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THE FIRST SYNTHESIS OF A C-9 CARBONYL MODIFIED BACCATIN III DERIVATIVE AND ITS CONVERSION TO NOVEL TAXOL® AND TAXOTERE® ANALOGUES

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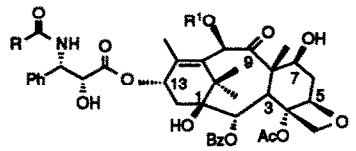
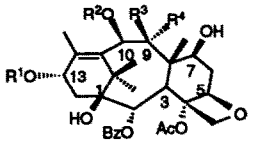
Abstract: Treatment of 7,10-ditrocbaccatin III with potassium *tert*-butoxide resulted in the formation of a cyclic carbonate. Its taxol and taxotere analogues were found to possess good microtubule assembly properties but exhibited poor *in vitro* cytotoxicity against B16 melanoma cells.

The naturally occurring diterpenoid taxol (**1a**) is considered to be the most promising new agent developed in recent years for cancer chemotherapy.[1] Its unique structural features, exciting biological activity, unprecedented mechanism of action and limited availability have attracted the attention of a diverse segment of the scientific community worldwide.[1-7] The interest in taxol has also stimulated an array of investigations aimed at modifying its structural features in search of more effective analogues and to understand better the structure activity relationships of this novel drug.[2-7]





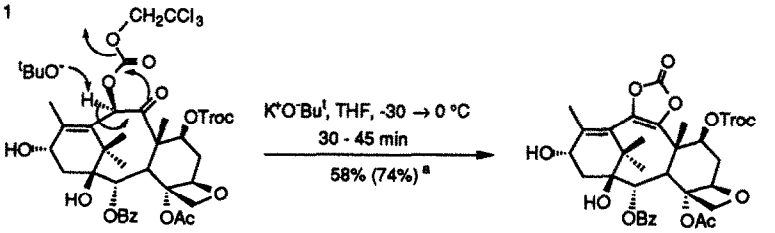
1 a R = Ph, R ^{1} = Ac (Taxol)
b R = *t*-BuO, R ^{1} = H (Taxotere)

2 R^1 = H, R^2 = Ac, R^3 = R^4 = O (Baccatin III)
3 R^1=R^2 = H, R^3/R^4 = O (10-Deacetylbaccatin III)
4 R^1=R^2 = Ac, R^3 = H, R^4 = OH (13-Acetyl-9-dihydrobaccatin III)

To date, a wide variety of taxol analogues, modified at both the C-13 phenylisoserine side chain and the taxane carbon skeleton have been reported.[2-7] However, the sterically hindered C-9 carbonyl group of both taxol and baccatin III (**2**) has defied modification.[3] Although 13-acetyl-9-dihydrobaccatin III (**4**) was found to occur naturally,[8] reactions aimed at synthesizing C-9 carbonyl modified baccatin analogues remained unsuccessful.[3,9] In the light of these observations, we decided on an indirect approach to overcome the low reactivity of the carbonyl center. We reasoned that a successful deprotonation of the activated methine hydrogen at C-10, using a suitable base, and subsequent trapping of the enolate anion should furnish a novel C-9 carbonyl modified baccatin III derivative for further studies. The results of our investigations are described herein.

We initially carried out the deprotonation studies on 10-deacetylbaccatin III (**3**), a more readily available, naturally occurring precursor for the semisynthesis of taxol.[10] Protection of the hydroxy groups at C-7 and C- 10 as their trichloroethyloxy carbonate (Troc) derivatives afforded the known baccatin III analogue **5**.[11] Initial attempts, using various bases (*viz.* NaH, BuLi, LDA, NaHMDS) and reaction conditions, failed to effect the expected deprotonation at C-10. However, when the reaction was carried out in the presence of potassium *tert*-butoxide (1.1 equiv), tlc monitoring showed the formation of a major product, which was identified as cyclic carbonate **6** (Scheme 1).[12,13] Presumably, deprotonation at C-10 (enolate formation) is followed by an intramolecular attack upon the trichloroethoxy carbonyl group resulting in product formation (Scheme 1).

Scheme 1



5 **6**

a. yield in parentheses is based on recovered starting material

Interestingly, the Troc- protection of the C-10 hydroxyl was found to be essential for the success of this reaction. Replacement of the Troc-group by acetyl or triethylsilyl protecting groups resulted in deesterification at the C-4 and/or C-2 positions under the above reaction conditions.[14] This difference in reactivity is probably due to the activation of the C-10 methine proton by the strong electron withdrawing Troc-group as well to the internal *in situ* trapping of the resulting enolate anion thereby favoring the product formation.

