



Taxol Photoaffinity Labels 2. Synthesis and Biological Evaluation of *N*-(4-Azidobenzoyl)-*N*-debenzoyltaxol, *N*-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-*N*-debenzoyltaxol, and 7-(4-Azido-2,3,5,6-tetrafluorobenzoyl)taxol

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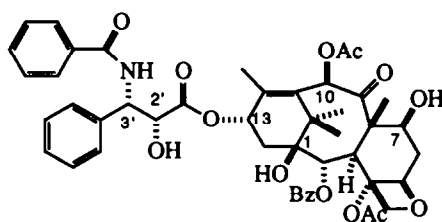
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Abstract: Biologically active photoaffinity labeled analogues of taxol (6, 8 and 9) have been synthesized utilizing (3*R*,4*S*)-3-hydroxy-4-phenyl-2-azetidinones, produced via the ester enolate-imine cyclocondensation reaction, and the diterpene baccatin III (2). Photoaffinity label 8 proved to be the most active taxol photoaffinity label synthesized to date in addition to having the most similar structure to that of the parent, taxol.

The naturally occurring antitumor agent taxol® (1) is one of the most important finds in cancer chemotherapy in recent years, eventually receiving FDA approval on December 29, 1992. Taxol has shown to be successful in the treatment of several cancers, most notably cisplatin refractory ovarian cancer.¹ This naturally occurring diterpene isolated from the Pacific yew² exhibits a unique mechanism of action. Taxol elicits its chemotherapeutic effects via the promotion of microtubule assembly and subsequent stabilization of the microtubule polymer which ultimately leads to cell death.³



taxol (1)

Recently we have reported the synthesis and biology of 7-(4-azidobenzoyl)taxol⁴ and we are now detailing our continuing studies of taxol photoaffinity labels. Our methodology has focused on the utilization of aryl azides as photoreactive nitrene precursors⁵ to develop taxol photoaffinity analogues designed to decipher the taxol binding site on the tubulin dimer.⁶⁻⁸ Utilizing semi-synthetic methodology developed in our laboratory,⁹⁻¹¹ we have synthesized three new photoaffinity labeled taxol analogues. The photoaffinity labels were placed on two sites at the molecule, the 7-hydroxy group and the *N*-benzoyl group of the C-13 side chain of taxol. Both positions can tolerate varying derivatizations without major effects on biological activity.^{12,13} The presence of the photolabels at different sites of the molecule could potentially provide detailed information pertaining to the site where the diterpene part of taxol and the biologically crucial phenylisoserine moiety bind.¹² The analogues 7-(4-azido-2,3,5,6-tetrafluorobenzoyl)taxol (6) and *N*-(4-azido-2,3,5,6-tetrafluorobenzoyl)-*N*-debenzoyltaxol (9) incorporate the advantages of the 4-azido-2,3,5,6-tetrafluorobenzoyl moiety. In contrast to nitrenes generated from aryl azides, which react only with nucleophiles, polyfluorinated aryl azides also insert into carbon-hydrogen bonds.¹⁴ The 4-azido-2,3,5,6-tetrafluorobenzoyl nitrene precursor does not undergo possible intramolecular

nitrene insertion resulting in the formation of dehydroazepines.¹⁴ The third photoaffinity label synthesized was *N*-(4-azidobenzoyl)-*N*-debenzoyletaxol (**8**).

CHEMISTRY

The semisynthetic approach to the photoaffinity analogues takes advantage of the naturally occurring taxane 10-deacetylbaccatin III^{9,12,13} and the synthetic utility of highly enantioenriched (3*R*,4*S*)-3-[(triisopropylsilyl)oxy]-4-phenyl-2-azetidinones (**10**), produced via the ester enolate-imine cyclocondensation.^{10,12,13,15} The synthetic pathway leading to 7-(4-azido-2,3,5,6-tetrafluorobenzoyl)taxol (**6**) was initiated via selective acylation of the 7-hydroxyl group of baccatin III (**2**) (Scheme I) in the presence of 4-azido-2,3,5,6-tetrafluorobenzoyl chloride, triethylamine, and dimethylaminopyridine (DMAP) producing **3** in 80% yield at 58% conversion. Acylation of the allylic hydroxyl group at the 13 position of baccatin III derivative **3** was accomplished utilizing 2-azetidinone **14**⁹ as the acylating agent in the presence of dimethylaminopyridine in pyridine and gave a diastereomeric mixture of **5** in 41% yield. Removal of the ethoxyethyl protecting group with 0.5% HCl/EtOH afforded final compound **6** in 75% yield.⁹

