SYNTHESIS OF NEOSAPONINS CARRYING OLIGOSACCHARIDES FROM NATURAL PRODUCTS

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Abstract----The natural oligosaccharide moieties, α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranose (fabatriose) and α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose (mimosatetraose), were respectively cleaved from soyasaponin I and julibrosides, and were linked to appropriate aglycones to give neosaponins. The cytotoxicity and hepatoprotective activity of the obtained neosaponins were assayed. The transglycosidation method developed here could be applied to synthesize novel bioactive glycosides.

Recent studies on glycobiology have revealed the important roles of cell surface glycoconjugates in cell-cell adhesions and signal transudations, *i. e.*, immune responses, viral and bacterial infection, regulation, differentiation, and development.[1] To investigate the intrinsic role of oligosaccharide moieties in the glycoconjugates, isomerically pure oligosaccharides have to be utilized in biological assays, although their preparation by linking monosaccharides is quite tedious due to the difficulty of regio- and stereochemical control in forming glycosidic linkages. Furthermore, practical cleaving and reattaching procedures, which are well established in the field of protein and gene technologies, are also required for oligosaccharides to develop the glycotechnology. Therefore, we here would like to report a new method to prepare neoglycoconjugates from naturally abundant glycosides by a cleaving and reattaching

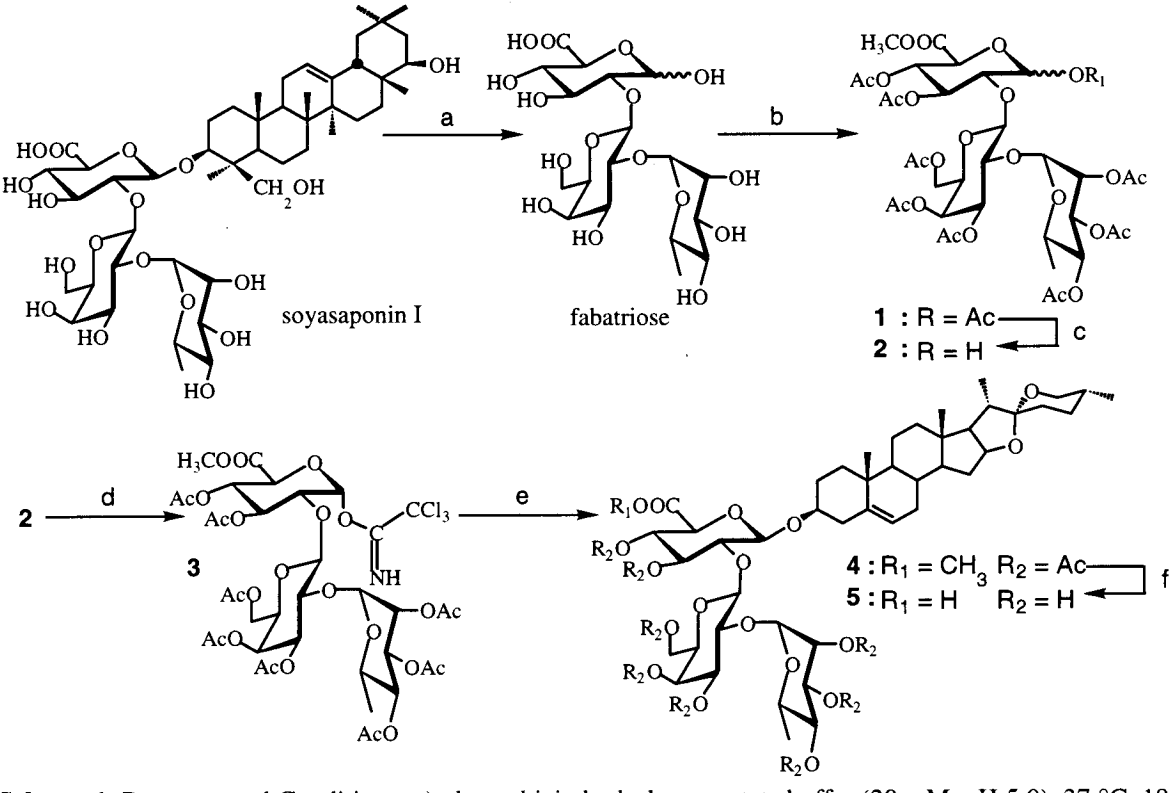
procedure.

Triterpenoid glycosides comprise one of the largest families of glycolipids such as glycosphingolipids and glycophospholipids. They are major components of traditional Chinese medicines and often have remarkable bioactivities, especially pharmacological activities,[2] *e. g.*, glycyrrhizin from licorice roots.[3] We recently found that α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl (β-fabatriosyl) oleanene glycosides from Fabaceae plants show a healing activity in experimental models of liver injuries.[4] This hepatoprotective activity is reduced when the β-fabatriosyl moiety is removed by acid hydrolysis.[5] On the other hand, julibroside III from *Albizia julibrissin* (Mimosoideae), which is a complex saponin and has an ester glycosidic bond as well as an ether glycosidic bond, is a cytotoxic triterpenoid glycoside.[6] Removing the ester glycosidic bond, α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl (mimosatetraosyl), dramatically decreased the cytotoxic activity.[7] Based on this background, β-fabatriose and mimosatetraose were chosen as an ether glycoside and an ester glycoside, respectively, for our first trial of chemical transglycosidation to prepare neosaponins.

Results and Discussion

Glycyrrhizin hydrolase (GHase) selectively cleaves an ether-linked *endo*-glucuronic acid at the C-3 position of triterpenoid.[8] The fabatriosyl moiety was cleaved by GHase from soyasaponin I, which is the major oleanene glucuronide contained in soy beans, for the study on our chemical transglycosidation of ether-linked oligosaccharides. The obtained fabatriose was methylated with CH_2N_2 and successively acetylated with Ac_2O and pyridine to give a compound (**1**) in overall 57 % yield (Scheme 1). The acetyl group at the C-1 position of the glucose in **1** was selectively deacylated with hydrazine acetate[9] to give methyl *O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-

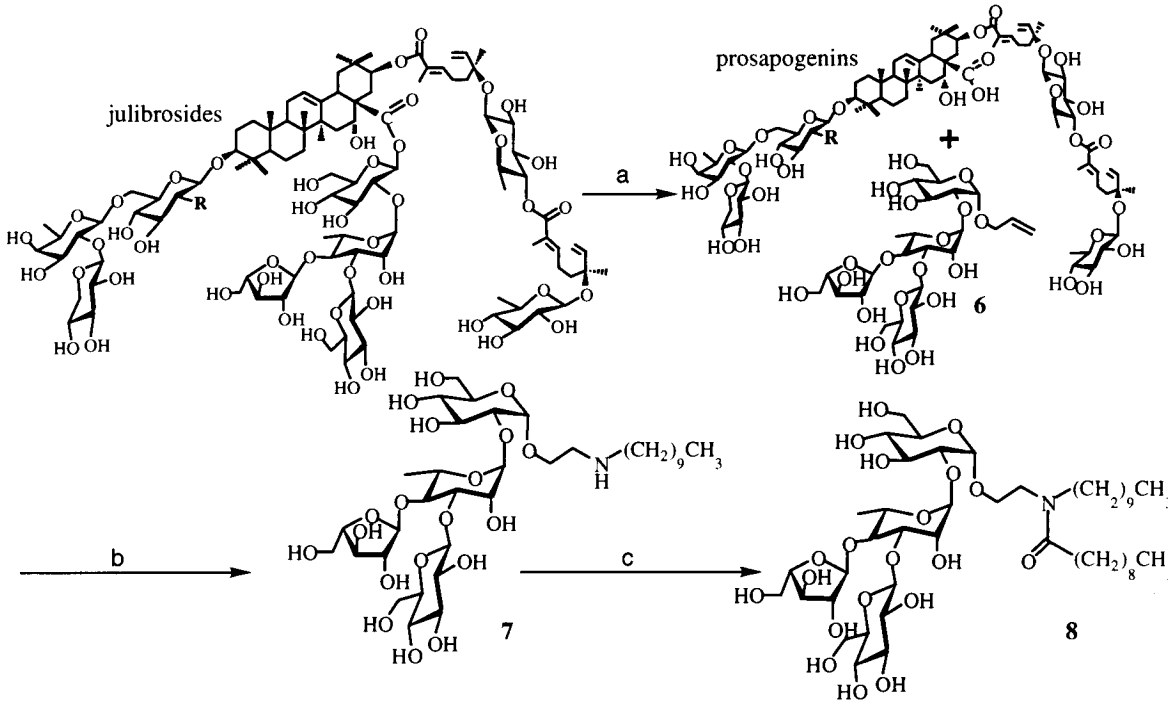
(1→2)-3,4-di-*O*-acetyl-D-glucopyranuronate (**2**) in 85 % yield. Reaction of **2** with trichloroacetonitrile in the presence of DBU provided the α-trichloroacetimidate (**3**) [10] quantitatively. The glycosyl donor (**3**) and diosgenin, a representative aglycone of cytotoxic steroidal glycosides,[11] were treated with $BF_3 \cdot Et_2O$ to afford a fully protected diosgenin β-fabatrioside (**4**) in 64 % yield without any detectable production of the α-fabatrioside. After deprotection in the usual manner, β-fabatiosyl diosgenin (**5**) was obtained.[12]



