Synthesis of pennogenyl saponin analogs using three methods

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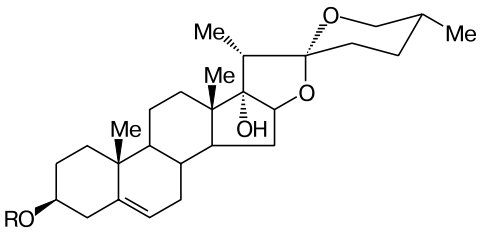
The synthesis of pennogenyl saponins and related compounds using three popular methods of glycosylation has been reported for the first time. Glycosyl halides, glycosyl trichloroacetimidates, and thioglycosides were used as glycosyl donors in the reactions with pennogenin as the glycosyl acceptor. The reactions occur selectively with the C(3)OH group due to the difference in steric accessibility of the hydroxyl groups at the C(3) and C(17) atoms of pennogenin. This makes it possible to synthesize a series of pennogenyl saponins without C(17)OH group protection.

Key words: pennogenyl saponins, pennogenin, glycosylation.

Steroid saponins represent a class of structurally and biologically diverse glycosides that are distributed in many species of plants and possess different biological activities. The structural diversity of saponins lies in their sapogenin and sugar constituents.[1] Among these saponins, compounds called pennogenyl saponins are known.



R = Glc, Rha(1→2)Glc, Rha(1→2)Glc, Rha(1→2)Glc
4 4
↑ ↑
Ara1 Rha(1→4)Rha1

In medicine they are used as haemostatic agents and promoters for shrinkage of uterus in clinic.[1] They also exhibit antibiotic and antitumor activity.[2,3] Pennogenyl saponins are steroid derivatives with the hydroxyl group at the C(17) atom. Perhaps, it is to this feature that their biological activity is related as in other C(17)OH-containing steroids, for example, cephalostatines[4] and OSW-1 (see Ref. 5). According to some data, the C(17)OH group in the aglycon can enhance the protective effect against certain diseases.[6]

Very few pennogenyl saponins can be obtained from natural sources, and it is extremely difficult to isolate them.[6,7] Therefore, further investigations into biological functions and quality control of the clinically used saponin preparations are problematic. Therefore, synthesis of this kind of saponins is topical. Now the synthesis and biosynthesis[8] of pennogenin became possible. It seems reasonable to reveal whether or not pennogenyl saponins can be synthesized.

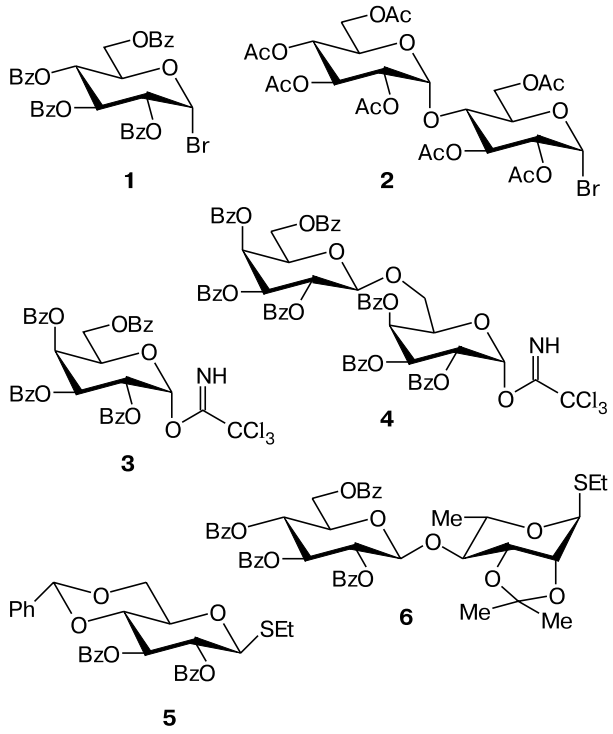
Major synthetic challenges are presented by the synthesis of glycosides in general, as the glycosidic bond is required to be formed in the correct anomeric configuration, and only one hydroxyl group in the aglycon should react. Protection of all other hydroxyl groups and other potential competing nucleophiles is essential. Thus, in the synthesis of these saponins, the glycosidic bond should be formed by glycosylation of a certain hydroxyl group of sapogenin with a glycosyl donor. In the course of the reaction, it is essential to remove such molecules as H_2O, HBr, HF, EtSH, *etc.*