The preparation of **8** (Scheme I) involved the synthesis of the appropriately functionalized (3*R*,4*S*)-3-hydroxy-4-phenyl-2-azetidinone **13** (Scheme II). β -Lactam **10** was acylated in the presence of 4-azidobenzoyl chloride, triethylamine and a catalytic amount of dimethylaminopyridine to form **11** in 71% yield.⁹ Deprotection of the hydroxyl group was accomplished with pyridinium hydrogen fluoride in acetonitrile in 85% yield. 3-Hydroxy-2-azetidinone **12** was then reprotected as its triethylsilyl ether to afford β -lactam **13** in 82% yield. The conversion of the protecting group from the triisopropylsilyl ether to the triethylsilyl ether (or ethoxyethyl (EE) derivative; *vide supra*) was essential as the triisopropyl groups elicited excessive sterical demand in the forthcoming acylation of the 13-hydroxyl group of 7-triethylsilylbaccatin III (**4**).⁹ The triethylsilyl group also proved to be superior to the ethoxyethyl protecting group due to the simplified identification of the synthetic intermediates, as it avoided the formation of diastereomeric mixtures, and also provided a more facile deprotection. Acylation of 7-triethylsilyl protected baccatin III (**4**) with β -lactam **13** in the presence of sodium hydride^{10,16} proceeded in 62% yield. Quantitative deprotection of the silyl ether using pyridinium hydrogen fluoride in acetonitrile produced the photoaffinity labeled taxol analogue *N*-(4-azidobenzoyl)-*N*-debenzoyletaxol (**8**). Photoaffinity labeled *N*-(4-azido-2,3,5,6-tetrafluorobenzoyl)-*N*-debenzoyletaxol (**9**) was synthesized by a different synthetic pathway, which is described in detail in the communication following this letter.^{11,17}

BIOLOGICAL EVALUATION

The ability of the photoaffinity analogues **6**, **8** and **9** to promote microtubule assembly *in vitro* was compared to that of taxol (**1**) (Table 1).¹² 7-(4-Azido-2,3,5,6-tetrafluorobenzoyl)taxol (**6**) had a much reduced activity and was less active than 7-(4-azidobenzoyl)taxol (Table 1). Analogue *N*-(4-azidobenzoyl)-*N*-debenzoyletaxol (**8**), on the other hand, was about half as active as taxol.

Table 1. Biological evaluation of taxol photoaffinity labels in the microtubule assembly assay and their cytotoxicity against the B16 melanoma cell line

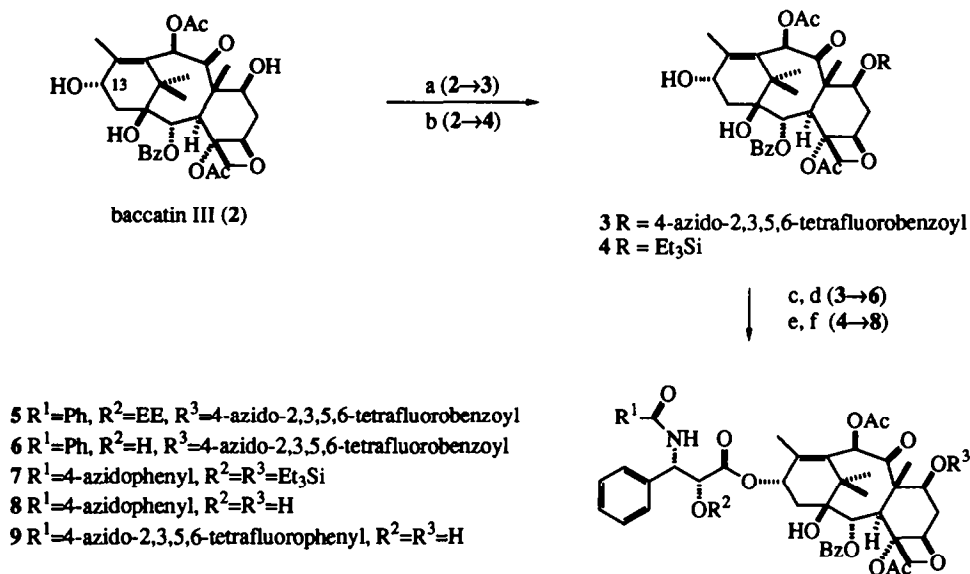
taxol photoaffinity analogue	stimulation of microtubule assembly ED ₅₀ /ED ₅₀ (taxol) ^a	inhibition of cell proliferation ED ₅₀ /ED ₅₀ (taxol) ^b
7-(4-azidobenzoyl)taxol ⁹	21.4	30
6	>22.4 ^c	not tested
8	2.0	4.6
9	4.6	14.8

^aED₅₀ is the concentration which causes polymerization of 50% of the tubulin present in 15 min. at 37 °C.

