Analysis of Conjugated Bile Acids in Human Biological Fluids. Synthesis of Hyodeoxycholic Acid 3- and 6-Glycosides and Related Compounds[1)]

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The glucuronide, glucoside and *N*-acetylglucosaminide conjugates of hyodeoxycholic acid were synthesized. In addition, murideoxycholic acid 3-glycosides and some of their C-5 epimeric analogs were also prepared. The principal reactions used are 1) the Koenigs–Knorr condensation reaction of 3-oxo-6α-hydroxy and 6-oxo-3α-hydroxy esters with an appropriate α-acetohalosugar catalyzed by cadmium carbonate in benzene under reflux, 2) reduction of the resulting bile acid glycoside methyl ester-acetates with *tert*-butylamine–borane complex, and 3) subsequent hydrolysis with aqueous lithium hydroxide.

Keywords hyodeoxycholic acid; murideoxycholic acid; glucuronide; glucoside; *N*-acetylglucosaminide; Koenigs–Knorr condensation reaction

The glycosidic conjugates of bile acids are of considerable interest in view of their biosynthetic and metabolic roles. Bile acid glucuronides, one of the earliest known glycosidic conjugates, have been reported to be present in various human biological fluids.[2-7)] In addition to bile acid glucuronides, glucosides[8-10)] and *N*-acetylglucosaminides[11,12)] have recently been identified as novel bile acid conjugates in human urine.

In addition, a number of 6-hydroxylated bile acids have been characterized in biological samples from patients with hepatobiliary diseases, and in fetuses and newborn infants.[4,5,13)] As far as the 6-hydroxylated bile acids are concerned, Zimniak *et al.*[14,15)] and Marschall *et al.*[16)] have investigated the *in vitro* glucuronidation of hyodeoxycholic acid (HDCA; 3α,6α-dihydroxy-5β-cholan-24-oic acid) with microsomes from human and rat liver, demonstrating the formation of HDCA 6-glucuronide. In more recent work, Radominska *et al.*[17)] have reported that HDCA is also glucosidated at the 6-position in human liver microsomes. In common bile acids, without a 6-hydroxyl group, such as chenodeoxycholic and cholic acids, the glycosyl moieties are attached to C-3.[5,14,18)]

The above findings indicate the existence of a new metabolic pathway, namely 6-glycosidation in humans. A particular interest in this topic prompted us to prepare authentic specimens of the glycosidic conjugates of HDCA. This paper describes the synthesis of the glucuronide, glucoside and *N*-acetylglucosaminide conjugates of HDCA (**1**G_1—G_3, **2**G_1—G_3; see Chart 1). In addition, murideoxycholic acid (MDCA; 3α,6β-

