Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 12, pp 2251–2265, 1997

PII: S0968-0896(97)00170-3
0968-0896/97 $17.00 + 0.00

Synthesis of Pavoninin-1, a Shark Repellent Substance, and its Structural Analogues toward Mechanistic Studies on their Membrane Perturbation

Yuki Ohnishi and Kazuo Tachibana*
Department of Chemistry, School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Abstract—Pavoninin-1 (**1**), which was isolated from a defense secretion of the sole *Pardachirus* spp. as an ichthyotoxic and a shark repellent principle, and its structural analogue **2** were synthesized, where glycosylation using an 2-azidoglycosyl sulfoxide (**10**) afforded the corresponding β-glycoside exclusively in high yield. Introduction of the α,β-unsaturated ketone system in the ring A of **1** was achieved by phenylselenenylation of dihydropavoninin-1 (**3**) and subsequent oxidative elimination without protection of the hydroxyl groups in the sugar portion. The mode of action of these glycosides was evaluated for their perturbation on phosphatidylcholine liposomal membrane, using the fluorescent dye leakage method. The results revealed that membrane affinity does not parallel membrane perturbation but rather compensates it, and the spatial arrangement of hydrophobic and hydrophilic regions within a molecule is likely to reflect on the difference in potency of action among them. © 1997 Elsevier Science Ltd.

Introduction

Steroid saponins are widely distributed over terrestrial plants and some marine invertebrates, and are known to possess various bioactivities including hemolytic and ichthyotoxic actions. Many of such biological activities are putatively related to their surfactant properties, and thus attributable to disruption of plasma membrane, leading to loss of its barrier function. Most of these saponins possess a mono- or oligosaccharide unit attached at C3 of the steroidal skeletons. Soles of the genus *Pardachirus* also produce steroid monoglycosides, pavoninins[1] and mosesins,[2] as their defense substances with hemolytic, ichthyotoxic, and shark-repellent activities, in addition to the other peptidic principle, pardaxins.[3] These steroid glycoside molecules are structurally unique in having a β-glycoside attached at C7 or C15 of their steroid aglycons (cf. pavoninin-1; **1** in Figure 1). These sites of glycosylation furnish the molecules with an amphiphilic arrangement in an axial or transverse direction with regard to the steroidal skeleton,[4] in contrast to common saponins with their amphiphilic dipoles along the longitudinal direction. Possibly reflecting such a difference, a commercial saponin (E. Merck) displayed much weaker shark repellent activity[5] whereas its hemolytic and ichthyotoxic potency was comparable to pavoninins and mosesins. In addition, these monoglycosides permeabilized artificial liposomes without cholesterol, while the commercial saponin did so only when the lipid bilayer contained cholesterol (unpublished results). To clarify the relationship between the intramolecular direction of the amphiphilic dipole and the mode of the membrane-disruptive activity, we adopted pavoninin-1 (**1**) as a typified example of the new type, and its analogue **2** with a regionally inverted structure to mimic conventional saponins as a control model compound (Figure 2). In this article, we report synthesis of these two molecules via stereoselective β-glycosidation using the sulfoxide-activated method as a key step, and the obtained correlation between direction of such amphiphilic dipoles and action of these glycosides including a dihydro analogue of **1** (**3**) and **2** toward liposomal lipid bilayer membrane. In the synthesis of **1**, efficiency of our strategy to introduce an olefin subsequent to glycosylation, as well as its limitation in the following synthesis of **2**, are also discussed.

*Corresponding author; Department of Chemistry, School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan. Tel: +81-3-3812-2111 ext. 4366; Fax: +81-3-5800-6898; E-mail: ktachi@chem.s.u-tokyo.ac.jp
Key words: pavoninin-1, steroid glycosides, lipid bilayer membrane, permeability enhancement, amphiphilic dipole.

Results

Synthesis of pavoninin-1

Synthesis of the aglycon alcohol of pavoninin-1 from (25*R*)-26-hydroxycholesterol, originated from diosgenin, has recently been reported.[6] Our experience in structure determination, however, showed as an aglycon with an enone functionality in the ring A, this is so unstable under acidic conditions as to yield the dienone readily, thus being unsuitable as a glycosyl acceptor. In order to overcome this problem as well as to facilitate deprotection of benzyl ethers in the sugar moiety, construction of the enone system was put off after the glycosylation in the synthetic planning. Synthesis of the

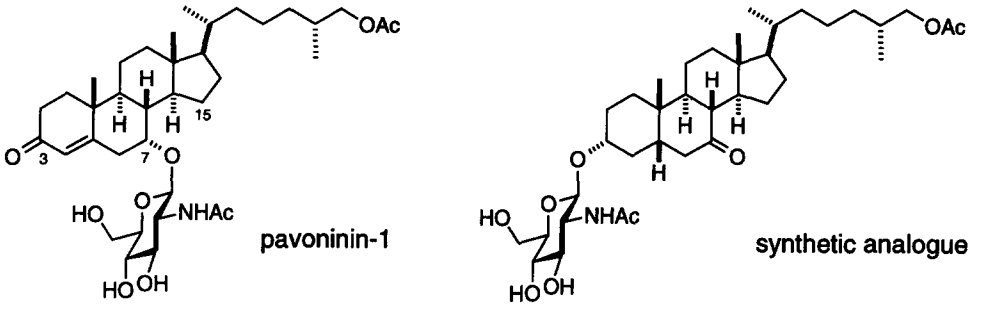


Figure 1. Structure of pavoninin-1 (**1**) and its synthetic analogue (**2**).

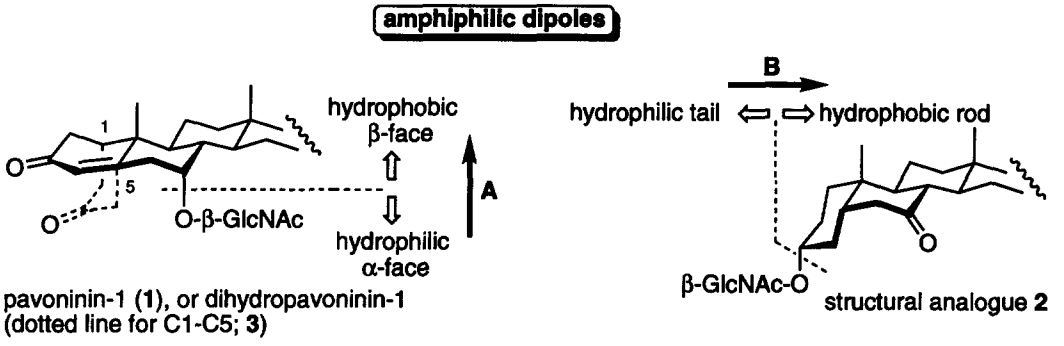
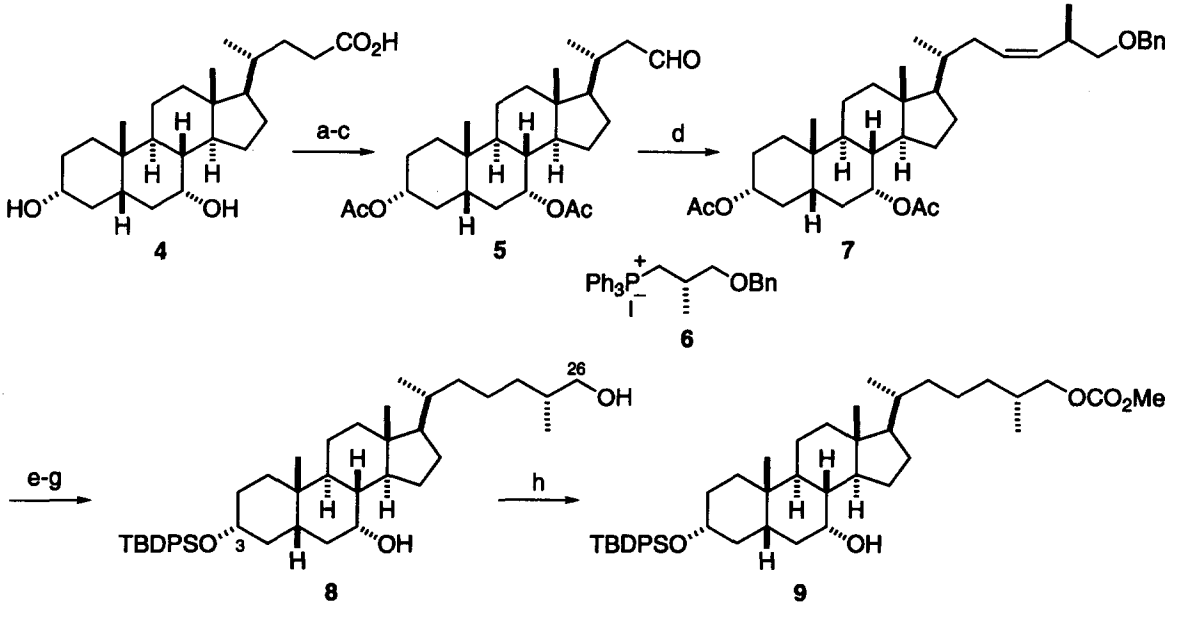


Figure 2. Amphiphilic dipoles of steroid glycosides; A: axial dipole, B; logitudinal dipole.

aglycon part started with commercially available chenodeoxycholic acid **4**, being suitably oxygenated in its steroid skeleton (Scheme 1). Elongation of the side chain was carried out following Gargiulo et al.[7] with some modifications. Namely, acetylation of **4** was followed by photochemical decarboxylation/iodination to give an iodide (70%), which was oxidized with dimethylsulfoxide (DMSO) and collidine at 150 °C to



Scheme 1. Reagents and conditions: (a) Ac_2O, pyridine, DMAP (cat.), 88%; (b) $Pb(OAc)_4$, I_2, hv, CCl_4, reflux, 70%; (c) DMSO, 2,4,6-collidine, 150 °C, 90%; (d) **6**, NaHMDS, 0 °C to rt, then **5**, THF, 0 °C, 65%; (e) $LiAlH_4$, THF, rt, 92%; (f) TBDPSCl, imidazole, DMF, rt; (g) H_2, $Pd(OH)_2/C$, EtOH, 83% (2 steps); (h) $ClCO_2Me$, pyridine, CH_2Cl_2, 0 °C, 95%.

provide the aldehyde **5** in 90% yield. Wittig homologation of **5** with the ylide, generated from phosphonium salt **6**[7] furnished the *Z*-olefin **7** in 65% yield. Reductive cleavage of the acetyl groups from **7** with $LiAlH_4$ to the corresponding diol (92%) and selective reprotection of the less hindered secondary alcohol at C3 as its *tert*-butyldiphenylsilyl (TBDPS) ether were followed by simultaneous catalytic hydrogenation/hydrogenolysis to afford the diol **8** in 83% yield for the two steps. The liberated hydroxyl group at C26 in **8** was reprotected to give methyl carbonate **9** in 95% yield to be used as the glycosyl acceptor.

The C7α hydroxy group in cholate-type steroids is highly hindered due to 1,3-diaxial positioning of the C4 methylene in the ring A and two angular hydrogens. Thus a glycosyl sulfoxide was adopted as a known efficient donor for unreactive substrates.[8] The glycosyl donor **10** was prepared from an anomeric mixture of 2-azido-2-deoxy-D-glucopyranose tetraacetate **11**, which was readily available from tri-*O*-acetyl-D-glucal by the known method,[9] in four steps (Scheme 2). Treatment of **11** with PhSH in the presence of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 under reflux afforded phenyl thioglycoside **12** in 77% yield. Replacement of the acetyl groups in **12** with benzyl groups followed by oxidation with *m*-chloroperbenzoic acid (*m*CPBA) provided sulfoxide **10** in the form of an anomeric mixture (α:β, 4:1) in 69% yield for the three steps.

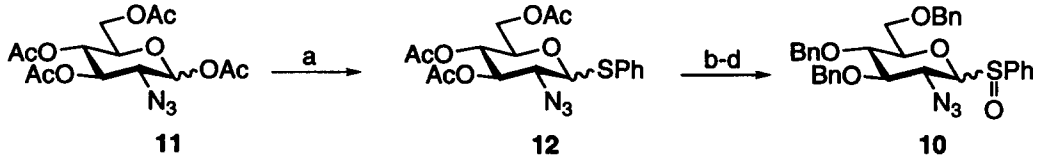
Scheme 3 summarizes the rest of the synthesis using the above two components, leading to pavoninin-1 (**1**). The highly hindered C7α hydroxyl group in **9** was glycosylated under the conditions of Kahne et al.[8] Thus, treatment of **9** and sulfoxide **10** with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DtBMP) at or below −20 °C in CH_2Cl_2 resulted in the stereoselective formation of β-glycoside **13** in 97% yield.[10] Considering that β-selectivity in corresponding glycosylation with perbenzylated glucosyl sulfoxide was reported to be 3:1,[8] exclusive formation of the β-glycoside in the present result implies significantly less steric hindrance of the neighboring azide group compared to benzyloxyl, when the acceptor substitutes at the anomeric carbon of the α-glycosyl triflate putatively as the true glycosyl donor in an S_N2-like manner.[11] Another possible contribution of the azide group to this high β-selectivity might be inductive instabilization of the oxonium cation, which would preferentially yield the α-anomer as the product, since the azide is known not to display the neighboring participation.

Removal of the silyl group from **13** with hydrofluoric acid in acetonitrile gave alcohol **14** in 96% yield. Oxidation of **14** with pyridinium dichromate (PDC) in CH_2Cl_2 gave the corresponding ketone (95%), which was protected as dimethyl ketal **15** with camphorsulfonic acid (CSA) and $CH(OMe)_3$ in $MeOH–CH_2Cl_2$ in 96% yield. Reduction of the azide group in **15** and the removal of the methoxycarbonyl group with $LiAlH_4$ were followed by acetylation to afford protected dihydropavoninin-1 (**16**) in 89% yield. Hydrogenolytic deprotection of the benzyl groups in the amino sugar moiety and subsequent acid hydrolysis of the ketal provided dihydropavoninin-1 (**3**).

