ORGANIC LETTERS

2006
Vol. 8, No. 11
2301−2304

Semisynthesis and Biological Evaluation of a Novel D-Seco Docetaxel Analogue[#]

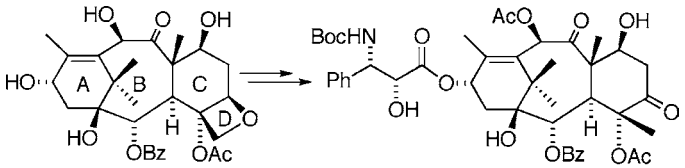
Sylviane Thoret, Françoise Guéritte, Daniel Guénard, and Joëlle Dubois*

Institut de Chimie des Substances Naturelles-CNRS, avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

joelle.dubois@icsn.cnrs-gif.fr

Received March 3, 2006

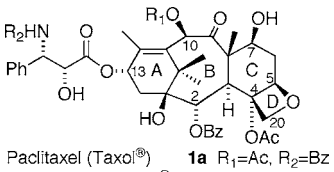
ABSTRACT



10-deacetylbaccatin III → **4**

A 4-methyl-5-oxo docetaxel analogue has been prepared starting from 10-deacetylbaccatin III. This new D-seco docetaxel analogue is slightly less potent than docetaxel at microtubule stabilization in vitro and has about 1/1000th the cytotoxicity of docetaxel. The lack of improved activity for this compound compared to other D-modified taxoids confirms that a C-5 oxygen atom is not required for biological activity.

Paclitaxel (Taxol, **1a**)[1] and its semisynthetic analogue docetaxel (Taxotere, **1b**) are important anticancer agents useful for the treatment of breast, ovarian, and non-small lung cancers[2] and are also active against prostate cancer.[3] They act by stabilizing microtubules, thereby blocking cell-cycle progression during mitosis.[4]



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

Since their discovery, these compounds have been the subject of intense chemical, biological, and clinical investigations. Especially, extensive SAR studies have shown that the C-13 side chain, the ester groups at C-2 and C-4, and the rigid core to which these moieties are attached are essential for biological activity.[5] The contribution of the oxetane D-ring to the biological activity of taxoids has also been the subject of extensive studies in the past decade. With respect to microtubule binding, it may have two functions: (i) it contributes to rigidify the taxoid core and thereby impose a specific orientation of the C-2, C-4, and C-13 side chains, or/and (ii) the oxygen atom at C-5 is involved in a stabilizing dipolar or hydrogen-bonding interaction with an amino acid of the tubulin binding site. Many derivatives have been designed to elucidate the actual contribution of the oxetane ring in microtubule binding.

Either D-modified or D-seco derivatives have been synthesized. In the first series, the oxygen atom has been substituted by nitrogen,[6] sulfur,[7] or selenium[7a] atoms or

[#] Dedicated to Pr Pierre Potier.

* To whom correspondence should be addressed. Phone: 33(0)-169823058. Fax: 33(0)169077247.

(1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325−2327.

(2) Guénard, D.; Guéritte-Voegelein, F.; Lavelle, F. *Curr. Pharm. Des.* **1995**, *1*, 95−112.

(3) De Wit, R. *Eur. J. Cancer* **2005**, *41*, 502−507.

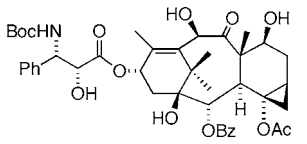
(4) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665−667.

(5) For recent reviews, see: (a) Guéritte, F. *Curr. Pharm. Des.* **2001**, *7*, 1229−1249. (b) Kingston, D. G. I.; Jagtap, P. J.; Yuan, H.; 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, C., Eds.; Springer-Verlag: Wien & New York, 2002; Vol. 84, pp 53−225.

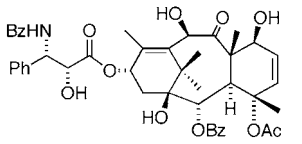
(6) Marder-Karsenti, R.; Dubois, J.; Bricard, L.; Guénard, D.; Guéritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631−6637.

(7) (a) 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. (b) 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.

