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SYNTHESIS AND ANTITUMOR PROPERTIES OF NOVEL 14-β-HYDROXYTAXOL AND RELATED ANALOGUES

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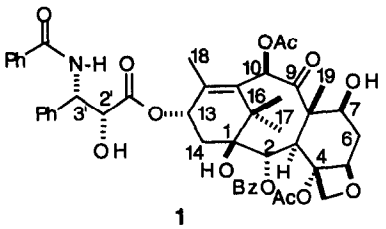
Abstract: *Novel analogues of TAXOL® with an additional hydroxyl group at the C-14 position were synthesized and evaluated as antitumor agents.*

TAXOL® (paclitaxel) (**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 paclitaxel 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] The clinical importance of paclitaxel has spurred the synthesis of a variety of novel analogues with the goal of designing more effective drugs and understanding the features of the paclitaxel binding site on microtubules.[4]



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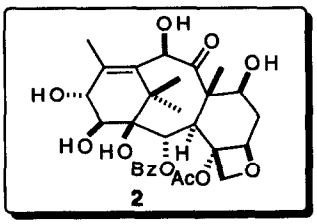
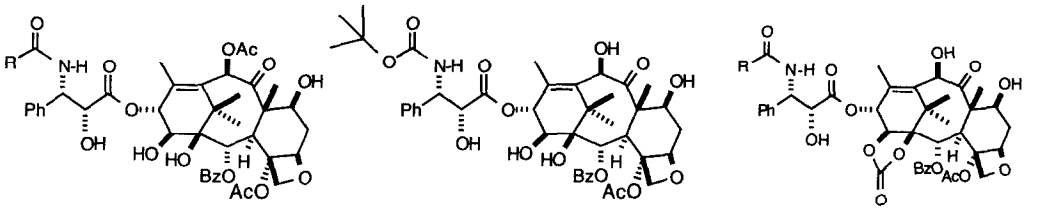


2

Recently, Appendino *et al.*, reported the isolation of 14-β-hydroxy-10-desacetylbaccatin (**2**), a novel diterpenoid from the needles of *Taxus wallichiana* Zucc.[5] In view of our on-going studies on paclitaxel, we envisioned the possibility of synthesizing and evaluating the biological profiles of 14-β-hydroxytaxol (**3**) and related analogues **4** and **5**.