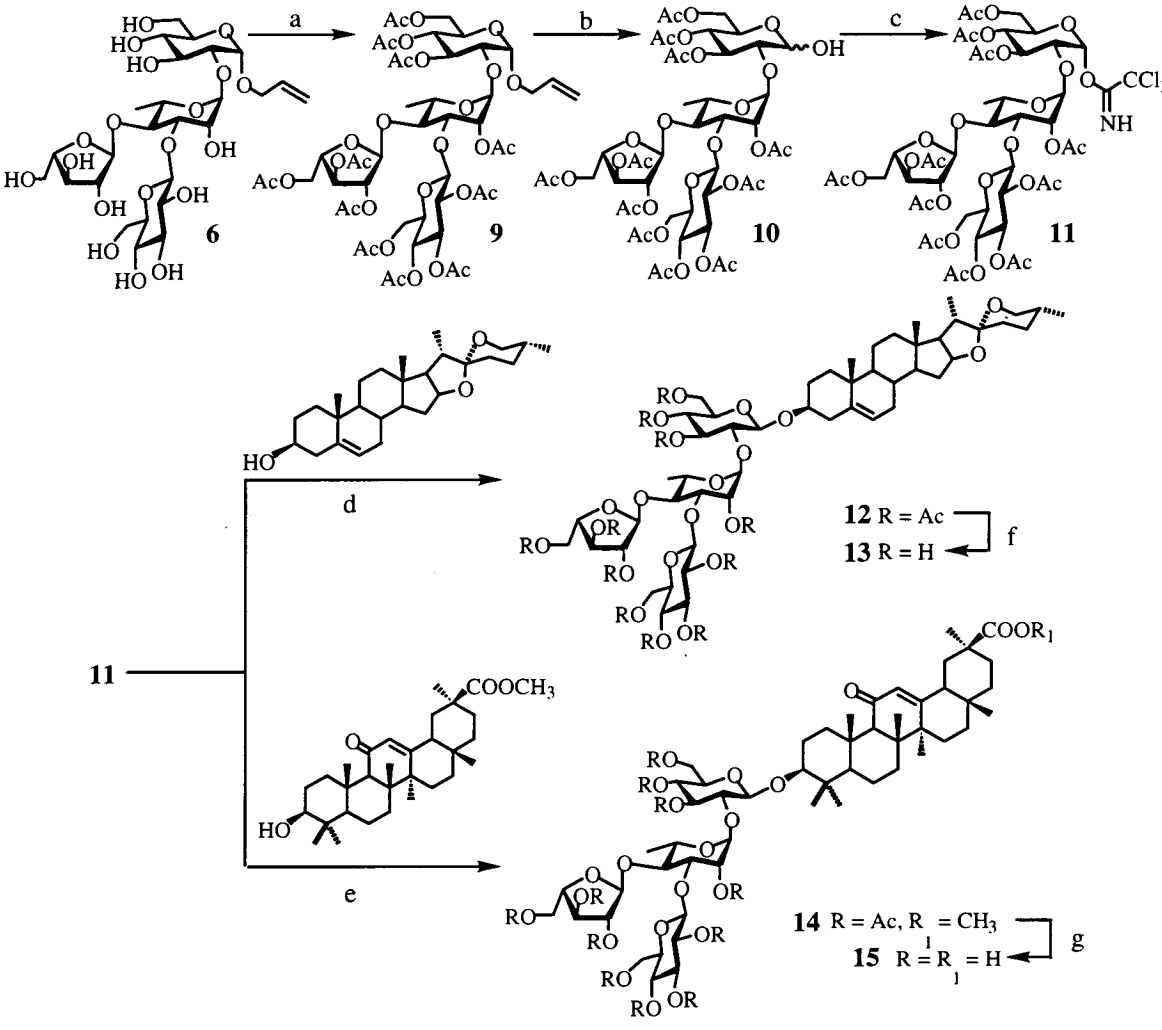
Scheme 1. Reagents and Conditions: a) glycyrrhizin hydrolase, acetate buffer (20 mM, pH 5.0), 37 °C, 18 h, quantitative; b) i. CH_2N_2 / ether, CH_3OH, 0 °C, ii. Ac_2O, DMAP, pyridine, rt, 2.5 h, overall 57 %; c) $NH_2NH_2 \cdot AcOH$, DMF, 60 °C, 10 min, 85 %; d) CCl_3CN, DBU, CH_2Cl_2, -5 °C, 2 h, quantitative; e) diosgenin, MS 4 Å, $BF_3 \cdot Et_2O$, CH_2Cl_2, -20 °C, 4 h, 64 %; f) 1*N* NaOH-EtOH (1:2), reflux, 1.5 h, 85 %.

Incidentally, the ester glycosidic bonds of julibrosides were cleaved with lithium iodide [13] in the presence of 2,6-lutidine and allyl alcohol to give the allyl α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-glucopyranoside (allyl α-mimosatetraoside) (**6**) (Scheme 2). During the reaction, mimosatetraosyl iodide was presumably formed as an intermediate which

spontaneously reacted with allyl alcohol by SN2 reaction. In this reaction, prosapogenins were obtained along with **6** without cleavage of any other glycosidic linkage. It is noteworthy that alkaline hydrolysis of julibrosides failed to afford mimosatetraose. As many double-chain neoglycolipids[14] mimicking glycosyl ceramides such as bissulfone lactosides,[15] double-tailed amide glycoside,[16] and dipalmitoylphosphatidylamine glycoside,[17] have shown to have interesting biological activities, the obtained allyl mimosatetraoside (**6**) is converted to a double-tailed amide glycoside. The compound (**6**) was ozonolyzed to afford the aldehyde, which was treated with decylamine in the presence of sodium cyanoborohydride[18] to give 1-decylaminoethyl-2-*O*-mimosatetraoside (**7**) (overall 80 % yield). This compound (**7**) was acylated with decanoyl chloride in a biphasic solution of THF / 2M NaOAc[19] to afford *N*-decanoyl-1-decylaminoethyl-2-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranoside (**8**) in 85 % yield.[20]



Scheme 2. Reagents and Conditions: a) LiI, 2, 6-lutidine, allyl alcohol, reflux, 18 h, 70 %; b) i. O_3, CH_3OH then $(CH_3)_2S$, -78 °C, ii. $CH_3(CH_2)_9NH_2$, $NaBH_3CN$, CH_3OH, rt, 72 h, overall 76 %; c) $CH_3(CH_2)_8COCl$, 2 M $NaHCO_3$, THF, rt, 1.5 h, 85 %.



Scheme 3. Reagents and Conditions: a) Ac_2O, pyridine, rt, 52 h, 82 %; b) $Pd[P(Ph)_3]_4$, 80 °C, 1 h, quantitative; c) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 94 %; d) diosgenin, MS 4Å, $BF_3 \cdot Et_2O$, CH_2Cl_2, -10 °C, 4 h, 66 % (α: β ; 1 : 4); e) methylglycyrrhetinate, MS 4Å, $BF_3 \cdot Et_2O$, CH_2Cl_2, -20 °C, 4 h, 70 % (α : β ; 1 : 3); f) 1*N* NaOH-EtOH (1:1), rt, 8 h, 94 %; g) 1*N* NaOH-EtOH (1:1), reflux, 8 h, 97 %.

