INVESTIGATIONS OF THE CHEMISTRY OF TAXOL

by

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ABSTRACT

Several C-7 and C-13 diazirinyl taxol analogs have been synthesized as potential photoaffinity-labeled derivatives for studying the nature of the binding site of taxol on polymerized tubulin. One analog has been prepared in both deuterium- and tritium-labeled versions.

Methods were developed to selectively hydrolyze the C-2 benzoate of taxol which have allowed for the preparation of a variety of C-2 modified taxol derivatives. The C-2 taxol analogs were tested in several cell culture assays, and substantial increases in potency were observed with many of these derivatives, suggesting that the C-2 benzoate may play a crucial role in taxol's activity.

Reaction of 2'-tert-butyldimethylsilyl-7-triethylsilyl taxol with Triton B™ selectively hydrolyzed the C-2 benzoate and the C-4 acetate respectively. This is the first disclosure of the C-4 deacetylation of taxol carrying an intact C-13 side chain.

Several oxetane ring opened taxol analogs were synthesized via reaction of taxol with electrophilic reagents. The reaction of these oxetane ring-opened analogs have revealed some interesting rearrangements and functional group transfer reactions.
TO CINDY
ACKNOWLEDGMENTS

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I. HISTORICAL

1.1 Introduction

Originating in various yew (Taxus) species, the taxane diterpenoids (taxoids) are a group of more than 125 secondary metabolites that share in an unusual carbocyclic framework as depicted in 1.1 (The convention used for numbering the taxene skeleton reflects the system adopted by IUPAC).2

Extensive chemical degradation experiments performed on the constituents of various Taxus species (primarily Taxus baccata) in the 1950's and 60's uncovered their chemical constitution3 and detailed $^1$H and $^{13}$C NMR spectroscopic studies have yielded their structure and relative stereochemistry.4 The application of optical rotary dispersion and circular dichroism to taxoids 5 helped deduce their absolute configuration, which was further confirmed by X-ray crystallographic analysis on a select few taxoids.6

The tricyclic ring system of the taxoids possesses a flexible and cup-shaped conformation, best exemplified in Figure 1, which is not immediately obvious from its two-dimensional representation. The six membered A ring adopts a distorted boat conformation cis-fused to a highly functionalized eight membered B ring, which in turn has a boat-chair conformation. The conformation of the
remaining trans-fused C-ring, containing an angular methyl group at C-8, is highly dependent on the functionality located at C-4,(5). All known taxoids

\[ \text{Figure 1. 3-D representation of the taxene nucleus with an oxetane at C-4,(5).} \]

are also characterized by the presence of a tetrasubstituted bridgehead olefin at C-11,(12), which would be expected to impose significant ring strain to the molecule. Semi-empirical molecular mechanics calculations performed on the

\[ \text{Figure 2. Effect of the C-11,(12) olefin on strain.} \]
hydrocarbons shown in Figure 2, however, have revealed that the double bond in these systems is remarkably unstrained despite its bridgehead character, and that it does not contribute much to the overall strain of the molecule.\textsuperscript{7} Taxene systems appear to be examples of "hyperstable olefins"\textsuperscript{8}-structures that are associated with negative olefin strain values and therefore achieve stabilization through unsaturation (compare 1.2-1.3 and 1.4-1.5). Furthermore, MM2 calculations have shown that this structural unit is stabilized by conformational effects despite the considerable increase in strain energy associated with the addition of the geminal dimethyl groups.

### 1.2 Classification of Naturally Occurring Taxoids

Numerous ring oxygenation arrays exist at nearly every position with the exception of C-3 and C-6. Based on several discrete oxygenation patterns localized in the C-4,(20) region, sub-classification is feasible. For example, taxusin (1.6)\textsuperscript{9} is the simplest member of a class of approximately 50 taxoids which accommodate a C-4,(20) double bond. Members of this class always carry an $\alpha$-oxygenated substituent at C-5, and a corresponding $\beta$-oxygenated substituent at C-10. Taxagine (1.7)\textsuperscript{10}, a related member of this class, also possesses the C-4,(20) olefin along with an unusual C-12,(16)-oxido bridge, and a
pndant cinnamoyl ester side chain at C-5. The second class, which consists of taxoids that contain a C-4,(20) epoxide, such as baccatin I (1.8)\(^{11}\), are not as common, but account for approximately twenty of the naturally occurring taxoids. As with the C-4,(20) olefin series, C-5 is always oxygenated, along with C-2, C-9, C-10, and C-13.

![Chemical structures](image1)

1.8  
1.9

The third class of taxoids revolves around compounds embodying an oxetane ring fused at C-4,(5) and in most cases, a bridgehead C-1 \(\beta\)-hydroxyl group. Baccatin III (1.9)\(^{12}\) and seventeen related analogs of this series are potential semisynthetic precursors to the most important class of taxoids which includes the promising anticancer agent taxol (1.10)\(^{13}\) that carries a \(\beta\)-phenylisoserine ester side chain at C-13. Fifteen other congeners of the taxol series have either \(\beta\)-xylosyl substituents at C-7\(^{14}\) or differ in the nature of the C-13 side chain.

![Chemical structures](image2)

1.10  
1.11
as does the closely related cephalomannine (1.11). Other miscellaneous taxoids which have been isolated include taxagifine III (1.12), taxinine K (1.13) (the C-3,(11) transannular congener of taxinine A), and the alkaloid taxine A (1.14) which does not have the normal taxene skeleton. The isolation of an AB ring contracted taxoid from *Taxus chinensis*, taxchinin A (1.15), has recently been reported, and may very well be a biogenetic precursor or a tertiary metabolite.
of the normal taxoid series. Similar AB ring contracted taxenes have been prepared synthetically from other naturally occurring taxoids,\textsuperscript{21} including taxol.\textsuperscript{22}

1.3 The Emergence of Taxol

Among the many taxoids that have been isolated to date, one member has emerged as the most auspicious member of its class. Taxol was isolated in small amounts from the bark of the western yew, \textit{Taxus brevifolia}, in the late 1960's by Monroe Wall and his co-workers from the Research Triangle Institute.\textsuperscript{13} Its structural determination was facilitated by mild methanolysis to yield the N-benzoyl-\(\beta\)-phenylisoserine methyl ester side chain 1.16 and the corresponding tetraol (10-deacetylbaccatin III) 1.17 (Scheme 1). X-ray analysis performed on the \(p\)-bromobenzoate of 1.16 and the C-7,10-bisidoacetate of 1.17 unambiguously confirmed taxol's structure.

\begin{center}
\includegraphics[width=\textwidth]{scheme1.png}
\end{center}

\textit{Scheme 1}. Methanolysis of taxol.

The initial finding that taxol possessed potent antileukemic and tumor inhibitory properties in the P-388 mouse leukemia assay, and the fact that it was the first compound manifesting the taxene ring system which had been
demonstrated to have such activity was not entirely sufficient to fuel widespread appeal. The problems associated with its low aqueous solubility and its limited supply posed a dilemma and delayed its initial development as a clinically useful drug. However, renewed interest was generated when it was shown that taxol exhibited excellent activity against murine B16 melanoma and the MX-1 mammary tumor xenograft in athymic mice.\textsuperscript{23} Moreover, pioneering work by Horwitz in 1979 revealed that taxol exhibited a unique mechanism of action, acting as a tubulin polymerization promotor.\textsuperscript{24}

These factors coupled with its intriguing structural complexity has launched taxol into the forefront of research from both a clinical and synthetic standpoint. Extensive reviews on the chemistry,\textsuperscript{1a,1b,25} attempted syntheses,\textsuperscript{26} and clinical activity\textsuperscript{27} of taxol and its analogs have appeared, and will not be discussed in great length. The mode of action of taxol with respect to its biological receptor, the microtubule, will be described along with relevant structure-activity relationships.

1.4 The Tubulin-Microtubule System.

A requisite of normal cellular division is the creation and disappearance of the mitotic spindle apparatus, which originates in the cytoplasm at prophase. The mitotic spindle, composed of microtubules and microfilaments, not only determines a cell's shape, but is directly implicated in a variety of cellular functions including organelle movements, cell motility, and chromosome segregation during cell division.\textsuperscript{18} Microtubules are proteinaceous organelles, present in nearly all eukaryotic cells, composed of two similar but distinct proteins, α- and β-tubulin, each with a relative molecular weight of ~ 50,000 as
determined by their amino acid sequences. Tubulin exists as dimers measuring 8 x 4 nm and align in a head to tail fashion to form chains of subunits or protofilaments. Microtubules are in a dynamic equilibrium with tubulin dimers (Figure 3), and the direction of this equilibrium toward microtubule assembly or disassembly appears to be determined by signals generated during specific cell cycle phases by intracellular regulators such as calcium and guanosine triphosphate. The tubulin-microtubule system is undoubtedly one of the most fascinating systems of molecular self-assembly in eukaryotic cells, and studies in vitro have helped to understand the conditions required for this highly regulated process.

Figure 3. The tubulin-microtubule equilibrium system.
1.5 Regulation of Tubulin-Microtubule Assembly

The process of \textit{in vitro} assembly of microtubules is believed to exhibit three sequential steps: a nucleation event, propagation of microtubule growth, and a termination step.\textsuperscript{32} The assembly process exhibits a critical concentration ($C_r$) required to trigger assembly, dependent on the presence of microtubule associated proteins (MAP's),\textsuperscript{33} GTP, magnesium ions, along with other regulatory factors.\textsuperscript{34}

The dynamics of the elongation step appears to be determined by successive additions of tubulin subunits to the growing pole and disassembly from the opposite end. Under assembly conditions, the rate of incorporation of tubulin into microtubules is greater than the rate of disassembly from the microtubules.\textsuperscript{35} Thus, the effective concentration of free tubulin decreases until it reaches the critical concentration ($C_r$) for assembly, and this process approaches a steady state. Table 1 summarizes \textit{in vivo} and \textit{in vitro} regulatory factors which interact with this system to promote either assembly or disassembly, and is by no means exhaustive.
Table 1. Regulatory factors of the tubulin-microtubule system.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Interaction</th>
<th>Mechanism</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>GTP</td>
<td>- binds to tubulin</td>
<td>Evidence supports GTP exerting conformational change upon binding to tubulin inducing assembly. GTP hydrolysis accompanies assembly, but GTP hydrolysis not required for assembly.</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>- one exchangeable site, one nonexchangeable site.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg^{2+} ions</td>
<td>- one high affinity binding site on tubulin, with multiple weak binding sites</td>
<td>The high concentrations of Mg(II) required to induce assembly suggest weak binding is responsible for promoting assembly, possibly altering charge distribution on protein</td>
<td>37</td>
</tr>
<tr>
<td>MAP's (MAP1, MAP2, Tau)</td>
<td>MAP2 and Tau binding localized on acidic carboxyl-terminal moiety of alpha tubulin</td>
<td>Possibly act as nucleation centers for initiation of microtubule formation or cross linking the microtubule to other cell components.</td>
<td>38</td>
</tr>
</tbody>
</table>

Factors which promote microtubule disassembly.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Interaction</th>
<th>Through the interaction of calmodulin with MAPs, direct interaction with tubulin.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca^{2+} ions</td>
<td>- One high affinity binding site on tubulin with multiple weak binding sites.</td>
<td></td>
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Table 1 somewhat oversimplifies what is known of the tubulin-microtubule system. The vast amount of data gathered from decades of research on microtubules and tubulin still leaves innumerable questions unanswered. How do microtubules form from tubulin, and what intermediate steps are involved? What are the thermodynamics of assembly? What are the roles of the regulatory factors such as GTP and MAP's? How are assembly and disassembly interrelated? Can observations in vitro be compared to those in vivo, where microtubules are often seen to assemble at specific sites of the cell (microtubule-organizing centers)? Although these questions have not been answered, the study of microtubules has been profoundly influenced by the availability of a large
and diverse collection of pharmacological agents which are potent inhibitors of microtubule function. The interaction of these agents with the tubulin-microtubule system are fundamental towards advancing the understanding of possible mechanisms regulating organized microtubule assembly.

1.6 The Tubulin-Microtubule System as a Chemotherapeutic Target

The tubulin-microtubule system has emerged as one of the most strategic subcellular targets of anticancer chemotherapeutics. Tubulin is a receptor for a number of pharmaceutical agents that were classified originally as “spindle poisons”. In addition to disrupting normal microtubule function in the mitotic apparatus of dividing eukaryotic cells, these drugs affect a large number of other diverse cellular functions that are dependent, either directly or indirectly, on microtubules.

Tubulin itself was originally identified as the subunit protein of microtubules on the basis of its specific binding to the plant alkaloid colchicine (1.18). Colchicine was shown to be a potent inhibitor of microtubule assembly,

![Colchicine Structure](image)

binding specifically and stoichiometrically to the β-tubulin subunit with a dissociation constant of $1.9 \times 10^{-7}$ M. This tubulin-colchicine complex has the
ability to self-associate in vitro, but the resulting polymers exhibit characteristics distinct from microtubules due to topological alterations in the interaction domains of tubulin subunits. Binding induces a slight conformational change in the β-subunit such that addition of a colchicine-tubulin subunit destabilizes it and blocks the addition of further unligated subunits.

The antineoplastic activity of the vinca alkaloids in cells has also been attributed to microtubule disruption. Vincristine (1.19) and vinblastine (1.20) bind to tubulin and prohibit microtubule assembly at low concentrations and cause tubulin aggregation at higher concentrations.

Other known antimitotic agents shown to interact directly with the tubulin subunits are the natural product podophyllotoxin and the synthetic drug oncodazole. These compounds along with colchicine and the vinca alkaloids express their activity by arresting cells at mitosis due to the disruption of the equilibrium between tubulin and microtubules, thereby destroying the cytoskeletal framework for chromosome separation and causing normal cell division to cease. On a subcellular level, these agents are extremely potent, and despite their promise, none demonstrate broad antineoplastic spectra.
1.7 Taxol's Mechanism of Action

The unusual mechanism of action of taxol was largely deduced by Horwitz and co-workers at the Albert Einstein College of Medicine in the late 1970's. These researchers demonstrated that taxol binds preferentially to microtubules rather than tubulin, with saturation occurring at a stoichiometry approaching 1 mole of taxol per mole of polymerized tubulin dimer.\(^ {45} \) The binding of taxol to microtubules is reversible, and the apparent binding constant is approximately \( 1.0 \times 10^{-6} \) M.\(^ {46} \) Unlike the normal spindle poisons which induce microtubule disassembly, taxol promotes microtubule assembly and stabilizes microtubules by shifting the dynamic equilibrium toward assembly, thus preventing disassembly. Taxol concentrations as low as 0.05 \( \mu \)M promote microtubule assembly \textit{in vitro}, decrease the lag time for assembly, and decrease the critical concentration of tubulin required for microtubule assembly.\(^ {47} \) Remarkably, this occurs either in the presence or absence of factors previously found to be essential for this process, such as GTP or MAP's.\(^ {48} \) Microtubules treated with taxol are stable even after treatment with calcium ions or low temperatures, conditions which promote disassembly.\(^ {49} \) All the evidence suggests that the taxol binding site is on the microtubule. Moreover, the taxol binding site is distinct from the exchangeable GTP site and the binding site for colchicine, podophyllotoxin, or vinblastine.\(^ {48b} \)

As seen by electron microscopy and indirect immuno-fluorescence, microtubules in cells treated with taxol show two morphological effects.\(^ {4} \) First, cells form prominent arrays of microtubules that are aligned in parallel bundles, which are formed during all phases of the cell cycle.\(^ {50} \) Second, taxol induces formation of abnormal spindle asters during mitosis.\(^ {51} \) A study of the
relationship between microtubule organizing centers (MTOC-kinetochores, basal bodies) and the assembly of microtubules in taxol-treated cells shows multiple asters which are independent of any MTOC. When these cells are treated with nocodazole (which disassembles microtubules), the only remaining microtubules are those attached to the centrosomes and kinetochores, demonstrating that taxol, by favoring the assembly of tubulin, abrogates the role of MTOC. Disruption of the mitotic spindle is the best explanation for the cytotoxic effects of taxol. Chromosomal non-disjunction and mitotic arrest were observed in biopsy and autopsy specimens from a variety of organs from patients treated with taxol.

1.8 Clinical Data

As mentioned previously, the decision to begin preclinical development of taxol in 1977 was based on biological data which had accumulated since its initial isolation, especially its potent activity against B16 melanoma. This decision was strengthened with Horwitz’s discovery of taxol’s unique mechanism of action, and the development of taxol at this time was warranted.

Preclinical toxicology studies established the toxic effects due to taxol administration, which were most evident in tissues with rapid cell turnover, including lymphatic, hematopoietic, and reproductive tissues. Because of its limited water solubility, taxol was formulated in Cremophor EL (polyethoxylated castor oil) as the vehicle, which itself exhibited inherent toxicity. Administered without taxol, it produced vasodilation, lethargy, hypotension, and death in dogs.

Phase I clinical trials have shown that patients whom were given this formulation experienced serious hypersensitivity reactions in several instances,
both with other drugs, and with taxol. Protocols have been developed (i.e. pretreatment with antihistamines) to minimize these allergic reactions. The dose limiting toxicities of taxol to emerge from Phase I trials are neutropenia, mucositis, and the hypersensitivity reactions mentioned above.

In early phase I trials, antineoplastic activity was observed in several tumor systems including melanoma, refractory ovarian cancer, non-small cell lung cancer, colon cancer, head and neck carcinoma, and acute leukemia.

Phase II clinical trials yielded exciting results from patients suffering from advanced refractory ovarian cancer; a 40% overall response rate in a group of 40 patients with taxol treatment. Particularly encouraging was the fact that these responses were observed in heavily pre-treated patients, considered to be resistant to cisplatin. Recently, very good response rates have been reported with breast cancer patients.

The introduction of taxol to the current realm of antineoplastic agents marks a major step towards cancer treatment, and is one of the most exciting anticancer drugs to be developed in the last twenty years.
1.9 Structure-Activity Relationships of Taxol Analogs

1.9.1. Introduction

Once a new lead molecule has been uncovered, the modern practice of drug research is to synthesize the most biologically active yet least toxic derivative. One wishes to obtain the maximal amount of information content (its biological response in various systems with respect to the parent drug as well as other derivatives) from each derivative prepared.

Interactions of drugs (ligands) with their receptors are very specific, and in most cases, only a portion of the drug may be involved in the site of action. Additional groups which the molecule contains may interfere with the appropriate interactions. One approach to lead modification is to delete functional portions of the molecule, or modify them in such a fashion as to determine which are essential and which are superfluous. Structural modifications are the key to activity and potency manipulations, which is evident from the following structure-activity relationships (SAR) of taxol. Taxol's SAR will be subclassified into four major regions as denoted in Figure 4.

*Figure 4.* Convention used for subclassifying the SAR of taxol.
The numerous assays which have been utilized by various investigators to assess the activity of taxol analogs somewhat complicates the discussion of structure-activity relationships. However, three of the more common assays have been used and found to be reliable in gauging the activity of both taxol and its analogs.

1. Microtubule assembly assay.  

As discussed in Section 1.7, taxol acts as an antimitotic agent by promoting the assembly of tubulin to microtubules. The microtubules formed are stabilized, and become resistant to disassembly by calcium ions or low temperatures. The assay developed measures the initial rate of either microtubule assembly or microtubule disassembly relative to taxol.

2. Mammalian cytotoxicity.

Cell culture toxicity of taxol analogs have been ascertained by using KB cells (human carcinoma of the nasopharynx), J774-2 cells (mouse macrophage-like cell line), P-388 cells (mouse lymphocytic leukemia cell line) or HL-60 cells (leukemia cell line). Activity measurements of taxol and its analogs obtained from these cell lines usually correlate to the activities measured in the tubulin assay.

3. Mouse assays.

Animal tests have been performed in the P-388 leukemia, B16 melanoma, and human tumors as xenografts in mice.
1.9.2. The Side Chain

In the earliest studies on the structure-activity relationships of taxol, Wall showed that the C-13 side chain played a critical role in the biological activity of taxol. Tested in the KB cell assay as separate entities, both the tetraol (1.18) and the corresponding methyl ester side chain (1.17), were approximately $10^3$ and $10^5$ orders of magnitude less active than taxol, respectively.\(^{13}\) This finding has been echoed with the biological testing of many other taxoids lacking the C-13 side chain (with or without an oxetane ring). In all cases, the data imply that baccatin III derivatives are all much less potent agents than taxol with respect to cytotoxicity and assembling tubulin.\(^ {59}\) This does not imply that only the side chain is responsible for taxol's activity, since other functional units located on the taxene nucleus (oxetane ring, 2-benzoate) contribute largely to taxol's biological activity. This will be discussed in future chapters.

Although the taxoids in the baccatin III series do not exhibit significant activity, they are readily available precursors for the hemi-synthesis of taxol and C-13 side chain modified taxol analogs. One of the first partial syntheses of taxol was accomplished by Potier and his co-workers (Scheme 2).\(^{60}\) Reaction of 7,10-bistrichloroethyloxy carbonylbaccatin III (1.21) with cinnamoyl chloride in the presence of AgCN afforded the C-13 cinnamoyl ester (1.22), which was reacted under Sharpless hydroxyamination conditions using N-chloro-N-sodio-$t$-butyl-carbamate to yield the N-$t$-BOC derivative (1.23), along with its respective regio- and diastereo-isomers. Cleavage of the N-$t$-BOC group was accomplished with trimethylsilyl iodide to furnish the free amine (1.24). Selective benzoylation followed by removal of the trichloroethyloxy carbonyl protecting groups with
Scheme 2. Potier's synthesis of a taxol and taxotere™ precursor.

Zinc in acetic acid afforded 10-deacetyltaxol (1.25). Similarly, taxol was prepared utilizing the same sequence, but starting with 7-trichloroethyloxy carbonylbaccatin III. Although the synthetic route had its limitations, it did result in the synthesis of N-debenzoyl-N-t-butoxycarbonyl-10-deacetyltaxol (1.26) (called taxotere™ or RP56976), which was shown to be superior to taxol in both
the cytotoxic KB cell assay as well as the tubulin assembly assay, and has been currently selected for further biological studies in Europe.\textsuperscript{62} This synthetic route has also enabled the same researchers to prepare a multitude of taxol and 10-deacetyltaxol analogs via the key compound 1.24 as well as the regio- and diastereoisomeric analogs derived from an improved hydroxyamination reaction 1.22$\rightarrow$1.23.\textsuperscript{63} Table 2 summarizes the structure-activity relationships of C-2'-C-3' modified side chains of the 10-deacetyltaxol series, while Table 3 contains data on the taxol series. Table 4 consists of limited data available on side chains modified at the C-3' phenyl. To simplify the discussion, activity data in the given assays are made relative to taxol; thus, numbers smaller than unity correspond to more active analogs.
Table 2. SAR of 10-deacetyltaxol derivatives modified at C-2'-C-3'.

![Chemical Structure]

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>R_4</th>
<th>Tubulin-Disassembly inhibition (IC_{50}^{rel})</th>
<th>Cytoxicity (EC_{50}^{rel})</th>
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\( a \) Data obtained from reference 63. \( b \) Concentration of drug leading to 50% inhibition of the rate of microtubule disassembly. IC_{50}^{taxol} = 0.5 mM. \( c \) Measured in the P388 cell culture assay. EC_{50}^{rel} = EC_{50}^{taxol} / EC_{50}^{taxol} \( \text{EC}_{50}^{taxol} = 0.27 \mu g/ml \).
Table 3. SAR of taxol derivatives modified at C-2'-C-3'.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Tubulin-Disassembly inhibition a</th>
<th>EC₅₀ b</th>
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<td>1</td>
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<td>1.4</td>
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<td>(1.5)²</td>
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<td>OH</td>
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<td>-</td>
<td>63</td>
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a Concentration of drug leading to 50% inhibition of the rate of microtubule disassembly. IC₅₀ taxol = 0.5 mM. b Measured in the J774.2 cell line. EC₅₀ rel = EC₅₀ / EC₅₀ taxol. EC₅₀ taxol = 0.27 μg/ml. c B16-melanoma cell culture assay.
Table 4. SAR of taxol derivatives modified at C-3'-phenyl.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>Tubulin-assembly inhibition</th>
<th>Cyto-toxicity EC₅₀</th>
<th>Ref.</th>
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</table>

a The initial slope in the assembly assay is determined at 35° in the presence of 15μM drug. Relative slope is then the initial slope (taxol)/initial slope (analog); taxol's initial slope = 3.57. b Measured in the J774.2 cell line. EC₅₀ = concentration of drug leading to 50% inhibition of cell growth. Taxol = 9 x 10⁻² μmol/L in this cell line. c In L1210 cells. d B16-melanoma cells.

Some trends can be drawn from the above structure-activity relationships with respect to the side chain:

1. Hydrolysis of the C-10 acetate to the corresponding alcohol does not lead to any significant loss of potency (compare 1.25 and 1.10).

2. Acylation of the C-2' hydroxy leads to a decrease in activity in the tubulin assay, but not in the cytotoxic assay (1.53). It is postulated that acyl groups at this site are readily hydrolyzed in vivo to the corresponding 2'-hydroxyl compound. These derivatives are not very potent tubulin disassembly inhibitors, but are quite cytotoxic, and a large number of water soluble prodrugs of taxol have been prepared based on this facile hydrolysis.⁶⁷b,⁶⁹ Hydrolytically stable groups such as the t-
butyldimethylsilyl ether (1.54) destroys cytotoxicity supporting this theory.

3. Removal of the C-2' hydroxy leads to decreases in activity.

4. No significant loss of potency was observed for analogs differing in the nature of the 3'S,-amido group when the benzoyl group was replaced with a hydrophobic functionality. For instance, the tigloyl (1.11) and tosyl (1.28) groups preserved the activity, while the t-butoxy-carbonyl group increased the activity two-fold (1.26 and 1.49). However, when the C-3' is simply an amine (1.52), loss of activity occurs.

5. Replacement of the C-3'-phenyl group with a hydrogen reduced activity markedly (1.64 to 1.67), while para-ring substitution reduced activity marginally (1.68 and 1.69), thus showing the necessity for a phenyl substituent at this position.

6. Maximal potency is obtained when the configuration at C-2',C-3' is that of the normal taxoid series (2'R,3'S) although, the 2'S, 3'S series having 3'-N-amido groups retain potency.

