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Chemo-enzymatic synthesis of ester-linked taxol–oligosaccharide conjugates as potential prodrugs

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Abstract

7-Glycolylpaclitaxel 2″-*O*-α-glucooligosaccharides, novel taxol (paclitaxel) prodrugs of ester-linked oligosaccharide series compounds, were synthesized by chemo-enzymatic procedures, including enzymatic transglycosylations with α-glucosidase and cyclodextrin glucanotransferase. The water-solubility of 7-glycolylpaclitaxel 2″-*O*-α-glucopentaoside was 2.7 mM, which was 6.8 thousand-fold higher than that of paclitaxel. C-7 modification of paclitaxel with a longer oligosaccharide chain decreased the in vitro cytotoxicity of paclitaxel against KF human ovarian cancer cells.

Taxol (paclitaxel), which is a taxane diterpenoid isolated from *Taxus brevifolia*, shows cytotoxic activity against leukemia cells and inhibitory action against a variety of tumors such as ovarian cancer, and has been recognized as one of the most effective and widely used anticancer agents.[1] It presents shortcomings such as low solubility in water and toxicity to normal tissues despite its effective pharmacological activities. Paclitaxel prodrugs, which incorporate acids or amino acids, have attracted considerable attention, because ester and amide linkages improve the water-solubility of paclitaxel and can be hydrolyzed by hydrolytic enzymes in the living body.[2] However, acid or amino acid conjugates lack tumor selectivity. To improve drug selectivity toward tumor cells, many efforts to chemically synthesize paclitaxel prodrugs designed containing a transport system have been made. An interesting approach for drug delivery is the use of saccharide based transporters,[3,4] and there have been several reports on the synthesis of paclitaxel–sugar conjugates.[3] In addition, saccharide conjugation drastically enhances the water-solubility of aglycone molecule.[5] In the present Letter, we describe the synthesis of highly water-soluble ester-linked oligosaccharide conjugates of paclitaxel, i.e., 7-glycolylpaclitaxel 2″-*O*-α-glucooligosaccharides [α-maltooligosaccharides; α-Glc-1→(4-α-Glc-1→)$_{n-1}$4-α-glucosides ($n = 1$–4)]. We also show the cytotoxic activity of paclitaxel–sugar conjugates toward KF human ovarian cancer cells.

Paclitaxel was used as the starting material for the synthesis of paclitaxel–sugar conjugates **4**–**8** (Scheme 1).

First, 7-glycolylpaclitaxel 2″-*O*-α-D-glucopyranoside (**4**) was synthesized by chemo-enzymatic methods, including stereoselective α-glucosylation with α-glucosidase. 2′-Hydroxyl group of paclitaxel was initially protected with triethylsilyl (TES) group to give 2′-TES ester of paclitaxel (Scheme 1). Carboxymethyl α-D-glucopyranoside (**1**) was prepared by stereoselective α-transglucosylation to glycolic acid (0.02 mol) from maltose (0.2 mol) with α-glucosidase (500 U) (Toyobo Co. Ltd) in 100 mL of DMSO–H_2O (1:4, v/v) at 40 °C for 24 h in 37% yield. Carboxymethyl α-D-glucopyranoside (**1**) was benzylated with BnBr/NaH in DMF at room temperature for 12 h, followed by stirring with KOH (1.5 equiv) to give carboxymethyl

