*n*-pentane) gave **5** as a white solid (85 mg, 94%): HRMS *m/e* calcd for $(M + 1)^+$ $C_{40}H_{48}NO_{13}$, 750.3126; found, 750.3119; $[\alpha]_D$ −82.4° (*c* = 3.8, chloroform).

***N*-(4-Azidobenzoyl)-*N*-debenzoyltaxol (6).** To a stirred solution of **5** (10 mg, 0.0134 mmol) in ethyl acetate (0.5 mL) were added saturated $NaHCO_3$ solution (aq, 0.5 mL) and *p*-azidobenzoyl chloride (4.8 mg, 0.0268 mmol) in ethyl acetate. (*p*-Azidobenzoyl chloride was prepared from *p*-azidobenzoic acid[25,26] by refluxing with thionyl chloride for 5 h.) After 5 min, the reaction mixture was extracted with ethyl acetate (2 × 10 mL), washed with brine, and dried over anhydrous Na_2SO_4. Removal of the solvent *in vacuo* and purification of the crude product by flash column chromatography (ethyl acetate/hexane = 1:1) gave **6** (8.6 mg, 72%) as a white solid: HRMS *m/e* calcd for (M + 1) $C_{47}H_{51}N_4O_{14}$, 895.3402; found, 895.3413; $[\alpha]_D$ −31.5° (*c* = 0.2, CH_2Cl_2).

***N*-(4-Azido-[3,5-^{3}H]benzoyl)-*N*-debenzoyltaxol (7).** To a stirred solution of succinimidyl 4-azido-[3,5-^{3}H]benzoate (0.0017 mg/0.25 mL, 250 μCi) was added succinimidyl 4-azidobenzoate (1.3 mg, 0.005 mmol) in 5% NaOH (aq, 2 mL). After 3 h at 35 °C, the reaction mixture was cooled to 0 °C, acidified to pH = 1 with 6 N HCl (aq), extracted with ethyl acetate, and dried over anhydrous $MgSO_4$. The crude product was converted to the corresponding acid chloride by refluxing with thionyl chloride (2 mL) for 5 h. After complete removal of thionyl chloride (vacuum pump for several hours), the crude acid chloride was dissolved in ethyl acetate (1 mL) and added to a stirred solution of *N*-debenzoyltaxol (**5**) (5.5 mg, 0.0073 mmol) in ethyl acetate (1 mL) and saturated $NaHCO_3$ (aq, 0.5 mL) at room temperature. After 5 min, the reaction mixture was extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4. After removal of the solvent *in vacuo* and purification of the crude product by flash column chromatography (ethyl acetate/hexane = 6:4), the solvent was removed again under reduced pressure. The product was then dissolved in a small amount of CH_2Cl_2 and precipitated by addition of an excess of *n*-pentane. Compound **7** (109 μCi, 43.6%) was obtained as a white solid. A comparison of the ^{1}H NMR spectrum of **7** with that of compound **6** verified its structure.

Photolabeling of Tubulin. In a typical photolabeling experiment, tubulin and [^{3}H]AzB-taxol were incubated for 20 min at 37 °C in 0.1 M Pipes, 1 mM EGTA, 1 mM $MgSO_4$, 1 mM dithiothreitol, and 0.5 mM GTP, pH 6.9. Aliquots of 250 μL of the AzB-taxol-induced microtubules were then placed in 24-well plates (each well 1.7 cm in diameter) at room temperature and exposed to radiation for different periods of time at a distance of 6 cm from a RPR lamp equipped with a 2 mm thick Vycor filter. The sample was then centrifuged in a Beckman TL-100 centrifuge at 40000*g* for 10 min at 37 °C. The pellets were treated with 0.5 mL of 50% ethanol, and the precipitated protein was collected by centrifugation and washed several times with 0.5 mL of 50% ethanol until the last wash showed no traces of radioactivity. The final precipitate, devoid of noncovalently bound [^{3}H]AzB-taxol, was then dissolved in 0.5 mL of 0.1 M NaOH, and the protein concentration and the radioactivity contents were determined.

After covalent incorporation of [^{3}H]AzB-taxol into tubulin, the precipitate obtained after washing with 50% ethanol was also dissolved in electrophoresis sample buffer and SDS–PAGE was performed using a 7.5% running gel with a 3% stacking gel.[27] The gels were stained with Coomassie blue, and the α- and β-bands were cut out, dissolved by incubation in 0.5 mL of 30% H_2O_2 at 65 °C,[28] and assayed for radioactivity.

