Potential bile acid metabolites. 23. Syntheses of 3-glucosides of nonamidated and glycine- and taurine-amidated bile acids[1]

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Abstract The 3-glucosides of nonamidated lithocholic, chenodeoxycholic, ursodeoxycholic, deoxycholic, and cholic acids, and their double conjugate forms with glycine and taurine were synthesized. The key reactions used were *1)* β-D- glucosidation at C-3 by the Koenigs-Knorr condensation reaction of 3α-hydroxylated bile acid methyl (or *p*-nitrophenyl) esters with 1α-bromo-1-deoxy-2, 3, 4, 6-tetra-O-acetyl-D-glucopyranose in the presence of cadmium carbonate in refluxing benzene; *2)* indirect and direct amidations at C-24 by the activated *p*-nitrophenyl ester and by the diethylphosphorylcyanide methods, respectively, using glycinate ester and taurine as coupling agents; and *3)* simultaneous alkaline hydrolysis of the hydroxyl-protecting and ester groups in both the sugar and aglycone moieties. **—Iida, T., S. Nishida, Y. Yamaguchi, M. Kodake, F. C. Chang, T. Niwa, J. Goto, and T. Nambara.** Potential bile acid metabolites. 23. Syntheses of 3-glucosides of nonamidated and glycine- and taurine-amidated bile acids. *J. Lipid Res.* 1995. **36:** 628–638.

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Bile acid conjugates are of substantiated interest in biosynthetic and metabolic studies of bile acids. Particular attention has been focused on the physiological significance of glycosidation of bile acids. At present, three types of glycosidic conjugation are known in bile acid metabolism of humans: glucuronidation (1, 2), *N*-acetylglucosaminidation (3, 4), and glucosidation (5–7).

Of the three glycosidic conjugations, bile acid glucosides have recently been demonstrated to be formed in human liver microsomes by a glucosyltransferase and to occur as constituents of human urine in health and liver diseases (5, 8). The existence of bile acid glucosides has been recognized by evidence based on enzymatic liberation of bile acids from biological materials by β-glucosidase (9). More direct proof for their existence had to await chemical synthesis and demonstration of identity of the isolated compounds with synthetic ones. In addition, the availability of synthetic authentic specimens may serve for identifying unknown bile acids present in biological materials.

For our series of studies on new and scarce potential bile acid metabolites, we have recently reported the preparation of the 3- and 6-glycoside (glucuronide, glucoside and *N*-acetylglucosaminide) conjugates of hyodeoxycholic and murideoxycholic acids (10). The present paper describes extension of the program to synthesize the 15 possible 3-glucosides of nonamidated chenodeoxycholic (CDCA; **4a**), deoxycholic (DCA; **4b**), cholic (CA; **4c**), lithocholic (LCA; **4d**), and ursodeoxycholic (UDCA; **4e**) acids (**1a–e**), and their double conjugate forms with glycine (**2a–e**) and taurine (**3a–e;** as sodium salt) (**Chart 1**). (Structures designated as "4" are the same as the "1" structures with 3α-OH instead of the 3-glucoside and with R_3 equal to OH.) As all of the corresponding bile acid 3-glucuronides (11) and 3-*N*-acetylglucosaminides (12) have already been synthesized recently, with the publication of this work, the entire set of the 45 theoretically pos-

Abbreviations and trivial names: IR, infrared; ^{1}HNMR, proton nuclear magnetic resonance; TLC, thin-layer chromatography; LCA, lithocholic acid, 3α-hydroxy-5β-cholan-24-oic acid; CDCA, chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholan-24-oic acid; UDCA, ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholan-24-oic acid; DCA, deoxycholic acid, 3α,12α-dihydroxy-5β-cholan-24-oic acid; CA, cholic acid, 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; 3-glucoside, 3α-O-(β-D-glucosyl)-; 3-glucoside tetraacetate, 3α-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-; DCC, N,N'-dicyclohexylcarbodiimide; DEPC, diethylphosphoryl cyanide. The various compounds in Chart 1 and Schemes 1 and 2 are designated by a **bold face** number.

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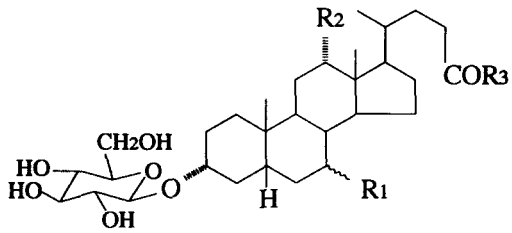


	R1	R2
1a~3a	α-OH	H
1b~3b	H	OH
1c~3c	α-OH	OH
1d~3d	H	H
1e~3e	β-OH	H

R3 = OH, $NHCH_2COOH$ or $NHCH_2CH_2SO_3Na$

(1, unconjugate ; 2, glycine conjugate ; 3, taurine conjugate)

Chart 1.

sible 3-glycosides of the 5 prominent naturally occurring bile acids have now been prepared, characterized, and recorded in the literature.

