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SYNTHESIS AND BIOLOGICAL EVALUATION OF WATER SOLUBLE TAXOIDS BEARING SUGAR MOIETIES

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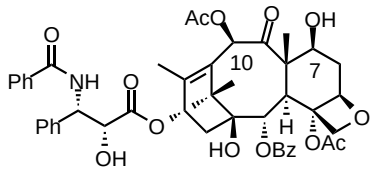
Abstract- Synthesis and biological evaluation of a variety of water soluble taxoids with sugar moieties are described.

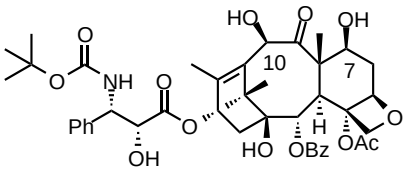
Paclitaxel (**1a**) has been recognized as the most exciting anticancer agent for the treatment of ovarian and breast cancer. However, its intrinsic poor water solubility compromises the use as a drug for clinical trials, which stimulates intensive research activities toward the synthesis and structure-activity relationship (SAR) studies of water soluble paclitaxel derivatives.[1]

Several years ago, we launched systematic SAR investigation of water soluble paclitaxel prodrugs that exhibit superior water solubility as well as the higher level of cytotoxicity to that of **1a**. On designing such compounds that meet the above demands, sugars seemed to be the first-rate candidate as hydrophilic functionalities to attach to the specific sites of **1a** and to be expected to exert additional biological activities of themselves. However, the conventional glycosylation protocols were not likely to be effective because **1a** might be susceptible of the core skeletal rearrangements, the oxirane cleavage, and so forth by the inevitable use of Lewis acids. Consequently, tethering a sugar to a hydroxyl group in **1a** by means of an ester linkage seemed to be the most effective strategy.

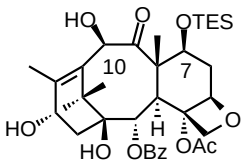
We wish to describe here the synthesis and the biological evaluation of a new array of water soluble taxoids (**3**) and (**4**), which feature an ester linkage to C-7 or C-10 hydroxyl group of taxoids with a variety of w-glycosyloxycarboxylic acids (n=0-8).



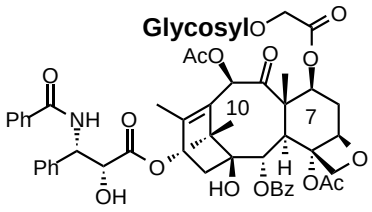
Paclitaxel (1a)



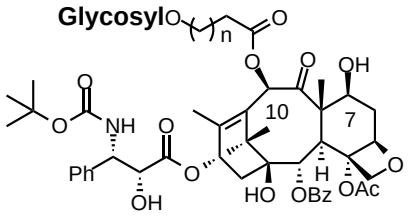
Docetaxel (1b)



7-TES-10-Deacetyl baccatin III (2)