Acknowledgment. We gratefully acknowledge the support from the National Institutes of Health (CA 55141) and the J. R. and Inez Jay Research Fund at the University of Kansas. Support is also acknowledged from the Scientific Education Partnership, which is funded through the Marion Merrell Dow Foundation, and the Kansas Health Foundation for postdoctoral fellowships awarded to G. C. B. Harriman and D. Dasgupta. The authors would like to acknowledge Professor R. Hanzlik for his assistance with the preparation of radioactive derivative **7**. We thank Dr. D. G. Vander Velde for his assistance with NMR assignments. A mixture of taxol and cephalomannine was provided to use for these studies by the National Cancer Institute.

Note Added in Proof. After submission of this manuscript, several articles on photoaffinity analogues of taxol have appeared,[29–31] including one describing the synthesis of compound **7**.[29]

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra and assignments for compounds **2**–**6** (13 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. The Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor Agent from *Taxus Brevifolia*. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
(2) Suffness, M. Taxol: From Discovery to Therapeutic Use. *Annu. Rep. Med. Chem.* **1993**, *28*, 305–314.
(3) Georg, G. I.; Ali, S. M.; Zygmunt, J.; Jayasinghe, L. R. Taxol: A Novel Antitumor Agent. *Exp. Opin. Ther. Pat.* **1994**, *4*, 109–120.
(4) Schiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of Microtubule Assembly *in vitro* by Taxol. *Nature* **1979**, *277*, 665–667.
(5) Parness, J.; Horwitz, S. B. Taxol Binds to Polymerized Tubulin in Vitro. *J. Cell Biol.* **1981**, *91*, 479–487.
(6) Georg, G. I.; Harriman, G. C. B.; Park, H.; Himes, R. H. Taxol Photoaffinity Labels 2. Synthesis and Biological Evaluation of Baccatin III 13-(*N*-4-Azidobenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserinate and 7-[4-Azido-2,3,5,6-tetrafluorobenzoyl)taxol. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 487–490.
(7) Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Harriman, G. C. B.; Hepperle, M.; Park, H.; Himes, R. H. Schotten-Baumann Acylation of Baccatin III 13-[(2′*R*,3′*S*)-3-Phenylisoserinate]: An Efficient Route to *N*-Acyl Taxol Analogues and their Microtubule Assembly Activity. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 335–338.
(8) Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. Synthesis of Biologically Active Taxol Analogues with Modified Phenylisoserine Side Chains. *J. Med. Chem.* **1992**, *35*, 4230–4237.
(9) Georg, G. I.; Cheruvallath, Z. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. An Efficient Semisynthesis of Taxol from (3*R*,4*S*)-*N*-Benzoyl-3-[(*t*-butyldimethylsilyl)oxy]-4-phenyl-2-azetidinone and 7-(Triethylsilyl)baccatin III. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2467–2470.
(10) Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R. Efficient and Practical Asymmetric Synthesis of the Taxol C-13 Side Chain, *N*-Benzoyl-(2*R*,3*S*)-3-phenylisoserine, and its Analogues via Chiral 3-Hydroxy-4-aryl-β-lactams through Chiral Ester Enolate-Imine Cyclocondensation. *J. Org. Chem.* **1991**, *56*, 1681–1683.
(11) Denis, J.-N.; Green, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. A Highly Efficient, Practical Approach to Natural Taxol. *J. Am. Chem. Soc.* **1988**, *110*, 5917–5919.
(12) Himes, R. H.; Burton, P. R.; Gaito, J. M. Dimethyl Sulfoxide-induced Self-assembly of Tubulin Lacking Associated Proteins. *J. Biol. Chem.* **1977**, *252*, 6222–6228.
(13) Georg, G. I.; Harriman, G. C. B.; Himes, R. H.; Mejillano, M. R. Taxol Photoaffinity Label: 7-(*p*-Azidobenzoyl)taxol. Synthesis and Biological Evaluation. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 735–738.
(14) Carboni, J. M.; Farina, V.; Rao, S.; Hauck, S. I.; Horwitz, S. B.; Ringel, I. Synthesis of a Photoaffinity Analog of Taxol as an Approach to Identify the Taxol Binding Site on Microtubules. *J. Med. Chem.* **1993**, *36*, 513–515.
(15) Rimoldi, J. M.; Kingston, D. G. I.; Chaudhary, A. G.; Samaranayake, G.; Grover, S.; Hamel, E. Modified Taxols, 9. Synthesis and Biological Evaluation of 7-Substituted Photoaffinity Analgoues of Taxol. *J. Nat. Prod.* **1993**, *56*, 1313–1330.
(16) Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. 3′-(*p*-Azidobenzamido)taxol Photolabels the N-terminal 31 Amino Acids of β-Tubulin. *J. Biol. Chem.* **1994**, *269*, 3132–3134.