EXPERIMENTAL PROCEDURES

Melting points (mp) were determined on a micro hot stage apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1600 Series FTIR as KBr tablets. Proton nuclear magnetic resonance (^{1}HNMR) spectra were obtained on JEOL FX-90Q and JEOL GSX-500 instruments at 90 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 thin-layer chromatography (TLC) was performed on precoated silica gel (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. 1α-Bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (α-acetobromoglucose) was prepared as previously described (13, 14). CA, DCA, and LCA were purchased from Wako Pure Chemical Industries, LTD. (Osaka, Japan). CDCA and UDCA were kindly donated by Tokyo Tanabe Co. (Tokyo, Japan). All compounds were dried by azeotropic distillation prior to use.

General procedure for the Koenigs-Knorr reaction

To a solution of bile acid ester (500 mg) in anhydrous benzene (25 ml) were added α-acetobromoglucose (500 mg) and freshly prepared cadmium carbonate ($CdCO_3$; 500 mg), and the resulting mixture was azeotropically refluxed with stirring. After 1 and 3 h, additional amounts of α-acetobromoglucose (250 mg) and $CdCO_3$ (250 mg) were added in several portions, and the mixture was further refluxed for several hours (total reaction time, 4–6 h); the reaction was monitored by TLC. The precipitate was removed by filtration and washed with EtOAc. The filtrate and washings were combined and evaporated down to dryness under reduced pressure, and an oily residue obtained was subjected to column chromatography on silica gel (45 g). Elution with benzene–acetone 98:2–96:4 (v/v) and recrystallization of the eluate gave the bile acid 3α-glucoside tetraacetate.

Methyl chenodeoxycholate 3-glucoside tetraacetate-7-formate (6f)

The formate-methyl ester **5f** (500 mg) [prepared in three steps from **4a** (15)] was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from aqueous acetone gave **6f** as colorless amorphous solid; yield, 426 mg (48%); mp, 165–167°C. IR ν_{max} cm^{-1}: 1758, 1228, 1180, 1040. Analysis calculated for $C_{40}H_{60}O_{14}$: C, 62.81; H, 7.91. Found: C, 62.95; H, 8.07.

Methyl deoxycholate 3-glucoside tetraacetate-12-formate (6g)

The formate-methyl ester **5g** (500 mg) [prepared from **4b** (15)] was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from aqueous MeOH gave **6g** as colorless thin plates; yield, 463 mg (53%); mp, 149–151°C. IR ν_{max} cm^{-1}: 1756, 1724, 1230, 1039. Analysis calculated for $C_{40}H_{60}O_{14} \cdot 1/2H_2O$; C, 62.08; H, 7.94. Found: C, 62.27; H, 8.07.

Methyl cholate 3-glucoside tetraacetate-7,12-diformate (6h)

The formate-methyl ester **5h** (500 mg) [prepared from **4c** (15)] was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from CH_2Cl_2–hexane gave **6h** as colorless thin plate; yield, 456 mg (54%); mp, 211–212°C. IR ν_{max} cm^{-1}: 1759, 1718, 1236, 1193, 1044. Analysis calculated for $C_{41}H_{60}O_{16}$: C, 60.87; H, 7.49. Found: C, 60.60; H, 7.47.

Chenodeoxycholic acid 3-glucoside (1a)

A solution of the glucoside acetate-methyl ester **6f** (300 mg) in 5% methanolic KOH (9 ml) was refluxed for 1 h. The reaction mixture was diluted with water, neutralized with 5% H_2SO_4 and then extracted with EtOAc. The EtOAc layer was washed with saturated brine, dried with Drierite, and evaporated to give an oily residue. The oil was recrystallized from EtOAc–hexane as colorless amorphous solid; yield, 198 mg (91%); mp, 146–148°C. IR ν_{max} cm^{-1}: 3388, 1716, 1074, 1044. Analysis calculated for $C_{30}H_{50}O_9 \cdot 1/2H_2O$; C, 63.92; H, 9.12. Found: C, 63.88; H, 9.23.