With over 50 side chain modified analogs prepared to date, maximum biological activity is reached with either the naturally occurring side chain or the N-tert-butoxycarbonyl side chains. Solution conformational $^1$H nmr analyses performed on taxol and taxotere by Guenard and co-workers suggests that the most active 2'R,3'S configuration occupies a conformation in which the benzoate group at C-2 holds the side chain in a specific orientation due to hydrophobic interactions between this group an the N-amido group at C-3' (This will be
discussed in greater detail in results and discussion section.\textsuperscript{70} This effect coupled with hydrogen bonding effects between 2'-OH-3'NH and 2'OH-1-C=O (see Figure 5) gives rise to a specific organization of the hydroxyl and phenyl groups at C-2' and C-3'.\textsuperscript{71} Conversely, the unnatural configuration 2'S, 3'R isomers possess different conformations with no hydrophobic interactions between the side chain and the taxene skeleton.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.png}
\caption{Preferred conformation of the methyl ester side chain of taxol (re-created from reference 71).}
\end{figure}

In view of the increased activity seen in the semisynthetic analog taxotere, baccatin III or 10-deacetyl!baccatin III may ultimately prove to be of greater value than taxol, based on the increased bioavailability from renewable sources of the baccatin III series. For example, efficient conversion of the readily available 10-deacetyl!baccatin III (1.17) and baccatin III (1.9) to taxol and taxotere has been achieved via the chiral synthesis of N-acyl-(2'R,3'S)-3-phenylisoserine side chains (1.70 and 1.71).\textsuperscript{72}
At least 10 reports have recently appeared describing the chiral synthesis of acyclic side chains, β-lactams, and oxazinones as latent precursors for the preparation of taxol and taxotere via various C-13 coupling protocols.\textsuperscript{73}

1.9.3 The Northern hemisphere

1.9.3.1 C-7 Hydroxy

Previous SAR studies have shown that modification of the β-hydroxy at C-7 has only secondary effects on taxol’s activity, as shown in Table 5. Acylation with hydrophobic groups decreases potency slightly in comparison with the corresponding hydrophilic groups such as a xylosyl substituent (1.74). Epimerization of the C-7 hydroxy through a base catalyzed retro-aldol
Table 5. SAR at C-7.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>R₁</th>
<th>R₂</th>
<th>Tubulin-Disassembly Inhibition</th>
<th>Cytoxicity&lt;sup&gt;b&lt;/sup&gt; EC&lt;sub&gt;50&lt;sup&gt;rel&lt;/sup&gt;&lt;/sub&gt;</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxol</td>
<td>OH</td>
<td>H</td>
<td>1</td>
<td>1</td>
<td>67a, 74</td>
</tr>
<tr>
<td>1.72</td>
<td>OAc</td>
<td>H</td>
<td>2.0</td>
<td>1.3</td>
<td>69</td>
</tr>
<tr>
<td>1.73</td>
<td>OCO(CH₂)₂CO₂H</td>
<td>H</td>
<td>1.0</td>
<td>-</td>
<td>59</td>
</tr>
<tr>
<td>1.74</td>
<td>O-xylose</td>
<td>H</td>
<td>0.4</td>
<td>-</td>
<td>75</td>
</tr>
<tr>
<td>1.75</td>
<td>H</td>
<td>OH</td>
<td>3.0</td>
<td>1.5</td>
<td>76</td>
</tr>
<tr>
<td>1.76</td>
<td>=O</td>
<td>H</td>
<td>-</td>
<td>&gt;10⁴</td>
<td>25b</td>
</tr>
<tr>
<td>1.77</td>
<td>H</td>
<td>H</td>
<td>-</td>
<td>0.025</td>
<td>76</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentration of drug leading to 50% inhibition of the rate of microtubule disassembly. IC₅₀ taxol = 0.5 μM. <sup>b</sup> Measured in the P388 cell culture assay. EC₅₀<sub>rel</sub> = EC₅₀ / EC₅₀ taxol; EC₅₀ taxol = 0.27 μg/ml.

reaction decreases activity slightly (see 1.75), while oxidation leads to a drastic loss of activity (1.76). Since 7-oxo taxol readily leads to the ring opened product (1.78) under mild acidic or basic conditions,<sup>77</sup> the activity of this analog is probably a reflection of the corresponding oxetane opened product.

From the limited data at C-7, it's apparent that this functional group contributed
little to the overall activity of taxol. The successful preparation of 7-deoxytaxol (1.77) supports this assumption. This compound, prepared via SnBu₃H reduction of xanthate 1.79, followed by hydrolysis of the C-2'-triethylsilyl group, was approximately 40 times more cytotoxic than taxol in the P388 cell culture assay.⁷⁶ Maximal activity is achieved when this position is devoid of functionality, and the synthesis of this compound represents a new class of taxol analogs in terms of potency.

1.9.3.2 C-9 Ketone

Limited SAR data are available for taxol analogs modified at C-9, due to the lack of reactivity of this hindered ketone. Only one taxol analog to date, 9α-dihydrotaxol (1.80) has been synthesized, albeit by stepwise functionalization
of the natural product 13-acetyl-9α-dihydrobaccatin III (1.81), already containing a reduced functionality at C-9. This compound exhibits slightly greater activity in the tubulin assay with respect to taxol, although no data exists on its cytotoxicity.

1.9.3.3 C-10 Acetate

As mentioned in the preceding chapter, the nature of the functionality at C-10 appears to contribute little to the overall activity of taxol and its analogs. However, the recent preparation of 10-deacetoxytaxol (1.82) and 10-dehydroxytaxotere (1.83) by Kingston’s group reveals that 1.82 is as active as taxol, but 1.83 is amazingly 100 times more active than taxol in the P388 cell culture assay. 79

1.82

1.83

1.9.3.4 C-11,12 Olefin

Reactions performed on taxol had shown the tetrasubstituted double bond to be completely inert to hydrogenation and ozonolysis, limiting modification at this area. 80 However, the Bristol group has recently prepared the allylic transposed olefin in an attempt to modify C-10. Treatment of 2',7-bistrichloro-
ethylchloroformyl-10-deacetyltaxol (1.84) with Yarovenko’s reagent (ClFHCCF₂NEt₂) did not afford any 10-fluoro derivative, but gave rise to diene-one (1.85) through simultaneous elimination and double bond transposition (Scheme 3).³¹ Diene-one was hydrogenated to yield 10-deacetoxytaxol (1.82). Another minor product was identified as the fluorinated derivative (1.86). Both 1.85 and 1.86 are approximately eight times less cytotoxic than taxol in the HCT116 human colon carcinoma assay, but retain activity in the tubulin assay. The above results indicate that modification in the C-11,(12) region may lead to slight losses in biological activity.

Scheme 3. Reaction of 2’,7-dichloroethoxy carbonyltaxol with Yarovenko’s reagent.
1.9.4 The Southern Hemisphere

The minimal functional requirements for maximal biological activity along the lower periphery of taxol have not yet been determined, but $^1$H nmr solution studies indicate that the C-2 benzoate provides stabilization of the C-13 side chain via through-space interactions.\textsuperscript{70} It has also been proposed that the lower region of the molecule accommodates a hydrophobic binding site on the microtubule.\textsuperscript{25d}

1.9.4.1 C-1 hydroxy

No information is available on the C-1 bridgehead hydroxy group's contribution to activity, since all attempts to acetylate C-1 even under drastic acetylation conditions have been fruitless.

1.9.4.2 C-2-benzoate

In order to systematically derivatize this position, hydrolysis to the corresponding C-2 alcohol has to be performed, preferably in a selective fashion. Of the four ester functionalities located around the highly oxygenated core of taxol, the C-10 acetate and the C-13 phenylisoserine ester are readily hydrolyzed upon treatment with mild base (1\%-NaHCO$_3$-MeOH).\textsuperscript{12} Moreover, the hydroxyl

Figure 6. Identification of base sensitive functionalities on taxol.
group at C-7 is readily epimerized under these conditions, exemplifying the need for C-7 protection with base stable groups (Figure 6).

A recent synthesis of the first C-2 modified taxol analog, 2-debenzoyloxytaxol (1.87) has appeared. Prepared in a nine step protocol from baccatin III, it was found to be approximately 120 times less active than taxol in the HCT116 human colon cancer cell line assay. Moreover, its ability to assemble tubulin was below measurable levels.

Recently, Kingston and co-workers achieved hydrolytic cleavage of the C-2 benzoate via stepwise deacylation of 2-hexahydro-7-triethylsilylbaccatin III (1.88) with sodium methoxide in methanol. Under these rather vigorous conditions, C-10 acetate hydrolysis occurs first yielding 1.89, followed by the C-4 tertiary acetate (presumably through intramolecular acetate transfer from C-4 to C-13 and hydrolysis) affording tetraol 1.90, and finally the C-2 cyclohexylcarboxylate giving rise to pentaol 1.91 (Scheme 4). Prolonged base treatment led to a new
Scheme 4. Deacylation of 7-triethylsilyl-2-hexahydrobaccatin III.

rearranged product, isomeric with the fully deacylated pentaol, and was identified as tetrahydrofuran 1.92-(called isobaccatin III). The formation of this

product is consistent with attack of the C-2-alkoxide (generated from hydrolysis of cyclohexylcarboxylate) onto C-20, leading to oxetane ring opening with
retention of configuration at C-5. Stepwise acylations of pentaol 1.91 led to 7-triethylsilyl-13-acetyl-4-deacetylbaccatin III (1.93); the order of reactivity of the alcohols towards reacylation was shown to be C-13 > C-2 > C-10. Attempts to acetylate the C-4 hydroxy group were only successful when the C-2 hydroxy was acetylated (1.94 → 1.95); when C-2 was benzoylated, C-4 was inert to acetylation (Scheme 5). The tertiary C-1 hydroxyl group also remained inert to the acylation conditions performed.

![Chemical structures](image)

**Scheme 5.** C-2 benzoate inhibition to C-4 acetylation.

Attempts directed towards synthesizing C-2 debenzoyl analogs has also appeared by Farina and Chen. Treatment of 7,13-diacetylbaccatin III (1.96) with
tributyltin methoxide in NMP in the presence of lithium chloride induced selective debenzoylation in the presence of the four acetates, and chemoselectivity was attributed to the ability of the neighboring C-1 hydroxy to coordinate the tin reagent (see 1.97) and direct it intramolecularly (Scheme 6). Unfortunately, the putative intermediate 1.98 could not be trapped, and exclusive isobaccatin III formation predominated yielding 1.99.

While deacylation and reacylation of the baccatin III series was being investigated, Drs. A. A. L. Gunatilaka and A. G. Chaudhary in Kingston's group gained access to the C-2 debenzoyl taxol series. Treatment of taxol with excess t-butoxycarbonyl anhydride/ dimethylaminopyridine in acetonitrile led to
Scheme 7. Reaction of taxol with excess t-butoxycarbonylanhydride.

formation of 2', 7,N-tri-t-butoxycarbonyltaxol (1.100). Reaction for a prolonged time yielded isolatable 1, 2', 7, N-tetra-t-butoxycarbonyltaxol (1.101) (the first report of acylation of the C-1 tertiary hydroxy group) which was subsequently converted back to 1.100 upon dilute acid workup (Scheme 7). Reaction of 1.100 with aqueous LiOH in THF under controlled conditions surprisingly
Scheme 8. Selective C-2-debenzoylation of taxol.

gave rise to selective debenzoylation of the C-2 ester yielding 1.102 along with a lesser amount of tetrahydrofuran 1.103 (Scheme 8). Chemoselectivity was attributed to the "protecting effects" of the bulky i-butoxy groups (which are base stable) diminishing hydrolytic cleavage of the side chain or the C-10 acetate. Cleavage of the benzoate through coordination of LiOH with the C-1 hydroxy thus directing the base is another contributing factor, because when 1.101 (i-boc protection at C-1) is reacted under these conditions, no C-2 debenzoylation was observed. This route established the first method for the selective cleavage of the C-2 benzoate on taxol, containing an intact C-13 side chain. Regeneration followed by deprotection of the t-Boc groups with formic acid afforded taxol, albeit in low yield. Unfortunately, 2-debenzoyltaxol could not be prepared, for it readily underwent tetrahydrofuran formation leading to opening of the oxetane ring under basic as well as acidic conditions. It is important to mention at this time that isotaxol 1.104 is approximately 1.2 \times 10^4 times less active than taxol in
the P-388 cytotoxicity assay, which is not surprising since this analog does not contain an intact oxetane ring.

Another approach to selective debenzoylation discovered by Dr. M. Ghpure in Kingston's group involved reacting taxol with sodium hydroxide under phase transfer conditions. Treatment of 2',7-bis(triethylsilyl)taxol (1.105)

\[ \text{Scheme 9. C-2 debenzoylation under phase transfer conditions.} \]

at room temperature with NaOH (aq) / benzene / tetrabutylammonium hydrogen sulfonate afforded 2'-7-bis(triethylsilyl)-2-debenzoyltaxol (1.106) in 50% yield along with unreacted starting material (Scheme 9). Under the phase transfer conditions, no formation of isotaxol was detected, but 13% of 7-triethylsilylbaccatin III was formed, denoting that C-13 side chain hydrolysis is a competing reaction. The 2-debenzoyl analog 1.106 was then re-benzoylated
using excess benzoic acid, dicyclohexylcarbodiimide and a catalytic amount of pyrrolidinopyridine in toluene at 60° giving rise to 1.105, thus confirming the selectivity of the debenzoylation step. The silyl ether protecting groups were easily removed with 5%HCl:MeOH, thus obviating the difficulty in deprotec ing the t-Boc groups in the earlier scheme. This route allowed for the synthesis of an extensive array of C-2 substituted analogs (see results and discussion section).

In conjunction with investigating structure-activity relationships at C-2, analog 1.107 was prepared by Dr. Ashok Chaudhary in Kingston's group as a candidate for photoaffinity labeling studies. It was anticipated that 1.107 would mimic the benzoate, and give rise to an analog which exhibited good tubulin binding characteristics-an essential requirement for the photoaffinity labeling experiment. However, this analog showed greatly reduced tubulin activity.

Another photoaffinity probe has also been synthesized at C-2 which surprisingly exhibited enhanced activity in the tubulin assay relative to taxol (see Table 6). 2-debenzoyl-2-(meta-azido)benzoyltaxol (1.109) was successfully
prepared by Dr. Ashok Chaudhary, via a three step protocol starting from 2-debenzoyl-2'-7-bis(triethylsilyl)taxol (1.106).

Table 6. Tubulin-disassembly assay data of 1.109.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (rel)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxol</td>
<td>1.0</td>
</tr>
<tr>
<td>1.109 (meta-azidobenzoyltaxol)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$ = concentration of drug leading to a 50% inhibition of the rate of microtubule disassembly. IC$_{50}$ (rel) = IC$_{50}$/IC$_{50}$ (taxol). IC$_{50}$ (taxol) = 0.42 μM.

By a more elaborate procedure, the ring-tritiated analog (1.110) was also synthesized. The details of this synthesis will not be discussed in any great length, but the fascinating feature of this synthesis is that coupled product 1.108 survives the relatively harsh tritium incorporation and diazotization conditions, affording 1.110 (Scheme 10).
Scheme 10. Chaudhary’s synthesis of meta-azidobenzoyltaxol analogs.

1.9.4.3 C-4 Acetate

Structure-activity relationship studies of taxol at C-4 parallel that of C-2; hydrolysis of the ester to the corresponding alcohol must initially be performed. Selectivity of C-4 acetate cleavage has been problematic, due to the tertiary nature of this functionality, and also because of competing hydrolytic cleavage of the remaining ester functionalities. To date, two groups have reported C-4 acetate hydrolysis, albeit on protected baccatin III derivatives. As mentioned in
Section 1.9.4.2, Kingston and co-workers have prepared pentaol 1.91 via hydrolysis of 2-hexahydro-7-triethylsilylbaccatin III (1.88). Through a series of reacylations and subsequent C-13 side chain coupling, 1.91 was transformed into 4-deacetyltaxol (1.111) in 7 steps (Scheme 11). This analog was approximately 1.5 times less active than taxol in the P-388 cytotoxicity assay, which suggests that the acetate at C-4 does not contribute much to taxol’s activity. Potier has also achieved hydrolysis of the C-4 acetate by reacting 7-triethylsilyl-10-deacetylbaccatin III (1.112) with LiAlH₄ (Scheme 12). This afforded 29% of 2-debenzoylated product (1.113), 6% of 4-deacetylated product (1.114), and 51% of unreacted starting material.
It has been proposed that C-4 acetate hydrolysis in the baccatin III series is mediated through acyl group transfer via the C-13 hydroxy, followed by C-13 acetate cleavage. Supporting evidence for this acyl transfer is manifested in Scheme 13. Dr. M. Gharpure in Kingston’s group has reacted 7-triethylsilylbaccatin III (1.115) with NaOH (aq)/benzene/tetrabutylammonium-

\[ 1.115 \xrightarrow{NaOH (aq.)} 1.116 \]

\[ 1.117 \xrightarrow{NaOH (aq.)} 1.118 \]

Scheme 13. Gharpure’s reaction of substituted baccatin III derivatives with NaOH.

hydrogen sulfate, and these conditions afforded 2-debenzoyl-4-deacetyl-7-triethylsilylbaccatin III (1.116). Reacted under the same conditions, 7,13-(bistriethylsilyl)baccatin III (1.117) gave rise exclusively to 2-debenzoyl-4-deacetyl-7,13-bis(triethylsilyl)baccatin III (1.118). Similarly, when 2',7-bis(triethylsilyl)taxol (1.105) is reacted under the above conditions for an extended time (bypassing selective C-2 debenzoylation), the major product isolated is 1.116 (Scheme 14).
Scheme 14. Consequences of NaOH/PTC reaction with extended time.

Clearly, all evidence to date supports the necessity for a free hydroxy at C-13 to effect C-4 deacetylation.

1.9.5 Oxetane Ring

As with the side chain, the oxetane ring has been shown to play a crucial role in taxol’s activity. The importance of an intact ring in securing correct binding on the microtubule has been proposed.\(^\text{86}\) Moreover, Potier and co-workers have shown by using activity-guided purification of Taxus extracts that no molecule was selected lacking an oxetane ring or a C-2-benzoate substituent, in spite of the presence of such derivatives in the crude plant extract.\(^\text{25d}\) Structure-activity modifications of taxol and derivatives lacking an intact oxetane ring parallel this finding.

As mentioned previously, oxidation of C-7 with Jones reagent followed by treatment with base or silica gel purification leads to a ring opened taxol derivative 1.78, which is \(10^4\) times less biologically active.\(^\text{87}\) The reaction of taxol with triethoxonium tetrafluoroborate gives rise to a ring opened compound 1.119, which is also \(2 \times 10^4\) less cytotoxic than taxol.\(^\text{22}\) Reactions involving
transformations of the oxetane ring will be discussed in the results and discussion section.

\[ \text{Diagram 1.119} \]

1.9.6 Ring Modifications

Several ring modified taxol derivatives have been prepared which differ in the nature of the AB ring system. Kingston was the first to report a novel AB ring contracted product, A-nortaxol (1.120), which was shown surprisingly, to be almost as active as taxol in the tubulin assay, but showed decreased potency in

\[ \text{Diagram 1.120} \]

the P-388 cytotoxicity assay.\textsuperscript{22} Molecular modeling of A-nortaxol shows this compound to have similar 3-dimensional spatial characteristics with respect to taxol, which might explain its good tubulin activity. A transannular B-ring contracted taxol derivative 1.121 has been prepared by irradiation of taxol in CCl\textsubscript{4}.\textsuperscript{88} Intramolecular hydrogen transfer from C-3 to C-12 occurs followed by transannular bond formation giving rise to 1.121. This analog also exhibited
reduced cytotoxicity (>100 less than taxol), and the authors attributed this decreased activity to dramatic changes in topology arising from ring contraction.

1.10 Perspective

It is not surprising that taxol has not yielded to a total synthesis, based on the unusual rearrangements occurring under the mildest of conditions. The ease of intramolecular functional group transfer reactions which transpire upon treatment with acid or base manifests the global complexity of the molecule.

The interaction between functional domains makes interpretation of structure-activity relationship data not only difficult, but, in many cases, misleading. However, this enormous database of SAR data generated in the past decade have led to the detection of three functional domains on taxol required for its activity: the C-13 side chain, the C-2 benzoate, and the oxetane ring. How these functionalities interact with taxol’s receptor, the microtubule, remains a mystery.

Because of the clinical importance of taxol, the development of improved analogs is a matter of urgency. One approach to meet this need is to gain an understanding of the nature of the taxol binding site on the microtubule. If this can be successfully accomplished in sufficient detail to allow a three-dimensional
map of the binding domain to be developed, then it might be possible to design specific taxol analogs which would fit this binding site, and thus show significant biological activity. Photoaffinity labeling was thus chosen as a viable approach to defining taxol's binding site.
II. RESULTS AND DISCUSSION

2.1. Photoaffinity Labeling

Although there is a vast wealth of information concerning the interaction of taxol with microtubules at the cellular level, little is known about taxol's mechanism of action at the molecular level. This can be largely attributed to the lack of a knowledge of the tertiary structure of tubulin, or more importantly, the microtubule. Low resolution X-ray diffraction studies of hydrated cytoplasmic microtubules at 18 Å have been accomplished, but the information obtained at this resolution did not reveal any detailed aspects of structure with respect to previously generated electron microscopy images. Moreover, even if the tertiary structure of the monomeric tubulin subunits were to be determined, there is no guarantee that this would correlate to the structure of tubulin in its polymerized form. Also, no advances have been made in generating crystalline taxol-microtubule complexes suitable for X-ray analysis.

In light of this, photoaffinity labeling was chosen as a suitable technique for the identification of the taxol binding site(s) on the microtubule. Photoaffinity labeling has emerged as a powerful method for investigating drug-receptor interactions in the absence of direct physical methods such as X-ray diffraction. A photoaffinity reagent is a modified ligand in which a photosensitive group masks a highly reactive intermediate, usually a carbene or nitrene. Binding of the photoaffinity reagent with its receptor is performed and subsequent photolysis of the receptor-photoaffinity reagent complex generates a highly reactive species, which in turn covalently links the ligand with regions of the receptor in contact with it, irrespective of the nature of the amino acid residues
present (in the case of proteins). The covalently bound ligand-receptor complex is then degraded by proteolysis or chemical cleavage to identify regions of the polypeptide labeled by the reagent. In this fashion, domains of the receptor which are in contact with the ligand can be identified, and give some insight into the forces of interaction that binds ligands to their receptors (see Figure 7).

\[\text{binding} \rightarrow \text{hv} \rightarrow \text{R} \]
\[R = \text{Receptor} \]
\[L = \text{Ligand} \]
\[\cdot = \text{Photoactivatable group} \]

*Figure 7.* The photoaffinity labeling experiment.

Photoaffinity labeling has previously been applied to analysis of the interactions of tubulin with vinblastine\textsuperscript{91}, GTP\textsuperscript{92}, colchicine\textsuperscript{93}, and ATP and GTP derivatives.\textsuperscript{94} One study of direct photoaffinity labeling of tubulin by taxol has appeared, which indicated selective binding of taxol to β-tubulin.\textsuperscript{95} Since in this study taxol did not carry a specific photoaffinity label, the specific nature of the binding of irradiated drug to tubulin is unknown, and it may be partially random. A short communication describing the synthesis of a C(7) substituted azidonitrobenzoic acid analog of taxol has also appeared.\textsuperscript{96} An approach using specifically labeled active taxol analogs should yield more detailed information about points of taxol-tubulin contact in the binding site(s) if peptide sequences
can be identified, and thus facilitate the understanding of minimal structural requirements of taxol with respect to its biological activity.

2.1.1 Selection of a Photoaffinity Reagent

In the light of the preceding discussion, we elected to investigate the synthesis and tubulin binding characteristics of taxol carrying specific photoaffinity labels.

The photoaffinity labeling experiment begins with the selection of an appropriate photoaffinity reagent. The reagent should be stable not only to storage but to the conditions under which the experiments are performed. It should be relatively simple to synthesize, and should be amenable to radioisotopic incorporation (preferably at later stages in the synthesis).

There are a variety of photoactivatable reagents which have been used with success in photoaffinity labeling experiments. Table 7 shows some of the more common reagents available along with their characteristics.
Table 7 Nitrone and carbene precursors used for labeling receptors.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Structure</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>Nitrone Precursors</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Aryl azides</td>
<td><img src="image" alt="Structure" /></td>
<td>Many bifunctional derivatives available with good absorption properties</td>
<td>Long lived intermediates</td>
<td>97</td>
</tr>
<tr>
<td>Alkoxy carbonyl azides</td>
<td>ROCON$_3$</td>
<td>Generate highly reactive nitrones.</td>
<td>Rarely used because of low ε.</td>
<td>98</td>
</tr>
<tr>
<td>Alkyl azides</td>
<td>RN$_3$</td>
<td>Availability</td>
<td>Imine formation predominates, Low ε</td>
<td>99</td>
</tr>
<tr>
<td>Carbene Precursors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazocarbonyl</td>
<td>RCN$_2$COR$_1$</td>
<td>Ease of synthesis.</td>
<td>RR$_1$ C=C=O formation via Wolff rearrangement</td>
<td>100</td>
</tr>
<tr>
<td>derivatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trifluoro methylphenyl di azirines</td>
<td><img src="image" alt="Structure" /></td>
<td>No long lived intermediates, Good absorption properties</td>
<td>Requires lengthy synthesis</td>
<td>101</td>
</tr>
</tbody>
</table>

Perhaps the most common photoactivatable reagents utilized are the aryl azides. Although their utility as photoaffinity reagents is beyond dispute, aryl azides have several disadvantages. The nitrines derived from them are not reactive enough to undergo photochemical intermolecular insertion into carbon-hydrogen bonds. Hydrophobic binding sites on the protein may be entirely hydrocarbon in nature and therefore be inert to the insertion process. Long lived
electrophilic intermediates derived from singlet aryl nitrenes such as cycloheptatetraenes and benzazirines have also been observed in several photolysis experiments (Scheme 15).\textsuperscript{102} The formation of these putative electrophilic species may lead to nonspecific labeling via reaction with nucleophilic residues. A further disadvantage of aryl and alkyl azides is that they absorb maximally in a spectral region where photochemical damage to proteins and nucleic acids occurs.\textsuperscript{103}

Diazirines are a class of compounds which have been exploited in response to the deficiencies of aryl azides. Smith and Knowles first suggested that diazirines would make suitable photoaffinity reagents, and prepared 3-aryl-3-H-diazirine (2.1).\textsuperscript{104} Unfortunately, this compound formed the linear diazo isomer 2.2 as well as the expected carbene 2.3 when irradiated. In 1980, Brunner and his co-workers introduced 3-(trifluoromethyl)-3-aryl-3H-diazirine
The carbenes derived from this compound underwent efficient intermolecular insertion into carbon-hydrogen bonds (50% insertion into cyclohexane forming adduct 2.6, and 65% of the diazirine was converted directly to the carbene 2.5 (Scheme 16)). Like other diazirines they are efficiently photolyzed at wavelengths outside the protein absorption region (355 nm), and are stable to acidic, basic, and reducing conditions. Although the carbene insertion products from diazirine photolabels can undergo elimination reactions leading to loss of the labeled moiety, their use under controlled conditions promises to provide the best labeling of tubulin. The diazirine class of carbene precursors was thus chosen to be prime candidates for preparing labeled taxol derivatives for the photoaffinity labeling experiment.
2.1.2 Synthesis of Photoaffinity Labels

We initially embarked upon the synthesis of [2-nitro-4-[3-(trifluoro-methyl)-3H-diazirin-3-yl]] phenoxyacetic acid (2.17), a reagent introduced in 1989 by Kanaoka and his colleagues.\textsuperscript{107} This diazirine has excellent spectroscopic properties, along with potential for radioisotopic incorporation. Although the synthetic route leading to the preparation of this reagent had appeared, no experimental data has been published to date.

The synthesis began with protection of readily available \( p \)-bromophenol. The ethoxymethyl group was used as the base stable protecting group, which would be removed at a later stage in the synthesis. Phenol 2.7 was treated with NaH in DMF to form the phenolate sodium salt, which was reacted with chloromethylethylether at room temperature to afford bromoether 2.8 in 84\%.

\[
\begin{align*}
\text{Br} & \quad \text{NaH} & \quad \text{ClCH}_2\text{OCH}_2\text{CH}_3 & \quad \text{Br} \\
\text{OH} & \quad & \text{OCH}_2\text{OCH}_2\text{CH}_3 & \quad 2.7 & \quad 2.8
\end{align*}
\]
The trifluoroacetophenone derivative 2.10, a key intermediate for the synthesis of diazirine groups, was obtained by trifluoroacetylation of the lithium salt 2.9 (generated from n-butylithium) with N-trifluoroacetylpiridine in 66% yield.\textsuperscript{98} An improved procedure was also utilized involving the addition of CuBr\textsubscript{2}-SMe\textsubscript{2} to the lithium salt 2.9, followed by addition of trifluoroacetic anhydride.\textsuperscript{108} This procedure gave rise to a 78% yield of 2.10 along with obviating the need for the preparation of trifluoroacetylpiridine. Compound 2.10 could either be distilled or carried through the next step without purification, without a dramatic effect on yield.

Oxime formation was accomplished using hydroxylamine hydrochloride in refluxing pyridine/ethanol to afford a mixture of syn-anti oxime isomers 2.11 in 82% yield. Reaction of oxime 2.11 with excess p-toluenesulfonylchloride in refluxing pyridine gave rise to tosyloxime 2.12 in 77% yield. Reductive
cyclization of the tosyl oxime with anhydrous NH$_3$ in ether yielded 96% of cyclic diaziridine 2.13. Yields were dramatically increased if a large excess of NH$_3$ was used, and if the reaction mixture was warmed to room temperature with the system equipped with a dry ice condenser to essentially reflux the ammonia.