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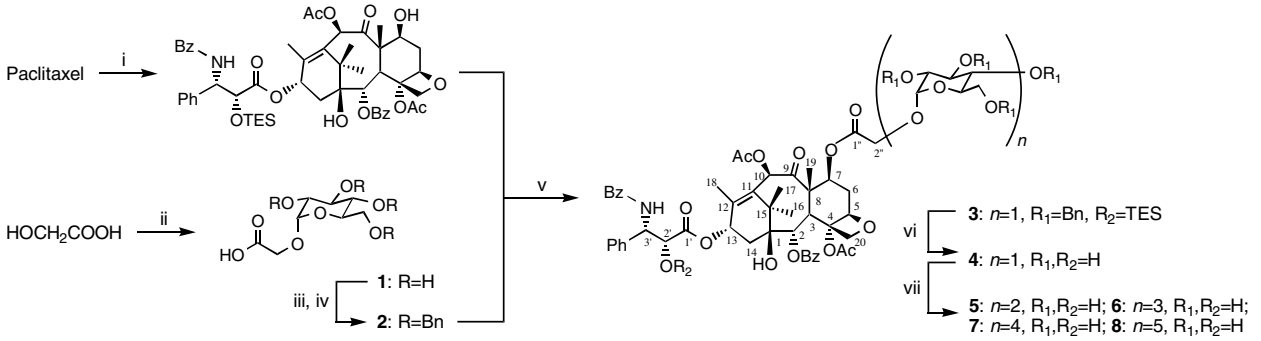

Scheme 1. Reagents and conditions: (i) TESCl, imidazole, DMAP; (ii) α-glucosidase, maltose, DMSO–H_2O (1:4, v/v); (iii) BnBr, NaH, DMF; (iv) KOH; (v) EDCI, DMAP, CH_2Cl_2; (vi) H_2, Pd black, HOAc–H_2O (9:1, v/v); (vii) CGTase, starch, Na–Pi buffer (25 mM).

2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (**2**). The yield of **2** was 89%. The coupling of 2′-TES ester of paclitaxel with **2** (1.2 equiv) in the presence of EDCI/DMAP in CH_2Cl_2 at room temperature for 12 h gave **3** in 95% yield. Deprotection of both TES and benzyl groups with Pd black in HOAc–H_2O (9:1, v/v) yielded 7-glycolylpaclitaxel 2″-*O*-α-D-glucopyranoside (**4**) in 97% yield.

Next, 7-glycolylpaclitaxel 2″-*O*-α-glucooligosaccharides, i.e., α-maltooligosaccharides, were synthesized from 7-glycolylpaclitaxel 2″-*O*-α-D-glucopyranoside (**4**) by enzymatic α-(1→4)-glucooligosaccharide formation with cyclodextrin glucanotransferase (CGTase). Glycosylation of **4** (1 mmol) with CGTase (700 U) (Amano Pharmaceutical Co. Ltd) in the presence of soluble starch (20 g) in 50 mL of 25 mM sodium phosphate buffer (pH 7.0) at 40 °C for 24 h yielded oligosaccharide products, four of which could be isolated by preparative HPLC [**5** (17% yield), **6** (14%), **7** (11%), and **8** (7%)] (Fig. 1). The structures of these four new compounds were established by HRFABMS and NMR analyses. The assignment of each signal in the NMR spectra of products **5**–**8** was performed by H–H COSY, C–H COSY, NOE, HMQC, and HMBC analyses, and products were identified as 7-glycolylpaclitaxel 2″-*O*-α-glucobioside (**5**), 7-glycolylpaclitaxel 2″-*O*-α-glucotrioside (**6**), 7-glycolylpaclitaxel 2″-*O*-α-glucotetraoside (**7**), and 7-glycolylpaclitaxel 2″-*O*-α-glucopentaoside (**8**).[6]

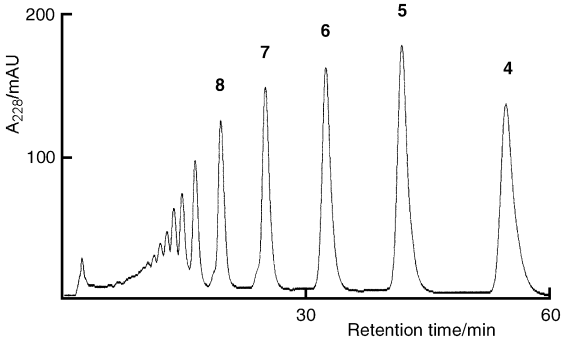

Fig. 1. HPLC analysis of 7-glycolylpaclitaxel 2″-*O*-α-glucooligosaccharides **5**–**8** produced by CGTase-catalyzed glycosylation of **4**.

