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Syntheses of Novel C-9 and C-10 Modified Bioactive Taxanes

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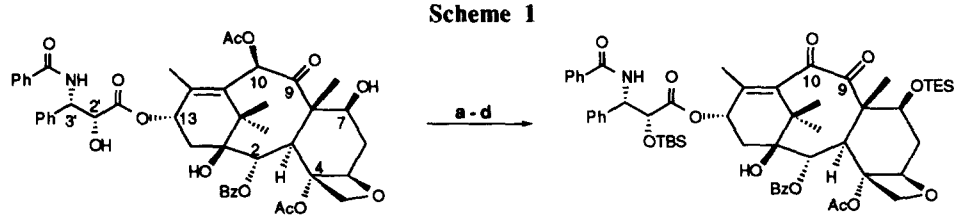
Abstract : A method has been developed for the syntheses of hitherto unreported C-9 and C-10 modified taxanes. These analogs demonstrated excellent to good cytotoxicity against B16 melanoma cells.

Paclitaxel (Taxol®, **1**), an antimitotic agent of natural origin,[1] has been an exciting discovery in the field of cancer chemotherapy.[2] The promising clinical activity, structural complexity and unique mode of action has made paclitaxel a subject of intense synthetic investigations and structure-activity relationship studies.[3] In continuation of our ongoing program aimed at modification of functionalities at the baccatin core,[4-8] we were interested in studying C-9 keto modified analogs of paclitaxel. Klein and collaborators have recently reported on the semisynthesis of 9α-hydroxypaclitaxel and analogs, starting from naturally occurring 13-acetyl-9α-hydroxybaccatin III.[9] These analogs exhibited comparable cytotoxicity and improved water solubility indicating the importance of C-9 keto modified taxanes in designing new generations of potent analogs. Unfortunately, the C-9 carbonyl group is highly unreactive towards conventional reducing agents and only a few methods have been reported for the reduction at the C-9 position.[10-13] In the light of these observations we contemplated a different approach and decided to utilize the hydroxy (acetoxy) group at C-10 towards activation of the C-9 carbonyl. We reasoned that, conversion of the C-10 acetate to a keto group will enhance the reactivity of the adjacent C-9 center and also will reduce steric bulk around C-9 thereby facilitating reactions at the C-9 carbonyl.

Scheme 1



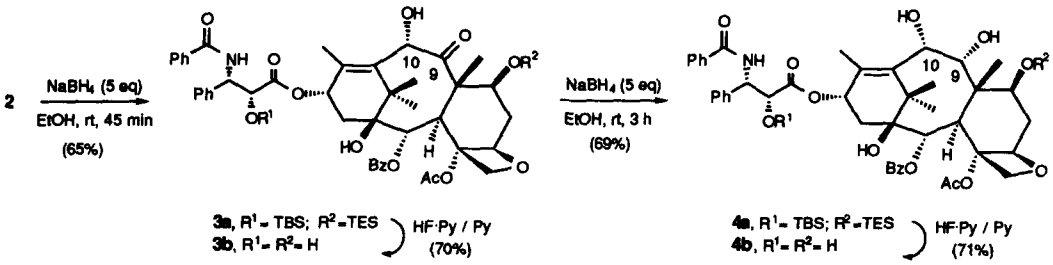
1 **2**

a. TBSCl, imidazole, CH_2Cl_2, 93%. b. $N_2H_4{\cdot}H_2O$, EtOH, 82%.
c. TESCl, DMAP, CH_2Cl_2, 90%. d. Pr_4NRuO_4, NMO,CH_2Cl_2, 79%.

The synthesis of 10-ketopaclitaxel analog **2** was carried out as shown in Scheme 1. Selective deacetylation of paclitaxel at C-10 was performed according to our recently developed protocol,[14] where initial protection of the 2'-hydroxy group as its *tert*-butyldimethylsilyl (TBS) ether followed by hydrazinolysis of the C-10 acetate afforded the corresponding 10-deacetyl derivative. Selective protection of the C-7 hydroxy group as its triethylsilyl (TES) ether and subsequent oxidation of the C-10 hydroxy group yielded 10-keto analog **2** in good overall yield. Having synthesized 10-keto intermediate **2**, we proceeded to study its reactivity towards reduction. Thus, when **2** was reacted with NaBH4 (5 eq) in ethanol the only product formed was the corresponding 10α-

hydroxy analog **3a** (Scheme 2), formed by stereoselective reduction of the C-10 carbonyl group. The site of reduction and stereochemistry of the product was assigned on the basis of cross-peak connectivities in an HMBC-NMR experiment and NOE studies. The alpha stereochemistry at C-10 can be rationalized via an approach of the nucleophile from the less hindered convex side of the molecule affording 10-*epi* paclitaxel analog **3a**.

