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Deletion of the Oxetane Ring in Docetaxel Analogues: Synthesis and Biological Evaluation

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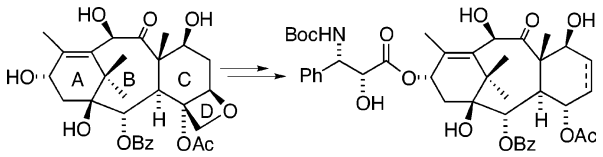
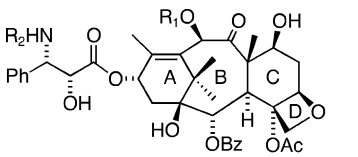
ABSTRACT



Two new docetaxel analogues have been prepared starting from 10-deacetylbaccatin III. Both derivatives lack the oxetane D-ring but possess the 4α-acetoxy group, which is important for biological activity. The influence of a more or less constrained C-ring was evaluated by adding, or not adding, a double bond in this ring. Both compounds were found to be equally less active than docetaxel in biological assays.

Paclitaxel, a complex diterpenoid **1a**, and its semisynthetic analogue docetaxel **1b** are the most important anticancer drugs developed in the past decade for the treatment of ovarian, breast, and nonsmall cell lung cancers.[1] The unique mechanism of action of paclitaxel and its densely functionalized, stereochemically rich framework has elicited a great deal of interest in structure−activity relationship studies for the development of more potent analogues. These studies have shown that the C13 ester side chain and the ester groups at C2 and C4 are essential for biological activity.[2]



Paclitaxel (Taxol®) **1a** R_1=Ac, R_2=Bz
Docetaxel(Taxotere®) **1b** R_1=H, R_2=Boc

In the past 10 years, significant efforts have been made to establish the actual contribution of the unique oxetane D-ring in the interaction with microtubules and cytotoxicity. Several synthesized D-ring analogues[3] have been found to show some interaction with microtubules, though generally to a lesser extent than paclitaxel or docetaxel, while the D-seco analogues were weakly cytotoxic[4] or totally inactive.[5]

(1) (a) *Paclitaxel in Cancer Treatment*; McGuire, W. P., Rowinsky, E. K., Eds.; Marcel Dekker: New York, 1995. Vol. 8, pp 1−349.

(2) For general reviews on taxoid chemistry and structure−activity relationship studies, see: (a) *Taxol: Science and Applications*; Suffness, M., Ed.; CRC Press: Boca Raton, FL, 1995. (b) *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series 583; American Chemical Society: Washington, DC, 1995. (c) *The Chemistry and Pharmacology of Taxol and its Derivatives;* Farina, V., Ed.; Elsevier: Amsterdam, 1995. (d) Kingston, D. G. I. *J. Nat. Prod* **2000**, *63*, 726−734. (e) Kingston, D. G. I.; Yuan, H.; Jagtap, P. J.; Samala, L. The Chemistry of Taxol and Related Taxoids. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, Ch., Eds.; Springer-Verlag: New York, 2002; Vol. 84.

(3) (a) Marder-Karsenti, R.; Dubois, J.; Bricard, L.; Guénard, D.; Guéritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631−6637. (b) Gunatilaka, A. A. L.; Ramdayal, F. D.; Sarragiotto, M.; Kingston, D. G. I.; Sackett, D. L.; Hamel, E.; *J. Org. Chem.* **1999**, *64*, 2694−2703. (c) Mercklé, L.; Dubois, J.; Place, E.; Thoret, S.; Guéritte, F.; Guénard, D.; Poupat, C.;Ahond, A.; Potier, P. *J. Org. Chem* **2001**, *66*, 5058−5065. (d) Dubois, J.; Thoret, S.; Guéritte, F.; Guénard, D. *Tetrahedron Lett.* **2000**, *41*, 3331−3334.

(4) (a) Beusker, P. H.; Veldhuis, H.; Van den Bossche, B. A. C.; Scheeren, H. W. *Eur. J. Org. Chem.* **2001**, 1761−1768. (b) Beusker, P. H.; Veldhuis, H.; Brinkhorst, J.; Hetterscheid, D. G. H.; Feichter, N.; Bugaut, A.; Scheeren, H. W. *Eur. J. Org. Chem.* **2003**, 689−705.