A characteristic feature of pennogenyl saponins is a glycosidic bond between the C(3)OH group and a carbohydrate residue; no bond with the C(17)OH group exists. Therefore, the elucidation of the fact of a possibility of glycosylation of the C(17)OH group is very important. The lack of this reaction would considerably facilitate synthesis of pennogenyl saponins. It should be expected that the activity of the tertiary C(17)OH group in pennogenin is extremely low because of steric hindrance. The results of studies on the synthesis of six analogs of natural pennogenyl saponins from synthetic pennogenin are reported in the present communication. Three types of monosaccharide and disaccharide donors **1**—**6** were used[9] as glycosyl donors.

Glycosides **7**—**12** were obtained upon glycosylation (Scheme 1, Table 1).

From the results obtained we can conclude that the glycosylation occurs only at the C(3)OH group and the nonprotected C(17)OH group is not reactive, as we

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 10, pp. 1789—1792, October, 2006.
1066-5285/06/5510-1856



assumed taking into account its steric hindrance. This characteristic of pennogenin can bring much convenience in the synthesis of other pennogenyl glycosides.

It can also be inferred that the glycosylation with glycosyl trichloroacetimidates is preferential due to mild conditions, short reaction time, and high yields. This agrees with published data.[16]

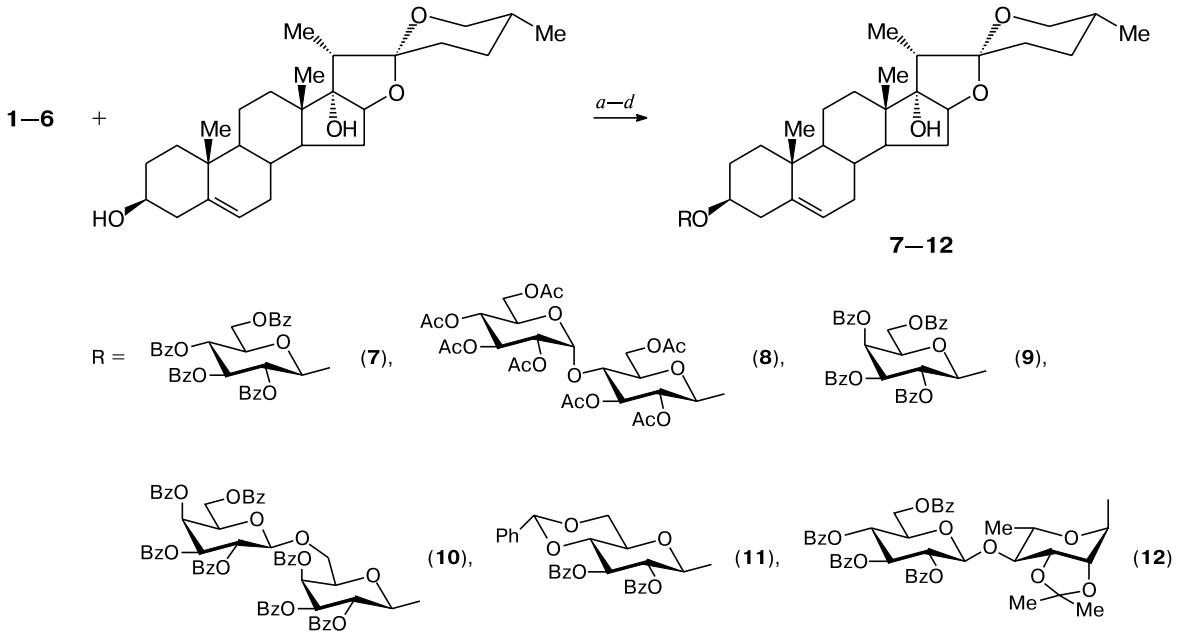
Table 1. The results of glycosylation of pennogenin