Of the several reported methods for the oxidation of NH-NH functional groups to the corresponding unsaturated N=N (t-tert-butyl hypochlorite, iodine), formation of the diazirine was best accomplished with freshly prepared Ag$_2$O $^{199}$ to afford 2.14 in near quantitative yield. This stage of the synthesis required all subsequent reactions to be performed under minimal light due to the photolytic reactivity of diazirines.

With the diazirine unit formed, nitration concomitant with deprotection of 2.14 was performed with nitric acid-acetic anhydride at -30$^\circ$C for two hours in the
absence of solvent. This gave an optimized yield of 42% of nitrophenol 2.15.

Most other common nitrating methods failed to give rise to any significant product, and led mainly to decomposition. Higher temperatures led to formation of dinitrated product along with decomposition.

The next step was to alkylate the phenol with methylbromoacetate, a compound which would serve two purposes. First, it would function as the "linker group" for attachment to taxol. Second, [\(^{14}\)C]-methylbromoacetate is commercially available for introduction of an isotope, necessary for detection in the photoaffinity experiment. Such a compound would greatly facilitate the isolation and sequencing of peptides and identification of reactive amino acid residues, which is required to define the taxol binding site. As a precaution, we intended to incorporate the \(^{14}\)C-isotope into the photoaffinity label, and not into taxol itself. Because most labels are attached through an ester or amide functionality, as in this case, cleavage of the taxol-label during photolysis or protein degradation would lead to loss of the isotope if it is incorporated on taxol (Figure 8B). Identification of the peptide fragment would not be possible since the isotope resides on taxol, which is no longer attached to the receptor fragment through the label. This would not be the case if the isotope is incorporated on the
label (Figure 8A). If cleavage of the taxol-label bond occurs, the receptor fragment could still be identified (if a covalent bond is formed before cleavage) since the isotope resides on the label.\textsuperscript{110}

Alkylation of the nitrophenol with methylbromoacetate proved to be more difficult than anticipated, due to the presence of the ortho nitro substituent. After numerous thwarted attempts at alkyling 2.15, Cs\textsubscript{2}CO\textsubscript{3} in a minimal amount of DMF at room temperature in the presence of excess methylbromoacetate gave rise to the O-alkylated product 2.16 in 50\% yield. Hydrolysis of the ester with NaOH afforded carboxylic acid 2.17, which was converted to the
corresponding N-hydroxysuccinimide ester 2.18 for comparison with previously synthesized material. The spectroscopic profile of 2.18 matched the published data exactly.\textsuperscript{107}
2.1.3 Preparation of Photoaffinity Labeled Taxol Analogs

One of the requirements of preparing a useful photoaffinity labeled analog is that the modified analog should resemble the original molecule, since gross alterations to the molecule could unacceptably reduce its affinity for a receptor.

Utilizing previously generated structure-activity relationships of taxol as a guide, several functional regions could serve as useful sites of attachment for diazirine 2.17. The C-2'-hydroxyl group was eliminated as a viable site since acylation leads to a significant loss of activity in the tubulin assay. In earlier work, Kingston showed that taxol analogs carrying acyl groups at C-7 retain most of the activity of taxol, and we thus elected to initially utilize the 7-position for attachment of the diazirine. Although it might not be the most strategic site for attachment of a label, it is the most readily accessible functionality to initiate the photoaffinity labeling experiment.

The effect of introducing a bulky aroyl group at C-7 was determined by preparing 7-benzoyltaxol (2.19). The activity of 2.19 was determined in an assay similar to that first described by Latas. Briefly, microtubule protein was allowed to polymerize at 38°C in the presence of various concentrations of drug, and the rate of depolymerization was determined after rapid cooling to 0°C. In this initial assay the concentration of 7-benzoyltaxol required to reduce the depolymerization rate by 50% was twice that of taxol itself (IC50 value). Thus, 7-aroyl substituted taxol derivatives appeared to be promising as valid taxol analogs. Moreover, In 1991, Samaranake also prepared taxol analog 2.20 as a useful entry into photoaffinity labeling of taxol. This analog was about 30% as
active as taxol in reducing the disassembly rate of polymerized microtubule protein.

Although 2.20 did stabilize microtubules, its synthesis did not lend itself to the preparation of carbon-14 or tritium labeled derivatives. The phenoxydiazirine 2.17 was expected to fulfill the requirements necessary for radioisotopic labeling.

Any scheme to prepare C-7 analogs of taxol must begin with protection of the more reactive C-2'-hydroxy. Selective protection of the C-2' hydroxy was achieved by reaction of taxol with 2.5 equivalents of triethylsilyl chloride/imidazole in CH$_2$Cl$_2$ to afford silyl ether 2.21 in 92% yield. Acylation of 2'-triethylsilyltaxol with diazirine 2.17 using dicyclohexylcarbodiimide and a catalytic amount of pyrrolidinopyridine yielded 68% of ester 2.22. Deprotection of the silyl ether with 5% HCl in MeOH gave rise to 7-[[2-nitro-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]] phenoxyacetyl taxol (2.23) in 75% yield (Scheme 17).
Scheme 17. Preparation of a C-7-photoaffinity labeled taxol analog.

Analog 2.23 was characterized by both low and high resolution mass spectral analysis, and further by its $^1\text{H}$ nmr spectrum. The downfield shift of the C-7 proton from 4.40 ppm to 5.65 ppm and the appearance of the AB quartet arising from the diastereotopic methylene protons at 4.75 and 4.90 ppm confirmed C-7 esterification.

Analog 2.23 showed comparable tubulin activity (see Appendix) and this set the stage for the synthesis of a radiolabeled version. The steps leading to the synthesis of 2.23 proceeded in fair overall yield, but the key alkylation step 2.15 to 2.16 could only be accomplished in good yield when an excess of methyl bromoacetate was used. It is crucial that this alkylation step work in high yields.
to insure complete consumption of $^{14}$C-labeled methyl bromoacetate, since it is commercially available in quantities of only 2.8 mg. Numerous control reactions were investigated utilizing unlabeled methyl bromoacetate as the limiting reagent, but all failed to give rise to any isolatable O-alkylated product. This pathway was deemed to be unsuitable and was discontinued. This unfortunate problem with the alkylation step could be circumvented by preparing a label devoid of the nitro substituent.

A second label synthesized was 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenoxy]acetic acid (2.24), lacking the nitro group, which could be

![Chemical Structure 2.24](image)

prepared from an intermediate of the previous reaction pathway. At first glance, acid deprotection of the ethylmethoxyether group of diazirine 2.14 would

![Chemical Structure 2.14](image) → ![Chemical Structure 2.25](image)

lead directly to phenol 2.25, which simply could be alkylated with methylbromoacetate. However, when 2.14 was reacted with HCl-HOAc, the
phenol generated was unstable both to workup conditions and purification on silica gel, and could not be isolated.

With the inherent instability of phenol 2.25, O-alkylation in situ via generation of the phenol, or more appropriately, the phenolate anion seemed like a viable alternative. This would require an O-silyl ether as the precursor

\[
\text{F}_3\text{C}^+ \text{N}^\text{+} \xrightarrow{\text{RX}} \text{F}_3\text{C}^+ \text{N}^\text{-} \xrightarrow{\text{R-X}} \text{F}_3\text{C}^+ \text{O}\text{-}
\]

Scheme 18. Alkylation via silyl ether precursor.

which could then be treated with fluoride reagents in the presence of the alkylating reagent (Scheme 18). Before this was accomplished, some protecting group manipulation was performed. Tosyl oxime 2.12 was reacted with HCl-HOAc to yield 2.26, which was silylated with tert-butyldiphenylsilylchloride yielding 2.27 in 80% yield. Reductive cyclization of tosyl oxime 2.27 afforded
28% of diaziridine 2.28 along with 65% of O-desilyated product 2.26. Both triethylsilyl and tert-butyldimethylsilyl groups were initially used, but these protecting groups did not survive the ammonia reaction and led entirely to O-desilylated product. Oxidation of 2.28 with Ag₂O yielded diazirine 2.29, which was then treated with CsF-methyl bromoacetate in acetonitrile affording 50% of O-alkylated diazirine 2.30. This reaction worked well with an excess of methyl bromoacetate, but yields were reduced dramatically when a 1:1 stoichiometry of silyl ether-methyl bromoacetate was used. Moreover, when 2.29 was treated with CsF in the absence of the alkylating reagent for 1 hour, followed by quenching with methyl bromoacetate, at least 5 products were produced, none corresponding to 2.30. It is quite possible that the phenolate anion
generated led to the spontaneous formation of an azo type compound through opening of the diazirine ring (Scheme 18).

Scheme 19. Putative pathway leading to decomposition of diazirine.

A similar chemical destruction of a diazirine ring been observed in glucosides carrying a diazirine on the anomeric carbon 2.31 (Scheme 19). Either through the speculated carbene or ylide formation, they were shown to spontaneously react with 1 equivalent of alcohols (i.e. isopropanol) at room temperature to afford diastereomeric mixtures of acetics 2.32.

Scheme 20. Spontaneous generation of glucosydic carbenes.
Although this route led to an undesirable radiolabeling scheme, the diazirine carbocyclic acid 2.24 was prepared and coupled to 2'-triethylsilyltaxol (2.21). Acid deprotection of the 2'-silyl group gave rise to labeled derivative 2.33. This analog exhibited reduced tubulin activity with respect to taxol and analog 2.23 (see Appendix).

Due to the problems associated with O-alkylation of the para-diazirine series, we then turned to the meta analog of 2.24, 3-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenoxy]acetic acid (2.34). This compound was prepared by the published procedure except that an isopropyl ether protecting group was used in place of the methoxymethyl ether of the literature method.

The general scheme is outlined below. Meta-bromophenol was treated with NaH in DMF followed by addition of isopropylidide to furnish 2.35. The series of reactions leading to isopropyl ether diazirine 2.36 were conducted in the same
fashion as in the para-series. Compound 2.36 was then reacted with 1.5 equivalents of boron trichloride in CH₂Cl₂ at 0°C for 1.5 hours and gave rise to a near quantitative yield of phenol 2.37, which was relatively stable to workup procedures and purification. The key alkylation step to form the ester 2.38 from the phenol 2.37 proceeded in 90% yield in the presence of cesium carbonate and 1.1 equivalents of methyl bromoacetate and could thus readily be used to prepare [¹⁴C] labeled material from [¹⁴C] labeled methyl bromoacetate. Hydrolysis of ester 2.38 with NaOH in THF yielded 90% of 2.34; however, H nmr data in CDCl₃ from the published synthesis differed slightly in the chemical shift values. Crystallization of the final product with chloroform-hexane allowed for unambiguous structural confirmation by X-ray crystallography (Figure 8). This is only the third report of an X-ray structure of a diazirine.
The taxol derivative 2.39 was then prepared via 2'-triethylsilylexal (2.21) in the usual manner. As expected, compound 2.39 exhibited reduced tubulin activity (See Appendix).

![Chemical Structure](image)

The relatively good tubulin activity and the potential for synthesis of a radiolabeled version indicated taxol derivative 2.39 to be the analog of choice for initial studies of photoaffinity labeling of polymerized tubulin by taxol. Even with the success in the O-alkylation experiment in the meta-series, a second option for isotopic labeling was also investigated, since the acidic α-protons of 2.34 or 2.38 could in principle allow the preparation of [3H] labeled derivatives by deprotonation and reprotonation. This changeover in the isotopic labeling
protocol was justified based on the quality of the $^{14}$C-methylbromoacetate received from the supplier, which appeared to have undergone decomposition based on some initial radioisotopic incorporation experiments using this material.

Deprotonation of the ester 2.38 or the acid 2.34 proved more difficult than anticipated. Although phenoxyacetic acid can readily be deprotonated to form the dilithium salt, both in our hands and others,\textsuperscript{117} this reaction initially failed when applied to the acid 2.34. Treatment of the ester 2.38 with lithium diisopropylamide (LDA) alone also failed to give useful deuterium incorporation after a D$_2$O quench. After numerous failed attempts, treatment of the ester 2.38 with LDA (1.1 eq) at -70\textdegree, followed by a D$_2$O:HMPA quench, yielded the $\alpha$-monodeuterated acid 2.40 (via \textit{in situ} ester hydrolysis) in 50\% yield with 45\% label incorporation. The percent of deuterium incorporation was determined by $^1$H nmr analysis of coupled product 2.41. The diastereotopic methylene protons
which appear as an AB quartet at 4.51 and 4.86 ppm in the unlabeled sample collapse to two singlets at 4.50 and 4.82 ppm when a deuterium atom is incorporated, and the ratio of the singlets to the AB quartet signals gives a good indication of the extent of deuterium incorporation.

Figure 10. Evidence for H-D exchange on coupled product 2.41.
(Figure 10). Since the model reaction worked well with D₂O on triplicate runs, the [³H]-labeled version was then prepared by treatment of the ester 2.38 with LDA followed by a HMPA/[³H]₂O quench, affording tritiated carboxylic acid 2.42.

Starting with a specific activity of 90 mCi/mmol of [³H]₂O, the resulting product 2.42 had a specific activity of 5.4 mCi/mmol, denoting a significant isotope effect. A portion of [³H]-2.42 was coupled to the 7-position of taxol in the usual manner giving rise to [³H]-taxol analog 2.43. Chromatographic profiles of the isotopically labeled sample were identical to that of non-isotopically labeled material. This compound had a specific activity of 2.55 mCi/mmol, which was sufficient for detection in the photoaffinity labeling experiment.
<table>
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<tr>
<th>Protons on</th>
<th>Taxol</th>
<th>2.23</th>
<th>2.33</th>
<th>2.39</th>
<th>2.50*</th>
<th>2.52</th>
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<td>4.94 (br d, 8.5)</td>
<td>4.87 (br d)</td>
<td>4.93 (br d)</td>
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<td>2.67 (m)</td>
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<td>6.24 (br t)</td>
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<td>2.36 (m)</td>
<td>2.35 (m)</td>
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<td>2.21 (m)</td>
<td>2.22 (m)</td>
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<td>4.33 (d, 8.5)</td>
<td>4.09 (d, 8.3)</td>
<td>4.19 (d, 8.4)</td>
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<td>4.18 (d, 8.5)</td>
<td>4.17 (d, 8.5)</td>
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<td>8.10 (d, 8.5, 1.3)</td>
<td>8.10 (d, 8.6, 1.4)</td>
<td>8.00 (d, 8.0)</td>
<td>7.98 (8.6, 1.2)</td>
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<td>7.75 (d, 8.5, 1.4)</td>
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<td>7.77 (d, 8.6, 1.3)</td>
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<td>2.19 (s)</td>
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<td>4.52 (d, 16.2)</td>
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<tr>
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<td>7.70 (d, 2.3)</td>
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<td>6.95 (d, 8.8)</td>
<td>7.35 (d, 8.8, 8.8)</td>
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<tr>
<td>C-20</td>
<td>-</td>
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* Measured in CDCl₃ at 270 MHz unless otherwise noted. Chemical shifts (δ) are expressed in parts per million from Me₄Si and coupling constants (J) in hertz. "* Multiplicity: s=single, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad. * 200 MHz frequency. * * C-16 and C-17 Methyl signals can be interchanged within columns. * * Hidden under acetate or methyl signals. * * Hidden under aromatic protons.
2.1.4 Biological Evaluation and The Photoaffinity Labeling Experiment

The biological evaluation of the taxol analogs and the photoaffinity labeling experiments were conducted by Dr. Ernest Hamel at the National Cancer Institute (For a detailed discussion of Dr. Hamel's experiments, see Appendix).

Hamel investigated the interaction of tritiated analog 2.43 with tubulin by harvesting the analog-microtubule complex by centrifugation, followed by illumination at 302 nm. The protein was denatured, and total protein and radiolabeled recovered were quantified, and a portion of each sample was examined by micro-gel filtration chromatography to determine the amount of 2.43 bound to the denatured protein. In summary, superstoichiometric amounts of 2.43 appeared to form a covalent bond with tubulin, and competition experiments with taxol failed to reduce the extent of covalent bond formation, indicating that the reactions were for the most part non-specific. Moreover, illumination failed to enhance covalent bond formation, which could imply that the diazirine could be chemically reacting with tubulin before irradiation.

Another possibility is that the C-7 ester is hydrolyzing during the assembly process, and the label is lost before irradiation. This might be the reason for the inability of the diazirine analogs to assemble tubulin at low concentrations and temperatures.
2.1.5 Preparation of a Side Chain Photoaffinity Labeled Taxol Analog.

Although the C-7 diazirinyltaxol analogs did not prove to be effective as suitable photoaffinity reagents, other sites of taxol were utilized as potential labeling sites. Based on limited structure-activity relationships of N-substituted derivatives of taxol, replacement of the N-benzoyl group with other N-acyl groups preserves tubulin activity (see Section 1.9.2).

A recent method describing the preparation of the synthetic side chain has appeared by Greene and co-workers,\textsuperscript{72a,73c} starting from readily available \textit{S}-phenylglycine. Side chain \textbf{2.44} was synthesized in an analogous fashion, however, this compound was very unstable and decomposed rapidly upon isolation. This had also been observed by Greene, who suggests storing this compound in a solution of toluene at -20°C.\textsuperscript{72a} However, this didn't alleviate the problem associated with its inherent instability.\textsuperscript{118} This problem was circumvented by combining Commercon's with Greene's procedure utilizing an acetonide protection system,\textsuperscript{72b} instead of the labile ethylvinyl ether protecting group at C-2'. In this fashion, the synthetic side chain was prepared in four
steps. Lithium aluminum hydride reduction of 5-phenylglycine to the corresponding alcohol followed by N-acylation in situ with t-butoxycarbonyl anhydride afforded 2.45 in 75% yield. Swern oxidation of 2.45 using oxalyl chloride-DMSO followed by addition of vinylmagnesium bromide at ambient temperature yielded 55% yield of a 96:4 ratio of allylic alcohols, with the syn-isomer predominating (2.46). Adduct 2.46 was then protected as the acetonide with 2-methoxypropene in the presence of pyridinium p-toluenesulfonic acid affording 2.47 in quantitative yield. Oxidation of the pendant olefin to the carboxylic acid was achieved with sodium periodate-
RuCl₃ and afforded protected side-chain 2.48 in 72% yield. The spectroscopic data of this compound were in accordance with the literature values.²²b

Before the synthetic side chain was coupled to baccatin III, the more reactive C-7 hydroxy group was protected as the triethylsilyl ether with triethylsilylchloride/imidazole to give 2.49 in 75% yield. DCC-DMAP coupling of 7-triethylsilylbaccatin III (2.49) with 1.5 equivalents of carboxylic acid 2.48 in toluene at 60°C yielded the C-13 esterified analog 2.50 in 96% yield. The downfield shift of the C-13 proton from 4.9 to 6.2 ppm in the ¹H nmr spectrum confirmed C-13 esterification. Formic acid treatment of 2.50 in the absence of solvent at ambient temperature furnished the fully deprotected amino alcohol.
2.51 in one step. Amino alcohol 2.51 was not purified due to retention on silica gel, but the proton nmr of crude 2.51 showed that it clearly consisted of only one product, and the shifts of the 2' and 3' protons were in accord with amino alcohol prepared by Potier's group. 2.51 was then reacted carefully with 1 equivalent of benzoic acid-DCC-PP, yielding taxol, which was identical to the natural product. The use of 1 equivalent of carboxylic acid is crucial so as to avoid concomitant acylation at C-2' OH. With the route established, amino alcohol 2.51 was coupled with the diazirine 2.38, yielding the N-photoaffinity labeled derivative 2.52.
The activity of 2.52 in the tubulin assay surprisingly showed decreased activity, compared with taxol, very similar to that of the C-7 analogs. Nonetheless, the tritiated derivative 2.53 was synthesized, and studies are currently underway investigating this analog for its potential use in the photoaffinity labeling experiment.
3.1 Modifications in the Southern Hemisphere of Taxol

3.1.1 Modifications at C-2

In an ongoing effort by Kingston's group to delineate structural features of taxol which contribute to its biological activity, chemistry was developed which gained access into C-2 modified taxols. Up to 1977, it has been shown that some 40% of all reported compounds incorporate an unfused benzene ring. Moreover, it has been stated that "50% of drug-oriented patents are concerned with substituted benzenes". 118, 120

With a method established for selectively cleaving the C-2 benzoate, taxol was converted to 2-debenzoyl-2-acyltaxol derivatives by acylation of 2-debenzoyl-2'-7-bis(triethylnyl) taxol 1.106 (obtained from reaction of 2'-7-bis(triethylnyl) taxol with NaOH-phase transfer conditions-see Scheme 9) with a variety of carboxylic acids in the presence of DCC-PP, followed by deprotection of the silyl groups yielding the 2-acyl analogs (Scheme 21). Optimal conditions for the success of the coupling step required at least 20 equivalents of DCC-carboxylic acid and a catalytic amount of pyrrolidinopyridine, in 0.15 ml
of toluene for every 10 mg of substrate. Most of the reactions required heating to 60\(^{\circ}\), although a few acylation reactions were performed at ambient temperature. If the reactions are performed under dilution conditions, or fewer equivalents of carboxylic acid is used, isotaxol formation is predominant, and C-5 acylated products are formed (Scheme 22). The reaction mixtures containing the coupled products were simply filtered over a silica gel plug in an appropriate solvent system, (to remove the insoluble dicyclohexylurea by-product) and subjected to acid deprotection.
Scheme 22. Consequences of dilution in attempts to acylate C-2.

Initially, random (non-benzoyl) esters were prepared at C-2, to assess whether a benzoate type ester is required at C-2 for biological activity. Based on the activity of taxotere (containing the N-t-butoxycarbonyl group), a similar derivative was prepared at C-2. Reaction of 2',7-bis(triethylsilyl)-2-debenzoyltaxol (1,106) with t-butoxycarbonylanhydride and pyrrolidinopyridine for 20 hours at room temperature in toluene gave rise to two products in a 4:1 ratio, which were chromatographically inseparable. However, deprotection of the triethylsilyl groups from the mixture allowed for their separation. The minor product was identified as 2-debenzoyl-2-t-butoxycarbonyltaxol (3.1) based on
its $^1$H nmr spectrum. Relative to 2-debenzoyl-2',7-(bistriethyl)silyltaxol (1.106), the downfield shift of the C-2 proton from 3.9 ppm to 5.34 ppm denoted C-2 esterification, and the appearance of the methyl signals of the t-butoxy group at 1.6 ppm confirmed the presence of this group. The major product was identified as 2-debenzoyl-1,2-carbonyltaxol (3.2). The C-2 proton's shift from 3.9 ppm to 4.5 ppm and the lack of the methyl signal from the t-butoxycarbonyl group suggested a cyclic carbonate. Moreover, a downfield shift of one of the methylene C-14 protons from approximately 2.2 ppm to 2.8 ppm denoted C-1 functionalization. The formation of this product must arise from initial formation of the C-2-t-butoxycarbonyl ester, which under basic conditions cyclizes concomitant with loss of t-butanol. To further verify the cyclic product, 2-debenzoyl-2',7-bis(triethylsilyl)taxol was reacted with excess triphosgene (expected to lead solely to carbonate formation) in pyridine at room temperature.
for 8 hours. Acid deprotection of the triethylsilyl groups afforded 3.2. Molecular modeling of 2-debenzoyl-2-t-butoxycarbonyltaxol reveals that the 1-hydroxy and the 2-carbonyl ester functionalities are in close spatial proximity (~2.5 Å), and the C1(O)-C1(C)-C2(C)-C2(O) dihedral angle is approximately 72° (see Figure 11). Cyclization to the carbonate is thus feasible based on the model.

Figure 11. Molecular model of 3.2. (protons and side chain hidden for clarity).
High and low resolution mass spectral analysis of 3.1 and 3.2 were in accord with their molecular composition.

Both of these derivatives in addition to a wide variety of functionally diverse C-2 modified analogs were synthesized and tested in several bioassays. The data from the P-388 cell line (mouse lymphocytic leukemia) was performed by Dr. William Lichter at the University of Miami School of Medicine, and the HL-60 human leukemia cell line was performed by Dr. Ernest Hamel at the National Cancer Institute. The activity of each derivative is given relative to taxol; compounds with an (ED$_{50}$/ED$_{50}$ (taxol)) value of less than 1 are more active than taxol in these assays. NT corresponds to analogs which were not tested in the assay under question.
Table 9. SAR of modified taxols at C-2.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>EC$_{50}^c$ P-388 cell line</th>
<th>IC$_{50}^d$ HL-60 leukemia cells</th>
<th>Prepared by</th>
</tr>
</thead>
<tbody>
<tr>
<td>taxol</td>
<td>Ph</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3.3</td>
<td>CH$_3$</td>
<td>28</td>
<td>166</td>
<td>b</td>
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<tr>
<td>3.4</td>
<td>C$<em>4$H$</em>{10}$(straight chain)</td>
<td>1.6</td>
<td>83</td>
<td>a</td>
</tr>
<tr>
<td>3.1</td>
<td>OC(CH$_3$)$_3$</td>
<td>&gt;28</td>
<td>NT</td>
<td>a</td>
</tr>
<tr>
<td>3.2</td>
<td>1,2-carbonate</td>
<td>22</td>
<td>166</td>
<td>a</td>
</tr>
<tr>
<td>3.5</td>
<td><img src="image" alt="Thiophene" /></td>
<td>4</td>
<td>NT</td>
<td>a</td>
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<tr>
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<td>a</td>
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<td><img src="image" alt="Thiophene" /></td>
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</tr>
</tbody>
</table>

$^a$ John Rimoldi.  $^b$ Dr. Milind Gharpure.  $^c$ The cytotoxic results are expressed in µg/ml required to cause 50% inhibition of cell growth. EC$_{50}$ = EC$_{50}$ (analog) / EC$_{50}$ (taxol).  $^d$ Results are expressed in nM required to cause 50% inhibition of cell growth. IC$_{50}$ = IC$_{50}$ (analog) / IC$_{50}$ (taxol). IC$_{50}$ (taxol) = 6 nM.
All of the analogs tested in Table 9 exhibited reduced activity with respect to taxol in both cell lines. However the magnitude of the diminished activity was accentuated in the HL-60 cell line assay relative to the P-388 assay. Based on this small core of data, it appears that drastic deviation from a benzoyl-type group leads to a loss of potency (see 3.2 and 3.3). The 3-thiophene (3.5) and furoyl analogs (3.7) exhibited comparable activity to taxol in the P-388 assay. Although these were not tested in the HL-60 assay, they might possibly exhibit similar profiles as valerate ester 3.4 (good activity in P-388, poor activity in HL-60).