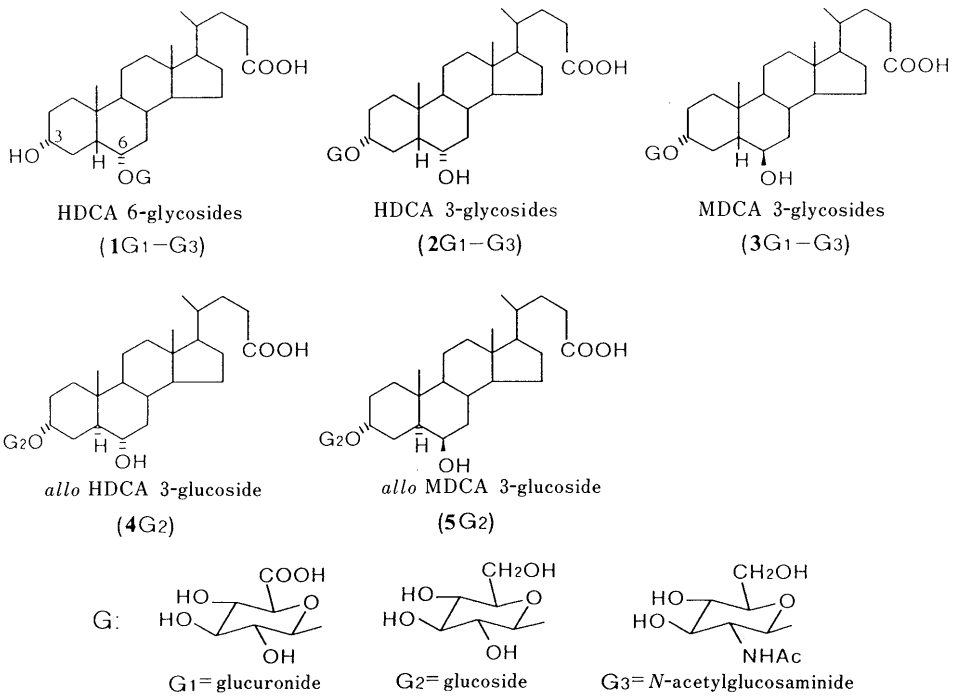

Chart 1

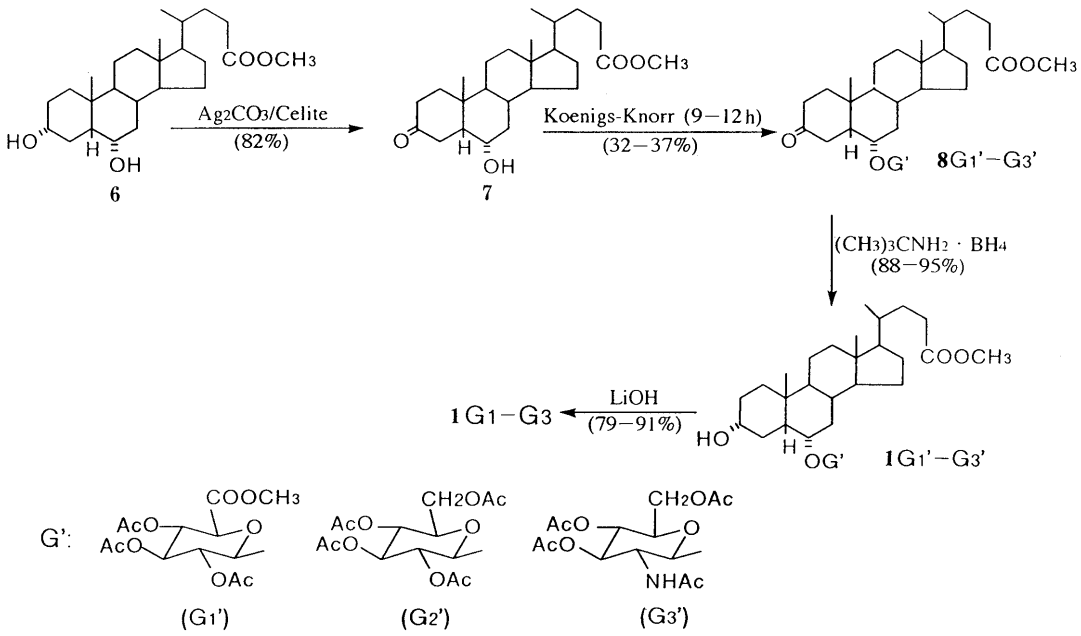

Chart 2

dihydroxy-5β-cholan-24-oic acid) 3-glycosides (**3**G_1—G_3) and some of their 5α-epimers (**4**G_2, **5**G_2), by-products of an exploratory reaction for the synthesis of the HDCA 3-glycosides, were isolated and characterized.

As summarized in Charts 2—4, the starting compounds for our syntheses are the 3- and 6-oxo ester derivatives (**7**, **9**) of **6**, which were prepared by selective oxidation with silver carbonate on Celite and potassium chromate, respectively.[19] The key reactions used are 1) the cadmium carbonate-catalyzed Koenigs–Knorr condensation reaction of the oxo esters with an appropriate α-acetohalosugar, 2) reduction of the resulting glycosidic conjugates of the ketones with *tert*-butylamine–borane complex, and 3) subsequent hydrolysis with LiOH. The α-acetohalosugars used in this study are methyl 1α-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-D-glucopyranosyluronate (methyl α-acetobromoglucuronate), 1α-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (α-acetobromoglucose) and 2-acetamido-1α-chloro-1,2-dideoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose (α-acetochloroglucosamine).

Koenigs–Knorr condensation of **7** and **9** with methyl α-acetobromoglucuronate in the presence of cadmium carbonate in boiling anhydrous benzene provided the bile acid 6- and 3-glucuronide methyl ester-acetates (**8**G'_1, **10**G'_1), respectively. The corresponding oxo bile acid 6- and 3-glucoside tetraacetates (**8**G'_2, **10**G'_2) and *N*-acetylglucosaminide derivatives (**8**G'_3, **10**G'_3) were similarly prepared by condensation with α-acetobromoglucose and α-acetochloroglucosamine, respectively.

Thin-layer chromatography (TLC) showed that the formation of a β-glycosidic linkage and the reaction product depended on the structure of the substrates and the reaction time. Glycosidation at C-3 in **9** took place smoothly and relatively rapidly (*ca.* 4—5 h) to give the desired 6-oxo-3α-glycoside methyl ester-acetates (**10**G'_1—G'_3) in good yields (50—57%). To purify the products by column chromatography, we used silica-gel as an adsorbent since this does not produce allomerization at C-5 (see below).[19] However, similar glycosidation at C-6 in **7** was much slower (12—13 h) than at C-3, and the desired 3-oxo-6α-glycoside derivatives (**8**G'_1—G'_3) were obtained in 32—37% yields. On being submitted to glucosidation for 24 h, **9** underwent a surprising allomerization at C-5 to give the corresponding 3α-glucosidated 5α ("*allo*") product (**11**G'_2) (yield, 53%). The A/B-*trans* structure in **11**G'_2 was characterized by the appearance of a singlet signal in the ^{1}H-NMR spectrum at 0.71 ppm due to the 19-methyl protons; this signal is deshielded by 0.12 ppm and resonates at 0.83 ppm in the corresponding 5β (A/B-*cis*) isomer (**10**G'_2), in accord with previous findings for non-glycosidated 6-oxo-3α-hydroxy analogs.[20] The allomerization to the thermodynamically more stable 5α form can be explained in terms of enolization of the 6-ketone to C-5.[21]

The high equatorial selectivity found in our previous reduction of 3-oxo bile acids with *tert*-butylamine–borane complex[22] suggested its utility for the preparation of **1**G'_1—G'_3. Indeed, when the 3-oxo-6α-glycoside derivatives (**8**G'_1—G'_3) were reduced in this way, the equatorially-oriented 3α-hydroxy compounds were obtained by direct recrystallization of the crude reaction products in good yields (88—94%); no 3β-epimer could be detected.

Similar reduction of the 6-oxo-3α-glycosides (**10**G'_1—G'_3) yielded 6-hydroxy epimeric mixtures (**2**G'_1 *vs.* **3** G'_1, **2**G'_2 *vs.* **3**G'_2 and **2**G'_3 *vs.* **3**G'_3) in which the 6β/6α ratios were *ca.* 1 : 1.3—1 : 1.5. Column chromatography of the mixtures on fine-particle silica-gel (230—400 mesh) successfully resolved the epimers; in each case, the less polar major product (fr. 1) was identified as the 6β-hydroxylated compound (**3**G'_1—G'_3) and the more polar one (fr. 2) as the corresponding 6α-epimer (**2**G'_1—G'_3).