Donor	Conditions	Product	Yield (%)
1 [10]	*a*	**7**	69
2 [11]	*b*	**8**	83
3 [12]	*c*	**9**	93
4 [13]	*c*	**10**	90
5 [14]	*d*	**11**	45
6 [15]	*d*	**12**	82

Experimental

Solvents were purified according to standard procedures. Thin-layer chromatography was carried out on plates with a fixed layer of silica gel HF (0.5 mm, Qingdao, Shandong, China) with detection by charring with a 10% solution (v/v) of H_2SO_4 in EtOH or by UV detector. Column chromatography was conducted on a column (8×100 mm or 10×240 mm) packed with silica gel (100—200 mesh, Qingdao, Shandong, China). Molecular sieves (Shanghai Yueji Daily Desiccant Factory, China) were washed with water and calcined in a muffle at 500 °C. Optical rotations were determined with an Ema Optical Works polarimeter. NMR spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$ with Me_4Si as the internal standard. Mass spectra were recorded on HP5989 and VG Quattro spectrometers. Elemental analyses were performed on a Perkin—Elmer Model 2400 instrument.

Scheme 1



Reagents and conditions: *a*. AgOTf (1.1 equiv.), −20 °C → ~20 °C, 2 h. *b*. $CdCO_3$/MeCN, 65 °C.
c. TMSOTf (0.05 equiv.), molecular sieves 4Å, CH_2Cl_2, ~20 °C. *d*. NIS, AgOTf, molecular sieves 4Å, CH_2Cl_2, −30 °C.

Pennogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (7). A mixture of glycosyl bromide **1** (99 mg, 0.15 mmol), pennogenin (54 mg, 0.125 mmol), and molecular sieves 4Å (33 mg) in dry CH_2Cl_2 (5 mL) was stirred at −20 °C under argon. Then a solution of AgOTf (30 mg) in anhydrous toluene (1 mL) was added, and the mixture was stirred for 4 h at the same temperature. The system was diluted with CH_2Cl_2 (5 mL) and filtered. The filtrate was washed with brine and $NaHCO_3$ solution, dried with $MgSO_4$, and concentrated. Chromatography of the residue on a silica gel column (light petroleum—AcOEt, 6 : 1) gave compound **7** as white crystalline substance in a yield of 86.9 mg (69%), $[\alpha]_D^{20}$ −10.3 (*c* 0.32, $CHCl_3$), R_f 0.28 (light petroleum—AcOEt, 4 : 1). Found (%): C, 72.75; H, 6.68. $C_{61}H_{68}O_{13}$. Calculated (%): C, 72.62; H, 6.75. ^{1}H NMR, δ: 7.27—8.01 (m, 20 H, Ph); 5.88 (t, 1 H, H(3′)$_{Glc}$, J = 9.5 Hz); 5.61 (t, 1 H, H(4′)$_{Glc}$, J = 9.6 Hz); 5.49 (dd, 1 H, H(2′)$_{Glc}$, J = 9.7 Hz); 5.22 (s, 1 H, H(6)); 4.93 (d, 1 H, H(1′)$_{Glc}$, J = 7.9 Hz); 4.51—4.60 (m, 2 H, H_a(6′)$_{Glc}$, H_b(6′)$_{Glc}$); 4.14 (m, 1 H, H(5′)$_{Glc}$); 3.96 (t, 1 H, H(16)); 3.36—3.51 (m, 3 H, H_a(26), H_b(26), H(3)); 2.15 (m, 2 H); 0.91 (s, 3 H, C(19)H_3); 0.89 (d, 3 H, C(21)H_3, J = 7.0 Hz); 0.78 (s, 6 H, C(18)H_3, C(27)H_3). Mass spectrum (ESI), *m/z*: 1032 [M + Na].

Pennogenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-D-glycopyranoside (8). A mixture of pennogenin (37 mg, 0.0868 mmol), dry $CdCO_3$ (37 mg, 0.217 mmol), and anhydrous MeCN (2 mL) was refluxed under argon until water was completely removed from the system. Then the temperature was lowered to ~20 °C and glycosyl donor **2** (122 mg, 0.174 mmol) was added rapidly. The mixture was stirred for 2—3 h at 65 °C under argon until all pennogenin disappeared (TLC). Then the reaction mixture was diluted with CH_2Cl_2 (5 mL) and filtered through a layer of diatomite. The filtrate was concentrated and the residue was purified by column chromatography on silica gel (light petroleum—acetone, 4 : 1). The yield was 75 mg (83%), white crystalline substance, $[\alpha]_D^{20}$ +91.3 (*c* 0.28, $CHCl_3$), R_f 0.25 (light petroleum—acetone, 3 : 1). Found (%): C, 60.78; H, 7.33. $C_{53}H_{76}O_{21}$. Calculated (%): C, 60.69; H, 7.25. ^{1}H NMR, δ: 5.41 (d, 1 H, H(6), J = 3.9 Hz); 5.31—5.39 (m, 2 H, H(3″)$_{Glc}$, H(1″)$_{Glc}$); 5.24 (t, 1 H, H(3′)$_{Glc}$, J = 9.3 Hz); 5.04 (t, 1 H, H(4″)$_{Glc}$, J = 9.6 Hz); 4.86 (dd, 1 H, H(2″)$_{Glc}$, J = 9.9 Hz, J = 3.3 Hz); 4.79 (dd, 1 H, H(2′)$_{Glc}$, J = 9.3 Hz, J = 7.8 Hz); 4.61 (d, 1 H, H(1′)$_{Glc}$, J = 7.8 Hz); 4.38—4.46 (m, 1 H, H_a(6″)$_{Glc}$); 4.20—4.27 (m, 2 H, H(5″)$_{Glc}$, H_b(6″)$_{Glc}$); 3.93—4.06 (m, 4 H, H(4′)$_{Glc}$, H_a(6′)$_{Glc}$, H_b(6′)$_{Glc}$, H(16)); 3.66 (m, 1 H, H(5′)$_{Glc}$); 3.33—3.49 (m, 3 H, H(3), H_a(26), H_b(26)); 1.95, 1.96, 1.99, 2.00, 2.02, 2.10, 2.16 (all s, 3 H each, OAc); 0.98 (s, 3 H, C(19)H_3); 0.96 (d, 3 H, C(21)H_3, J = 6.9 Hz); 0.79 (s, 6 H, C(18)H_3, C(27)H_3). Mass spectrum (ESI), *m/z*: 1072 [M + Na].

Pennogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (9). A mixture of trichloroacetimidate **3** (110 mg, 0.148 mmol), pennogenin (37 mg, 0.0868 mmol), and molecular sieves 4Å (33 mg) in dry CH_2Cl_2 (3 mL) was stirred under argon at ~20 °C. Then a solution of TMSOTf in CH_2Cl_2 (0.1 mol L^{-1}, 80 μL, 0.09 equiv.) was added. The reaction mixture was stirred for 1 h, quenched with Et_3N (10 μL), and concentrated. The residue was purified by column chromatography on silica gel (light petroleum—AcOEt, 6 : 1). The yield was 81 mg (93%), white crystalline substance, $[\alpha]_D^{20}$ +41.3 (*c* 0.32, $CHCl_3$), R_f 0.29 (light petroleum—AcOEt, 4 : 1). Found (%): C, 72.75; H, 6.68. $C_{61}H_{68}O_{13}$. Calculated (%): C, 72.62; H, 6.75. ^{1}H NMR, δ: 7.25—8.11 (m, 20 H, Ph); 5.96 (d, 1 H, H(4′)$_{Gal}$, J = 3.3 Hz); 5.77 (dd, 1 H, H(2′)$_{Gal}$, J = 10.2 Hz, J = 8.4 Hz); 5.58 (dd, 1 H, H(3′)$_{Gal}$, J = 10.2 Hz, J = 3.3 Hz); 5.22 (d, 1 H, H(6)); 4.90 (d, 1 H, H(1′)$_{Gal}$, J = 8.4 Hz); 4.65 (dd, 1 H, H_a(6′)$_{Gal}$, J = 11.1 Hz, J = 6.9 Hz); 4.25—4.41 (m, 2 H, H_b(6′)$_{Gal}$, H(5′)$_{Gal}$); 3.95 (t, 1 H, H(16)); 3.36—3.51 (m, 3 H, H_a(26), H_b(26), H(3)); 0.98 (s, 3 H, C(19)H_3); 0.96 (d, 3 H, C(21)H_3, J = 7.0 Hz); 0.80 (d, 3 H, C(27)H_3, J = 6.0 Hz); 0.78 (s, 3 H, C(18)H_3). Mass spectrum (ESI), *m/z*: 1032 [M + Na].