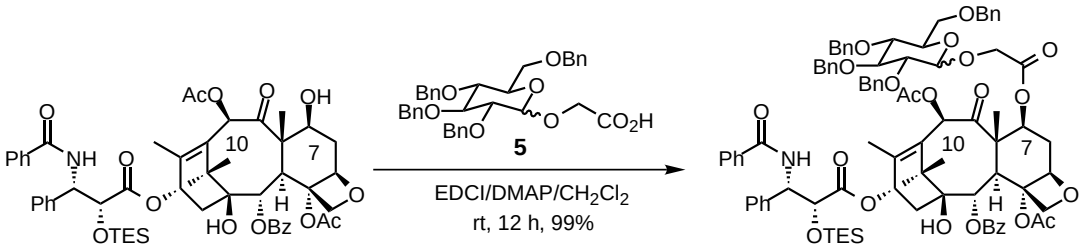
3



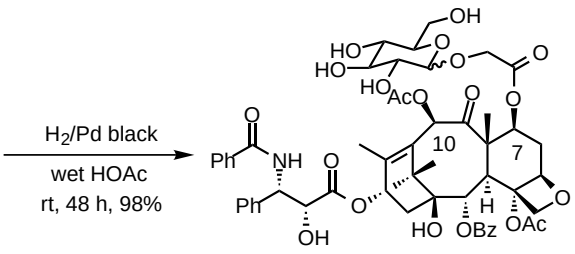
4

In the earlier stage of our project, C-7 modified taxoids (**3**) bearing various types of sugars were synthesized. Scheme 1 shows the synthetic outline of glucose-linked paclitaxel (**3a**). Condensation of 2'-TES-**1a** with glucosyloxyacetic acid (**5**)[2] (1.2 eq) [EDCI/DMAP, CH_2Cl_2, rt, 12 h] provided **6** in 99% yield. Both the triethylsilyl and benzyl groups were successfully removed by the use of Pd black in wet acetic acid to give **3a**[3] in 98% yield. Analogously, compounds (**3b**) (galactose), (**3c**) (mannose), and (**3d**) (xylose) were synthesized as a diastereomeric mixture of anomers except for **3c**.[4]

Scheme 1



2'-TES-1a **6**



3a

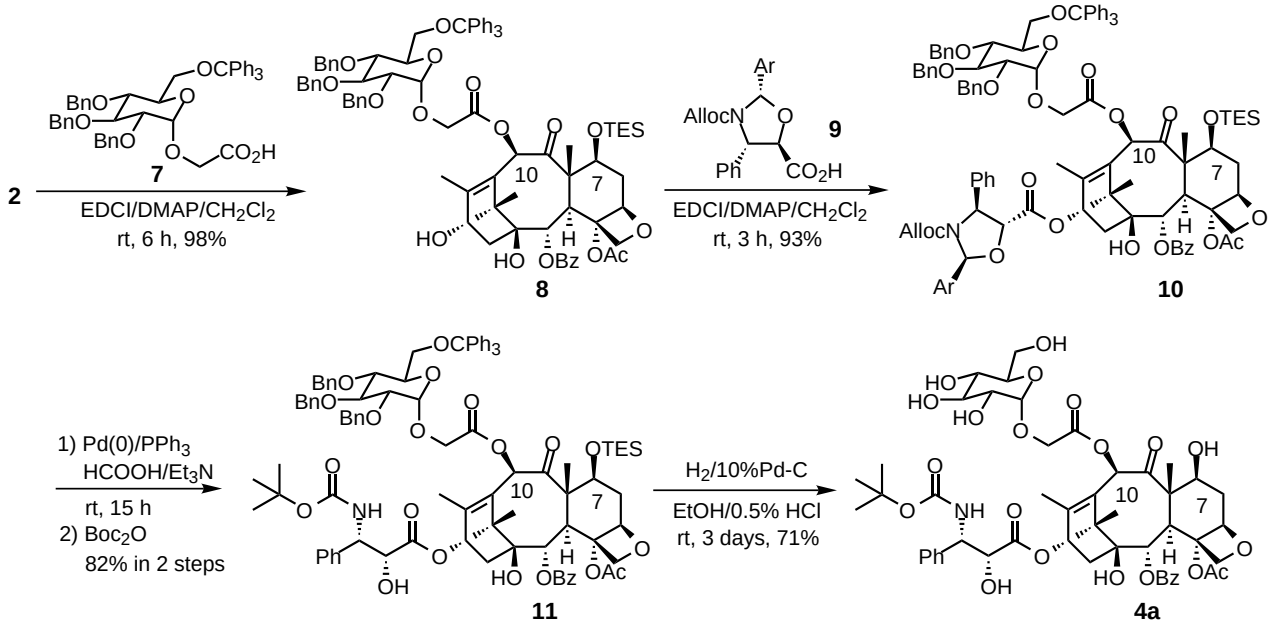
The water solubility[5] of **3a-3d** thus obtained ranges from 58 to 258 times of **1a** as shown in Table 1. Disappointingly their *in vitro* cytotoxicity (IC_{50})[6] against P388 leukemia cells was much lower than that of **1a**.