Elimination of the protecting groups was carried out by treatment with LiOH in aqueous methanol under mild conditions, yielding the desired HDCA 6- and 3-glycosides

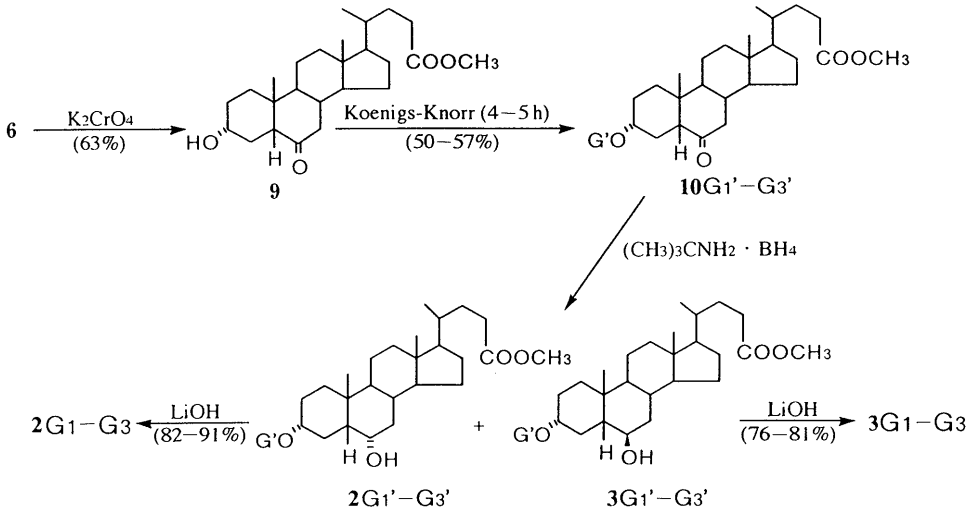

Chart 3

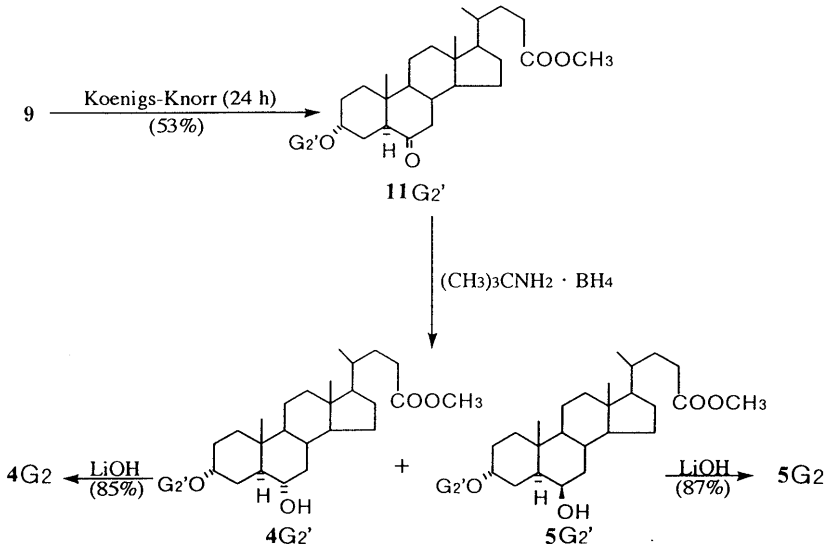

Chart 4

($\mathbf{1}G_1$—G_3, $\mathbf{2}G_1$—G_3), MDCA 3-glycosides ($\mathbf{3}G_1$—G_3) and *allo* HDCA and *allo* MDCA 3-glucosides ($\mathbf{4}G_2$, $\mathbf{5}G_2$, respectively) in reasonable yields.

The IR and ^{1}H-NMR spectral data for the glucuronides, glucosides and *N*-acetylglucosaminides indicated formation of the β-glycosidic linkage. In particular, the anomeric proton signal of the sugar moieties in the ^{1}H-NMR spectra appeared at 4.30—4.60 ppm as a doublet ($J=7$—8 Hz), indicating a *trans*-diaxial relationship with the vicinal 2′-protons. Further definitive characterization of the structures of these compounds by two-dimensional NMR is being conducted in our laboratories, and the details will be reported elsewhere.

The biological aspects of the metabolic conjugation of HDCA and its 6β-epimer is extremely interesting. The availability of these authentic samples may be helpful for studying the conjugation of bile acids in patients with hepatobiliary diseases.

Experimental

Melting points (mp) were determined on an electric micro hot stage and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1600 Series FTIR spectormeter as KBr disks. ^{1}H-NMR spectra were obtained on JEOL FX-90Q, Hitachi R-3000 and JEOL GSX-500 instruments at 90, 300 and 500 MHz, respectively, with $CDCl_3$ containing 0.1% Me_4Si as the solvent, unless otherwise specified; chemical shifts are expressed in δ(ppm) relative to Me_4Si. Analytical TLC was performed on precoated silica-gel plates (20 cm × 20 cm, 0.25 mm layer thickness; Merck, Darmstadt, Germany) using EtOAc–hexane–AcOH (50 : 50 : 1—10 : 40 : 2, v/v/v) or $CHCl_3$–MeOH–AcOH (22 : 8 : 3, v/v/v) as the developing solvent. α-Acetobromoglucuronate, α-acetobromoglucose and α-acetochloroglucosamine were prepared by published methods.[23] HDCA was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan); it was converted to the methyl ester (**6**) by the usual method.

The desired bile acid glycosides ($\mathbf{1}G_1$—G_3, $\mathbf{2}G_1$—G_3, $\mathbf{3}G_1$—G_3, $\mathbf{4}G_2$, $\mathbf{5}G_2$) were synthesized from **6** as shown in Charts 2—4. The individual steps were carried out as follows.

General Procedure for the Koenigs–Knorr Reaction To a solution of the 6α-hydroxy-3-oxo ester **7** (or 3α-hydroxy-6-oxo ester **9**) (1.0 g) in anhydrous benzene (50 ml) were added freshy prepared cadmium carbonate (1.0 g), α-acetohalosugar (1.0 g) and a quantity of molecular sieve (1.0 g). The resulting mixture was azeotropically refluxed with stirring. After 1 and 3 h, additional quantities of α-acetohalosugar (500 mg) and cadmium carbonate (500 mg) were added, and the mixture refluxed for several more hours; the reaction was monitored by TLC using hexane–EtOAc–AcOH (50 : 50 : 1, v/v/v) as the developing solvent.

The precipitate was removed by filtration and washed with EtOAc. The filtrate and washings were combined and evaporated to dryness under reduced pressure and the oily residue obtained was subjected to column chromatography on silica-gel (50 g). Elution was carried out with benzene–acetone (96 : 4—92 : 8, v/v) and the material in the eluate was recrystallized from an appropriate solvent to gave the 3- and 6-oxo bile acid glycoside methyl ester-acetates (**8G′$_1$**—**G′$_3$**, **10G′$_1$**—**G′$_3$**, **11G′$_2$**).