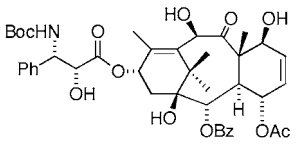
10.1021/ol060531d CCC: $33.50
Published on Web 04/27/2006



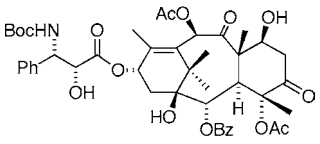
2



3



4



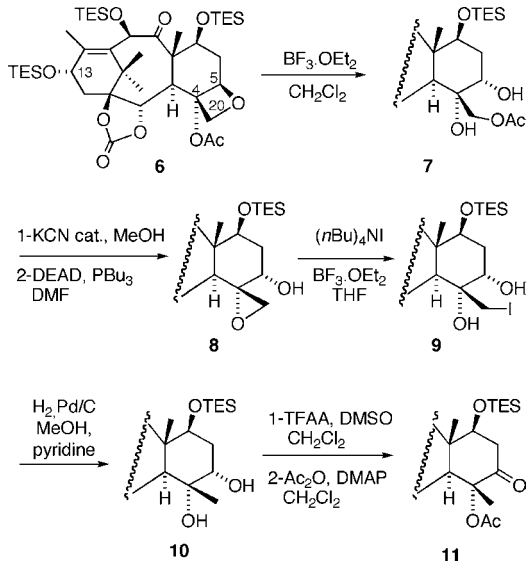
5

deleted.[8] In the D-seco series, only compounds devoid of an oxygen atom at the C-5 position and/or of the C-4 acetyl group have been synthesized.[9]

The most active compounds in both series are 5(20)-deoxydocetaxel **2**[8] and the 4-methyl paclitaxel analogue **3**[9f] which are almost as active as their parent compounds on the tubulin assay but are much less cytotoxic. The closely related compound **4** that lacks the 20-methyl group has dramatically reduced microtubule-stabilizing activity as well as low cytotoxicity. It has been suggested that this activity loss could come from the modified C-ring conformation and acetoxy orientation.[9e] Therefore, it has been proposed from all these results that the D-ring is not necessary for tubulin activity as long as the overall conformation of the taxane skeleton, as well as the position of the C-4 acetoxy group relative to the C-13 side chain, is maintained.[9e,f] However, compounds **2** and **3** are less cytotoxic than their parent compounds (100- and 400-fold respectively), and we wondered if the absence of the oxygen atom at C-5 could be responsible for this reduced cytotoxicity. Therefore, to evaluate the importance of the C-5 oxygen atom on biological activity, we designed and synthesized the D-seco compound **5**, bearing a methyl group at C-4 and a ketone at C-5.

Our starting material was 10-deacetylbaccatin III (DAB) suitably modified to allow the construction of the C-5 ketone together with the C-4 β-methyl group. The oxetane ring of compound **6**, obtained according to the reported procedure,[10] was opened with $BF_3{\cdot}OEt_2$ to afford compound **7** bearing an oxygen in the C-5 position (Scheme 1). Compound **7** was deacetylated under mild conditions, and the C-20 OH was reduced under the same conditions as those described by Barboni et al.[9f] Formation of the epoxide **8** occurred in high yield under the same conditions as those reported for the synthesis of 5(20)deoxydocetaxel.[9e] Conditions for the epoxide opening with an iodide ion had to be modified for compound **8** because the reported conditions afforded the C-20 iodo derivative **9** in very poor yield. The use of THF as solvent instead of CH_2Cl_2 and 2 equiv of Lewis acid gave compound **9** in good yield, and this was hydrogenolyzed to afford compound **10** in 85% yield. The C-5 hydroxyl group was then oxidized to ketone using trifluoroacetic anhydride-activated DMSO in CH_2Cl_2, and finally, the C-4 OH was acetylated under classical conditions (Scheme 1). Compound **11** was thus obtained in seven steps from modified 10-deacetylbacatin III **6** with 21% overall yield. It should be noted that only this sequence of reactions can afford compound **11** from **6** in acceptable yield because oxidation of the C-5 OH prior to deacetylation gives rise to unexpected side reactions during acetyl removal.

Scheme 1



Whereas the C-1,C-2 carbonate of **11** was readily opened by phenyllithium in good yield (Scheme 2), further modifications to complete the synthesis of the docetaxel analogue **5** were troublesome.

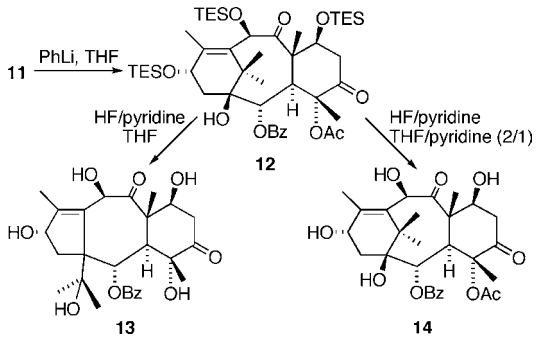
The main difficulty lies on the removal of the protecting groups. Under the usual conditions to remove the silyl protecting groups on taxoids (HF/pyridine complex), the A-ring of compound **12** underwent a Wagner−Meerwein-type rearrangement that has already been observed on taxoids but under more acidic conditions (Scheme 2).[11]

(8) Dubois, J.; Thoret, S.; Guéritte, F.; Guénard, D. *Tetrahedron Lett.* **2000**, *41*, 3331−3334.