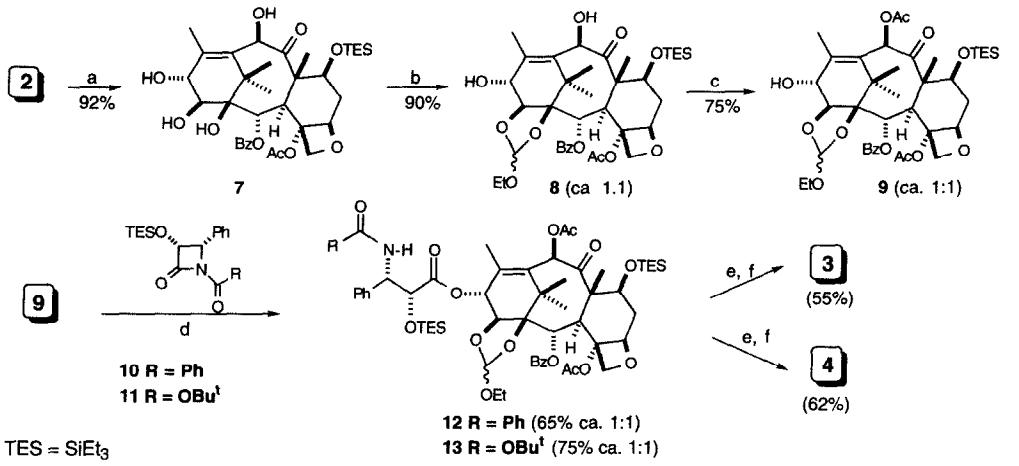
3 R = Ph
4 R = OBu^t

5

6 R = Ph or ^{t}BuO

During the course of our studies, Ojima and co-workers reported analogues of paclitaxel and Taxotere™ (docetaxel) synthesized from **2**. Of particular interest was the cyclic carbonate **6** (R=OBu^t) which exhibited interesting *in-vitro* cytotoxicity in the human ovarian cell line and was found to be more potent than paclitaxel in human breast and colon cell lines.[6] However, no *in-vivo* antitumor activity was reported on this compound. Recent studies on structure-activity relationships (SAR) suggest that functionalities in the southern half of the molecule are important to retain the antitumor properties of paclitaxel.[4] In view of these data and in search for compounds with better biological profiles than paclitaxel, we were interested in synthesizing new C-14 paclitaxel analogues. Herein, we wish to report the synthesis and antitumor properties of novel 14-β-hydroxytaxol and related analogues.

Scheme 1



10 R = Ph
11 R = OBu^t

12 R = Ph (65% ca. 1:1)
13 R = OBu^t (75% ca. 1:1)

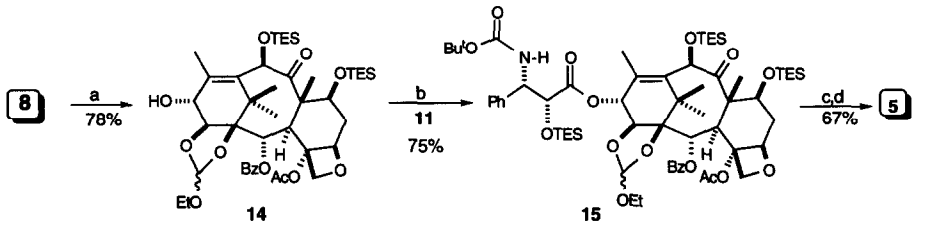
TES = $SiEt_3$

Conditions: (a) TESCl, imidazole, DMF, rt, 5h. (b) $CH(OEt)_3$, cat-PPTS, THF, 16h, rt. (c) LiHMDS (1.0 eq), AcCl,-40°C to 0°C, THF, 60 min. (d) LiHMDS (1.0 eq), **10** or **11**, THF, -40°C to 0°C, 60 min. (e) 6*N* HCl (10 equiv), CH_3CN, -5°C, 5.0 h; (f) NH_4OH (15*M*, 10 equiv), THF, 0°C, 3-4h.

The key features of our synthesis (Scheme 1) were the selective protection of the three hydroxyl groups (C-7, C-10, and C-14) followed by the introduction of the phenylisoserine side chain at the C-13 position. The C-7 hydroxyl group in **2** was protected as the silyl ether by the treatment of triethylsilyl chloride in conjunction

with imidazole in DMF at ambient temperature. For further synthetic manipulation, it was necessary to protect the C-14 hydroxyl group since attempts to protect the C-10 hydroxyl in the presence of a free C-14 hydroxyl were unsuccessful. After diligently evaluating a number of protecting groups, the C1-C14 hydroxyl groups were protected as ethoxymethylene acetal.[7] Hence, treatment of **7** with triethylorthoformate in THF at room temperature afforded the acetal **8** as a 1:1 mixture of diastereomers. Chemoselective acetylation at C-10 was achieved in high yield by selectively deprotonating the C-10 hydroxyl of **8** using 1.0 equiv of LiHMDS followed by the addition of acetyl chloride to afford **9**. No acetylation was observed at the C-13 position. Next, the phenylisoserine side chain was introduced at the C-13 position using previously described β-Lactam chemistry to synthesize **12**.[8] The chiral β-lactams **10** and **11** were synthesized using the procedure described by Farina.[9] Finally, under acidic conditions, the two silyl groups and the acetal functionality were removed to afford the C-14 monoformate intermediate which was subsequently hydrolyzed under mild basic conditions (NH_4OH at 0°C for 3 hours)[10] to afford our target compound **3**. Following the similar protocol, the 10-acetyl derivative **4** was also synthesized. Next the synthesis of 14-β-hydroxytaxotere (**5**) was undertaken; the key step in the synthesis was selective protection of the C-10 hydroxyl group. Attempts to protect both the C-7 and C-10 hydroxyls as silyl ethers in one pot under standard silylating protocol were unsuccessful; a mixture of di and tri silyl ethers were isolated. However, treatment of **8** with 1.0 equiv of LiHMDS followed by the addition of triethylsilyl chloride, selectively, protected the C-10 hydroxyl in the presence of a C-13 hydroxyl group. Subsequent steps were straightforward, and involved the intoduction of the amino acid side chain followed by deprotection to afford **5** in modest yield (Scheme 2).[11]