Table 1 Water solubility and cytotoxicity of **3**

3 (Glycosyl)	Water solubility (μg/mL)	Cytotoxicity ($IC_{50}/IC_{50(\mathbf{1a})}$)
Paclitaxel (**1a**)	0.4	1
3a (glucose)	23	3.3
3b (galactose)	68	3.8
3c (α-mannose)	103	18.3
3d (xylose)	32	2.7

Then, we turned our attention to C-10 modified taxoids (**4**) (n=0) whose amino group protection is simultaneously replaced by a more hydrophilic carbamate as is seen in docetaxel (**1b**). The synthesis of compound (**4a**) possessing a-glucose is represented in Scheme 2. Condensation of 7-TES-10-deacetylbaccatin III (**2**) with a-glucosyloxyacetic acid (**7**) [7] (1.1 eq) [EDCI/DMAP, CH_2Cl_2, rt, 6 h] proceeded in a highly regioselective manner to give **8** in 98% yield. Then, **8** was condensed with a side chain precursor (**9**)[8] (1.2 eq) [EDCI/DMAP, CH_2Cl_2, rt, 3 h] to afford **10** in 93% yield. The amino group protection of **10** was cleaved very smoothly by the treatment with an excess of HCO_2H/Et_3N (5 eq/10 eq) in the presence of $Pd(OAc)_2/PPh_3$ catalyst (2 mol%/4 mol%) (THF, rt, 15h)[9] to give a free amine which, without purification, was led to **11** in 82% after treatment with Boc_2O. Finally, the all protecting groups in **11** were simultaneously removed by hydrogenation (H_2/10% Pd-C/EtOH/0.5% HCl, rt, 3 days) to produce **4a**[10] in 71% yield.

Scheme 2

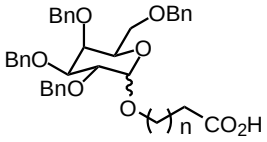


Compounds (**4b**) (b-glucose), (**4c**) (a-galactose), (**4d**) (b-galactose), and (**4e**) (a-mannose) were synthesized by the same procedures. Both the water solubility and *in vitro* cytotoxicity against P388 leukemia cells of **4a-4e** were listed in Table 2. The 18 to 74 fold solubility of **1b** was achieved. Of particular interest was that compounds (**4a-4d**) showed considerably stronger cytotoxicity than **1b**.

Table 2 Water solubility and cytotoxicity of **4** (n=0)

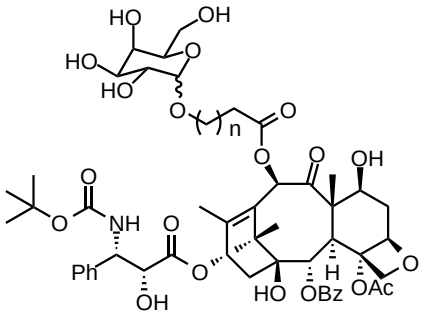
4 (Glycosyl)	Water solubility(μg/mL)	Cytotoxicity ($IC_{50}/IC_{50(\mathbf{1b})}$)
Docetaxel (**1b**)	14	1
4a (α-glucose)	332	0.46
4b (β-glucose)	253	0.38
4c (α-galactose)	482	0.50
4d (β-galactose)	301	0.63
4e (α-mannose)	1039	3.1

To investigate the effect of the tether length on the water solubility and the cytotoxicity, we prepared a new array of taxoids (**4f-4j**) (n=2~4, 6, 8) by condensing w-galactosyloxy carboxylic acids (**12**) (n=2~4, 6, 8) followed by the same synthetic procedures of **4a** as depicted in Scheme 2.