Paclitaxel–sugar conjugates **4**–**8** were tested for their water-solubility.[7] The water-solubility of **4** was 21 μM, which was 53-fold higher than that of paclitaxel (Table 1). The water-solubility of paclitaxel–sugar conjugates increased in the order **4**, **5**, **6**, **7**, and **8**. The solubility of **8** was about 6.8 thousand-fold higher than that of paclitaxel.

The sensitivity of KF (paclitaxel-sensitive) and HAC-2 (paclitaxel-resistant) human ovarian adenocarcinoma cells to paclitaxel or paclitaxel–sugar conjugates **4**–**8** was examined by the MTT assay, and IC_{50} values of each test compounds are summarized in Table 2.[8,9] Two compounds, 7-glycolylpaclitaxel 2″-*O*-α-glucoside (**4**) and 7-glycolylpaclitaxel 2″-*O*-α-glucobioside (**5**), showed strong cytotoxicity against KF cells. The cytotoxic activity decreased in the order **4**, **5**, **6**, **7**, and **8**. On the other hand, no cytotoxic activity toward HAC-2 cells was found for all derivatives. Paclitaxel–sugar conjugates **4**–**8** were individually incubated with KF cells.[10] Compounds **4** and **5** were converted to a small amount of paclitaxel in 3% and 1%, respectively, whereas trace or no paclitaxel was obtained in the cases of **6**–**8**. These findings indicated that all derivatives were cytotoxic themselves, and that C-7 modification of paclitaxel with a longer oligosaccharide chain decreased its cytotoxicity. The oligosaccharide based transporters have been reported to serve to target drug to a specific organ in the living body, liver,[4] in which oligosaccharide moiety could be cleaved by hydrolytic enzymes. Thus, paclitaxel derivatives having a longer oligosaccharide, which show low cytotoxicity, would be potential antitumor prodrugs.[2d]

The ester-linked paclitaxel–sugar conjugates, novel taxol prodrugs of oligosaccharide-linked series compounds with a

Table 1
Water-solubility of paclitaxel and paclitaxel–sugar conjugates **4**–**8**

Compound	Water-solubility[a] (μM)	Fold
Paclitaxel	0.4	1
4	21	53
5	3.0×10^2	7.5×10^2
6	7.6×10^2	1.9×10^3
7	1.6×10^3	4.1×10^3
8	2.7×10^3	6.8×10^3

[a] Water-solubility was measured at 25 °C.

Table 2
IC_{50} values of paclitaxel and paclitaxel–sugar conjugates **4**–**8**[a]

Cell line	Paclitaxel (nM)	**4** (nM)	**5** (nM)	**6** (nM)	**7** (nM)	**8** (nM)
KF	131	170	855	1320	1906	2152
HAC-2	>3000	>3000	>3000	>3000	>3000	>3000

[a] IC_{50} values in KF or HAC-2 ovarian cancer cells.

high water-solubility, were synthesized by chemo-enzymatic procedures, including enzymatic glycosylations with α-glucosidase and CGTase. Recently, Mizukami and co-workers reported that the water-solubility of curcumin was drastically enhanced by a glycosyl conjugation.[5] The water-solubility of curcumin digentiobioside was 20 million-fold higher than that of curcumin. In this study, glycosyl conjugation effectively enhanced the water-solubility of paclitaxel, for example, the water-solubility of 7-glycolylpaclitaxel 2″-*O*-α-glucopentaoside, which has five glucose residues, was 6.8 thousand-fold higher than that of paclitaxel. We should emphasize that paclitaxel–sugar conjugates with a shorter saccharide at C-7 position would be useful water-soluble antitumor agents, that is, 7-glycolylpaclitaxel 2″-*O*-α-glucoside and 7-glycolylpaclitaxel 2″-*O*-α-glucobioside showed strong cytotoxicity against KF human ovarian cancer cells. Also paclitaxel derivatives with a longer oligosaccharide chain at C-7 position would act as potential prodrugs with a low cytotoxicity. The present chemo-enzymatic method is very useful for the practical preparation of ester-linked drug-oligosaccharide conjugates as highly water-soluble and inactive derivatives. Further studies on the therapeutic value of paclitaxel–sugar conjugates and on their hydrolysis process in the living body are currently in progress.