General Procedure for Reduction of the *tert*-Butylamine–Borane Complex Reduction of 6-Ketones **10G′$_1$**—**G′$_3$** and **11G′$_2$**: To a stirred solution of the 6-ketone (500 mg) in CH_2Cl_2 (25 ml) was added *tert*-butylamine–borane complex (250 mg). The mixture was stirred at room temperature for 2 h and then acidified with 3 N HCl. The CH_2Cl_2 layer was washed with water, dried with Drierite, and evaporated. The crude reduction products were estimated from TLC evidence to be a 6α/6β-mixture of the corresponding 6-hydroxy epimers. The 6-epimeric mixture was purified by chromatography on a column of silica-gel (230—400 mesh; 70 g) using a benzene–acetone mixture (94 : 6—90 : 10, v/v) as the eluent. In all cases, the less polar fraction (fr. 1) was identified as the 6β-hydroxy compounds (**3G′$_1$**—**G′$_3$**, **5G′$_2$**) and the more polar fraction (fr. 2) as the corresponding 6α-epimers (**2G′$_1$**—**G′$_3$**, **4G′$_2$**).

Reduction of 3-Ketones **8G′$_1$**—**G′$_3$**: The 3-ketone (500 mg) was reduced with *tert*-butylamine–borane complex as described above. After being treated in the same way, the oily product, which on TLC consisted essentially of a single component, was purified by multiple recrystallizations from an appropriate solvent. In all cases, the products were identified as the 3α-hydroxylated compounds (**1G′$_1$**—**G′$_3$**).

General Procedure for LiOH Hydrolysis To a solution of bile acid glycoside methyl ester–acetate (200 mg) in MeOH (20 ml) an aqueous solution (8 ml) of 2 M LiOH was added dropwise, and the mixture was stirred at room temperature for 4 h. After most of the solvent had been evaporated under reduced pressure, the reaction product was diluted with water, neutralized with 5% HCl and evaporated to dryness. The residue obtained was redissolved in anhydrous EtOH (20 ml) and the insoluble material was filtered off and washed with EtOH. The combined mother liquor (filtrate) was evaporated and the residue was recrystallized from an appropriate solvent to give the desired acids (**1G$_1$**—**G$_3$**, **2G$_1$**—**G$_3$**, **3G$_1$**—**G$_3$**, **4G$_2$**, **5G$_2$**).

Methyl 6α-Hydroxy-3-oxo-5β-cholanoate 6-Glucuronide Methyl Ester-triacetate (8G′$_1$) The 3-oxo-6α-hydroxy ester **7** (prepared from **6** by Ag_2CO_3/Celite oxidation[19]) was subjected to the Koenigs–Knorr reaction (12 h) with methyl α-acetobromoglucuronate and recrystallization of the crude product from EtOAc–hexane gave **8G′$_1$** as a colorless amorphous solid; yield, 654 mg (37%); mp 160—162 °C. IR ν_{max} cm^{-1}: 1756, 1728, 1713, 1244, 1222, 1054, 1040. ^{1}H-NMR (90 MHz) δ: 0.67 (s, 3H, 18-CH_3), 0.91 (d, 3H, J=6.3 Hz, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 2.01, 2.12 (s, 9H, $COCH_3$), 3.66, 3.76 (s, each 3H, $COOCH_3$), 4.03 (br m, 1H, 6β-H), 4.59 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{38}H_{56}O_{13}$: C, 63.31; H, 7.83. Found: C, 63.20; H, 7.66.

Methyl 6α-Hydroxy-3-oxo-5β-cholanoate 6-Glucoside Tetraacetate (8G′$_2$) The ester **7** was subjected to the Koenigs–Knorr reaction (12 h) with α-acetobromoglucose and recrystallization of the crude product from EtOAc–hexane gave **8G′$_2$** as a colorless amorphous solid; yield, 586 mg (32%); mp 143—145 °C. IR ν_{max} cm^{-1}: 1757, 1713, 1229, 1040. ^{1}H-NMR (90 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.92 (d, 3H, J=5.4 Hz, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 1.99, 2.02, 2.08, 2.11 (s, each 3H, $COCH_3$), 3.66 (s, 3H, $COOCH_3$), 4.00 (br m, 1H, 6β-H), 4.55 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{58}O_{13}$: C, 63.73; H, 7.96. Found: C, 63.60; H, 7.92.

Methyl 6α-Hydroxy-3-oxo-5β-cholanoate 6-*N*-Acetylglucosaminide Triacetate (8G′$_3$) The ester **7** was subjected to the Koenigs–Knorr reaction (13 h) with α-acetochloroglucosamine and recrystallization of the crude product from acetone–hexane gave **8G′$_3$** as a colorless amorphous solid; yield, 581 mg (32%); mp 220—221 °C. IR ν_{max} cm^{-1}: 1745, 1715, 1377, 1051, 1033, 1015. ^{1}H-NMR (300 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.92 (d, 3H, J=6.3 Hz, 21-CH_3), 0.99 (s, 3H, 19-CH_3), 2.00, 2.02, 2.03, 2.08 (s, each 3H, $COCH_3$), 3.67 (s, 3H, $COOCH_3$), 4.03 (br m, H, 6β-H), 4.81 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{59}NO_{12}\cdot 1/4H_2O$: C, 63.44; H, 8.12; N, 1.90. Found: C, 63.42; H, 7.83; N, 1.80.

Methyl 3α-Hydroxy-6-oxo-5β-cholanoate 3-Glucuronide Methyl Ester-triacetate (10G′$_1$) The 3α-hydroxy-6-oxo ester **9** (prepared from **6** by potassium chromate oxidation[19]) was subjected to the Koenigs–Knorr reaction (4 h) with methyl α-acetobromoglucuronate and recrystallization of the crude product from EtOAc–hexane gave **10G′$_1$** as a colorless amorphous solid; yield, 928 mg (52%); mp 113—117 °C. IR ν_{max} cm^{-1}: 1758, 1703, 1218, 1037. ^{1}H-NMR (300 MHz) δ: 0.64 (s, 3H, 18-CH_3), 0.83 (s, 3H, 19-CH_3), 0.93 (d, 3H, J=6.3 Hz, 21-CH_3), 2.02, 2.05 (s, 9-H, $COCH_3$), 3.54 (br m, 1H, 3β-H), 3.67, 3.75 (s, each 3H, $COOCH_3$), 4.61 (d, 1H, J=7.8 Hz, anomeric H). *Anal.* Calcd for $C_{38}H_{56}O_{13}$: C, 63.31; H, 7.83. Found: C, 63.32; H, 7.76.