Conversion of **3** to pavoninin-1 (**1**) was successfully carried out without protection of the sugar portion. Treatment of **3** with PhSeCl[12] in EtOAc gave 4β-selenenylated ketone **17** as the major product in 30% yield, after separation from the other products and unreacted **3**.[13] Treatment of the isolated **17** with hydrogen peroxide in aqueous THF afforded pavoninin-1 (**1**) in 82% yield. The spectroscopic data for the synthetic pavoninin-1 were identical in all respects with those of the natural product.

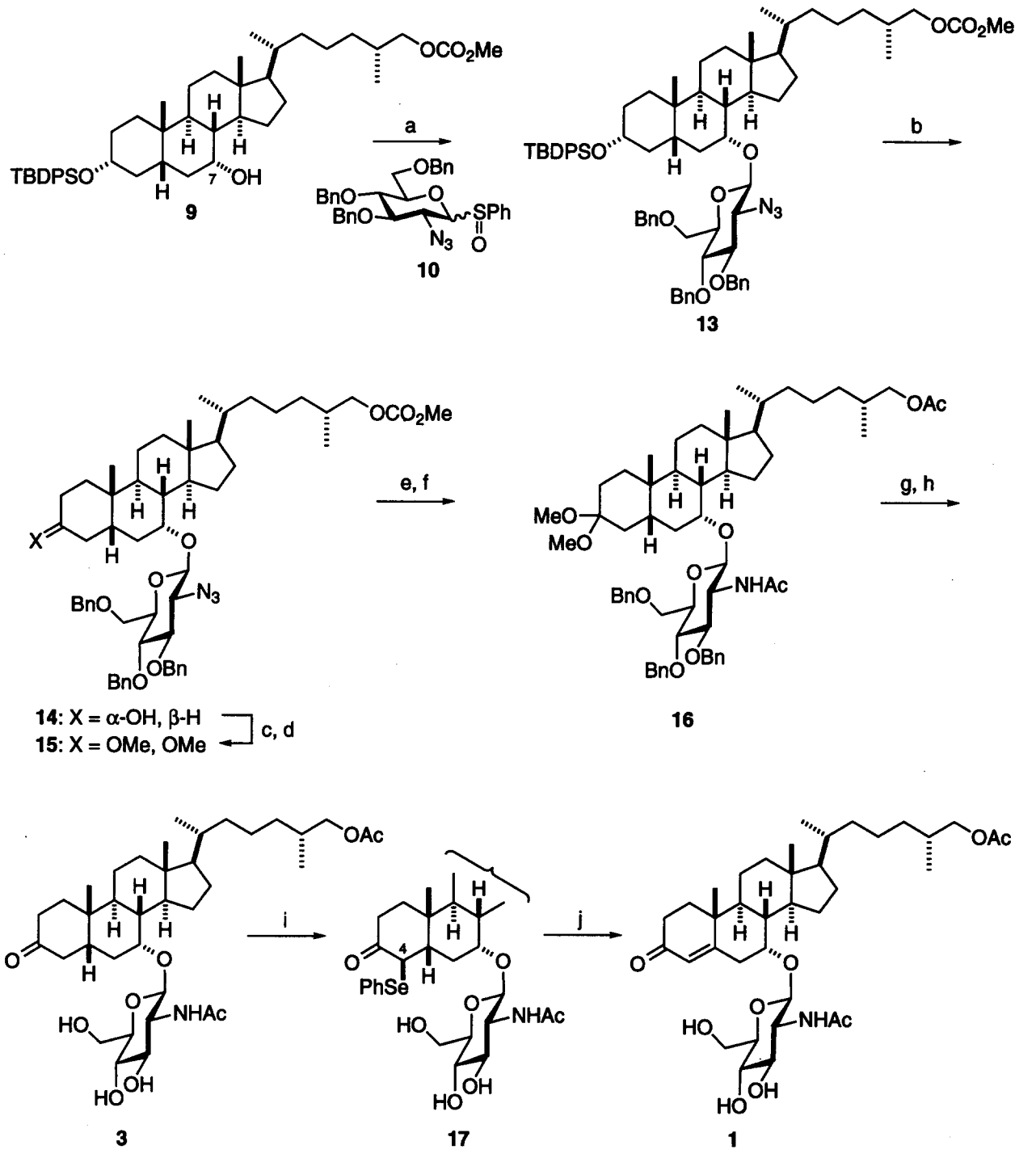
Synthesis of 2, a structural isomer of dihydropavoninin-1 (3)

Synthesis of compound **2**, with its inverted A/B ring moiety relative to **3**, started with the *Z*-olefin **7**, as shown in Scheme 4. Prior to deacetylation, hydrogenation of **7** to alcohol **18** (98%) and reprotection as its TBDPS ether were followed by selective hydrolysis of the acetate at C3 to give the alcohol **19** in 90% two-step yield. The glycosylation of **19** with the sulfoxide **10**, under the same conditions as described above, afforded β-glycoside **20** stereoselectively in 70% yield. Treatment of **20** with $LiAlH_4$, followed by selective acetylation of the resultant amine, afforded the alcohol **21** in 54% yield for the two steps. PDC oxidation of **21** (81%) after replacement of the silyl ether to an acetate furnished the ketone **22** in 88% two-step yield, and removal of the benzyl groups from **22** provided glycoside **2** in 87% yield. Introduction of the olefin at 5,6 positions was originally planned to obtain the enone whose relation to **1** would correspond to that between **2** and **3**. All



11 12 10

Scheme 2. Reagents and conditions: (a) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, reflux, 77%; (b) NaOMe, MeOH, rt, 94%; (c) NaH, BnBr, TBAI, DMF, rt, 89%; (d) mCPBA, CH_2Cl_2, −78 to 0 °C, 83%.

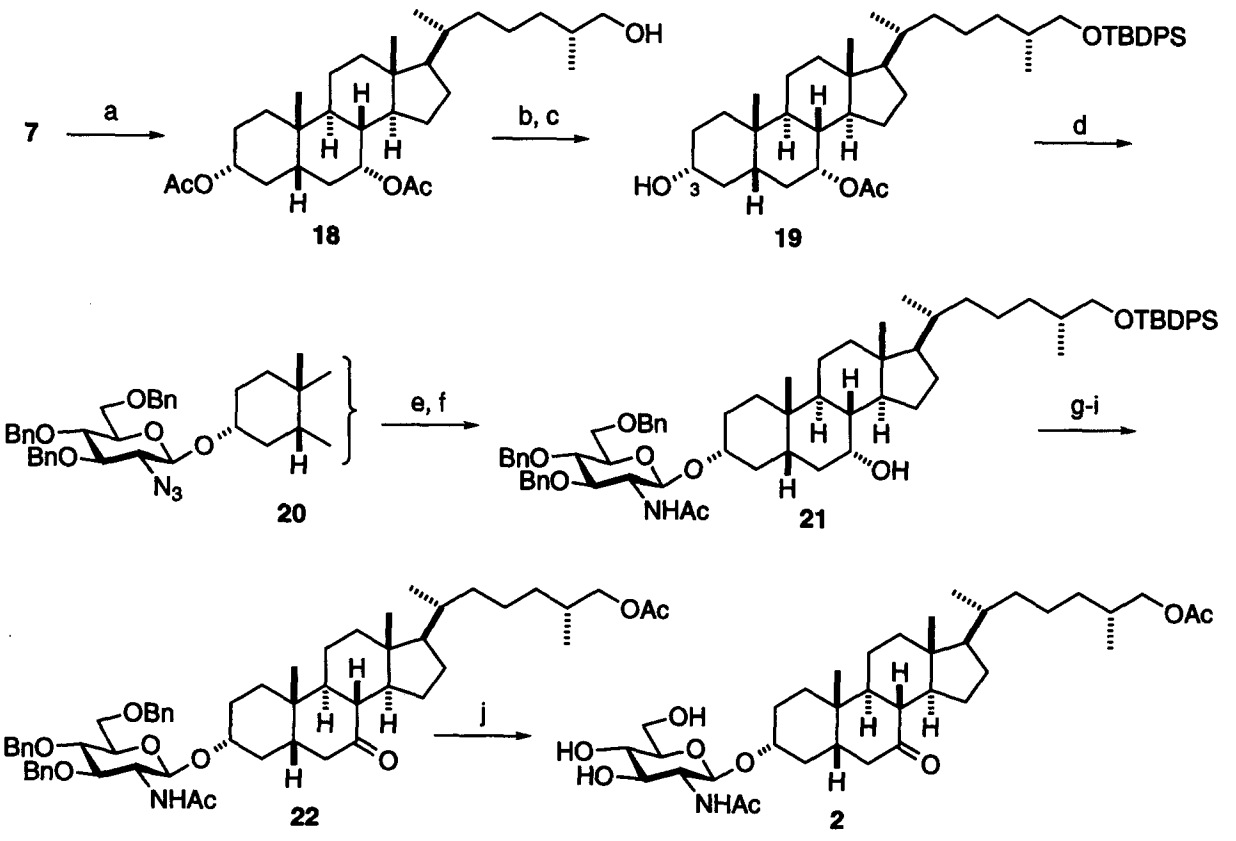


Scheme 3. Reagents and conditions: (a) **10**, Tf_2O, DtBMP, CH_2Cl_2, then **9**, −78 to −20 °C, 97%; (b) 46% HF aq, MeCN, rt, 96%; (c) PDC, MS4A, CH_2Cl_2, rt, 95%; (d) $CH(OMe)_3$, CSA, MeOH, CH_2Cl_2, rt, 96%; (e) $LiAlH_4$, THF, rt; (f) Ac_2O, pyridine, DMAP (cat.), CH_2Cl_2, rt, 89% (two steps); (g) H_2, $Pd(OH)_2/C$, MeOH, rt; (h) AcOH, H_2O, rt, 87% (two steps); (i) PhSeCl, EtOAc, rt, 30%; (j) 30% H_2O_2, THF, 0 °C, 82%.

attempts to selenenylate at C6, however, failed in this case presumably due to stereochemical congestion.[12] Since dihydropavoninin-1 (**3**) was expected to behave similarly to pavoninin-1 (**1**), while **2** serves as a model for typical saponins, the permeabilizing action of **1**, **2**, and **3** toward lipid bilayer membrane was investigated at any rate using artificial liposomes as described in the following.

Permeability enhancement by 1, 2, and 3 on liposomal membrane

Permeability enhancement by the external substrate was evaluated by the fluorescent dye leakage assay, which has been commonly used in mechanistic studies of surfactant peptides,[14–18] using small unilamellar vesicles (SUV) prepared from egg-yolk phosphatidylcholine



Scheme 4. Reagents and conditions: (a) H_2, $Pd(OH)_2/C$, EtOAc–MeOH, rt, 98%; (b) TBDPSCl, imidazole, CH_2Cl_2, rt; (c) NaOMe, MeOH, rt, 90% (two steps); (d) **10**, Tf_2O, DtBMP, CH_2Cl_2, −78 °C, then **19**, 70%; (e) $LiAlH_4$, THF, rt; (f) Ac_2O, pyridine, MeOH–CH_2Cl_2, 0 °C, 54% (two steps); (g) PDC, MS4A, CH_2Cl_2, rt, 81%; (h) TBAF, THF, rt; (i) Ac_2O, DMAP (cat.), pyridine–CH_2Cl_2, rt, 88% (two steps); (j) H_2, $Pd(OH)_2/C$, EtOH, rt, 87%.

(PC) where calcein was trapped inside. Since calcein is known to increase fluorescence intensity when it is diluted, the permeability increase of the liposomal membrane can be obtained as that of the fluorescence intensity (defined as the leakage extent in the following text). The leakage observed by the total rupture of liposomes by Triton X-100 was adopted as 100% leakage. In order to assess the respective contributions of membrane-binding affinity of a substrate molecule, and bilayer perturbing ability per membrane-bound molecule to observed permeability enhancement, a binding isotherm was also drawn for each substrate from dose–response correlations obtained in the leakage experiments performed at various concentrations of liposomes.[16–19]

Figure 3 shows four dose–response curves for pavoninin-1 (**1**) at different lipid concentrations when the fluorescence increase was recorded arbitrarily 1 min after addition of the sample to the liposome suspension. These curves shift to the right as the lipid concentration increases, by apparent dilution of the membrane-bound substrate by the additional volume of the lipid bilayer. Since permeability of the liposomal bilayer to a solute depends only on the chemical composition of the membrane, under the reasonable assumption of equibrium at this time scale, the molar ratio of membrane-bound substrates ($[S]_b$) to phospholipid ([L]), defined here as $r = [S]_b/[L]$, should be constant at a given leakage extent in the four curves regardless of lipid concentrations.[16–19] As a measured amount of added glycoside is the sum of the free and membrane-bound fractions, the material balance gives rise to the following first-order equation:

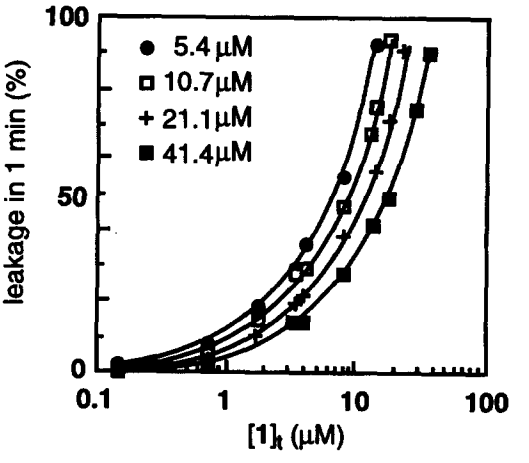


Figure 3. Dependency of calcein leakage from PC liposomes by pavoninin-1 (**1**) on lipid concentrations.

$$[S]_t = [S]_f + [S]_b = [S]_f + \mathbf{r} \cdot [L]$$

Using this equation, the unmeasurable values $[S]_f$ and **r** are obtained as the intercept and the slope, respectively, by plotting $[S]_t$ versus [L] for any leakage extent of interest. A series of **r** and corresponding $[S]_f$ values were estimated with the least square calculations for leakage extents of 20–75% on the basis of the four dose–response curves in Figure 3 (Figure 4).