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6. *Spectral data for* **4**–**8**; product **4**: HRFABMS: *m*/*z* 1096.3050 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD, δ in ppm): δ 1.09 (3H, s, H-16), 1.15 (3H, s, H-17), 1.78 (3H, s, H-19), 1.81 (1H, m, H-6β), 1.87 (3H, s, H-18), 2.00 (1H, dd, *J* = 15.6, 9.2 Hz, H-14a), 2.15 (3H, s, CH_3 in 10Ac), 2.23 (1H, dd, *J* = 15.6, 9.2 Hz, H-14b), 2.37 (3H, s, CH_3 in 4Ac), 2.58 (1H, m, H-6α), 3.29–3.78 (8H, m, H-2″, 2a, 3a, 4a, 5a, 6a), 3.90 (1H, d, *J* = 7.2 Hz, H-3), 4.18 (3H, m, H-7, 20), 4.75 (1H, d, *J* = 5.2 Hz, H-2′), 4.95 (1H, d, *J* = 3.2 Hz, H-1a), 5.01 (1H, d, *J* = 9.2 Hz, H-5), 5.63 (2H, m, H-2, 3′), 6.15 (1H, t, *J* = 9.2 Hz, H-13), 6.21 (1H, s, H-10), 7.28 (1H, t, *J* = 7.6 Hz, *p*-H in Ph), 7.39–7.58 (9H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph), 7.65 (1H, t, *J* = 7.6 Hz, *p*-H in OBz), 7.85 (2H, d, *J* = 8.0 Hz, *o*-H in NBz), 8.10 (2H, d, *J* = 8.0 Hz, *o*-H in OBz); ^{13}C NMR (100 MHz, CD_3OD, δ in ppm): δ 11.3 (C-19), 14.7 (C-18), 20.6 (CH_3 in 10Ac), 22.1 (C-16), 23.1 (CH_3 in 4Ac), 26.7 (C-17), 34.1 (C-6), 36.3 (C-14), 44.5 (C-3, C-15), 57.2 (C-3′), 57.7 (C-8), 62.4 (C-6a), 65.9 (C-2″), 71.4 (C-7, C-13), 72.1 (C-4a), 73.6 (C-5a), 74.1 (C-2a), 74.8 (C-2′), 75.1 (C-3a), 75.7 (C-2), 76.6 (C-10), 77.2 (C-20), 78.8 (C-1), 81.8 (C-4), 85.0 (C-5), 100.6 (C-1a), 128.3 (*o*-C in NBz, *o*-C in Ph), 128.9 (*p*-C in NBz), 129.5 (*m*-C in OBz, *m*-C in Ph), 131.1 (*m*-C in NBz, q-C in OBz), 132.7 (*o*-C in OBz, *p*-C in Ph), 134.1 (C-11), 134.5 (q-C in Ph), 135.4 (*p*-C in OBz), 139.8 (q-C in NBz), 142.1 (C-12), 167.4 (C=O in OBz), 170.1 (C=O in NBz), 170.9 (C-1″), 171.3 (C=O in 4Ac), 171.9 (C=O in 10Ac), 174.3 (C-1′), 203.2 (C-9). Product **5**: HRFABMS: *m*/*z* 1258.3451 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.10 (3H, s, H-16), 1.16 (3H, s, H-17), 1.78 (3H, s, H-19), 1.81 (1H, m, H-6β), 1.87 (3H, s, H-18), 2.01 (1H, dd, *J* = 15.4, 9.2 Hz, H-14a), 2.15 (3H, s, CH_3 in 10Ac), 2.23 (1H, dd, *J* = 15.4, 9.2 Hz, H-14b), 2.37 (3H, s, CH_3 in 4Ac), 2.58 (1H, m, H-6α), 3.30–3.79 (14H, m, H-2″, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b, 6a, 6b), 3.90 (1H, d, *J* = 7.2 Hz, H-3), 4.19 (3H, m, H-7, 20), 4.75 (1H, d, *J* = 5.2 Hz, H-2′), 4.97 (1H, d, *J* = 3.2 Hz, H-1a), 5.01 (1H, d, *J* = 9.0 Hz, H-5), 5.22 (1H, d, *J* = 3.6 Hz, H-1b), 5.62 (2H, m, H-2, 3′), 6.15 (1H, t, *J* = 9.0 Hz, H-13), 6.21 (1H, s, H-10), 7.28 (1H, t, *J* = 7.2 Hz, *p*-H in Ph), 7.38–7.59 (9H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph), 7.65 (1H, t, *J* = 7.6 Hz, *p*-H in OBz), 7.85 (2H, d, *J* = 7.6 Hz, *o*-H in NBz), 8.10 (2H, d, *J* = 7.6 Hz, *o*-H in OBz); ^{13}C NMR (CD_3OD): δ 11.3 (C-19), 14.7 (C-18), 20.6 (CH_3 in 10Ac), 22.2 (C-16), 23.1 (CH_3 in 4Ac), 26.7 (C-17), 34.2 (C-6), 36.3 (C-14), 44.5 (C-3, C-15), 57.2 (C-3′), 57.7 (C-8), 62.4 (C-6b), 62.5 (C-6a), 65.9 (C-2″), 71.4 (C-7, C-13), 72.0 (C-4b), 73.3 (C-5a), 73.9 (C-2a), 74.8 (C-2′), 75.1 (C-2b, C-3a), 75.3 (C-3b, C-5b), 75.7 (C-2), 76.6 (C-10), 77.2 (C-20), 78.8 (C-1), 81.0 (C-4a), 81.8 (C-4), 85.0 (C-5), 100.5 (C-1a), 100.7 (C-1b), 128.3 (*o*-C in NBz, *o*-C in Ph), 128.9 (*p*-C in NBz), 129.6 (*m*-C in OBz, *m*-C in Ph), 131.2 (*m*-C in NBz, q-C in OBz), 132.7 (*o*-C in OBz, *p*-C in Ph), 134.1 (C-11), 134.5 (q-C in Ph), 135.4 (*p*-C in OBz), 139.9 (q-C in NBz), 142.2 (C-12), 167.5 (C=O in OBz), 170.0 (C=O in NBz), 170.9 (C-1″), 171.3 (C=O in 4Ac), 172.0 (C=O in 10Ac), 174.3 (C-1′), 203.2 (C-9). Product **6**: HRFABMS: *m*/*z* 1420.3877 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.09 (3H, s, H-16), 1.16 (3H, s, H-17), 1.78 (3H, s, H-19), 1.81 (1H, m, H-6β), 1.87 (3H, s, H-18), 2.00 (1H, dd, *J* = 15.6, 9.2 Hz, H-14a), 2.15 (3H, s, CH_3 in 10Ac), 2.24 (1H, dd, *J* = 15.6, 9.2 Hz, H-14b), 2.37 (3H, s, CH_3 in 4Ac), 2.58 (1H, m, H-6α), 3.29–3.80 (20H, m, H-2″, 2a, 2b, 2c, 3a, 3b, 3c, 4a, 4b, 4c, 5a, 5b, 5c, 6a, 6b, 6c), 3.90 (1H, d, *J* = 7.0 Hz, H-3), 4.17 (3H, m, H-7, 20), 4.75 (1H, d, *J* = 5.4 Hz, H-2′), 4.98 (1H, d, *J* = 3.2 Hz, H-1a), 5.00 (1H, d, *J* = 9.2 Hz, H-5), 5.19 (1H, d, *J* = 3.6 Hz, H-1b), 5.23 (1H, d, *J* = 3.6 Hz, H-1c), 5.63 (2H, m, H-2, 3′), 6.15 (1H, t, *J* = 9.2 Hz, H-13), 6.21 (1H, s, H-10), 7.28 (1H, t, *J* = 7.3 Hz, *p*-H in Ph), 7.40–7.59 (9H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph), 7.66 (1H, t, *J* = 7.6 Hz, *p*-H in OBz), 7.85 (2H, d, *J* = 8.0 Hz, *o*-H in NBz), 8.10 (2H, d, *J* = 7.6 Hz, *o*-H in OBz); ^{13}C NMR (CD_3OD): δ 11.3 (C-19), 14.7 (C-18), 20.7 (CH_3 in 10Ac), 22.1 (C-16), 23.1 (CH_3 in 4Ac), 26.7 (C-17), 34.1 (C-6), 36.3 (C-14), 44.6 (C-3, C-15), 57.1 (C-3′), 57.7 (C-8), 62.3 (C-6b, C-6c), 62.5 (C-6a), 66.0 (C-2″), 71.4 (C-7, C-13), 71.9 (C-4c), 73.5 (C-5a), 74.0 (C-2a), 74.4 (C-5b), 74.7 (C-2b), 74.8 (C-2′), 75.1 (C-2c, C-3a), 75.3 (C-3b, C-3c, C-5c), 75.7 (C-2), 76.6 (C-10), 77.2 (C-20), 78.8 (C-1), 81.1 (C-4a, C-4b), 81.8 (C-4), 85.0 (C-5), 100.5 (C-1a), 100.6 (C-1b, C-1c), 128.3 (*o*-C in NBz, *o*-C in Ph), 129.0 (*p*-C in NBz), 129.5 (*m*-C in OBz, *m*-C in Ph), 131.2 (*m*-C in NBz, q-C in OBz), 132.7 (*o*-C in OBz, *p*-C in Ph), 134.1