In order to expand the usage of ester-linked oligosaccharides, the mimosatetraose was transferred to glycyrrhetinic acid[21] and diosgenin.[11] The former is a typical oleanene-type triterpenoid and the latter is the aglycone of anti-tumor active dioscin. Allyl glycoside (**6**) was converted into trichloroacetimidate for the glycosylation, after protection of the hydroxyl groups. Acetylation of **6** with acetic anhydride and pyridine in the presence of 4-dimethylaminopyridine gave an acetate (**9**) (Scheme 3). Deallylation of **9**

with tetrakis(triphenylphosphin)palladium in acetic acid gave a compound (**10**) in quantitative yield.[22] Conversion of **10** into a glycosyl donor was achieved stereoselectively by treatment with trichloroacetonitrile and DBU to give a trichloroacetimidate (**11**) in 94 % yield. Reaction of **11** with diosgenin in the presence of $BF_3 \cdot Et_2O$ afforded the desired, fully protected glycoside (**12**) as a mixture of α and β anomers (1:4) in 66 % yields. Deacetylation of **12** was attained with 1*N* NaOH-EtOH (1:1) in 94 % yield, and recrystallization from CH_3OH gave a pure β anomer of **13**. In the same way, reaction of **11** with methyl glycyrrhetinate in the presence of $BF_3 \cdot Et_2O$ afforded the fully protected glycoside (**14**) as a mixture of α and β anomers (1:3) in 70 % yield. Purification of the mixture by column chromatography over micro-Bondapak C_{18} (CH_3CN:H_2O= 4:1) afforded pure β anomer (**14**). Deacylation of **14** gave the target glycoside (**15**) in 97 % yield.

The cytotoxicity of the obtained neoglycolipids (**5**, **7**, **8**, **13**, and **15**) toward PC-6 and P388 cell lines was tested (Table 1). The compounds (**8**, **13**, and **15**) showed some cytotoxicity even though they were less active than cisplatin (CDDP) and dioscin. Apparently, mimosatetraose plays an important role for the cytotoxicity.

Table 1. IC_{50} (μg/mL) of Neosaponins (**5**, **7**, **8**, **13**, and **15**).

Samples	PC-6	P388
CDDP	0.08	0.01
dioscin	1.1	0.4
5	>50	>50
7	>50	>50
8	38.8	2.2
13	7.7	9.1
15	>50	9.2

Hepatoprotective activity of the compounds (**5**, **13**, and **15**) was also assayed *in vitro* toward an immunological liver injury model (Table 2).[4] Since the compound (**5**) showed the activity, the β-fabatriosyl moiety apparently is important for the hepatoprotective effect.

Table 2. Hepatoprotective activity of Glycyrrhizin, Soyasaponin I, **5**, **13**, and **15** toward *in vitro* immunological liver injury on primary cultured rat hepatocytes.

	Concentration (μg/mL)	*n*	Protection (%)
Glycyrrhizin	200	4	5 ± 3 %
	500	4	25 ± 6 % **
Soyasaponin I	200	4	13 ± 9 %
	500	4	54 ± 6 % **
5	90	4	15 ± 9 % *
	200	4	37 ± 2 % **
	500	4	58 ± 4 % **
13	200	4	16 ± 6 %
	500	4	30 ± 6 % **
15	200	4	10 ± 3 %
	500	4	19 ± 3 % **

Significantly different from Reference, effective * $p < 0.05$, ** $p < 0.01$

In conclusion, the method described above is generally useful to prepare novel glycosides by a cleaving and reattaching procedure in the case that glycoconjugates have an *endo*-glucuronic or ester glycosidic linkage, and the procedure could disclose the biological roles of the sugar moiety of many other glycolipids at the molecular level.

EXPERIMENTAL

General. The fresh stem bark of *Albizzia julibrissin* DURAZZ was collected in the Botanical Garden of Kumamoto University. The optical rotations were measured with a JASCO DIP-1000KUY automatic digital polarimeter. IR spectra were recorded with a JEOL JIR-6500W FT-IR spectrometer. ^{1}H and ^{13}C-NMR spectra were measured with a JEOL EX-270 and/or α-500 FT-NMR spectrometer and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as the internal standard. The FAB-MS was measured with a JEOL DX-300 and/or SX102A spectrometer. The HR FAB-MS were measured with a JEOL DX-303 HF spectrometer and taken in a glycerol, triethylene glycol and *m* -nitrobenzyl alcohol matrix. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). Column chromatography was carried out on Kieselgel 60 (70-230 mesh and 230-400 mesh), Diaion HP-20, MCI gel CHP-20P

(Mitsubishi Chemical Industries), micro-Bondapak C_{18} (Waters), Sephadex LH-20 (Pharmacia), Amberlite IR-120B, and Amberlite MB-3 (Organo). Soyasaponin I was purified from commercially available saponin (from soy beans, Wako Pure Chemical Industries Ltd.) by chromatographies over MCI gel CHP-20P, silica gel and micro-Bondapak C_{18}. Glycyrrhizin hydrolase from *Aspergillus niger* GRM3 was gifted from Maruzen Kasei CO., Ltd.

Purification of Soyasaponin I. Saponin from soy beans (20 g) purchased from Wako Pure Chemical Industries Ltd. was further purified by column chromatography over MCI gel CHP-20P eluting with H_2O, 50% CH_3OH, and CH_3OH, successively. The CH_3OH eluate was subjected to SiO_2 column chromatography ($CHCl_3$:CH_3OH:H_2O=7:3:0.5) and further purification was attained by column chromatography over micro-Bondapak C_{18} (H_2O→70% CH_3OH) to afford a pure soyasaponin I (1.08 g).

Methyl *O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→2)-1,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (1). Glycyrrhizin hydrolase from *Aspergillus niger* GRM3 (3.0 mL: Activity, Glycyrrhizin 570 mg/mL/h) was added to a solution of soyasaponin I (292 mg, 0.31 mmol) in Tween 80 (1 mL), ethanol (4 mL) and an acetate buffer (pH 4.5, 50 mL), and the mixture was incubated at 37 °C for 18 h. After the reaction, the reaction mixture was heated at 80 °C for 1 min and the precipitate was filtered off, and the filtrate was subjected to column chromatography over Diaion HP-20 and eluted with H_2O and CH_3OH. The H_2O eluate was deionized by column chromatography over Sephadex LH-20 (95% CH_3OH), and the solvent was evaporated off. The residue was dissolved in H_2O and passed through column chromatography of Amberlite IR120B (H^+ form) and the eluate was lyophilized to afford fabatriose with a trace of amount of contaminates. Without further purification, the lyophilized residue was used for the next reaction. Excess amount of the CH_2N_2 in Et_2O was added to a solution of crude fabatriose (144 mg) in CH_3OH (5 mL) at 0 °C. The reaction was quenched by adding a small amount of acetic acid and the organic solvent was evaporated off. A solution

of the residue (153 mg) and 4-dimethylaminopyridine (catalytic amount) in acetic anhydride-pyridine (1:1, 2 mL) was stirred for 2.5 h at rt. The reaction mixture was poured into ice water and extracted with EtOAc, and the extract was washed with brine, dried over $MgSO_4$, and volatiles were then evaporated *in vacuo*. The residue was chromatographed over SiO_2 (hexane:EtOAc=1:1) to give **1** (157 mg, overall 57 % yield) as an amorphous powder; $[\alpha]_D$ +13.7° (*c* 1.18, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.21 (d, 3H, *J* = 6.1 Hz, rha-H-6), 2.02, 2.03, 2.07, 2.12×2, 2.13×2, 2.14, 2.17 (each s, 3H, C<u>H</u>$_3$CO), 3.74 (s, 3H, gluA-6 COOC<u>H</u>$_3$), 3.76 (*m* , 1H, gal-H-2), 3.90 (*m* , 2H, gal-H-5, rha-H-5), 4.08 (dd, 1H, *J* = 3.1, 9.8 Hz, gluA-H-2), 4.12 (*m* , 2H, gal-H-6, 6'), 4.37 (d, 1H, *J* = 10.4 Hz, gluA-H-5), 4.58 (d, 1H, *J* = 7.3 Hz, gal-H-1), 4.92 (d, 1H, *J* = 1.2 Hz, rha-H-1), 4.95 (dd, 1H, *J* = 3.0, 9.8 Hz, gal-H-3), 5.03 (*m* , 1H, rha-H-2), 5.04 (t, 1H, *J* = 9.8 Hz, rha-H-4), 5.13 (*m* , 1H, rha-H-3), 5.15 (dd, 1H, *J* = 9.8, 10.4 Hz, gluA-H-4), 5.34 (d, 1H, *J* = 3.0 Hz, gal-H-4), 5.40 (t, 1H, *J* = 9.8 Hz, gluA-H-3), 6.30 (d, 1H, *J* = 3.1 Hz, gluA-H-1); ^{13}C-NMR ($CDCl_3$): Table 3; Positive FAB-MS *m/z* 917 $[M+Na]^+$; HR positive FAB-MS 917.2537 $[M+Na]^+$ ($C_{37}H_{50}O_{25}Na$, Calcd for 917.2539).

