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STRUCTURE–ACTIVITY RELATIONSHIPS STUDY AT THE 3′-N POSITION OF PACLITAXEL-PART 1: SYNTHESIS AND BIOLOGICAL EVALUATION OF THE 3′-(*t*)-BUTYLAMINOCARBONYLOXY BEARING PACLITAXEL ANALOGS

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Abstract: An efficient syntheses of the 3′-N isomeric paclitaxel analogs, **4** and **5**, are described. A highly diastereoselective Sharpless asymmetric dihydroxylation reaction is utilized to establish the required (2′*R*,3′*S*) stereochemistry on the C-13 side chain. Both of the 3′-N modified analogs **4** and **5** were found to be cytotoxic in vitro. Analog **4** also displayed comparable in vivo activity to that of paclitaxel in the ip M-109 tumor model.

Paclitaxel (Taxol®**1**), a highly oxygenated natural diterpenoid,[1] has shown great clinical promise against a wide variety of solid tumors such as ovary, breast, lung, head, neck, and melanoma.[2] It has recently received FDA approval for the treatment against *cis*-platinum refractory ovarian cancer and metastatic breast cancer. Among the present clinically used antitumor agents, paclitaxel is unique in its mode of action. Unlike other tubulin interacting agents such as colchicine and the vinca alkaloids, which via inhibiting microtubule assembly, paclitaxel promotes microtubule assembly and stabilizes the polymer thus formed, also hindering depolymerization.[3] It is believed that paclitaxel exerts its antitumor efficacy via disruption of the nornal dynamic equilibrium between cellular tubulin and polymerized microtubules.

Docetaxel **3**, a side chain analog of paclitaxel, has also shown promising antitumor activity and has recently received approval for clinical use.[4] Careful examination of the side chain SAR reveals that replacement of the 3′-benzamide moiety present in paclitaxel with 3′-(*t*)-butyl carbamate leads to a series of side chain analogs possessing increased potency, as exemplified by 10-acetyl docetaxel **2**[5] and docetaxel **3**.[6] This observation has provided further incentive for SAR modification at the side chain 3′-N position.[7] In this connection, analogs containing 3′-N-Boc(*t*) *isosteres* in the side chain including 3′-(*t*)-butyl urea[8] and 3′-(*t*)-butyl thiocarbamate[9] have been described. In this letter, we wish to report the synthesis of another type of side chain 3′-N-Boc(*t*) mimic, the *isomeric* 3′-N-Boc(*t*) carbamate analogs, as represented by **4** and **5** shown in Figure 1. These compounds are prepared from a 2′,3′ diol precursor rather than an amino alcohol based side chain.

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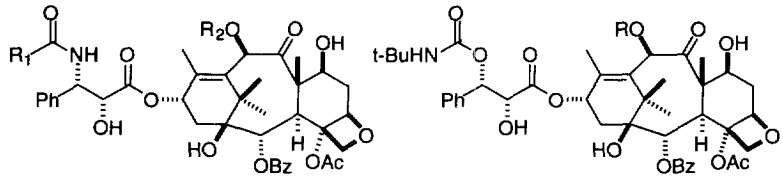
Figure 1