(C-11), 134.5 (q-C in Ph), 135.4 (*p*-C in OBz), 139.8 (q-C in NBz), 142.1 (C-12), 167.5 (C=O in OBz), 170.1 (C=O in NBz), 171.0 (C-1″), 171.3 (C=O in 4Ac), 172.1(C=O in 10Ac), 174.3 (C-1′), 203.2 (C-9). Product **7**: HRFABMS: *m*/*z* 1582.4395 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.10 (3H, s, H-16), 1.16 (3H, s, H-17), 1.78 (3H, s, H-19), 1.80 (1H, m, H-6β), 1.87 (3H, s, H-18), 2.00 (1H, dd, $J = 15.6$, 9.0 Hz, H-14a), 2.15 (3H, s, CH_3 in 10Ac), 2.23 (1H, dd, $J = 15.6$, 9.0 Hz, H-14b), 2.37 (3H, s, CH_3 in 4Ac), 2.59 (1H, m, H-6α), 3.25–3.80 (26H, m, H-2″, 2a, 2b, 2c, 2d, 3a, 3b, 3c, 3d, 4a, 4b, 4c, 4d, 5a, 5b, 5c, 5d, 6a, 6b, 6c, 6d), 3.90 (1H, d, $J = 7.6$ Hz, H-3), 4.18 (3H, m, H-7, 20), 4.75 (1H, d, $J = 5.2$ Hz, H-2′), 4.99 (1H, d, $J = 3.2$ Hz, H-1a), 5.02 (1H, d, $J = 9.2$ Hz, H-5), 5.20 (2H, d, $J = 3.6$ Hz, H-1b, 1c), 5.25 (1H, d, $J = 4.0$ Hz, H-1d), 5.63 (2H, m, H-2, 3′), 6.15 (1H, t, $J = 9.2$ Hz, H-13), 6.21 (1H, s, H-10), 7.28 (1H, t, $J = 7.6$ Hz, *p*-H in Ph), 7.39–7.58 (9H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph), 7.66 (1H, t, $J = 7.6$ Hz, *p*-H in OBz), 7.85 (2H, d, $J = 7.6$ Hz, *o*-H in NBz), 8.11 (2H, d, $J = 7.2$ Hz, *o*-H in OBz); ^{13}C NMR (CD_3OD): δ 11.3 (C-19), 14.7 (C-18), 20.7 (CH_3 in 10Ac), 22.1 (C-16), 23.1 (CH_3 in 4Ac), 26.7 (C-17), 34.1 (C-6), 36.3 (C-14), 44.5 (C-3, C-15), 57.3 (C-3′), 57.7 (C-8), 62.4 (C-6b, C-6c, C-6d), 62.5 (C-6a), 66.0 (C-2″), 71.5 (C-7, C-13), 71.9 (C-4d), 73.6 (C-5a), 74.1 (C-2a), 74.4, 74.5 (C-2b, C-2c, C-5b, C-5c), 74.8 (C-2′), 75.1, 75.2, 75.3 (C-2d, C-3a, C-3b, C-3c, C-3d, C-5d), 75.9 (C-2), 76.6 (C-10), 77.2 (C-20), 78.9 (C-1), 81.0, 81.1 (C-4a, C-4b, C-4c), 81.8 (C-4), 85.2 (C-5), 100.5 (C-1a), 100.6, 100.7 (C-1b, C-1c, C-1d), 128.5 (*o*-C in NBz, *o*-C in Ph), 128.9 (*p*-C in NBz), 129.5 (*m*-C in OBz, *m*-C in Ph), 131.2 (*m*-C in NBz, q-C in OBz), 132.7 (*o*-C in OBz, *p*-C in Ph), 134.1 (C-11), 134.6 (q-C in Ph), 135.4 (*p*-C in OBz), 140.0 (q-C in NBz), 142.2 (C-12), 167.4 (C=O in OBz), 170.2 (C=O in NBz), 170.9 (C-1″), 