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SYNTHESIS AND CYTOTOXIC ACTIVITY OF NOVEL 10-ALKYLATED DOCETAXEL ANALOGS

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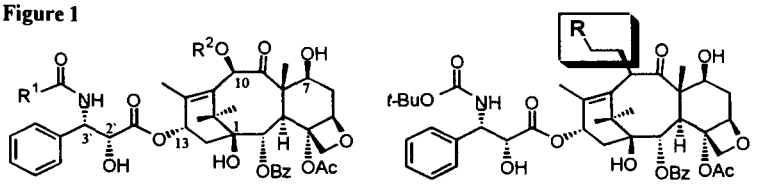
Abstract: An alkylation method of docetaxel at the C-10 position has been established by a radical coupling reaction using a 10-xanthate derivative of 7-*O*-TES-10-deacetylbaccatin III and appropriate alkenes. In addition the cytotoxic activity of 10-alkylated docetaxel analogs was evaluated. Among these analogs, a derivative having a methoxycarbonyl group at the end of the alkyl moiety exhibited more potent cytotoxic activity than docetaxel.

Paclitaxel (**1**, Taxol®),[1] a complex diterpenoid isolated in small quantities from the bark of *Taxus brevifolia*, is currently considered one of the most exciting leads in cancer chemotherapy. Paclitaxel has been approved by the FDA for the treatment of advanced ovarian (December 1992) and breast cancer (April 1994). On the other hand, docetaxel (**2**, Taxotere®)[2] is a semisynthetic analog that exhibits a slightly better activity than paclitaxel in *in vitro* and *in vivo* experimental models.[3] Docetaxel has recently been approved by the FDA for the treatment of breast cancer (May 1996).[4] Both taxoids act as potent mitosis inhibitors with a unique mechanism of action (Fig. 1).[5]

Figure 1



Paclitaxel (**1**: R^1 = Ph, R^2 = Ac)
Docetaxel (**2**: R^1 = *t*-BuO, R^2 = H)

3: R = polar substituents

These two drugs have excellent efficacy against solid tumors. However, their low water-solubility requires that they should be co-injected with a detergent, Cremophor EL or Tween 80. These detergents may exhibit untoward hypersensitivity reactions (hypotension, bronchospasm, urticaria, etc.) to patients, in addition to their complicating injection.[6]

To solve the problem of low water-solubility, several research groups have synthesized and evaluated water-soluble taxoids such as esterase- or phosphatase-cleavable prodrugs.[7] Both the C-2' and C-7 hydroxy functionalities were initially utilized for prodrug synthesis. However, these prodrugs are liable to exhibit

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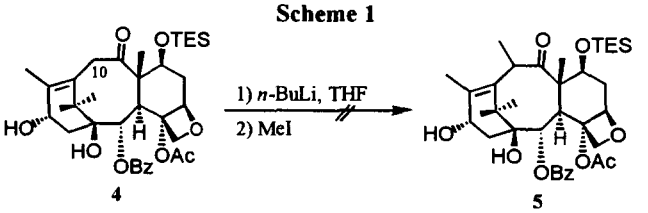
unstable efficacy because of variation in the enzymatic activity among patients.

Within this background, we have been seeking a water-soluble non-prodrug analog that overcomes the drawbacks of both taxoids. Kingston et al. have previously reported that 10-deoxy analog of docetaxel (**2**) exhibited significantly improved *in vitro* cytotoxic activity.[8] On the basis of this result, we designed the docetaxel analogs which have a polar substituent at the end of the alkyl moiety at the C-10 position. Herein, we report the synthesis and cytotoxic activity of several 10-alkylated docetaxel analogs **3**.

Chemistry

There have been no reported methods of introducing alkyl group to the C-10 position, so we tried two methods for the alkylation. One is to use the C-10 anion of the 10-deoxybaccatins, the other is a radical alkylation method.

