

SYNTHESIS AND BIOLOGICAL EVALUATION OF WATER SOLUBLE TAXOIDS BEARING SUGAR MOIETIES

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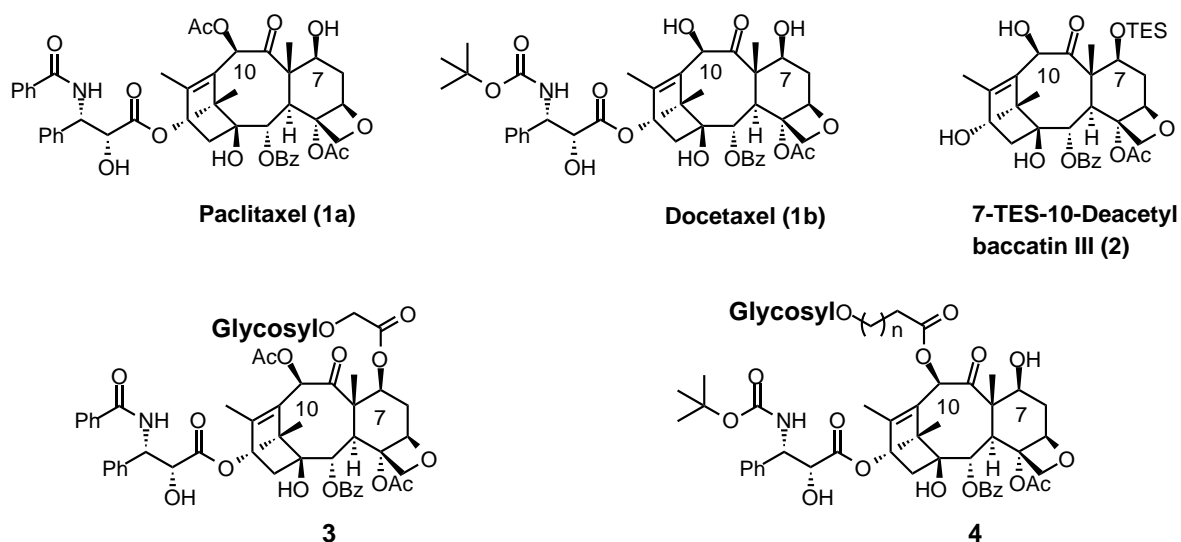
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Abstract- Synthesis and biological evaluation of a variety of water soluble taxoids with
sugar moieties are described.

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

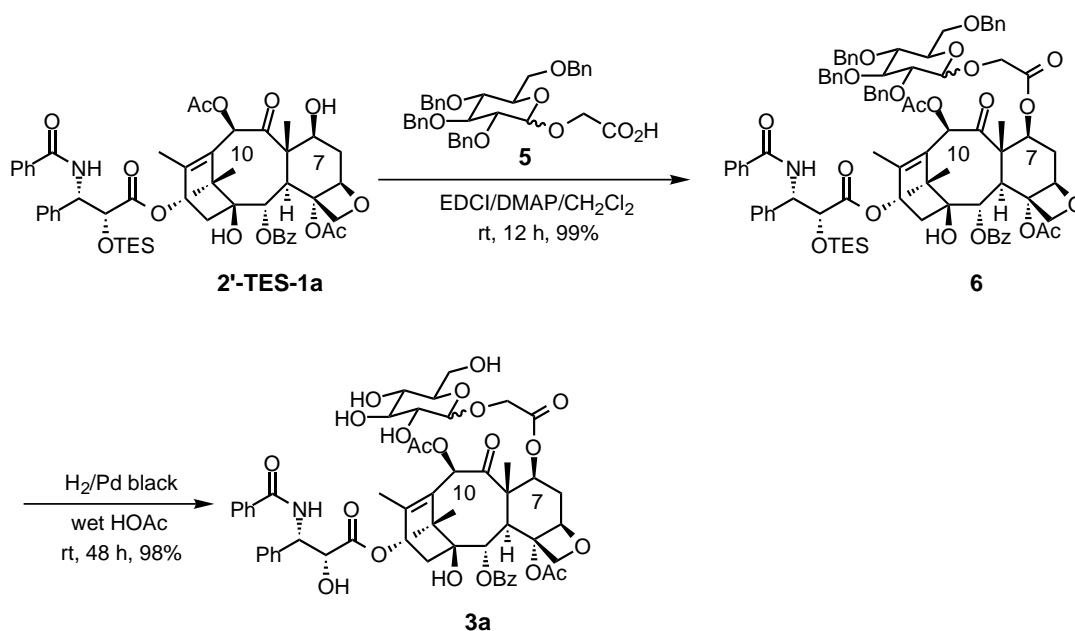
Several years ago, we launched systematic SAR investigation of water soluble paclitaxel prodrugs that exhibit superior water solubility as well as the higher level of cytotoxicity to that of **1a**. On designing such compounds that meet the above demands, sugars seemed to be the first-rate candidate 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).



