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Synthesis of a Photoreactive Taxol Side Chain

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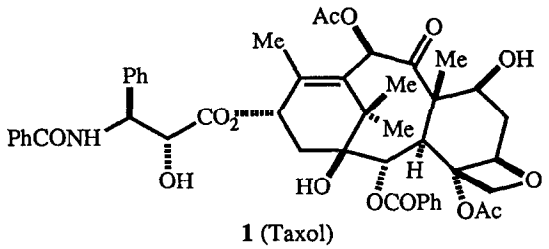
Abstract: The photoreactive taxol side chain, (2*R*,3*S*)-(-)-N-(4'-azido-benzoyl-3-phenylisoserine methyl ester (6), was prepared in four steps from (2*R*,3*R*)-(+)-methyl 3-phenylglycidate (2).

Taxol (**1**), a potent antimitotic natural product isolated from the bark of the western yew, *Taxus brevifolia*, has shown promising clinical activity against ovarian cancer.[1-3] Although the unique abilities of taxol to promote the assembly of tubulin to microtubules and to stabilize the intact microtubule assembly against depolymerization have been well documented *in vitro*, very little is known about the location and molecular characteristics of the protein binding site(s) for taxol.[3-5]



1 (Taxol)

Photoaffinity labelling is an excellent tool for the identification and characterization of ligand-specific mediators of many biological and pharmacological phenomena.[6,7] Significant progress has been made over the past decade in understanding the interaction of biologically active compounds with neuroreceptors, hormone receptors, transport proteins, nucleic acids and nucleotides, and enzymes through use of the photoaffinity labelling technique. Surprisingly little has been done with tubulin or tubulin/microtubule binding agents, however. Two well known antimitotic agents, colchicine and vinblastine, have been converted to photoreactive derivatives and employed in photoaffinity labelling studies of tubulin.[8-11] Kingston, *et al.* reported recently a photoreactive

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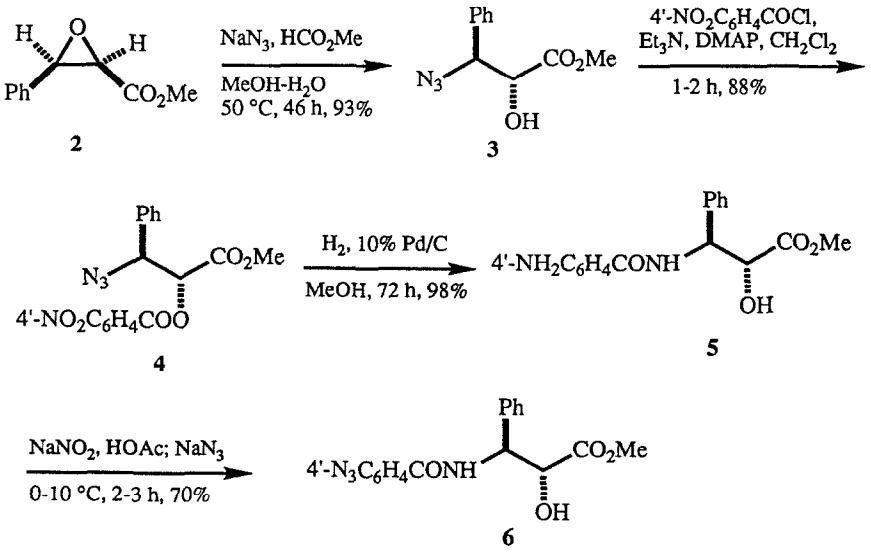
derivative of taxol that incorporated a C-ring 7-azibenzoyl group.[3] The aromatic rings of the taxol side chain are potentially two additional sites for photoreative functional group incorporation, particularly since this modification would be expected to have a minimal affect upon the biological activity of the taxol or taxane analog.[1-3,5,12-14] Herein we wish to report the synthesis of a photoreactive taxol side chain that bears an azido group at the 4'-position of the N-benzoyl ring.

Our synthesis of the photoreactive taxol side chain, (2*R*,3*S*)-(-)-N-(4'-azidobenzoyl)-3-phenylisoserine methyl ester (**6**), is outlined in Scheme 1. Subjection of the (+)-glycidic ester **2** to the sodium azide ring opening methodology of Denis *et al.* delivered the (2*R*,3*S*)-azido alcohol **3** in 93% yield on an 0.15 mol scale.[15,16] The reported tedious chromatographic purification of **3** could be avoided, however, through a modified workup procedure in which the reaction solvent mixture was concentrated and the product extracted into ether. The azido alcohol **3** obtained upon evaporation of the extract was sufficiently pure (>95% by ^{1}H NMR) for subsequent use. Incorporation of an azidobenzamide group into the taxol side chain relied upon the known propensity for O to N aroyl group transfer.[15] In this regard, **3** was converted first to the 4'-nitrobenzoate **4** by treatment with 4-nitrobenzoyl chloride and triethylamine in the presence of a catalytic amount of N,N-dimethylaminopyridine. Catalytic reduction of **4** over 10% palladium on carbon produced O-(4'-aminobenzoyl)-3-phenylisoserine methyl ester, which rearranged spontaneously over the course of 2 days to the 4'-aminobenzamide **5**. Compound **5** was



