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FIRST SYNTHESIS AND CYTOTOXIC ACTIVITY OF NOVEL DOCETAXEL ANALOGS MODIFIED AT THE C18-POSITION

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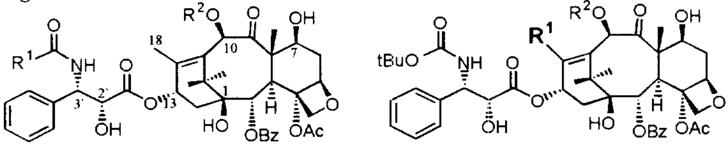
Abstract: To investigate the effect of the C-18 position of docetaxel in its cytotoxic activity, 18-modified docetaxel analogs were synthesized for the first time from the 18-brominated baccatin derivatives. All of these analogs were found to be less active than docetaxel in cytotoxic activity against four test cell lines. © 1997 Elsevier Science Ltd.

Paclitaxel (**1**, Taxol®),[1] a diterpene natural product isolated by M. C. Wani *et al.* from *Taxus brevifolia*, has shown exceptional efficacy in cancer chemotherapy and has been approved by the FDA for the treatment of advanced ovarian and breast cancer in December 1992 and April 1994, respectively. On the other hand, docetaxel (**2**, Taxotere®),[2] a semisynthetic paclitaxel analog, was approved by the FDA for the treatment of breast cancer in May 1996.[3] Docetaxel has shown slightly better activity when compared to paclitaxel in several in vivo tumor models.[4] Both taxoids possess a unique mechanism of action as promoters of tubulin assembly and inhibitors of microtubule disassembly (Fig. 1).[5]

Figure 1



Paclitaxel (**1**: R^1 = Ph, R^2 = Ac)
Docetaxel (**2**: R^1 = tBuO, R^2 = H)

3: R^1 = CH_2CN, CH_2CH_3, CHO, etc.
R^2 = H or Ac

In order to obtain new compounds with improved biological activity and to study the structure-activity relationships in this series, a number of structural modifications have been performed on both taxoids.[6] These modifications have revealed the essential role of the ester groups at C-2, C-4 and C-13 and the oxetane ring as well as of the oxygenated functions at C-7 and C-10. So far, however, no information is available concerning the role of C-18 position in biological activity. That is probably due to the absence of suitable strategy for modification at the C-18 position. With this background, we have launched a program aimed at the modification of C-18 position. Herein, we report the synthesis and cytotoxic activity of novel C-18 modified docetaxel analogs **3**.

Chemistry

We first attempted bromination at the C-18 allylic position of 7-TES-10-deacetylbaccatin III (**4a**)[7] or 7,10-bis(TES)-10-deacetylbaccatin III (**4b**)[8] with NBS, but it resulted in generally complicated mixtures of products (Table 1).

Scheme 1

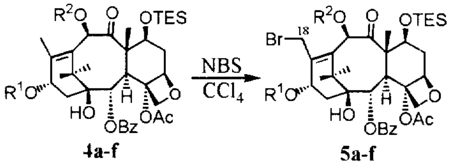
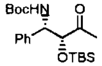
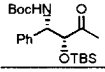


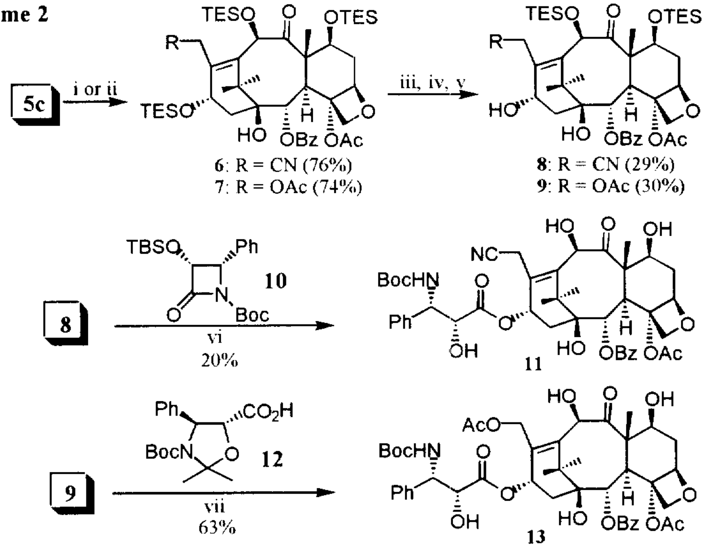
Table 1

Substrate	R¹	R²	Product	Yield (%)
4a	H	H	**5a**	0
4b	H	TES	**5b**	0
4c	TES	TES	**5c**	76
4d	TES	Ac	**5d**	47
4e		TES	**5e**	60
4f		Ac	**5f**	45

Therefore, we decided to attempt the same reaction using 7, 10, 13-tris(TES)-10-deacetylbaccatin III (**4c**), which was synthesized from **4a** by reference to Chen's method.[9] Indeed, treatment of **4c** with NBS gave the desired C-18 brominated baccatin derivative **5c**[10] in 76% yield (Scheme 1). Similarly, 7,13-bis(TES)-baccatin III (**4d**)[11] and 2'-TBS-protected taxoids (**4e**,**f**)[12] bearing C-13 side chain gave compounds **5d**-**f** in moderate yields, respectively.