Having attained the primary objective of synthesizing a C-9 carbonyl modified baccatin III derivative, we next proceeded to convert **6** to the corresponding taxol and taxotere analogues so as to study the effect of this additional ring on biological activity. Thus, the coupling of **6** with the *N,O*-protected side chain precursor **7**[15] under standard reaction conditions afforded product **8** (Scheme 2). *N,O*-Deprotection of the oxazolidine ring then yielded the amino alcohol **9**, a common precursor for both the taxol and taxotere analogues. Introduction of the benzoyl or the *tert*-butoxycarbonyl group at the 3'-amino group was carried out under standard reaction conditions,[16] resulting in the corresponding 7-Troc protected taxol or taxotere analogues **10a** and **10b** in good yields. Deprotection of the 7-hydroxy group completed the syntheses of the C-9 keto modified analogues of taxol (**11a**) and taxotere (**11b**).[17]

Interestingly, the *in vitro* microtubule assembly activity of compounds **11a** and **11b** were promising, but their cytotoxicity against B16 melanoma cells was found to be poor (Table I).[18] Taxol structure activity studies have lead to the suggestion that modifications at C-10 may not have a major impact on taxol bioactivity.[7] Our studies, however, suggest that structural changes at C-9/C-10 can have a pronounced

effect on cytotoxcity. The fact that **11a** and **11b** had microtubule-assembling properties but did not display significant cytotoxicity is presumably due to a difference in uptake or metabolism of these derivatives in comparison to taxol and taxotere.

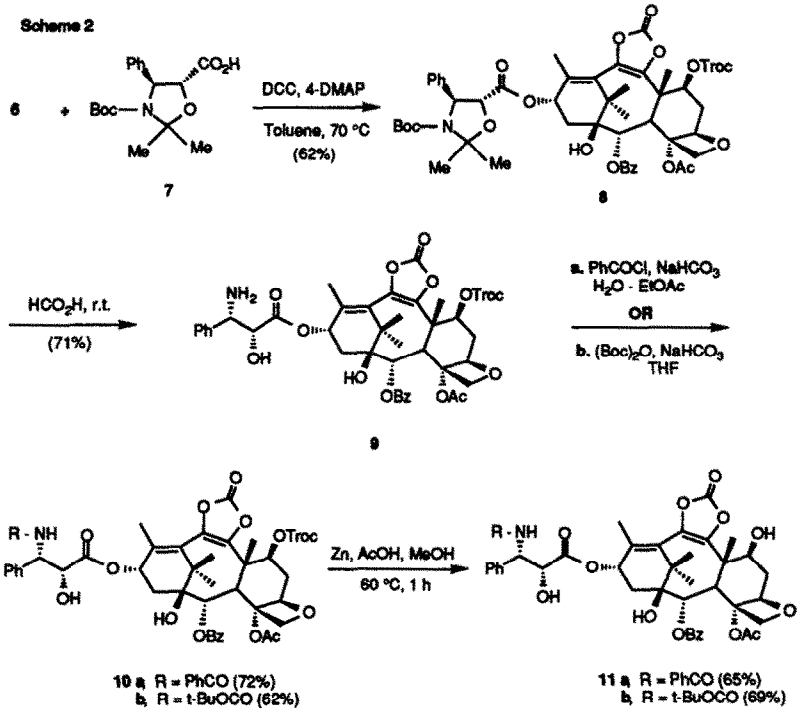


Table I. Biological activities of **1a**, **1b**, **11a** and **11b**.

Analogue	Microtubule Assembly (ED_{50}/ED_{50} taxol)[a]	B16 melanoma (ED_{50}/ED_{50} taxol)[b]
1a (taxol)	1	1
11a	2.4	>34.5
11b	0.5	16.4

[a]ED_{50} = Concentration in mM which reduces the supernatant protein concentration by 50%.
[b]ED_{50} = Concentration in nM which produces 50% inhibition of proliferation after a 40 h incubation.

In conclusion, the strategy of overcoming the unreactivity of the C-9 keto group of the taxane skeleton via its enolization was successfull and provided a pathway for the synthesis of a novel class of taxol analogues. Efforts are underway to utilize the above reaction in further modification at both the C-9 and

C-10 positions of the baccatin III moiety. Our studies also suggest that modifications at C-10 and C-9 of taxol may have a more significant influence on cytotoxicity than previously recognized.

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+ Address for correspondence

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