The relationship between the obtained $[S]_f$ and **r**, termed as the binding isotherm, was drawn to obtain the apparent binding constant, K_{app} (= $\mathbf{r}/[S]_f$), as the slope, together with those obtained for the glycosides **2** and **3** in the same manner (Figure 5a). Contrary to our expectation, where dihydropavoninin-1 (**3**) would behave similarly to pavoninin-1 (**1**), while 3-*O*-glycoside **2** would be different in that it should require cholesterol in the lipid bilayer to enhance its permeability, the permeabilizing potency and membrane-binding behavior of **3** turned out to be similar to **2** despite the different positions of glycosylation between **2** and **3**. The K_{app} values at the infinite dilution were estimated from the linear range of binding isotherms and summarized for three steroid glycosides **1**, **2**, and **3** in Table 1.

The relationships between **r** and the leakage extent for the three substrates are shown in Figure 5b, illustrating the intrinsic disruptive ability of the membrane-bound substrate. This intrinsic ability is independent from the liposome concentration, and thus from the membrane-binding affinity of each glycoside. The molar ratio of membrane-bound substrate to the lipid causing 50% leakage is defined here as $\mathbf{r}_{50}$ for convenience in quantitative comparison. The $\mathbf{r}_{50}$ values for **1**, **2**, and **3** are also shown in Table 1, where the lower $\mathbf{r}_{50}$ value means a stronger disruptive ability. Here, the order of membrane permeabilizing ability was shown to be **1**, **3**, and then **2**, as if it compensates their membrane-binding affinity.

Although 3-*O*-glycoside **2** was shown to enhance the permeability of the lipid bilayer even without cholesterol, the leakage assay was repeated with **1–3** in the same manner but using SUV prepared from PC, containing 20% cholesterol, in relative molality to PC to see whether **2** shows a remnant of conventional 3-*O*-glycosidic saponins by associating with membranal cholesterol in its disruptive action. As the results show in Table 1, none of the three glycosides displayed

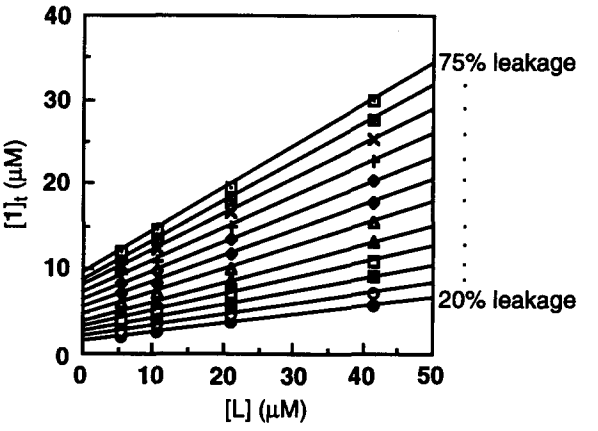


Figure 4. Relations between [L] and $[S]_t$ to estimate unbound 1 ($[S]_f$) and **r** for series of % leakage.

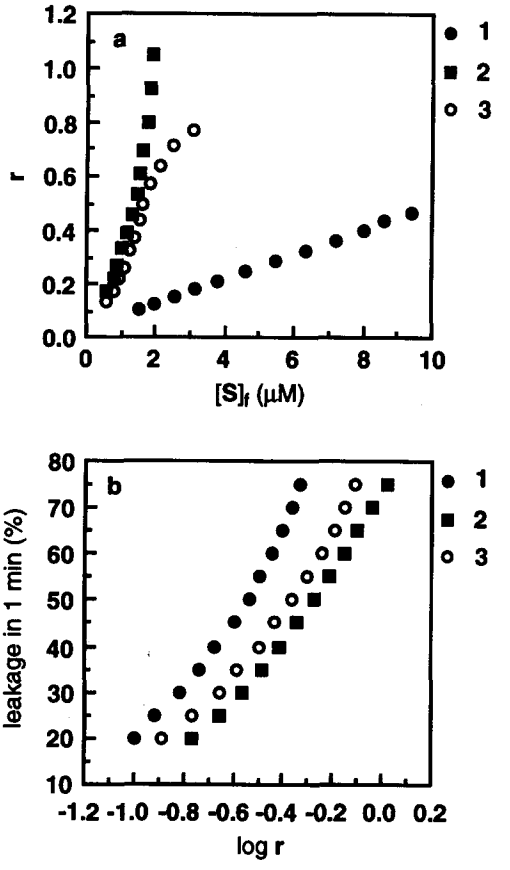


Figure 5. (a) Binding isotherms for three steroid glycosides **1**, **2** and **3** with PC liposomes. (b) Membrane permeabilizing ability of the bound glycosides.

Table 1. Membrane-binding affinity (K_{app}) and 50% effective binding to disrupt the bilayer ($\mathbf{r}_{50}$) of **1–3**, and their ichthyotoxicity to medaka

Substrate	K_{app} (μM^{-1})[a]	r_{50}[a]	K_{app} (μM^{-1})[b]	r_{50}[b]	Ichthyotoxicity[c]
1	0.047	0.26	0.044	0.26	++
2	0.29	0.51	0.16	0.48	–
3	0.28	0.40	0.15	0.39	+

[a]PC liposome.
[b]PC liposome containing 20% cholesterol.
[c]See text.

increased activity in comparison to the case with liposomes with PC only. Membrane-binding affinity of glycosides **2** and **3** somewhat dropped instead, probably due to rigidification of the bilayer structure by participation of cholesterol. Reflection of the rigidification by cholesterol on the decreased membrane-binding affinity of the substrates, rather than on resistance to permeability enhancement by the bound substrates, conforms to our previous observation for a peptidic pardaxin.[18]

Ichthyotoxicity

To correlate the $\mathbf{r}_{50}$ values obtained here in a model system to activity in a biological system, the ichthyotoxicity of glycosides **2** and **3** was examined on the Japanese killifish (medaka; *Oryzias latipes*). The results showed that dihydropavoninin-1 (**3**) was lethal at 17 μg/mL with death time around 1 h, while **2** exhibited no effect on the fish at 33 μg/mL. When the results were compared to the value on **1** (8.5 μg/mL as LC_{1h}),[1] **3** practically inherited the ichthyotoxicity of **1** though at a somewhat reduced level. This difference in ichthyotoxicity between **2** and **3** reflected our original speculation, as is the $\mathbf{r}_{50}$ obtained in the present liposomal experiments as far as the order is concerned.

Discussion

The result obtained above for pavoninin-1 (**1**) was quite straightforward in that it binds to the outer layer of lipid bilayer only shallowly, thus with a small binding constant, while a single bound molecule stretches on a large surface area of a liposome. The resultant imbalance of the area between the outer and inner layers of the bilayer creates tension on the membrane structure, eventually leading to a forced flip-flop,[19] or temporary channel formation,[20] during which solutes in the liposomes diffuse out, as proposed for the mechanisms of action for amphiphilic peptides.

In contrast, the 3-*O*-glycoside (**2**) thrusts itself in the outer layer of a liposome along its longitudinal direction. Its deeper and yet narrower interaction with phospholipid molecules permits a larger binding constant, but causes less tension to the bilayer structure. Considering its still superior activity to conventional saponins, the binding direction of **2** within the outer layer of liposomes must be somewhat tilted from the perpendicular axis of the lipid bilayer due to the bent structure at its A/B ring region and/or α-direction of the glycosyl residue. An equilibrium may exist as well between the tilted and straight positionings relative to phospholipid molecules, the latter of which may make the glycoside cramped. The lack of promoted activity in **2** toward liposomes containing cholesterol can be explained by the same reasons. Namely, **2** binds to the outer layer a little but significantly too shallowly to associate with the cholesterol molecules buried in the outer layer for the same structural reason(s), or the tilted positioning may prevent such association.

The difference observed between binding isotherms of **1** and its dihydro analogue **3** is uninterpretable from the chemical structural point of view, and must await further experimental design. Although an earlier saturation of binding by **3** compared to **2** (Figure 5a) indicates that **2** still retains the character of **1** in a wide interacting area at the surface of liposomes, the bend at the A/B ring of **3** must make it different from **1** in its binding to the bilayer, as yet to be envisaged. The effective binding plot (Figure 5b), however, reflects an intermediate character of **3** between **1** and **2**. It also reflects the order of ichthyotoxicity, where the difference between **2** and **3** appears to be emphasized. While SUV are considered to be more sensitive to distortion caused by the binding of external substrate molecules, permeability enhancement toward smaller inorganic ions are more significant in biological activity at the cellular level, causing depolarization at the plasma membrane of a certain kind of cells, the malfunction of which becomes fatal to the test fish, for instance. Therefore, the small difference between **2** and **3** on Figure 5b may become significant in a biological system, and the plot for **2** should become rather closer to that of **1** in permeability enhancement to smaller solutes when one notices the difference in **r** at small leakage extents in this figure. This tendency for small leakage is, in practice, more distinct in the plot using liposomes containing 20% cholesterol (data not shown). This correspondence between **r** and ichthyotoxicity also suits a reasonable inference that small effective binding values, rather than membrane-binding affinity, become more important in a bioassay with confined systems such as the present case, where two fish of 2–3 cm were confined in 30 mL water.

Experimental Section

Synthesis

General methods. The starting materials and all the reagents were purchased from standard vendors and used without purification unless otherwise noted. All reactions sensitive to air or moisture were carried out under nitrogen or argon atmosphere with freshly distilled solvents under anhydrous conditions, unless otherwise noted. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Methanol was distilled from $Mg(OMe)_2$.

Analytical thin-layer chromatography (TLC) was developed on E. Merck silica gel 60 F_{256} plates (0.25 mm thickness), and spots were visualized with aqueous cerium sulfate-phosphomolybdic acid followed by heating. Column chromatography was performed on E. Merck silica gel 60 (60–230 mesh) or florisil (60–100 mesh, Kanto Chemical Co., Inc.) at ambient pressure. Flash column chromatography was carried out using E. Merck silica gel 60 (230–400 mesh). Celite was purchased from Kanto Chemical Co., Inc. (celite 545). Solvent compositions are in v/v.

Infrared spectra were recorded on Horiba FT200 or JASCO 300 FTIR and ν_{max}'s are presented. NMR spectra were obtained on a JEOL A500 spectrometer (^{1}H at 500 MHz, and ^{13}C at 125 MHz) at 300 K. ^{1}H chemical shifts are presented only for distinct signals and those relevant to the structural alteration in parts per million (δ) adjusted to $CHCl_3$ at 7.24 ppm, CHD_2OD at 3.30 ppm, or CHD_2CN at 1.93 ppm as an internal reference. ^{13}C chemical shifts were referenced with the $^{13}CDCl_3$ at 77.0 ppm, $^{13}CD_3OD$ at 49.0 ppm, or $CD_3{}^{13}CN$ at 118.0 ppm. Optical rotations were measured on a JASCO DIP370 digital polarimeter. Mass spectra were obtained on a JEOL SX-102A mass spectrometer on fast-atom bombardment (FAB) mode with *m*-nitrobenzyl alcohol as the matrix with NaI as the additive to promote molecular ions as $(M + Na)^+$.

23-Oxo-24-nor-5β-cholane-3α,7α-diyl diacetate (5). To a solution of chenodeoxycholic acid (**4**; 2.04 g, 5.09 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in pyridine (30 mL) was added Ac_2O (10 mL, 0.106 mol) at 0 °C and the mixture was left to stand at room temperature for 4 h. After the solvent was evaporated in vacuo, the residue was dissolved in EtOAc (200 mL) and washed with 1M HCl and then brine. Evaporation of the solvent and purification by silica gel column chromatography (50% EtOAc in hexane) gave 3,7-di-*O*-acetylchenodeoxycholic acid (2.17g, 88%) as a white solid: R_f 0.4 (50% EtOAc in hexane); $[\alpha]^{23}_D$ +13.2° (*c* 1.16; $CHCl_3$); IR (KBr) 3683, 2492, 1737, 1446, 1378, 1253, 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.86 (brdt, *J* = 5.5, 3.0 Hz, 7-H), 4.57 (m, 3-H), 2.03 (3H, s, OAc), 2.00 (3H, s, OAc), 0.91 (3H, d, *J* = 7.4 Hz, 20-Me), 0.91 (3H, s, 10-Me), 0.63 (3H, s, 13-Me); ^{13}C-NMR ($CDCl_3$) δ 179.2, 170.7, 170.5, 74.2, 71.3, 55.7, 42.7, 40.9, 39.5, 37.9, 35.2, 34.9, 34.8, 34.6, 34.1, 30.7, 28.0, 26.8, 23.5, 22.7, 21.6, 20.6, 18.2, 11.7; FABMS *m/z* 499 $(M + Na)^+$.

A solution of the above diacetate (7.20 g, 15.1 mmol) and lead tetraacetate (9.11 g, 20.5 mmol) in CCl_4 (120 mL), freshly distilled from P_2O_5, was irradiated by a 300 W tungsten lamp under reflux with bubbling N_2 for 20 min. To the solution was added iodine (10.90 g, 42.6 mmol) in CCl_4 (140 mL) dropwise and the mixture was refluxed for 70 min. Insoluble materials were filtered, and the filtrate was extracted with CH_2Cl_2 (500 mL) and washed with saturated aqueous Na_2SO_3 (600 mL). Drying over Na_2SO_4, evaporation of the solvent, and purification by silica gel column chromatography (10–20% EtOAc in hexane) gave 23-iodo-24-nor-5β-cholane-3α,7α-diyl diacetate (5.88 g, 70%) as a white solid: R_f 0.2 (10% EtOAc in hexane); $[\alpha]^{24}_D$ +26.2° (*c* 1.11; $CHCl_3$); IR (KBr) 2940, 2871, 1733, 1378, 1253, 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.86 (brdt, *J* = 5.5, 2.7 Hz, 7-H), 4.57 (m, 3-H), 3.28 (dt, *J* = 9.4, 3.9 Hz, 23-Ha), 3.08 (dt, *J* = 9.4, 8.6 Hz, 23-Hb), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc), 0.91 (3H, s, 10-Me, and 3H, d, *J* = 6.4 Hz, 20-Me), 0.63 (3H, s, 13-Me); ^{13}C-NMR ($CDCl_3$) δ 170.6, 170.4, 74.2, 71.2, 55.7, 50.4, 42.8, 40.4, 40.3, 39.5, 37.9, 34.9, 34.8, 34.6, 34.1, 31.3, 28.0, 26.8, 23.5, 22.7, 21.6, 21.5, 17.8, 11.7, 4.9; HRMS (FAB) *m/z* calcd for $C_{27}H_{43}O_4INa$ $(M + Na)^+$ 581.2104, found 581.2095.