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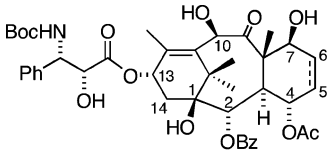
Interestingly, calculations by the Snyder group using a minireceptor approach and a refined model of the paclitaxel-β-tubulin binding pocket predicted that derivatives with oxygenated substituents at C4 and an appropriately placed double bond maintain paclitaxel-like activity.[6]

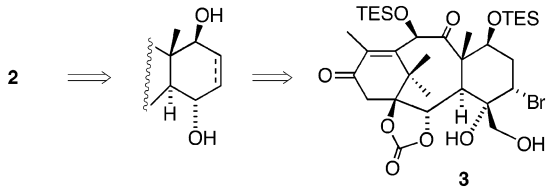
In the present communication, we report our continuing endeavor toward the synthesis of D-ring-modified analogues of docetaxel. Our target was the synthesis of the simple D-seco docetaxel analogue **2a** lacking the oxetane ring. This kind of compound has been predicted by the Snyder group's calculations to be well accommodated in the β-tubulin binding pocket.[6] To evaluate the influence of a more constrained C-ring on the biological activity, the synthesis of 5,6-dehydro derivative **2b** has also been designed.



2a : 5,6-saturated
2b : 5,6-dehydro

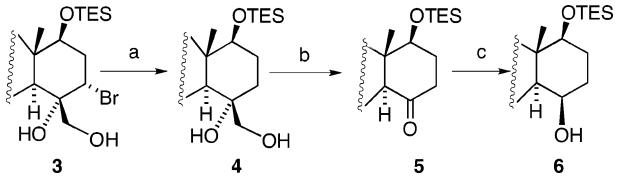
We chose to proceed via our key intermediate **3**, prepared from 10-deacetylbaccatin III and successfully used for the synthesis of our D-modified docetaxel analogues.[3a,c,d] This compound can be debrominated or dehydrobrominated and the 1,2-diol transformed by oxidative cleavage and stereoselective reduction to the α-C4 alcohol. Subsequent acetylation and introduction of the C2 and C13 functionalities could afford the desired compounds **2a** and **2b** (Scheme 1).

Scheme 1



After a number of experiments with several reducing agents, debromination of **3** was achieved with Pd/C in MeOH under an atmosphere of hydrogen. Oxidative cleavage of the vicinal diol in **4** with $Pb(OAc)_4$ gave the ketone **5**, reduction of which with sodium borohydride proceeded with complete facial selectivity yielding the undesired β-hydroxide **6** as previously reported[4a,7] (Scheme 2). Mitsunobu reaction on the β-hydroxide to obtain acetylation with inversion of configuration proved to be unsuccessful.

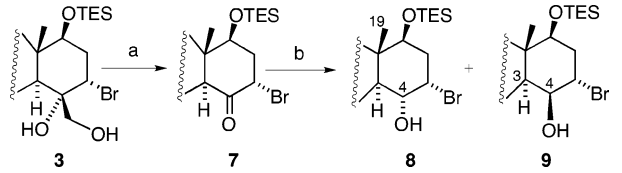
Scheme 2 [a]



[a] Reagents and conditions: (a) Pd/C, MeOH, H_2, rt (98%); (b) $Pb(OAc)_4$, CH_3CN, 0 °C (98%); (c) $NaBH_4$, MeOH, 0 °C (80%).