Deoxycholic acid 3-glucoside (1b)

Treatment of the glucoside acetate-methyl ester **6g** (300 mg) with 5% methanolic KOH in the manner described for **1a** and recrystallization of the crude product from EtOAc–hexane gave **1b** as colorless amorphous solid; yield, 211 mg (97%); mp, 192–194°C. IR ν_{max} cm^{-1}: 3406, 1709, 1076, 1037. Analysis calculated for $C_{30}H_{50}O_9 \cdot 21/3H_2O$: C, 60.38; H, 9.23. Found: C, 60.46; H, 8.94.

Cholic acid 3-glucoside (1c)

Treatment of the glucoside acetate-methyl ester **6h** (300 mg) with 5% methanolic KOH in the manner described for **1a** and recrystallization of the crude product from aqueous MeOH gave **1c** as colorless needles; yield, 190 mg (90%); mp, 164–166°C. IR ν_{max} cm^{-1}: 3396, 1703, 1075, 1036. Analysis calculated for $C_{30}H_{50}O_{10} \cdot H_2O$: C, 61.20; H, 8.90. Found: C, 60.95; H, 9.18.

p-Nitrophenyl chenodeoxycholate 7-formate (8f)

To a stirred suspension of the formate-acid **7f** (3.0 g) [prepared in two steps from **4a** (16)] in anhydrous dioxane (30 ml) was added slowly a solution of *p*-nitrophenol (1.5 g) and *N,N'*-dicyclohexylcarbodiimide (DCC; 2.6 g) in anhydrous EtOAc (70 ml), and the mixture was stirred overnight at room temperature. After removal of the precipitate by filtration, the filtrate was evaporated and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dried with Drierite, and evaporated to give a pale yellow oil. The oil was chromatographed on a column of silica gel (90 g). Elution with C_6H_6/EtOAc (1:1, v/v) gave the title compound **8f** which was recrystallized from aqueous MeOH as light yellow amorphous solid; yield, 3.21 g (83%); mp, 126–129°C. IR ν_{max} cm^{-1}: 3330, 1720, 1523, 1347, 1179. Analysis calculated for $C_{31}H_{43}O_7N \cdot 1/2H_2O$: C, 67.61; H, 8.05; N, 2.54. Found: C, 67.74; H, 7.76; N, 2.95.

p-Nitrophenyl deoxycholate 12-formate (8g)

The formate-acid **7g** (3.0 g) [prepared from **4b** (16)] was converted to its *p*-nitrophenyl ester **8g** by the method described for the preparation of **8f;** yield, 3.09 g (80%); mp, 118–120°C (light yellow amorphous solid from MeOH). IR ν_{max} cm^{-1}: 3380, 1715, 1525, 1348, 1207, 1183. Analysis calculated for $C_{31}H_{43}O_7N \cdot 1/4H_2O$: C, 68.17; H, 8.03; N, 2.56. Found: C, 68.27; H, 7.87; N, 2.77.

p-Nitrophenyl cholate 7,12-diformate (8h)

The formate-acid **7h** (3.0 g) [prepared from **4c** (16)] was converted to its *p*-nitrophenyl ester **8h** by the method described for the preparation of **8f;** yield, 2.82 g (72%); mp, 154–156°C (light yellow thin plates from MeOH). IR ν_{max} cm^{-1}: 3359, 1716, 1522, 1344, 1185. Analysis calculated for $C_{32}H_{43}O_9N$: C, 65.62; H, 7.40; N, 2.39. Found: C, 65.31; H, 7.46; N, 2.46.

p-Nitrophenyl chenodeoxycholate 3-glucoside tetraacetate-7-formate (9f)

The formate-*p*-nitrophenyl ester **8f** (1.0 g) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from aqueous acetone gave **9f** as colorless amorphous solid; yield, 900 mg (56%); mp, 214–218°C. IR ν_{max} cm^{-1}: 1758, 1720, 1527, 1368, 1230, 1042. Analysis calculated for $C_{45}H_{61}O_{16}N$: C, 61.98; H, 7.07; N, 1.61. Found: C, 62.04; H, 7.09; N, 1.78.

p-Nitrophenyl deoxycholate 3-glucoside tetraacetate-12-formate (9g)

The formate-*p*-nitrophenyl ester **8g** (1.0 g) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from acetone–hexane gave **9g** as colorless needles; yield, 790 mg (49%); mp, 196–197°C. IR ν_{max} cm^{-1}: 1757, 1718, 1526, 1348, 1224. Analysis calculated for $C_{45}H_{61}O_{16}N$: C, 61.98; H, 7.07; N, 1.61. Found: C, 61.74; H, 6.91; N, 1.83.

p-Nitrophenyl cholate 3-glucoside tetraacetate-7,12-diformate (9h)

The formate-*p*-nitrophenyl ester **8h** (1.0 g) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from acetone–hexane gave **9h** as colorless amorphous solid: yield, 693 mg (44%); mp, 210–214°C. IR ν_{max} cm^{-1}: 1758, 1717, 1526, 1348, 1227, 1179, 1040. Analysis calculated for $C_{46}H_{61}O_{18}N \cdot 1/2H_2O$: C, 59.73; H, 6.76; N, 1.51. Found: C, 59.84; H, 6.82; N, 1.54.