Methyl *O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,4-di-*O*-acetyl-α-D-glucopyranuronate (2). A mixture of **1** (135 mg, 151 μmol) and hydrazine acetate (20 mg, 217 μmol) in DMF (2 mL) was stirred for 10 min at 60 °C under a nitrogen atmosphere. After completion of the reaction, the mixture was diluted with EtOAc. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. The volatiles were evaporated *in vacuo*, and the residue was chromatographed over SiO_2 (benzene:acetone=7:1) to give **2** (110 mg, 85%) as an amorphous powder; $[\alpha]_D$ +22.8° (*c* 0.48, $CHCl_3$); IR (KBr): ν 3465 (OH), 1751 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.21 (d, 3H, *J* = 5.9 Hz, rha-H-6), 1.98, 2.02, 2.03, 2.07, 2.11, 2.12×2, 2.13 (each s, 3H, C<u>H</u>$_3$CO), 3.34 (d, 1H, *J* = 3.7 Hz, gluA-1-O<u>H</u>), 3.74 (s, 3H, gluA-6 COOC<u>H</u>$_3$), 3.86 (dd , 1H, *J* = 7.9, 10.3 Hz gal-H-2), 3.90 (*m* , 1H, gal-H-5), 3.97 (dd, 1H, *J* = 3.7, 9.8 Hz, gluA-H-2), 4.09 (*m* , 1H, rha-H-5), 4.13, 4.14 (each

m , 1H, gal-H-6, 6'), 4.61 (d, 1H, *J* = 9.8 Hz, gluA-H-5), 4.62 (d, 1H, *J* = 7.9 Hz, gal-H-1), 4.95 (br s, 1H, rha-H-1), 4.96 (dd, 1H, *J* = 3.7, 9.8 Hz, gal-H-3), 5.05 (t, 1H, *J* = 9.8 Hz, rha-H-4), 5.07 (*m* , 1H, rha-H-2), 5.13 (t, 1H, *J* = 9.8 Hz, gluA-H-4), 5.15 (*m* , 1H, rha-H-3), 5.37 (d, 1H, *J* = 3.7 Hz, gal-H-4), 5.44 (d, 1H, *J* = 3.7 Hz, gluA-H-1), 5.47 (t, 1H, *J* = 9.8 Hz, gluA-H-3); ^{13}C-NMR ($CDCl_3$): Table 3; Positive FAB-MS *m/z* 875 $[M+Na]^+$; HR positive FAB-MS 875.2435 $[M+Na]^+$ ($C_{35}H_{48}O_{24}Na$, Calcd for 875.2433).

Methyl *O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,4-di-*O*-acetyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (3). A solution of **2** (105 mg, 123 μmol), Cl_3CCN (0.6 mL, 5.98 mmol), and DBU (20 μL, 135 μmol) in CH_2Cl_2 (1 mL) was stirred for 2 h at -5 °C and then evaporated *in vacuo*. The residue was chromatographed over SiO_2 (benzene:acetone=15:1) to give **3** (122 mg, quantitative) as an amorphous powder; $[\alpha]_D$ +16.0° (*c* 0.43, $CHCl_3$); IR (KBr): ν 1751 (C=O), 1678 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.21 (d, 3H, *J* = 5.9 Hz, rha-H-6), 1.98, 2.02×2, 2.08×2, 2.11, 2.12×2 (each s, 3H, CH_3CO), 3.73 (s, 3H, gluA-6 $COOCH_3$), 3.79 (*m* , 1H, gal-H-2), 3.90 (*m* , 2H, gal-H-5, rha-H-5), 4.08 (dd, 1H, *J* = 3.6, 9.6 Hz, gluA-H-2), 4.09 (*m* , 2H, gal-H-6, 6'), 4.44 (d, 1H, *J* = 10.2 Hz, gluA-H-5), 4.65 (d, 1H, *J* = 7.6 Hz, gal-H-1), 4.94 (br s, 1H, rha-H-1), 4.98 (dd, 1H, *J* = 3.0, 9.9 Hz, gal-H-3), 5.01 (*m* , 1H, rha-H-2), 5.02 (t, 1H, *J* = 9.9 Hz, rha-H-4), 5.15 (*m* , 1H, rha-H-3), 5.21 (dd, 1H, *J* = 9.6, 10.2 Hz, gluA-H-4), 5.35 (d, 1H, *J* = 3.0 Hz, gal-H-4), 5.40 (t, 1H, *J* = 9.6 Hz, gluA-H-3), 6.61 (d, 1H, *J* = 3.6 Hz, gluA-H-1), 8.75 (s, 1H, -C=NH); ^{13}C-NMR ($CDCl_3$): Table 3; Negative FAB-MS *m/z* 1018 $[M+Na]^+$; HR negative FAB-MS 1018.1525 $[M+Na]^+$ ($C_{37}H_{48}NO_{24}Cl_3$, Calcd for 1018.1529).

Methyl [Diosgenyl *O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,4-di-*O*-acetyl-β-D-glucopyranoid] uronate (4). To a mixture of **3** (111 mg, 111 μmol), diosgenin (100 mg, 241 μmol), and powdered 4 Å molecular sieves (1 g) in CH_2Cl_2 (2.8 mL), $BF_3{\cdot}Et_2O$ (30 μL, 244 μmol) was added at -20°C under nitrogen atmosphere. The mixture was

stirred for 4 h at -20°C, then for 1 h at 0°C. After the completion of the reaction, the reaction mixture was diluted with $CHCl_3$. The precipitates appeared were filtrated off and washed thoroughly with $CHCl_3$. The filtrate and $CHCl_3$ used for the washing were combined and washed successively with sat. aq. $NaHCO_3$ and H_2O, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed over SiO_2 (benzene:acetone=20:1) to give **4** (88 mg, 64%) as an amorphous powder; $[\alpha]_D$ -64.4° (*c* 0.42, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.79 (s, 3H, H-18), 0.80 (d, 3H, *J* =6.0 Hz, H-27), 0.97 (d, 3H, *J* =7.3 Hz, H-21), 1.03 (s, 3H, H-19), 1.23 (d, 3H, *J* = 6.1 Hz, rha-H-6), 1.98, 1.99, 2.03, 2.05, 2.10×2, 2.12×2, (each s, 3H, C$\underline{H}_3$CO), 3.37, 3.47 (each *m* , 1H, H-26, 26'), 3.59 (*m* , 1H, H-3), 3.73 (s, 3H, gluA-6 COOC$\underline{H}_3$), 3.79 (dd, 1H, *J* = 7.9, 9.8 Hz, gal-H-2), 3.87 (*m* , 1H, gal-H-5), 3.92 (dd, 1H, *J* = 5.5, 7.3 Hz, gluA-H-2), 4.11 (*m* , 2H, gal-H-6, 6'), 4.12 (d, 1H, *J* = 9.8 Hz, gluA-H-5), 4.13 (*m* , 1H, rha-H-5), 4.41 (*m* , 1H, H-16), 4.68 (d, 1H, *J* = 7.9 Hz, gal-H-1), 4.69 (d, 1H, *J* = 5.5 Hz, gluA-H-1), 4.96 (br s, 1H, rha-H-1), 4.97 (dd, 1H, *J* = 3.1, 9.8 Hz, gal-H-3), 5.07 (t, 1H, *J* = 9.8 Hz, rha-H-4), 5.11 (*m* , 1H, rha-H-2), 5.17 (dd, 1H, *J* = 7.3, 9.8 Hz, gluA-H-3), 5.19 (*m* , 1H, rha-H-3), 5.32 (t, 1H, *J* = 9.8 Hz, gluA-H-4), 5.34 (br s, 1H, H-6), 5.37 (d, 1H, *J* = 2.4 Hz, gal-H-4) ; ^{13}C-NMR ($CDCl_3$): Table 3; Positive FAB-MS *m/z* 1271 $[M+Na]^+$; HR positive FAB-MS 1271.5477 $[M+Na]^+$ ($C_{62}H_{88}O_{26}$, Calcd for 1271.5461).