1 R_1 = Ph, R_2 = Ac ***(Paclitaxel)***
2 R_1 = t-BuO, R_2 = Ac
3 R_1 = t-BuO, R_2 = H ***(Docetaxel)***

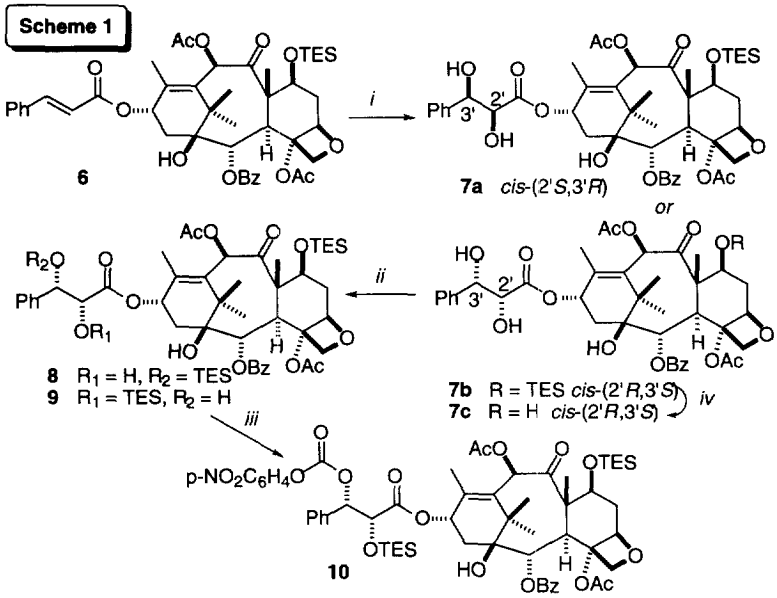
4 R = Ac
5 R = H ***(iso-Docetaxel)***

The synthetic route employed for the preparation of the C-3′-*para*-nitrophenyl carbonate derivative **10**, a key intermediate for the target molecules **4** and **5**, is shown in Scheme 1. Diastereoselective dihydroxylation of the C-13 cinnamoyl derivative **6**[10] was achieved according to Sharpless protocol (K_2OsO_4/NMO/$(DHQ)_2PHAL$),[11] and provided 85% of the desired (2′*R*,3′*S*) diol diastereomer **7b** along with trace amounts of its isomer **7a**. The use of [$(DHQD)_2PHAL$] as the chiral ligand in this asymmetic dihydroxylation reaction resulted in reverse in diastereoselectivity. In this case, the (2′*S*,3′*R*) bearing isomer **7a** was obtained as the major product in 62% yield. *Thus, a method for the highly diastereoselective dihydroxylation of the C-13 cinnamoyl side chain is firmly established.*[12]

With *cis*-(2′*R*,3′*S*) diol **7b** in hand, side chain mono-silylation was attempted under standard conditions (TESCl/imidazole/DMF/0 °C). Under these conditions, two products in a 4:1 ratio were isolated in 42% overall yield along with ~20% unreacted starting material. On the basis of ^{1}HNMR analysis,[13] the major product was identified as the C2′-TES bearing isomer **9**, while the minor product was assigned to structure **8**, a C3′-TES containing isomer. The observed regioselectivity (C2′>C3′ towards silylation) here is in good agreement with that obtained with side chain (2′*R*,3′*S*) diol methyl ester.[14] Conversion of the C2′-TES bearing derivative **9** to the corresponding C-3′ carbonate **10** was affected in the usual manner (Hunig's base/*p*-nitrophenyl chloroformate). Alternatively, removal of the C-7 TES protecting group afforded the side chain diol **7c** for biological evaluation.

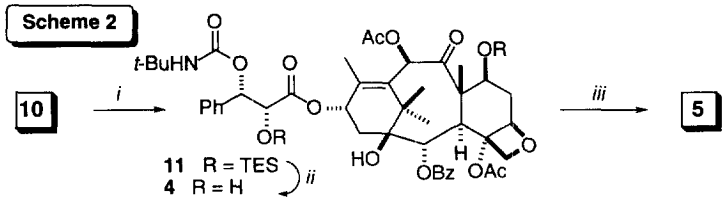
Using the key intermediate **10**, the synthesis of **4** and iso-docetaxel **5** were completed, as shown in Scheme 2. Reaction of a THF solution of **11** with *t*-butylamine provided C3'-deamino-(*t*) butyl carbamate derivative **12**, which after desilylation of C-2′ and C-7, provided the final target **4**. Removal of the C-10 acetyl group from **4** was accomplished regiospecifically using Zheng's methodology,[15] and afforded the desired iso-docetaxel **5** in 51% yield.