(17) McLaughlin, J. L.; Miller, R. W.; Powell, R. G.; Smith, C. R., Jr. 19-Hydroxybaccatin III, 10-Deacetylcephalomannine, and 10-Deacetyltaxol: New Antitumor Taxanes from *Taxus wallichiana*. *J. Nat. Prod.* **1981**, *44*, 312–319.
(18) Miller, R. W.; Powell, R. G., Smith, C. R., Jr.; Arnold, E.; Clardy, J. Antileukemic Alkaloids from *Taxus wallichiana Zucc*. *J. Org. Chem.* **1981**, *46*, 1469–1474.
(19) Guéritte-Voegelein, F.; Guénard, D.; Lavelle, F.; Le Goff, M.-T.; Mangatal, L.; Potier, P. Relationships Between the Structure of Taxol Analogues and their Antimitotic Activity. *J. Med. Chem.* **1991**, *34*, 992–998.
(20) 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*-phenylisoserinate) and Baccatin III 13-(*N*-Benzoyl-(2′*R*,3′*S*)-3′-(*p*-chlorophenyl)isoserinate). *Bioorg. Med. Chem. Lett.* **1992**, *2*, 295–298.
(21) Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R. Semisynthesis and Biological Activity of Taxol Analogues: Baccatin III 13-(*N*-benzoyl-(2′*R*,3′*S*)-3′-(*p*-tolyl)isoserinate), Baccatin III 13-(*N*-(*p*-toluoyl)-(2′*R*,3′*S*)-3′-phenylisoserinate), Baccatin III 13-(*N*-benzoyl-(2′*R*,3′*S*)-3′-(*p*-trifluoromethylphenyl)isoserinate), and Baccatin III 13-(*N*-(*p*-trifluoromethylbenzoyl)-(2′*R*,3′*S*)-3′-phenylisoserinate). *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1751–1754.
(22) Rao, S.; Horwitz, S. B.; Ringel, I. Direct Photoaffinity Labeling with Taxol. *J. Natl. Cancer Inst.* **1992**, *84*, 785–788.
(23) Tiwari, S. C.; Suprenant, K. A. *A* pH and Temperature-Dependent Cycling Method that Doubles the Yield of Microtubule Protein. *Anal. Biochem.* **1993**, *215*, 96–103.
(24) Algaier, J.; Himes, R. H. The Effect of Dimethyl Sulfoxide on the Kinetics of Tubulin Assembly. *Biochim. Biophys. Acta* **1988**, *954*, 235–243.
(25) Smith, P. A. S.; Brown, B. B. The Reaction of Aryl Azides with Hydrogen Halides. *J. Am. Chem. Soc.* **1951**, *73*, 2438–2441.
(26) Lewis, R. V.; Roberts, M. F.; Dennis, E. A.; Allison, W. S. Photoactivated Heterobifunctional Cross-Linking Reagents Which Demonstrate the Aggregation State of Phospholipase A_2. *Biochemistry* **1977**, *16*, 5650–5654.
(27) Laemmli, U. K. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* **1970**, *227*, 680–685.
(28) Czarnecki, J. J.; Abbott, M. S.; Selman, B. R. Photoaffinity Labeling with 2-Azidoadenosine Diphosphate of A Tight Nucleotide Binding Site on Chloroplast Coupling Factor 1. *Proc. Natl. Acad. Sci.* U.S.A. **1982**, *79*, 7744–7748.
(29) Swindell, C. S.; Heerding, J. M.; Krauss, N. E.; Horwitz, S. B.; Rao, S. Characterization of Two Taxol Photoaffinity Analogues Bearing Azide and Benzophenone-Related Photoreactive Substituents in the A-Ring Side Chain. *J. Med. Chem.* **1994**, *37*, 1446–1449.
(30) Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I. Unexpectedly Facile Hydrolysis of the 2-Benzoate Group of Taxol and Syntheses of Analogs with Increased Activities. *J. Am. Chem. Soc.* **1994**, *116*, 4097–4098.
(31) Combeau, C.; Commerçon, A.; Mioskowski, C.; Rousseau, B.; Aubert, F.; Goeldner, M. Predominant Labeling of β-over α-Tubulin from Porcine Brain by a Photoactivatable Taxoid Derivative. *Biochemistry* **1994**, *33*, 6676–6683.