***O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucopyranuronic acid diosgenin (5).** 1*N* NaOH (2 mL) was added to a solution of **4** (52 mg, 42 μmol) in EtOH (4 mL), and the mixture was refluxed for 1.5 h. The reaction mixture was diluted with H_2O and subjected to column chromatography over MCI gel CHP20P and eluted with H_2O and CH_3OH, successively. The CH_3OH eluate was evaporated *in vacuo*, and the residue was chromatographed over SiO_2 ($CHCl_3$:CH_3OH:H_2O= 7:3:0.5) to afford **5** (32 mg, 85%) as an amorphous powder; $[\alpha]_D$ -84.5° (*c* 0.18, CH_3OH); ^{1}H-NMR (C_5D_5N): δ 0.70 (d, 3H,*J* =5.5 Hz, H-27), 0.81 (s, 3H, H-18), 0.90 (s, 3H, H-19), 1.13 (d, 3H,*J* =6.7 Hz, H-21), 1.73 (d, 3H, *J* = 6.1 Hz, rha-H-6), 1.94 (t, 1H, *J* = 6.7 Hz, H-17), 1.97, 2.01 (each *m* , 1H, H-15),

2.15 (*m* , 1H, H-2'), 2.59, 2.83 (each *m* , 1H, H-4), 3.50, 3.59 (each *m* , 1H, H-26), 3.95 (*m* , 1H, H-3), 4.01 (t, 1H, *J* = 6.1 Hz, gluA-H-3), 4.20 (dd, 1H, *J* = 3.7, 9.7 Hz, gal-H-3), 4.29 (t, 1H, *J* = 9.2 Hz, rha-H-4), 5.00 (*m* , 1H, rha-H-5), 5.30 (d, 1H, *J* = 6.7 Hz, gluA-H-1), 5.37 (br s, 1H, H-6), 5.66 (d, 1H, *J* = 7.3 Hz, gal-H-1), 6.31 (s, 1H, rha-H-1); ^{13}C-NMR (C_5D_5N): Table 3; Positive FAB-MS *m/z* 921 $[M+Na]^+$; HR positive FAB-MS *m/z* 921.4472 $[M+Na]^+$ ($C_{45}H_{70}O_{18}Na$, Calcd for 921.4460).

Extraction of Julibrosides. The fresh stem bark of *Albizzia julibrissin* DURAZZ (6.3 kg) was extracted with CH_3OH (10 L) for 4 h under reflux, and the procedure was repeated twice. The combined CH_3OH extract (379 g) was partitioned between 1-BuOH and H_2O. The aqueous layer (294 g) was subjected to column chromatography over MCI gel CHP-20P ($H_2O \rightarrow 80\%$ CH_3OH) to give julibrosides (116 g).

Allyl α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranoside (6). To a solution of a solution of julibrosides (10 g) in allyl alcohol (30 mL) and 2,6-lutidine (50 mL), lithium iodide (5 g, 37 mmol) was added at rt under nitrogen atmosphere. The mixture was refluxed for 18 h and then diluted with 50% aqueous CH_3OH (100 mL), and subjected to column chromatography of Amberlite MB-3 (350 mL) and eluted with methanol. The eluate was evaporated *in vacuo* and the residue was chromatographed over Diaion HP-20 (gel: 200 mL; solvent: 30% aqueous CH_3OH 1 L, CH_3OH 1 L). The 30% aqueous CH_3OH fraction was evaporated *in vacuo* and purified by column chromatography over SiO_2 column chromatography ($CHCl_3$:CH_3OH:H_2O= 6:4:1) to give **6** (1.82 g, *ca.*70%) as an amorphous powder; $[\alpha]_D$ -73.1° (*c* 0.12, CH_3OH); ^{1}H-NMR (C_5D_5N): δ 1.66 (d, 3H, *J* = 4.9 Hz, rha-H-6), 4.89 (d, 1H, *J* =8.0 Hz, β-glc-H-1), 5.14 (br d, 1H, *J* =10.2 Hz, C<u>H</u>$_2$=CH-), 5.38 (br d, 1H, *J* =15.5 Hz, C<u>H</u>$_2$=CH-), 5.38 (br s, 1H, α-glc-H-1), 5.69 (br s, 1H, rha-H-1), 6.08 (br s, 1H, ara(f)-H-1), and 6.05 (*m*, 1H, CH_2=C<u>H</u>-); ^{13}C-NMR (C_5D_5N): Table 4; Positive FAB-MS *m/z* 683 $[M+Na]^+$, 661 $[M+H]^+$; HR positive FAB-MS *m/z* 661.2557 $[M+H]^+$ ($C_{26}H_{45}O_{19}$, Calcd for 661.2555).

Decylaminoethyl-2-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-

rhamnopyranosyl-(1→2)-α-D-glucopyranoside (7). To a solution of **6** (151 mg, 229 μmol) in CH_3OH (50 mL) O_3 gas was bubbled at –78 °C until the solution color turned slightly blue. After excess O_3 was removed with N_2, dimethyl sulfide (1 mL, 13.6 mmol) was added to the reaction mixture with elevating the reaction temperature to rt, and the organic solvent was evaporated. The gummy residue was used without any purification as the crude aldehyde. 5 *N* HCl-CH_3OH (95 μL) was added to a solution of decylamine (280 μL, 1.41 mmol) in CH_3OH (3 mL), and then the crude aldehyde and $NaBH_3CN$ (57 mg, 907 μmol) were added to the solution. The resulting solution was stirred for 72 h and the solution was evaporated *in vacuo*. The residue was purified by column chromatography over SiO_2 ($CHCl_3$:CH_3OH:H_2O:NH_4OH= 6:4:0.8:0.2) to give **7** (140 mg, 76%) as an amorphous solid; $[\alpha]_D$ -13.5° (*c* 0.11, CH_3OH); IR (KBr): ν 3402 (OH), 3371 (NH) cm^{-1}; ^{1}H-NMR (C_5D_5N): δ 0.85 (t, 3H, *J* =7.3 Hz, CH_3-), 1.21~1.34 (16H, -CH_2-), 1.72 (d, 3H, *J* = 5.5 Hz, rha-H-6), 5.04 (d, 1H, *J* =7.9 Hz, β-glc-H-1), 5.43 (d, 1H, *J* =3.7 Hz, α-glc-H-1), 5.79 (s, 1H, rha-H-1), and 6.14 (d, 1H, *J* =2.4 Hz, ara(f)-H-1); ^{13}C-NMR (C_5D_5N): Table 4; Positive FAB-MS *m/z* 804 $[M+H]^+$; HR positive FAB-MS *m/z* 804.4236 $[M+H]^+$ ($C_{35}H_{66}NO_{19}$, Calcd for 804.4229).

***N*-Decanoyl-1-decylaminoethyl-2-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranoside (8).** Decanoyl chloride (13.6 μL, 66 μmol) was added to a solution of **7** (34 mg, 42 μmol) in 2M sodium acetate-THF (2:3, 5 mL), and the reaction mixture was stirred for 1.5 h at rt. The reaction mixture was directly passed through column chromatography over MCI gel CHP-20P (100 mL) and washed with H_2O (500 mL) and eluted with CH_3OH (300 mL), and the CH_3OH eluate was evaporated *in vacuo*. The residue was purified by column chromatography over SiO_2 column chromatography ($CHCl_3$:CH_3OH:H_2O:NH_4OH=7:3:0.3:0.1~6:4:0.8:0.2) to give **8** (34 mg, 85%) as an amorphous solid; $[\alpha]_D$ -40.2° (*c* 1.01, CH_3OH); IR (KBr): ν 3402 (OH), 1622 (amide) cm^{-1}; ^{1}H-NMR (C_5D_5N) at 50°C: δ 0.84 (t, 6H, *J* =6.6 Hz, CH_3-), 1.23~1.34

(34H, -C$\underline{H}_2$-), 1.69 (d, 3H, J = 5.2 Hz, rha-H-6), 5.10 (d, 1H, J =7.6 Hz, β-glc-H-1), 5.36 (d, 1H, J =3.6 Hz, α-glc-H-1), 5.67 (s, 1H, rha-H-1), and 6.09 (br s, 1H, ara(f)-H-1); ^{13}C-NMR (C_5D_5N): Table 4; Positive FAB-MS *m/z* 958 $[M+H]^+$; HR positive FAB-MS *m/z* 958.5544 $[M+H]^+$ ($C_{45}H_{84}NO_{20}$, Calcd 958.5587).

Allyl-*O*-(2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl)-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (9). A mixture of **6** (670 mg, 1.02 mmol) and 4-dimethylaminopyridine (16 mg, 130 μmol) in acetic anhydride-pyridine (1:1, 20 mL) was stirred for 52 h at rt, and then volatiles were evaporated *in vacuo*. The residue was chromatographed over SiO_2 (hexane:EtOAc=1:1) to give **9** (914 mg, 82%) as an amorphous powder; $[\alpha]_D$ -18.1° (*c* 0.13, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.23 (d, 3H, J = 5.3 Hz, rha-H-6), 1.97, 1.99, 2.02, 2.03, 2.06, 2.09×3, 2.10×2, 2.21 (each s, 3H, C$\underline{H}_3$CO), 4.74 (br s, 1H, rha-H-1), 4.81 (d, 1H, J =7.6 Hz, β-glc-H-1), 4.98 (d, 1H, J =3.7 Hz, α-glc-H-1), 5.27 (br d, 1H, J =10.9 Hz, C$\underline{H}_2$=CH-), 5.31 (br d, 1H, J =14.2 Hz, C$\underline{H}_2$=CH-), 5.47 (s, 1H, ara(f)-H-1), and 5.95 (*m*, 1H, CH_2=C$\underline{H}$-); ^{13}C-NMR ($CDCl_3$): Table 4; Positive FAB-MS *m/z* 1145 $[M+Na]^+$, 1123 $[M+H]^+$; HR positive *m/z* FAB-MS 1123.3781 $[M+H]^+$ ($C_{48}H_{67}O_{30}$, Calcd for 1123.3717).