To a solution of the above iodide (1.44 g, 2.58 mmol) in DMSO (45 mL), freshly distilled from CaH_2, 2,4,6-collidine was added and the mixture was left to stand at 150 °C for 1.5 h. The reaction was quenched with H_2O, and the solution was extracted with CH_2Cl_2 (200 mL) and washed with 1M HCl, H_2O, saturated aqueous $NaHCO_3$, and brine in this sequence. Drying over Na_2SO_4, concentration, and purification by silica gel column chromatography (10–20% EtOAc in hexane) gave aldehyde **5** (1.04 g, 90%): R_f 0.2 (20% EtOAc in hexane); $[\alpha]^{23}_D$ +3.4° (*c* 0.98; $CHCl_3$); IR (thin film) 2941, 2871, 2711, 1734, 1379, 1254, 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.73 (dd, *J* = 3.4, 1.2 Hz, CHO), 4.86 (brdt, *J* = 5.8, 3.1 Hz, 7-H), 4.56 (m, 3-H), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc), 1.00 (3H, d, *J* = 6.4 Hz, 20-Me), 0.91 (3H, s, 10-Me), 0.68 (3H, s, 13-Me); ^{13}C-NMR ($CDCl_3$) δ 203.3, 170.6, 170.4, 74.1, 71.1, 55.8, 50.4, 42.8, 40.9, 39.4, 37.9, 34.9, 34.8, 34.6, 31.6, 31.3, 28.4, 26.8, 23.5, 22.7, 21.6, 21.5, 20.6, 20.0, 11.7; FABMS *m/z* 469 $(M + Na)^+$.

(*Z*,25*R*)-26-benzyloxy-5β-cholest-22-ene-3α,7α-diyl diacetate (7). To a suspension of [(2*S*)-3-benzyloxy-2-methylpropyl]trimethylphosphonium iodide (**6**; 1.61 g, 2.91 mmol) in THF (10 mL) was added 1M $NaN(SiMe_3)_2$ in THF (2.6 mL, 2.6 mmol) at 0 °C and the mixture was stirred at room temperature for 30 min, then cooled again to 0 °C. A solution of aldehyde **5** (647.4 mg, 1.45 mmol) in THF (12 mL) was added to the above ylide solution. The mixture was stirred at 0 °C for 1 h and the reaction was quenched with saturated aqueous NH_4Cl. The solution was diluted with EtOAc (200 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$, H_2O, and then brine. Evaporation of the solvent and purification by silica gel column chromatography (10% EtOAc in hexane) gave *Z*-olefin **7** (562.3 mg, 65%) as a colorless oil: R_f 0.3 (20% EtOAc in hexane); $[\alpha]^{26}_D$ −12.2° (*c* 1.19; $CHCl_3$); IR (thin film) 2939, 2868, 1734, 1655, 1369, 1242, 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.33–7.24 (5H, m, Ph), 5.36 (m, 23-H), 5.20 (dd, *J* = 11.0, 9.5 Hz, 24-H), 4.86 (brd, *J* = 2.5 Hz, 7-H), 4.57 (m, 3-H), 4.50 (d, *J* = 12.0 Hz, CHaPh), 4.47 (d, *J* = 12.0 Hz, CHbPh), 3.92 (dd, *J* = 9.2, 6.5 Hz, 26-Ha), 3.22 (dd, 9.2, 7.0 Hz, 26-Hb), 2.76 (m, 25-H), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc), 0.97 (3H, d, *J* = 6.5 Hz, 20-Me), 0.91 (3H, s, 10-Me), 0.89 (3H, d, *J* = 7.0 Hz, 25-Me) 0.68 (3H, s, 13-Me); ^{13}C-NMR ($CDCl_3$) δ 170.6, 170.4, 138.7, 133.3, 128.8, 128.2, 127.4, 75.3, 74.1, 72.8, 71.2, 56.1, 50.4, 42.7, 40.9, 39.4, 37.9, 36.3, 34.9, 34.8, 34.0, 33.9, 32.4, 31.3, 28.2, 26.8, 23.6, 22.7, 21.5, 21.4, 20.6, 18.7, 17.7, 11.7; FABMS *m/z* 615 $(M + Na)^+$.

(25*R*)-3α-*tert*-butyldiphenylsiloxy-5β-cholestane-7α,26-diol (8). To a suspension of $LiAlH_4$ (132.3 mg, 3.48 mmol) in THF (10 mL) was added a solution of olefin **7** (949.9 mg, 1.86 mmol) in THF (40 mL) at 0 °C

and the mixture was stirred at room temperature for 2 h. After cooling to 0 °C, the reaction was quenched with EtOAc and the mixture was extracted with EtOAc (300 mL), and washed with 0.5M HCl, H_2O, and then brine. Evaporation of the solvent and purification by silica gel column chromatography gave (*Z*,25*R*)-26-benzyloxy-5β-cholest-22-ene-3α,7α-diol (749.9 mg, 92%) as a colorless oil: R_f 0.2 (50% EtOAc in hexane); $[\alpha]^{29}_D$ −25.7° (*c* 0.65; $CHCl_3$); IR (thin film) 3394, 2927, 2865, 1454, 1369, 1111, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34–7.25 (5H, m, Ph), 5.37 (m, 23-H), 5.20 (dd, *J* = 10.7, 9.8 Hz, 24-H), 4.51 (d, *J* = 12.0 Hz, CHaPh), 4.47 (d, *J* = 12.0 Hz, CHbPh), 3.84 (brdt, *J* = 5.8, 3.4 Hz, 7-H), 3.44 (m, 3-H), 3.30 (dd, *J* = 9.2, 6.4 Hz, 26-Ha), 3.23 (dd, *J* = 9.2, 7.3 Hz, 26-Hb), 2.77 (m, H-25), 0.97 (3H, d, *J* = 6.7 Hz, 20-Me), 0.90 (3H, d, *J* = 7.6 Hz, 25-Me), 0.89 (3H, s, 10-Me), 0.64 (3H, s, 13-Me); ^{13}C-NMR ($CDCl_3$) δ 138.7, 133.3, 128.8, 128.3, 127.5, 127.4, 75.3, 72.9, 72.0, 68.6, 56.0, 50.5, 42.7, 41.5, 39.9, 39.5, 39.4, 36.4, 35.3, 35.0, 34.6, 34.0, 32.8, 32.4, 30.7, 28.4, 23.8, 22.8, 20.6, 18.7, 17.8, 11.8; FABMS *m/z* 531 $(M + Na)^+$.

To a solution of the above diol (355.5 mg, 0.70 mmol) and imidazole (99.2 mg, 1.45 mmol) in DMF (10 mL) *tert*-butyldiphenylsilyl chloride (0.24 mL, 0.92 mmol) was added and the mixture was let stand at room temperature for 17 h then reaction was quenched with brine. The reaction mixture was extracted with EtOAc (200 mL), and the extract was washed with 1M HCl, H_2O, and then brine (30 mL). Evaporation of the solvent and purification by silica gel column chromatography (10% EtOAc in hexane) gave the 3α-silyl ether with an inseparable minor component (651.4 mg), which was used in the next reaction without further purification: R_f 0.6 (10% EtOAc in hexane); ^1H NMR ($CDCl_3$) δ 7.72–7.32 (15H, m, 3 × Ph), 5.38 (m, 23-H), 5.22 (dd, *J* = 10.5, 9.5 Hz, 24-H), 4.53 (d, *J* = 12.0 Hz, CHaPh), 4.49 (d, *J* = 12.0 Hz, CHbPh), 3.81 (brd, *J* = 2.5 Hz, 7-H), 3.46 (m, 3-H), 3.32 (dd, *J* = 9.5, 6.5 Hz, 26-Ha), 3.25 (dd, *J* = 9.5, 8.0 Hz, 26-Hb), 2.80 (m, 25-H), 1.04 (9H, s, *t*-Bu), 1.01 (3H, d, *J* =7.0 Hz, 20-Me), 0.91 (3H, d, *J* =7.0 Hz, 25-Me), 0.79 (3H, s, 10-Me), 0.63 (3H, s, 13-Me); FABMS 769 *m/z* $(M + Na)^+$.

A solution of the above crude silyl ether (651.4 mg) in EtOH (20 mL) was stirred with $Pd(OH)_2/C$ (67.5 mg) under hydrogen atmosphere for 4 days. The catalyst was removed by filtration and the solvent was evaporated. The residue was purified by silica gel column chromatography (5–30% EtOAc in hexane) to give alcohol **8** (381.3 mg, 83% from the 3α,7α-diol) as a colorless oil: R_f 0.2 (20% EtOAc in hexane); $[\alpha]^{29}_D$ +15.5° (*c* 0.26; $CHCl_3$); IR (thin film) 3396, 2933, 2859, 1459, 1375, 1219, 1099 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67–7.32 (10H, m, 2 × Ph), 3.80 (brdt, *J* = 6.0, 3.0 Hz, 7-H), 3.51–3.39 (3H, m, 3-H and 26-H_2), 1.02 (9H, s, *t*-Bu), 0.90 (6H, d, *J* = 7.0 Hz, 20- and 25-Me), 0.78 (3H, s, 10-Me), 0.62 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 135.8, 135.0, 134.9, 129.3, 128.3, 127.4, 73.6, 68.6, 68.5, 56.1, 50.5, 42.6, 41.4, 39.8, 39.6, 39.4, 36.1, 35.7, 35.7, 35.3, 35.0, 34.7, 33.5, 32.8, 30.9, 28.3, 27.0, 23.7, 23.3, 22.7, 20.8, 19.1, 18.6, 16.5, 11.7; FABMS *m/z* 681 $(M + Na)^+$.

(25*R*)-3α-*tert*-butyldiphenylsilyloxy-7α-hydroxy-5β-cholestan-26-yl methyl carbonate (9). To a solution of alcohol **8** (477.9 mg, 0.73 mmol) in CH_2Cl_2 (12 mL) and pyridine (0.5 mL) was added $ClCO_2Me$ (70 mL, 0.91 mmol), and the mixture was left to stand at 0 °C for 4 h. The reaction was quenched with 1 M HCl, and the solution was diluted with EtOAc (300 mL) and washed with H_2O, then brine. Evaporation of the solvent and purification by flash column chromatography (10% EtOAc in hexane) gave **9** (495.5 mg, 95%) as a colorless oil: R_f 0.7 (30% EtOAc in hexane); $[\alpha]^{26}_D$ +14.9° (*c* 0.60; $CHCl_3$); IR (thin film) 3537, 2935, 2861, 1745, 1456, 1377, 1273, 1174, 1097 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67–7.32 (10H, m. 2 × Ph), 4.00 (dd, *J* = 10.5, 6.0 Hz, 26-Ha), 3.91 (dd, *J* = 10.5, 7.0 Hz, 26-Hb), 3.80 (brdt, *J* = 5.5, 2.5 Hz, 7-H), 3.76 (3H, s, CO_2Me), 3.44 (m, 3-H), 1.02 (9H, s, *t*-Bu), 0.92 (3H, d, *J* = 7.0 Hz, 20-Me), 0.89 (3H, d, *J* = 7.0 Hz, 25-Me), 0.78 (3H, s, 10-Me), 0.62 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 156.0, 135.8, 135.1, 134.9, 129.3, 127.4, 73.6, 73.2, 68.6, 56.1, 54.6, 50.5, 42.7, 41.4, 39.9, 39.6, 39.4, 36.0, 35.7, 35.3, 35.0, 34.7, 33.4, 32.8, 32.6, 30.9, 28.3, 27.0, 23.7, 23.2, 22.7, 20.6, 19.1, 18.6, 16.5, 11.7; FABMS *m/z* 739 $(M + Na)^+$.

2-Azido-1,2-dideoxy-1-phenylthio-D-glucopyranose triacetate (12). To a solution of the anomeric mixture of 2-azido-2-deoxy-D-glucopyranose tetraacetate **11** (6.87 g, 18.4 mmol) and PhSH (2.3 mL, 22.4 mmol) in CH_2Cl_2 (60 mL) was added $BF_3{\cdot}OEt_2$ (9.0 mL, 77.3 mmol) and the mixture was refluxed for 10 h. The solution was diluted with EtOAc (500 mL), and washed with 1M HCl, saturated aqueous $NaHCO_3$, H_2O, and brine. Evaporation of the solvent and purification by flash column chromatography (0–10% EtOAc in toluene) gave thioglycoside **12** (5.88 g, 77% as the anomeric mixture of α:β, 4:1) as a brown syrup: R_f 0.2 (20% EtOAc in hexane); $[\alpha]^{25}_D$ +116.5° (*c* 1.15; $CHCl_3$); IR (thin film) 3014, 2951, 2112, 1747, 1221 cm^{-1}; ^1H NMR ($CDCl_3$) α-anomer: δ 7.58–7.28 (5H, m, Ph), 5.62 (d, *J* = 5.5 Hz, 1-H), 5.32 (dd, *J* = 10.4, 9.2 Hz, 3-H), 5.02 (dd, *J* = 10.4, 9.2 Hz, 4-H), 4.57 (ddd, *J* = 10.1, 5.0, 2.4 Hz, 5-H), 4.27 (dd, *J* = 12.5, 5.0 Hz, 6-Ha), 4.06 (dd, *J* = 10.4, 5.5 Hz, 2-H), 4.01 (dd, *J* = 12.5, 2.4 Hz, 6-Hb), 2.08 (3H, s, OAc), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc); β-anomer: δ 7.58–7.28 (5H, m, Ph), 5.05 (dd, *J* = 9.8, 9.5 Hz, 3-H), 4.90 (dd, *J* = 9.8, 9.5 Hz, 4-H), 4.47 (d, *J* = 10.4 Hz, 1-H), 4.21 (dd, *J* = 12.2, 4.9 Hz, 6-Ha), 4.15 (dd, *J* = 12.2, 2.4 Hz, 6-Hb), 3.67 (ddd, *J* = 9.8, 4.9, 2.4 Hz, 5-H), 3.39 (dd, *J* = 10.4, 9.8 Hz, 2-H), 2.06 (3H, s, OAc), 2.04 (3H, s, OAc), 1.99 (3H, s, OAc); ^{13}C NMR ($CDCl_3$) α-anomer: δ 170.5, 169.8, 134.1, 132.2, 129.2, 128.1, 86.5, 72.0, 68.7, 68.5, 61.9, 61.6, 20.61, 20.56, 20.52; β-anomer: δ 170.5, 169.8, 169.6, 132.4, 130.2, 129.1, 128.9, 85.8, 75.8, 74.4, 68.1, 62.6, 62.0, 20.61, 20.56, 20.52; FABMS *m/z* 446 $(M + Na)^+$.