171.3 (C=O in 4Ac), 172.1 (C=O in 10Ac), 174.3 (C-1′), 203.2 (C-9). Product **8**: HRFABMS: *m*/*z* 1744.4733 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 1.10 (3H, s, H-16), 1.16 (3H, s, H-17), 1.78 (3H, s, H-19), 1.82 (1H, m, H-6β), 1.87 (3H, s, H-18), 2.01 (1H, dd, $J = 15.6$, 9.2 Hz, H-14a), 2.15 (3H, s, CH_3 in 10Ac), 2.23 (1H, dd, $J = 15.6$, 9.2 Hz, H-14b), 2.37 (3H, s, CH_3 in 4Ac), 2.59 (1H, m, H-6α), 3.26–3.83 (32H, m, H-2″, 2a, 2b, 2c, 2d, 2e, 3a, 3b, 3c, 3d, 3e, 4a, 4b, 4c, 4d, 4e, 5a, 5b, 5c, 5d, 5e, 6a, 6b, 6c, 6d, 6e), 3.90 (1H, d, $J = 7.2$ Hz, H-3), 4.19 (3H, m, H-7, 20), 4.75 (1H, d, $J = 5.2$ Hz, H-2′), 4.99 (1H, d, $J = 3.2$ Hz, H-1a), 5.01 (1H, d, $J = 9.6$ Hz, H-5), 5.20 (3H, m, H-1b, 1c, 1d), 5.25 (1H, d,$J = 4.0$ Hz, H-1e), 5.63 (2H, m, H-2, 3′), 6.15 (1H, t, $J = 9.0$ Hz, H-13), 6.21 (1H, s, H-10), 7.28 (1H, t, $J = 7.6$ Hz, *p*-H in Ph), 7.37–7.58 (9H, m, *m*-H in NBz, *p*-H in NBz, *m*-H in OBz, *o*-H in Ph, *m*-H in Ph), 7.65 (1H, t, $J = 7.6$ Hz, *p*-H in OBz), 7.85 (2H, d, $J = 8.2$ Hz, *o*-H in NBz), 8.11 (2H, d, $J = 8.0$ Hz, *o*-H in OBz); ^{13}C NMR (CD_3OD): δ 11.3 (C-19), 14.7 (C-18), 20.6 (CH_3 in 10Ac), 22.1 (C-16), 23.3 (CH_3 in 4Ac), 26.7 (C-17), 34.1 (C-6), 36.3 (C-14), 44.5 (C-3, C-15), 57.3 (C-3′), 57.7 (C-8), 62.4 (C-6b, C-6c, C-6d, C-6e), 62.5 (C-6a), 65.8 (C-2″), 71.4 (C-7, C-13), 72.0 (C-4e), 73.5 (C-5a), 73.9 (C-2a), 74.4, 74.5 (C-2b, C-2c, C-2d, C-5b, C-5c, C-5d), 74.8 (C-2′), 75.1, 75.2, 75.3 (C-2e, C-3a, C-3b, C-3c, C-3d, C-3e, C-5e), 75.7 (C-2), 76.6 (C-10), 77.2 (C-20), 78.5 (C-1), 81.0, 81.1 (C-4a, C-4b, C-4c, C-4d), 81.8 (C-4), 85.0 (C-5), 100.5 (C-1a), 100.6, 100.7 (C-1b, C-1c, C-1d, C-1e), 128.3 (*o*-C in NBz, *o*-C in Ph), 129.1 (*p*-C in NBz), 129.5 (*m*-C in OBz, *m*-C in Ph), 131.0 (*m*-C in NBz, q-C in OBz), 132.7 (*o*-C in OBz, *p*-C in Ph), 134.0 (C-11), 134.5 (q-C in Ph), 135.4 (*p*-C in OBz), 139.9 (q-C in NBz), 142.2 (C-12), 167.5 (C=O in OBz), 170.3 (C=O in NBz), 170.9 (C-1″), 171.3 (C=O in 4Ac), 172.0 (C=O in 10Ac), 174.3 (C-1′), 203.2 (C-9).