Ethyl glycochenodeoxycholate 3-glucoside tetraacetate-7-formate (10f)

To a stirred solution of the glucoside acetate-*p*-nitrophenyl ester **9f** (300 mg) in anhydrous pyridine (3 ml) was added dropwise glycine ethyl ester hydrochloride (150 mg) dissolved in dry pyridine (3 ml), and the mixture was stirred overnight at room temperature. The reaction mixture was poured onto water and extracted with EtOAc. The combined EtOAc layer was washed with water, dried with Drierite, and evaporated to give an oily residue. Chromatography of the oil on a column of silica gel (10 g) and elution with benzene–acetone (98:2, v/v) afforded the title compound **10f** which was recrystallized from aqueous MeOH as colorless amorphous solid; yield, 217 mg (75%); mp, 148–150°C. IR ν_{max} cm^{-1}: 1755, 1717, 1228, 1039. Analysis calculated for $C_{43}H_{65}O_{15}N$: C, 61.78; H, 7.84; N, 1.67. Found: C, 61.50; H, 7.79; N, 1.73.

Ethyl glycodeoxycholate 3-glucoside tetraacetate-12-formate (10g)

The glucoside acetate-*p*-nitrophenyl ester **9g** (300 mg) was treated with glycine ethyl ester hydrochloride, followed by column chromatographic purification as described for the preparation of **10f;** yield, 250 mg (87%);

Although this compound was homogeneous according to TLC and ^{1}HNMR analyses, it could not be crystallized. IR ν_{max} cm^{-1} 1755, 1717, 1224, 1039. Analysis calculated for $C_{43}H_{65}O_{15}N \cdot 2H_2O$: C, 59.22; H, 7.97; N, 1.61. Found: C, 59.14; H, 7.72; N, 1.63.

Ethyl glycocholate 3-glucoside tetraacetate-7,12-diformate (10h)

The glucoside acetate-*p*-nitrophenyl ester **9h** (300 mg) was treated with glycine ethyl ester hydrochloride, followed by column chromatographic purification as described for the preparation of **10f.** Recrystallization of the product from aqueous acetone gave **10h** as colorless needles; yield, 234 mg (81%); mp, 210–212°C. IR ν_{max} cm^{-1}: 1756, 1715, 1230, 1186, 1041. Analysis calculated for $C_{44}H_{65}O_{17}N \cdot 1/2H_2O$: C, 59.45; H, 7.48; N, 1.58. Found: C, 59.24; H, 7.43; N, 1.65.

Glycochenodeoxycholic acid 3-glucoside (2a)

A solution of the glycoconjugate ester-glucoside acetate **10f** (200 mg) in 5% methanolic KOH (6 ml) was refluxed for 1 h. The reaction mixture was diluted with water (13 ml) and then neutralized with 5% H_2SO_4. The solvent was evaporated under reduced pressure, and the reaction product was dissolved in absolute EtOH (2 ml). The insoluble material was filtered off and washed with EtOH. The combined mother liquor was evaporated to dryness to give the title compound **2a,** which was recrystallized from MeOH/Et_2O as colorless amorphous solid; yield, 112 mg (77%); mp, 156–159°C. IR ν_{max} cm^{-1}: 3384, 1652, 1075, 1036. Analysis calculated for $C_{32}H_{53}O_{10}N \cdot 4H_2O$: C, 56.20; H, 8.99; N, 2.05. Found: C, 56.31; H, 8.70; N, 2.02.

Glycodeoxycholic acid 3-glucoside (2b)

The glycoconjugate ester-glucoside acetate **10g** (200 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **2a,** yielded the crude acid. Recrystallization from MeOH/Et_2O gave the desired **2b** as colorless needles; yield, 116 mg (79%); mp, 213–216°C. IR ν_{max} cm^{-1}: 3386, 1604, 1083, 1042. Analysis calculated for $C_{32}H_{53}O_{10}N \cdot 5H_2O$; C, 54.76; H, 9.05; N, 2.00. Found: C, 54.38; H, 8.90; N, 1.96.