We then decided to carry out the oxidative cleavage before removal of the bromine, as we hypothesized that the presence of the C5-α bromine would sufficiently hinder the α face of the molecule to facilitate hydride delivery from the β-face during the reduction of the C4-ketone. Oxidative cleavage of compound **3** afforded the desired ketone **7** in high yield, and as expected, reduction of the C4-ketone with sodium borohydride was less stereoselective, affording 30% of the α-hydroxide **8** along with 50% of the β-isomer **9** (Scheme 3). The stereochemistry of the C4 hydroxyl group was

Scheme 3 [a]



[a] Reagents and conditions: (a) $Pb(OAc)_4$, CH_3CN, 0 °C (99%); (b) $NaBH_4$, MeOH, 0 °C, **8** (30%) and **9** (50%) or BH_3·Me_2S, (*R*)-2-methyl CBS-oxazaborolidine, CH_2Cl_2, −20 °C, **8** (63%) and **9** (22%).

determined by NOE experiments on both compounds **8** and **9**. Compound **8** showed interactions between H4/H2 and H4/$CH_3$19, characteristic of the β-orientation of H4, whereas compound **9** exhibited the same correlations (H4/H3 and H4/H14α) as compound **6**.

To increase the yield of the α-isomer **8**, several other reducing agents (L- and K-Selectride, lithium triethylborohydride, lithium tri-*tert*-butoxyaluminohydride, lithium borohydride) were tried but did not give the desired reduction product, in many cases because of concomitant debromination. Modification of the reaction conditions (solvent, temperature) did not really improve the α-isomer yield. Finally, the best result was obtained with $BH_3.Me_2S$ in the presence of (*R*)-2-methyl CBS-oxazaborolidine catalyst,[8] which afforded the desired α-isomer in 63% yield along with 22% of the β-isomer.

Acetylation of **8** with acetic anhydride/DMAP proved to be facile, affording the acetate **10** in high yield. It should be noticed that, using the same conditions, compound **9** has also

(5) (a) Barboni, L.; Dutta, A.; Dutta, D.; Georg, G. I.; Vander Velde, D. G.; Himes, R. H.; Wang, M.; Snyder, J. P. *J. Org. Chem.* **2001**, *66*, 3321−3329. (b) Samaranayake, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114−5119.

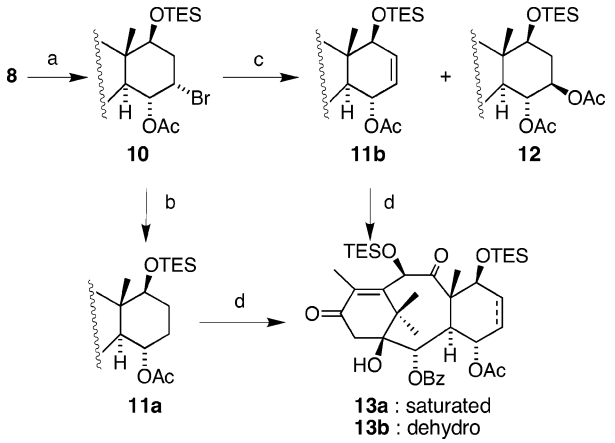
(6) Wang, M.; Cornett, B.; Nettles, J.; Liotta, D. C.; Snyder, J. P. *J. Org. Chem.* **2000**, *65*, 1059−1068.

(7) β-Configuration of the C4 hydroxyl group was proved by NOESY experiments, which showed H4/H3 and H4/H14α correlations.

(8) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, *109*, 5551−5553.

been easily acetylated, contrary to what was reported by Beusker et al. on their compound deprived of any functionality at C5.[4a] Therefore, the presence of the C5-α-Br alters the conformation of the molecule to render the 4β-hydroxy group sterically less hindered. Debromination of **10** by hydrogen in the presence of Pd/C afforded **11a** in good yield. Dehydrobromination proved to be more difficult. A number of bases (DBU, DBN, lithium or potassium carbonate, etc.) and experimental conditions (solvent, temperaure) were tested for this reaction. Compound **11b** was obtained with the best yield by heating **10** in DMF in the presence of potassium acetate. However this reagent afforded also the 5-acetyl derivative **12** as a side product. This intermolecular substitution of the 5-α-bromine has never been reported before, but this may be due, in our case, to the absence of a methyl or methylene group at position 4-β. Then, the C1−C2 carbonate of **11a** and **11b** was readily opened by phenyllithium as previously described[3a] (Scheme 4).