Scheme 2



Conditions: (a) LiHMDS (1.0 eq), TESCl,-40°C to 0°C, THF, 60 min. (b) LiHMDS (1.0 eq), THF, -40°C to 0°C, 60 min. (c) 6 *N* HCl (10 equiv) CH_3CN, -5°C, 5.0 h. (d) NH_4OH (15 M, 10 equiv), THF, 0°C, 3-4h.

The 14-β-hydroxyl analogues **3-5** were evaluated in tubulin polymerization and *in-vitro* cytotoxicity assays. The *in-vitro* cytotoxicity experiments were performed using the HCT116 human colon carcinoma cell lines.[12] 14-β-hydroxytaxol (**3**) was also evaluated *in-vivo* in experiments performed against a paclitaxel sensitive murine M109 solid tumor model.[13] The results are summarized in Tables I and II. The analogues **4** and **5** exhibited *in-vitro* antitumor properties comparable to paclitaxel and docetaxel. On the other hand, 14-β-hydroxytaxol (**3**) was less potent than paclitaxel in the *in-vitro* cytotoxicity assays and in promoting microtubule assembly (Table I). Furthermore, the analogue **3** failed to exhibit any significant *in-vivo* antitumor activity (T/C= <125%) in two separate experiments, when evalauted at doses ranging from 12.5-200 mg/Kg/inj

compared to concomitantly evaluated paclitaxel (maximum T/C values of 153% and 194%) in the M109 i.p./i.p tumor model system. The nearly 4 gm reduction in body weight caused by the highest doses evaluated (data not shown) suggest that further dose escalation of analogue **3** would have resulted in unacceptable toxicity. In view of *in-vitro* and *in-vivo* experiments, the presence of an additional hydroxyl group at the C-14 position seems to have a deleterious effect on the biological activity as compared to the parent compound.

Table I

Paclitaxel	2.4	4.1 ± 1.6
Docetaxel	2.0	2.6 ± 0.4
14-β-Hydroxytaxol (**3**)	8.0	8.9 ± 1.9
14-β-Hydroxy-10-acetyltaxotere (**4**)	2.3	7.3 ± 3.0
14-β-Hydroxytaxotere (**5**)	2.4	2.9 ± 0.5

a = Drug concentration required to inhibit cell proliferation to 50% vs. untreated cells (incubated at 37°C for 72 h).
b = Effective concentration capable of inducing polymerization of tubulin (incubated at 37°C for 30 min) at an initial rate of 0.01 optical density/minute, as measured at 350 nm.[3] *The results are the average of three determinations.*