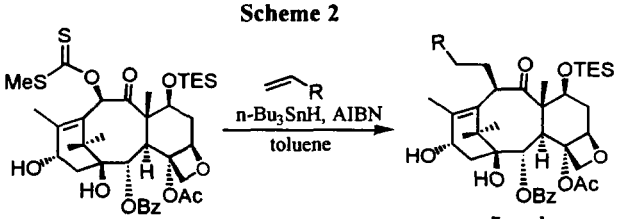
Scheme 1



4 **5**

We first attempted the anion method using 10-deoxybaccatin derivative **4**, but this approach resulted either in no reaction or in formation of a complex mixture (Scheme 1). Therefore, we decided to explore the radical alkylation (Scheme 2). We hypothesized that the C-10 radical would be generated as an intermediate of the deoxygenation reaction of the 10-xanthate baccatin. If the radical can be subsequently trapped with an appropriate alkene,[9] the alkylation and the characterization of the alkyl moiety will be accomplished at the same time.

Scheme 2



6 **7a–d**

Table 1

Run	Product	R	Yield (%)
1	**7a**	CO_2Et	22
2	—	CO_2-*t*-Bu	0
3	**7b**	CN	58
4	**7c**	CHO	39
5	**7d**	$COCH_3$	35

The results of the radical coupling are shown in Table 1. After examining several reaction conditions, we found the following procedure to be favored: to a solution of compound **6**[8] (200 mg, 0.267 mmol), acrylonitrile (200 μL, 3.30 mmol) and AIBN (catalyst amount) in toluene (3 mL) were added, and then acrylonitrile (200 μL, 3.30 mmol) and tributyltin hydride (123 μL, 0.457 mmol) in toluene (0.3 mL) separately and simultaneously at a rate of 10 μL/min at 80 °C under nitrogen atmosphere. After the addition was completed, the reaction mixture was concentrated under reduced pressure. The residue was purified with preparative thin layer chromatography using $CHCl_3$: acetone (95 : 5) as an

eluent to give the 10-(2'-cyanoethyl) derivative **7b** (109 mg, 58%) as an amorphous foam (run 3).

Under these conditions, when the R is a bulky group such as *tert*-butyl, **4** was obtained instead of the coupling product (run 2). In all cases, each compound (**7a-c** or **d**) was isolated as a single isomer. In order to determine the stereochemistry of the newly constructed carbon-carbon bond, we measured the NOESY spectrum of **10b** (Scheme 3). The cross peak was observed between C-10 proton and C-18 proton. According to the minimized 3D-structure of **10b** and **4**,[10] the α-H of C-10 is close to the C-18 proton, hence β-H of C-10 is rather far from C-18 proton; therefore the alkyl group is oriented at β-position as shown in Fig. 2. This result agrees with the fact that the α-face of **4** is covered by TES group; hence the β-face has much space to react (Fig. 3).

Fig. 2

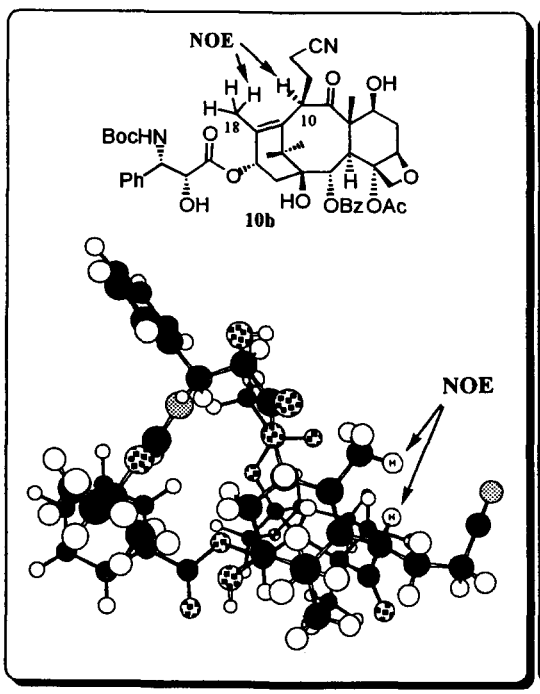
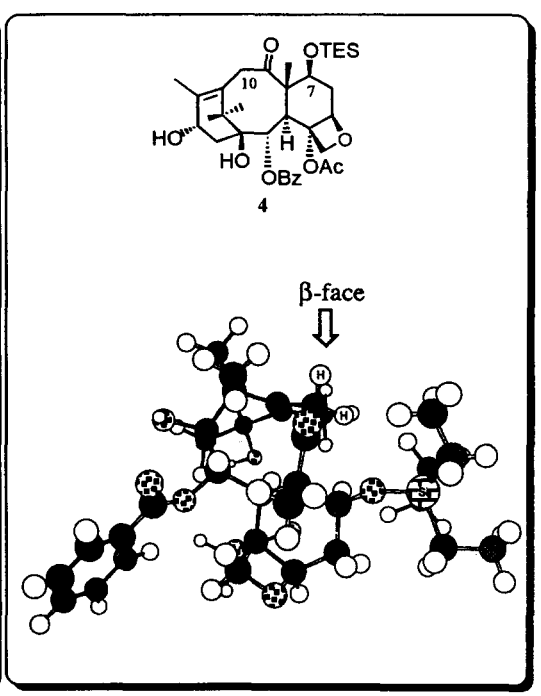
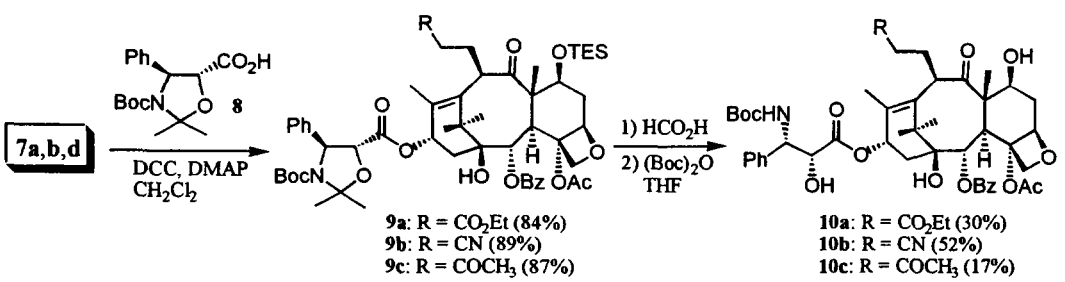