2-Azido-3,4,6-tri-*O*-benzyl-1,2-dideoxy-1-phenylsulfinyl-D-glucopyranose (10). To a solution of **12** (3.89 g, 13.1 mmol) in MeOH (15 mL) was added 1 M NaOMe in MeOH (3 mL). The reaction mixture was left to stand at room temperature for 5 h, and neutralized with Dowex 50W-X8 acidic resin (200–400 mesh). The resin was filtered, and the filtrate was concentrated. The residue was purified by flash column chromatography (5–10% MeOH in $CHCl_3$) to give the deacetylated triol (2.58 g, 94% as the anomeric mixture of α:β, 4:1) as a colorless syrup: R_f 0.5 (10% MeOH in $CDCl_3$); $[\alpha]^{27}_D$ +145.3° (*c* 0.98; $CHCl_3$); ^1H NMR (CD_3OD) α-anomer: δ 7.59–7.25 (5H, m, Ph), 5.56 (d, *J* = 5.2 Hz, 1-H), 4.10 (m, 5-H), 3.76–3.72 (3H, m, 2-H and 6-H2), 3.65 (dd, *J* = 10.4, 8.7 Hz, 3-H), 3.42, (dd, *J* = 9.8, 8.7 Hz, 4-H), β-anomer: δ 7.59–7.25 (5H, m, Ph), 4.53 (d, *J* = 10.1 Hz, 1-H), 3.86 (dd, *J* = 12.0, 1.8 Hz, 6-Ha), 3.67 (dd, *J* = 12.0, 5.2 Hz, 6-Hb), 3.39 (dd, *J* = 9.5, 9.2 Hz, 3-H), 3.31–3.27 (2H, m, 4- and 5-H), 3.12 (dd, *J* = 10.1, 9.2 Hz, 2-H); ^{13}C NMR (CD_3OD) α-anomer: δ 133.5, 130.1, 128.7, 89.2, 74.9, 74.7, 71.9, 65.4, 62.2; β-anomer: δ 135.4, 133.8, 130.0, 129.0, 87.3, 82.2, 78.5, 71.2, 67.1, 62.7; FABMS *m/z* 320 $(M + Na)^+$.

To a solution of the above triol (548.8 mg, 1.85 mmol) in DMF (20 mL) was added 60% w/v NaH suspension in mineral oil (398.7 mg, 10.4 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min, and benzyl bromide (1.08 mL, 8.41 mmol) and tetra-*n*-butylammonium iodide (141.4 mg, 0.38 mmol) were added to the solution. The reaction mixture was further stirred at room temperature for 4 h. The reaction was quenched with MeOH (5 mL), and the mixture was diluted with EtOAc (200 mL), washed with H_2O and then brine. Evaporation of the solvent and purification by silica gel column chromatography (10% EtOAc in hexane) gave the tribenzyl ether (932.6 mg, 89% as the anomeric mixture of α:β, 4:1) as a white solid: R_f 0.2 (10% EtOAc in hexane); $[\alpha]^{25}_D$ +80.5° (*c* 1.39; $CHCl_3$); IR (thin film) 3032, 2891, 2110, 1105 cm^{-1}; ^1H NMR ($CDCl_3$) α-anomer: δ 7.58–7.15 (15H, m, 3 × Ph), 4.91–4.51 (6H, m, 3 × CH_2Ph), 5.59 (d, *J* = 5.5 Hz, 1-H), 4.34 (ddd, *J* = 10.0, 5.0, 2.0 Hz, 5-H), 3.93 (dd, *J* = 10.2, 5.0 Hz, 6-Ha), 3.82–3.72 (3H, m, 2-, 3- and 4-H), 3.61 (dd, *J* = 10.2, 2.0 Hz, 6-Hb); β-anomer: δ 7.58–7.15 (15H, m, 3 × Ph), 4.91–4.51 (6H, m, 3 × CH_2Ph), 4.39 (d, *J* = 10.0 Hz, 1-H), 3.75–3.70 (2H, m, 6-H_2), 3.59 (dd, *J* = 9.5, 9.0 Hz, 4-H), 3.49 (dd, *J* = 9.5, 9.0 Hz, 3-H), 3.45 (m, 5-H), 3.32 (dd, *J* = 10.0, 9.5 Hz, 2-H); ^{13}C NMR ($CDCl_3$) α-anomer; δ 137.8, 137.78, 137.67, 133.6, 132.1, 129.0, 128.49, 128.45, 128.4, 128.1, 127.8, 127.7, 87.3, 81.8, 78.3, 75.7, 75.1, 73.4, 71.8, 68.3, 64.1; β-anomer: δ 137.8, 137.78, 137.67, 133.5, 129.0, 128.4, 128.2, 127.9, 127.7, 127.61, 127.59, 127.56, 85.9, 85.1, 79.3, 77.5, 75.9, 75.0, 68.7, 65.1; FABMS *m/z* 590 $(M + Na)^+$.

To a solution of the above thioglycoside tribenzyl ether (992.1 mg, 1.75 mmol) in CH_2Cl_2 (8 mL) was added mCPBA (332.2 mg, 1.93 mmol) at −78 °C. The mixture was allowed to warm up to 0 °C and then neutralized with saturated aqueous $NaHCO_3$. The mixture was diluted with CH_2Cl_2 (120 mL) and washed with saturated aqueous $NaHCO_3$ and then brine. Drying over $MgSO_4$, evaporation of the solvent, and purification by silica gel column chromatography (3–25% EtOAc in benzene) gave sulfoxide **10** (849.2 mg, 83% as the anomeric and diastereomeric sulfoxide mixture; α:β, 4:1) as a colorless syrup: R_f 0.2 (10% EtOAc in benzene); $[\alpha]^{28}_D$ −38.8° (*c* 1.07; $CHCl_3$); IR (thin film) 3030, 2868, 2112, 1099 cm^{-1}; ^1H NMR ($CDCl_3$) the major diastereomer of α-anomers; δ 7.73–7.15 (15H, m, 3 × Ph), 4.81 (d, *J* = 11.0 Hz, CHPh), 4.73 (d, *J* = 11.0 Hz, CHPh), 4.68 (d, *J* = 11.3 Hz, CHPh), 4.62 (d, *J* = 4.3 Hz, 1-H), 4.47 (d, *J* = 11.3 Hz, CHPh), 4.40 (d, *J* = 11.9 Hz, CHPh), 4.31 (d, *J* = 11.9 Hz, CHPh), 4.25–4.21 (2H, m, 2- and 3-H), 4.04 (ddd, *J* = 8.9, 4.6, 2.5 Hz, 5-H), 3.65 (dd, *J* = 8.9, 5.8 Hz, 4-H), 3.51 (dd, *J* = 10.8, 4.6 Hz, 6-Ha), 3.41 (dd, *J* = 10.8, 2.5 Hz, 6-Hb); ^{13}C NMR ($CDCl_3$) the major diastereomer of α-anomers; δ 141.5, 137.6, 137.55, 137.45, 137.2, 128.9, 128.5, 128.1, 125.3, 93.9, 79.1, 76.3, 76.0, 74.3, 74.0, 73.3, 68.3, 60.9; FABMS *m/z* 606 $(M + Na)^+$.

(25*R*)-7α-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)-3α-*tert*-butyldiphenylsilyloxy-5β-cholestan-26-yl methyl carbonate (13). Sulfoxide **10** (84.5 mg, 0.14 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (33.4 mg, 0.16 mmol) were azeotropically dried with toluene, dissolved in CH_2Cl_2 (7 mL), and a triflic anhydride (0.025 mL, 0.15 mmol) was added at −78 °C. The mixture was left to stand at the same temperature for 5 min. To this mixture, a solution of alcohol **9** (84.1 mg, 0.12 mmol) in CH_2Cl_2 (6 mL) was added. The reaction mixture was allowed to warm up to −20 °C and then the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was diluted with EtOAc (140 mL) and washed with brine. Evaporation of the solvent and purification by silica gel column chromatography (5–10% EtOAc in hexane) gave β-glycoside **13** (134.0 mg, 97%) as a colorless oil: R_f 0.7 (10% EtOAc in hexane); $[\alpha]^{23}_D$ +1.66° (*c* 1.03; $CHCl_3$); IR (thin film) 2931, 2862, 2114, 1743, 1450, 1365, 1265, 1103 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72–7.21 (25H, m, 5 × Ph), 4.92–4.52 (6H, m, 3 × CH_2Ph), 4.40 (d, *J* = 7.5 Hz, 1′-H), 4.01 (dd, *J* = 9.2, 6.5 Hz, 26-Ha), 3.99 (brd, *J* = 3.0 Hz, 7-H), 3.92 (dd, *J* = 9.2, 6.0 Hz, 26-Hb), 3.76 (3H, s, CO_2Me), 3.73–3.65 (2H, m, 6′-H_2), 3.63 (t, *J* = 10.0 Hz, 4′-H), 3.49–3.37 (4H, m, 3-, 2′-, 3′-, and 5′-H), 1.04 (9H, s, *t*-Bu), 0.93 (3H, d, *J* = 7.0 Hz, 25-Me), 0.90 (3H, d, *J* = 6.0 Hz, 20-Me), 0.80 (3H, s, 10-Me), 0.61 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 156.0, 138.4, 138.2, 138.1, 135.95, 135.90, 135.8, 135.1, 135.0, 129.4, 129.31, 129.27, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 97.1, 83.3, 77.9, 75.39, 75.36, 75.0, 73.5, 73.3, 73.22, 73.20, 73.17, 71.5, 68.9, 67.4, 56.0, 54.6, 49.8, 42.3, 41.2, 39.5, 39.0, 38.4, 36.0, 35.7, 35.3, 35.0, 33.5, 33.1, 32.7, 33.6, 28.3, 27.9, 27.0, 26.9, 23.24, 23.21, 22.7, 20.7, 19.1, 18.6, 16.6, 11.7; HRMS (FAB) *m/z* calcd for $C_{72}H_{95}O_9N_3SiNa$ $(M + Na)^+$ 1196.6735, found 1196.6719.

(25*R*)-7α-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)-3α-hydroxy-5β-cholestan-26-yl methyl carbonate (14). To a solution of silyl ether **13** (431.7 mg, 0.37 mmol) in MeCN (30 mL) was added 46% aqueous HF (1 mL, 23 mmol) dropwise at 0 °C. The solution was left to stand at room temperature for 3.5 h and the reaction was quenched with saturated aqueous $NaHCO_3$ at 0 °C. The mixture was extracted with $CHCl_3$ (250 mL), concentrated, and purified by flash column chromatography (10–40% EtOAc in hexane) to give alcohol **14** (331.9 mg, 96%) as a colorless oil: R_f 0.2 (20% EtOAc in hexane); $[\alpha]^{24}_D$ −16.9° (*c* 0.84; $CHCl_3$); IR (thin film) 3479, 2929, 2112, 1747, 1454, 1360, 1269, 1074 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36–7.19 (15H, m, 3 × Ph), 4.89–4.50 (6H, m, 3 × CH_2Ph), 4.32 (d, *J* = 7.9 Hz, 1′-H), 4.01 (dd, *J* = 10.4, 5.8 Hz, 26-Ha), 3.93–3.62 (3H, m, 26-Hb and 4′-H), 3.76 (3H, s, CO_2Me), 3.73 (3H, m, 4′-H and 6′-H_2), 3.47–3.34 (4H, m, 3-, 2′-, 3′-, and 5′-H), 0.93 (3H, d, *J* = 6.7 Hz, 25-Me), 0.91 (3H, s, 20-Me), 0.90 (3H, d, *J* = 6.7 Hz, 10-Me), 0.63 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 156.0, 138.2, 138.1, 138.0, 128.4, 128.3, 127.90, 127.88, 127.79, 127.74, 127.5, 127.4, 97.7, 83.5, 77.9, 75.5, 75.0, 74.9, 73.6, 73.2, 73.1, 71.7, 68.9, 67.4, 55.8, 54.6, 49.6, 42.5, 41.4, 39.30, 39.26, 39.1, 36.0, 35.7, 35.4, 35.0, 33.5, 32.9, 32.6, 30.5, 29.0, 28.3, 23.2, 23.1, 22.7, 20.6, 18.6, 16.5, 11.7; HRMS (FAB) *m/z* calcd for $C_{56}H_{77}O_9N_3Na$ $(M + Na)^+$ 958.5558, found 958.5562.

