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Studies Toward Structure-Activity Relationships of *Taxol* ®: Synthesis and Cytotoxicity of *Taxol* ® Analogues with C-2' Modified Phenylisoserine Side Chains†

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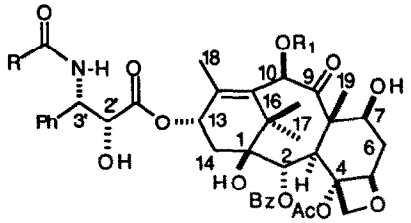
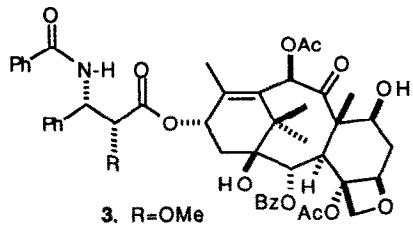
Abstract: *Analogues of taxol with a modified phenylisoserine side chains were synthesized and evaluated as potential cytotoxic agents.*

Taxol (1), a complex antineoplastic diterpene isolated from *Taxus brevifolia* ,[1] has recently been approved for the treatment of metastatic carcinoma of the ovary.[2] The cytotoxicity of taxol is related to microtubule-mediated interruption of mitosis which occurs by inducing tubulin polymerization and forming extremely stable and nonfunctional microtubules abnormally resistant to depolymerization.[3]

Study of natural and semisynthetic congeners of taxol has demonstrated that an intact taxane ring and an ester C-13 side chain are required for cytotoxicity as Baccatin III and *N*-benzoyl-(2*R*, 3*S*)-3-phenylisoserine are devoid of significant antitumor activity.[4] Taxol derivatives with simplified side chains displayed no cytotoxicity or significantly lower biological activity than taxol and the stereochemistry *2'R* and *3'S* was also found to be critical for optimal activity.[5a-d] Derivatives with substituted phenyl rings at the 3'-position displayed cytotoxicity comparable to taxol.[6] Interestingly, replacing the *N*-benzoyl group with *tert*-butyloxycarbonyl group at the C-3' position of the C-10 desacetyl derivative of taxol has resulted in an analogue, *Taxotere*® (2), which is currently undergoing clinical trials in the United States and Europe.[5c]



1. Taxol : R=Ph, R_1=Ac
2. Taxotere: R=^{t}BuO , R_1 = H

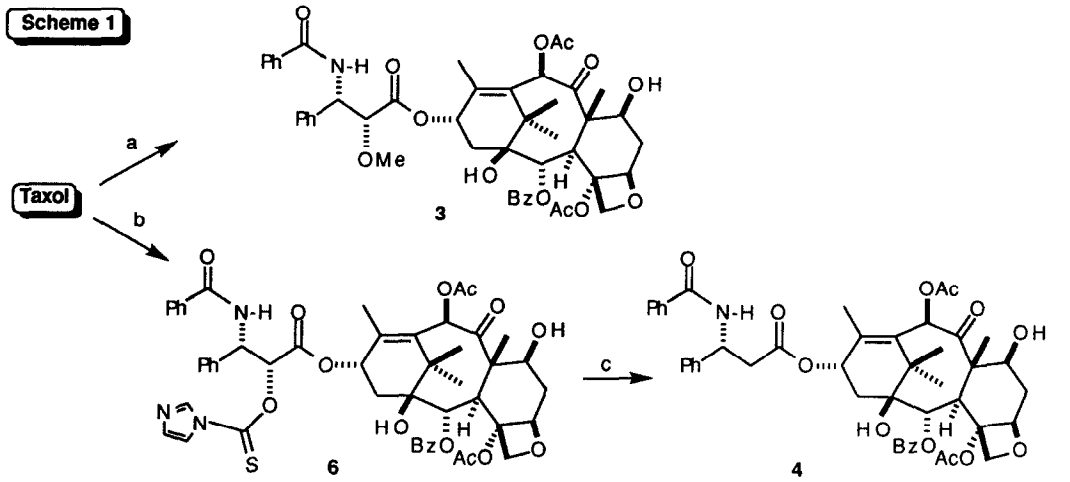


3. R=OMe
4. R=H
5. R=F

In order to better define the structure-activity relationships of taxol for the design of more effective drugs and to understand the features of the taxol binding site on microtubules we were interested in the role of the 2'-hydroxyl group. Based upon the X-ray structure of *Taxotere* ® [7], Swindell has suggested that the 2'-hydroxyl

is stabilizing the amino acid side chain conformation for proper binding to the receptor via intramolecular hydrogen bonding between the ester carbonyl, the 2'-hydroxyl, and the 3'-NH groups.[5d] However, we cannot rule out the possibility of direct participation of the 2'-OH group in an intermolecular hydrogen bonding at the receptor site. So far modifications at C-2' position have only included prodrugs of taxol.[8] Simple analogues like 2'-desoxytaxol or the 2'-methyl ether have not been reported in the literature. We felt that the biological properties of these compounds might lead to some insight into the role of 2'-hydroxyl group, therefore, we decided to synthesize 2'-methoxytaxol (**3**) and 2'-desoxytaxol (**4**). Furthermore, we were also interested in synthesizing and evaluating 2'-fluorotaxol (**5**) as a potential cytotoxic agent. Isosteric groups are often applied extensively in drug design[9] and **5** can be viewed as an isostere of taxol. Herein, we describe the synthesis and cytotoxicity results of these new 2'-modified analogues of taxol.