Fig. 3



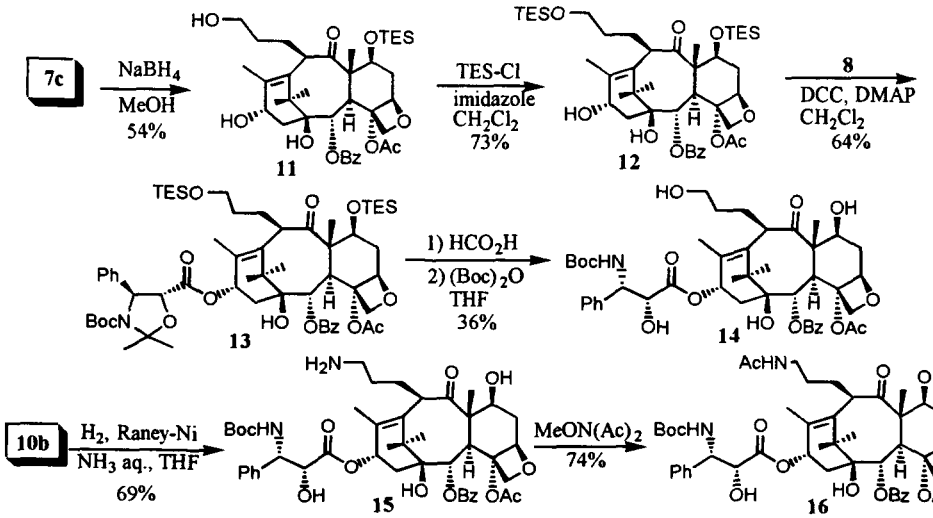
Next, we converted these alkylated compounds **7a,b,d** to the docetaxel analogs **10a – c** using protected β-phenylisoserine **8**[11] as the side chain precursor (Scheme 3).