With the limited structure-activity relationships from this group of derivatives, it was postulated that the minimal functional requirement at C-2 for maximal biological activity is benzoyl-like in nature. To support this hypothesis, a large number of C-2-benzoate esters were synthesised, and their activities determined. The following tables are arranged according to the ring substitution patterns.
Table 10. Taxol analogs modified at C-2 as benzoate esters-mono-substituted.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>ED₅₀ₐ</th>
<th>IC₅₀ₐ</th>
<th>Prepared by</th>
</tr>
</thead>
<tbody>
<tr>
<td>taxol</td>
<td>H</td>
<td>H</td>
<td>1</td>
<td>1</td>
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### Para series

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<tr>
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<th>IC₅₀ₐ</th>
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<td>3.8</td>
<td>H</td>
<td>F</td>
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<td>3.9</td>
<td>H</td>
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<tr>
<td>3.10</td>
<td>H</td>
<td>SMe</td>
<td>&gt;1⁴</td>
<td>NT</td>
<td>a</td>
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<tr>
<td>3.11</td>
<td>H</td>
<td>COCH₃</td>
<td>&gt;1⁴</td>
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<tr>
<td>3.12</td>
<td>H</td>
<td>N₃</td>
<td>&gt;1.7 x 1⁵</td>
<td>&gt;1.6 x 1³</td>
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</tr>
<tr>
<td>3.13</td>
<td>H</td>
<td>CN</td>
<td>&gt;28</td>
<td>166</td>
<td>a</td>
</tr>
<tr>
<td>3.14</td>
<td>H</td>
<td>CF₃</td>
<td>&gt;28</td>
<td>NT</td>
<td>a</td>
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<tr>
<td>3.15</td>
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<td>NO₂</td>
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### Meta series

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<th>Prepared by</th>
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<td>3.17</td>
<td>Cl</td>
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<td>0.8</td>
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<td>3.18</td>
<td>H</td>
<td>H</td>
<td>0.03</td>
<td>5</td>
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<td>3.19</td>
<td>CN</td>
<td>H</td>
<td>0.13</td>
<td>1</td>
<td>c</td>
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<td>15</td>
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<td>3.22</td>
<td>N₃</td>
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<td>H</td>
<td>0.0004</td>
<td>0.3</td>
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<tr>
<td>3.25</td>
<td>NH₂</td>
<td>H</td>
<td>1500</td>
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<td>c</td>
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<tr>
<td>3.26</td>
<td>OPh</td>
<td>H</td>
<td>4.3</td>
<td>NT</td>
<td>c</td>
</tr>
</tbody>
</table>

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* John Rimoldi.  
* Dr. Ashok Chaudhary.  
* Dr. Milind Gharpure.  
* The cytotoxic results are expressed in μg/ml required to cause 50% inhibition of cell growth.  
  EC₅₀ = EC₅₀ (analog) / EC₅₀ (taxol).  
* Results are expressed in nM required to cause 50% inhibition of cell growth.  
  IC₅₀ = IC₅₀ (analog) / IC₅₀ (taxol).  
  IC₅₀ (taxol) = 6 nM.
Table 11. Taxol analogs modified at C-2 as benzoate esters-di- and tri-substituted.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>EC₅₀ᵃ</th>
<th>IC₅₀ᵇ</th>
<th>Prepared by</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>P-388</td>
<td>HL-60</td>
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</tr>
<tr>
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<td>H</td>
<td>H</td>
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<td>1</td>
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**Di-substituted**

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<tr>
<td>3.27</td>
<td>NO₂</td>
<td>I</td>
<td>H</td>
<td>750</td>
<td>NT</td>
<td>c</td>
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<td>3.28</td>
<td>NH₂</td>
<td>I</td>
<td>H</td>
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<tr>
<td>3.29</td>
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<td>Cl</td>
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<td>F</td>
<td>F</td>
<td>H</td>
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<td>NT</td>
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<td>3.31</td>
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<td>H</td>
<td>NO₂</td>
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<td>0.3</td>
<td>b</td>
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**Tri-substituted**

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<tbody>
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<td>3.33</td>
<td>OCH₃</td>
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<td>OCH₃</td>
<td>OCH₃</td>
<td>0.5</td>
<td>NT</td>
<td>a</td>
</tr>
</tbody>
</table>

ᵃ John Rimoldi. ᵇ Dr. Milind Gharpure. ᶜ Dr. Ashok Chaudhary. ⁴ The cytotoxic results are expressed in μg/ml required to cause 50% inhibition of cell growth. EC₅₀ = EC₅₀ (analog) / EC₅₀ (taxol). ⁶ Results are expressed in nM required to cause 50% inhibition of cell growth. IC₅₀ = IC₅₀ (analog) / IC₅₀ (taxol). IC₅₀ (taxol) = 6 nM.
There appears to be a significant variance in potency when comparing the data from the P-388 and the HL-60 cell lines (Figure 12). The analogs which showed excellent activity in the P-388 assay (>10^3 more active) were only at best three times more active than taxol in the HL-60 assay. On the contrary, analogs which showed marginal decreases in activity in the P-388 assay were orders of magnitude less active in the HL-60 assay. Conclusions which are extracted from this data set will be discussed with respect to both assays.

![Diagram](image)

*Figure 12. Variance in potency values relative to taxol.*

The biological data for the analogs prepared in Tables 10 and 11 reveal some interesting patterns:

1. Foremost, in the monosubstituted series, their appears to be an adverse steric effect with substitution at the *para* position, which is operative irrespective of the nature of the substituent. The only equipotent member of the *para* series is 4-fluorobenzoyltaxol (3.8) which would contribute the least to steric effects. Conversely, nearly all of the *meta* substituted analogs showed an increase in potency relative to taxol. Moreover, comparison of *para* vs. *meta* analogs for
similar substituents reveals that every meta analog is more potent than its para counterpart. (Compare 3.8 and 3.16, 3.9 and 3.17, 3.12 and 3.22, 3.13 and 3.19, 3.14 and 3.20, 3.15 and 3.21). Astonishingly, several meta substituted analogs (3.22 and 3.24) showed increased activities in the P-388 assay on the order of $10^3$ to $10^5$.

2. The di- and tri-substituted series show variable activities based on the substitution patterns. Remarkably, the 3,4,5-trimethoxybenzoyltaxol analog (3.34) is nearly equipotent to taxol, while the di-substituted analogs 3.27 and 3.28 are much less active. 3,5-difluorobenzoyl taxol (3.31) is incredibly $10^5$ orders of magnitude more active in the P-388 assay.

Figure 13 is a 3-dimensional representation of taxol re-created from Potiers analysis of the preferred conformation of taxol in solution. It is postulated that hydrophobic interactions arising between the C-2 benzoate and the 3'-N-benzoate set the side chain in a postion so the 2'-hydroxyl and 3'-phenyl groups can interact with tubulin residues leading to a stabilization of the taxol-tubulin complex. The distance between the C-2 benzoate and 3'-N-benzoate was measured to be approximately 4.5 to 5.5 angstroms.
The increased activity observed in the C-2-meta -benzoyl analogs could partially be due to an enhanced interaction with active amino acid residues located on the binding site of the microtubule, thus stabilizing the taxol-receptor complex. Conversely, para substituion, which leads to decreases in activity, induces unfavorable steric interactions with amino acid residues during binding. However, this alone cannot completely account for the observed increase in potency, since compounds 3.29 (3,4-dichlorobenzoyltaxol) and 3.34 (3,4,5-trimethoxybenzoyltaxol) are near equipotent as taxol, and these analogs contain groups at the para position.

Enhancement of hydrophobic interactions between the C-2 meta-benzoyl moiety and the side chain is another factor which could lead to an overall
conformational change in taxol. Free rotation of the C-2 benzoate (which normally exists on the unsubstituted ring) might be prohibited with meta substitution, and this barrier to rotation could lead to an increase in the through space interactions between the C-2- benzoate and the side chain. In the final analysis, the observed increases in potency from C-2 modification might be directly related to the ability of this group to directly induce a positive effect on the side chain conformation, leading to a global stabilization of the drug-receptor complex. Clearly, these sorts of interactions complicate systematic structure-activity relationship studies.
### Table 12  \(^1\)H nmr spectra of selected C-2 modified taxol analogs\(^{a,b}\)

<table>
<thead>
<tr>
<th>Protons on</th>
<th>Taxol</th>
<th>1.105</th>
<th>1.106</th>
<th>3.1</th>
<th>3.2</th>
<th>3.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-2</td>
<td>5.67 (d, 7.1)</td>
<td>5.70(^c)</td>
<td>3.92 (ps, 5.8)</td>
<td>5.34 (d, 6.9)</td>
<td>4.48(^c)</td>
<td>5.41 (d, 6.9)</td>
</tr>
<tr>
<td>C-3</td>
<td>3.79 (t, 7.0)</td>
<td>2.83 (d, 7.0)</td>
<td>3.49 (d, 6.8)</td>
<td>3.85 (d, 6.5)</td>
<td>3.43 (d, 5.8)</td>
<td>3.67 (d, 6.8)</td>
</tr>
<tr>
<td>C-5</td>
<td>4.94 (br d, 9.0)</td>
<td>4.94 (br d, 9.0)</td>
<td>4.98 (br d)</td>
<td>4.95 (br d)</td>
<td>4.98 (br d)</td>
<td>4.34 (br d, 9.5)</td>
</tr>
<tr>
<td>C-6</td>
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<td>2.50 (m)</td>
<td>2.50 (m)</td>
<td>2.53 (m)</td>
<td>2.62 (m)</td>
<td>2.55 (m)</td>
</tr>
<tr>
<td>C-7</td>
<td>1.88 (m)</td>
<td>1.86 (m)</td>
<td>1.86 (m)</td>
<td>1.95 (m)</td>
<td>1.95 (m)</td>
<td>1.87 (m)</td>
</tr>
<tr>
<td>C-10</td>
<td>4.40 (m)</td>
<td>4.48 (dd, 10.6, 6.6)</td>
<td>4.42 (dd, 10.6, 6.7)</td>
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<td>4.36 (m)</td>
<td>4.35 (m)</td>
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<tr>
<td>C-13</td>
<td>6.27 (s)</td>
<td>6.45 (s)</td>
<td>6.37 (s)</td>
<td>6.16 (s)</td>
<td>6.28 (s)</td>
<td>6.24 (s)</td>
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<tr>
<td>C-14</td>
<td>6.23 (br t)</td>
<td>6.24 (br t)</td>
<td>6.20 (br t)</td>
<td>6.38 (br t)</td>
<td>6.19 (br t)</td>
<td>6.20 (br t)</td>
</tr>
<tr>
<td>C-16,17-Ch(^\alpha)</td>
<td>2.35 (m)</td>
<td>2.32 (m)</td>
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<td>1.13 (s)</td>
<td>1.80 (d, 11.4)</td>
<td>1.75 (d, 11.4)</td>
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<tr>
<td></td>
<td>2.28 (m)</td>
<td>2.25 (m)</td>
<td>1.10 (s)</td>
<td>1.25 (s)</td>
<td>1.38 (s)</td>
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<tr>
<td>C-18-Ch(^\alpha)</td>
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<td>1.24 (s)</td>
<td>1.25 (s)</td>
<td>1.70 (s)</td>
<td>1.80 (d, 11.4)</td>
<td>1.80 (d, 11.4)</td>
<td>1.75 (d, 11.4)</td>
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<tr>
<td>C-19-Ch(^\alpha)</td>
<td>1.79 (d, 11.1)</td>
<td>2.05 (br s)</td>
<td>1.95 (br s)</td>
<td>1.70 (br s)</td>
<td>1.80 (d, 11.1)</td>
<td>1.75 (d, 11.1)</td>
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<tr>
<td>C-19-Ch(^\beta)</td>
<td>1.68 (s)</td>
<td>1.70 (s)</td>
<td>1.51 (s)</td>
<td>1.64 (s)</td>
<td>1.75 (s)</td>
<td>1.64 (s)</td>
</tr>
<tr>
<td>C-20</td>
<td>4.30 (d, 8.4)</td>
<td>4.32 (d, 8.0)</td>
<td>4.62 (br s)(^c)</td>
<td>4.56 (d, 8.2)</td>
<td>4.53 (d, 8.2)</td>
<td>4.34 (d, 8.2)</td>
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<tr>
<td></td>
<td>4.19 (d, 8.5)</td>
<td>4.22 (d, 8.0)</td>
<td>4.56 (br s)(^c)</td>
<td>4.53 (d, 8.2)</td>
<td>4.53 (d, 8.2)</td>
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<tr>
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<td>4.70 (dd, 5.1)</td>
<td>4.62(^c)</td>
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<tr>
<td>C-3(^\prime)</td>
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<td>5.70(^c)</td>
<td>5.63 (br dd)</td>
<td>5.72 (dd, 8.7, 2.8)</td>
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<td>5.72 (dd, 8.7, 2.8)</td>
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<tr>
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<td>7.10 (d, 8.9)</td>
<td>7.08 (d, 9.5)</td>
<td>7.01 (d, 8.7)</td>
<td>6.89 (d, 8.7)</td>
<td>6.94 (d, 8.9)</td>
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<tr>
<td>C-2-Obz (ortho)</td>
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<td>8.13 (dd, 8.5, 1.4)</td>
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<td>7.3-7.5 (m)</td>
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</tbody>
</table>

\(^{a}\)Measured in CDCl\(_3\) at 270 MHz. Chemical shifts are expressed in parts per million from Me\(_4\)Si and coupling constants in hertz.

\(^{b}\)Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, ps=poorly resolved. Overlapping peaks are indicated in the same row.

\(^{c}\)Values for C-16 and C-17 methyl signals can be interchanged within columns. Hidden under acetate or methyl signals.
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<td>5.56 (d, 7.1)</td>
<td>5.65 (d, 7.1)</td>
<td>5.63 (d, 7.1)</td>
<td>5.65 (d, 7.1)</td>
<td>5.55 (d, 7.1)</td>
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<tr>
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<td>3.80 (d, 7.1)</td>
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<td>6.27 (s)</td>
<td>6.27 (s)</td>
<td>6.27 (s)</td>
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<tr>
<td>C-14 (α, β)</td>
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<td>6.21 (br t)</td>
<td>6.27 (br t)</td>
<td>6.27 (br t)</td>
<td>6.25 (br t)</td>
<td>6.27 (br t)</td>
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<tr>
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<td>1.13 (s)</td>
<td>1.13 (s)</td>
<td>1.13 (s)</td>
<td>1.14 (s)</td>
</tr>
<tr>
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<td>1.25 (s)</td>
<td>1.24 (s)</td>
<td>1.24 (s)</td>
<td>1.24 (s)</td>
<td>1.25 (s)</td>
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<tr>
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<tr>
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<td>1.68 (s)</td>
<td>1.68 (s)</td>
<td>1.69 (s)</td>
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<td>C-2'</td>
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<td>4.42 (d, 8.3)</td>
<td>4.48 (d, 8.7)</td>
<td>4.17 (d, 8.4)</td>
<td>4.19 (d, 8.3)</td>
<td>4.19 (d, 8.2)</td>
</tr>
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<td>4.26 (d, 8.3)</td>
<td>4.28 (d, 8.7)</td>
<td>4.27 (d, 9.4)</td>
<td>4.30 (d, 8.3)</td>
<td>4.25 (d, 9.2)</td>
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<td>4.79 (dd, 5.1, 2.5)</td>
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<td>4.80 (dd, 5.0, 2.5)</td>
<td>4.80 (dd, 5.0, 2.5)</td>
<td>4.81 (dd, 5.0, 2.4)</td>
</tr>
<tr>
<td>NBz (ortho)</td>
<td>7.73 (dd, 8.6, 1.3)</td>
<td>7.74 (dd, 8.6, 1.3)</td>
<td>7.72 (dd, 8.6, 1.5)</td>
<td>7.72 (dd, 8.6, 1.4)</td>
<td>7.73 (dd, 8.6, 1.4)</td>
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<td>7.30-7.50 (m)</td>
<td>7.35-7.50 (m)</td>
</tr>
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<td>3.51 (d, 5.0)</td>
<td>3.47 (5.0)</td>
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<td>2.39 (s)</td>
<td>2.39 (s)</td>
<td>2.40 (s)</td>
<td>2.41 (s)</td>
</tr>
<tr>
<td>10-0Ac</td>
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<td>2.23 (s)</td>
<td>2.24 (s)</td>
<td>2.23 (s)</td>
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<td>8.02 (d, 8.6)</td>
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*Measurements in CDCl₃ at 270 MHz. Chemical shifts δ are expressed in parts per million from Me₄Si and coupling constants J in hertz.  
*Multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, p = pseudo triplet. Values for C-16 and C-17 methyl groups can be interchanged within columns. *Hidden under acetate or methyl signals.
Table 14  $^1$H nmr spectra of selected C-2 modified taxol analogs$^{a,b}$

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<th>Protons on</th>
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<th>3.14</th>
<th>3.29</th>
<th>3.34*</th>
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<td>5.62 (d, 7.1)</td>
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<td>3.82 (d, 7.2)</td>
<td>3.80 (d, 7.2)</td>
<td>3.80 (d, 7.1)</td>
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<tr>
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<td>4.95 (br d)</td>
<td>4.95 (br d)</td>
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<td>4.42 (m)</td>
<td>4.38 (m)</td>
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</tr>
<tr>
<td>C-13</td>
<td>6.27 (s)</td>
<td>6.27 (s)</td>
<td>6.26 (s)</td>
<td>6.27 (s)</td>
</tr>
<tr>
<td>C-14 (α, β)</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>C-16-17-CH$_3$$^c$</td>
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<td>1.14 (s)</td>
<td>1.13 (s)</td>
<td>1.13 (s)</td>
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<tr>
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<td>1.25 (s)</td>
<td>1.24 (s)</td>
<td>1.23 (s)</td>
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<tr>
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<td>1.53 (br s)</td>
<td>1.41 (d, 1.1)</td>
<td>1.40 (s)</td>
</tr>
<tr>
<td>C-20</td>
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<td>1.69 (s)</td>
<td>1.68 (s)</td>
<td>1.69 (s)</td>
</tr>
<tr>
<td>C-2'</td>
<td>4.20 (br s)</td>
<td>4.19 (d, 8.2)</td>
<td>4.16 (d, 8.1)</td>
<td>4.20 (d, 8.4)</td>
</tr>
<tr>
<td>3'-NH</td>
<td>4.26 (d, 8.2)</td>
<td>4.27 (d, 3.1)</td>
<td>4.39 (d, 9.5)</td>
<td>4.39 (d, 9.5)</td>
</tr>
<tr>
<td>NBz (ortho)</td>
<td>6.90 (d, 9.1)</td>
<td>6.94 (d, 9.1)</td>
<td>6.93 (d, 8.8)</td>
<td>6.97 (d, 9.0)</td>
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<tr>
<td>ArH</td>
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<td>7.70-7.93 (m)</td>
<td>7.71 (d, 8.5, 1.5)</td>
<td>7.70 (br d) 8.6</td>
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<tr>
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<td>7.36-7.37 (m)</td>
<td>7.35-7.52 (m)</td>
<td>7.32-7.50 (m)</td>
<td>7.3-7.6 (m)</td>
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<td>3.47 (d, 5.3)</td>
<td>3.50 (d, 5.1)</td>
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<tr>
<td>10'-OAc</td>
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<td>2.24 (s)</td>
<td>2.24 (s)</td>
<td>2.24 (s)</td>
</tr>
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<td>8.27 (d, 8.2)</td>
<td>8.27 (d, 8.2)</td>
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<tr>
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<td>7.81 (d, 8.4)</td>
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$^a$Measured in CDCl$_3$ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me$_4$N$^+$ and coupling constants (J) in hertz. $^b$Multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad, p = pseudo triplet. $^c$Values for C-16 and C-17 methyl signals can be interchanged within solutions. $^*$Holden under acetate or methyl signals. **Run at a frequency of 200 MHz.
3.1.2 C-4 Deacetylation of Taxol

Based on the increased potency observed in the C-2 modified taxol series, an attempt at enhancing the selectivity of C-2 benzoate cleavage was undertaken. The conditions previously used to effect C-2 ester hydrolysis have been conducted in basic media using biphasic solvent systems that generally require high concentrations of base. This leads to competing ester hydrolysis and side reactions that are sometimes unavoidable, even under controlled conditions. It was rationalized that by utilizing lower base concentrations, selective C-2 benzoate hydrolysis could be achieved while minimizing side reactions, thus increasing the yield of this reaction. A way to accomplish this is to use near-equimolar amounts of bases which are soluble in non-polar solvents.

Initially, the C-2' and C-7 hydroxyl groups of taxol were protected to prevent facile side chain cleavage and to preclude epimerization. A t-butyldimethylsilyl protecting group was chosen for the C-2'-OH instead of the triethylsilyl group, to provide a bulky steric environment which could impede C-13 side chain cleavage. Since the C-7-OH of taxol is unreactive towards protection with the t-butyldimethylsilyl group, the triethylsilyl group was utilized at C-7. 2'-t-Butyldimethylsilyl-7-triethylsilyltaxol (3.35) was synthesized.
in one pot by initially reacting taxol with 10 equivalents of t-butyldimethylsilyl chloride / imidazole in a minimal amount of DMF at \(80^\circ\) for 2 hours, followed by room temperature addition of 10 equivalents of triethylsilylchloride/imidazole. This afforded 92% of 3.35, which was treated with 3 equivalents of Triton B (40% benzyltrimethylammonium hydroxide in methanol) in \(\text{CH}_2\text{Cl}_2\) at \(0^\circ\) for 1 minute. The starting material was completely consumed, and four products were formed. Dilute acid workup followed by chromatographic separation by preparative TLC yielded, as one of the envisioned products, 2'-t-butyldimethylsilyl-7-triethylsilyl-2-debenzoyltaxol (3.36). The \(^1\text{H} \text{nmr} \) spectrum confirmed that hydrolysis of the
benzoate had occurred by the disappearance of the C-2 benzoate aromatic protons, along with the upfield shift of the C-2 proton. The integrity of all of the corresponding side chain protons, along with the C-13 proton located at 6.2 ppm, established the presence of an intact C-13 side chain. The methyl singlets at at 2.1 and 2.4 ppm denoted intact acetates at C-10 and C-4. High and low resolution mass spectral analysis, along with correlation of the proton nmr spectrum of 3.36 with 2',7-bis(triethylsilyl)-2-debenzoyltaxol (1.106) further substantiated this structure.

The 1H nmr spectrum from the second major product isolated from the Triton B reaction verified the integrity of the C-13 side chain and the C-10 acetate, from their diagnostic signals, along with the C-2' and C-7 methyl and methylene signals from the silyl protecting groups. Absent from this spectrum were the signals for the aromatic C-2 benzoate protons (indicative of hydrolysis at C-2) in addition to the C-4-acetate methyl signal at 2.4 ppm (indicative of C-4 hydrolysis). Mass spectral analysis gave a molecular weight of 936, corresponding to loss of a benzoate and an acetate. The data is in accord with structure 3.37, 2'-i-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-4-deacetyl-taxol. Moreover, the C-7 proton of 3.37 had shifted downfield from 4.5 ppm to 4.1 ppm, (which has been observed when C-4 is devoid of an acetate) and the appearance of a sharp singlet at 4.7 ppm, exchangeable with D2O, was assigned to the C-4-OH group.

Formed in lesser amounts was tetrahydrofuran derivative 3.38 arising from attack of the C-2-OH group on C-20, leading to oxetane ring opening. Confirmation of this structure is based on the diagnostic C-5 proton. This proton only appears as a broad or sharp dd (around 5 ppm) when the oxetane ring is
intact. Otherwise, it collapses to a broad triplet or multiplet; in the present case, this proton was observed as a broad triplet.

The fourth product was identified as 2-debenzoyl-4-deacetyl-7-triethylsilylbaccatin III (1.116) by $^1$H nmr comparison with previously synthesized material. Cleavage of the C-13 side chain, C-2 benzoate, and the C-4 acetate groups had occurred.

The formation of 3.37 is remarkable, since it had previously been assumed that a free C-13 hydroxy is necessary for C-4-deacetylation. Just as incredible, all products isolated contained an intact C-10 acetate. Based on the relative yields of the four products, it appears that selectivity could be achieved by varying conditions. Thus, Table 15 shows the order of deacylation determined by lowering the equivalents of base and temperature so as to effect selective ester hydrolysis.

**Table 15. Determination of order of ester hydrolysis.**

<table>
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<tr>
<th>Entry</th>
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<th>Product distribution (%)</th>
</tr>
</thead>
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<td>equivalents of Triton B</td>
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<tr>
<td>1</td>
<td>3.0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1 min @ -78; 15 min warm to RT</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>1 min @ -78; 5 min warm to RT</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>1 min @ -78; 15 min warm to RT</td>
</tr>
</tbody>
</table>

*a 20 mg of 3.35 in 1.5 ml of dry CH$_2$Cl$_2$. b Isolated yields.*
The Triton B reaction is very sensitive to conditions, especially at 0\(^{\circ}\). At this temperature, reaction times longer than 1 minute led to increased formation of 3.38 and 1.116. The rate of the reaction was slowed by lowering the temperature of addition of Triton B, which in effect led to selective C-2 benzoate hydrolysis (entry 4). Moderately high yields of C-2, C-4 deacylated product 3.37 could be obtained by extended reaction time with 2 eq. of Triton B at -78\(^{\circ}\) C (entry 5).

Scheme 23 shows the representative order of deacylations. C-2 hydrolysis occurs initially, affording 3.36, which is the substrate leading to C-4 deacetylated product 3.37, since all of the products isolated were devoid of the C-2 benzoate (analogously, no formation of C-4 deacetylated product was detected which retained the benzoate at C-2). The fate of 3.37 is that it can either undergo C-13 side chain cleavage giving rise to baccatin III derivative 1.116, or isomerize to tetrahydrofuran derivative 3.38.
Scheme 23. Order of deacetylations from the Triton B reaction.

The course of this reaction can be easily monitored by thin layer chromatography. Figure 14 shows the chromatographic profile during the reaction, which also substantiates the order of ester cleavages. The most interesting aspect in relation to the chromatographic profile is that the C-2 debenzoylated product 3.36 is *more* polar that the C-2 debenzoyl, C-4 deacetyl product 3.37. The reversal in polarity on silica gel can be expained by hydrogen bonding effects between the C-4 OH and the carbonyl group of the C-13 ester.
Hydrogen bonding has been observed in the baccatin III series between the C-13 OH and the C-4 acetate (Figure 15 A), which accounts for the forcing conditions necessary to acetylate the C-13-OH. Hydrogen bonding in product 3.37 is simply a result of the transposition of the ester and hydroxy functionalities (Figure 15 B).

Figure 14. Monitoring the Triton B reaction by TLC.

Figure 15. Hydrogen bonding effects between the C-4 and C-13 functionalities.
In order to determine whether Triton B or the nature of the protecting group at the C-2' OH accounted for the unexpected selectivity of the hydrolysis reaction, 2',7-bis(triethylsilyl)taxol (1.105) was reacted with Triton B (Scheme 24) under the conditions shown in Table 15, entry 5. These conditions afforded 2 major products, which were identified as 7-triethylsilylbaccatin III (2.49), and 7-triethylsilyl-2-debenzoyltaxol (3.39). Although this reaction was not investigated to any further extent, clearly there is another reaction pathway operating. A possible course of reaction is shown in Scheme 24. Initially, deprotection of the C-2' silyl group occurs, followed by competition between C-13 side chain cleavage or C-2 debenzoylation, affording 2.49 or 3.39. In conclusion, the success of the Triton B reaction is dependent upon a stable protecting group at C-2' OH, which impedes
C-13 side chain hydrolysis. This permits another pathway to operate, namely C-2 and C-4 ester hydrolysis.

Since the secondary C-2 OH is more reactive than the tertiary C-4 OH, compound 3.37 was benzyolated with benzoic acid/DCC/PP in toluene at 80° to afford 2′-t-butyldimethylsilyl-7-triethylsilyl-4-deacetyltaxol (3.40). Representative changes in the 1H nmr spectrum of 3.40 relative to 3.37 are the downfield shift of C-2 proton from 3.8 to 5.7 ppm, and the appearance of the aromatic benzoyl protons at 8.1 ppm.

Attempts to acetylate 3.40 under common acetylation conditions (acetic anhydride / pyridine/ DMAP/ elevated temperatures) failed. This parallels Kingston's finding; when C-2 is benzyolated, C-4 is inert to acetylation. In order
to circumvent this, 3.37 was reacted with triphosgene/pyridine or t-butoxycarbonyl anhydride/pyrrolidinopyridine to afford the cyclic carbonate 3.41, which would serve as a protecting group at C-2 (and C-1) to facilitate C-4 acetylation.