Glycocholic acid 3-glucoside (2c)

The glycoconjugate ester-glucoside acetate **10h** (200 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **2a,** yielded the crude acid. Recrystallization from MeOH/EtOAc gave the desired **2c** as colorless amorphous solid; yield, 120 mg (84%); mp, 190–193°C. IR ν_{max} cm^{-1}: 3385, 1595, 1075, 1039. Analysis calculated for $C_{32}H_{53}O_{11}N \cdot 3H_2O$: C, 56.37; H, 8.72; N, 2.05. Found: C, 56.11; H, 8.57; N, 2.00.

Taurochenodeoxycholic acid 3-glucoside tetraacetate-7-formate (11f)

Taurine (80 mg) in water (7 ml) was added to a solution of the glucoside acetate-*p*-nitrophenyl ester **9f** (300 mg) in pyridine (25 ml) and the mixture was stirred at room temperature for 6 h. The resulting solution was concentrated under reduced pressure and the oily residue was chromatographed on a column of silica gel (15 g). Elution with CH_2Cl_2/MeOH (5:1–2:1, v/v) afforded the title compound **11f** which was recrystallized from EtOAc/hexane as colorless amorphous solid; yield, 233 mg (79%); mp, 251–253°C. IR ν_{max} cm^{-1}: 3473, 1757, 1720, 1654, 1546, 1226, 1043. Analysis calculated for $C_{41}H_{63}O_{16}NS \cdot 2\ 1/2H_2O$: C, 54.53; H, 7.59; N, 1.55. Found: C, 54.39; H, 7.45; N, 1.53.

Taurodeoxycholic acid 3-glucoside tetraacetate-12-formate (11g)

The glucoside acetate-*p*-nitrophenyl ester **9g** (300 mg) was converted to the taurine derivative **11g** by the method described for the preparation of **11f;** yield, 258 mg (87%); Although this compound was homogeneous according to TLC and ^{1}HNMR analyses, it could not be crystallized. IR ν_{max} cm^{-1}: 3414, 1757, 1651, 1557, 1227, 1042. Analysis calculated for $C_{41}H_{63}O_{16}NS \cdot 2H_2O$: C, 55.08; H, 7.55; N, 1.57. Found: C, 55.18; H, 7.38; N, 1.37.

Taurocholic acid 3-glucoside tetraacetate-7,12-diformate (11h)

The glucoside acetate-*p*-nitrophenyl ester **9h** (300 mg) was converted to the taurine derivative **11h** by the method described for the preparation of **11f;** yield, 263 mg (89%); Although this compound was homogeneous according to TLC and ^{1}HNMR analyses, it could not be crystallized. IR ν_{max} cm^{-1}: 3417, 1756, 1718, 1645, 1558, 1228, 1180, 1043. Analysis calculated for $C_{42}H_{63}O_{18}NS \cdot H_2O$: C, 54.83; H, 7.12; N, 1.52. Found: C, 54.51; H, 6.90; N, 1.72.

Taurochenodeoxycholic acid 3-glucoside sodium salt (3a)

A solution of the tauroconjugate-glucoside acetate **11f** (200 mg) in 5% methanolic KOH (12 ml) was refluxed for 1 h. The reaction mixture was diluted with water (3 ml) and then neutralized with 5% H_2SO_4. Most of the solvent was evaporated under reduced pressure, and the residue was dissolved in absolute EtOH (2 ml). The insoluble material was filtered off and washed with EtOH. The combined mother liquor was evaporated to give an oily residue. The oil dissolved in water (2 ml) was subjected to ion-exchange chromatography on Dowex-50W-X8 (Na^+ form; 800 mg; Muromachi Kagaku Kogyo, Tokyo, Japan) and eluted with water. Recrystallization of the product from EtOH gave **3a** as colorless amorphous solid; yield, 128 mg (80%); mp, 235–237°C (dec.). IR

ν_{max} cm^{-1}: 3430, 1645, 1206, 1047. Analysis calculated for $C_{32}H_{54}O_{11}NNaS \cdot 2\ H_2O$: C, 53.39; H, 8.12; N, 1.94. Found: C, 53.33; H, 8.10; N, 2.00.

Taurodeoxycholic acid 3-glucoside sodium salt (3b)

The tauroconjugate-glucoside acetate **11g** (200 mg) was treated with 5% methanolic KOH, followed by Dowex-50W-X8 (Na^+ form) column as described for the preparation of **3a.** The crude salt **3b** was recrystallized from EtOH as colorless amorphous solid; yield, 116 mg (73%); mp, 184–186°C. IR ν_{max} cm^{-1}: 3430, 1641, 1205, 1045. Analysis calculated for $C_{32}H_{54}O_{11}NNaS \cdot 1/2H_2O$: C, 55.47; H, 8.00; N, 2.02. Found: C, 55.36; H, 8.00; N, 2.00.