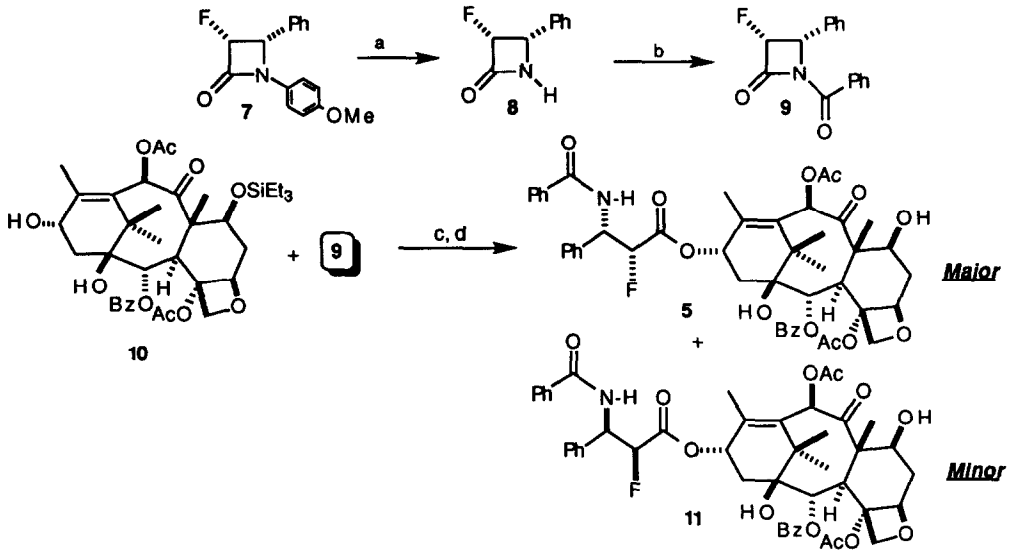
Chemistry: Treatment of taxol (**1**) with silver oxide and methyl iodide in acetone at ambient temperature for 4 days afforded the desired compound **3**. For the synthesis of 2'-desoxytaxol (**4**) we relied on the Barton's deoxgenation.[10] Hence, treatment of **1** with 1,1-thiocarbonyldiimidazole produced imidazolide **6** which upon subsequent treatment with tributyltin hydride afforded the desired desoxy compound **4** (Scheme 1).



The synthesis of 2'-fluorotaxol was accomplished following the methodology developed by Holton to synthesize taxol from baccatin III.[11] The racemic *cis* 3-fluoroazetidinone **7** was prepared by ketene-imine chemistry,[12] followed by the removal of the *p*-anisyl moiety under oxidative conditions to afford **8**.[13] Further treatment of **8** with benzoyl chloride afforded the *N*-benzoyl derivative **9**, a key synthon for elaboration of the side chain. Treatment of 7-triethylsilylbaccatin (**10**)[14] with *n*-BuLi at low temperature followed by the addition of **9** afforded the silyl protected fluorotaxols as a mixture of diastereomers with a bias for the desired *2'R,3'S* isomer

(ca. 3:1). Desilylation provided the fluorotaxols **5** and **11** separable by flash chromatography (silica: acetone/methylene chloride 3:30)(Scheme 2).[15]

Scheme 2



Reaction Conditions: a. CAN,CH_3CN, 0°C, 4h (67%); b. BzCl, i-Pr_2NEt, cat-DMAP, CH_2Cl_2, 0°C, 60 min (90%) c. *n*-BuLi, THF, -40°C to -0°C, 60 min (61%).; d. 6N HCl, CH_3CN, -5°C, 3h (82%).

The absolute stereochemistry of the major and minor diastereomers was confirmed by nuclear Overhauser enhancements (nOe) experiments. Upon irradiating the C-4 OAc methyl protons of the major isomer **5**, nOe's were observed on the C-3' and C-2' protons suggesting the *2'R and 3'S stereochemistry*. No nOe was observed on the C-3' proton of the minor isomer. Similar nOe's have been observed in taxol.[16]

Biology: Analogues of taxol were evaluated by a cytotoxicity assay in HCT116 human colon carcinoma cell line by a standard protocol [17] and the results are shown below:

Compound	*HCT116 IC_{50} [a] (μM)*
Taxol (**1**)	0.004
2'-Methoxytaxol (**3**)	0.866
2'-Desoxytaxol (**4**)	0.297
2'-Fluorotaxol (**5**)	0.475

a= Drug concentration required to inhibit cell proliferation to 50% vs. untreated cells (incubated at 37°C for 72 h)

Discussion: Replacing the hydrogen bond donor C-2'-hydroxyl group with the hydrogen bond acceptor methoxyl group resulted in considerable loss of cytotoxicity. The desoxygenated taxol derivative **4** retained some

activity compared to **3** but was 75 fold less active than taxol. Similarly, replacing the hydroxyl group with a hydrogen bond acceptor, fluorine,[18] also resulted in over 100 fold loss of cytotoxicity when compared to taxol. The observed loss could be attributed either to the failure of the phenylisoserine side chain to adopt a *"preferred conformation"* required for effective binding or to the need for participation of the 2'-hydroxyl group in intermolecular hydrogen bonding at the receptor site. We are continuing our endeavors to answer these questions and further work will be reported in future publications from our laboratories. In conclusion, our study has demonstrated the significance of the 2'-hydroxyl group as a hydrogen bond donor for the optimal antimitotic activity of taxanes.

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References and Notes

† TAXOL® (Paclitaxel) is a registered trademark of Bristol-Myers Squibb Company.

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