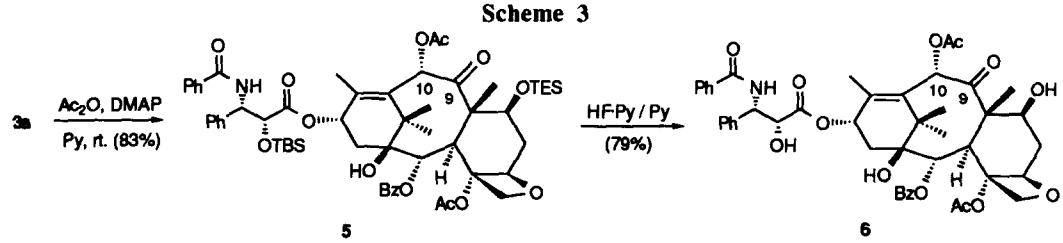
Scheme 2



Interestingly, reaction of **2** with excess $NaBH_4$ for prolonged time showed the formation of a more polar minor product (18%) along with the expected 10α-hydroxy analog **3a** (54%). On the basis of spectral and analytical data the new product was found to be the C-9, C-10 di-reduced analog **4a**. The cis-relationship between the C-9 and C-10 substituents was confirmed by NOE experiments and the coupling constant between H-9 and H-10 ($J_{H\text{-}9,H\text{-}10}$ = 3.7 Hz). *To the best of our knowledge compound **4a** is the first example of a taxane where the C-9 and C-10 substituents are present in a cis-alpha configuration.* The surprising ease of reduction of the C-9 carbonyl can probably be attributed to the inversion of stereochemistry at the neighboring C-10 where the bulkier hydroxy group is now at the concave face of the molecule thereby facilitating the approach of the incoming nucleophile from the less hindered convex face resulting in the formation of **4a**. The yield of **4a** could be improved considerably by first isolating the mono-reduced product **3a** and then reacting it with $NaBH_4$ (Scheme 2) to obtain the di-reduced product, which also confirmed the intermediacy of **3a** in product formation. The 10-*epi* analog **3a** and the C-9, C-10 dihydroxy analog **4a** on deprotection under standard conditions afforded the C-9 and C-10 modified paclitaxel analogs **3b** and **4b** (Scheme 2).

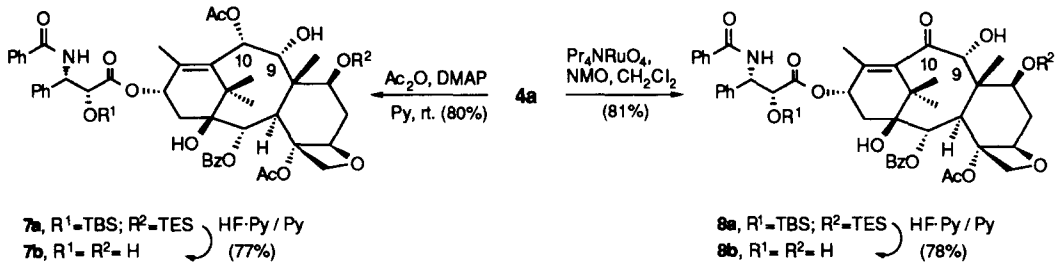
The efficiency of the above method in performing regio- and stereoselective reduction of the carbonyl groups at C-9 and C-10 encouraged us to undertake further modifications at these positions. Thus, with a view to synthesize hitherto unreported 10-*epi*-paclitaxel (**6**, Scheme 3), the 10α–hydroxy paclitaxel analog **3a** was treated with excess acetic anhydride and DMAP in pyridine yielding the 2',7-diprotected 10-*epi* -paclitaxel analog **5** in high yield.

Scheme 3



Subsequent removal of the silyl protecting groups afforded 10-*epi* paclitaxel (**6**) in good overall yield. It is worth mentioning that our initial efforts to synthesize 10-*epi*-paclitaxel under Mitsunobu reaction conditions were not successful, showing the usefulness of the present method. Synthetic modifications of the 9,10-dihydroxy analog **4a** were next investigated. Reaction of **4a** with an excess of acetic anhydride and DMAP in pyridine afforded the corresponding 10-acetylated analog **7a** (Scheme 4) in high yield. Attempted acylation of the C-9 hydroxy group under the above reaction condition was not successful. Deprotection of the 2' and 7 silyl groups of **7a** furnished 9α-hydroxy-10-*epi*-paclitaxel (**7b**) in good yield.

Scheme 4



The selective acylation of the C-10 hydroxyl in preference to the hydroxy group at C-9 opened up interesting possibilities towards further modification of the paclitaxel B-ring. Thus, on oxidation with a catalytic amount of tetrapropylammonium perruthenate in the presence of *N*-methylmorpholine-*N*-oxide (NMO) the expected 10-keto analog **8a** (Scheme 4), formed by selective oxidation at C-10, was obtained in 81% yield. Deprotection under standard reaction conditions then afforded the novel 9,10-keto transposed 9α-hydroxy-10-ketopaclitaxel (**8b**) in good overall yield.

The C-9 and C-10 modified taxanes[15] **3b**, **4b**, **6**, **7b**, and **8b** were evaluated for their ability to promote the assembly of microtubules and for their cytotoxicity against B16 melanoma cells.[16]

Table: *In vitro* biological evaluation of analogs **3b**, **4b**, **6**, **7b**, and **8b**

Compound	Microtubule assembly[a] ED_{50}/ED_{50} (paclitaxel)	B16 melanoma cytotoxicity[b] ED_{50}/ED_{50} (paclitaxel)
1	1.0	1.0
3b	0.75	0.62
4b	0.79	4.5
6	0.42	0.73
7b	1.2	3.6
8b	1.2	1.2

[a]ED_{50} = concentration which causes polymerization of 50% of the tubulin present, in 15 min at 37 °C. Relative to paclitaxel = 1.0. [b]ED_{50} = concentration which produces 50% inhibition of proliferation after 40 h of incubation. Relative to paclitaxel = 1.0.

Both the C-10 epi taxanes, **3b** and **6**, proved to be quite active and displayed increased activity in the microtubule assembly assay and cytotoxicity relative to paclitaxel (Table). The C-9, C-10 dihydroxylated

analogs **4b** and **7b** also exhibited microtubule assembly properties comparable to paclitaxel, however in the B16 melanoma cytotoxicity assay both analogs were 3-4 times less cytotoxic. The keto transposed analog **8b** was found to be comparable to paclitaxel in both assays.

In view of the above results it is reasonable to assume that stereochemical inversion of the oxygen functionality at C-10 has a positive effect on biological activity and opens up the possibility of synthesizing analogs with improved cytotoxic activity through such modifications. Further studies are in progress.

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