Studies are currently underway to effect acetylation of 3.41 at C-4 for the preparation of C-4 modified taxol analogs for analysis of structure-activity relationships at this position.
<table>
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<th>Protons on</th>
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<th>3.37</th>
<th>3.38</th>
<th>3.40</th>
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<td>C-2</td>
<td>5.69 (d, 7.0)</td>
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<td>4.95 (br d)</td>
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<td>4.80 (dd, 9.6, 3.6)</td>
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<td>2.51 (m)</td>
<td>2.50 (m)</td>
<td>2.40 (m)</td>
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<td>C-7</td>
<td>1.88 (m)</td>
<td>1.90 (m)</td>
<td>2.00 (m)</td>
<td>2.00 (m)</td>
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<td>C-10</td>
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<td>4.41 (dd, 10.3, 6.6)</td>
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<td>C-13</td>
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<td>C-14</td>
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<td>C-16,17-CH₃</td>
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<td>1.62 (s)</td>
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<td>1.56 (s)</td>
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<tr>
<td>C-20</td>
<td>4.19 (d, 8.3)</td>
<td>4.63 (br s)</td>
<td>4.46 (d, 8.6)</td>
<td>4.40 (d, 8.6)</td>
<td>3.70 (d, 9.9)</td>
</tr>
<tr>
<td>C-2'</td>
<td>4.30 (d, 8.4)</td>
<td>4.71 (d, 8.6)</td>
<td>4.73 (d, 8.6)</td>
<td>3.90 (d, 9.9)</td>
<td>4.30 (d, 8.2)</td>
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<td>C-3'</td>
<td>4.67 (d, 2.1)</td>
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<td>3'-NH</td>
<td>7.74 (d, 9.0, 2.1)</td>
<td>5.67 (dd, 9.2, 1.6)</td>
<td>5.62 (s)</td>
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<td>5.60 (dd, 9.1, 1.4)</td>
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<td>7.05 (d, 9.2)</td>
<td>7.05 (d, 9.2)</td>
<td>7.05 (d, 9.2)</td>
<td>7.05 (d, 9.2)</td>
</tr>
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<td>NBz (ortho)</td>
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<td>7.74 (dd, 8.3, 1.6)</td>
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<td>A'H</td>
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<td>7.26-7.60 (m)</td>
<td>7.26-7.60 (m)</td>
<td>7.26-7.60 (m)</td>
<td>7.26-7.60 (m)</td>
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<tr>
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<tr>
<td>16-OAc</td>
<td>2.16 (s)</td>
<td>2.14 (s)</td>
<td>2.14 (s)</td>
<td>2.14 (s)</td>
<td>2.14 (s)</td>
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<td>-S=O</td>
<td>0.52 (d, 7.9)</td>
<td>0.57 (d, 7.8)</td>
<td>0.60 (d, 7.8)</td>
<td>0.55 (d, 7.7)</td>
<td>0.52 (d, 7.7)</td>
</tr>
<tr>
<td>-SCH₂CH₂</td>
<td>0.92 (t, 7.8)</td>
<td>0.92 (t, 7.8)</td>
<td>0.93 (t, 7.8)</td>
<td>0.90 (t, 7.7)</td>
<td>0.95 (t, 7.8)</td>
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<tr>
<td>-Si(CH₃)₂</td>
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<td>-0.07 (s)</td>
<td>-0.08 (s)</td>
</tr>
<tr>
<td>-Si(CH₃)₂</td>
<td>0.80 (s)</td>
<td>0.83 (s)</td>
<td>0.83 (s)</td>
<td>0.83 (s)</td>
<td>0.83 (s)</td>
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<tr>
<td>Other</td>
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<td>2.79 (d, 5.9)</td>
<td>2.79 (d, 5.9)</td>
<td>2.79 (d, 5.9)</td>
<td>2.79 (d, 5.9)</td>
</tr>
</tbody>
</table>

*Measured in CDCl₃ at 270 MHz. Chemical shifts (δ) are expressed in parts per million from Me₄Si and coupling constants (J) in hertz. Multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, ps = pseudo triplet. Overlapping peaks within same column. Values for C-16 and C-17 methyl signals can be interchanged within columns. Hidden under acetox or methyl signals. Hidden under aromatic protons.
4.1 Modifications of the Oxetane Ring

4.1.1 Introduction

One of the unique features of taxol is the oxetane ring at C-4,5, which has been implicated to play a major role taxol’s activity, since taxol analogs devoid of the oxetane ring display significantly decreased biological activity ($>10^3$), in both the tubulin assay and the \textit{in vitro} cytotoxicity assay.\textsuperscript{25a} A few of these analogs lacking the oxetane ring are shown in Figure 16.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure16.png}
\caption{Biologically inactive oxetane ring opened analogs.}
\end{figure}

Dreiding models of analogs 1.88 and 4.1 reveal that these oxetane-ring opened analogs exhibit C-ring flexibility, which is absent when the oxetane ring is intact. If strict conformational requirements are necessary for maximal interactions between taxol and its receptor, this conformational change resulting from ring-opening might account for its diminished biological activity. This
dramatic increase in activity associated with an intact oxetane ring relative to its ring opened analogs has prompted us to further investigate modification of the D-ring. In order to prepare biologically active derivatives for structure-activity relationships at this region of the molecule, a necessity exists for an intact fused ring system at C-4(5). The initial strategy was to prepare analogs which retained the four-membered ring system at C-4(5), but differed solely in the nature of the heteroatom (oxetane to azetidine—see Scheme 25). This heteroatom exchange would provide access to taxol analogs which could further be modified via reaction of the azetidine nitrogen with alkylating or acylating reagents allowing for systematic structure-activity relationships at this site, which would otherwise be impossible.

![Scheme 25. Oxetane to azetidine transformation.](image)

The sequence of steps leading to the synthesis of the azetidine would initially begin with cleavage of the oxetane ring. The oxetane ring has been shown to be very susceptible to ring opening by reaction with triethoxonium tetrafluoroborate\(^2\) and tin (IV) chloride\(^1\) leading to ring opened analogs \(1.119\) and \(4.1\). The formation of these products is consistent with the mechanism proposed initially by Kingston and co-workers and more recently elaborated by Chen (Scheme 26).\(^2\),\(^1\) This mechanism parallels that from Weinstein's classical
studies on neighboring group acetate participation during solvolysis reactions.\textsuperscript{122} The initial step involves complexation of the oxetane ring oxygen with the Lewis acid $4.2 \rightarrow 4.3$ followed by neighboring group participation inducing oxetane ring opening giving rise to the stabilized acetonium intermediate $4.4$. The fate of acetonium ion $4.4$ is that it can be trapped to give the unstable hemi-ortho ester $4.5$ leading to the C-5 acetylated product $4.6$, or it can interconvert to another isomeric acetonium ion $4.8$ through ortho ester $4.7$. This leads to the corresponding C-20 acetylated product $4.9$. The formation of either $4.6$ or $4.9$ has been shown to be highly dependent on the nature of the Lewis acid utilized and the conditions under which the reactions are performed. One important observation obtained from these studies was that none of the products isolated retained the acetate at C-4, irrespective of the Lewis acid used.

\textbf{Scheme 26.} Mechanism of formation of regioisomeric ring opened analogs.
Under more vigourous conditions such as refluxing acetyl chloride, Aβ-ring contraction occurs concomitant with oxetane ring opening (Scheme 27). A similar transformation has been observed by reaction of taxol with boron tribromide. This necessitates the use of mild and selective electrophilic reagents to prevent this competing reaction from occurring.

![Scheme 27. Reaction of taxol with refluxing acetyl chloride.](image)

4.1.2 Reaction of Taxol with Trimethylsilylhalides.

The use of trimethylsilyl iodide and trimethylsilyl bromide as effective reagents for the cleavage of cyclic ethers, particularly epoxides and oxetanes, is well documented. These reagents react with oxetanes to afford the corresponding O-trimethylsilyl alkyl halides with halogen substitution occurring at the least substituted carbon (in the case of epoxides). Although the regioselectivity of the reaction of TMS-halides with oxetanes has not been extensively investigated, it was anticipated that reaction of either of these reagents with taxol would afford one of two halide substituted products 4.10 or 4.12 (Scheme 28). Formation of the α-C-5 halogenated product 4.10 would be ideal, serving as the precursor for introduction of nitrogen via azide 4.11, followed by reductive cyclization with triphenylphosphine affording the required azetidine.
Reaction of 2'-t-butyldimethylsilyl-7-triethylsilyltaxol 3.35 with 1.5 equivalents of trimethylsilyl iodide CH₂Cl₂ at -78°C led to the rapid formation of one major product, identified as 4.13. Mass spectral analysis gave a molecular ion at m/z 968 in 4.13, corresponding to cleavage a silyl group, relative to the starting material 3.35. The 2'-t-butyldimethylsilyl group was absent in the ¹H nmr spectrum. ¹H nmr analysis also revealed two diagnostic olefin signals at 4.85 and 4.75 ppm indicative of AB ring contraction. Oxetane ring opening had occurred, since the C-5 proton which normally appears as a broad doublet at 4.95 ppm, had shifted to a broad triplet at 3.7 ppm. This chemical shift of the C-5 proton also indicates the lack of an acetate at this position, for when it is acetylated, this proton shifts to 5.5 ppm. The sharp AB-quartet at 4.2 is indicative of C-20 acetylation, as well as the location of the primary C-20 acetate at 1.6 ppm. Molecular mass is consistent with loss of H₂O from ring contraction, addition of H₂O from oxetane ring opening, and cleavage of the t-butyldimethylsilyl group.
Treatment of 3.35 with trimethylsilyl bromide at -40 °C in CH₂Cl₂ also gave rise to 4.13. Participation of the C-4 acetate via intermediate 4.7 appears to be the operative mechanism leading to oxetane ring opening, with unavoidable AB ring contraction occurring. Reaction of 3.35 with trimethylsilyl chloride led to extensive decomposition.

4.1.3 Reaction of Taxol with Triphenylcarbenium Tetrafluoroborate.

When 3.35 was treated with triphenylcarbenium tetrafluoroborate in CH₂Cl₂ at 0°C, the formation of two products occurred within 30 minutes.
The major product was identified as 4.14, in which the oxetane ring had undergone cleavage, along with loss of the C-2'-t-butyldimethylsilyl group. The second product, formed in lesser amounts, was characterized as product 4.15, which had retained the silyl protecting group at C-2'. Interestingly, this reaction did not lead to any AB ring contracted products. Products 4.14 and 4.15 containing the C-20 acetate are of little utility, since in order to reform a ring system, C-20 must be devoid of functionality. This would require selective methods to hydrolyze this acetate in the presence of the remaining C-2, C-10, and C-13 esters.

4.1.4 Reaction of Taxol with Trimethylsilyl Azide-Zinc Iodide.

Reaction of 3.35 with a large excess of trimethylsilylazide-ZnI$_2$ in CH$_2$Cl$_2$ at room temperature for 4 hours resulted in the formation of a single product, less polar than the starting material. $^1$H nmr analysis of this product revealed that the oxetane ring had undergone cleavage, based on the peak shape of the C-5 proton. Other features of the spectrum were the upfield shift of the C-20 proton to 3.5 ppm and the appearance of a D$_2$O exchangeable singlet at 4.2 ppm. The product resulting from expected azide attack at C-5 (4.16) or C-20 (4.17) was ruled out since the molecular mass of 1172 corresponded to addition of an oxygen and a trimethylsilyl group. The lack of olefin signals at 4.7 and 4.9 ppm was indicative that AB ring contraction had not occurred.
The molecular mass and $^1$H nmr spectral analysis are in accordance with structure 4.18. The formation of this compound can best be explained by ZnI$_2$ complexation of the oxetane oxygen followed by C-4 acetate participation to induce opening of the ring, forming the stable acetoxonium ion of type 4.4, which is the species which reacts with trimethylsilyl azide and undergoes silylation of the C-20 hydroxy group. The acetoxonium ion intermediate can is then simply trapped with water upon workup to give the unstable hemi-ortho ester which leads to product 4.18.
With three differentiated silyl protecting groups, the C-20 trimethylsilyl ether was selectively removed by treatment of 4.18 with 10% citric acid in methanol\textsuperscript{127} at room temperature for 20 minutes, yielding 92% of 4.19. This reaction also verified the placement of the trimethylsilyl group in 4.18, since the only major change occurring in the $^{1}$H nmr spectrum of 4.19 relative to 4.18 is the collapse of the sharp C-20 AB-quartet at 3.5 ppm, now additionally coupled to the C-20 OH, as seen by D$_{2}$O exchange and COSY experiments. Once again, compound 4.19 is not an ideal substrate to perform heteroatom substitution and subsequent ring closure, due to the presence of the C-5 acetate.

Compound 4.19 was then treated with 1% sodium bicarbonate in methanol to determine the order of ester hydrolysis, in an attempt to deacetylate the ester at C-5. Within minutes, the complete conversion of the starting material to a more polar product on tlc had occurred. Analysis of the $^{1}$H nmr spectrum revealed that drastic changes in both the C-20 and C-2 regions had taken place. Most notable was the upfield shift of the C-2 proton, coupled with the C-3 and a D$_{2}$O exchangeable proton, denoting debenzylation at C-2. However the appearance of the ortho aromatic protons from the benzoate indicated that the benzoate had not hydrolyzed. The large downfield shift of the C-20 protons suggested that an intramolecular transfer of the benzoyl group from C-2 to C-20
had occurred, affording product 4.20. A similar type of transfer reaction has also

![Chemical structure](image)

been documented by Halsall on a naturally occurring taxoid.\textsuperscript{11} Molecular models of 4.20 indicate that the C-2 benzoate is in close proximity to the C-20 hydroxyl group in ring opened oxetane analogs. Further confirmation of structure 4.20 resides in the fact that the molecular mass of remains unchanged relative to 4.19, suggestive that a functional group migration had occurred.

Compound 4.20 was then subjected to benzoylation conditions with meta-chlorobenzoic acid, to further verify a free hydroxy at C-2. Compound 4.20 was reacted with excess meta-chlorobenzoic acid-DCC-pyrrolidinopyridine at 60\textdegree{}C in toluene which afforded C-2 benzoylated analog 4.21. Because of the large excess
of benzoylating reagent used, this compound could not be completely purified, so it was treated with 5% HCl in methanol for 20 minutes to selectively deprotect the C-7 triethylsilyl group. This afforded compound 4.22 which clearly showed corresponding downfield shifts of C-2 and C-3 in its $^1$H nmr spectrum relative to 4.20, thus verifying C-2 benzoylation. Figure 17 contains the 3.0 to 6.5 ppm region of the $^1$H nmr spectrums of compounds 4.19, 4.20, and 4.22. One feature worth noting is the large chemical shift difference between the C-20 diastereotopic methylene protons (5.35 and 4.5 ppm) in compound 4.20 containing a C-20 benzoate and a hydroxyl group at C-2.
Figure 17. $^1$H nmr changes manifested from C-2 to C-20 benzoyl transfer.
Treatment of the C-20 silyl ether 4.18 or the C-20 hydroxy derivative 4.19 with two equivalents of Triton B at -78°C in CH₂Cl₂ also induced C-2 to C-20 benzoyl transfer, giving rise to compound 4.20.

4.1.5 Reaction of Taxol with Trimethylsilyl Cyanide-Zinc Iodide.

When bis-silyl protected taxol 3.35 was reacted at room temperature with trimethylsilyl cyanide and zinc iodide¹²⁸ in CH₂Cl₂ for 30 minutes, a single product was formed in 86% yield with a higher polarity on silica gel. Interestingly, the ¹H nmr spectrum of this product was similar to, but not identical with that of silyl ether 4.18. The major difference in this product resided in the chemical shift of the C-3 proton, which appeared 0.9 ppm downfield relative to 4.18. A small upfield shift of the C-20 protons was also evident. Moreover, the 4-OH proton was absent from this spectrum.
The structure could not be unambiguously confirmed solely by its $^1$H nmr or COSY analysis, but the identity of the product from the trimethylsilyl azide reaction was established as 4.23 by a combination of $^{13}$C nmr experiments. Analysis of the $^{13}$C decoupled spectrum, the $^{13}$C DEPT spectrum, and the HETCOR spectrum led to the following assignments shown in Figure 18.

*Figure 18. Carbon assignments for compound 4.23.*
Convincing evidence for structure 4.23 resides from the $^{13}$C spectrum, which showed only four carbonyl resonances (rather than five) in the region 166-171 ppm, and were assigned to the carbonyl groups of the C-13 ester, C-10 acetate, C-2 benzoate and the N-benzoate. The DEPT sequence revealed two quaternary carbon resonances in an unusual region at 118 ppm and 98 ppm. The carbon resonance at 118 is indicative of a nitrile carbon, which generally appears at 110-120 ppm. The quaternary carbon at 98 ppm was assigned to the carbon bearing the nitrile. FAB mass spectral analysis yielded a molecular ion at $m/z$ [M+Na]$^+$ of 1204, consistent with the proposed structure.

The formation of this product confers direct evidence for the acetoxonium ion intermediate. The mechanism of formation of this product is shown in Scheme 29.

Scheme 29. Mechanism of formation of product 4.23.
Complexation of the zinc with the oxetane oxygen allows for facile C-4 acetate participation inducing ring opening to yield the stable acetoxonium intermediate 4.26. Before this intermediate has an opportunity to equilibrate to the other acetoxonium ion, it reacts with trimethylsilyl cyanide to afford C-20 silylated intermediate 4.27, which is then trapped by cyanide to yield the product.

Chen and co-workers have recently trapped the acetoxonium ion by reaction of baccatin III with trifluoroacetic acid and phenyldimethylsilylane, to give a mixture of the corresponding acetals 4.29 and 4.30 (Scheme 30).\textsuperscript{123}

\begin{center}
\begin{tikzpicture}
\node[anchor=west] (a) at (0,0) {\includegraphics[width=0.4\textwidth]{4.26.png}};
\node[anchor=west] (b) at (0,0) {\includegraphics[width=0.4\textwidth]{4.27.png}};
\end{tikzpicture}
\end{center}

\textit{Scheme 30.} Trapping of acetoxonium ions by dimethylphenylsilane.

The placement of the trimethylsilyl group of 4.23 was confirmed by selective deprotection with 10\% citric acid in methanol to yield the corresponding O-desilated product 4.31. This compound did not undergo any C-2 to C-20 benzoyl transfer under basic conditions, which might be a direct consequence of the fused ring system at C-4-C-5. Reaction of 4.31 with 5\% HCl-MeOH for 2 hours at room
temperature effected the deprotection of the silyl groups at C-2' and C-7 to give compound 4.32.

![Chemical structures](image)

4.31

4.32
Table 17. $^1$H nmr spectra of oxetane ring modified taxol analogs.\(^a,b\)

<table>
<thead>
<tr>
<th>Protons on</th>
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<th>4.19</th>
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<th>4.22</th>
<th>4.23</th>
<th>4.32</th>
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<td>C-2</td>
<td>5.60 (d, 5.5)</td>
<td>5.65 (d, 5.5)</td>
<td>5.20 (dd, 10.1, 6.1)</td>
<td>5.71 (d, 5.5)</td>
<td>5.58 (d, 7.6)</td>
<td>5.55 (d, 7.8)</td>
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<tr>
<td>C-3</td>
<td>3.91 (d, 5.5)</td>
<td>3.90 (d, 5.5)</td>
<td>3.49 (d, 6.1)</td>
<td>4.02 (d, 5.4)</td>
<td>3.30 (d, 7.5)</td>
<td>3.05 (d, 7.7)</td>
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<td>5.04 (bri)</td>
<td>5.27 (bri)</td>
<td>5.46 (bri)</td>
<td>5.48 (bri)</td>
<td>5.03 (m)</td>
<td>4.37 (m)</td>
</tr>
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<td>2.10 (m)</td>
<td>1.80 (m)</td>
<td>2.05 (m)</td>
<td>1.80 (m)</td>
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<td>4.30 (dd, 11.4, 4.1)</td>
<td>4.23 (dd, 11.1, 4.8)</td>
<td>4.30 (m)</td>
<td>4.20 (m)</td>
<td>4.13 (m)</td>
</tr>
<tr>
<td>C-7</td>
<td>6.63 (s)</td>
<td>6.53 (s)</td>
<td>6.57 (s)</td>
<td>6.54 (s)</td>
<td>6.41 (s)</td>
<td>6.34 (s)</td>
</tr>
<tr>
<td>C-8</td>
<td>6.13 (m)</td>
<td>6.07 (m)</td>
<td>6.09 (br t)</td>
<td>5.10 (m)</td>
<td>6.31 (br t, 10.8)</td>
<td>6.34 (m)</td>
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<tr>
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<td>3.22 (dd, 15.2, 5.7)</td>
<td>2.98 (dd, 15.3, 6.2)</td>
<td>1.05 (dd, 15.5, 5.5)</td>
<td>2.90 (dd, 15.2, 9.8)</td>
<td>2.90 (dd, 15.6, 9.5)</td>
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<tr>
<td>C-16-NH</td>
<td>1.11 (s)</td>
<td>1.11 (s)</td>
<td>1.10 (s)</td>
<td>1.16 (s)</td>
<td>1.21 (s)</td>
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<tr>
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<td>1.19 (s)</td>
<td>1.19 (s)</td>
<td>1.16 (s)</td>
<td>1.21 (s)</td>
<td>1.22 (s)</td>
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<tr>
<td>C-18</td>
<td>2.23 (s)</td>
<td>2.33 (s)</td>
<td>2.23 (s)</td>
<td>2.26 (s)</td>
<td>2.05 (s)</td>
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<tr>
<td>C-19</td>
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<td>1.25 (s)</td>
<td>1.31 (s)</td>
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<tr>
<td>C-20</td>
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<td>3.48 (br d, 11.0)</td>
<td>3.37 (d, 11.7)</td>
<td>4.30 (d, 12.0)</td>
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<tr>
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<td>4.48 (d, 1.3)</td>
<td>4.53 (d, 1.4)</td>
<td>4.54 (d, 1.4)</td>
<td>4.54 (d, 1.4)</td>
<td>4.54 (d, 1.4)</td>
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</tr>
<tr>
<td>C-22</td>
<td>5.23 (dd, 8.4, 1.3)</td>
<td>5.26 (dd, 9.0, 1.4)</td>
<td>5.21 (dd, 8.8, 1.7)</td>
<td>5.26 (br d, 9.0)</td>
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<td>5.75 (dd, 9.3, 2.7)</td>
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<td>C-23</td>
<td>7.18 (d, 8.8)</td>
<td>7.18 (d, 8.8)</td>
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<td>6.90 (d, 8.8)</td>
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<tr>
<td>C22 (ortho)</td>
<td>8.19 (dd, 8.4, 1.6)</td>
<td>8.10 (dd, 8.5, 3.5)</td>
<td>8.08 (dd, 8.3, 1.4)</td>
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<td>7.77 (dd, 8.5, 3.5)</td>
<td>7.62 (dd, 9.3, 1.0)</td>
<td>7.78 (dd, 9.3, 1.1)</td>
<td>7.75 (8.1, 1.5)</td>
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<tr>
<td>4-OH</td>
<td>4.15 (s)</td>
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<td>-</td>
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<tr>
<td>SO2CH3</td>
<td>3.58 (q, 7.5)</td>
<td>3.58 (q, 7.7)</td>
<td>3.54 (q, 7.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4-SO2CH3</td>
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<td>3.92 (t, 7.1)</td>
<td>3.91 (t, 7.6)</td>
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<td>SiMe3</td>
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<td>-</td>
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<td>1-Butyl</td>
<td>0.04 (s)</td>
<td>0.02 (s)</td>
<td>0.79 (s)</td>
<td>-0.36 (s)</td>
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<td>-</td>
</tr>
<tr>
<td>Other</td>
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<td>-0.12 (dd, 1.8)</td>
<td>5.06 (ps, 3.0)</td>
<td>2.13 (s)</td>
<td>5.02 (dd, 3.3, 2.0)</td>
<td>2.20 (s)</td>
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</table>

\(^a\) Measured in CDCl₃. Chemical shifts (d) are expressed in parts per million from Me₄Si and coupling constants (\(J\) in hertz). \(^b\) Multiplicity: s=singlet. d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. \(^c\) Hidden under aromatic protons. \(^d\) Values in the same column are interchangeable. \(^e\) Hidden under methyl signals.
III. CONCLUSIONS

5.1 Photoaffinity Labeling

Throughout the course of this project, it became clear that the diazirine labels were not suitable reagents for probes in the photoaffinity labeling experiment. These somewhat bulky groups must impede binding during the assembly process, which limit their utility as useful probes. The most likely candidate for further studies is 2-debenzoyl-(2-meta-azidobenzoyl) taxol, which retains tubulin activity relative to taxol. Similar meta-azidobenzoyl analogs should be prepared at C-7 and the C-13 side chain to assess their relative tubulin activity.

5.2 C-2 Modified Analogs

Structure activity relationship analysis at C-2 reveals that a benzoate-type functionality at C-2 is a necessary requirement for maximal biological activity. The ring-substitution is crucial, with preference favoring meta- over para-substitution. These studies have generated useful information directed towards synthesizing more potent taxol analogs.
5.3 C-4 Functionalization

The success of the Triton B reaction in selectively deacetylating C-4 in the presence of the C-13 side chain has implications in preparing C-4 modified analogs. It appears that C-4 re-acetylation is not possible with a benzoate at C-2. The 1,2-carbonate linkage could possibly be the best substrate for attempts at reacylating C-4.

5.4 Possible Methods to Circumvent Competing Reactions During Oxetane Ring-Opening

Although the oxetane to azetidine transformation was unsuccessful, the oxetane ring-opening reactions have led to the following conclusions: 1. C-4 acetate participation is unavoidable. 2. C-2 to C-20 benzoate transfer occurs under mild basic conditions. 3. AB ring contraction occurs under stronger lewis acid conditions.

In order to successfully synthesize oxetane analogs, one must begin with a modified taxol derivative that will prohibit the competing reactions from occurring. One such derivative is shown in Figure 19.
Figure 19. Substrate for future oxetane ring opening reactions.

The advantages of beginning with this specific substrate is two-fold. First, this compound can be readily synthesized via the Triton B reaction, in approximately 47% overall yield starting with taxol. Second, this substrate includes all of the necessary features to perform oxetane ring opening reactions while obviating the competing side reactions, as described below.

1. Beginning with a substrate devoid of an acetate at C-4 should allow for the "normal" oxetane ring cleavage reactions to take place, since acetate participation through acetoxonium ion formation will no longer be possible.
2. The facile AB ring contraction can be avoided by protection at C-1 with an acid stable protecting group. Since the C-1 hydroxyl group has only been functionalized with two groups to date, the 1,2 carbonate protection system is the most likely candidate. This system might also prevent the C-2 to C-20 benzoate transfer chemistry from occurring.
3. The TBDMS protecting group at C-2' is required to successfully deacetylate C-4 via the Triton B reaction.
4. C-7 protection is required to alleviate epimerization at this site under basic conditions. Both the C-7 and C-2'-silyl groups are removed by treatment with 5% HCl-MeOH.

This substrate would also serve its utility for functionalization at C-4.
IV. EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.- All non-aqueous reactions were performed in oven-dried glassware under a positive pressure of argon. THF and diethyl ether were distilled from Na/benzophenone ketyl. CH₂Cl₂, toluene, and CH₃CN were distilled from CaH₂. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ¹H nmr spectra were obtained on a Bruker WP-270 spectrometer operating at 270 MHz, and ¹³C nmr spectra were obtained on a Varian Unity 400 spectrometer operating at 100.57 MHz. All nmr spectra were recorded in CDCl₃ with TMS as an internal standard, unless otherwise noted. IR data were obtained on a Perkin-Elmer 283B infrared spectrophotometer using NaCl plates. Analytical TLC was performed on silica gel 60 F₂₅₄ plates (E. Merck), 0.2-mm layer. Preparative TLC was performed on silica gel GF plates (20 x 20 cm), 500 or 1000 mm thick (Analtech). The taxol derivatives were extracted from silica gel with EtOAc, and dissolved in CHCl₃ after removal of solvent. Hexane was then added to precipitate the compound. Taxol derivatives were visualized on TLC plates by spraying with a vanillin / H₂SO₄ solution and then heating.

Radioactivity measurements were performed by liquid-scintillation counting with a Beckman LS3800 liquid scintillation system. ³H₂O was purchased from ICN radiochemicals.

The phrase "worked up by standard methods" refers to diluting the reaction with an excess of organic solvent (i.e. CH₂Cl₂, EtOAc, ethyl ether), successive washing with H₂O and brine, drying over Na₂SO₄, and evaporating the solvent in vacuo unless otherwise noted.
1-BROMO-4-ETHOXYMETHOXYBENZENE [2.8]-15.0 g (86.7 mmol, 1 eq.) of p-bromophenol [2.7] in 40 ml of dimethylformamide (DMF) was added dropwise to a slurry of 2.29 g (95.3 mmol, 1.1 eq.) of dry sodium hydride in 100 ml of DMF at 15\(^{\circ}\). This was stirred for 2 hours at room temperature, and 9.0 g of chloromethylethylether (95.3 mmol, 1.1 eq) was added dropwise to the solution. After stirring for 12 hours the mixture was worked up by standard methods and the crude product was distilled to give 2.8 as a clear liquid (16.8 g, 84\%): b.p. 107\(^{\circ}\) / 3.5 mm Hg; ir (NaCl) 2950, 2860, 1580, 1475, 1220, 1145, 1090, 1070, 980, 820 cm\(^{-1}\); \(^{1}\)H nmr (270 MHz, CDCl3) 1.21 (3H, t, J=7.0, CH\(_3\)), 3.70 (2H, q, J=7.0, OCH\(_2\)CH\(_3\)), 5.18 (2H, s, OCH\(_2\)O), 6.92 (2H, d, J=9.0, ArH), 7.37 (2H, d, J=9.0, ArH); eims m/z (rel. int.) [M\(^+\)] 232, 230 (10), 174 (20), 59 (100); hreims m/z [M\(^+\)] 229.9938 (C\(_9\)H\(_{11}\)BrO\(_2\) requires 229.9942).