(25*R*)-7α-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)-3,3-dimethoxy-5β-cholestan-26-yl methyl carbonate (15). To a solution of alcohol **14** (200.0 mg, 0.21 mmol) in CH_2Cl_2 (20 mL) with molecular sieve 4A was added pyridinium dichromate (248.2 mg, 0.66 mmol) and the mixture was stirred at room temperature for 4.7 h. The reaction mixture was directly purified by florisil column chromatography (100% EtOAc) to afford (25*R*)-7α-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)-3-oxo-5β-cholestan-26-yl methyl carbonate (190.4 mg, 95%) as a colorless oil: R_f 0.5 (30% EtOAc in hexane); $[\alpha]^{26}_D$ −8.9° (*c* 1.24; $CHCl_3$); IR (thin film) 2933, 2866, 2110, 1747, 1714, 1454, 1271, 1059 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36–7.19 (15H, m, 3 × Ph), 4.88–4.50 (6H, m, 3 × CH_2Ph), 4.30 (d, *J* = 7.9 Hz, 1′-H), 4.04–4.00 (2H, m, 7-H and 26-Ha), 3.92 (d, *J* = 10.4, 6.7 Hz, 26-Hb), 3.76 (3H, s, CO_2Me), 3.73–3.63 (3H, m, 4′-H and 6′-H_2), 3.46 (dd, *J* = 9.5, 9.2 Hz, 3′-H), 3.39–3.35 (2H, m, 2′- and 5′-H), 3.27 (dd, *J* = 15.5, 14.3 Hz, 4α-H), 2.44 (dt, *J* = 14.3, 4.9 Hz, 2α-H), 2.21 (brdd, *J* = 15.5, 3.0 Hz, 4β-H), 2.14 (brd, *J* = 14.3 Hz, 2β-H), 1.02 (3H, s, 10-Me), 0.94 (3H, d, *J* = 6.7 Hz, 25-Me), 0.92 (3H, d, *J* = 6.4 Hz, 20-Me), 0.68 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 212.8, 155.9, 138.1, 137.9, 128.4, 128.3, 127.9, 127.82, 127.75, 127.72, 127.5, 127.4, 97.4, 83.4, 77.8, 75.4, 75.0, 74.9, 73.5, 73.1, 72.6, 68.8, 67.0, 55.8, 54.5, 49.5, 44.7, 42.9, 42.5, 39.2, 39.0, 36.8, 36.7, 35.9, 35.6, 35.3, 33.4, 32.6, 28.2, 23.2, 23.0, 21.8, 21.0, 18.6, 16.5, 11.7; HRMS (FAB) *m/z* calcd for $C_{56}H_{77}O_9N_3Na$ $(M + Na)^+$ 956.5401, found 956.5414.

To a solution of the above ketone (240.3 mg, 0.26 mmol) in CH_2Cl_2–MeOH (1:2, 15 mL) were added $CH(OMe)_3$ (1.0 mL, 9.14 mmol) and camphorsulfonic acid (15.5 mg, 0.07 mmol). The mixture was left to stand at room temperature for 2 h and the reaction was quenched with saturated aqueous $NaHCO_3$. The solution was diluted with EtOAc (200 mL) and washed with H_2O and then brine. Evaporation of the solvent and purification by silica gel column chromatography afforded dimethyl ketal **15** (242.0 mg, 96%) as a colorless oil: R_f 0.6 (30% EtOAc in hexane); $[\alpha]^{25}_D$ −9.9° (*c* 1.23; C_6H_6); IR (thin film) 2935, 2110, 1747, 1454, 1271, 1101, 1055 cm^{-1}; ^1H NMR (CD_3CN) δ 7.38–7.23 (15H, m, 3 × Ph), 4.82–4.49 (6H, m, 3 × CH_2Ph), 4.47 (d, *J* = 7.9 Hz, 1′-H), 3.98–3.95 (2H, m, 7-H and 26-Ha), 3.88 (dd, *J* = 10.4, 6.7 Hz, 26-Hb), 3.70 (dd, *J* = 11.0, 4.0 Hz, 6′-Ha), 3.69 (3H, s, CO_2Me), 3.65 (dd, *J* = 11.0, 1.8 Hz, 6′-Hb), 3.56 (dd, *J* = 9.8, 8.9 Hz, 4′-H), 3.49 (dd, *J* = 9.8, 8.9 Hz, 3′-H), 3.42 (ddd, *J* = 9.8, 4.0, 1.8 Hz, 5′-H), 3.30 (dd, *J* = 9.8, 7.9 Hz, 2′-H), 3.13 (3H, s, OMe-a), 3.05 (3H, s, OMe-b), 2.17 (dd, *J* = 14.0, 13.6 Hz, 4α-H), 0.93 (3H, s, 10-Me), 0.91 (3H, d, *J* = 6.4 Hz, 20-Me), 0.89 (3H, d, *J* = 6.7 Hz, 25-Me), 0.65 (3H, s, 13-Me); ^{13}C NMR (CD_3CN) δ 156.8, 139.63, 139.58, 139.4, 129.3, 129.1, 129.0, 128.9, 128.7, 128.6, 128.5, 101.3, 98.0, 84.0, 79.1, 75.8, 75.7, 75.5, 74.1, 73.6, 73.4, 69.8, 68.2, 57.0, 55.2, 50.8, 47.6, 47.5, 43.3, 40.6, 40.0, 39.8, 36.8, 36.6, 36.56, 36.1, 34.2, 33.8, 33.6, 33.4, 29.1, 28.7, 27.9, 24.0, 23.8, 23.2, 21.7, 19.1, 16.8, 12.2; HRMS (FAB) *m/z* calcd for $C_{58}H_{81}O_{10}N_3Na$ $(M + Na)^+$ 1002.5820, found 1002.5816.

(25*R*)-7α-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosaminyloxy)-3,3-dimethoxy-5β-cholestan-26-yl acetate (16). To a suspension of $LiAlH_4$ (77.2 mg, 2.0 mmol) in THF (20 mL) was added a solution of ketal **15** (194.5 mg, 0.20 mmol) in THF (30 mL) at 0 °C and the mixture was let stand at room temperature for 6 h. The reaction was quenched with wet Et_2O at 0 °C and insoluble material was filtered through celite. The filtrate was dried over Na_2SO_4, and evaporated to give the 26-*O*-deacetylated aminoglycoside (R_f 0.15, 50% EtOAc in hexane, stained by dragendorff), which was acetylated as described below without further purification.

To a solution of the above amine, and a catalytic amount of DMAP in pyridine (8 mL), Ac_2O (2 mL, 21 mmol) was added and the mixture was left to stand at room temperature for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$, and the mixture was diluted with EtOAc (200 mL) and washed with H_2O and then brine. Evaporation of the solvent and purification by florisil column chromatography (20–40% EtOAc in hexane) gave acetate **16** (173.1 mg, 89% from **15**) as a colorless oil: R_f 0.5 (50% EtOAc in hexane); $[\alpha]^{24}_D$ +22.5° (*c* 1.22; C_6H_6); IR (thin film) 3288, 2933, 2868, 1739, 1651, 1556, 1454, 1365, 1238, 1099, 1057 cm^{-1}; ^1H NMR (CD_3CN) δ 7.34–7.22 (15H, m, 3 × Ph), 6.40 (d, *J* = 9.2 Hz, NH), 4.75–4.49 (7H, m, 1′-H and 3 × CH_2Ph), 3.88 (dd, *J* = 10.7, 5.8 Hz, 26-Ha), 3.82–3.78 (2H, m, 7-H and 26-Hb), 3.72–3.64 (3H,

m, 2′-H and 6′-H_2), 3.60 (dd, J = 10.1, 8.7 Hz, 3′-H), 3.54 (dd, J = 9.6, 8.7 Hz, 4′-H), 3.40 (ddd, J = 9.6, 4.3, 2.1 Hz, 5′-H), 3.09 (3H, s, OMe-a), 3.04 (3H, s, OMe-b), 1.96 (3H, s, OAc), 1.90 (3H, s, NAc), 0.91 (6H, d, J = 6.4 Hz, 20- and 25-Me), 0.91 (3H, s, 10-Me), 0.64 (3H, s, 13-Me); ^{13}C NMR (CD_3CN) δ 171.7, 170.8, 139.9, 139.70, 139.66, 129.29, 129.26, 129.2, 128.95, 128.88, 128.63, 128.57, 128.4, 101.3, 99.2, 84.3, 79.5, 75.7, 75.6, 75.3, 75.1, 74.1, 70.3, 70.0, 56.9, 56.4, 50.5, 47.6, 47.4, 43.4, 40.3, 40.1, 40.0, 36.8, 36.6, 36.1, 36.0, 34.4, 34.0, 33.3, 33.1, 30.0, 29.2, 28.8, 24.2, 23.9, 23.8, 23.2, 21.6, 21.1, 19.1, 17.0, 12.4; FABMS m/z 1002 $(M + Na)^+$.

(25*R*)-7α-(*N*-acetyl-β-D-glucopyranosaminyloxy)-3-oxo-5β-cholestan-26-yl acetate (5β-dihydropavoninin-1; 3). A solution of the above acetate **16** (169.6 mg, 0.17 mmol) in MeOH (20 mL) was stirred with $Pd(OH)_2$/C (49.8 mg) at room temperature under hydrogen atmosphere for 20 h. Removal of the catalyst by filtration followed by evaporation of the solvent gave the debenzylated glucosaminide, which was dissolved in AcOH:H_2O (5:1, 24 mL). The solution was stirred at room temperature for 2.5 h, and the solvent was evaporated as the azeotrope with toluene. The residue was dissolved in EtOAc (150 mL), and washed with saturated aqueous $NaHCO_3$, H_2O, and then brine. Evaporation of the solvent and purification by flash column chromatography (5–15% MeOH in $CHCl_3$) afforded dihydropavoninin-1 **3** (99.7 mg, 87% from **16**): R_f 0.4 (15% MeOH in $CHCl_3$); $[\alpha]^{24}_D$ +17.4° (*c* 0.86; $CHCl_3$); IR (thin film) 3357, 2931, 2866, 1739, 1712, 1651, 1377, 1240, 1078 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.51 (d, J = 8.2 Hz, 1′-H), 4.04 (brd, J = 1.8 Hz, 7-H), 3.94 (dd, J = 10.7, 6.1 Hz, 26-Ha), 3.88 (dd, J = 11.6, 2.4 Hz, 6′-Ha), 3.85 (dd, J = 10.7, 6.7 Hz, 26-Hb), 3.76 (dd, J = 11.6, 6.1 Hz, 6′-Hb), 3.62 (dd, J = 10.2, 8.2 Hz, 2′-H), 3.46 (dd, J = 10.2, 8.6 Hz, 3′-H), 3.30 (dd, J = 9.8, 8.6 Hz, 4′-H), 3.26–3.20 (2H, m, 4a- and 5′-H), 2.03 (3H, s, OAc), 1.90 (3H, s, NAc), 1.03 (3H, s, 10-Me), 0.95 (3H, d, J = 6.4 Hz, 20-Me), 0.93 (3H, d, J = 7.0 Hz, 25-Me), 0.64 (3H, s, 13-Me); ^{13}C NMR (CD_3OD) δ 216.0, 173.8, 173.0, 98.6, 77.7, 76.3, 73.8, 72.4, 70.6, 63.2, 57.7, 57.1, 50.4, 49.9, 46.5, 44.7, 43.7, 40.54, 40.50, 38.0, 37.7, 37.2, 37.0, 36.4, 34.9, 34.3, 33.8, 29.7, 29.4, 24.3, 24.2, 23.4, 22.2, 22.1, 20.8, 19.3, 17.1, 12.3; HRMS (FAB) m/z calcd for $C_{37}H_{61}O_9NNa$ $(M+Na)^+$ 686.4244, found 686.4235.

4β-Phenylselenenyl-5β-dihydropavoninin-1 (17). To a solution of dihydropavoninin-1 (**3**; 58.6 mg, 0.088 mmol) in EtOAc (8 mL), freshly distilled from P_2O_5, was added PhSeCl (18.0 mg, 0.094 mmol), and the mixture was left to stand at room temperature for 2 h. The reaction was quenched with H_2O, and the mixture was diluted with EtOAc (100 mL) and washed with H_2O. Evaporation of the solvent and purification by flash column chromatography (3–15% MeOH in $CHCl_3$) gave a selenenyl ketone **17** (20.8 mg, 30%) as a glassy solid: R_f 0.3 (10% MeOH in $CHCl_3$); $[\alpha]^{23}_D$ +21.6° (*c* 0.64; $CHCl_3$); IR (thin film) 3357, 2933, 1732, 1658, 1545, 1375, 1242, 1074 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.56–7.26 (5h, m, Ph), 4.65 (d, J = 7.9 Hz, 1′-H), 4.32 (d, J = 8.9 Hz, 4-H), 4.17 (brdt, J = 4.9, 2.8 Hz, 7-H), 3.95–3.89 (2H, m, 26- and 6′-Ha), 3.84 (dd, J = 10.7, 6.7 Hz, 26-Hb), 3.68 (m, 5′-H), 3.60 (dd, J = 10.4, 7.9 Hz, 2′-H), 3.50 (m, 3′-H), 3.28–3.27 (2H, m, 4′-H and 6′-Hb), 2.02 (3H, s, OAc), 1.95 (3H, s, NAc), 0.94–0.91 (9H, m, 10-, 20-, and 25-Me), 0.67 (3H, s, 13-Me); ^{13}C NMR (CD_3OD) δ 209.6, 174.3, 173.1, 137.6, 130.1, 129.7, 129.3, 128.6, 99.0, 77.9, 75.9, 72.9, 72.6, 70.6, 63.3, 58.2, 57.0, 56.5, 50.4, 48.3, 43.6, 40.6, 40.3, 37.3, 37.2, 37.0, 36.6, 35.5, 35.0, 34.9, 33.8, 29.4, 28.3, 24.4, 24.2, 23.7, 22.9, 22.0, 20.8, 19.3, 17.1, 12.2; HRMS (FAB) m/z calcd for $C_{43}H_{65}O_9NSeNa$ $(M + Na)^+$ 842.3722, found 842.3728.