(9) (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. (c) Beusker, P. H.; Veldhuis, H.; Van den Bossche, B. A. C.; Scheeren, H. W. *Eur. J. Org. Chem.* **2001**, 1761−1768. (d) 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. (e) Deka, V.; Dubois, J.; Thoret, S.; Guéritte, F.; Guénard, D. *Org. Lett.* **2003**, *5*, 5031−5034. (f) Barboni, L.; Giarlo, G.; Ricciutelli, M.; Ballini, R.; Georg, G. I.; VanderVelde, D. G.; Himes, R. H.; Wang, M.; Lakdawala, A.; Snyder, J. P. *Org. Lett.* **2004**, *6*, 461−464.

(10) Ojima, I.; Lin, S.; Inoue, T.; Miller, M. L.; Borella, C. P.; Geng, X.; Walsh, J. J. *J. Am. Chem. Soc.* **2000**, *122*, 5343−5353.

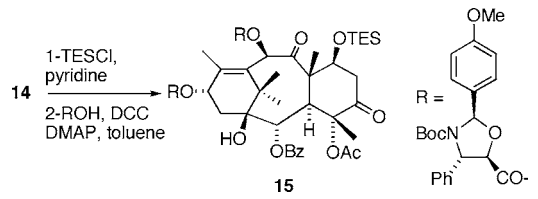
Scheme 2



Many other deprotection conditions (HCOOH, PTSA, HCl, TFA, and CAN) were tried, leading either to rearranged compound **13** or to starting material **12**. We suspected compound **12** to be more sensitive to an acidic medium than other DAB derivatives, and so we added pyridine to the HF/pyridine complex to buffer the reaction medium. As expected, the fully deprotected compound **14** was obtained in 85% yield under these conditions (Scheme 2).

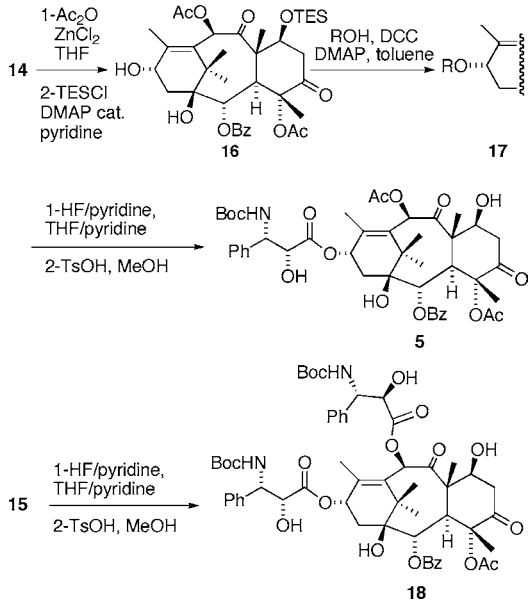
The next step in our synthesis was to introduce the docetaxel side chain selectively at C-13. It has been reported that a sterically bulky group at C-7, such as TES, prevents enzymatic acylation at C-10.[12] Furthermore, the phenylisoserine side chain has been introduced selectively at C-13 on 2-debenzoyl 4-deacetyl 7-TES DAB[13a] and 2-debenzoyl 7-TES DAB.[13b] We thought the chemical acylation by the protected docetaxel side chain after selective silylation at the C-7 OH of compound **14** would be worth trying.[14] However, under our usual conditions, both the C-10 and C-13 hydroxyl groups were acylated affording compound **15** (Scheme 3).

Scheme 3



Because 10-acetyldocetaxel is as active as docetaxel, we decided to acetylate the C-10 OH first according to the reported procedure[15] and then to protect the C-7 alcohol with a TES group to introduce the docetaxel side chain selectively at the C-13 position. Surprisingly, the formation of the 7-triethylsilyl-10-acetylbaccatin III derivative **16** proved to be difficult. Silylation conditions had to be modified, and the addition of a catalytic amount of DMAP together with heating were the only conditions that were able to afford **16**. After acylation of the C-13 OH, full deprotection of compound **17** was carried out in two steps: removal of the TES group and then opening of the oxazolidine ring of the docetaxel side chain (Scheme 4).

Scheme 4



Compound **15** was also deprotected under the same conditions, affording compound **18** for biological evaluation (Scheme 4).

Then, biological activities of compounds **5** and **18** were evaluated on a cold-induced microtubule disassembly assay and in an antiproliferative assay on KB cell lines.