To investigate the effect of the C-18 position of docetaxel (**1**) in cytotoxic activity, we converted the C-18 brominated compounds **5c**-**f** to several kind of docetaxel analogs **11**, **13**, and **18**-**22** as shown in Scheme 2-4.

Scheme 2

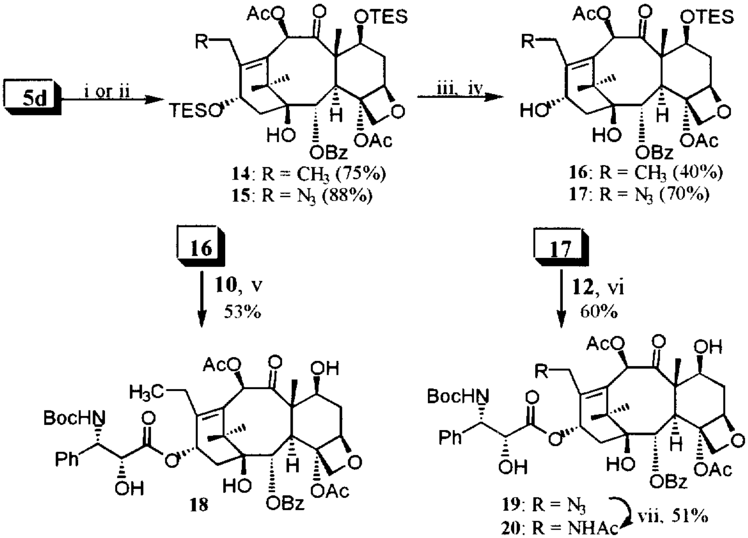


Reagents and Conditions: (i) KCN, 18-Crown-6, MeCN, reflux; (ii) *n*-Bu_4NOAc, acetone, reflux; (iii) HF-Py, pyridine; (iv) TES-Cl, imidazole, DMF; (v) TES-Cl, *n*-BuLi, THF, -78 °C; (vi) NaHMDS, THF, -78 °C; then (iii); (vii) DCC, DMAP, AcOEt; then cat. TsOH, MeOH.

Compound **5c** was subjected to replacement reaction with KCN or *n*-Bu_4NOAc[13] to give the 18-cyano (**6**) and 18-acetoxy (**7**) baccatin derivatives, respectively. Intermediates **8** and **9** were obtained in 29% and 30% overall yields, respectively, using a sequence of desilylation and selective resilylation at C-7 and C-10 positions. Final side chain attachment onto compounds **8** and **9**, using *β*-lactam **10**[14] or protected *β*-phenylisoserine **12**[15] as the side chain precursor, followed by deprotection, gave the 18-cyano (**11**) and 18-acetoxy (**13**) docetaxel (Scheme 2), respectively.

The 18-Methyl (**18**) and 18-azido (**19**) analogs were synthesized from compounds **16** and **17**, which were derived from 7,13-protected-18-bromo-baccatin III **5d** in several steps, using the side chain precursor **10** or **12** in a similar manner as above (Scheme 3). The treatment of **19** with activated Zn gave the 18-acetamido analog **20**.

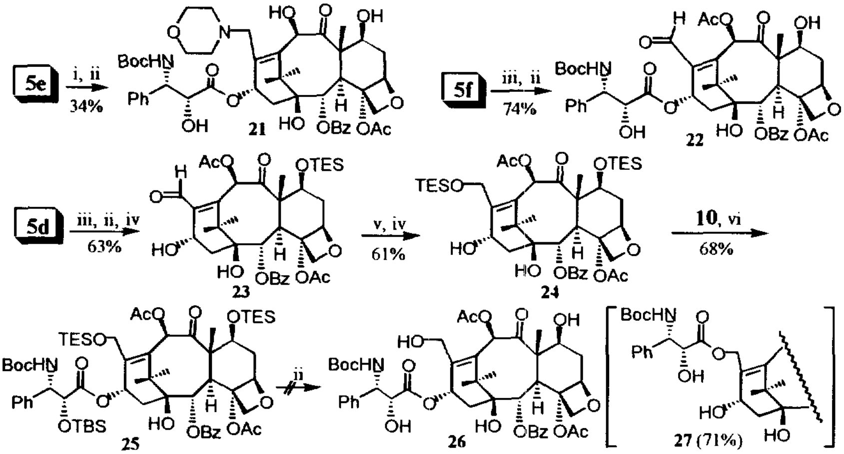
Scheme 3



Reagents and Conditions: (i) Me_2CuLi, THF, -78 °C; (ii) NaN_3, DMF, 60 °C; (iii) HF-Py, pyridine, rt; (iv) TES-Cl, imidazole, DMF, 0 °C; (v) NaHMDS, THF, -78 °C; then (iii); (vi) DCC, DMAP, AcOEt, rt; then cat. TsOH, MeOH, rt; (vii) Zn, AcOH, Ac_2O, rt.