Pavoninin-1 (1). To a solution of selenenyl ketone **17** (14.9 mg, 0.018 mmol) in THF (3 mL) was added 30% H_2O_2 (0.5 mL, 7.5 mmol) dropwise at 0 °C over 10 min. The mixture was allowed to warm up to room temperature and left to stand for 1 h. The reaction mixture was diluted with EtOAc (40 mL) and washed with H_2O and then brine. Evaporation of the solvent and purification by flash column chromatography (100% EtOAc to 5% MeOH in $CHCl_3$) afforded pavoninin-1 (**1**) (9.9 mg, 82%); R_f 0.4 (15% MeOH in $CHCl_3$); $[\alpha]^{23}_D$ +17.5° (*c* 0.26; $CHCl_3$); ^{1}H NMR (CD_3OD) δ 5.74 (brs, H-4), 4.42 (d, J = 8.2 Hz, 1′-H), 4.02 (brdt, J =4.8, 2.9 Hz, 7-H), 3.97 (dd, J = 10.7, 6.1 Hz, 26-Ha), 3.89–3.83 (2H, m, 26-Hb and 6′-Ha), 3.69 (dd, J = 11.6, 5.8 Hz, 6′-Hb), 3.59 (dd, J = 10.4, 8.2 Hz, 2′-H), 3.44 (dd, J = 10.4, 8.9 Hz, 3′-H), 3.32 (dd, J = 9.8, 8.9 Hz, 4′-H), 3.27–3.23 (m, 5′-H), 2.03 (3H, s, OAc), 1.79 (3H, s, NAc), 1.22 (3H, s, 10-Me), 0.94 (3H, d, J = 7.3 Hz, 25-Me), 0.92 (3H, d, J = 6.7 Hz, 20-Me), 0.73 (3H, s, 13-Me); ^{13}C NMR (CD_3OD) δ 202.0, 173.5, 173.4, 173.1, 126.7, 99.1, 77.7, 75.9, 74.0, 72.3, 70.6, 63.2, 57.23, 57.21, 50.6, 46.2, 43.4, 40.7, 40.4, 39.7, 37.3, 37.2, 37.0, 36.5, 34.85, 34.77, 33.8, 29.3, 24.22, 24.18, 23.6, 21.9, 20.8, 19.2, 17.4, 17.1, 12.2; HRMS (FAB) m/z calcd for $C_{37}H_{59}O_9NNa$ $(M + Na)^+$ 684.4088, found 684.4058.

(25*R*)-26-hydroxy-5β-cholestane-3α,7α-diyl diacetate (18). A solution of *Z*-olefin **7** (421.9 mg, 0.71 mmol) in EtOAc–MeOH (2:3, 20 mL) was stirred with $Pd(OH)_2$/C (72.0 mg) at room temperature under hydrogen atmosphere for 2 days. The catalyst was removed by filtration and the solvent was evaporated. The residue was purified by flash column chromatography (10–40% EtOAc in hexane) to give alcohol **18** (351.6 mg, 98%) as a colorless oil: R_f 0.2 (20% EtOAc in hexane); $[\alpha]^{27}_D$ +18.0° (*c* 1.01; $CHCl_3$); IR (thin film) 3463, 2937, 2870, 1738, 1732, 1466, 1439 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.84 (brdt, J = 5.5, 3.0 Hz, 7-H), 4.55 (m, 3-H), 3.46 (dd, J = 10.5, 6.0 Hz, 26-Ha), 3.39 (dd, J = 10.5, 6.5 Hz, 26-Hb), 2.02 (3H, s, OAc), 2.00 (3H, s, OAc), 0.89 (3H, d, J = 6.0 Hz, 20-Me), 0.88 (3H, d, J = 6.5 Hz, 25-Me), 0.87 (3H, s, 10-Me), 0.61 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.4, 74.2, 71.3, 68.5, 56.1, 50.4, 42.6, 40.9, 39.5, 37.9, 36.1, 35.8, 35.7, 34.9, 34.8, 34.6,

34.0, 33.5, 31.3, 28.1, 26.8, 23.5, 23.4, 22.7, 21.6, 21.4, 20.6, 18.6, 16.5, 11.7; FABMS *m/z* 527 $(M + Na)^+$.

(25*R*)-26-*tert*-butyldiphenylsiloxy-3α-hydroxy-5β-cholestan-7α-yl acetate (19). To a solution of alcohol **18** (227.7 mg, 0.45 mmol) and imidazole (76.0 mg, 1.12 mmol) in CH_2Cl_2 (20 mL) *tert*-butyldiphenylsilyl chloride (0.30 mL, 1.15 mmol) was added and the mixture was left to stand at room temperature for 24 h. The reaction was quenched with H_2O, and the mixture was diluted with EtOAc (200 mL) and washed with 1 M HCl, saturated aqueous $NaHCO_3$, H_2O, and then brine. Evaporation of the solvent and purification by silica gel column chromatography (10% EtOAc in hexane) gave crude silyl ether with an inseparable minor component (655.2 mg), which was used without further purification: R_f 0.6 (10% EtOAc in hexane); ^1H NMR ($CDCl_3$) δ 7.66–7.34 (10H, m, 2 × Ph), 4.87 (brdt, *J* = 5.8, 3.4 Hz, 7-H), 4.58 (m, 3-H), 3.48 (dd, *J* = 9.8, 5.8 Hz, 26-Ha), 3.42 (dd, *J* = 9.8, 6.4 Hz, 26-Hb), 2.02 (3H, s, OAc), 2.01 (3H, s, OAc), 1.03 (9H, s, *t*-Bu), 0.92 (3H, d, *J* = 6.7 Hz, 25-Me), 0.90 (3H, d, *J* = 6.7 Hz, 20-Me), 0.88 (3H, s, 10-Me), 0.63 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.4, 135.6, 134.1, 129.4, 127.5, 74.2, 71.3, 69.0, 56.1, 50.4, 42.6, 41.0, 39.5, 37.9, 36.1, 35.7, 35.6, 34.9, 34.8, 34.6, 34.1, 33.5, 31.3, 28.1, 26.8, 23.6, 23.4, 22.7, 21.6, 21.4, 20.6, 19.3, 18.6, 16.8, 11.7; FABMS *m/z* 765 $(M + Na)^+$.

To a solution of the above crude silyl ether (655.2 mg) in MeOH (30 mL) NaOMe (1 M solution in MeOH, 5 mL) was added. The reaction mixture was left to stand at room temperature for 23 h, and the reaction was quenched with saturated aqueous NH_4Cl. The solution was diluted with EtOAc (200 mL) and washed with H_2O and then brine. Evaporation of the solvent and purification by silica gel column chromatography (5–50% EtOAc in hexane) gave alcohol **19** (283.9 mg, 90% from **18**) as a colorless oil: R_f 0.1 (10% EtOAc in hexane); $[\alpha]^{25}_D$ +4.0° (*c* 1.14; $CHCl_3$); IR (thin film) 3415, 2933, 2860, 1730, 1466, 1433, 1378, 1155, 1109, 1076, 1018 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66–7.34 (10H, m, 2 × Ph), 4.86 (brdt, *J* = 5.5, 3.0 Hz, 7-H), 3.51–3.45 (2H, m, 3-H and 26-Ha), 3.42 (dd, *J* = 10.0, 6.5 Hz, 26-Hb), 2.03 (3H, s, OAc), 1.03 (9H, s, *t*-Bu), 0.91 (3H, s, 10-Me), 0.89 (3H, d, *J* = 7.0 Hz, 25-Me), 0.88 (3H, d, *J* = 7.0 Hz, 20-Me), 0.62 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 170.6, 135.6, 134.1, 129.4, 127.5, 71.8, 71.4, 69.0, 56.1, 50.4, 42.6, 41.1, 39.5, 38.9, 37.9, 36.1, 35.7, 35.6, 35.2, 34.7, 34.1, 33.5, 31.4, 30.6, 28.1, 26.8, 23.6, 23.4, 22.7, 21.6, 20.6, 19.3, 18.6, 16.8, 11.7; FABMS *m/z* 723 $(M + Na)^+$.

(25*R*)-26-*tert*-butyldiphenylsilyloxy-3α-(2-azido-3,4,-6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)-5β-cholestan-7α-yl acetate (20). Sulfoxide **10** (82.3 mg, 0.14 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (50.4 mg, 0.25 mmol) were azeotropically dried with toluene, and dissolved in CH_2Cl_2 (7 mL). To this solution triflic anhydride (0.03 mL, 0.18 mmol) was added at −78 °C and left to stand at −78 °C for 5 min, then a solution of alcohol **19** (76.1 mg, 0.11 mmol) in CH_2Cl_2 (7 mL) was added. The reaction mixture was left to stand at the same temperature for 30 min and the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was diluted with EtOAc (120 mL) and washed with H_2O, aqueous NH_4Cl, and then brine. Evaporation of the solvent and purification by flash column chromatography (10–30% EtOAc in hexane) gave β-glycoside **20** (88.8 mg, 70%) as a colorless oil: R_f 0.1 (10% EtOAc in hexane); $[\alpha]^{27}_D$ −0.88° (*c* 1.01; $CHCl_3$); IR (thin film) 2933, 2860, 2110, 1732, 1454, 1248, 1111, 1026 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68–7.16 (25H, m, 5 × Ph), 4.90–4.51 (7H, m, 7-H and 3 × CH_2Ph), 4.39 (d, *J* = 7.5 Hz, 1′-H), 3.71 (dd, *J* = 10.7, 1.5 Hz, 6′-Ha), 3.67 (dd, *J* = 10.7, 5.0 Hz, 6′-Hb), 3.61–3.56 (2H, m, 3- and 4′-H), 3.49 (dd, *J* = 10.0, 6.0 Hz, H-26a), 3.46–3.37 (4H, m, 26-Hb, 2′-, 3′-, and 5′-H), 2.02 (3H, s, OAc), 1.06 (9H, s, *t*-Bu), 0.92–0.89 (9H, m, 10-, 20-, and 25-Me), 0.64 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 170.8, 138.0, 137.9, 135.6, 134.1, 129.4, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.5, 100.1, 83.1, 79.3, 77.7, 75.4, 75.0, 74.9, 73.4, 71.2, 69.0, 68.8, 66.3, 56.0, 50.3, 42.6, 41.2, 39.5, 37.9, 36.7, 36.1, 35.7, 34.9, 34.0, 33.5, 31.4, 28.1, 26.9, 23.6, 23.3, 22.8, 21.6, 20.7, 19.3, 18.6, 16.8, 11.7; FABMS *m/z* 1181 $(M + Na)^+$.

(25*R*)-26-*tert*-butyldiphenylsilyloxy-7α-hydroxy-5β-cholestan-3α-yl *N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosaminide (21). To a solution of azidoglycoside **20** (88.8 mg, 0.076 mmol) in THF (7 mL) $LiAlH_4$ (37.8 mg, 1.0 mmol) was added at 0 °C and the mixture was stirred at room temperature for 4.5 h. The reaction was quenched with EtOAc and the insoluble material was filtered through celite. The filtrate was concentrated to give the 7-*O*-deacetylated aminoglycoside (R_f 0.5, 50% EtOAc in hexane), which was acetylated as described below without further purification.

To a solution of the above aminoglycoside in MeOH–CH_2Cl_2 (1:1, 10 mL) were added Ac_2O (0.2 mL, 2.1 mmol) and pyridine (0.5 mL), and the mixture was left to stand at room temperature for 1 h. The reaction mixture was diluted with EtOAc (160 mL) and washed with H_2O, 1M HCl, and then brine. Evaporation of the solvent and purification by flash column chromatography (10–30% EtOAc in hexane) gave alcohol **21** (46.2 mg, 54% from **20**) as a colorless oil: R_f 0.6 (50% AcOEt in hexane); $[\alpha]^{26}_D$ +11.1° (*c* 0.66; $CHCl_3$); IR (thin film) 3502, 2930, 2859, 1652, 1454, 1372, 1112, 1074, 1028 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66–7.18 (25H, m, 5 × Ph), 5.62 (d, *J* = 7.0 Hz, NH), 5.05 (d, *J* = 8.0 Hz, 1′-H), 4.82–4.53 (6H, m, 3 × CH_2Ph), 4.31 (dd, *J* = 10.0, 8.5 Hz, 3′-H), 3.79 (brd, *J* = 2.5 Hz, 7-H), 3.74 (dd, *J* = 10.5, 2.0 Hz, 6′-Ha), 3.66 (dd, *J* = 10.5, 4.5 Hz, 6′-Hb), 3.59–3.40 (5H, m, 3-, 4′-, 5′-H, and 26-H_2), 3.10 (ddd, *J* = 10.1, 8.0, 7.0 Hz, 2′-H), 1.84 (3H, s, NAc), 1.04 (9H, s, *t*-Bu), 0.89 (3H, d, *J* = 6.5 Hz, 25-Me), 0.87 (3H, d, *J* = 6.5 Hz, 20-Me), 0.86 (3H, s, 10-Me), 0.62 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 170.4, 138.6, 138.3, 138.1, 135.6, 134.2, 129.4, 128.43, 128.37, 128.3, 127.9, 127.8,

127.7, 127.5, 97.4, 80.2, 79.1, 78.9, 74.8, 74.7, 74.6, 73.4, 69.2, 69.0, 68.4, 58.5, 56.1, 50.4, 42.6, 41.5, 39.7, 39.4, 37.4, 36.1, 35.70, 35.68, 35.2, 35.1, 34.7, 33.5, 32.8, 28.3, 27.0, 26.9, 23.71, 23.67, 23.3, 22.8, 20.6, 19.3, 18.6, 16.8, 11.7; FABMS *m/z* 1154 $(M + Na)^+$.