12

(n=2~4, 6, and 8)



4f-4j

(n=2~4, 6, and 8)

The water solubility of **4f-4j** thus obtained is listed in Table 3. Interestingly, **4f** (n=2), **4g** (n=3), and **4h** (n=4) were found to be more soluble than **4a-4d** (n=0). On the contrary to these observations, the *in vitro* cytotoxicity[11] of **4f-4j** against B16 melanoma cells was much lower than that of **1b**.

Table 3 Water solubility and cytotoxicity of **4f-4j**

4 (n)	Water solubility(μg/mL)	Cytotoxicity [IC_{50} (ng/mL)]
Docetaxel (**1b**)	14	<3.9
4f (n=2)	712	700
4g (n=3)	928	700
4h (n=4)	525	155
4i (n=6)	95	360
4j (n=8)	10	-*

* Not measured.

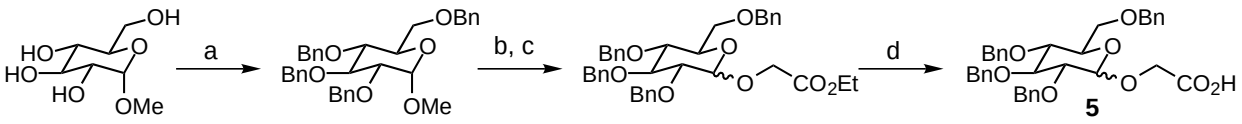
In conclusion, we succeeded in exploiting a variety of taxoids with the superior water solubility to **1a** or **1b** by tethering a sugar moiety by an ester linkage at 7- or 10-position. Above all, 10-modified compounds (**4a**-**4d**) showed the satisfactory improvement in both water solubility and cytotoxicity in comparison with **1b**. These encouraging observations have much stimulated our challenge to SAR study of taxoids. Further investigation on their biological activity *in vivo* and biochemical behaviors will be reported in due course.

ACKNOWLEDGMENTS

This work was supported by the Grant-in-Aid for Scientific Research on Priority Area No. 08245103 from the Ministry of Education, Science, Sports and Culture, of Japanese Government.

REFERENCES AND NOTES

1. For a review: *Taxol Science and Application*, ed. by M. Suffness, CRC:Boca Raton, FL, 1995.
2. Compound (**5**) was prepared as follows:

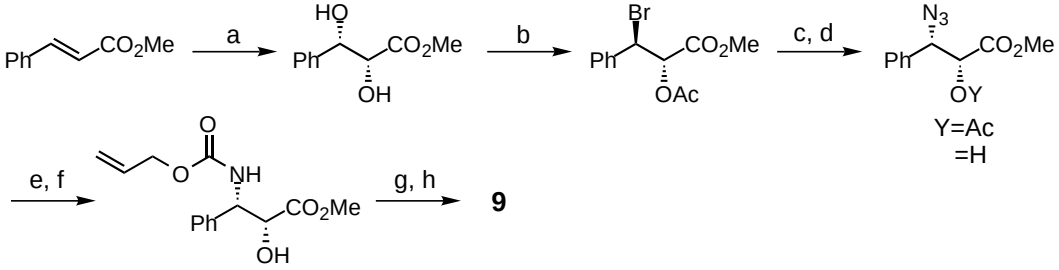


a) BnBr/NaH/DMF, rt, 12 h, 90% b) H_2SO_4/HOAc, 100 °C, 3 h, 85% c) $HOCH_2CO_2Et$/*p*-TsOH/PhH, reflux, 3 h d) KOH, 75% in 2 steps