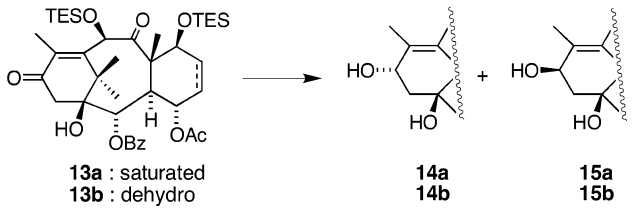
Scheme 4 [a]



[a] Reagents and conditions: (a) Ac_2O, pyridine, DMAP, CH_2Cl_2, 40 °C, (86%); (b) H_2, Pd/C, MeOH, (88%); (c) KOAc, DMF, 80 °C, **11b** (48%) and **12** (12%); (d) PheLi, THF, −78 °C, **13a** (67%) and **13b** (61%).

Contrary to our earlier results,[3a,c,d] the reduction of the C13 ketone with $NaBH_4$ was no longer stereoselective. The saturated derivative **13a** led to the C13-α and C13-β isomers in 30 and 47% yields, respectively, whereas the unsaturated analogue **13b** gave rise to the same isomers in 45 and 25% yields, respectively[9] (Scheme 5). In that case, modification of the reducing agent or addition of a chiral reagent could not improve the stereoselectivity. In the literature, borohydride reduction of the C13-ketone leading to the C13-β-hydroxy group has only been reported for 4-deacetyl-7-(triethylsilyl)-13-oxo-baccatin III, where the 4-hydroxy group participates in a transannular delivery of the hydride.[10] The difference in the stereoselectivity for the saturated and

Scheme 5 [a]

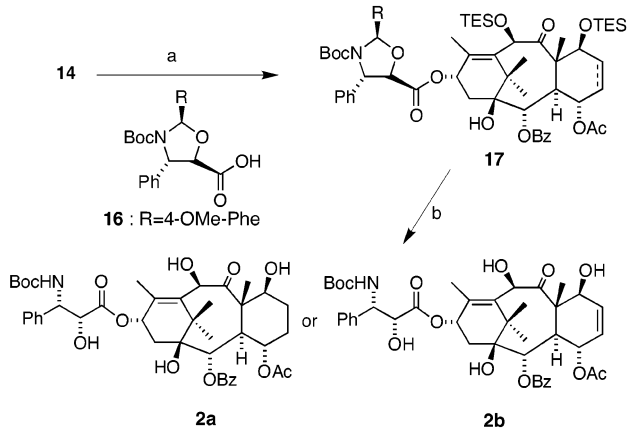


[a] Reagents and conditions: $NaBH_4$, THF/MeOH (6:1), rt, **14a** (30%) and **15a** (47%); **14b** (45%) and **15b** (25%).

dehydro compounds shows that the double bond induces a conformational change in the C-ring and probably a different position of the 4-acetyl group that restrains the accessibility of the hydride to the α face.

Esterification was then realized with the 2-(4-OMe)phenyl 1,3-oxazolidine of *N*-Boc-phenylisoserine **16**, DCC, and DMAP in toluene at room temperature, affording **17** in good yield. Removal of all the protective groups in one step with *p*-toluenesulfonic acid in methanol afforded the desired compounds **2a** and **2b** (86 and 50%, respectively) (Scheme 6).

Scheme 6 [a]



[a] Reagents and conditions: (a) **16**, DCC, DMAP, toluene, rt **17a** (93%), **17b** (88%); (b) PTSA, MeOH, rt, **2a** (86%), **2b** (50%).

These compounds were found to be less active than docetaxel, 70 times less in the microtubule disassembly assay[11] and up to a 1000 times less cytotoxic against KB cell lines.[12] No difference was observed between the two compounds, indicating that an additional constraint on the C-ring is not sufficient to restore the activity. It is to be noted that these derivatives are the first D-seco analogues retaining an interaction, though comparatively weak, with microtubules.

(9) The stereochemistry of each compounds was assigned by NOE experiments.

(10) Hoemann, M. Z.; Vander Velde, D. G.; Aubé, J.; Georg, G. I. *J. Org. Chem.* **1995**, *60*, 2918−2921.

(11) Lataste, H.; Sénilh, V.; Wright, M.; Guénard, D.; Potier, P. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4090−4094.