(25*R*)-3α-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosaminyloxy)-7-oxo-5β-cholestan-26-yl acetate (22). To a solution of alcohol **21** (46.2 mg, 0.041 mmol) in CH_2Cl_2 (7 mL) with molecular sieve 4A pyridinium dichromate (56.3 mg, 0.15 mmol) was added and the mixture was stirred at room temperature for 1 h. The supernatant of the reaction mixture was directly applied on a silica gel column and purified by the column chromatography (50% EtOAc in hexane) to afford (25*R*)-26-*tert*-butyldiphenylsilyloxy-3α-(*N*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosaminyloxy)-5β-cholestan-7-one (37.4 mg, 81%) as a colorless oil: R_f 0.8 (50% EtOAc in hexane); $[\alpha]^{26}_D$ +0.94° (*c* 0.48; $CHCl_3$); IR (thin film) 3230, 2933, 2862, 1709, 1651, 1454, 1454, 1211, 1113, 1072, 1007 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55–7.07 (25H, m, 5 × Ph), 5.48 (d, *J* = 7.0 Hz, NH), 4.95 (d, *J* = 8.0 Hz, 1′-H), 4.70 (d, *J* = 11.0 Hz, CHPh), 4.66 (d, *J* = 11.0 Hz, CHPh), 4.53–4.41 (4H, m, 2 × CH_2Ph), 4.14 (dd, *J* = 10.0, 8.5 Hz, 3′-H), 3.62 (brd, *J* = 10.5 Hz, 6′-Ha), 3.55 (dd, *J* = 10.5, 4.5 Hz, 6′-Hb), 3.59-3.40 (5H, m, 3-, 4′-, 5′-H, and 26-H_2), 3.10 (ddd, *J* = 10.0, 8.0, 7.0 Hz, 2′-H), 1.84 (3H, s, NAc), 1.04 (9H, s, *t*-Bu), 0.89 (3H, d, *J* = 6.5 Hz, 25-Me), 0.87 (3H, d, *J* = 6.5 Hz, 20-Me), 0.86 (3H, s, 10-Me), 0.62 (3H, s, 13-Me); ^{13}C NMR ($CDCl_3$) δ 211.6, 170.4, 138.5, 138.3, 138.1, 135.6, 134.1, 129.4, 128.44, 128.39, 128.3, 127.8, 127.7, 127.5, 97.4, 80.2, 79.1, 78.9, 74.8, 74.7, 74.6, 73.4, 69.2, 69.0, 68.4, 58.5, 56.1, 50.4, 42.6, 41.5, 39.7, 39.4, 37.4, 36.1, 35.70, 35.68, 35.2, 35.1, 34.7, 33.5, 32.8, 28.3, 27.0, 26.9, 23.71, 23.67, 23.3, 22.8, 20.6, 19.3, 18.6, 16.8, 11.7; FABMS *m/z* 1152 $(M + Na)^+$.

To a solution of the above ketone silyl ether (62.8 mg, 0.056 mmol) in THF (7 mL) tetra-*n*-butylammonium fluoride (1M in THF, 0.18 mL, 0.18 mmol) was added at room temperature. The solution was left to stand at room temperature for 15.5 h and the reaction was quenched with H_2O. The mixture was diluted with EtOAc (80 mL) and washed with saturated aqueous NH_4Cl, $NaHCO_3$, H_2O, and then brine. Evaporation of the solvent gave the crude desilylated alcohol.

To a solution of the above crude alcohol and a catalytic amount of DMAP in pyridine-CH_2Cl_2 (7 mL) Ac_2O (0.7 mL) was added, and the mixture was left to stand at room temperature for 24 h. The solution was diluted with EtOAc (100 mL) and washed with 1M HCl, saturated aqueous $NaHCO_3$, H_2O, and then brine. Evaporation of the solvent and purification by flash column chromatography (10–40% EtOAc in hexane) gave acetate **22** (45.5 mg, 88% from the siloxyketone) as a colorless oil: R_f 0.7 (50% EtOAc in hexane); $[\alpha]^{25}_D$ +1.2° (*c* 0.69; $CHCl_3$); IR (thin film) 3278, 2935, 2860, 1738, 1711, 1651, 1454, 1238, 1119, 1072 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65–7.03 (15H, m, 3 × Ph), 5.60 (d, *J* = 7.5 Hz, NH), 5.04 (d, *J* = 8.0 Hz, 1′-H), 4.80 (d, *J* = 11.0 Hz, CHPh), 4.76 (d, *J* = 11.0 Hz, CHPh), 4.63–4.51 (4H, m, 2 × CH_2Ph), 4.24 (dd, *J* = 10.0, 8.0 Hz, 3′-H), 3.92 (dd, *J* = 10.2, 6.0 Hz, 26-Ha), 3.82 (dd, *J* = 10.2, 6.5 Hz, 26-Hb), 3.72 (dd, *J* = 10.5, 1.5 Hz, 6′-Ha), 3.65 (dd, *J* = 10.5, 4.5 Hz, 6′-Hb), 3.59–3.51 (3H, m, 3-, 4′-, and 5′-H), 3.09 (ddd, *J* = 10.0, 8.0, 7.5 Hz, 2′-H) , 2.04 (3H, s, OAc), 1.81 (3H, s, NAc), 1.14 (3H, s, 10-Me), 0.89 (3H, d, *J* = 6.5 Hz, 20-Me), 0.88 (3H, d, *J* = 6.5 Hz, 25-Me), 0.62 (3H, s, 13-Me) ; ^{13}C NMR ($CDCl_3$) δ 211.6, 171.3, 170.4, 138.5, 138.2, 138.1, 128.43, 128.38, 128.33, 127.84, 127.81, 127.7, 127.5, 97.5, 80.0, 78.9, 77.6, 74.7, 74.5, 73.4, 69.6, 69.1, 58.2, 55.0, 49.4, 48.9, 46.0, 45.3, 42.6, 42.4, 38.9, 36.0, 35.6, 35.2, 33.9, 33.7, 32.4, 28.4, 26.4, 24.9, 23.6, 23.2, 23.0, 21.6, 21.0, 18.7, 16.7, 12.0 ; FABMS *m/z* 956 $(M + Na)^+$.

(25*R*)-3α-(*N*-acetyl-β-D-glucopyranosaminyloxy)-7-oxo-5β-cholestan-26-yl acetate (2). A solution of acetate 22 (32.6 mg, 0.035 mmol) in EtOH (5 mL) was stirred with $Pd(OH)_2/C$ (12.8 mg) at room temperature under hydrogen atmosphere for 2 days. The catalyst was removed by filtration and the solvent removed by evaporation, followed by purification by flash column chromatography (7–20% MeOH in $CHCl_3$) gave deprotected glycoside **2** (20.2 mg, 87%) as a colorless oil: R_f 0.2 (10% MeOH in $CHCl_3$); $[\alpha]^{27}_D$ −36.9° (*c* 0.36; $CHCl_3$); IR (thin film) 3390, 2935, 2866, 1739, 1709, 1647, 1458, 1238, 1155, 1074 cm^{-1}; ^1H NMR (CD_3OD) δ 4.52 (d, *J* = 8.0 Hz, 1′-H), 3.93 (dd, *J* = 10.5, 6.0 Hz, 26-Ha), 3.87–3.83 (2H, m, 26-Hb and 6′-Ha), 3.67–3.60 (2H, m, 3-H and 6′-Hb), 3.52 (dd, *J* = 10.0, 8.0 Hz, 2′-H), 3.46, (dd, *J* = 10.0, 8.5 Hz, 3′-H), 3.27 (dd, *J* = 10.0, 8.0 Hz, 4′-H), 3.23 (m, 5′-H), 2.02 (3H, s, OAc), 1.95 (3H, s, NAc), 1.21 (3H, s, 10-Me), 0.94 (3H, d, *J* = 7.0 Hz, 25-Me), 0.92 (3H, d, *J* = 6.5 Hz, 20-Me), 0.69 (3H, s, 13-Me); ^{13}C NMR (CD_3OD) δ 215.0, 173.7, 173.1, 101.1, 79.3, 77.9, 75.9, 72.2, 70.6, 62.9, 57.8, 56.5, 50.6, 50.4, 47.5, 46.3, 44.3, 43.8, 40.4, 37.2, 36.9, 36.42, 36.35, 34.9, 34.8, 33.7, 29.5, 27.7, 25.8, 24.3, 23.4, 23.0, 22.8, 20.8, 19.2, 17.1, 12.5; HRMS (FAB) *m/z* calcd for $C_{37}H_{61}O_9NNa$ $(M + Na)^+$ 686.4244, found 686.4257.

Liposome assay

Materials and instruments. Water, distilled once, was used for preparation of the buffer solutions. Egg-yolk phosphatidylcholine (egg PC) and cholesterol were purchased from Sigma and Kanto Chemical Co., Inc., respectively. Phospholipid B-Test Wako and 3,3′-bis[*N*,*N*-di(carboxymethyl)aminomethyl]fluorescein (calcein) were from Wako Pure Chemical Industries, Ltd. Polyethylene glycol mono-*p*-octylphenyl ether (Triton X-100) was from Tokyo Chemical Industry Co., Ltd.

Ultra-violet absorption was measured with a Hitachi U-3500 instrument. Fluorescence was recorded on a Hitachi 650-60 spectrophotometer.

Preparation of liposomes. Egg-yolk phosphatidylcholine (20 mg) was dissolved in 5 mL of redistilled $CHCl_3$. After removal of the solvent at 30 °C and then with a vacuum pump for 2 h, the filmy residue was hydrated with 10 mM Tris–HCl buffer containing 60 mM calcein (pH 7.0). This was then vortexed for 10 min, forming multilamellar vesicles (MLV). The suspension of MLV was continuously sonicated in the ice bath for 30 min to form SUV. The calcein-entrapping SUV were isolated from the outer calcein solution and residual MLV on a Sepharose 4B (Pharmacia-LKB) column with 10 mM Tris–HCl/150 mM NaCl buffer (pH 7.0). The concentration of the phospholipid forming SUV was then determined by choline oxidase and colorimetry with phenol at 505 nm using the phospholipid B-Test, Wako. Liposomes containing cholesterol were similarly prepared.

Leakage measurement. A liposome solution diluted with the above Tris–HCl/NaCl buffer to 1–2 mL was put in a cuvette and placed in a fluorescence spectrophotometer. A substrate solution in 10–20 μL 50% aqueous *n*-PrOH was added to this with stirring, to result in the release of trapped-calcein. The fluorescence change was observed at excitation and emission wavelengths of 490 and 520 nm, respectively. The fluorescence intensity corresponding to 100% leakage was obtained by adding a drop of 10% (v/v) Triton X-100 aqueous solution in each leakage experiment. The liposomal permeability was expressed in terms of the increase of fluorescence by leaked calcein according to the following equation:

$$I\ (\%) = 100\ (I_x - I_0)/(I_{100} - I_0)$$

where I_{100} refers to the fluorescence intensity at the 100% release, and I_0 and I_x represent the fluorescence intensities before and after incubation with a test sample, respectively. No appreciable leakage of calcein was observed when the liposomes were incubated with 1% *n*-PrOH, the largest amount used in the experiments. All the measurements were carried out at 25 °C.

Ichthyotoxicity

A solution of a weighed sample in *n*-PrOH (60 μL) was added in the 5 mM Tris–HCl buffer solution (30 mL), where two individuals of killifish were transferred. No effect was observed on the fish exposed to 0.5% *n*-PrOH in the buffer, or in a solution of **2** at a final concentration of 33 μg/mL for 12 h. In a solution of **3** at 17 μg/mL, an individual died in 50 min, and another in 92 min. In that of 8.5 μg/mL, one died in 120 min and another in 12 h.

Acknowledgements

The authors are grateful to Dr M. Sasaki, Mr Y. Fujimori, and Ms C. Kaise, of this laboratory, for discussion and preliminary experiments in the synthesis of pavoninin-1. This work was supported by the Grant-in-Aid for Scientific Research on a Priority Area, 'Natural Supramolecules', No. 06240101 from the Ministry of Education, Science, Sports, and Culture, Japan.

References

1. (a) Tachibana, K.; Sakaitani, M.; Nakanishi, K. *Science* **1984**, *226*, 703–705. (b) Tachibana, K.; Sakaitani, M.; Nakanishi, K. *Tetrahedron* **1985**, *41*, 1027–1037.

2. Tachibana, K.; Gruber, S. H. *Toxicon* **1988**, *26*, 839–853.

3. Thompson, S. A.; Tachibana, K.; Nakanishi, K.; Kubota, I. *Science* **1986**, *233*, 341–343.

4. Tachibana, K. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer: Berlin, 1988; Vol. 2, pp. 117–138.

5. Gruber, S. H.; Zlotkin, E.; Nelson, D. R. In *Toxins, Drugs, and Pollutants in Marine Animals*; Bolis, L.; Zadunaisky, J.; Gilles, R., Eds.; Springer: Berlin, 1984; pp. 26–42.

6. Kim, H.-S.; Kim, I.-C.; Lee, S.-O. *Tetrahedron* **1997**, *53*, 8129–8136.

7. Gargiulo, D.; Blizzard, T. A.; Nakanishi, K. *Tetrahedron* **1989**, *45*, 5423–5432.

8. Kahne, D.; Walker, S.; Cheng, Y.; Engen, D. V. *J. Am. Chem. Soc.* **1989**, *111*, 6881–6882.

9. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984**, 1826–1847.

10. Assignment of β-configuration at the anomeric position is based on the *J* value for the 1′-H doublet signal of 7.5 Hz, whereas the typical values for 2-azido-2-deoxy-α-glucopyranosides are around 5 Hz (unpublished results from this laboratory).

11. Crich, D.; Sun, S. *J. Org. Chem.* **1997**, *62*, 1198–1199.

12. Sharpless, K. B.; Lauer, R. F.; Teranishi, A. Y. *J. Am. Chem. Soc.* **1973**, *95*, 6137–6139.

13. Phenylselenenylation of **3** gave mixture of 17, 2-selenenylketone, 1,4-diselenenylketone and unreacted **3** in the ratio of ca. 37:26:5:32 based on the ^{1}H NMR spectrum of the mixture. They were separable by silica gel column chromatography.

14. Katu, T.; Kobayashi, H.; Hirota, T.; Fujita, Y.; Sato, K.; Nagai, U. *Biochim. Biophys. Acta* **1987**, *899*, 159–170.

15. Barrow, C. J.; Nakanishi, K.; Tachibana, K. *Biochim. Biophys. Acta* **1992**, *1112*, 235–240.

16. Matsuzaki, K.; Nakai, S.; Handa, T.; Takaishi, Y.; Fujita, T.; Miyajima, K. *Biochemistry* **1989**, *28*, 9392–9398.

17. Matsuzaki, K.; Harada, M.; Funakoshi, S.; Fujii, N.; Miyajima, K. *Biochim. Biophys. Acta* **1991**, *1063*, 162–170.

18. Konoki, K.; Sasaki, M.; Tachibana, K. In *Peptide Chemistry* **1992**; Yanaihara, N., Ed., ESCOM: Leiden, 1993, pp. 402–405.

19. Hu, M.; Konoki, K.; Tachibana, K. *Biochim. Biophys. Acta* **1996**, *1299*, 252–258.

20. Matsuzaki, K.; Murase, O.; Tokuda, H.; Funakoshi, S.; Fujii, N.; Miyajima, K. *Biochemistry* **1994**, *33*, 3342–3349.

(*Received in Japan* 3 *March* 1997; *accepted* 21 *August* 1997)