1-(4-ETHOXYMETHOXYPHENYL)-2,2,2-TRIFLUOROETHANONE [2.10]- A 250 ml round bottomed flask was charged with 150 ml of ether and 10.0 g of protected bromophenol [2.8] (43.3 mmol, 1.0 eq). The flask was cooled to -40\(^{\circ}\), and 19.0 ml of 2.5 M n-butyl lithium (47.6 mmol, 1.1 eq) was added dropwise. The mixture was stirred for 2 hours. A solution containing 8.62 g (47.6 mmol, 1.1 eq) of trifluoroacetyl piperidine [synthesized by reacting 1.2 equivalents of piperidine in ether with 1 equivalent of trifluoroacetic anhydride in the presence of 1 equivalent of triethylamine, washing with 0.1 N aqueous HCl and distillation in vacuo ; b.p. 63-64\(^{\circ}\)/ 4.5 mm Hg] in 40 ml of ether was added dropwise at -40\(^{\circ}\). After 4 hours at this temperature the cooling bath was removed, the mixture was hydrolyzed with 25 ml of saturated aqueous NH\(_4\)Cl, and worked up by standard methods. The crude product was carried through the next step without
purification or distilled to give 2.10 as a clear liquid (7.1 g, 66%): b.p. 116-117°/6.0 mm Hg; ir (NaCl) 2960, 2880, 1700, 1590, 1310, 1240, 1140, 970, 930, 840, 760, 730 cm⁻¹; ¹H nmr (270 MHz, CDCl₃) 1.22 (3H, t, J=7.0, CH₃), 3.73 (2H, q, J=7.0, OCH₂CH₃), 5.32 (2H, s, OCH₂O), 7.15 (2H, d, J=9.0, ArH), 8.05 (2H, d, J=9.0, ArH); ¹³C nmr 14.92 (CH₃), 64.90 (OCH₂CH₃), 92.92 (OCH₂O), 116.50 (Ar-C=H), 117.0 (CF₃, J_C-F=291.7 Hz), 123.64 (Ar-C=COF₃), 132.53 (Ar-C-H), 163.32 (Ar-C-OR), 179.25 (CO): eims m/z (rel. int.) [M]+ 248 (5), 121 (35), 59 (100); hreims m/z [M]+ 248.0668 (C₁₁H₁₁F₃O₃ requires 248.0660).

1-(4-ETHOXYMETHOXYPHENYL)-2,2,2-TRIFLUOROETHANONE OXIME [2.11]-Hydroxylamine hydrochloride (4.90 g, 70.6 mmol, 2.5 eq) was added to 7.0 g of ketone 2.10 (28.2 mmol, 1.0 eq) dissolved in 50 ml of pyridine and 25 ml of absolute EtOH. This was refluxed for 6 hours, followed by removal of the solvent in vacuo. The residue was dissolved in ether, and the solution was washed successively with 0.1 N HCl, water, and brine. The ether layer was dried over Na₂SO₄ and concentrated to yield a crude liquid which was purified by Si gel flash chromatography (5% EtOAc/95% CH₂Cl₂). The product was obtained as a thick pale yellow liquid (6.1 g, 82%): ir (NaCl) 3349, 3070, 2951, 2360, 1677, 1607, 1513, 1444, 1242, 1153, 1081, 1003, 923, 838 cm⁻¹; ¹H nmr (270 MHz, CDCl₃) 1.23 (3H, t, J=7.0, CH₃), 3.74 (2H, q, J=7.0, OCH₂CH₃), 5.26 (2H, s, OCH₂O), 7.13 (2H, d, J=9, ArH), 7.54 (2H, d, J=9, ArH), 9.87 and 10.00 (syn- and anti OH); eims m/z (rel. int.) [M]+ 263 (10), 233 (2), 218 (4), 59 (100); hreims m/z [M]+ 263.0772 (C₁₁H₁₂F₃NO₃ requires 263.0769).
O-(p-TOLUENESULFONYL)-1-(4-ETHOXYMETHOXYPHENYL)-2,2,2-TRIFLUOROETHANONE OXIME [2.12]- Compound 2.11 (6.1 g, 23.2 mmol) dissolved in 40 ml of pyridine was treated with 6.6 g of p-toluenesulfonyl chloride (34.8 mmol, 1.5 eq), and the mixture was refluxed for 3 hours. The pyridine was removed in vacuo, and the reaction worked up by standard methods. Si gel flash chromatography (30%CH2Cl2/ 70%hexanes) afforded 2.12 as a white solid (7.5 g, 77%): m.p. 39-41 °C; 1H nmr (270 MHz, CDCl3) 1.23 (3H, t, J = 7.2, CH2CH3), 2.47 (3H, s, ArCH3), 3.73 (2H, q, J=7.2, OCH2CH3), 5.26 (2H, s, OCH2O), 7.10 (2H, d, J=9.0, ArH), 7.38 (4H, m, ArH), 7.89 (2H, d, J=8.4, ArH); eims m/z (rel. int.) [M+] 417 (1), 248 (25), 155 (80), 91 (100), 65 (30), 59 (100). hreims m/z [M]+ 417.0858 (C18H18F3NO5S requires 417.0857).

3-(4-ETHOXYMETHOXYPHENYL)-3-(TRIFLUOROMETHYL)-DI AZIRIDINE [2.13]- A 500 ml three-necked round bottomed flask was equipped with a dry ice condenser and a gas inlet. Tosyl oxime 2.12 (6.1 g), dissolved in 100 ml of ether, was added to the flask and cooled to -78°. Approximately 75 ml of anhydrous NH3 was condensed into the flask, and the solution was stirred for 1 hour at -78°. The cooling bath was removed, the gas inlet was replaced with a drying tube to prevent pressure buildup, and the condenser maintained at -78°. The solution was stirred at ambient temperature while the NH3 refluxed for 2 hours. The condenser was removed and the NH3 was allowed to evaporate. The remaining residue was dissolved in ether and worked up by standard methods. Si gel flash chromatography (1% EtOAc/ 99% CHCl3) yielded the product as a clear oil (3.7 g, 96%): 1H nmr (270 MHz, CDCl3) 1.21 (3H, t, J = 7.1, CH3), 2.16 (1H, br d, NH), 2.74 (1H, br d, NH), 3.71 (2H, q, J=7.1, OCH2CH3), 5.23 (2H, s,
OCH$_2$O), 7.07 (2H, d, $J=8.4$, ArH), 7.53 (2H, d, $J=8.4$, ArH); eims m/z (rel. int.) [M$^+$] 262 (12), 261 (40), 217 (15), 183 (10), 59 (100); hreims m/z [M]$^+$ 262.0933 (C$_{11}$H$_{13}$F$_3$N$_2$O$_2$ requires 262.0929).

3-(4-ETHOXYMETHOXYPHENYL)-3-(TRIFLUOROMETHYL)-3H-DIAZIRINE [2.14] - Silver oxide (13.1 g, 56.5 mmol, 4.0 eq) was prepared by dropwise addition of 4.5 g of NaOH (112.9 mmol, 8.0 eq) in 50 ml of H$_2$O to a boiling solution of 19.2 g of silver nitrate (112.9 mmol, 8.0 eq) in 150 ml of H$_2$O. The precipitated Ag$_2$O was filtered, washed successively with H$_2$O, acetone and ether and used immediately. It was added in the dark to 3.7 g of diaziridine [2.13] (14.1 mmol, 1 eq) in 50 ml of ether. The mixture was stirred at room temperature for 1 hour, and filtered through Celite. The ether was removed by evaporation in vacuo and purification by flash chromatography (30% CH$_2$Cl$_2$ / 70% hexanes) afforded 2.14 as a yellow liquid (3.6 g, 98%); $^1$H nmr (270 MHz, CDCl$_3$) 1.20 (3H, t, $J=7.1$, -CH$_3$), 3.70 (2H, d, $J=7.1$, OCH$_2$CH$_3$), 5.22 (2H, s, OCH$_2$O), 7.05 (2H, d, $J=9.0$, ArH), 7.14 (2H, d, $J=9.0$, ArH).

3-(4-HYDROXY-3-NITROPHENYL)-3-(TRIFLUOROMETHYL)-3H-DIAZIRINE [2.15] - HNO$_3$ (2.78 g, 70% w/w, 30.8 mmol, 4.0 eq) was added to 15.7 g of acetic anhydride (154.0 mmol, 20 eq) at 150$^\circ$. After 15 minutes at 150$^\circ$ the temperature was reduced to -30$^\circ$, and 2.0 g of diazirine [2.14] (7.70 mmol, 1 eq) was added in the dark. No starting material was detected after 2.5 hours by tlc. The flask was warmed to 0$^\circ$, and 25 ml of H$_2$O was slowly added. Workup by standard methods followed by flash chromatography (40% CH$_2$Cl$_2$ / 60%hexanes) yielded nitrophenol [2.15] as a bright yellow oil, which solidified below 0$^\circ$ (800 mg, 42%):
ir (NaCl) 3220, 1610, 1530, 1480, 1410, 1310, 1150, 980, 820 cm⁻¹; **1H nmr** (270 MHz, CDCl₃) 7.23 (1H, d, J=8.9, ArH), 7.51 (1H, dd, J= 8.9, 2.2, ArH), 7.95 (1H, d, J=2.2, ArH), 10.50 (1H, s, OH); **cims m/z** (rel. int.) [MH]+ 248 (25), 232 (5), 218 (10), 190 (15), 85 (100), 81 (80).

**METHYL-2-NITRO-4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL] PHENOXYACETATE [2,16]**- To 500 mg of nitrophenol [2,15] (2.02 mmol, 1 eq) was added 2.48 g of methyl bromoacetate (16.2 mmol, 10 eq) and 2.5 ml of CH₃CN. Solid Cs₂CO₃ (2.63 g, 8.08 mmol, 4.0 eq) was added at room temperature in the dark. After 1 hour the mixture was diluted with ether. H₂O was added, and the mixture acidified with 2N aqueous HCl. Workup by standard methods followed by Si gel flash chromatography (hexanes:CHCl₃:EtOAc 11:4.5:1) yielded a pale yellow oil (200 mg, 40%): **1H nmr** (270 MHz, CDCl₃) 3.80 (3H, s, -CH₃), 4.85 (2H, s, OCH₂), 7.09 (1H, d, J=8.9, ArH), 7.41 (1H, dd, J=8.9, 2.4, ArH), 7.71 (1H, d, J=2.4, ArH).

**2-NITRO-4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXY ACETIC ACID [2,17]**- Ester 2.16 (200 mg) dissolved in 10 ml of THF was reacted with 1.2 ml of 2 N aqueous NaOH at room temperature in the dark for 1 hour. The solution was diluted with ethyl acetate and washed with water. The basic aqueous layer was acidified into fresh ethyl acetate with 1 N HCl to pH=2, and the aqueous layer was washed several times with ethyl acetate to extract the carboxylic acid. The organic layers were combined, washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield 2.17 as a pale yellow solid (180 mg,
94%): m.p. 128-130°; ^1H nmr (270 MHz, CDCl₃) 4.86 (2H, s, OCH₂O), 7.05 (1H, d, J=8.9, ArH), 7.44 (1H, dd, J=8.9, 2.4, ArH), 7.74 (1H, d, J=2.4, ArH); ^13C nmr 66.38 (CH₂), 117.16 (ArC-H), 120.79, 121.96, 124.66 (ArC-H), 124.81, 132.90 (ArC-H), 141.33, 153.01, 168.66; cims m/z (rel. int.) [MH]^+ 306 (100), 278 (40), 260 (60), 246 (50), 232 (65), 203 (70), 175 (50), 103 (40), 85 (50); hrcims m/z [MH]^+ 306.0338 (C₁₀H₇F₃N₃O₅ requires 306.0334).

SYNTHESIS OF 2.18 VIA COUPLING OF 2.17 WITH N-HYDROXY-SUCCINIMIDE-
To a solution of 50 mg (0.16 mmol) of carboxylic acid 2.17 in 1.0 ml of acetonitrile was added 36 mg (0.16 mmol) of dicyclohexylcarbodiimide followed by 15.7 mg (0.16 mmol) of N-hydroxy succinimide, and a catalytic amount of dimethylaminopyridine. This was stirred at room temperature for 12 hours, and then filtered over silica gel to remove the urea by-product. The silica gel was washed several times with EtOAc and evaporated to dryness. Purification by preparative tlc (20% EtOAc/80% hexanes) yielded 2.18, as a pale yellow solid m.p. 108-109° C (dec.); ^1H nmr (270 MHz, CDCl₃) 2.86 (s, CH₂), 5.13 (s, OCH₂), 7.14 (d, J = 9.1, ArH), 7.51 (dd, J = 3.2, 9.1, ArH), 7.71 (d, J = 3.2, ArH). The spectral data are in accord with the product previously described.

2'-TRIETHYLSILYL TAXOL [2.21]- To a solution of 50 mg of taxol (0.059 mmol, 1 eq) in 2.0 ml of dry CH₂Cl₂ was added 10 mg of imidazole (0.147 mmol, 2.5 eq) and 25 ml of triethylsilyl chloride at room temperature. Workup by standard methods followed by preparative tlc (40%EtOAc/ 60% hexanes) yielded 2'-triethylsilyltaxol (52 mg, 92%): m.p. 157-159° C; ^1H nmr (270 MHz, CDCl₃) 0.47 (6H, q, J = 8.0, SiCH₂CH₃), 0.82 (9H, t, J=8.0, SiCH₂CH₃), 1.13 (3H, s, CH₃), 1.25
(3H, s, CH₃), 1.68 (3H, s, CH₃), 1.90 (3H, s, CH₃), 2.20 (3H, s, C-10-OAc), 2.53 (3H, s, C-4-OAc), 3.82 (1H, d, J = 7.0, C-2), 4.21 and 4.32 (2H, ABq, J = 8.5, C-20), 4.32, (1H, m, C-7), 4.69 (1H, d, J=2.0, H-2'), 4.97 (1H, br. d, J = 7.8, C-5), 5.68 and 5.71 (2H, m, H-2 and H-3'), 6.27 (1H, m, C-13), 6.29 (1H, s, C-10), 7.12 (1H, d, J = 8.9, NH), 7.32-7.60 (11H, m, ArH), 7.73 (2H, d, J = 8.5, NHCOArH), 8.12 (2H, d, J = 8.5, C-2-OCOArH); fabms [MNa]+ 991 (3), 990 (5), 531 (10), 422 (100), 354 (40), 237 (50).

2'-TRIETHYLSILYL-7-[[2-NITRO-4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]]PHENOXY] ACETYL TAXOL [2.22]- 2'-Triethylsilyltaxol (2.21) (25.0 mg, 0.029 mmol, 1 eq) was dissolved in 1.0 ml of CH₂Cl₂. Acid 2.17 (26 mg, 0.088 mmol, 3 eq) was added at room temperature, followed by 18.0 mg of dicyclohexylcarbodiimide (0.088 mmol, 3 eq) and a catalytic amount of 4-pyrolidinopyridine. The mixture was stirred in the dark for 1 hour. The solvent was evaporated, and the crude residue was purified by preparative tlc (35% EtOAc/ 65% hexanes) to yield the coupled product (22 mg, 68%). ¹H nmr (270 MHz, CDCl₃) 0.48 (6H, q, J = 8.0, SiCH₂CH₃), 0.83 (9H, t, J=8.0, SiCH₂CH₃), 1.09 (3H, s, CH₃), 1.25 (3H, s, CH₃), 1.69 (3H, s, CH₃), 1.88 (3H, s, CH₃), 2.20 (3H, s, C-10-OAc), 2.53 (3H, s, C-4-OAc), 2.68 (1H, m, C-6), 3.93 (1H, d, J = 7.0, C-2), 4.20 and 4.34 (2H, ABq, J = 8.5, C-20), 4.68 (1H, d, J=1.8, H-2'), 4.75 and 5.00 (2H, ABq, J = 16.3, CH₂), 4.95 (1H, br. d, C-5), 5.65 (1H, m, C-7), 5.68 and 5.70 (2H, m, H-2 and H-3'), 6.20 (1H, s, C-10), 6.27 (1H, m, C-13), 7.12 (1H, d, J = 8.9, NH), 7.30-7.50 (11H, m, ArH), 7.73 (2H, d, J = 8.5, NHCOArH), 8.12 (2H, d, J = 8.5, C-2-OCOArH).
7-[2-NITRO-4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXY-ACETYL]
TAXOL [2.23] - Compound 2.22 (22 mg) was added to 1.0 ml of a 5% HCl: MeOH solution at room temperature and stirred for 10 minutes. Workup by standard methods followed by preparative tlc (45% EtOAc/ 35% hexanes) yielded 2.23 (15 mg, 75%): m.p. 212-218\(^{\circ}\) C;\(^{1}\)H nmr (270 MHz, CDCl\(_3\)) (see Table 8); fabms m/z (rel. int.) [M]\(^{+}\) 1141 (1), 240 (10), 105 (100); hrfabms m/z [MH\(^{+}\)] 1141.3490 (C\(_{57}\)H\(_{56}\)F\(_{3}\)N\(_{4}\)O\(_{18}\) requires 1141.3542).

O-(P-TOLUENESULFONYL)-1-(4-HYDROXYPHENYL)-2,2,2-TRIFLUORO-
ETHANONE OXIME [2.26]- Protected tosyl oxime 2.12 (3.2 g) was added to a mixture of 10 ml of THF, 4.0 ml of isopropanol, and 5.0 ml of 8 N aqueous HCl. After stirring at room temperature for 22 hours, the solution was worked up by standard methods. Si gel flash chromatography (20% EtOAc/ 80% hexanes) afforded 2.26 as a white solid (2.31 g, 84%): m.p. 104-106\(^{\circ}\) C;\(^{1}\)H nmr (270 MHz, CDCl\(_3\)) 2.47 (3H, s, ArCH\(_3\)), 5.9 (1H, s, OH), 6.90 (2H, d, j=9.0, ArH), 7.39 (4H, n, ArH), 7.89 (2H, d, j=9.0, ArH); cims m/z (rel. int.) [MH\(^{+}\)] 360 (65). 188 (100). 155 (80), 91 (30); hrcims m/z [MH\(^{+}\)] 360.0530 (C\(_{15}\)H\(_{13}\)F\(_{3}\)NO\(_{4}\)S requires 360.0517).

O-(P-TOLUENESULFONYL)-1-(4-T-BUTYLDIPHENYLSILOXY-PHENYL)-2,2,2-TRI-
FLUOROETHANONE OXIME [2.27]- Phenol 2.26 (2.3 g, 6.4 mmol, 1 eq) was dissolved in 15 ml of anhydrous DMF. Imidazole (653 mg, 9.6 mmol, 1.5 eq) was added at room temperature, followed by addition of 2.64 g of t-butyldiphenylsilylchloride (9.6 mmol, 1.5 eq). The solution was stirred for 5 hours, and worked up by standard methods. Flash chromatography (30% CH\(_{2}\)Cl\(_{2}\)/ 70% hexanes) yielded the silyl-protected tosyl oxime as a clear oil (3.44
g, 90%): $^1$H nmr (270 MHz, CDCl$_3$)  1.10 (9H, s, t-bu), 2.45 (3H, s, CH$_3$), 6.80 (2H, d, $J$=9.0, ArH), 7.23-7.87 (16H, m, ArH); eims m/z (rel. int.) [M]$^+$ 597 (2), 540 (100), 370 (70), 353 (40), 300 (20), 91 (20).

3-(4-t-BUTYLDIPHENYLSILOXYPHENYL)-3-(TRIFLUOROMETHYL)-DIAZIRIDINE [2.28]- The protected tosyl oxime 2.27 (3.4 g) dissolved in 50 ml of ether was added to a 250 ml round-bottomed flask equipped with a dry ice condenser and gas inlet. The flask and condenser apparatus was cooled to -78°C, and 30 ml of anhydrous NH$_3$ was admitted through the condenser. This was stirred for 1 hour at -78°C, and the cooling bath was removed. A drying tube was connected to the inlet, and the NH$_3$ refluxed at room temperature for 2 hours. The condenser was removed, and the NH$_3$ was evaporated. Workup by standard methods followed by flash chromatography (1% EtOAc/ 99% CH$_2$Cl$_2$) afforded the diaziridine (2.28) as a colorless oil (712 mg, 28%) along with 1.5 g of O-desilylated product: $^1$H nmr (270 MHz, CDCl$_3$)  1.10 (9H, s, t-Bu), 2.05 (1H, br d, NH), 2.62 (1H, br d, NH), 6.75 (2H, d, $J$= 8.7, ArH), 7.32-7.72 (12H, m, ArH); eims m/z (rel. int.) [M]$^+$ 442 (10), 385 (100), 300 (15), 222 (10), 57 (10).

3-(4-t-BUTYLDIPHENYLSILOXYPHENYL)-3-(TRIFLUOROMETHYL)-3H-DIAZIRINE [2.29]- Freshly prepared Ag$_2$O (1.50 g, 6.45 mmol, 4.0 eq) was added in the dark to 710 mg of diaziridine 2.28 (1.61 mmol, 1 eq) in 10 ml of ether. The mixture was stirred at room temperature for 1 hour and filtered through Celite. Evaporation of ether yielded the product as a pale yellow liquid (680 mg, 96%): $^1$H nmr (270 MHz, CDCl$_3$)  1.08 (9H, s, t-Bu), 6.74 (2H, d, $J$=9.0, ArH), 6.92 (2H, d, $J$=9.0, ArH), 7.33-7.43 (6H, m, ArH), 7.66-7.70 (4H, m, ArH); fabms m/z (rel. int.)
[M+] 440 (6), 412 (65), 383 (10), 307 (11), 197 (44), 135 (100); hrfabms [M]+ 440.1513 (C24H23F3N2OSi requires 440.1531).

4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXYACETATE [2.30]- To 700 mg of diazirine 2.29 (1.59 mmol, 1 eq) and 25 ml of dry CH3CN was added 1.46 g of methyl bromoacetate (9.55 mmol, 6 eq), followed by addition of 1.4 g of CsF (9.55 mmol, 6 eq). The mixture was stirred in the dark at room temperature for 4 hours. Workup by standard methods followed by preparative tlc (hexanes/CHCl3/EtOAc 11:4.5:1.0) yielded 2.30 as a clear liquid (196 mg, 46%): 1H nmr (270 MHz, CDCl3) 3.80 (3H, s, CH3), 4.64 (2H, s, OCH2), 6.90 (2H, d, J=9.0, ArH), 7.17 (2H, d, J=9.0,ArH). Mass spectral analysis did not yield an identifiable molecular ion.

4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXYACETIC ACID [2.24]- Aqueous 2N NaOH (0.4 ml) was added to a solution of 2.30 (190 mg) in 10 ml of THF. After stirring in the dark for 1 hour at room temperature, the solution was diluted with ethyl acetate and water. The basic aqueous layer was separated, acidified into fresh ethyl acetate with 1 N HCl, and washed several times with EtOAc. The organic layers containing the carboxylic acid were combined, dried over Na2SO4, and concentrated to yield 2.24 as a white solid (170 mg, 94%): m.p. 92-940 C; 1H nmr (270 MHz, CDCl3) 4.68 (2H, s, OCH2), 6.90 (2H, d, J=9.0, ArH), 7.17 (2H, d, J=9.0,ArH). cims m/z (rel. int.) [MH]+ 261 (50), 233 (90), 232 (100), 215 (30), 175 (40), 145 (40); hrcims [M-28]+ 232.0338 (C10H7F3O3 requires 232.0347)
2'-Triethylsilyltaxol (2.21) (25.0 mg, 0.029 mmol, 1 eq) was dissolved in 1.0 ml of CH₂Cl₂. Acid 2.24 (20 mg, 0.088 mmol, 3 eq) was added at room temperature, followed by 18.0 mg of dicyclohexylcarbodiimide (0.088 mmol, 3 eq) and a catalytic amount of 4-pyrrolidinopyridine. The mixture was stirred in the dark for 1 hour. The solvent was evaporated, and the crude residue was passed through a short column of silica gel (35% EtOAc/65% hexanes) and evaporated to dryness. Treatment of the partially purified product with 1.0 ml of 5%HCl/MeOH followed by workup in the usual manner and preparative tlc (45% EtOAc/hexanes) yielded 2.33 as a pale yellow amorphous solid (73%): ¹H nmr (270 MHz, CDCl₃) (see Table 8); fabms m/z (rel. int.) [M⁺] 1096 (40), 1069 (15), 1018 (15), 812 (45), 753 (100).

3-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOL [2.37]- To a solution of 1.18 g of isopropyl diazirine 2.36 dissolved in 25.0 ml of anhydrous CH₂Cl₂ at 0 °C was added 7.2 ml of boron trichloride (1.0 M solution in CH₂Cl₂). After stirring at this temperature for 1 hour, 25 ml of water was carefully added dropwise, and the resulting mixture was extracted several times with CH₂Cl₂, dried over Na₂SO₄, and concentrated to yield 900 mg (90%) of 2.37 as a pale yellow oil. This material decomposed rapidly when exposed to air or light for extended periods of time. ¹H nmr(270 MHz, CDCl₃) 5.34 (br. s, OH), 6.67 (bs, ArH), 6.72 (d, j = 8.0, ArH), 6.88 (dd, j = 8.0, 2.5, ArH), 7.25 (dd, j = 8.0, 8.1, ArH).

4-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXYACETATE [2.38]- To 200 mg (1.0 mmol) of phenol 2.37 dissolved in 2.0 ml of dry CH₃CN was added
166 mg (1.1 mmol) of methylbromoacetate and 357 mg (1.1 mmol) of Cs₂CO₃.
After stirring at room temperature for 2 hours, the reaction mixture was diluted
with EtOAc, and filtered over Celite. Evaporation in vacuo followed by
preparative tlc (20% EtOAc/80% hexanes) yielded 240 mg (90%) of 2.38 as a pale
yellow oil. ¹H nmr(270 MHz, CDCl₃) 3.71 (s, OCH₃), 4.7 (s, CH₂), 6.71 (br s.
ArH), 6.74 (d, J = 8.0, ArH), 6.90 (dd, J = 8.0, 2.1, ArH), 7.29 (dd, J = 8.0, 8.2, ArH).
eims m/z [M-28]+ 246 (50), 157 (100), 57 (40).

3-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXYACETIC ACID [2.34]-
Aqueous 2N NaOH (0.4 ml) was added to a solution of 2.30 (240 mg) in 10 ml of
THF. After stirring in the dark for 1 hour at room temperature, the solution was
diluted with ethyl acetate and water. The basic aqueous layer was separated,
acidified into fresh ethyl acetate with 1 N HCl, and washed several times with
EtOAc. The organic layers containing the carboxylic acid were combined, dried
over Na₂SO₄, and concentrated to yield 2.34 as a white solid (214 mg, 94%): m.p.
75-77⁰ C; ¹H nmr (270 MHz, CDCl₃) 4.70 (2H, s, OCH₂), 6.75 (1H, s, ArH), 6.85
(1H, d, J = 8.0, ArH), 6.95 (1H, d, J=8.0, ArH), 7.34 (1H, dd, J = 8.0, 8.0, ArH).
fabms m/z (rel. int.) [M]+ 260 (5), 232 (45), 215 (30), 175 (40), 157 (100), 145 (60), 63
(25), 57 (30).