Scheme 3



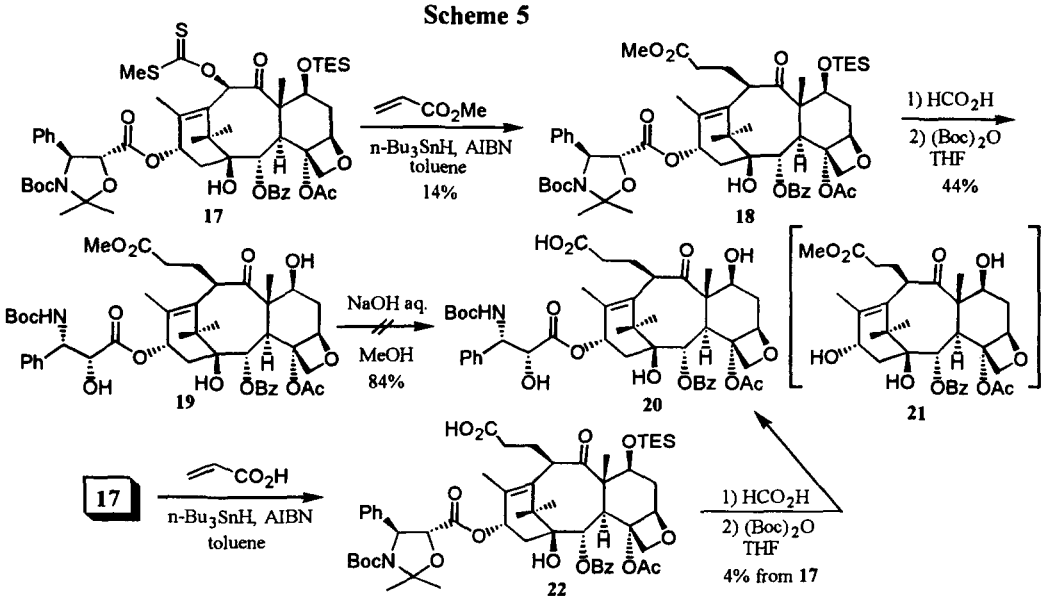
The 10-(3`-hydroxypropyl) analog **14** was synthesized from **12**, which was derived from the aldehyde **7c** in two steps, using **8** in a similar manner as above. The reduction of **10b** with Raney-Ni gave the 10-(3`-aminopropyl) analog **15** in 69% yield. Furthermore, **15** was converted to the acetamido analog **16** as shown in Scheme 4.

Scheme 4



An attempted synthesis of the carboxylic acid analog **20** using the methyl ester **19**, which was derived from the 10-xanthate derivative **17** in three steps, gave 13-*O*-deacylated compound **21**. Therefore, we tried direct conversion from acrylic acid and **17**. The yield of the radical coupling was poor, but we could obtain the desired analog **20** as shown in Scheme 5.

Scheme 5



Cytotoxic Activity

We evaluated cytotoxic activity of novel 10-alkylated docetaxel analogs against three cell lines (P388, PC-6, and PC-12). In order to obtain more meaningful comparisons of relative activities, docetaxel (**2**) was tested as a positive control. The results are presented in Table 2.

Table 2

Compound	Cytotoxic activity GI_{50} (ng/ml)[a]		
	P388	PC-6	PC-12
Docetaxel (**2**)	6.74	1.13	53.4
10a	4.31	1.35	12.0
10b	18.4	0.681	76.9
10c	7.12	0.266	32.4
14	15.5	0.777	67.0
15	1520	236	6820
16	71.8	10.5	634
19	3.30	0.771	24.4
20	1870	934	>10000

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

The activity of the analogs (**15**, **16**, **20**) which had polar substituents such as amino, acetamido, or carboxy groups in the alkyl moiety was significantly less than that of docetaxel, while the hydroxy (**14**) and cyano (**10b**) analogs were only moderately less active. The methyloxo (**10c**), ethoxycarbonyl (**10a**), and methoxycarbonyl (**19**) analogs showed fairly good activity, especially **19** exhibiting more potent activity than docetaxel. These results indicate that expression of the cytotoxic activity of the C-10 site is dependent on the substituent's polarity rather than on its steric demands.

In conclusion, we report a radical alkylation method for the C-10 position of baccatin derivatives, and established that alkylation could increase cytotoxic activity in some cases. Using this alkylation type derivatization, we would obtain a water-soluble non-prodrug taxoid with excellent cytotoxic activity.[14] Futher investigation of 10-alkylated taxoids is now under way.

References and notes.

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10. The 3D structures were calculated as follows: after the modification of X-ray structure of docetaxel,[12] based on the known average bond angles and lengths, the minimization was performed using CHARm[13] minimization option of the Quanta program (method: adopted basis Newton-Raphson; energy gradient tolerance: 0.001 Kcal/mol Å).
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14. The water-solubility data for 10-alkylated docetaxel analogs will be reported in detail in the near future.