Taurocholic acid 3-glucoside sodium salt (3c)

The tauroconjugate-glucoside acetate **11h** (200 mg) was treated with 5% methanolic KOH, followed by Dowex- 50W-X8 (Na^+ form) column as described for the preparation of **3a.** The crude salt **3c** was recrystallized from EtOH as colorless amorphous solid; yield, 112 mg (72%); mp, 195–197°C. IR ν_{max} cm^{-1}: 3406, 1644, 1215, 1045. Analysis calculated for $C_{32}H_{54}O_{12}NNaS \cdot 1/4H_2O$: C, 54.57; H, 7.80; N, 1.99. Found: C, 54.48; H, 7.70; N, 2.00.

p-Nitrophenyl lithocholate (12d)

LCA (**4d;** 5.0 g) was converted to its *p*-nitrophenyl ester **12d** by the method described for the preparation of **8f;** yield, 5.42 g (82%); mp, 158–160°C (colorless needles from aqueous MeOH) [lit. (17) mp, 156–158°C]. IR ν_{max} cm^{-1}: 1762, 1521, 1343, 1207. Analysis calculated for $C_{30}H_{43}O_5N$: C, 72.40; H, 8.71; N, 2.81. Found: C, 72.35; H, 8.72; N, 2.81.

p-Nitrophenyl ursodeoxycholate (12e)

UDCA (**4e;** 5.0 g) was converted to its *p*-nitrophenyl ester **12e** by the method described for the preparation of **8f;** yield, 5.80 g (89%). Although this compound was homogeneous according to TLC and ^{1}HNMR analyses, it could not be crystallized. IR ν_{max} cm^{-1}: 3327, 1767, 1524, 1346, 1112. Analysis calculated for $C_{30}H_{43}O_6N \cdot 1/4H_2O$: C, 69.54; H, 8.46; N, 2.70. Found: C, 69.69; H, 8.55; N, 2.84.

Methyl ursodeoxycholate 3-glucoside tetraacetate (14e)

UDCA (**4e**) was methylated by the usual MeOH/*p*-toluenesulfonic acid method. The methyl ester (1.0 g), subjected to the Koenigs-Knorr reaction and processed as described for the general procedure, afforded an oily product. Although this compound **14e** was homogeneous according to TLC and ^{1}HNMR analyses, it could not be crystallized; yield, 833 mg (46%). IR ν_{max} cm^{-1}: 3547, 1759, 1225, 1039. Analysis calculated for $C_{39}H_{60}O_{13} \cdot 4H_2O$: C, 57.91; H, 8.47. Found: C, 57.62; H, 8.27.

Lithocholic acid 3-glucoside (1d)

The *p*-nitrophenyl ester **12d** (1.0 g) was subjected to the Koenigs-Knorr reaction. After cooling the mixture, the precipitated solid was removed by filtration, and the filtrate was evaporated to give an oily residue. The oil was hydrolyzed with 5% methanolic KOH (30 ml) for 1 h, neutralized with 5% H_2SO_4 and then concentrated under reduced pressure. The residue obtained was redissolved in water (10 ml) and the aqueous solution was passed through a column of Cosmosil 140C_{18}-OPN (10 g; Nakarai Tesque, Tokyo, Japan). After successive washing with water and 20% MeOH, the desired compound was eluted with MeOH. Recrystallization of the eluate from MeOH/Et_2O gave **1d** as colorless amorphous solid; yield, 258 mg (26%); mp, 251–255°C. IR ν_{max} cm^{-1}: 3424, 1652, 1558, 1033. Analysis calculated for $C_{30}H_{50}O_8$: C, 66.88; H, 9.36. Found: C, 66.97; H, 9.26.

Ursodeoxycholic acid 3-glucoside (1e)

(a) The methyl ester-glucoside acetate **14e** (400 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **2a,** recrystallized from EtOH/EtOAc as colorless prism; yield, 250 mg (83%); mp, 214–216°C. IR ν_{max} cm^{-1}: 3387, 1711, 1078, 1023. Analysis calculated for $C_{30}H_{50}O_9 \cdot H_2O$; C, 62.91; H, 9.15. Found: C, 63.04; H, 8.85.

(b) The *p*-nitrophenyl ester **12e** (300 mg) was treated with α-acetobromoglucose/$CdCO_3$, followed by 5% methanolic KOH as described for the preparation of **1d.** Purification by Cosmosil 140C_{18}-OPN chromatography eluting with MeOH gave the desired **1e;** yield, 59 mg (18%). This compound was found to be identical, according to TLC and ^{1}HNMR comparisons, with **1e** prepared as described above (a).