Both compounds are less active than docetaxel **1b** and 5(20)deoxydocetaxel **2** (Table 1). Addition of an oxygen atom to the same position as in the oxetane ring does not improve the biological activity of D-seco taxoids. On the basis of NMR data, it can be stated that the overall conformation of compound **5** is identical to that of docetaxel. As described for docetaxel and the two biologically active D-modified taxoids, 5(20)deoxydocetaxel **2**[8] and compound **3**,[9f] the coupling constant between H-2 and H-3 is 7.0 Hz; H-13 has nearly equal coupling to both protons at C-14, and the signal is a broad triplet (J_{13-14} = 8 Hz). Therefore, the slight loss of activity may not be due to conformational changes in compound **5**.

(11) (a) Samaranaryake, G.; Magri, N. F.; Jitrangari, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114−5119. (b) Wahl, A.; Guéritte-Voegelein, F.; Guénard, D.; Le Goff, M.-T.; Potier, P. *Tetrahedron* **1992**, *48*, 6965−6974.

(12) Lee, D.; Kim, K.-C.; Kim, M.-J. *Tetrahedron Lett.* **1998**, *39*, 9039−9042.

(13) (a) 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 Applications*; Georg, I. G., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1995; pp 189−202. (b) Kingston, D. G. I. *Ibid.* pp 203-216.

(14) Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917−5919.

(15) Holton, R. A.; Zhang, Z.; Clarke, P. A.; Nadizadeh, H.; Procter, D. J. *Tetrahedron Lett.* **1998**, *39*, 2883−2886.

Table 1. Results of the Biological Evaluation of Compounds **5** and **18**

compound	microtubule-disassembly inhibitory activity[a] IC_{50}/IC_{50}(paclitaxel)	cytotoxicity against KB cell line[b] IC_{50} (nM)
1b	0.5	0.6
2	1.2	60
5	3.5	600
18	10	3000

[a] IC_{50} is the concentration that inhibits 50% of the rate of microtubule disassembly. The ratio IC_{50}/IC_{50}(paclitaxel) gives the activity with respect to paclitaxel. IC_{50}(paclitaxel) = 1 μM. [b] IC_{50} measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h of incubation.

Compound **18** is less active than **5** showing that the addition of a second docetaxel side chain at C-10 is detrimental to the activity. This result is not surprising because it has already been reported that the addition of a cinnamyl side chain at C-10 reduces the tubulin activity.[16]

In summary, a novel D-seco taxoid **5** bearing an oxygen atom at the same position as in the oxetane ring has been synthesized. The 10-deacetylbaccatin III derivative **12** displays unusal chemical behavior, in particular, an increased sensitivity to an acidic medium compared to the other D-modified DAB derivatives already described.[6,7b,8,9e,10] This reactivity has made the complete transformation of **12** to docetaxel analogue **5** very complicated.

Contrary to our expectation, addition of an oxygen atom at C-5 on D-seco taxoids does not improve the antiproliferative nor the microtubule-stabilizing activities. These activities are even slightly reduced in the tubuline assay and are lower by an order of magnitude for cytotoxicity. However, they are comparable to that of thia derivatives of docetaxel.[7b]

Taking the results on D-modified taxoids together, it can be concluded that the oxetane ring is not essential for tubulin binding. The contribution of the oxygen atom to microtubule interaction seems to be weak, in disagreement with the hypothesis of a hydrogen bond between the oxetane and Thr 274 in the binding site.[17] With regard to the oxetane binding site, it is to be noted that it may be relatively restricted because C-20 acetoxy derivatives[9a] and N-20 Me or N-20 Ac azetidine analogues[18] are completely inactive. The most important element for microtubule interaction is definitely the conformational properties of the taxane diterpene that can be preserved by the presence of either a small D-cycle (four- or three-membered ring) or a C-4 β-Me as in compounds **3** and **5**.

The absence of the oxetane ring is more crucial for cytotoxicity and may reflect other factors besides microtubule interaction. Addition of an oxygen atom was thought to increase the bioavailability of this D-seco derivative. Because no cytotoxicity improvement has been observed, other elements have to be further investigated to explain this reduced cytotoxicity of D-modified taxoids.

Acknowledgment. The authors thank Dr. A. Commerçon (Sanofi-Aventis) for a gift of the docetaxel side chain. J. F. Gallard and M. T. Martin are acknowledged for performing NMR spectra, and C. Gaspard is acknowledged for cytotoxicity evaluations. The authors are very grateful to Prof. G. Appendino (University of Torino, Italy) for helpful discussions on silyl group removal.

Supporting Information Available: Experimental procedures and spectral data for compounds **5** and **7**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL060531D

(16) Guénard, D.; Thoret, S.; Dubois, J.; Adeline, M.-T.; Wang, Q.; Guéritte, F. *Bioorg. Med. Chem.* **2000**, *8*, 14−156.

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

(18) Unpublished results. 5(20)-Azadocetaxel is 16-fold less active than docetaxel.