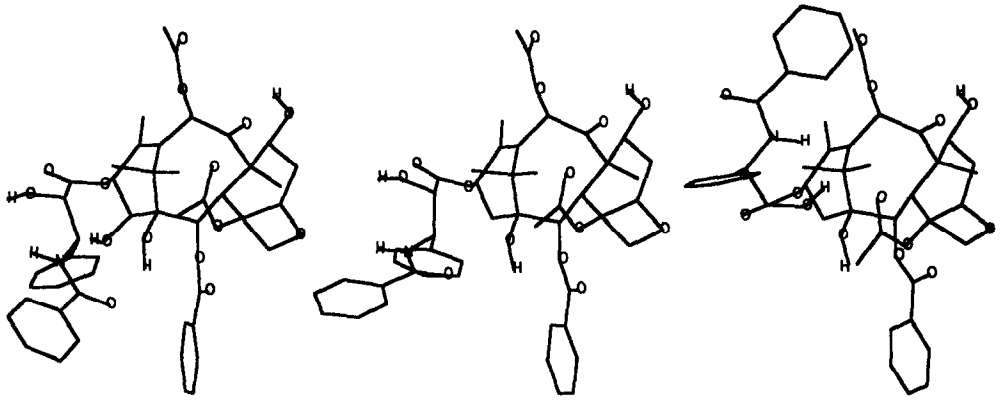
Table II

14-β-hydroxytaxol (3)	**Paclitaxel**
Expt. A; Expt. B	Expt. A; Expt. B
112 (25); 106 (12.5)	138 (40); 153 (40)
115 (50); 111 (25)	194 (60); 119 (60)
109 (100); 108 (50)	
106 (200); 114 (75)	

a = Murine lung carcinoma i.p. (intraperitoneal) implant model. b = % T/C refers to the percent of the median survival time of drug-treated time of drug treated mice (6 per dose) to vehicle treated controls. c = Dose administered i.p. on days 5 and 8; vehicle used: cremophor, ethanol and water. Significant activity in this tumor model is defined as a T/C >125.

To address the issue of reduced binding affinity of **3** to microtubules, we decided to study the conformation of this molecule. Molecular modeling studies (in vacuo) were performed on 14-β-hydroxytaxol (**3**) and the results were compared with paclitaxel **1**.[14] Based on these studies, we found that 14-β-hydroxytaxol (**3**) existed predominantly in one major conformation (**14-OHPT**, energy = -480.064 Kcal/mole) where as paclitaxel existed in two major conformations (**PT-I**, energy = -433.523 Kcal/mole; and less populated **PT-II**, energy = -432.793 Kcal/mole). These results were consistent with the crystal structure[15] of Taxotere™ and NMR/modeling studies recently reported.[16] The conformation of 14-β-hydroxytaxol (**14-OHPT**) was found very similar to **PT-I**; most likely, the C-14 hydroxyl group might be stabilizing the conformation by intermittent

hydrogen bonding with the C-1' and C-3' carbonyl groups. Since there are no conformational differences between **1** and **3**, the reduced binding affinity of this novel analogue **3** could be attributed to the free C-14 hydroxyl group which might be introducing steric and/or electronic factors incompatible with the β-tubulin binding site. Further studies on these interesting 14-hydroxyl analogues are in progress and will be reported in the near future.



14-OHPT **PT-I** **PT-II**

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References and Notes

TAXOL®, a chemical formulated material for the treatment of cancer, is a registered trademark of Bristol-Myers Squibb Company.

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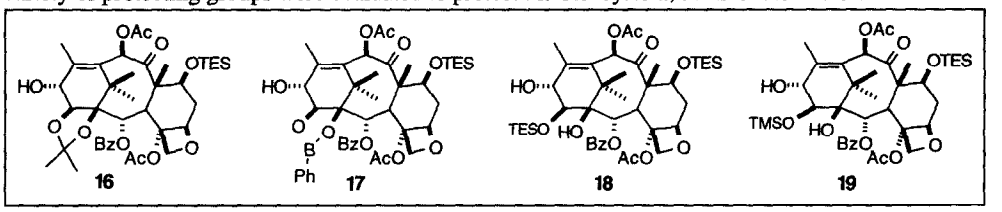
7. A variety of protecting groups were evaluated to protect the diol system, as illustrated below:



Under literature conditions (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*; Wiley: New York, 1991) we were unable to remove the acetonide (**16**) or phenyl boronate (**17**) without sacrificing the oxetane ring. The 14-β-hydroxyl group can be easily protected as silyl ethers (triethylsilyl ether **18** or trimethylsilyl ether **19**), but we were unable to introduce the amino acid side chain at C-13 on these C-14 silyl ethers employing the β-lactam chemistry.

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14. CHARMM molecular dynamics[a] was used to generate a nano second trajectory for **1** and **3**. Structures were collected every 0.5 psec providing 2000 viable conformations that are accessible along a free energy pathway. The structures were grouped into families and the average structure from each family was minimized with CHARMM followed by AM1[b]. The energies reported in the text are from AM1. a) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187-217. b) Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

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