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Chemical Transglycosylation of Functional Bioactive Glyco-linkages

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Abstract: The natural functional bioactive oligosaccharides moieties, mimosatetraose and fabatriose, were cut off from julibrosides and soyasaponin I by chemical and enzymatic methods, respectively, and were pasted to diosgenin to give neosaponins **1** and **2**. The obtained neosaponins were tested for cytotoxicity and hepatoprotective activity. This method could be applied to the synthesis of novel bioactive glycosides. © 1998 Elsevier Science Ltd. All rights reserved.

Recent studies on glycobiology have revealed the important roles of many glycoconjugates in immune response, viral and bacterial infection, regulation, differentiation, development, inflammation, cell adhesion and many other inter- and intracellular communication processes. Triterpenoidal saponins which are often found as major components in traditional Chinese medicines are also good examples of glycoconjugates with remarkable pharmaceutical and biological activities.

Julibrosides, isolated triterpenoid glycosides from *Albizia julibrissin*[1], show cytotoxicity against KB cell (IC_{50} 12.8 μg/ml). When their ester glycosides were removed, the cytotoxicity dramatically decreased.[2] This finding suggests that the ester glycosidic part, α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl (mimosatetraosyl) moiety, is the factor to exhibit cytotoxicity. On the other hand, during our course of studies on leguminous plants,[3] we found that α-L-rhamnopyranosyl -(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl (β-fabatriosyl) triterpenoidal saponins, for example soyasaponin I, are effective for experimental liver injuries.[3, 4] When the β-fabatriosyl moiety is removed by acid hydrolysis, the hepatoprotective activity is reduced.[5] Since some oligosaccharide parts of natural glycosides seemed to play an important role for the activities, we planned to synthesize neosaponins carrying the oligosaccharide components of the original glycosides and examine their biological activities. Cleaving and reattaching oligosaccharides, which are shown in this article, would be a promissing technology to investigate the biological functions of the oligosaccharide parts in saponins.

The ester glycosidic bond from julibrosides could be selectively cleaved with lithium iodide[6] in the presence of lutidine and allyl alcohol to give an allyl-mimosatetraoside. After acetylation and deallylation[7], the tetrasaccharide is converted to an α-trichloroacetimidate[8] and glycosylated to diosgenin, which is a representative mevalonate, using boron trifluoride etherate[9] as a promotor to afford the fully-protected glycoside as a mixture of α:β anomers (1:4, α-anomer; δ 5.24, d, J =3.0 Hz, β-anomer; δ 4.54, d, J =7.9 Hz) in 66 % yield. Deacetylation was effected in a conventional way, and recrystallization from MeOH gave a pure β anomer of **1**[10] (Scheme 1).