Pennogenyl 2,3,4,6-tetra-*O*-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (10). A mixture of trichloroacetimidate **4** (163 mg, 0.148 mmol), pennogenin (37 mg, 0.0868 mmol), and molecular sieves 4Å (33 mg) in dry CH_2Cl_2 (3 mL) was stirred under argon at ~20 °C. Further procedures were the same as described for the synthesis of compound **9**. The yield was 115 mg (90%), white crystalline substance, $[\alpha]_D^{20}$ −49.4 (*c* 0.22, $CHCl_3$), R_f 0.31 (toluene—AcOEt, 6 : 1). Found (%): C, 71.41; H, 5.99. $C_{88}H_{90}O_{21}$. Calculated (%): C, 71.26; H, 6.07. ^{1}H NMR, δ: 7.23—8.07 (m, 35 H, Ph); 5.88 (d, 1 H, H(4″)$_{Gal}$, J = 3.4 Hz); 5.84 (d, 1 H, H(4′)$_{Gal}$, J = 3.4 Hz); 5.72 (dd, 1 H, H(2″)$_{Gal}$, J = 8.9 Hz, J = 10.4 Hz); 5.60 (dd, 1 H, H(3″)$_{Gal}$, J = 3.4 Hz, J = 10.4 Hz); 5.54 (dd, 1 H, H(3′)$_{Gal}$, J = 3.4 Hz, J = 10.4 Hz); 5.45 (dd, 1 H, H(2′)$_{Gal}$, J = 8.0 Hz, J = 10.4 Hz); 5.20 (s, 1 H, H(6)); 5.03 (d, 1 H, H(1″)$_{Gal}$, J = 8.0 Hz); 4.90 (d, 1 H, H(1′)$_{Gal}$, J = 8.0 Hz); 4.40 (dd, 1 H, H_a(6″)$_{Gal}$, J = 5.4 Hz, J = 11.2 Hz); 4.08—4.27 (m, 4 H, H_b(6″)$_{Gal}$, H_a(6′)$_{Gal}$, H(5″)$_{Gal}$, H(5′)$_{Gal}$); 3.96—3.98 (m, 2 H, H_b(6′)$_{Gal}$, H(16)); 3.36—3.51 (m, 3 H, H_a(26), H_b(26), H(3)); 2.15 (m, 2 H); 0.97 (s, 3 H, C(19)H_3); 0.95 (d, 3 H, C(21)H_3, J = 7.0 Hz); 0.77 (d, 3 H, C(27)H_3, J = 6.0 Hz); 0.75 (s, 3 H, C(18)H_3). Mass spectrum (ESI), *m/z*: 1506 [M + Na].