7-[3-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]PHENOXYACETYL] TAXOL
[2.39]- To 20 mg of carboxylic acid 2.34 and 25 mg of 2'-triethylsilyltaxol (2.21) in
1.0 ml of CH₂Cl₂ was added 16.0 mg of dicyclohexylcarbodiimide and a catalytic
amount of pyrollolidinopyridine. This was stirred at room temperature for 30
minutes. The mixture was then filtered over a cotton plug to remove the urea by-
product, followed by preparative tlc (30% EtOAc/70% hexanes) to yield the coupled product (27 mg, 86%): m.p. 118-123°C; $^{1}$H nmr (270 MHz, CDCl$_3$)  0.48 (6H, m, SiCH$_2$CH$_3$), 0.83 (9H, t, J=8.0, SiCH$_2$CH$_3$), 4.51 and 4.86 (ABq, J = 16.20, PhOCH$_2$CO$_2$R), 5.62 (1H, m, H-7), 6.74 (1H, br s., ArH), 6.85 (1H, br dd, ArH), 6.95 (1H, dd, J = 2.5, 8.4); fabms m/z (rel. int.) [MH]$^+$ 1211 (1), 752 (21), 400 (30), 355 (50), 215 (100).

The coupled product was dissolved in 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 15 minutes. The solution was diluted with CH$_2$Cl$_2$, washed with water, brine, dried over Na$_2$SO$_4$, and concentrated to yield the crude product. Purification by column chromatography on silica gel (40% EtOAc/60% hexanes) yielded 2.39 as a white solid (19 mg, 78%): m.p. 190-192°C; $^{1}$H nmr (270 MHz, CDCl$_3$) (See table 7); fabms m/z (rel. int.) [M]$^+$ 1096 (40), 812 (45), 753 (100).

SYNTHESIS OF THE DEUTERATED ANALOG OF 3-[3-(TRIFLUORO-METHYL)-3H-DIAZIRIN-3-YL] PHENOXYACETIC ACID [2.40]- A 50 ml flame dried round-bottomed flask under argon was charged with 5 ml of THF and 250 mg (0.9125 mmol, 1.0 eq) of ester 2.38. This was cooled to -78°C, and 2.03 ml (1.00 mmol, 1.1 eq) of freshly prepared 0.5 M lithium diisopropylamine was added dropwise to the ester over a period of 2 minutes. After stirring for 15 minutes at -78°C, a solution of $^2$H$_2$O (0.05 ml) in 0.0625 ml of hexamethylphosphoramide (HMPA) was added. The cooling bath was removed and the reaction stirred at ambient temperature for 1 hour. The solution was then diluted with EtOAc, followed by H$_2$O. The solution was transferred to a separatory funnel, and the ethyl acetate layer was removed. The aqueous layer was washed with ethyl acetate twice, and
then acidified into fresh ethyl acetate with 1.0 N HCl. The organic layer was washed successively with H₂O and brine, and dried over Na₂SO₄. Concentration in vacuo yielded the deuterated carboxylic acid 2.40 (118 mg, 50%): m.p. 75-78°C; ¹H nmr analysis confirmed H-D exchange (~45%) by integration at 4.65 ppm with respect to aromatic protons.

7-[[α-²H]-[3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL]-PHENOXYACETYL]

TAXOL (2.41)- To 20 mg (0.077 mmol) of carboxylic acid 2.40 and 24.7 mg (0.026 mmol) of 2'-triethylsilyltaxol (2.21) in 1.0 ml of dry CH₂Cl₂ was added 16.0 mg of dicyclohexylcarbodiimide and a catalytic amount of DMAP. This was stirred at room temperature for 30 minutes. The CH₂Cl₂ was evaporated, and the residue partially purified by dissolving the residue in EtOAc, and filtering over silica gel (30% EtOAc/70% hexanes), which was dissolved in 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 15 minutes. The solution was diluted with CH₂Cl₂, washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield the crude product. Purification by silica gel preparative tlc yielded the deuterated product 2.41 as a white solid (17 mg, 66%). ¹H nmr analysis revealed approximately 45% deuterium incorporation by integration under the methylene proton peaks. ¹H nmr (270 MHz, CDCl₃) 4.51 and 4.86 (ABq, J = 16.20, PhOCH₂CO₂R), 4.50 and 4.85 singlets, 5.62 (1H, m, H-7), 6.74 (1H, br s., ArH), 6.85 (1H, br dd, ArH), 6.95 (1H, dd, J = 2.5, 8.4).
GENERAL METHOD FOR TRITIUM EXCHANGE REACTIONS. PREPARATION OF \([\alpha^3H]–[\text{3-TRIFLUOROMETHYL-3H-DIAZIRIN-3-YL] PHENOXY ACETIC ACID [2.42]}\)- A 100 ml flame dried round-bottomed flask under argon was charged with 20 ml of THF and 1.0 g (3.65 mmol, 1.0 eq) of ester 2.38. This was cooled to \(-78^\circ\) C, and 8.15 ml (4.00 mmol, 1.1 eq) of freshly prepared 0.5 M lithium diisopropylamine was added dropwise to the ester over a period of 2 minutes. After stirring for 15 minutes at \(-78^\circ\) C, a solution of \(^3\text{H}_2\text{O} \) (0.20 ml, 1.0 Ci, 90 mCi/mmol) in 0.25 ml of hexamethylphosphoramide (HMPA) was added. The cooling bath was removed and the reaction stirred at ambient temperature for 1 hour. The solution was then diluted with ethyl acetate, followed by water. The solution was transferred to a separatory funnel, and the ethyl acetate layer was removed. The aqueous layer was washed with ethyl acetate twice, and then acidified into fresh ethyl acetate with 1.0 N HCl. The organic layer was washed successively with water and brine, and dried over Na\(_2\)SO\(_4\). Concentration \textit{in vacuo} yielded the tritiated carboxylic acid 2.42 (500 mg, 53%): specific activity = 5.4 mCi/mmol.

7-[(\[\alpha^3H]-[\text{3-TRIFLUOROMETHYL-3H-DIAZIRIN-3-YL]-PHENOXY-ACETYL] TAXOL [2.43]})- To 20 mg of \(^3\text{H}\)-carboxylic acid 2.42 and 24.7 mg of 2’-triethylsilyltaxol in 1.0 ml of dry \(\text{CH}_2\text{Cl}_2\) was added 16.0 mg of dicyclohexylcarbodiimide and a catalytic amount of pyrrolidinopyridine. This was stirred at room temperature for 30 minutes. The mixture was filtered over silica gel, washed with EtOAc, and evaporated (specific activity = 3.8 mCi/mmol). The crude residue was dissolved in 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 15 minutes. The solution was diluted with \(\text{CH}_2\text{Cl}_2\), washed successively with H\(_2\)O and brine, dried over Na\(_2\)SO\(_4\), and

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concentrated to yield the crude tritiated product. Purification by preparative tlc (30% EtOAc/70% hexanes) yielded 2.43 as a white solid (18 mg, 78%). Specific activity= 2.55 mCi/mmols. The chromatographic profile matched exactly with that of non-tritiated compound 2.39.

SYNTHESIS OF (4S,5R)-N-BOC-2,2-DIMETHYL-4-PHENYL-5-OXAZOLIDINE CARBOXYLIC ACID [2.48]- The side chain intermediate (+)-1,1-dimethylethyl (N-((1S,2S)-2-hydroxy-1-phenyl-3-butenyl)amino)methanoate (2.46) was prepared according to the methodology of Greene, et. al. (Denis, J.-N., Correa, A., and Greene, A.E., J. Org. Chem., 56, 6939-6942 (1991)). Compound 2.46 (545 mg, 2.039 mmol) was dissolved in 20 ml anhydrous toluene, and to the stirring solution was added 2-methoxypropene (1.18 g, 8.0 equivalents), followed by pyridinium paratoluenesulfonate (20 mg, 0.04 equivalents). The reaction was allowed to proceed at 62 °C under argon for 4 hr. After cooling to room temperature and adding three drops of pyridine, the solvent was evaporated, and the crude product was purified by chromatography on silica gel (4% EtOAc/96% CH₂Cl₂), affording 468 mg (75%) of compound 2.47 as a thick oil. Eims or cims did not yield an identifiable molecular ion. \(^{1}H\) nmr(270 MHz, CDCl₃) 7.20 - 7.34 (m, 5H), 5.87 (ddd, \(J = 6.7, 10.4, 17.1\) Hz, 1H), 5.28 (m, 1H), 5.11 (m, 1H), 4.36 (br m, 1H), 4.25 (t, 1H), 1.74 and 1.73 (2s, 6H), 1.04 (br s, 9H).

The above cyclic acetal 2.47 (468.7 mg, 1.525 mmol) was dissolved in 6.0 ml 1:1 CH₃CN/CCl₄, and to the stirred solution was added 4.6 ml distilled water. To the biphasic mixture was added sodium bicarbonate (832.7 mg, 6.5 equivalents) followed by sodium periodate (1.794 g, 5.5 equivalents) added in small portions. After stirring the mixture for 10 minutes, RuCl₃ (50.6 mg, 0.16
equivalents) was added, and the reaction was allowed to proceed for 28 hr. At this time the reaction mixture was diluted with distilled water, followed by diethyl ether. The ether layer was separated and the aqueous layer was extracted twice with ether (20 ml). The aqueous layer was then treated with 1N HCl to pH 3, followed by extraction with CH₂Cl₂ (3 x 50 ml). The combined CH₂Cl₂ layers were washed with brine (2 x 50 ml), dried with Na₂SO₄, filtered, and the solvent evaporated, yielding 387.8 mg (78%) of the colorless, amorphous solid.

2.48. m.p. 135-138⁰C (lit. 137⁰C), [α]D +5.2 (c=1.1, CHCl₃). ¹H nmr (270 MHz, CDCl₃) 7.34 (m, 5H), 5.11 (1H, br d, C-3'), 4.52 (1H, d, J = 5.7, C-2'), 1.79 (s, 3H), 1.74 (s, 3H), 1.19 (9H, br s, C(CH₃)₃). cims m/z [MH]+ 322 (4), 306 (9), 278 (7), 266 (29), 250 (17), 222 (100), 206 (16), 146 (9), 121 (8), 91 (12).

7-TRIETHYLSILYLBACCATIN III [2.49]. Baccatin III (50 mg, 0.085 mmol) was dissolved in 5 ml of CH₂Cl₂ with stirring. Imidazole (58 mg, 0.85 mmol, 10 eq.) and triethylsilylchloride (135 µl, 0.854 mmol, 10 equivalents) was added and the reaction was allowed to proceed at room temperature for 4 hr. At this time the reaction was stopped by the addition of 40 ml distilled water, followed by 30 ml EtOAc. The aqueous layer was washed with additional EtOAc (2 x 10 ml). The combined organic layers were then washed with brine (3 x 10 ml), and after drying over Na₂SO₄, the solution was filtered and evaporated to yield the crude product which was subjected to column chromatography on silica gel (50% EtOAc /50% hexanes), affording 50 mg (83%) of pure 7-triethylsilylbaccatin III (2.49). ¹H nmr (270 MHz, CDCl₃) 0.58 (6H, q, J = 8.0, SiCH₂CH₃), 0.93 (9H, t. J=8.0, SiCH₂CH₃), 1.04 (3H, s, CH₃), 1.20 (3H, s, CH₃), 1.65 (3H, s, CH₃), 1.80 (3H, s, CH₃), 2.18 (3H, s, C-10-OAc), 2.25 (3H, s, C-4-OAc), 3.88 (1H, d, J = 7.0, C-2),

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4.10 and 4.30 (2H, ABq, J = 8.0, C-20), 4.49, (1H, dd, J = 7.0, 10.0, C-7), 4.83 (1H, m, C-13), 4.96 (1H, br. d, C-5), 5.63 (1H, d, J = 7.0, H-2), 6.46 (1H, s, C-10), 7.44-7.64 (3H, m, ArH), 8.11 (2H, dd, J = 8.5, 1.3, C-2-OCOArH); *fabms m/z* [MH]+ 701 (100), 679 (16), 669 (11), 661 (9), 655 (15), 641 (63), 623 (29), 611 (21), 593 (32), 587 (87), 581 (52), 563 (34).

COUPLING OF SYNTHETIC SIDE CHAIN WITH 7-TRIETHYLSILYL-BACCATIN III-
SYNTHESIS OF [2.50] - 7-triethylsilyl-baccatin III (2.49) (42mg, 0.06 mmol), carboxylic acid 2.48 (29 mg, 0.09 mmol), dicyclohexylcarbodiimide (20 mg, 0.09 mmol), and a catalytic amount of DMAP were added to 1.0 ml of anhydrous toluene. This was stirred at 60°C for 30 minutes, filtered over a plug of silica gel, washed with EtOAc, and concentrated to afford the crude residue. Purification on preparative tlc (35%, EtOAc/65% hexanes) yielded 57 mg (95%) of 2.50 as a glassy solid. *H nmr*(270 MHz, CDCl3) (see Table 8); *fabms m/z* (rel. int.) [MH]+ 1005 (100), 947 (30), 905 (52), 889 (35), 871 (20), 854 (65).

FORMIC ACID DEPROTECTION OF 2.50: SYNTHESIS OF N-DEBENZOYLTXAOL [2.51]- Formic acid (1.0 ml, 99%) was added to 30 mg of 2.50 and this stirred at room temperature for 2.5 hours. The formic acid was removed by evaporation in vacuo, and the resulting residue dissolved in EtOAc and washed several times with dilute NaHCO₃, water, and brine respectively. Drying over Na₂SO₄ and evaporation of the solvent afforded 2.51 (22 mg) which was directly used without purification. *H nmr*(270 MHz, CDCl3) 1.12 (3H, s, CH₃), 1.23 (3H, s, CH₃), 1.65 (3H, s, CH₃), 1.81 (3H, s, CH₃), 2.23 (6H, s, C-10-OAc, C-4-OAc), 3.74 (1H, d, J = 7.0, C-2), 4.13 and 4.26 (2H, ABq, J = 8.3, C-20), 4.30 (2H, m, C-2', C-3'), 4.40, (1H,
m, C-7), 4.92 (1H, br. d, C-5), 5.62 (1H, d, J = 7.0, H-2), 6.13 (1H, m, C-13), 6.27 (1H, s, C-10), 7.37-7.67 (8H, m, ArH), 8.05 (2H, dd, J = 8.5, 1.4, C-2-OCOArH).

REACTION OF AMINO ALCOHOL 2.51 WITH DIAZIRINE-SYNTHESIS OF N-DIAZIRINYL TAXOL [2.52]- To a solution containing 23 mg (0.030 mmol) of 2.51 in 1.0 ml of CH₂Cl₂ was added 7.4 mg (0.034 mmol) of dicyclohexyl-carbodiimide and 8.7 mg (0.034 mmol) of carboxylic acid 2.34. A crystal of pyrrolidinopyridine was added, and this stirred at room temperature for 75 minutes. The mixture was filtered over cotton, and applied directly to a preparative tlc plate and eluted (60% EtOAc/40% hexanes). This yielded 7.5 mg (25%) of 2.52 as a white solid.

2',7-BIS(TRIETHYLSILYL) TAXOL [1.105]- To 140 mg of taxol (0.164 mmol) in 5.0 ml of dry CH₂Cl₂ was added 115 mg of solid imidazole (1.64 mmol) and 250 µl of triethylsilylechloride (1.64 mmol). After stirring at room temperature for 1 hour, and the reaction was diluted with dichloromethane, and washed several times with water and brine respectively. The CH₂Cl₂ layer was dried over Na₂SO₄, evaporated, and subjected to preparative thin layer chromatography (SiO₂, 1000 µm, 30% EtOAc/70% hexanes) to yield 162 mg (92%) of 1.105 as a white powder. m.p. 122-124° C; ¹H nmr (270 MHz, CDCl₃) (see Table 12); fabms m/z [MNa-H]⁺ 1104 (100), 982 (10).

2-DEBENZOYL-2',7-BIS(TRIETHYLSILYL) TAXOL [1.106]- To a stirred solution of 1.105 (65 mg, 0.060 mmol) in benzene: CH₂Cl₂ (8:1.2 ml) and tetrabutylammoniumhydrogen sulfate (500 mg) was added 8.0 ml of aqueous 2N
NaOH at room temperature. The reaction mixture was stirred for 2 hours and then diluted with benzene. The organic layer was separated, washed with water and brine, and dried over Na₂SO₄. The crude product was purified by preparative tlc (50% EtOAc/50% hexanes) to yield 25 mg (45%) of 1.106 as a glassy solid, 25 mg of recovered 1.105, and 5.0 mg (13%) of 7-triethylsilylbaccatin III 2.49. ¹H nmr(270 MHz, CDCl₃) (see Table 12); fabms m/z [MNa]+ 1001 (10), 978 (100).

REACTION OF 2-DEBENZOYL-2'-7-BIS(TRIETHYLSILYL)TAXOLOL WITH DI-TERT-BUTYLDICARBONATE. - To 12.0 mg (0.012 mmol) of 1.106 dissolved in 150 µl of anhydrous toluene was added 54 mg (0.24 mmol) of tert-butoxycarbonylanhydride and 5 mg of pyrrolidinopyridine. The mixture was stirred at room temperature for 20 hours. The solution was applied directly to a preparative tlc plate (25% EtOAc/75% hexanes) to yield a mixture of two products. This mixture was dissolved in 1.0 ml of a 5% HCl/MeOH solution, and stirred at room temperature for 20 minutes. The solution was diluted with ethyl acetate, washed several times with water and brine respectively, dried over Na₂SO₄, and concentrated to yield the crude product which was purified by preparative tlc (45%, EtOAc/55% hexanes) to yield 1.58 mg of 2-debenzoyl-2-t-butoxycarbonyltaxol (3.1) and 3.8 mg of 2-debenzoyl-1,2-carbonyltaxol (3.2).

3.1: ¹H nmr(270 MHz, CDCl₃) (see Table 12); fabms m/z (rel. int.) [MNa]+ 873 (100), 850 (3).

3.2: ¹H nmr(270 MHz, CDCl₃) (see Table 12); fabms m/z (rel. int.) [M]+ 776 (20), 621 (3), 307 (15), 154 (100). hrfabms [M⁺] 775.2887 (C₄₁H₄₅N₁O₁₄ requires 775.2840).
REACTION OF 1.106 WITH TRIPHOSGENE/PyRIDINE-SYNTHESIS OF 2-DEBENZOYL-1,2-CARBONYLTAXOL [3.2]- To 10.0 mg (0.012 mmol) of 1.106 dissolved in 1.0 ml of CH₂Cl₂ was added 5 ml of pyridine and 3 mg of triphosgene. This stirred at room temperature for 8 hours, followed by dilution with dichloromethane, and successive washes with water and brine respectively. After drying over Na₂SO₄, and concentration, this was treated with 1.0 ml of 5% HCl in MeOH for 20 minutes at room temperature. The solution was diluted with ethyl acetate, washed several times with water and brine respectively, dried over Na₂SO₄, and concentrated to yield the crude product which was purified by preparative tlc (45% EtOAc/ 55% hexanes) to yield 4.5 mg of 3.2.

A GENERAL METHOD FOR THE PREPARATION OF 2-ACYL TAXOL ANALOGS- To a preformed mixture of dicyclohexylcarbodiimide (42 mg, 0.205 mmol, 20 eq.) and 20 equivalents of the corresponding carboxylic acid in 150 µl of anhydrous toluene is added a solution of 10 mg (0.010 mmol) of 2-debenzoyl-2'-7-bis(triethylsilyl)taxol (1.106) in 50 µl of anhydrous toluene. 5 mg of pyrrolidinopyridine is then added, and the slurry is stirred at 60° C for 20 hours. The mixture is then diluted with 1.0 ml of EtOAc, filtered through a pipette containing silica gel, and washed thoroughly with EtOAc. The EtOAc is evaporated, and the crude residue is treated with 1.0 ml of 5% HCl in MeOH for 20 minutes. The solution is then diluted with EtOAc, washed several times with water and brine respectively, and dried over Na₂SO₄. Concentration followed by preparative tlc purification on silica gel (EtOAc:hexanes) and extraction of the
products from silica gel with EtOAc yielded the corresponding 2-debenzoyl-2-acyl analogs. See Tables 12-14 for the \(^1\)H nmr assignments (270 MHz, CDCl\(_3\)).

2-debenzoyl-2-valenerytaxol (3.4): 5.0 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 834 (1), 621 (5), 307 (10), 154 (100). \textit{hrfabms [MH\(^+\)]} 834.3677 (C\(_{45}H_{56}NO_{14}\) requires 834.3700)

2-debenzoyl-2-(3-thiophenyl)taxol (3.5): 5.2 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 860 (1), 515 (4), 416 (5), 225 (10). \textit{hrfabms [MH\(^+\)]} 860.2952 (C\(_{45}H_{50}NO_{14}S\) requires 860.2952).

2-debenzoyl-2-(2-thiophenyl)taxol (3.6): 5.1 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 860 (1), 575 (0.5), 515 (5), 154 (100). \textit{hrfabms [MH\(^+\)]} 860.2950 (C\(_{45}H_{50}NO_{14}S\) requires 860.2952).

2-debenzoyl-2-(4-fluorobenzoyl)taxol (3.8): 4.0 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 872 (1), 621 (1), 460 (3), 307 (20), 154 (100). \textit{hrfabms [MH\(^+\)]} 872.3277 (C\(_{47}H_{51}NO_{14}F\) requires 872.3294).

2-debenzoyl-2-(4-chlorobenzoyl)taxol (3.9): 5.5 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 888 (66), 603 (30), 543 (100), 225 (30). \textit{hrfabms [MH\(^+\)]} 888.2982 (C\(_{47}H_{51}NO_{14}Cl\) requires 888.2998).

2-debenzoyl-2-(4-thiomethylbenzoyl)taxol (3.10): 3.6 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 901 (7), 899 (7), 429 (30), 126 (100).

2-debenzoyl-2-(4-acetylbenzoyl)taxol (3.11): 5.5 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 896 (8), 552 (15), 429 (35), 286 (100).

2-debenzoyl-2-(4-cyanobenzoyl)taxol (3.13): 6.0 mg, \textit{fabms m/z} (rel. int.) [M\(^+\)] 879 (4), 663 (10), 515 (40), 154 (70). \textit{hrfabms [MH\(^+\)]} 879.3317 (C\(_{48}H_{51}N_{2}O_{14}\) requires 879.3340).
2-debenzoyl-2-(4-trifluoromethylbenzoyl)taxol (3.14): 4.1 mg, **fabms** m/z (rel. int.) [M]+ 922 (13), 621 (15), 531 (6), 225 (18), 150 (100). **hrfabms** [MH+] 922.3225 (C_{48}H_{51}F_{3}NO_{14} requires 922.3262).

2-debenzoyl-2-(3,4-dichlorobenzoyl)taxol (3.29): 5.2 mg, **fabms** m/z (rel. int.) [M]+ 922 (14), 621 (10), 515 (10), 412 (5), 307 (17), 225 (40), 154 (100). **hrfabms** [MH+] 922.2571 (C_{48}H_{50}Cl_{2}NO_{14} requires 922.2608).

2-debenzoyl-2-(3,4,5-trimethoxybenzoyl)taxol (3.34): 4.3 mg, **fabms** m/z (rel. int.) [M]+ 944 (7), 621 (10), 599 (10), 286 (50), 225 (40), 195 (100).

**2'-**TERT-BUTYLDIMETHYLSILYL-7-TRIETHYLSILYLTAXOL (3.35) - Imidazole (107 mg, 1.58 mmol) and t-butyldimethylsilylchloride (238 mg, 1.58 mmol) was added to a solution of taxol (135 mg, 0.158 mmol) in 1.0 ml of DMF. This was heated at 60° for 2 hours. The solution was cooled to room temperature and an additional 107 mg (1.58 mmol) of imidazole and 150 µl of triethylysilylchloride was added. After stirring for an additional hour, the solution was diluted with ethyl acetate and washed successively with water and brine. After drying over Na_{2}SO_{4} and concentration in vacuo the crude material was purified by Si gel preparative tlc (30% EtOAc/ 70% hexanes) to yield 3.35 (92%) as a white solid. **m.p.** 128-130° C; **1H nmr** (270 MHz, CDCl_{3}) (see Table 18); **fabms** m/z (rel. int.) [MNa-H]+ 1104 (5), 705 (3), 422 (40), 354 (12), 105 (100); **hrfabms** [M+Na]^{+} 1104.4936 (C_{59}H_{79}NO_{14}Si_{2}Na requires 1104.4937).

**REACTION OF 2'-**TERT-BUTYLDIMETHYLSILYL-7-TRIETHYLSILYLTAXOL (3.35) WITH BENZYLTRIMETHYLLAMMONIUM HYDROXIDE (TRITON B). - To a solution of 20 mg of 3.35 dissolved in 1.0 ml of anhydrous CH_{2}Cl_{2} was added 3 equivalents (24
μl) of Triton B (40% w/w solution in MeOH) at room temperature. After 1 minute, the solution was diluted with CH₂Cl₂ and quenched with 1.0 ml of 0.1 N HCl. The dichloromethane layer was washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield the crude residue, which was purified by preparative tlc (40% EtOAc/ 60% hexanes) yielding the following products.

2'-tert-butyldimethylsilyl-7-triethylsilyl-2-debenzoyltaxol (3.36): 5.8 mg (33%) white powder, m.p. 133-135° C; ¹H nmr (270 MHz, CDCl₃) (see Table 16); fabms m/z (rel. int.) [MNa-H]⁺ 1000 (3), 400 (22), 354 (84), 177 (25), 105 (100), hrfabms [M+Na]⁺ 1000.4632 (C₅₂H₇₅NO₁₃Si₂Na requires 1000.4675).

2'-tert-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-4-deacetyltaxol (3.37): 5.9 mg, (34%), m.p. 128-132° C; ¹H nmr (270 MHz, CDCl₃) (see Table 16); fabms m/z (rel. int.) [M+Na]⁺ 985 (5), 400 (7), 354 (30), 177 (20), 105 (100), hrfabms [M+Na]⁺ 958.4551 (C₅₀H₇₃NO₁₂Si₂Na requires 958.4569).

7-triethylsilyl-2-debenzoyl-4-deacetylbaaccatin III (1.116): 1.0 mg (10%); ¹H nmr (270 MHz, CDCl₃) (see Table 16); fabms m/z (rel. int.) [M+Na]⁺ 577(20), 281 (15), 149 (32), 115 (100), hrfabms [M+Na]⁺ 577.2821 (C₈₂H₄₆O₉SiNa requires 577.2809).

2'-tert-butyldimethylsilyl-7-triethylsilyl-2-debenzoyl-4-deacetylisotaxol (3.38): 1.0 mg (5%); ¹H nmr (270 MHz, CDCl₃) (see Table 16).

2'-TERT-BUTYLDIMETHYLSILYL-7-TRIETHYLSILYL-2-DEBENZOYL-TAXOL [3.36]-To a solution of 20 mg of 3.35 dissolved in 1.0 ml of anhydrous CH₂Cl₂ was added 2 equivalents (17 μl) of Triton B (40% w/w solution in MeOH) at -78° C. After 5 minutes, the solution was diluted with CH₂Cl₂ and quenched with 1.0
ml of 0.1 N HCl. The dichloromethane layer was washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield the crude residue, which was purified by preparative tlc (50% EtOAc/ 60% hexanes) to yield 16 mg (90%) of 3.36.

2'-TERT-BUTYLDIMETHYLSILYL-7-TRIETHYLSILYL-2-DEBENZOYL-4-DEACETYL-
TAXOL [3.37]- To a solution of 20 mg of 3.35 dissolved in 1.0 ml of anhydrous CH₂Cl₂ was added 2 equivalents (17 µl) of Triton B (40% w/w solution in MeOH) at -78⁰ C. After 1 minute, the cooling bath was removed, and the reaction warmed to room temperature over 15 minutes, following the progress of the reaction by tlc. After the reaction was complete, the solution was diluted with CH₂Cl₂ and quenched with 1.0 ml of 0.1 N HCl. The dichloromethane layer was washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield the crude residue, which was purified by preparative tlc (40% EtOAc/ 60% hexanes) to yield 11 mg (63%) of 3.37.