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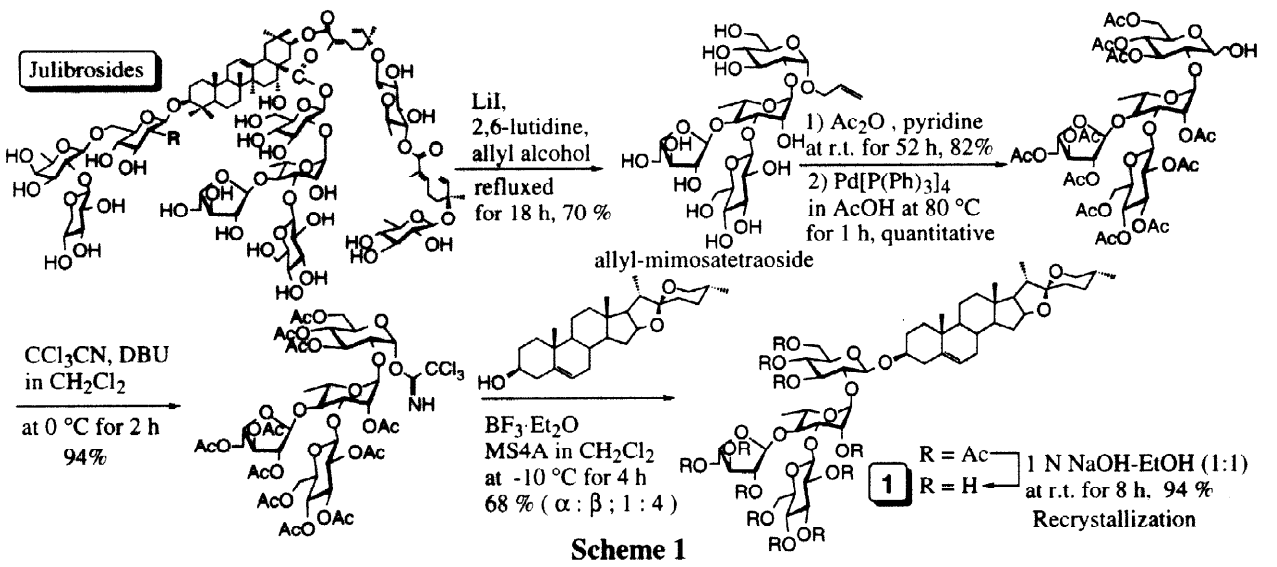
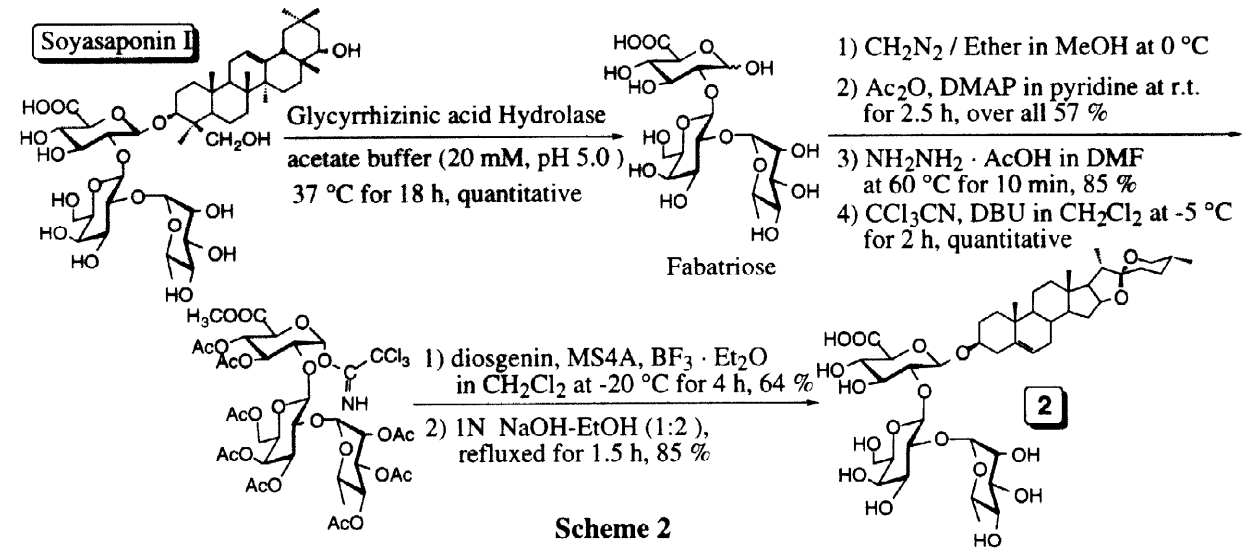


Scheme 1

Glycyrrhizine hydrolase (GHase) selectively cleaves an ether-linked *endo*-glucuronic acid at the C-3 position of triterpenoid.[11] The fabatriosyl moiety obtained by GHase from soyasaponin I[12] was converted to the imidate[13] after methylation of the carboxyl group of glucuronic acid, acetylation of the hydroxy groups, and deacetylation[14] of the reducing end of the fabatrioside, and glycosylated to diosgenin in the same manner described above to afford a diosgenin fabatrioside (**2**)[15] (Scheme 2).



Scheme 2

The cytotoxicity of the obtained neosaponins (**1** and **2**) was examined. Although they were less active than cisplatin (CDDP) and dioscin[16], **1** having mimosatetraose showed some cytotoxicity (Table 1). Next, the hepatoprotective activity of **1** and **2** was tested using an *in vitro* immunological liver injury model.[4a] Since **2** showed activity, the β-fabatriosyl moiety apparently is important for the hepatoprotective effect (Table 2). Thus, it is suggested that the oligosaccharides play an important role to promote the cytotoxic and hepatoprotective activity.

Table 1. GI50 (μg / ml) of Neosaponins (**1** and **2**)

Samples	PC-6	P388
CDDP	0.08	0.01
dioscin	1.1	0.4
1	7.7	9.1
2	>50	>50

Table 2. Hepatoprotective Activity of Glycyrrhizin, Soyasaponin I, **1**, and **2** 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 % **
1	200	4	16 ± 6 %
	500	4	30 ± 6 % **
2	90	4	15 ± 9 % *
	200	4	37 ± 2 % **
	500	4	58 ± 4 % **

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

It appears that the method described here is generally useful for the release of the oligosaccharide component of bioactive glycoconjugates with ester glycosidic linkages and *endo*-glucuronic linkage of triterpenoid and the reconstruction of the novel glycosides. Work is in progress to use this methodology to study the role of the oligosacchride component of bioactive glycoconjugates.