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, CH₂Cl₂, 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



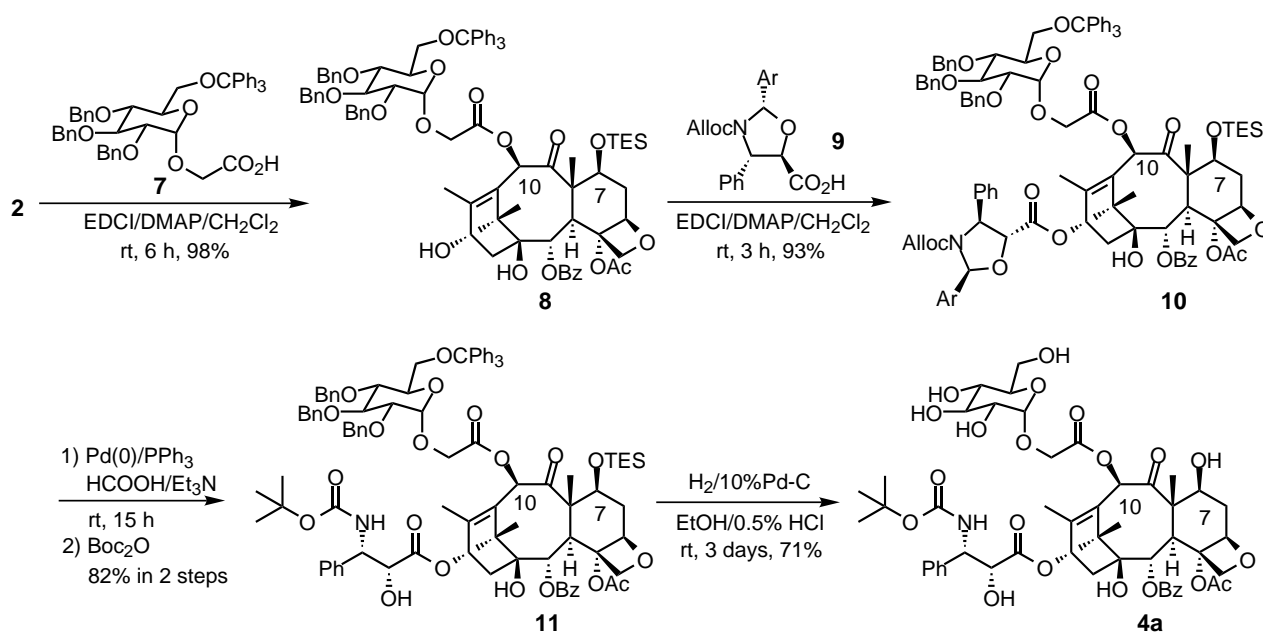
The water solubility⁵ of **3a-3d** thus obtained ranges from 58 to 258 times of **1a** as shown in Table 1. Disappointingly their *in vitro* cytotoxicity (IC₅₀)⁶ against P388 leukemia cells was much lower than that of **1a**.

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

3 (Glycosyl)	Water solubility ($\mu\text{g/mL}$)	Cytotoxicity ($\text{IC}_{50}/\text{IC}_{50(1a)}$)
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 α -glucose is represented in Scheme 2. Condensation of 7-TES-10-deacetylbaaccatin III (**2**) with α -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 $\text{HCO}_2\text{H}/\text{Et}_3\text{N}$ (5 eq/10 eq) in the presence of $\text{Pd}(\text{OAc})_2/\text{PPh}_3$ 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 ($\text{H}_2/10\% \text{Pd-C}/\text{EtOH}/0.5\% \text{HCl}$, rt, 3 days) to produce **4a**¹⁰ in 71% yield.