^bED₅₀ refers to the concentration which produces 50% inhibition of proliferation after 40 h incubation.

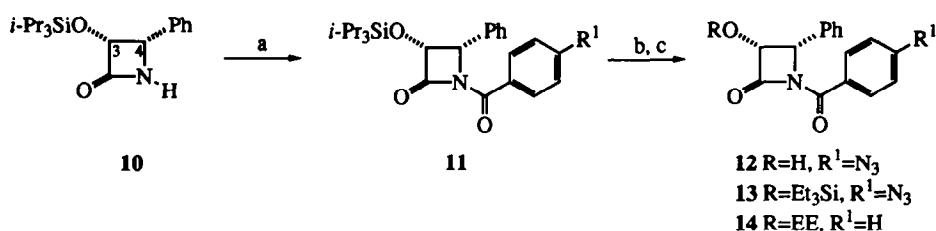
^c50% inhibition was not achieved at the ratio of the analogue to taxol indicated.

Scheme I



(a) 4-azido-2,3,5,6-tetrafluorobenzoyl chloride (1.6 equiv.), DMAP (cat.), Et₃N (2 equiv.), CH₂Cl₂, 0 °C, 24 h. (b) triethylsilyl chloride (20 equiv.), pyridine (excess), 25 °C, 24 h. (c) 14 (5 equiv.), DMAP (1 equiv.), pyridine, 25 °C, 58 h. (d) 0.5% HCl/EtOH, 0 °C, 48 h. (e) 13 (1.5 equiv.), NaH (50 equiv.), THF, 0 °C to 35 °C, 3 h. (f) pyridinium HF (excess), CH₃CN, 0 °C, 2 h.

Scheme II



(a) 4-azidobenzoyl chloride (1.5 equiv.), DMAP (cat.), Et₃N (excess), CH₂Cl₂, 25 °C, 45 min. (b) pyridinium HF (excess), CH₃CN, 25 °C, 12 h. (c) triethylsilyl chloride (2 equiv.), pyridine (excess), 25 °C, 12 h.

N-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-*N*-debenzoyltaxol (**9**) was less active than its nonfluorinated analogue **8**. The effect of the taxol derivatives **6**, **8**, and **9** on the proliferation of B16 melanoma cells in culture was also examined and similar results were obtained.

The reduced ability of photoaffinity taxol derivatives 7-(4-azidobenzoyl)taxol⁴ and its tetrafluoro derivative **6** to induce microtubule assembly and inhibit melanoma cell proliferation may be explained by the geometry of the azidobenzoyl photoaffinity label at the C-7 hydroxyl group (linear orientation of the azidoaryl moiety). An evaluation of related 7-substituted taxol photoaffinity analogues recently revealed that they were not suitable for photoaffinity labeling studies.⁷ A comparison of 7-(4-azidobenzoyl)taxol⁴ and analogue **6** demonstrated that the fluorine atoms in **6** significantly reduced biological activity. The effectiveness of analogues **8** and **9** in both the microtubule assembly assay as well as against the B16 melanoma cell lines demonstrates that these analogues may prove useful in the photochemical binding studies now in progress in our laboratory. Since both nitrene precursors (**8** and **9**) have only small structural differences compared to taxol, it is assumed that their binding is similar to that of taxol. Again it was observed that aryl azide **8** was more active than the corresponding tetrafluoroaryl derivative **9**.

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- (17) The 4-azidobenzoic acid chlorides utilized were generated from their respective acids in refluxing thionyl chloride. They were then subjected to purification via silica gel flash chromatography through a short silica gel bed. All of the newly synthesized derivatives were characterized by ¹H NMR, ¹³C NMR, IR, FABMS, high resolution MS, and optical rotation. The purity of **6** and **8** was also verified by HPLC analysis.

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