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8. An amorphous powder; $[\alpha]_D^{25}$ -13.8° (*c* 0.17, $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) 1.23 (3H, d, 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 (3H, each s, CH_3CO), 4.72 (1H, d, J=7.9 Hz, *exo*-glc H-1), 4.83 (1H, s, rha H-1), 5.44 (1H, br s, ara(*f*) H-1), 6.45 (1H, d, J=3.6 Hz, *endo*-glc H-1), 8.79 (1H, s, NH); δ_C ($CDCl_3$, 125 MHz) 94.5, 77.3, 70.9, 67.9, 70.1, 61.3 (*endo*-glc C-1~6), 99.7, 71.8, 77.1, 74.2, 67.3, 17.7 (rha C-1~6), 99.8, 71.8, 72.7, 67.5, 71.8, 61.4 (*exo*-glc C-

1~6), 105.4, 81.6, 77.2, 81.2, 63.3 (ara(*f*) C-1~6), 90.9 (-CCl_3), 161.0 (-CN=H); HR-FABMS (negative, NBA matrix) *m/z* 1225.2482 $(M)^-$ ($C_{47}H_{62}NO_{30}Cl_3$, Calcd for M, 1225.2423).

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10. **1** : Colorless needles ; mp 298 °C (decomp.); $[\alpha]_D^{25}$ -96.8° (*c* 0.14, MeOH); δ_H (C_5D_5N, 500 MHz) 0.71 (3H, d, *J*=5.0 Hz, H-27), 0.77 (3H, s, H-18), 1.14 (3H, d, *J*=6.9 Hz, H-21), 1.19 (3H, s, H-19), 1.83 (3H, d, *J*= 6.3 Hz, rha H-6), 5.07 (1H, d, *J*=7.6 Hz, *endo*-glc H-1), 5.42 (1H, d, *J*=7.9 Hz, *exo*-glc H-1), 5.56 (1H, br s, H-6), 6.26 (1H, br s, ara(*f*) H-1), 6.35 (1H, br s, rha H-1); δ_C (C_5D_5N, 125 MHz) 37.5, 30.2, 78.1, 39.9, 140.9, 122.0, 32.2, 31.6, 50.4, 37.2, 21.2, 39.1, 40.4, 56.7, 32.3, 81.1, 62.9, 16.5, 19.8, 42.0, 15.1, 109.3, 31.8, 29.3, 30.6, 66.9, 17.3 (C-1~27), 100.4, 79.4, 77.1, 71.8, 78.2, 62.5 (*endo*-glc C-1~6), 101.3, 71.9, 81.7, 79.8, 68.2, 18.8 (rha C-1~6), 105.7, 75.5, 78.2, 71.5, 78.2, 62.6 (*exo*-glc C-1~6), 110.9, 85.0, 78.4, 84.4, 62.6 (ara(*f*) C-1~6); HR-FABMS (positive, glycerol matrix) *m/z* 1039.5067 $(M+Na)^+$ ($C_{50}H_{80}O_{21}Na$, Calcd for M, 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.
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12. Soyasaponin I was obtained from the crude soyasaponin, which was purchased from Wako Pure Chemical Industies Ltd. (Japan), by purification of the column chromatograpy over silica gel and ODS.
13. An amorphous powder; $[\alpha]_D^{25}$ +16.0° (*c* 0.43, $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) 1.21 (3H, d, *J*= 5.9 Hz, rha H-6), 1.98, 2.02×2, 2.08×2, 2.11, 2.12×2 (3H, each s, CH_3CO-), 3.73 (3H, s, -$COOCH_3$), 3.79 (1H, *m* , gal H-2), 3.90 (2H, *m* , gal H-5, rha-H-5), 4.08 (1H, dd, *J*= 3.6, 9.6 Hz, glcA H-2), 4.09 (2H, *m* , gal H-6, 6'), 4.44 (1H, d, *J*= 10.2 Hz, glcA H-5), 4.65 (1H, d, *J*= 7.6 Hz, gal H-1), 4.94 (1H, br s, rha H-1), 4.98 (1H, dd, *J*= 3.0, 9.9 Hz, gal H-3), 5.01 (1H, *m* , rha H-2), 5.02 (1H, t, *J*= 9.9 Hz, rha H-4), 5.15 (*m* ,rha H-3), 5.21 (1H, dd, *J*= 9.6, 10.2 Hz, glcA H-4), 5.35 (1H, d, *J*= 3.0 Hz, gal H-4), 5.40 (1H, t, *J*= 9.6 Hz, glcA H-3), 6.61 (1H, d, *J*= 3.6 Hz, glcA H-1), 8.75 (1H, s, -C=NH); δ_C ($CDCl_3$, 125 MHz) 95.4, 75.5, 70.5, 70.0, 70.1, 167.4 (glcA C-1~6), 102.4, 73.7, 73.0, 67.3, 70.9, 61.3 (gal C-1~6), 97.9, 68.3, 69.8, 70.7, 67.1, 17.2 (rha C-1~6), 53.0 (-$COOCH_3$), 90.8 (CCl_3), 160.7 (-C=NH); HR-FABMS (positive, glycerol matrix) *m/z* 1018.1525 $(M+Na)^+$ ($C_{37}H_{48}NO_{24}Cl_3$, Calcd for M, 1018.1529).
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