The treatment of the 18-bromo-docetaxel derivative **5e** with morpholine in MeOH, followed by desilylation, gave analog **21** in 34% yield. Furthermore, compound **5f** was converted to the 12-formyl analog **22** by oxidative reaction with silver tetrafluoroborate in DMSO.[16] But, an attempt to synthesize the 18-hydroxy analog **26** from the 18-*O*-TES protected taxoid **25**, which was derived from **5d** in several steps, failed to give compound **27** accompanying transesterification of the C-13 side chain on **26**.

Scheme 4



Reagents and Conditions: (i) morpholine, MeOH, reflux; (ii) HF-Py, pyridine, rt; (iii) $AgBF_4$, Et_3N, DMSO, 80 °C; (iv) TESCl, imidazole, DMF, 0 °C; (v) $NaBH_4$, THF, 0 °C; (vi) NaHMDS, THF, -78 °C.

Cytotoxic Activity

We evaluated cytotoxic activity of novel C-18 modified docetaxel analogs against four cell lines (PC-6, PC-6/VCR, PC-12, P388). In order to obtain more meaningful comparisons of relative activities, docetaxel (**2**) was tested as the positive control. The results are presented in Table 2.

Table 2.

Entry	Compound	Cytotoxic activity GI_{50} (ng/ml)[a]			
		PC-6	PC-6/VCR	PC-12	P388
1	Docetaxel (**2**)	1.16	173	19.1	1.62
2	**11**	4.81	754	96.9	8.37
3	**13**	163	>1000	>1000	181
4	**18**	10.3	167	35.8	7.45
5	**19**	52.4	390	84.5	40.1
6	**20**	131	>1000	>1000	393
7	**21**	>1000	>1000	>1000	>1000
8	**22**	699	>1000	>1000	>1000

[a] Concentration that inhibited the growth of cells by 50% on 72 h continuous exposure for four cell lines [mouse leukemia (P388), human lung cancer cell lines (PC-6, PC-12), and vincristine resistant cancer cell line (PC-6/VCR)].

The acetoxy (**13**), acetamido (**20**), and morpholino (**21**) analogs, which had polar substituents at the C-18 position, showed remarkably decreased activity. In addition, the 12-formyl analog **22** was absolutely inactive against PC-6. Introduction of a cyano or an azido group decreased the activity against PC-6/VCR and PC-12 (resistant cancer cell line associated P-glycoprotein) (entries 2 and 5). The C-18 methyl analog **18** was 2 times less active than **2** against PC-12, but retained the same activity as **2** against PC-6/VCR. On the whole, the modification of the C-18 position on docetaxel showed a tendency to decrease in cytotoxic activity. These results lead us to conclude that the C-18 site has no steric allowance for expression of the cytotoxic activity.

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10. **General Bromination Procedure**: A mixture of **4c** (2.0 g, 2.25 mmol), NBS (441 mg, 2.48 mmol), and benzoylperoxide (10 mg) in CCl_4 (40 mL) was refluxed for 1 h. The reaction mixture was then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0.5% acetone/$CHCl_3$) to give compound **5c** (1.65 g, 76%) as a colorless crystalline solid. mp 202−206 °C. MS (FAB) *m/z* 967 (MH^+). *Anal.* Calcd for $C_{47}H_{77}BrO_{10}Si_3$: C, 58.42; H, 8.03. Found: C, 58.26; H, 7.83. IR (KBr) 3500, 2960, 2916, 2884, 1728 cm^{-1}. ^1H-NMR (400 MHz, $CDCl_3$, TMS as reference) δ: 0.60 (m, 6 H, TES), 0.78 (m, 12 H, TES), 1.00 (m, 27 H, TES), 1.14 (s, 3 H, H-16), 1.20 (s, 3 H, H-17), 1.63 (s, 3 H, H-19), 1.89 (m, 1 H, H-6), 2.20 (m, 2 H, H-14), 2.28 (s, 3 H, OAc), 2.47 (m, 1 H, H-6), 3.84 (d, J = 7 Hz, 1 H, H-2), 4.14 (d, J = 8 Hz, 1 H, H-20), 4.28 (d, J = 8Hz, 1 H, H-20), 4.31 (d, J = 10 Hz, 1 H, H-18), 4.38 (dd, J = 10 Hz, 7 Hz, 1 H, H-7), 4.56 (d, J = 10 Hz, 1 H, H-18), 4.84 (d, J = 8Hz, 1 H, H-5), 5.06 (t, J = 8 Hz, 1 H, H-13), 5.25 (s, 1 H, H-10), 5.59 (d, J = 7 Hz, 1 H, H-2), 7.46 (t, J = 8 Hz, 2 H, Bz), 7.59 (t, J = 8 Hz, 1 H, Bz), 8.07 (d, J = 8 Hz, 2 H, Bz).

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