3. ^{1}H NMR (500 MHz, $CDCl_3$) d 1.14 (s, 3H, 17-CH_3), 1.20 (s, 3H, CH_3), 1.81 (s, 3H, CH_3), 1.84 (s, 3H, CH_3), 2.17 (s, 3H, CH_3CO), 2.38 (s, 3H, CH_3CO), 2.25-2.35 (m, 2H), 2.5-2.7 (m, 2H), 3.3-3.9 (m, 5H), 4.1-4.5 (m, 4H), 4.85 (br, 1H, H2'), 4.95 (brd, J=9.1, 1H, H5), 5.5-5.8 (m, 3H), 6.1-6.2 (m, 2H), 7.3-7.6 (m, 11H, Ar, NH), 7.6-7.7 (m, 1H, Ar), 7.7-7.8 (m, 2H, Ar), 8.1-8.2 (m, 2H, Ar).
4. Galactosyloxy-, xylosyloxy-, and mannosyloxyacetic acids were prepared, respectively, by the same procedure depicted in reference 2. However, only the a-anomer was obtained in mannosyloxyacetic acid synthesis.
5. The water solubility was measured as follows: After a mixture of water (0.5 mL) and taxoid (2.5 mg) being stirred at room temperature for 18 h, the solution was filtered, and the filtrate was

analyzed by HPLC (ODS column).

6. P388 murine leukemia cells (5 10^3 cells) were cultured in a well of a 96-well microtestplate for 24 h and further incubated in the presence of serial 2-fold dilutions of each taxoid for 48 h in RPMI1640 medium supplemented with 10% fetal calf serum. At the termination of culture, the number of viable cells was determined by MTT assay.[10] The IC_{50} indicates the concentration showing 50% of compound free control value (absorbance at 540 nm).
7. T. Mandai, H. Okumoto, T. Oshitari, K. Nakanishi, K. Mikuni, K. Hara, and K. Hara, *Heterocycles*, 2000, **52**, 129.
8. Compound (**9**) was prepared according to the reported procedures.



a) Sharpless AD, 70% b) 33% HBr/HOAc, rt, 4 h c) NaN_3/DMF, 60 °C, 20 h d) MeOH/AcCl (cat.), rt, 12 h, 75% in 3 steps e) PPh_3/H_2O/THF, rt, 12 h f) $ClCO_2CH_2CH{=}CH_2/Et_2O$, $NaHCO_3$, rt, 12 h, 93% in 2 steps g) *p*-anisaldehyde dimethyl acetal/CSA, toluene, reflux, 5 h, 93% h) 6N NaOH/EtOH, rt, 3 h, 95%

Cf: (a) Z.-M. Wang, H. C. Kolb, and K. B. Sharpless, *J. Org. Chem*., 1994, **59**, 5104. (b) J. Deng, Y. Hamada, and T. Shioiri, *J. Am. Chem. Soc*., 1995, **117,** 7824. (c) I. Shiina, K. Saitoh, I. Fréchard-Ortuno, and T. Mukaiyama, *Chem. Lett*., 1998, 3.

9. For a review: J. Tsuji and T. Mandai, *Synthesis*, 1996, 1.
10. ^{1}H NMR spectrum (500 MHz, $CDCl_3/CD_3OD$=10/1) showed ambiguous broad peaks. However, it was observed that the ester linkage at C-10 of **4a** was gradually cleaved in whole human blood, human serum, or rat serum to release docetaxel (**1b**) and a-glucosyloxyacetic acid which were easily monitored by HPLC.
11. B16 murine melanoma cells (5 10^3 cells) were cultured in a well of a 96-well microtestplate for 24 h and further incubated in the presence of serial 2-fold dilutions of each taxoid for 48 h in Eagle's MEM medium supplemented with 10% fetal calf serum. At the termination of culture, the number of viable cells was determined by MTT assay.[12] The IC_{50} indicates the concentration showing 50% of compound free control value (absorbance at 540 nm).
12. H. Tada, O. Shiho, K. Kuroshima, M. Koyama, and K. Tsukamoto, *J. Immunol. Method*, 1986, **93,** 157.