O-(2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl)-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (10). Tetrakis(triphenylphosphin)palladium (450 mg, 389 μmol) was added to a solution of **9** (613 mg, 546 μmol) in acetic acid (6 mL) under a nitrogen atmosphere, and mixture was heated at 80°C for 1 h. After the reaction, the organic solvent was removed by azeotropic evaporation with toluene, and the residue was chromatographed over SiO_2 (toluene:EtOAc=1:1) to give **10** (590 mg, quantitative, α:β anomer =1:4, H-6 of Rha: α; δ 1.25, β; δ 1.27) as an amorphous powder; $[\alpha]_D$ -23.8° (*c* 0.65, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): α anomer; δ 1.25 (d, 3H, J = 5.6 Hz, rha-H-6), 1.97, 1.99, 2.02, 2.03, 2.07, 2.08, 2.09×2, 2.11,

2.12, 2.21 (each s, 3H, CH_3CO), 4.82 (d, 1H, J =7.9 Hz, β-glc-H-1), 4.83 (br s, 1H, rha-H-1), 5.33 (d, 1H, J =3.1 Hz, α-glc-H-1), 5.44 (s, 1H, ara(f)-H-1); ^{13}C-NMR ($CDCl_3$): Table 4; Positive FAB-MS *m/z* 1105 $[M+Na]^+$, 1083 $[M+H]^+$; HR positive FAB-MS *m/z* 1083.3405 $[M+H]^+$ ($C_{45}H_{63}O_{30}$, Calcd for 1083.3405).

***O*-(2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl)-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (11).** A solution of **10** (590 mg, 546 μmol), Cl_3CCN (1.4 mL, 13.9 mmol), and DBU (45 μL, 300 μmol) in CH_2Cl_2 (3 mL) was stirred for 2 h at 0 °C and then the organic solvent was evaporated *in vacuo*. The residue was chromatographed over SiO_2 (hexane:EtOAc=2:3) to give **11** (632 mg, 94%) as an amorphous powder; $[\alpha]_D$ -13.8° (*c* 0.17, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.23 (d, 3H, J = 5.3 Hz, rha-H-6), 1.96, 1.98, 2.02, 2.04, 2.06, 2.08×2, 2.09, 2.11×2, 2.19 (each s, 3H, CH_3CO), 4.72 (d, 1H, J =7.9 Hz, β-glc-H-1), 4.83 (s, 1H, rha-H-1), 5.44 (s, 1H, ara(f)-H-1), 6.45 (d, 1H, J =3.6 Hz, α-glc-H-1), 8.79 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$): Table 5; Negative FAB-MS *m/z* 1225 $[M-H]^-$; HR negative FAB-MS *m/z* 1225.2482 $[M]^-$ ($C_{47}H_{62}NO_{30}Cl_3$, Calcd for 1225.2423).

***O*-(2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl)-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-D-glucopyranosyl diosgenin (12).** To a mixture of **11** (550 mg, 450 μmol), diosgenin (750 mg, 1.81 mmol), and powdered 4 Å molecular sieves (6 g) in CH_2Cl_2 (20 mL), $BF_3{\cdot}Et_2O$ (113 μL, 919 μmol) was added at -10°C under nitrogen atmosphere. The mixture was stirred for 4 h at -10°C, then for 24 h at rt. After the completion of the reaction, the reaction mixture was diluted with $CHCl_3$. The precipitates appeared were filtrated off and washed thoroughly with $CHCl_3$. The filtrate and $CHCl_3$ used for the washing were combined and neutralized with Et_3N, and the organic solvent was evaporated *in vacuo*. The residue was chromatographed over SiO_2 (hexane:EtOAc=1:1) to give **12** (438 mg, 66%, α:β 1:4) as an amorphous

powder; $[\alpha]_D$ -73.1° (*c* 0.17, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): β anomer; δ 0.79 (br s, 3H, H-27), 0.80 (s, 3H, H-18), 0.98 (d, 3H, *J* =6.6 Hz, H-21), 1.03 (s, 3H, H-19), 1.25 (d, 3H, *J* = 6.3 Hz, rha-H-6), 1.97×2, 2.00, 2.02, 2.04, 2.06×2, 2.07, 2.09, 2.11, 2.19 (each s, 3H, CH_3CO), 4.54 (d, 1H, *J* =7.9 Hz, inner-glc-H-1), 4.77 (d, 1H, *J* =7.9 Hz, terminal-glc-H-1), 5.07 (s, 1H, rha-H-1), 5.38 (br s,1H, H-6), 5.47 (s, 1H, ara(f)-H-1); ^{13}C-NMR ($CDCl_3$): Table 5; Positive FAB-MS *m/z* 1479 $[M+H]^+$; HR positive FAB-MS *m/z* 1479.6470 $[M+H]^+$ ($C_{72}H_{103}O_{32}$, Calcd for 1479.6433).

α-L-Arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl diosgenin (13). 1*N* NaOH(10 mL) was added to a solution of **12** (405 mg, 274 μmol) in EtOH (10 mL), and the mixture was stirred for 8 h at rt. After the reaction, the reaction mixture was diluted with H_2O and subjected to column chromatography over MCI gel CHP20P (100 mL) eluted with H_2O (500 mL) and CH_3OH (300 mL). The CH_3OH eluate was evaporated *in vacuo* to give α/β mixture (**13**) (263 mg, 94%). The mixture was recrystallized from CH_3OH to afford pure β anomer of **13** as colorless needles (104 mg); $[\alpha]_D$ -96.8° (*c* 0.14, CH_3OH); mp 298°C (decomp); ^{1}H-NMR (C_5D_5N): δ 0.71 (d, 3H, *J* =5.0 Hz, H-27), 0.77 (s, 3H, H-18), 1.14 (d, 3H, *J* =6.9 Hz, H-21), 1.19 (s, 3H, H-19), 1.83 (d, 3H, *J* = 6.3 Hz, rha-H-6), 5.07 (d, 1H, *J* =7.6 Hz, inner-glc-H-1), 5.42 (d, 1H, *J* =7.9 Hz, terminal-glc-H-1), 5.56 (br s, 1H, H-6), 6.26 (br s, 1H, ara(f)-H-1), 6.35 (br s, 1H, rha-H-1); ^{13}C-NMR (C_5D_5N): Table 5; Positive FAB-MS *m/z* 1039 $[M+Na]^+$; HR positive FAB-MS *m/z* 1039.5067 $[M+Na]^+$ ($C_{50}H_{80}O_{21}Na$, Calcd for 1039.5090): *Anal.* Calcd for $C_{50}H_{80}O_{21}\cdot 1.5\ H_2O$: C, 57.51; H, 8.01. Found: C, 57.59; H, 7.89.

***O*-(2,3,5-Tri-*O*-acetyl-α-L-arabinofuranosyl)-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-D-glucopyranosyl methyl-glycyrrhetinate (14).** To a mixture of **11** (278 mg, 227 μmol), methyl glycyrrhetinate (1.10 g, 2.28 mmol), and powdered 4 Å molecular sieves (6.2 g) in CH_2Cl_2 (20 mL), $BF_3\cdot Et_2O$ (42 μL, 341 μmol) was added at -20°C under nitrogen atmosphere. The mixture was stirred for 4 h at -20°C. After the completion

of the reaction, the reaction mixture was diluted with $CHCl_3$. The precipitates appeared were filtrated off and washed thoroughly with $CHCl_3$. The filtrate and $CHCl_3$ used for the washing were combined and washed with sat. aq. $NaHCO_3$ solution and brine successively and dried over $MgSO_4$. The volatiles were evaporated *in vacuo* and the residue was chromatographed over SiO_2 ($CHCl_3$:EtOAc=5:1) to give α/β mixture of **14** (245 mg, 70%, α:β 1:3). These isomers were separated by column chromatography over micro-Bondapak C_{18} (gel: 100 mL, solvent: CH_3CN:H_2O=4:1) to afford pure β anomer of **14** (101 mg) as an amorphous powder; $[\alpha]_D$ +8.9° (*c* 0.11, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.82, 0.89, 1.05, 1.13×2, 1.15, 1.38 (each s, 3H, tertiary methyl), 1.26 (br s, 3H, rha-H-6), 1.98×2, 2.00, 2.02, 2.03, 2.05, 2.07, 2.08, 2.09, 2.13, 2.20 (each s, 3H, CH_3CO), 2.34 (s, 1H, H-9), 3.16 (dd, 1H, *J* =4.1, 10.8 Hz, H-3), 3.69 (s, 3H, -$COOCH_3$), 4.50 (d, 1H, *J* =7.6 Hz, inner-glc-H-1), 4.76 (d, 1H, *J* =7.9 Hz, terminal-glc-H-1), 5.07 (s, 1H, rha-H-1), 5.47 (s, 1H, ara(f)-H-1), 5.67 (s, 1H, H-12); ^{13}C-NMR ($CDCl_3$): Table 5; Positive FAB-MS *m/z* 1549 $[M+H]^+$; HR positive FAB-MS *m/z* 1549.6835 $[M+H]^+$ ($C_{76}H_{109}O_{33}$, Calcd for 1549.6850).