Methyl glycolithocholate (15d)

To a stirred suspension of **4d** (2.0 g) and glycine methyl ester hydrochloride (2.0 g) in *N,N'*-dimethylformamide (DMF; 40 ml) were added dropwise diethylphosphoryl cyanide (DEPC; 2.0 g) and triethylamine (8 ml). After the mixture was stirred at room temperature for 5 h, the solution was diluted with water to near turbidity and allowed to stand until crystallization was complete. The precipitated solid was filtered, washed with water, and recrystallized from EtOAc/hexane as colorless amorphous solid of **15d**; yield, 2.17 g (91%); mp, 149–153°C. IR ν_{max} cm^{-1}: 3422, 1745, 1652. Analysis calculated for $C_{27}H_{45}O_4N$: C, 72.44; H, 10.13; N, 3.13. Found: C, 72.65; H, 10.02; N, 2.98.

Methyl glycolithocholate 3-glucoside tetraacetate (16d)

(a) The *p*-nitrophenyl ester **12d** (1.0 g) was subjected to the Koenigs-Knorr reaction. After cooling the reaction mixture, the precipitated solid was removed by filtration, and the filtrate was evaporated to an oily residue. To a solution of the crude oil in dry pyridine (10 ml) was added

dropwise glycine methyl ester hydrochloride (1.0 g) in dry pyridine (10 ml), and the mixture was stirred overnight at room temperature. The reaction product was extracted with EtOAc, and the organic layer was washed with water to neutrality, dried over Drierite, and evaporated to give an oily product. Chromatography of the oil on a silica gel column (50 g) and elution with C_6H_6–EtOAc 7:3 (v/v) gave the title compound **16d** which was recrystallized from aqueous acetone as colorless amorphous solid: yield, 340 mg (22%); mp, 137–139°C. IR ν_{max} cm^{-1}: 1752, 1655, 1232, 1040. Analysis calculated for $C_{41}H_{63}O_{13}N$: C, 63.30; H, 8.16; N, 1.80. Found: C, 63.43; H, 8.06; N, 1.65.

(b) The glycoconjugate ester **15d** (500 mg), subjected to the Koenigs-Knorr reaction, afforded **16d** (yield, 417 mg, 48%), identical with that prepared above according to mp, TLC and ^{1}HNMR comparisons.

Methyl glycoursodeoxycholate 3-glucoside tetraacetate (16e)

The *p*-nitrophenyl ester **12e** (1.0 g) was treated with α-acetobromoglucose/$CdCO_3$, followed by glycine methyl ester hydrochloride as described for the preparation of **16d** (Method a). After being processed analogously, the oily product was purified by chromatography on a column of silica gel (60 g). Elution with C_6H_6–EtOAc 7:3 (v/v) gave the title compound **16e** (yield, 430 mg, 28%) which was homogeneous according to TLC and ^{1}HNMR analyses, but failed to crystallize. IR ν_{max} cm^{-1}: 3403, 1755, 1228, 1039. Analysis calculated for $C_{41}H_{63}O_{14}N \cdot 1/2H_2O$: C, 61.33; H, 8.03; N, 1.74. Found: C, 61.07; H, 7.86; N, 1.73.

Glycolithocholic acid 3-glucoside (2d)

The glycoconjugate ester-glucoside acetate **16d** (300 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **2a,** yielded the crude acid. Recrystallization from MeOH/Et_2O gave the desired **2d** as colorless amorphous solid; yield, 161 mg (70%); mp, 164–168°C. IR ν_{max} cm^{-1}: 3387, 1734, 1648, 1078, 1036. Analysis calculated for $C_{32}H_{53}O_9N \cdot H_2O$: C, 62.62; H, 9.03; N, 2.28. Found: C, 62.53; H, 9.09; N, 2.45.

Glycoursodeoxycholic acid 3-glucoside (2e)

The glycoconjugate ester-glucoside acetate **16e** (300 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **2a,** yielded the crude acid. Recrystallization from MeOH/EtOAc gave the desired **2e** as colorless amorphous solid; yield, 168 mg (73%); mp, 188–190°C. IR ν_{max} cm^{-1}: 3364, 1600, 1403, 1052, 1032. Analysis calculated for $C_{32}H_{53}O_{10}N \cdot 6H_2O$: C, 53.40; H, 9.10; N, 1.95. Found: C, 53.00; H, 8.98; N, 1.95.