(12) Da Silva, A. D.; Machado, A. S.; Tempête, C.; Robert-Gero, M. *Eur. J. Med. Chem.* **1994**, *29*, 149−152.

As previously described by Boge et al.,[13] the absence of the oxetane ring slightly modifies the overall conformation of the taxane skeleton. Our compounds **2a** and **2b** display changes similar to those of D-secopaclitaxel, especially for the A-ring as shown by H14 chemical shifts and coupling constants (Table 1).

Table 1. Comparison of Selected NMR Data and Coupling Constants of Compounds **2a** and **2b** in $CDCl_3$ with Docetaxel and D-Secopaclitaxel

	H14α	H14β	$J_{13,14\alpha}$	$J_{13,14\beta}$	$J_{2,3}$
docetaxel **1b**	2.28	2.18	8 Hz	8 Hz	7 Hz
D-secopaclitaxel[13]	3.08	2.45	4.4 Hz	10.3 Hz	5.3 Hz
2a	2.98	2.46	6 Hz	10 Hz	5.5 Hz
2b	2.88	2.45	6 Hz	10 Hz	6.5 Hz

The presence of the 5,6-double bond in the C-ring in **2b** does not change the A-ring conformation but induces only a modest conformational change, as seen with the H2,H3 coupling constant (Table 1). NOESY experiments have not shown many differences in the conformational behavior in solution except a much weaker NOE between H2′ and 4-OAc than in docetaxel, suggesting a slightly different position for the 4-acetoxy group that was confirmed by molecular modeling studies.

Contrary to 4-deacetyldocetaxel,[14] molecular modeling studies and NOE experiments have not shown any significant modification in the position of the side chain when the oxetane D-ring is absent. The overall conformation of these derivatives does not seem to be very different from that of docetaxel.

In conclusion, contrary to what was predicted by Snyder's model,[6] deletion of the oxetane ring is detrimental to biological activity. This small ring comprises three important features: an oxygen atom, a constrained four-membered ring, and a methylene group in a 1,3-diaxial interaction with the 19-methyl group. Deletion of the oxetane ring resulted in suppression of all these features. The oxygen atom has been shown not to be essential for tubulin interaction since 5(20)-deoxydocetaxel is equipotent to paclitaxel in the microtubule disassembly assay.[3d,15] The constraint imposed by the small ring actually influences the overall conformation of the diterpene moiety as seen by NMR data in D-secopaclitaxel[13] and in compounds **2**. An additional double bond on the C-ring is not sufficient to restore good affinity for microtubules, suggesting that a constrained C-ring is not the essential factor in the oxetane ring. The main difference between docetaxel and compounds **2** lies in the C-ring conformation and, therefore, in the orientation of the 4-acetoxy group. This result suggests that the biological activity is very sensitive to the position of this acetoxy group relative to the C13-side chain. In compounds **2**, the absence of a methylene or a methyl group at position 4β may be critical for the conformation of the C-ring. It is likely that the 1,3-diaxial interaction between the 19-methyl group and a 4β-bulky group would induce a conformation of the C-ring that better fits the tubulin binding site. To check this hypothesis, new D-seco compounds with a 20-methyl group and the 4α-acetoxy moiety are currently being synthesized in our laboratory.

Acknowledgment. The authors thank Dr. Alain Commerçon (Aventis-Pharma) for a gift of the docetaxel side chain. Jean-François Gallard is acknowledged for performing NOESY and ROESY experiments and Christiane Gaspard for cytotoxicity evaluations.

Supporting Information Available: Experimental procedures and full characterization data for compounds **2a**, **2b**, **4**−**15**, and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Guéritte-Voegelein, F.; Guénard, D.; Dubois, J.; Wahl; A.; Marder, R.; Müller, R.; Lund, M.; Bricard, L.; Potier, P. In *Taxane Anticancer Agents: Basic Science and Current Status;* Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series 583; American Chemical Society: Washington, DC, 1995; pp 189−202.

(15) However, 5(20)-deoxydocetaxel is about 100-fold less cytotoxic than docetaxel against KB cell line (IC_{50}(5(20)-deoxydocetaxel) = 0.06 μM).