α-L-Arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl glycyrrhetinic acid (15). 1*N* NaOH (5 mL) was added to a solution of β anomer of **14** (90 mg, 58 μmol) in EtOH (5 mL), and refluxed for 8 h. After the reaction, the reaction mixture was diluted with H_2O and subjected to column chromatography over MCI gel CHP20P (50 mL), and eluted with H_2O (250 mL) and CH_3OH (150 mL). The CH_3OH eluate was evaporated *in vacuo* to give **15** (60 mg, 97%); $[\alpha]_D$ +1.2° (*c* 1.19, CH_3OH); ^{1}H-NMR (C_5D_5N): δ 0.81, 1.09, 1.22, 1.23, 1.37, 1.43, 1.46 (each s, 3H, tertiary methyl), 1.76 (d, 3H, *J* =6.3 Hz, rha-H-6), 2.48 (s, 1H, H-9), 3.41 (*m*, 1H, H-3), 4.92 (d, 1H, *J* =7.3 Hz, inner-glc-H-1), 5.41 (d, 1H, *J* =7.9 Hz, terminal-glc-H-1), 6.00 (s, 1H, H-12), 6.25 (d, 1H, *J* =2.0 Hz, ara(f)-H-1), 6.36 (br s, 1H, rha-H-1); ^{13}C-NMR (C_5D_5N): Table 5; Negative FAB-MS *m/z* 1071 $[M-H]^-$; HR negative FAB-MS *m/z* 1071.5316 $[M-H]^-$ ($C_{53}H_{83}O_{22}$, Calcd for 1071.5376).

Cytotoxicity Bioassays against PC-6 and P388 cells.

The tetrazolium-based semiautomatic colorimetric assay (MTT assay) developed by Carmichael *et al.* [23] was used for the *in vitro* assay of cytotoxicity to PC-6 and P388 cells.

Hepatoprotective Activity Assay:

Preparation of primary cultured rat hepatocytes.

Liver cells were isolated according to a procedure developed by Berry and Friend.[24] The detailed procedure was described in the previous paper.[4]

Preparation of antiserum against the rat liver plasma membranes.

The rat liver plasma membranes were prepared according to a procedure developed by Loten *et al.*[25] The detailed procedure was described in the previous paper.[4]

Determination of hepatoprotective activity of Saponins toward *in vitro* Immunological Liver Injury.

One day after the isolated rat hepatocytes were plated, the cultured cells were exposed to the above prepared medium (300 μL) containing the antiserum against rat plasma membranes (40 μL/mL) and DMSO solution (4 μL) of the tested saponins [final concentration 0 (reference), 10, 30, 90, 200, 500 μM]. Forty minutes after rat plasma membranes were treated with antiserum, the enzyme activity (ALT) in the medium was determined.

Instrument and Bioassay Method

The ALT activity was assayed by autoanalyzer COBAS MIRA (Roche) using commercial kits based on the GPT assay method.[26]

Statistical analysis

The data are shown in mean ± SD (n=4). After analysis of variances, Tukey's test was employed to determine the significance of differences between reference and experimental samples.

Table 3. ^{13}C-NMR Data for Compound (**1~5**).

	1 [a]	**2** [a]	**3** [a]	**4** [a]	**5** [b]
GluA-1	91.1	92.7	95.4	99.5	101.5
2	74.1	75.4	75.5	76.2	81.7
3	70.4	70.4	70.5	73.7	76.4
4	70.1	70.3	70.0	69.4	72.8
5	70.2	68.5	70.1	72.3	78.2
6	167.4	168.4	167.4	168.1	172.8
$COO\underline{C}H_3$	52.9	52.8	53.0	52.7	
Gal-1	102.1	101.8	102.4	100.2	103.3
2	73.8	73.7	73.7	74.3	77.4
3	73.0	72.9	73.0	73.1	76.3
4	67.1	67.3	67.3	67.2	70.5
5	71.0	71.1	70.9	70.6	76.7
6	61.2	61.2	61.3	61.1	61.8
Rha-1	98.1	98.2	97.9	98.2	102.0
2	69.9	69.9	69.8	69.9	72.3
3	68.4	68.5	68.3	68.7	72.6
4	70.7	70.6	70.7	70.7	74.5
5	67.0	67.2	67.1	67.0	69.7
6	17.2	17.1	17.2	17.3	18.9
(Diosgenin) C-1		$\underline{C}NH$	160.7	37.1	37.5
2		$\underline{C}Cl_3$	90.8	29.2	30.0
3				79.2	79.0
4				39.8	39.9
5				140.4	141.1
6				121.8	121.5
7				31.9	32.2
8				31.4	31.7
9				50.1	50.3
10				36.9	37.0
11				20.6	21.1
12				38.7	39.2
13				40.3	40.5
14				56.5	56.7
15				32.1	32.3
16				80.8	81.1
17				62.1	62.9
18				16.3	16.3
19				19.4	19.4
20				41.6	42.0
21				14.5	15.0
22				109.3	109.3
23				31.4	31.9
24				28.8	29.3
25				30.3	30.6
26				66.9	66.9
27				17.1	17.3

a) $CDCl_3$, *b)* C_5D_5N

Table 4. ^{13}C-NMR Data for Compound (**6~11**).

	6 [a]	**7** [a]	**8** [a]	**9** [b]	**10** [b]	**11** [b]
Glc(endo)-1	98.0	99.5	99.5, 99.3 [c]	96.7	92.2	94.5
2	80.3	79.1	78.9	78.8	77.7	77.3
3	73.5	74.1	73.9	70.9	70.8	70.9
4	70.8	72.2	71.8	68.3	68.3	67.9
5	73.5	74.2	74.3,74.1 [c]	67.5	67.6	70.1
6	61.8	62.4	62.2	61.3	61.1	61.3
Rha-1	103.1	103.6	103.7	99.9	99.2	99.7
2	70.8	71.5	71.2	71.9	72.0	71.8
3	81.9	82.0	82.2, 82.1 [c]	76.3	75.8	77.1
4	78.3	80.5	80.6	74.5	74.4	74.2
5	68.0	68.6	68.4	67.0	67.4	67.3
6	18.3	18.8	18.7	17.9	17.8	17.7
Ara-1	110.1	110.8	110.8, 110.6 [c]	105.6	105.7	105.4
2	83.9	84.5	84.4	81.5	81.6	81.6
3	77.6	78.2	78.1	77.6	77.5	77.2
4	84.1	84.6	84.7	81.4	81.2	81.2
5	62.0	62.8	62.4	63.3	63.3	63.3
Glc(exo)-1	104.7	105.2	105.1	99.6	99.5	99.8
2	74.8	75.4	75.4, 75.3 [c]	71.9	72.2	71.8
3	77.5	78.0	78.1	72.7	72.7	72.7
4	71.7	71.4	71.2	68.0	68.1	67.5
5	77.2	78.0	77.8	71.8	71.8	71.8
6	62.0	62.5	62.3	62.0	62.0	61.4
Allyl-1	117.0			118.8		
2	134.5			133.2		
3	68.5			68.8		
-$\underline{C}H_2$O-glc		67.9	66.7, 66.5 [c]		-$\underline{C}Cl_3$	90.9
-N$\underline{C}H_2$		50.1	49.4		-$\underline{C}$=NH	161.0
-N$\underline{C}H_2$		49.6	47.4			
-CO			173.1, 172.7 [c]			
-CO$\underline{C}H_2$			46.3, 46.1			
-$\underline{C}H_2$		32.1, 30.4	33.4, 33.1			
		30.0×2	32.0, 29.9×2			
		29.9	29.8×2, 29.7			
		29.6, 27.7	29.5, 28.1			
		22.9	27.3, 27.0			
			26.0, 25.8			
			22.9			
$\underline{C}H_3$		14.3	14.2×2			

a) C_5D_5N, *b)* $CDCl_3$, *c)* each 1/2 C (Both *cis* and *trans* isomers of amide were observed even at 60 °C.)