Scheme 1

only slightly soluble in methanol and consequently the product crystallized directly from the reaction mixture. Introduction of the photolabile azido group was accomplished in 70% yield by mild oxidation of **5** with 1.1 mol equivalents of sodium nitrite in an acetic acid solution of sodium azide. The 4'-azido taxol side chain **6** exhibited strong absorptions in the IR spectrum at 2120 cm^{-1} and in the UV spectrum (EtOH) at 205 nm (log ε 4.43) and 269 nm (log ε 4.32).

In summary, the photoreactive taxol side chain **6** was prepared from (2*R*,3*R*)-(+)-methyl 3-phenylglycidate in 56% overall yield and in a form suitable for coupling to the baccatin III nucleus of taxol.[18,19] Furthermore, the 4'-aminobenzamide side chain **5** permits the introduction of a radiolabel into the molecule or the synthesis of other photoreactive analogs by condensation with commercial photoreactive derivatizing agents[6,7] such as N-hydroxysuccinimidyl-4-azidobenzoate and 6-(4-azido-2-nitrophenylamino)hexanoic acid N-hydroxysuccin-imide. Our continued efforts with the amino and azido side chains will be the subject of future reports.

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(17) All new compounds were fully characterized by IR, ^1H and ^{13}C NMR spectroscopy, optical rotation, and combustion analysis. Spectroscopic data for compounds **5** and **6** follow where the numbers in parentheses for the carbon resonances refer to the number of attached protons on that carbon atom as determined from the APT and DEPT - GL experiments.

(2*R*,3*S*)-(-)-N-(4'-Aminobenzoyl)-3-phenylisoserine methyl ester

(**5**): mp 205-207 °C (MeOH-$CHCl_3$); IR (KBr) 3480, 3380, 3340, 1735, 1630, 1610, 1535, 1510, 1440, 1355, 1320, 1295, 1195, 1087, 773, 708 cm^{-1}; ^1H NMR (MeOH-d_4, 300 MHz) δ 7.62 (ddd, 1 H, *J* = 8.8, 4.6, 2.3 Hz), 7.43-7.24 (m, 5 H), 6.68 (ddd, 1 H, *J* = 8.8, 4.6, 2.3 Hz), 5.56 (d, 1 H, *J* = 3.5 Hz), 4.87 (br s, 4 H), 4.59 (d, 1 H, *J* = 3.5 Hz), 3.70 (s, 3 H); ^{13}C NMR (MeOH-d_4, 75 MHz) 174.4 (0), 170.0 (0), 153.5 (0), 140.7 (0), 130.1 (1), 129.4 (1), 128.5 (1), 128.1 (1), 122.8 (1), 114.7 (1), 75.0 (1), 57.4 (1), 52.8 (3) ppm; $[\alpha]_D^{20}$ = -73.5 (*c* = 0.20, acetone).

(2*R*,3*S*)-(-)-N-(4'-Azidobenzoyl)-3-phenylisoserine methyl ester

(**6**): mp 163 °C dec. ($CHCl_3$-petroleum ether); IR (KBr) 3500, 3370, 2120, 1730, 1640, 1607, 1525, 1495, 1440, 1285, 1258, 1190, 1103, 850, 770, 710, 705 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (ddd, 1 H, *J* = 8.6, 4.3, 2.2 Hz), 7.47-7.29 (m, 5 H), 7.06 (ddd, 1 H, *J* = 8.6, 4.3, 2.2 Hz), 6.94 (br d, 1 H, *J* = 8.8 Hz), 5.72 (dd, 1 H, *J* = 8.8, 2.1 Hz), 4.63 (d, 1 H, *J* = 2.1 Hz), 3.85 (s, 3 H), 3.3 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 173.4 (0), 169.8 (0), 165.8 (0), 143.7 (0), 138.6 (0), 130.4 (0), 128.9 (1), 128.8 (1) 128.0 (1), 126.9 (1), 119.1 (1), 73.2 (1), 54.9 (1), 53.3 (3) ppm ; $[\alpha]_D^{20}$ = -51.1 (*c* = 0.09, $CHCl_3$)

Anal. Calcd for $C_{17}H_{16}O_4N_4$: C, 59.99; H, 4.74; N, 16.46. Found: C, 60.04; H, 5.10; N, 16.14.

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