Methyl 3α-Hydroxy-6-oxo-5β-cholanoate 3-Glucoside Tetraacetate (10G′$_2$) The ester **9** was subjected to the Koenigs–Knorr reaction (4 h) with α-acetobromoglucose and recrystallization of the crude product from EtOAc–hexane gave **10G′$_2$** as a colorless amorphous solid; yield, 908 mg (50%); mp 147—150 °C. IR ν_{max} cm^{-1}: 1756, 1701, 1228, 1054, 1033, 1016. ^{1}H-NMR (90 MHz) δ: 0.65 (s, 3H, 18-CH_3), 0.83 (s, 3H, 19-CH_3), 0.93 (d, 3H, J=6.3 Hz, 21-CH_3), 2.00, 2.02, 2.04, 2.05 (s, each 3H, $COCH_3$), 3.55 (br m, 1H, 3β-H), 3.66 (s, 3H, $COOCH_3$), 4.55 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{58}O_{13}\cdot 1/2H_2O$: C, 62.97; H, 7.99. Found: C, 62.79; H, 7.93.

Methyl 3α-Hydroxy-6-oxo-5β-cholanoate 3-*N*-Acetylglucosaminide Triacetate (10G′$_3$) The ester **9** was subjected to the Koenigs–Knorr reaction (5 h) with α-acetochloroglucosamine and recrystallization of the crude product from EtOAc–hexane gave **10G′$_3$** as a colorless amorphous solid; yield, 1.03 g (57%); mp 214—215 °C. IR ν_{max} cm^{-1}: 1747, 1702, 1237, 1044. ^{1}H-NMR (300 MHz) δ: 0.64 (s, 3H, 18-CH_3), 0.83 (s, 3H, 19-CH_3), 0.92 (d, 3H, J=6.3 Hz, 21-CH_3), 1.96, 2.02, 2.03, 2.06 (s, each 3H, $COCH_3$), 3.53 (br m, 1H, 3β-H), 3.67 (s, 3H, $COOCH_3$), 4.79 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{59}NO_{12}\cdot 1/4H_2O$: C, 63.44; H, 8.12; N, 1.90. Found: C, 63.42; H, 8.11; N, 1.94.

Methyl 3α-Hydroxy-6-oxo-5α-cholanoate 3-Glucoside Tetraacetate (11G′$_2$) The 3α-hydroxy-6-oxo ester **9** was subjected to the Koenigs–Knorr reaction (24 h) with α-acetobromoglucose and recrystallization of the crude product obtained from aqueous MeOH gave **11G′$_2$** as a colorless amorphous solid; yield, 968 mg (53%); mp 161—164 °C. IR ν_{max} cm^{-1}: 1748, 1706, 1228, 1040. ^{1}H-NMR (90 MHz) δ: 0.65 (s, 3H, 18-CH_3), 0.71 (s, 3H, 19-CH_3), 0.93 (d, 3H, J=5.4 Hz, 21-CH_3), 2.02, 2.05, 2.06 (s, 12H, $COCH_3$), 3.66 (s, 3H, $COOCH_3$), 3.98 (m, 1H, 3β-H), 4.51 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{58}O_{13}$: C, 63.74; H, 7.96. Found: C, 63.82; H, 8.08.

Methyl 3α,6α-Dihydroxy-5β-cholanoate 3-Glucuronide Methyl Ester-triacetate (2G′$_1$) and Its 6β-Epimer (3G′$_1$) These compounds were prepared from the 6-ketone **10G′$_1$** by the general reduction procedure.

(Fr. 1): **3G′$_1$**; yield, 220 mg (44%); mp 193—194 °C (colorless amorphous solid from acetone–hexane). IR ν_{max} cm^{-1}: 3569, 1761, 1226, 1040. ^{1}H-NMR (90 MHz) δ: 0.67 (s, 3H, 18-CH_3), 0.91 (d, 3H, J=5.4 Hz, 21-CH_3), 1.09 (s, 3H, 19-CH_3), 2.02, 2.04 (s, 9H, $COCH_3$), 3.50 (br m, 1H, 3β-H), 3.66, 3.75 (s, each 3H, $COOCH_3$), 3.71 (m, 1H, 6α-H), 4.64 (d, 1H, J=7.2 Hz, anomeric H). *Anal.* Calcd for $C_{38}H_{58}O_{13}$: C, 63.14; H, 8.09. Found: C, 62.91; H, 7.96.

(Fr. 2): **2G′$_1$**; yield, 167 mg (33%); mp 138—139/157—158 °C (colorless amorphous solid from EtOAc–hexane). IR ν_{max} cm^{-1}: 3505, 3372, 1755, 1250, 1224, 1046, 1030. ^{1}H-NMR (90 MHz) δ: 0.63 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.91 (d, 3H, J=5.4 Hz, 21-CH_3), 2.01, 2.04 (s, 9H, $COCH_3$), 3.60 (br m, 1H, 3β-H), 3.66, 3.75 (s, each 3H, $COOCH_3$), 4.01 (br m, 1H, 6β-H), 4.65 (d, 1H, J=7.2 Hz, anomeric H). *Anal.* Calcd for $C_{38}H_{58}O_{13}$: C, 63.14; H, 8.09. Found: C, 63.01; H, 8.03.

Methyl 3α,6α-Dihydroxy-5β-cholanoate 3-Glucoside Tetraacetate (2G′$_2$) and Its 6β-Epimer (3G′$_2$) These compounds were prepared from the 6-ketone **10G′$_2$** by the general reduction procedure.