Pennogenyl 4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-D-glucopyranoside (11). A mixture of thioglycoside **5** (73 mg, 0.14 mmol), pennogenin (50 mg, 0.117 mmol), and molecular sieves 4Å (50 mg) in dry CH_2Cl_2 (2 mL) was stirred for 1h at ~20 °C under argon and then cooled to −30 °C. *N*-Iodosuccinimide (40 mg, 0.176 mmol) and then immediately a solution of AgOTf (12 mg, 0.046 mmol) in anhydrous toluene were added. The mixture was stirred for 0.5 h, quenched with Et_3N (0.1 mL), filtered, and concentrated. The residue was chromatographed on silica gel (light petroleum—AcOEt, 6 : 1). The yield was 46 mg (45%), white crystalline solid, $[\alpha]_D^{20}$ +8.3 (*c* 0.24, $CHCl_3$), R_f 0.35 (light petroleum—AcOEt, 4 : 1). Found (%): C, 73.11; H, 7.01. $C_{54}H_{64}O_{11}$. Calculated (%): C, 72.97; H, 7.20. ^{1}H NMR, δ: 7.30—8.00 (m, 15 H, Ph); 5.75 (t, 1 H, H(3′)$_{Glc}$, J = 9.5 Hz); 5.53 (s, 1 H, PhC<u>H</u>); 5.43 (dd, 1 H, H(2′)$_{Glc}$, J = 7.9 Hz); 5.22 (d, 1 H, H(6), J = 5.2 Hz); 4.89 (d, 1 H, H(1′)$_{Glc}$); 4.34 (t, 1 H, H(4′)$_{Glc}$); 4.26 (dd, 1 H, H_a(6′)$_{Glc}$, J = 4.9 Hz, J = 12.8 Hz); 3.97—3.99 (m, 2 H, H_b(6′)$_{Glc}$, H(16)); 3.67 (m, 1 H, H(5′)$_{Glc}$); 3.40—3.60 (m, 3 H, H(3), H_a(26), H_b(26)); 0.97 (s, 3 H, C(19)H_3); 0.95 (d, 3 H, C(21)H_3, J = 7.0 Hz); 0.78 (d, 3 H, C(27)H_3, J = 6.0 Hz); 0.76 (s, 3 H, C(18)H_3). Mass spectrum (ESI), *m/z*: 888, 459, 105.

Pennogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (12) was synthesized according to a procedure similar to that for compound **11**. A mixture of thioglycoside **6** (217 mg, 0.1 mmol) and pennogenin (37 mg, 0.0868 mmol) was treated with *N*-iodosuccinimide (40 mg, 0.176 mmol) and AgOTf (12 mg, 0.046 mmol). The yield was 85 mg (82%), white crystalline substance, $[\alpha]_D^{20}$ −39.2 (*c* 0.33, $CHCl_3$), R_f 0.38 (light petro-

leum—AcOEt, 4 : 1). Found (%): C, 70.51; H, 6.95. $C_{70}H_{82}O_{17}$. Calculated (%): C, 70.35; H, 6.87. ^{1}H NMR, δ: 7.26—8.06 (m, 20 H, Ph); 5.89 (t, 1 H, $H(3'')_{Glc}$, J = 9.6 Hz); 5.63 (t, 1 H, $H(4'')_{Glc}$, J = 9.8 Hz); 5.46 (dd, 1 H, $H(2'')_{Glc}$, J = 8.0 Hz, J = 9.5 Hz); 5.27—5.34 (m, 2 H, H(6), $H(1'')_{Glc}$); 4.85 (s, 1 H, $H(1')_{Rha}$); 4.50—4.61 (m, 2 H, $H(6'')_{Glc}$); 4.05 (m, 1 H, $H(5'')_{Glc}$); 3.97 (t, 1 H, H(16)); 3.80—3.89 (m, 3 H, $H(2')_{Rha}$, $H(3')_{Rha}$, $H(5')_{Rha}$); 3.37—3.69 (m, 4 H, $H(4')_{Rha}$, $H_a(26)$, $H_b(26)$, H(3)); 1.16—1.45 (m, 9 H, CMe_2, Me); 0.98 (s, 3 H, $C(19)H_3$); 0.96 (d, 3 H, $C(21)H_3$, J = 7.0 Hz); 0.79 (s, 6 H, $C(18)H_3$, $C(27)H_3$). Mass spectrum (ESI), m/z: 1218 [M + Na], 1196 [M + 1].

This work financially supported by the Natural Science Foundation of China (Grant No. 30472135).

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Received May 2, 2006;
in revised form July 11, 2006