Scheme 2

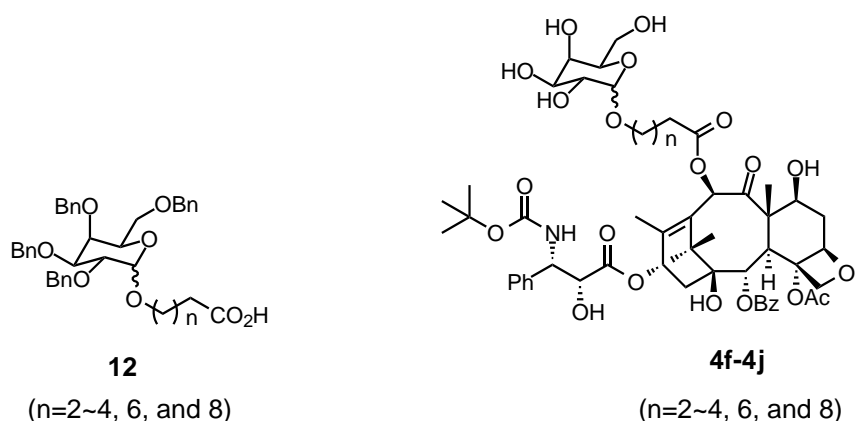


Compounds (**4b**) (β-glucose), (**4c**) (α-galactose), (**4d**) (β-galactose), and (**4e**) (α-mannose) were synthesized by the same procedures. Both the water solubility and *in vitro* cytotoxicity against P388 leukemia cells of **4a-4e** were listed in Table 2. The 18 to 74 fold solubility of **1b** 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 (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.



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* cytotoxicity¹¹ 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($\mu\text{g/mL}$)	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.

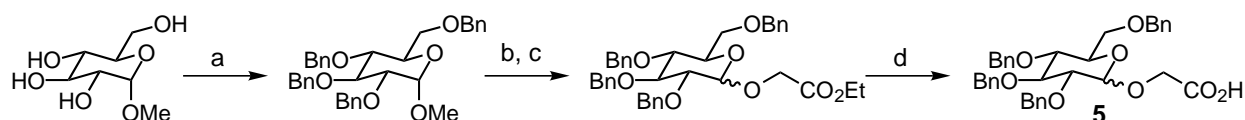
In conclusion, we succeeded in exploiting a variety of taxoids with the superior water solubility to **1a** or **1b** by tethering a sugar moiety by an ester linkage at 7- or 10-position. Above all, 10-modified compounds (**4a-4d**) showed the satisfactory improvement in both water solubility and cytotoxicity 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

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

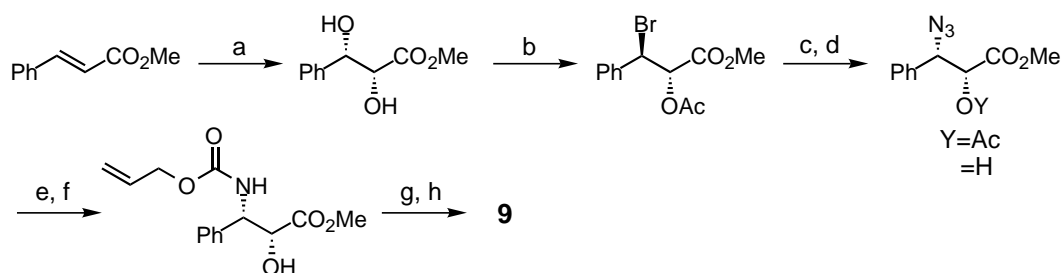


a) $\text{BnBr}/\text{NaH}/\text{DMF}$, rt, 12 h, 90% b) $\text{H}_2\text{SO}_4/\text{HOAc}$, 100 $^\circ\text{C}$, 3 h, 85% c) $\text{HOCH}_2\text{CO}_2\text{Et}/p\text{-TsOH}/\text{PhH}$, reflux, 3 h d) KOH , 75% in 2 steps

- ^1H NMR (500 MHz, CDCl_3) δ 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, H_2'), 4.95 (brd, $J=9.1$, 1H, H_5), 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).
- Galactosyloxy-, xylosyloxy-, and mannosyloxyacetic acids were prepared, respectively, by the same procedure depicted in reference 2. However, only the α -anomer was obtained in mannosyloxyacetic acid synthesis.
- 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).

- 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).
- T. Mandai, H. Okumoto, T. Oshitari, K. Nakanishi, K. Mikuni, K. Hara, and K. Hara, *Heterocycles*, 2000, **52**, 129.
- 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. Frécharde-Ortuno, and T. Mukaiyama, *Chem. Lett.*, 1998, 3.

- For a review: J. Tsuji and T. Mandai, *Synthesis*, 1996, 1.
- 1H 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.
- 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).
- H. Tada, O. Shiho, K. Kuroshima, M. Koyama, and K. Tsukamoto, *J. Immunol. Method*, 1986, **93**, 157.