(Fr. 1): **3G′$_2$**; yield, 231 mg (46%); mp 140—141 °C (colorless amorphous solid from EtOAc–hexane). IR ν_{max} cm^{-1}: 3436, 1758, 1226, 1039. ^{1}H-NMR (90 MHz) δ: 0.67 (s, 3H, 18-CH_3), 0.92 (d, 3H, J=5.4 Hz, 21-CH_3), 1.10 (s, 3H, 19-CH_3), 2.00, 2.02, 2.04, 2.08 (s, each 3H, $COCH_3$), 3.57 (br m, 1H, 3β-H), 3.66 (s, 3H, $COOCH_3$), 3.74 (m, 1H, 6α-H), 4.59 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{60}O_{13}$: C, 63.56; H, 8.21. Found: C, 63.29; H, 8.04.

(Fr. 2): **2G′$_2$**; yield, 153 mg (30%); mp 177—178 °C (colorless needles from EtOAc–hexane). IR ν_{max} cm^{-1}: 3540, 1757, 1228, 1038. ^{1}H-NMR (90 MHz) δ: 0.63 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.91 (d, 3H, J=5.4 Hz, 21-CH_3), 2.00, 2.02, 2.04, 2.08 (s, each 3H, $COCH_3$), 3.55 (br m, 1H, 3β-H), 3.66 (s, 3H, $COOCH_3$), 3.98 (br m, 1H, 6β-H), 4.59 (d, 1H, J=7.2 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{60}O_{13}$: C, 63.56; H, 8.21. Found: C, 63.33; H, 8.10.

Methyl 3α,6α-Dihydroxy-5β-cholanoate 3-*N*-Acetylglucosaminide Triacetate (2G′$_3$) and Its 6β-Epimer (3G′$_3$) These compounds were prepared from the 6-ketone **10G′$_3$** by the general reduction procedure.

(Fr. 1): **3G′$_3$**; yield, 258 mg (52%); mp 213—214 °C (colorless needles from aqueous MeOH). IR ν_{max} cm^{-1}: 3340, 1747, 1236, 1047, 1033.

^{1}H-NMR (300 MHz) δ: 0.67 (s, 3H, 18-CH_3), 0.91 (d, 3H, J=6.0 Hz, 21-CH_3), 1.09 (s, 3H, 19-CH_3), 1.95, 2.02, 2.03, 2.08 (s, each 3H, $COCH_3$), 3.56 (br m, 1H, 3β-H), 3.67 (s, 3H, $COOCH_3$), 3.70 (m, 1H, 6α-H), 4.88 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{61}NO_{12}\cdot 1/4H_2O$: C, 63.27; H, 8.37; N, 1.89. Found: C, 63.23; H, 8.21; N, 1.78.

(Fr. 2): **2G'$_3$**; yield, 183 mg (37%); mp 218—221 °C (colorless amorphous solid from acetone–hexane). IR ν_{max} cm^{-1}: 3667, 3276, 1742, 1240, 1053, 1033, 1017. ^{1}H-NMR (300 MHz) δ: 0.63 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.91 (d, 3H, J=7.0 Hz, 21-CH_3), 1.95, 2.03, 2.04, 2.09 (s, each 3H, $COCH_3$), 3.56 (br m, 1H, 3β-H), 3.67 (s, 3H, $COOCH_3$), 4.01 (br m, 1H, 6β-H), 4.87 (d, 1H, J=8.4 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{61}NO_{12}\cdot 1/2H_2O$: C, 62.88; H, 8.39; N, 1.88. Found: C, 62.87; H, 8.34; N, 1.63.

Methyl 3α,6α-Dihydroxy-5β-cholanoate 6-Glucuronide Methyl Ester-triacetate (1G'$_1$) This compound was prepared from the 3-ketone **8G'$_1$** by the general reduction procedure; yield, 441 mg (88%); mp 148—150 °C (colorless amorphous solid from EtOAc–hexane). IR ν_{max} cm^{-1}: 3577, 3446, 1756, 1231, 1040. ^{1}H-NMR (90 MHz) δ: 0.63 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.91 (d, 3H, J=5.4 Hz, 21-CH_3), 2.02, 2.06 (s, 9H, $COCH_3$), 3.52 (br m, 1H, 3β-H), 3.66, 3.75 (s, each 3H, $COOCH_3$), 4.05 (br m, 1H, 6β-H), 4.61 (d, 1H, J=7.2 Hz, anomeric H). *Anal.* Calcd for $C_{38}H_{58}O_{13}\cdot 1/4H_2O$: C, 62.75; H, 8.11. Found: C, 62.82; H, 8.05.

Methyl 3α,6α-Dihydroxy-5β-cholanoate 6-Glucoside Tetraacetate (1G'$_2$) This compound was prepared from the 3-ketone **8G'$_2$** by the general reduction procedure; yield, 460 mg (92%); mp 137—139 °C (colorless amorphous solid from EtOAc–hexane). IR ν_{max} cm^{-1}: 3480, 1754, 1223, 1034. ^{1}H-NMR (90 MHz) δ: 0.63 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.91 (d, 3H, J=5.4 Hz, 21-CH_3), 2.00, 2.02, 2.06, 2.08 (s, each 3H, $COCH_3$), 3.59 (br m, 1H, 3β-H), 3.66 (s, 3H, $COOCH_3$), 4.00 (br m, 1H, 6β-H), 4.56 (d, 1H, J=7.2 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{60}O_{13}$: C, 63.56; H, 8.21. Found: C, 63.30; H, 8.03.

Methyl 3α,6α-Dihydroxy-5β-cholanoate 6-*N*-Acetylglucosaminide Triacetate (1G'$_3$) This compound was prepared from the 3-ketone **8G'$_3$** by the general reduction procedure; yield, 470 mg (94%); mp 181—184 °C (colorless amorphous solid from CH_2Cl_2–hexane). IR ν_{max} cm^{-1}: 3378, 1748, 1236, 1045. ^{1}H-NMR (300 MHz) δ: 0.63 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.90 (d, 3H, J=6.3 Hz, 21-CH_3), 1.96, 2.02, 2.03, 2.08 (s, each 3H, $COCH_3$), 3.53 (br m, 1H, 3β-H), 3.67 (s, 3H, $COOCH_3$), 3.98 (br m, 1H, 6β-H), 4.89 (d, 1H, J=8.1 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{61}NO_{12}\cdot 1/2H_2O$: C, 62.88; H, 8.39; N, 1.88. Found: C, 62.91; H, 8.10; N, 1.70.