REACTION OF 2'-7-BIS(TRIETHYLSILYL)TAXOL WITH TRITON B- to a solution of 20 mg of 1.105 dissolved in 1.0 ml of anhydrous CH₂Cl₂ was added 2 equivalents (17 µl) of Triton B (40% w/w solution in MeOH) at -78⁰ C. After 1 minute, the cooling bath was removed, and the reaction warmed to room temperature over 15 minutes, following the progress of the reaction by tlc. After the starting material was consumed, the solution was diluted with CH₂Cl₂ and quenched with 1.0 ml of 0.1 N HCl. The dichloromethane layer was washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield the crude residue, which was purified by preparative tlc (45% EtOAc/60%.
hexanes) to yield 6 mg (38%) of 7-triethylsilyl-2-debenzoyltaxol 3.39 and 6.5 mg
(50%) of 7-triethylsilylbaccatin III 2.49. $^1$H nmr (270 MHz, CDCl$_3$) (3.39): 0.58 (q,
$J$=8.0, SiCH$_2$CH$_3$), 0.90 (t, $J$=8.0, -SiCH$_2$CH$_3$), 1.10 (s, CH$_3$), 1.18 (s, CH$_3$), 1.68 (s,
CH$_3$), 1.89 (s, CH$_3$), 2.15 (s, C-10 OAc), 2.21 (s, C-4 OAc), 2.70 (d, $J$= 5.2, C-2-OH),
3.42 (d, $J$=6.6, C-3), 3.9 (ps t, $J$ = 5.8, C-2), 4.38 (dd, $J$=10.5, 6.5, C-7), 4.60 (br dd,
C-20), 4.75 (br d, C-2'), 4.85 (br d, C-5), 5.70 (br d, C-3'), 6.2 (br t, C-13), 6.35 (s, C-
10), 6.87 (d, $J$ = 9.3, NH), 7.3-7.5 (m, ArH), 7.74 (dd, $J$ = 8.6, 1.3, NBz (ortho)).

2'-TERT-BUTYLDIMETHYLSIYL-7-TRIETHYLSIYL-4-DEACETYL-TAXOL (3.40)-
(5.0 mg, 0.005 mmol) of compound 3.37 dissolved in 25 µl of anhydrous toluene
was added to a slurry of dicyclohexylcarbodiimide (20 mg, 0.010 mmol) and
benzoic acid (10 mg, 0.010 mmol) in 100 ml of toluene. This was heated at 80° C
for 8 hours. After the reaction was complete, the crude mixture was diluted with
EtOAc, filtered over a pipette of silica gel, and washed with EtOAc. After
evaporation of the EtOAc, the residue was subjected twice to preparative tlc
(40%, EtOAc/ 60% hexanes) to yield 2.8 mg (50%) of benzyolated product 3.40. $^1$H nmr
(270 MHz, CDCl$_3$) (see Table 16). fabms m/z (rel. int.) [M]$^+$ 1040 (70), 982 (60),
964 (100).

2'-TERT-BUTYLDIMETHYLSIYL-7-TRIETHYLSIYL-2-DEBENZOYL-1,2-
CARBONYL-4-DEACETYL-TAXOL [3.41]. To 10.0 mg of 3.37 dissolved in 0.20 ml of
anhydrous toluene was added 25 mg of t-butoxycarbonyl anhydride and a
catalytic amount of pyrrolidinopyridine. This stirred at room temperature for 20
hours, and was directly applied to preparative tlc (25% EtOAc/ 75% hexanes).
This yielded 7.5 mg (74%) of 3.37 as a glassy solid. $^1$H nmr (270 MHz, CDCl$_3$)

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-0.25 (3H, s, SiCH₃), -0.10 (3H, s, SiCH₃), 0.57 (6H, q, J = 8.1, SiCH₂CH₃), 0.80 (9H, br s, C(CH₃)₃) 0.90 (9H, t, J = 8.1, -SiCH₂CH₃), 1.17 (3H, s, CH₃), 1.21 (3H, s, CH₃), 1.66 (3H, s, CH₃), 2.20 (6H, br s, C-10 OAc, CH₃), 2.50 (1H, m, C-6), 2.75 (1H, dd, J = 15.5, 5.7, C-14), 3.00 (1H, d, J = 7.4, C-3), 4.22 (1H, dd, J = 14.0, 6.6, C-7), 4.40 (1H, d, J = 6.6, C-20), 4.60 (3H, C-20, C-2, C-2'), 4.98 (1H, br dd, J = 10.6, 1.9, C-5), 5.25 (1H, br s, C-4 OH), 5.88 (2H, C-3', C-13), 6.44 (1H, s, C-10), 7.28-7.54 (8H, m, ArH), 7.74 (dd, J = 8.0, 1.7, NBz (ortho)). **fabms m/z** (rel. int.) [MH]+ 963 (100), 905 (50), 868 (60).

**REACTION OF 3.35 WITH TRIMETHYLSILYL IODIDE-SYNTHESIS OF 4.13**- To 10 mg of bis-silyl taxol 3.35 in 1.0 ml of CH₂Cl₂ at -78 °C was added 2 µl of trimethylsilyl iodide. Monitoring the reaction by tlc revealed that complete consumption of the starting material had occurred within 15 minutes. 1.0 ml of H₂O was added, and after separation and drying over Na₂SO₄, the solvent was evaporated. The crude residue was applied to silica gel preparative tlc (40% EtOAc/60% hexanes) and eluted to yield 5.2 mg (57%) of 4.13. **¹H nmr** (270 MHz, CDCl₃) (see Table 17). **fabms m/z** (rel. int.) [MNa]+ 991 (50), 968 (8).

Reaction of 3.35 with trimethylsilyl bromide at -40 °C for 2 hours using the same conditions as above also afforded 4.1 mg (45%) of 4.13 which matched identically to the product obtained from the trimethylsilyl iodide reaction.

**REACTION OF 3.35 WITH TRIPHENYLCARBENIUM TETRAFLUOROBORATE**- 20 mg (0.060 mmol) of triphenylcarbenium tetrafluoroborate was added at 0 °C to a solution containing 30 mg (0.028 mmol) of 3.35 in 1.5 ml of CH₂Cl₂. The solution turned bright yellow, and this stirred at this temperature for 30 minutes, as
monitored by tlc. The reaction mixture was applied directly to preparative tlc (35% EtOAc/65% hexanes) and eluted to yield 15 mg (55%) of 4.14 and 7.5 mg (23%) of 4.15.

REACTION OF 3.35 WITH TRIMETHYLSILYL AZIDE / ZINC IODIDE- [4.18]- To a solution of 3.35 (27 mg, 0.025 mmol) in CH₂Cl₂ (0.5 ml) was added ZnI₂ (25 mg) and 25 μl of trimethylsilyl azide. After stirring for 5 hours at room temperature, the mixture was applied directly to silica gel preparative tlc and eluted with 30% EtOAc/70% hexanes to yield 4.18 (20 mg, 69%) as an amorphous solid. ¹H nmr (270 MHz, CDCl₃) (see Table 17). fabms [M+Na]⁺ 1195 (3), 795 (2), 422, (5), 354 (13), 177 (10), 105 (100); hrfabms calculated for C₆₂H₈₉NO₁₅Si₃Na [M+ Na]+: 1194.5438, found 1194.5420.

DEPROTECTION OF 4.18 WITH CITRIC ACID-SYNTHESIS OF [4.19]- Compound 4.18 (20 mg, 0.017 mmol) was added to 1.0 ml of a 10% citric acid in MeOH solution at room temperature. After 30 minutes, the solution was diluted with EtOAc and washed successively with water and brine. Drying over Na₂SO₄ followed by concentration and silica gel preparative tlc (40% EtOAc/60% hexanes) yielded 4.19 (17 mg, 92%) as a white powder. ¹H nmr (270 MHz, CDCl₃) (see Table 17). fabms m/z (rel. int.) [M]+ 1100 (5), 683 (35), 623 (25), 519 (38), 354 (15), 105 (100). hrfabms calculated for C₅₉H₈₂NO₁₅Si₂: 1100.5223; found 1100.5222.

TREATMENT OF 4.19 WITH DILUTE AQUEOUS SODIUM BICARBONATE. Compound 4 (15 mg, 0.0136 mmol) was dissolved in 1.0 ml of a 1% NaHCO₃ in
MeOH:H₂O [3:1] at room temperature and stirred for 15 minutes. The solution was diluted with EtOAc, washed several times with water and brine successively, dried over Na₂SO₄, and concentrated. Purification by silica gel preparative tlc (40% EtOAc / 60% hexanes) afforded 14.5 mg (97%) of 4.20. ¹H nmr (270 MHz, CDCl₃) (see Table 17). fabms m/z (rel. int.): [M]+ 1100 (50), 1099 (90), 1098 (100), 1080 (30).

**BENZOYLATION OF 4.20 WITH META-CHLORBENZOIC ACID**- 40 mg (0.18 mmol) of dicyclohexylcarbodiimide and 29 mg (0.18 mmol) of 3-chlorobenzoic acid was added to a solution of 10 mg (0.009 mmol) of 4.20 dissolved in 100 µL of toluene. A catalytic amount of pyrrolidinopyridine was added, and the reaction mixture was stirred at 60 °C for 6 hours. Filtration over a cotton plug followed by evaporation yielded the crude residue, which was directly subjected to acid treatment (1.0 ml 5% HCl-MeOH) for 20 minutes. This was diluted with EtOAc, washed successively with water and brine, dried over Na₂SO₄, and concentrated to yield the crude product. Purification by preparative tlc (55% EtOAc/45% hexanes) yielded 2.5 mg of 4.22. ¹H nmr (270 MHz, CDCl₃) (see Table 17).

**REACTION OF 3.35 WITH TRIMETHYLSILYL CYANIDE / ZINC IODIDE**- [4.23]- To 35 mg (0.032 mmol) of 3.35 dissolved in 2.0 ml of CH₂Cl₂ was added 41.2 mg (0.129 mmol) of zinc iodide followed by 17.0 µl (0.129 mmol) of trimethylsilyl cyanide. This stirred at room temperature for 15 minutes, and the reaction mixture was applied directly to preparative tlc (30% EtOAc/70% hexanes) to yield 31 mg (80%) of 4.23 as a white powder. m.p. 125-128 °C; ¹H nmr (270 MHz, CDCl₃) (see Table 17); ¹³C nmr (CDCl₃, 100.5 MHz) -For assignments, see Figure
21. -5.8, -5.2, -1.3, 5.2, 6.7, 10.0, 14.5, 18.1, 20.8, 21.5, 25.5, 25.8, 26.7, 31.2, 35.2, 43.4, 53.3, 55.7, 59.3, 66.9, 70.8, 71.5, 74.7, 74.8, 75.3, 77.8, 79.3, 86.7, 98.1, 118.1, 126.3, 127.0, 127.9, 128.7, 128.75, 128.76, 129.5, 130.3, 131.7, 133.1, 133.5, 134.1, 138.3, 139.3, 166.7, 17.6, 169.4, 171.1, 202.0. \textbf{fabms} m/z (rel. int.) [MNa]^+ 1204 (70), 1176 (100), 1154 (70); \textbf{hrfabms} calculated for $C_{63}H_{89}N_2O_{14}Si_3Na$: 1204.5519; found 1204.5461.

\textbf{Deprotection of 4.23 with Citric Acid-Synthesis of [4.31]}- Compound 4.23 (20 mg, 0.017 mmol) was added to 1.0 ml of a 10% citric acid in MeOH solution at room temperature. After 45 minutes, the solution was diluted with EtOAc and washed successively with water and brine. Drying over Na$_2$SO$_4$ followed by concentration and silica gel preparative tlc (40%EtOAc/60% hexanes) yielded 4.31 (18 mg, 96%). \textbf{H nmr} (270 MHz, CDCl$_3$): -0.25 (3H, s, SiCH$_3$), -0.10 (3H, s, SiCH$_3$), 0.52 (6H, q, J=8.1, SiCH$_2$CH$_3$), 0.85 (9H, br s, C(CH$_3$)), 0.90 (9H, t, J=8.1, -SiCH$_2$CH$_3$), 1.19 (3H, s, CH$_3$), 1.21 (3H, s, CH$_3$), 1.58 (3H, s, CH$_3$), 2.15 (3H, s, CH$_3$), 2.20 (3H, s, CH$_3$), 2.31 (3H, s, CH$_3$), 2.90 (1H, dd, J = 15.7, 5.6, C-14), 3.12 (1H, d, J = 7.6, C-3), 3.70 (3H, m, C-20, C-20-OH), 4.10 (1H, dd, J =14.0, 2.5, C-7), 4.60 (1H, C-2', d, 1.9), 4.78 (1H, m, C-5), 5.60 (1H, d, J = 7.7, C-2), 5.68 (1H, dd, J = 9.0, 1.9, C-3'), 6.31 (1H, br t, C-13), 6.40 (1H, s, C-10), 7.04 (1H, d, J = 9.0, NH), 7.25-7.60 (11H, m, ArH), 7.77 (2H, dd, J = 8.4, 1.7, N8z (ortho)), 8.15 (2H, dd, J = 8.5, 1.1, C-2-OBz (ortho)).

\textbf{Deprotection of 4.31 with HCl/Methanol-Synthesis of [4.32]}- 12 mg of compound 4.31 was added to 1.0 ml of 5% HCl-MeOH and stirred at room temperature for 2 hours. The solution was diluted with EtOAc, washed
successively with water and brine, dried over Na$_2$SO$_4$, concentrated, and applied to preparative tlc (60% EtOAc/ 40% hexanes) to yield 7 mg of 4.32. $^1$H nmr (270 MHz, CDCl$_3$) (see Table 17). fabms m/z (rel. int.) [MH]$^+$ 881 (90), 853 (100).
V. REFERENCES


VI. APPENDIX

6.1 Hamel's experimental data.

Because nonspecific reactions can be extensive when photoaffinity labeling of proteins is performed, we decided to examine the interactions of the taxol analogues with purified tubulin as opposed to the microtubule protein (microtubules obtained from brain tissue and containing microtubule-associated proteins in addition to tubulin). This will eliminate the possibility of nonspecific interactions with nontubulin components of the microtubule.

The use of purified tubulin presents us with two major alternatives for examining the interactions of photoreactive taxol analogs with the protein. These are illustrated in Figure 20 with 10 µM tubulin ± 10 µM taxol. Curves 1

![Graph showing taxol-induced polymerization of purified tubulin under polymerizing (1.0M glutamate) and nonpolymerizing (0.1 M 4-morpholineethane sulfonate) conditions.](image)

Figure 20. Taxol-induced polymerization of purified tubulin under polymerizing (1.0M glutamate) and nonpolymerizing (0.1 M 4-morpholineethane sulfonate) conditions.

Curves 1 and 2: 1.0 M monosodium glutamate (2 M stock solution adjusted to pH 6.6 with HCl), 10 µM (1.0 mg/ml) tubulin, 4% (v/v) dimethyl sulfoxide, 50 µM GTP, and 10 µM taxol (curve 2 only). Curves 3 and 4: 0.1 M 4-morpholineethane sulfonate (1 M stock solution adjusted to pH 6.9 with NaOH), 10 µM (1.0 mg/ml) tubulin, 4% (v/v) dimethyl sulfoxide, 100 µM GTP, 0.5 mM MgCl₂, and 10 µM taxol (curve 4 only). Baselines were established with cuvette contents at 0°. At time zero the electronic temperature controller was set at 15°. Additional temperatures were set at the times indicated by the dashed lines.
(no taxol) and 2 (+ taxol) compare reaction mixtures in which 1.0 M glutamate replaces microtubule-associated proteins as an inducer of polymerization. In the absence of taxol no reaction occurred at 150°, while an extensive reaction occurred at 370°. With taxol, extensive polymerization occurred at the lower temperature. In the absence of taxol the polymer was cold labile, with about 80% of the total depolymerization reaction occurring at 100°. With taxol less than 50% depolymerization occurred, with the bulk of depolymerization occurring at 00° rather than 100°. Alternatively, one could use a low ionic strength reaction condition, such as 0.1 M 4-morpholine-ethanesulfonate-0.5 mM MgCl2 (pH 6.9), represented by curves 3 (no taxol) and 4 (+ taxol). Without taxol there was essentially no polymerization reaction. With taxol, polymerization occurred at 370° but not at 150°. With taxol there was little depolymerization at 100° and extensive, relatively slow depolymerization at 00°. In this reaction condition microtubule-associated proteins would induce a reaction similar to that represented by curve 1, and addition of taxol + microtubule-associated proteins would yield a reaction similar to that represented by curve 2.

Since depolymerization effects at low temperatures seemed to be the best method for quantitative comparison of its analogs with taxol,59 the glutamate system was chosen for initial evaluation. IC50 values for the concentration of drug which reduced the rate of depolymerization by 50% were obtained for taxol and compounds 2.19, 2.20, 2.23, 2.33, and 2.39. The values obtained are presented in Table 18, and typical experiments with taxol and compound 2.39
Table 18. IC₅₀ values of taxol and analogues in inhibiting tubulin polymer depolymerization a

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM) ± S. D.</th>
<th>IC₅₀ (analog) / IC₅₀ (taxol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxol</td>
<td>0.42 ± 0.09</td>
<td>1</td>
</tr>
<tr>
<td>2.19</td>
<td>0.65 ± 0.03</td>
<td>1.5</td>
</tr>
<tr>
<td>2.20</td>
<td>1.6 ± 0.3</td>
<td>3.8</td>
</tr>
<tr>
<td>2.23</td>
<td>1.9 ± 0.3</td>
<td>4.5</td>
</tr>
<tr>
<td>2.33</td>
<td>3.1 ± 0.6</td>
<td>7.4</td>
</tr>
<tr>
<td>2.39</td>
<td>2.8 ± 0.3</td>
<td>6.6</td>
</tr>
</tbody>
</table>

a Reaction mixtures (0.30 ml) contained 1.0 mg/ml (10 µM) purified tubulin, 1.0 M monosodium glutamate (2 M stock solution adjusted to pH 6.6 with HCl), 50 µM GTP, 4% (v/v) dimethyl sulfoxide, and varying concentrations of taxol or taxol analogue. Polymerization was initiated by a temperature jump from 0 to 37°C (the temperature jump took approximately 75 sec), and the temperature was maintained at 37°C for about 20 minutes. Depolymerization was initiated by a reverse jump to 0°C (which took about 6 min to complete), and the maximum rate of depolymerization (as measured by reduction in A₃50 or A₄20) was determined. IC₅₀ values were determined by interpolation. At least three independent determinations were performed with each compound.

are presented in Figs. 21A (taxol) and 8B (2.39), with reaction mixtures below (curve 2) and above (curve 3) the IC₅₀ compared to a control reaction (curve 1). Under this reaction condition an IC₅₀ value of 0.42 µM was obtained for taxol, and none of the analogs were as active as taxol itself. The IC₅₀ value obtained for 2.19 was 0.65 µM. The IC₅₀ values for the potential photoreactive compounds ranged from 1.6 µM for 2.20 to 3.1 µM for 2.33. Nevertheless, substoichiometric IC₅₀ values (i.e. lower than the tubulin concentration of 10 µM) were obtained with all compounds, which indicated they might have promise as photolabels. With compound 2.39 concentrations near the IC₅₀ value
for polymerization had relatively little effect on polymerization (Fig. 21B). This was also observed with taxol (Fig. 21A) and with the other analogs described here.

![Figure 21. Determination of IC50 values for inhibition of depolymerization.](image)

Reaction conditions are described in detail in legend of Table 18. A. Taxol. Concentrations as follows: curve 1, none; curve 2, 0.25 μM; curve 3, 0.5 μM. B. Compound 2.39. Concentrations as follows: curve 1, none; curve 2, 1.0 μM; curve 3, 3.0 μM. At 20 min, as indicated by the first interruption in the abscissa, the chart speed was changed from 30 cm/hr to 60 cm/hr and the reaction temperature was reduced to 0°C (see table 1 legend). At approximately 35 min, as indicated by the second interruption in the abscissa, the chart speed was changed back to 30 cm/hr.

With the successful preparation of compound 2.43, the radiolabeled version of 2.39, we began to investigate its incorporation into tubulin polymer. The initial strategy was to use the low ionic strength reaction condition to minimize the presence of polymer not containing taxol analog. Polymerization however was not induced with concentrations of 2.39 as high as 50 μM. The effects of 2.39, as
well as compounds 2.19, 2.20, 2.23, and 2.33, were examined in greater detail. As shown in Figure 22, with the exception of compound 2.19, these agents at 40 \( \mu \text{M} \) had little effect on tubulin polymerization in 1.0 M glutamate.

![Graph](image)

**Figure 22.** Effects of compounds 2.19, 2.20, 2.23, 2.33, and 2.39 on tubulin polymerization and depolymerization.

Reaction conditions were as described in the legend of Fig. 21. Compounds were added to the reaction mixtures as follows: curve 1, none; curve 2, 40 \( \mu \text{M} \) compound 2.19; curve 3, 40 \( \mu \text{M} \) compound 2.20; curve 4, 40 \( \mu \text{M} \) compound 2.23; curve 5, 40 \( \mu \text{M} \) compound 2.33; curve 6, 40 \( \mu \text{M} \) compound 2.39.

and they all failed to induce polymerization in 0.1 M 4-morpholineethane sulfonate. With compound 2.19, a slight reaction occurred at 100\(^\circ\), and a brisk polymerization occurred when the temperature was increased to 370\(^\circ\), with turbidity greatly exceeding that of the drug-free control and that observed with 10 \( \mu \text{M} \) taxol (Fig. 22, curve 2). With 10 \( \mu \text{M} \) compound 2.19 turbidity development and polymer stability was nearly as great as that observed with 40
μM drug. These compounds displayed variable effects on depolymerization, and the greatest stabilization occurred with compound 2.19 (equivalent to that obtained with taxol), the least occurred with compound 2.32.

The reduced stabilization that occurred with 40μM concentrations of compounds 2.20, 2.23, 2.33, and 2.39 was puzzling in view of the relatively low IC50 values for the rate of depolymerization obtained with these analogs. Compound 2.39 was studied over a range of concentrations, and extent of stabilization, as measured by retention of turbidity, was highly variable. Maximum, although incomplete, stabilization occurred at concentrations near stoichiometric with the tubulin concentration. This would indicate that there is one specific taxol binding site per tubulin molecule in polymer.46

Since compound 2.39 failed to induce polymerization of tubulin at low ionic strength but did stabilize the glutamate polymer, the interaction of radiolabeled 2.43 with tubulin in 1.0 M glutamate was investigated. Polymer formed in the presence of 2.43 was harvested by centrifugation, with and without a preincubation with taxol and with and without a period of illumination of the reaction mixture at 302 nm. These pellets were dissolved in guanidine hydrochloride to denature the tubulin. Total protein and radiolabel recovered were quantitated, and a portion of each sample was examined by micro-gel filtration chromatography1,41 to determine the amount of 2.43 bound to the denatured protein, which should be a measure of the extent of covalent reaction between tubulin and ligand. These studies are summarized in Table 19. In brief, superstoichiometric amounts of 2.43 appeared to form a covalent bond with tubulin. However, taxol failed to reduce the extent either of incorporation into pellet or of covalent bond formation, indicating that these reactions were largely
nonspecific. In addition, illumination failed to enhance the extent of the covalent reaction, indicating that our photolabel may be too reactive, with a reaction occurring in the low level of ambient light required to perform these experiments.

*Table 19. Evaluation of compound 2.43 as a photoreactive analogue of taxol a*

<table>
<thead>
<tr>
<th>Mol compound 2.43/mol tubulin (± S. D.)</th>
<th>Low ambient light b</th>
<th>UV illumination c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incorporated into polymer d</td>
<td>Covalently bound to tubulin e</td>
</tr>
<tr>
<td>- Taxol f</td>
<td>5.1 ± 0.3</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>+ Taxol f</td>
<td>5.1 ± 0.3</td>
<td>3.9 ± 0.1</td>
</tr>
</tbody>
</table>

a Each reaction mixture (1.0 ml final volume; all concentrations in terms of final volume) contained 0.5 mg/ml (5 μM) tubulin, 1.0 M monosodium glutamate (pH of 2.0 M stock solution adjusted to 6.6 with HCl), 0.5 mM MgCl₂, 100 μM GTP, and, if indicated, 50 μM taxol in a 0.975 ml volume. Reaction mixtures were prepared and maintained with minimal light in the room ("low ambient light"). After a 15 min incubation at 37°C compound 2.43 was added in 25 μl to a final concentration of 25 μM. Incubation was continued at 37°C for 15 min. Half the reaction mixtures were left in low ambient light at room temperature for 15 minutes; the other half were illuminated at 302 nm for 15 minutes. All reaction mixtures were centrifuged for 10 minutes at 45,000 rpm in a Beckman Ti70 rotor. The supernatants were aspirated and discarded, and the pellets were washed twice with a solution containing 1.0 M monosodium glutamate (pH 6.6) and 0.5 mM MgCl₂. The pellets were each dissolved in 0.5 ml of 3.0 M guanidine hydrochloride. This solution was used to determine the amount of protein and quantity of compound 2.43 in polymer and to determine the amount of compound 2.43 covalently linked to tubulin. Each data point was performed in triplicate with the average values and standard deviations (S. D.) presented.

b The reaction mixtures were prepared with as little room illumination as possible. A UVX photometer (manufactured by UVP, Inc. of San Gabriel, CA) with a 365 nm sensor measured an ambient intensity of less than 0.3 μW/cm².

c The samples were illuminated at 302 nm for 15 minutes with a model xx-15 lamp (manufactured by UVP, Inc.). The light source was at a distance of 3.5 cm from the surface of the reaction mixtures. Illumination was performed in a microtiter plate, with each well containing 0.25 ml. The UVX photometer with a 310 nm sensor measured a light intensity of 4.6 mW/cm².

d The amounts of radiolabel and protein in the polymer pellets were quantitated on aliquots of the guanidine hydrochloride solutions derived from the pellets.

e Aliquots of the pellet solutions were placed on syringe-columns of Sephadex G-50 (superfine) equilibrated with 3.0 M guanidine hydrochloride and processed by low speed centrifugation as described previously (43). Protein and radiolabel in the filtrates were determined. It is assumed that radiolabel that
remains associated with tubulin following denaturation and gel filtration represents compound 2.43 which has reacted covalently with the protein.

1 Taxol was present, as indicated, in the initial incubation prior to addition of compound 2.43 in an attempt to minimize the interaction of the analogue with tubulin.

In summary, we have examined five derivatives of taxol esterified at C(7) (compounds 2.19, 2.20, 2.23, 2.33, and 2.39). With the exception of compound 2.19, these are potential photoreactive agents, and one of them (2.39) was prepared in a radiolabeled form. The tritiated version of compound 2.39 appears to be highly reactive with tubulin, probably in low levels of ambient light, but specificity of the covalent reaction could not be demonstrated. An unanticipated finding from these studies was that the taxol analogs prepared had some, but not all of the properties of taxol. They were all deficient at inducing tubulin polymerization, both at low ionic strength at 37° and in glutamate at a reduced temperature (15°). If polymerization occurred, as at 37° in glutamate, all five derivatives stabilized the polymer that had formed. This stabilization occurred at low analog concentrations and was particularly evident at intermediate temperatures (i.e., 10° as opposed to 0°). The stabilization which occurred with compound 2.19 was essentially indistinguishable from that observed with taxol. These observations indicate that compound 2.19 and additional analogs modified at position C(7) merit careful evaluation for potential differences from taxol in antitumor spectrum and/or toxicity.
6.2 $^1$H and $^{13}$C nmr spectra of selected compounds
VITA

John Rimoldi was born on September 22, 1967 in Johnstown, Pennsylvania, were he obtained his primary education, and graduated from Conemaugh Valley High School in 1985. He received his Bachelor of Science degree in Chemistry in 1989 from The University of Pittsburgh at Johnstown, where he worked under the guidance of Dr. George B. Trimitsis. He then attended graduate school at Virginia Polytechnic Institute and State University in Blacksburg, Virginia. He was married to Cynthia Marie Guyan in August of 1991, in Johnstown, Pennsylvania. He was awarded his Ph.D. in Chemistry under the auspices of Dr. David G. I. Kingston in 1993. He is currently employed as a post-doctoral associate at Virginia Tech, working with Dr. Neal Castagnoli Jr. at the Harvey Peters Research Center for the Study of Parkinson's Disease and Disorders of the Central Nervous System.

[Signature]