7. Each test compound was stirred in water for 24 h at 25 °C. The mixture was centrifuged at 12,000*g* for 30 min at 25 °C. Concentration of each compound was estimated on the basis of the peak area from HPLC using calibration curves prepared by HPLC analyses of respective samples on a Crestpak C18S column (4.6 × 150 mm, JASCO) [solvent: MeOH–H_2O (2:3, v/v); detection: UV (228 nm); flow rate: 1.0 mL/min].
8. The sensitivity of KF and HAC-2 cells to paclitaxel or **4**–**8** was determined according to the previously reported method.[9] Cells were diluted with culture medium to the seeding density (10^5 cells/mL), suspended in 96-well tissue culture plates (100 μL/well), preincubated at 37 °C for 4 h, and then treated for 24 h with paclitaxel or **4**–**8** at various concentrations to obtain a dose–response curve for each compound. After incubation, 20 μL MTT solution (2.5 mg/mL) was added to each well and the plates were further incubated for 4 h. Absorbance at 570 nm was measured with a microplate reader model 450 (BIO-RAD). Dose–response curves were plotted on a semi-log scale as percentage of the cell numbers in control cultures not exposed to test compounds.
9. Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55.
10. To a 5-mL vial containing 1 mL of RPMI 1640 medium (Nissui Pharmaceutical Co. Ltd) and 20 mg of KF cells was added 5 μmol of each compound. The mixture was incubated at 37 °C for 24 h. The cells and medium were separated by centrifugation at 10,000*g* for 5 min. The cells were extracted with MeOH. MeOH extract was concentrated, and the residue was partitioned between H_2O and CH_2Cl_2. The medium was extracted with CH_2Cl_2. The CH_2Cl_2 fractions were combined, concentrated, and analyzed by HPLC. The yield of the product, paclitaxel, was calculated on the basis of the peak area from HPLC using a calibration curve provided by HPLC analyses of authentic paclitaxel. Yields of paclitaxel from compounds **4**–**8** were 3%, 1%, trace, 0%, and 0%, respectively.