Methyl 3α,6α-Dihydroxy-5α-cholanoate 3-Glucoside Tetraacetate (4G'$_2$) and Its 6β-Epimer (5G'$_2$) Treatment of the ester **11G'$_2$** (500 mg) with *tert*-butylamine–borane complex in the manner described above gave two reduction products.

(Fr. 1): **5G'$_2$**; yield, 203 mg (41%); mp 199—200 °C (colorless needles from CH_2Cl_2–hexane). IR ν_{max} cm^{-1}: 3585, 1749, 1226, 1036. ^{1}H-NMR (90 MHz) δ: 0.69 (s, 3H, 18-CH_3), 0.92 (d, 3H, J=5.4 Hz, 21-CH_3), 1.01 (s, 3H, 19-CH_3), 2.02, 2.08 (s, 12H, $COCH_3$), 3.66 (s, 3H, $COOCH_3$), 3.73 (m, 1H, 6α-H), 4.03 (m, 1H, 3β-H), 4.55 (d, 1H, J=7.2 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{60}O_{13}$: C, 63.56; H, 8.21. Found: C, 63.28; H, 8.26.

(Fr. 2): **4G'$_2$**; yield, 149 mg (30%); mp 150—154 °C (colorless amorphous solid from acetone–hexane). IR ν_{max} cm^{-1}: 3564, 1745, 1222, 1040. ^{1}H-NMR (90 MHz) δ: 0.65 (s, 3H, 18-CH_3), 0.78 (s, 3H, 19-CH_3), 0.92 (d, 3H, J=5.4 Hz, 21-CH_3), 2.03, 2.08 (s, 12H, $COCH_3$), 3.45 (br m, 1H, 6β-H), 3.66 (s, 3H, $COOCH_3$), 3.98 (m, 1H, 3β-H), 4.59 (d, 1H, J= 7.2 Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{60}O_{13}$: C, 63.56; H, 8.21. Found: C, 63.32; H, 8.19.

3α,6α-Dihydroxy-5β-cholanoic Acid 3-Glucuronide (2G$_1$) Hydrolysis of **2G'$_1$**, by the general procedure, afforded the corresponding acid **2G$_1$** which was recrystallized from MeOH–EtOAc to give a colorless amorphous solid; yield, 129 mg (82%); mp 244—246 °C. IR ν_{max} cm^{-1}: 3387, 1619, 1043. ^{1}H-NMR (500 MHz; CD_3OD) δ: 0.66 (s, 3H, 18-CH_3), 0.93 (s, 3H, 19-CH_3), 0.94 (d, 3H, J=6.0 Hz, 21-CH_3), 3.71 (br m, 1H, 3β-H), 4.00 (br m, 1H, 6β-H), 4.45 (d, 1H, J=7.5 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{48}O_{10}\cdot 2H_2O$: C, 59.58; H, 8.67. Found: C, 59.38; H, 8.54.

3α,6β-Dihydroxy-5β-cholanoic Acid 3-Glucuronide (3G$_1$) Hydrolysis of **3G'$_1$**, by the general procedure, afforded the corresponding acid **3G$_1$** which was recrystallized from MeOH–EtOAc to give a colorless amorphous solid; yield, 141 mg (89%); mp 251—252 °C. IR ν_{max} cm^{-1}: 3404, 1625, 1028. ^{1}H-NMR (500 MHz) δ: 0.69 (s, 3H, 18-CH_3), 0.95 (d, 3H, J=6.5 Hz, 21-CH_3), 1.10 (s, 3H, 19-CH_3), 3.72 (m, 1H, 6α-H), 3.77 (br m, 1H, 3β-H), 4.44 (d, 1H, J=8.0 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{48}O_{10}\cdot 2\,1/2H_2O$: C, 58.71; H, 8.70. Found: C, 58.57; H, 8.51.

3α,6α-Dihydroxy-5β-cholanoic Acid 3-Glucoside (2G$_2$) Hydrolysis of **2 G'$_2$**, by the procedure manner, afforded the corresponding acid **2G$_2$** which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 136 mg (91%); mp 195—198 °C. IR ν_{max} cm^{-1}: 3385, 1651, 1078, 1032. ^{1}H-NMR (300 MHz; $CDCl_3$ + 20% CD_3OD) δ: 0.65 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 0.92 (d, 3H, J=7.0 Hz, 21-CH_3), 3.62 (br m, 1H, 3β-H), (6β-H overlaps with H_2O signal), 4.40 (d, 1H, J=7.5 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{50}O_9\cdot 1\,1/2H_2O$: C, 61.94; H, 9.18. Found: C, 61.69; H, 9.25.

3α,6β-Dihydroxy-5β-cholanoic Acid 3-Glucoside (3G$_2$) Hydrolysis of **3G'$_2$**, by the general procedure, afforded the corresponding acid **3G$_2$** which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 123 mg (81%); mp 207—210 °C. IR ν_{max} cm^{-1}: 3406, 1652, 1077, 1023. ^{1}H-NMR (300 MHz; $CDCl_3$ + 20% CD_3OD) δ: 0.68 (s, 3H, 18-CH_3), 0.93 (d, 3H, J=6.3 Hz, 21-CH_3), 1.10 (s, 3H, 19-CH_3), 3.66 (br m, 1H, 3β-H), 3.71 (m, 1H, 6α-H), 4.41 (d, 1H, J=7.8 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{50}O_9\cdot 1\,1/2H_2O$: C, 61.94; H, 9.18. Found: C, 61.85; H, 8.93.

3α,6α-Dihydroxy-5β-cholanoic Acid 3-*N*-Acetylglucosaminide (2G$_3$) Hydrolysis of **2G'$_3$**, by the general procedure, afforded the corresponding acid **2G$_3$** which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 128 mg (80%); mp 167—169 °C. IR ν_{max} cm^{-1}: 3374, 1715, 1652, 1204, 1054, 1032. ^{1}H-NMR (500 MHz) δ: 0.65 (s, 3H, 18-CH_3), 0.90 (s, 3H, 19-CH_3), 0.93 (d, 3H, J=6.0 Hz, 21-CH_3), 2.01 (s, 3H, $COCH_3$), 3.53 (br m, 1H, 3β-H), 3.98 (br m, 1H, 6β-H), 4.58 (d, 1H, J=7.0 Hz, anomeric H). *Anal.* Calcd for $C_{32}H_{53}NO_9\cdot H_2O$: C, 62.61; H, 9.03; N, 2.28. Found: C, 62.47; H, 8.97; N, 1.99.