Scheme 1



Reagents and Conditions: (i) cat.K_2OsO_4/NMO/t-BuOH+THF/$(DHQ)_2$PHAL, 85% of (**7b**); or $(DHQD)_2$PHAL, 62% of (**7a**); (ii) TESCl/ imidazole/DMF/0° C, 8% (**8**) & 36% (**9**), plus ~20% S.M.; (iii) for **9** to **10**: $EtPr_2N$/ClC(O)OC_6H_4-NO_2-*p*/ CH_2Cl_2/0 °C, 95%; (iv) 6 N HCl/ CH_3CN/0 °C, 76% (**7c**).

Scheme 2



Reagents and Conditions: (i) t-$BuNH_2$/THF/rt, 94%; (ii) 6 N HCl/CH_3CN/0 °C, 81%; (iii) 30%H_2O_2/THF/$NaHCO_3$/rt, 51%.

Derivatives **4**, **5**, and **7c** were evaluated in vitro in a tubulin polymerization assay[16] and cytotoxicity assay against human colon cancer cell lines (HCT-116).[17] The results are shown in Table 1. The side chain diol **7c** was devoid of any activity in both the tubulin polymerization assay and the in vitro cytotoxicity assay. Surprisingly, however, the same compound **7c** was reported to be only three fold less potent than paclitaxel in the microtubule disassembly assay by French workers in 1991.[18] The reason for this discrepancy is not clear.

Table 1. Biological evaluation of novel side chain analogs (**7c**, **4**, and **5**)

Compound	*Tubulin Polym. ratio*[a]	*Cytotoxicity ratio*[b]	*IC_{50} (nM) HCT-116*	*T/C (%) M-109 (mg/kg/inj.)*[c]
Paclitaxel **1**	1.0	1.0	2.0	166–228 (60)
7c	>100	>100	>200	--
4	1.5	3.3	6.6	166 (50)
5	7.8	5.1	10.3	--

[a]Potency of analog relative to paclitaxel ($EC_{0.01}$). [b]IC_{50} (analog) vs. IC_{50} (paclitaxel). [c]Dosed at day 5 and 8.

The C-3′-(*t*)-butylaminocarbonyloxy analog **4** was 1.5-fold and 3.3-fold less potent than paclitaxel **1** in the tubulin polymerization assay and the in vitro cytotoxicity assay, respectively. In the subsequent in vivo experiment[19] against the ip implanted M-109 lung carcinoma, analog **4** showed equivalent in vivo activity to paclitaxel, with the T/C (%) value of 166 (50 mg/kg/injection). *Thus, to the best of our knowledge, analog **4** represents the first example of a C-3′-oxygenated paclitaxel analog, which retained in vivo activity.*

Unlike its C-3′-(*t*)-butylcarbamate conterpart, docetaxel **3**, the C-10 deacetyl derivative **5** was found to be slightly less cytotoxic than **4**. This compound was not evaluated in vivo.

In summary, we have devised a distereoselective route for the preparation of the novel class of C-3′-oxygenated paclitaxel analogs. In contrast to the previous report by French workers, the side chain diol **7c** was found to be essentially inactive in vitro, thus establishing the necessity of the 3′-carbamate moiety. The initial in vivo activity observed with **4**, a representative C-3′-deamino-3′-hydroxylated paclitaxel derivative, thus suggests that further work in this series may be warranted.

Acknowledgment

We are indebt to Dr. S. E. Klohr for the measurements of high resolution mass spectra.

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13. The structures of **7b** and **4** were confirmed by their NMR spectroscopic data. The regiochemistry at the C-3′ position for compound **4** was determined by the HMBC spectrum, which showed the carbon–proton three bond couplings between the C-3′ proton (6.01 ppm) and the *ortho* carbon (126.4 ppm) of the C-3′ phenyl ring, and between the C-3′ proton and the carbonyl carbon (125.3 ppm) of the C-3′-*t*-butyl aminocarbonyloxy moiety.

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