Table 5. ^{13}C-NMR Data for Compound (**12~15**).

	12 [a]	**13** [b]	**14** [a]	**15** [b]
C-1	37.2	37.5	39.5	39.9
2	29.6	30.2	26.0	26.6
3	79.2	78.1	90.5	88.9
4	39.7	39.9	39.5	39.9
5	140.3	140.9	55.7	55.8
6	122.1	122.0	17.4	17.6
7	31.8	32.2	32.7	32.9
8	31.4	31.6	44.0	44.2
9	50.1	50.4	61.8	62.1
10	36.9	37.2	36.8	37.3
11	20.6	21.2	200.0	199.7
12	38.4	39.1	128.5	128.6
13	40.3	40.4	168.6	169.8
14	56.5	56.7	45.4	45.6
15	32.1	32.3	26.5	26.8
16	80.8	81.1	26.4	26.8
17	62.1	62.9	31.8	32.2
18	16.3	16.5	48.4	48.8
19	19.4	19.8	41.1	41.8
20	41.6	42.0	43.2	43.5
21	14.5	15.1	31.1	31.6
22	109.3	109.3	37.7	38.5
23	31.4	31.8	27.6	28.2
24	28.8	29.3	16.5	16.8
25	30.3	30.6	16.7	17.0
26	66.9	66.9	18.7	18.8
27	17.1	17.3	23.4	23.6
28			28.5	28.8
29			28.3	28.7
30			176.9	179.4
		COOMe	51.8	
Glc(endo)1	99.7	100.4	104.1	105.7
2	72.7	79.4	72.7	79.2
3	74.7	77.1	75.5	77.5
4	68.8	71.8	69.3	72.0
5	71.6	78.2	71.6	78.1
6	61.3	62.5	61.7	62.4
Rha-1	97.9	101.3	96.1	101.1
2	71.8	71.9	72.1	71.2
3	76.1	81.7	76.6	81.5
4	74.3	79.8	74.2	79.5
5	66.7	68.2	66.6	68.5
6	17.8	18.8	17.7	18.8
Ara(f)-1	105.7	110.9	105.6	110.6
2	81.3	85.0	81.3	85.0
3	77.5	78.4	77.7	78.3
4	81.4	84.4	81.9	84.5
5	63.4	62.6	63.4	62.7
Glc(exo)1	99.6	105.7	99.7	105.7
2	71.8	75.5	71.8	75.5
3	72.7	78.2	72.6	78.3
4	68.0	71.5	68.1	71.6
5	71.7	78.2	71.7	78.3
6	62.2	62.6	62.5	62.6

a) $CDCl_3$, *b)* C_5D_5N

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REFERENCES AND NOTES

1. A. Kobata, 'Biology of Carbohydrates', Vol. 2, ed. by V. Ginsburg and P. W. Robins, Wiley, New York, 1984, p. 87; T. Feizi, *Nature*, 1985, **314**, 53; M. Fukuda, *Biochem. Biophys. Acta*, 1985, **780**, 119; S. Hakomori, *Adv. Cancer Res.*, 1989, **52**, 257; A. Varki, *Glycobiology*, 1993, **3**, 97.
2. H. Safayhi and E.-R. Sailer, *Planta Medica*, 1997, **63**, 487; R. Hostettmann, and A. Marston, 'Saponins', Cambridge University Press, 1995; C-R. Yang and O. Tanaka, 'Advances in Plant Glycosides, Chemistry and Biology', Elsevier, 1999.
3. S. Shibata, *Shoyakugaku Zasshi*, 1986, **40**, 1.
4. J. Kinjo, *Natural Medicine*, 1996, **50**, 79; T. Arao, M. Udayama, J. Kinjo, T. Funakoshi, S. Kojima, and T. Nohara, *Biol. Pharm. Bull.*, 1997, **20**, 988; H. Miyao, T. Arao, M. Udayama, J. Kinjo, and T. Nohara, *Planta Medica*, 1998, **64**, 5; J. Kinjo, M. Imagire, M. Udayama, T. Arao, and T. Nohara, *Planta Medica*, 1998, **64**, 233; T. Arao, M. Udayama, J. Kinjo, and T. Nohara, *Planta Medica*, 1998, **64**, 413; J. Kinjo and T. Nohara, 'Towards Natural Medicine Research in the 21st Century', ed. by H. Ageta, N. Aimi, Y. Ebizuka, T. Fujita, and Y. Honda, Elsevier, Tokyo, 1998, p. 237.
5. T. Ikeda, M. Udayama, M. Okawa, T. Arao, J. Kinjo, and T. Nohara, *Chem. Pharm. Bull.*, 1998, **46**, 359.
6. T. Ikeda, S. Fujiwara, J. Kinjo, T. Nohara, Y. Ida, J. Shoji, T. Shingu, R. Isobe, and T. Kajimoto, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 3483.
7. T. Ikeda, S. Fujiwara, K. Araki, J. Kinjo, T. Nohara, and T. Miyoshi, *J. Nat. Prod.*, 1997, **60**, 102.

8. T. Muro, T. Kuramoto, K. Imoto, and S. Okada, *Agric. Biol. Chem.*, 1986, **50**, 687; Y. Sakaki, T. Morita, T. Kuramoto, K. Mizutani, R. Ikeda, and O. Tanaka, *Agric. Biol. Chem.*, 1988, **52**, 207.

9. G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, 1975, **39**, 368.

10. R. R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 731; R. R. Schmidt, *Advan. Carbohydr. Chem. and Biochem.*, 1994, **50**, 21.

11. T. Nakamura, C. Komori, Y. Lee, F. Hashimoto, S. Yahara, T. Nohara, and A. Ejima, *Biol. Pharm. Bull.*, 1996, **19**, 564.

12. T. Ikeda, T. Kajimoto, J. Kinjo, K. Nakayama, and T. Nohara, *Tetrahedron Lett.*, 1998, **39**, 3513.

13. K. Ohtani, K.Mizutani, R. Kasai, and O. Tanaka, *Tetrahedron Lett.*, 1984, **25**, 4537.

14. Y.-C. Lee and R. T. Lee, 'Neoglycoocnjugates', Academic Press, 1994.

15. G. Magnusson, S. Ahlfors, J. Dahmen, K. Jansson, U. Nilsson, G. Noori, K. Stenvall, and A. Tjornebo, *J. Org. Chem.*, 1990, **55**, 3932.

16. B. D. Read, R. A. Demel, H. Wiegandt, and L. L. M. van Deenen, *Biochim. Biophys. Acta*, 1977 **470**, 325.

17. M. S. Stoll, T. Mizuochi, R. A. Childs, and T. Feizi, *Biochem. J.*, 1988, **256**, 661.

18. R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897.

19. R. R. Schmidt and P. Zimmermann, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 725.

20. T. Ikeda, T. Kajimoto, T. Nohara, J. Kinjo, and C.-H. Wong, *Tetrahedron Lett.*, 1995, **36**, 1509.

21. R. S. H. Finney and G. F. Somers, *J. Pharm. Pharmacl.*, 1958, **10**, 613; L. M. Atherden, *Biochem. J.*, **69**, 1958, 75; A. Kumagai, S. Yano, M. Otomo, and K. Takeuchi, *Endocrinol. Japan*, 1957, **4**, 17; M. W. Whitehouse, P. D. G. Dean, and T. G. Halsall, *J. Pharm. Pharmacol.*, 1967, **19**, 533.

22. K. Nakayama, K. Uoto, K. Higashi, T. Soga, and T. Kusama, *Chem. Pharm. Bull.*, 1992, **40**, 1718.

23. J. Carmichael, W. DeGraff, A. F. Gazdar, J. D. Minna, and J. B. Mitchell, *Cancer Res.*, 1987, **47**, 936.

24. M. N. Berry and D. S. Friend, *J. Cell. Biol.*, 1969, **43**, 506.

25. E. G. Loten and J. C. Redshaw-Loten, *Anal. Biolchem.*, 1986, **154**, 183.

26. W. Heerspink, J. C.Hafkenscheid, H. Siepel, J. van der Ven-Jongekryg, and C.C. Dijt, *Enzyme*, 1980, **25**, 333.

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