3α,6β-Dihydroxy-5β-cholanoic Acid 3-*N*-Acetylglucosaminide (3G$_3$) Hydrolysis of **3G'$_3$**, by the general procedure, afforded the corresponding acid **3G$_3$** which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 123 mg (76%); mp 177—179 °C. IR ν_{max} cm^{-1}: 3378, 1709, 1652, 1268, 1078, 1027. ^{1}H-NMR (500 MHz) δ: 0.69 (s, 3H, 18-CH_3), 0.94 (d, 3H, J=6.0 Hz, 21-CH_3), 1.09 (s, 3H, 19-CH_3), 2.01 (s, 3H, $COCH_3$), 3.59 (br m, 1H, 3β-H), 3.70 (m, 1H, 6α-H), 4.60 (d, 1H, J=8.0 Hz, anomeric H). *Anal.* Calcd for $C_{32}H_{53}NO_9\cdot 2\,1/2H_2O$: C, 60.40; H, 9.11; N, 2.20. Found: C, 60.49; H, 8.95; N, 1.90.

3α,6α-Dihydroxy-5β-cholanoic Acid 6-Glucuronide (1G$_1$) Hydrolysis of **1G'$_1$**, by the general procedure, afforded the corresponding acid **1G$_1$** which was recrystallized from MeOH–EtOAc to give a colorless amorphous solid; yield, 128 mg (85%); mp 222—225 °C. IR ν_{max} cm^{-1}: 3405, 1638(s), 1034. ^{1}H-NMR (500 MHz; CD_3OD) δ: 0.66 (s, 3H, 18-CH_3), 0.93 (s, 3H, 19-CH_3), 0.94 (d, 3H, J=6.0 Hz, 21-CH_3), 3.59 (br m, 1H, 3β-H), 4.10 (br m, 1H, 6β-H), 4.35 (d, 1H, J=7.5 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{48}O_{10}\cdot 3H_2O$: C, 57.86; H, 8.74. Found: C, 57.85; H, 8.49.

3α,6α-Dihydroxy-5β-cholanoic Acid 6-Glucoside (1G$_2$) Hydrolysis of **1G'$_2$**, by the general procedure, afforded the corresponding acid **1G$_2$** which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 119 mg (79%); mp 184—187 °C. IR ν_{max} cm^{-1}: 3387, 1711, 1078, 1039. ^{1}H-NMR (300 MHz) δ: 0.65 (s, 3H, 18-CH_3), 0.90 (s, 3H, 19-CH_3), 0.93 (d, 3H, J=6.3Hz, 21-CH_3), 3.55 (br m, 1H, 3β-H), (6β-H overlaps with H_2O signal), 4.33 (d, 1H, J=7.5 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{50}O_9\cdot 1\,1/2H_2O$: C, 61.94; H, 9.18. Found: C, 61.70; H, 8.89.

3α,6α-Dihydroxy-5β-cholanoic Acid 6-*N*-Acetylglucosaminide (1G$_3$) Hydrolysis of **1G'$_3$**, by the general procedure, afforded the corresponding acid **1G$_3$** which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 147 mg (91%); mp 212—215 °C. IR ν_{max} cm^{-1}: 3396, 1652, 1318, 1079, 1056, 1030. ^{1}H-NMR (500 MHz; CD_3OD) δ: 0.64 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.93 (d, 3H, J=6.0 Hz, 21-CH_3), 2.01 (s, 3H, $COCH_3$), 3.58 (br m, 1H, 3β-H), 3.94 (br m, 1H, 6β-H), 4.52 (d, 1H, J=8.0 Hz, anomeric H). *Anal.* Calcd for $C_{32}H_{53}NO_9\cdot 2H_2O$: C, 60.83; H, 9.09; N, 2.22. Found: C, 60.79; H, 9.06; N, 2.49.

3α,6α-Dihydroxy-5α-cholanoic Acid 3-Glucoside (4G$_2$) Hydrolysis of **4G'$_2$**, by the general procedure, afforded the corresponding acid **4G$_2$** which was recrystallized from EtOH–acetone to give a colorless amorphous solid; yield, 128 mg (85%); mp 233—236 °C. IR ν_{max} cm^{-1}: 3418, 1652, 1081, 1046. ^{1}H-NMR (300 MHz; $CDCl_3$ + 10% CD_3OD) δ: 0.64 (s, 3H, 18-CH_3), 0.79 (s, 3H, 19-CH_3), 0.92 (d, 3H, J=6.0 Hz, 21-CH_3), (3β- and 6β-H overlapped with other signals), 4.31 (d, 1H, J=8.0 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{50}O_9\cdot 2\,1/2H_2O$: C, 60.08;

H, 9.24. Found: C, 59.65; H, 8.96.

3α,6β-Dihydroxy-5α-cholanoic Acid 3-Glucoside ($5G_2$) Hydrolysis of $5G'_2$, by the general procedure, afforded the corresponding acid $5G_2$ which was recrystallized from EtOH–EtOAc to give a colorless amorphous solid; yield, 131 mg (86%); mp 206—209 °C. IR ν_{max} cm^{-1}: 3358, 1715, 1081, 1044. ^{1}H-NMR (300 MHz; $CDCl_3$ + 10% CD_3OD) δ: 0.70 (s, 3H, 18-CH_3), 0.93 (d, 3H, J = 6.0 Hz, 21-CH_3), 1.00 (s, 3H, 19-CH_3), (6α-H overlapped with other signals), 4.07 (m, 1H, 3β-H), 4.33 (d, 1H, J = 8.0 Hz, anomeric H). *Anal.* Calcd for $C_{30}H_{50}O_9 \cdot 1\,1/2H_2O$: C, 61.94; H, 9.18. Found: C, 62.10; H, 8.99.

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References and Notes

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