Taurolithocholic acid 3-glucoside sodium salt (3d)

To a stirred suspension of the unconjugated 3α-glucoside **1d** (250 mg) and taurine (250 mg) in dry DMF (10 ml) were added dropwise DEPC (250 mg) and triethylamine (1 ml). After further stirring for 2 h at room temperature, the mixture was adjusted to pH 12 with 1 M NaOH and loaded on an Amberlite XAD-2 (5 ml; Rohm and Haas, Philadelphia, PA) column, which was washed with water. Elution with MeOH afforded the desired **3d** which was recrystallized from EtOH/hexane as colorless amorphous solid; yield, 195 mg (63%); mp, 178–180°C. IR ν_{max} cm^{-1}: 3421, 1750, 1596, 1221, 1033. Analysis calculated for $C_{32}H_{54}O_{10}NNaS \cdot 2H_2O$: C, 54.60; H, 8.31; N, 1.99. Found C, 54.50; H, 8.35; N, 2.01.

Tauroursodeoxycholic acid 3-glucoside sodium salt (3e)

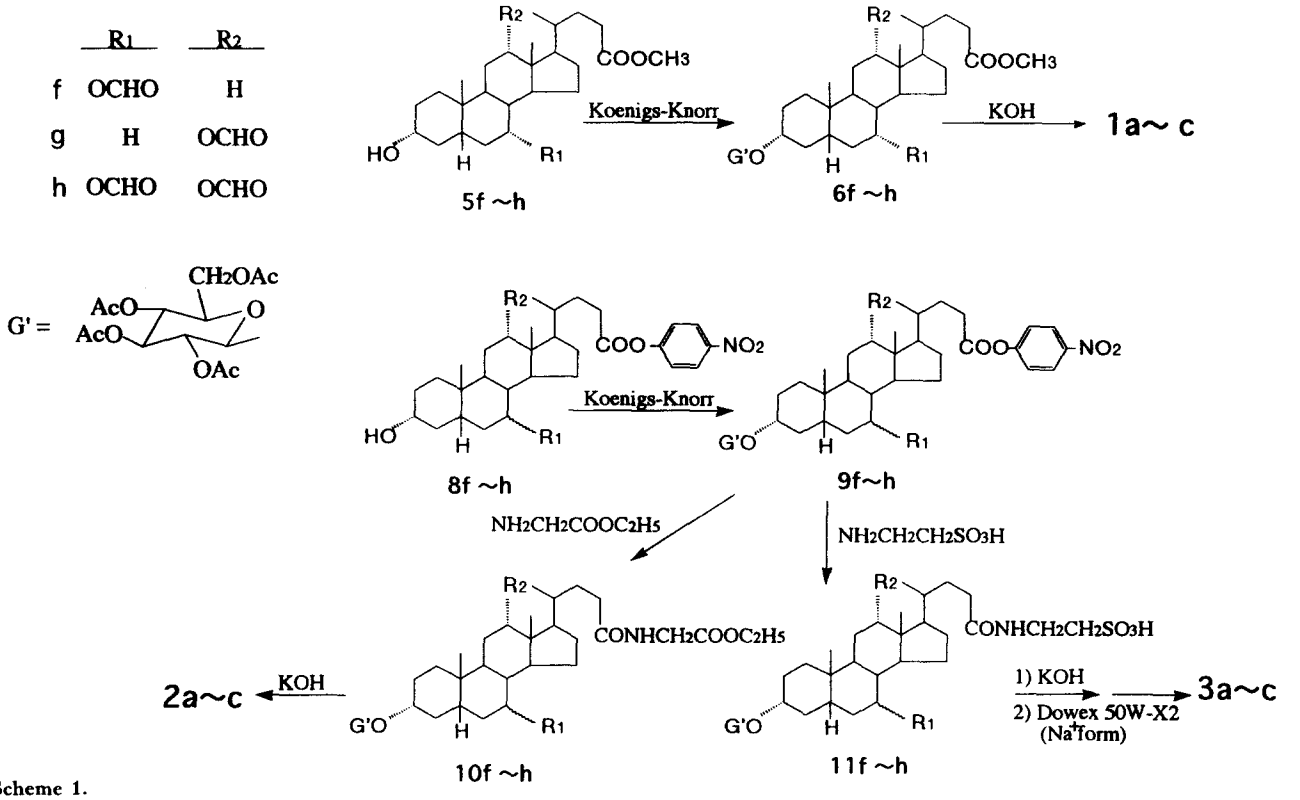
The unconjugated 3α-glucoside **1e** (200 mg) was treated with taurine/DEPC/triethylamine, followed by 1 M NaOH as described for the preparation of **3d.** After being processed analogously, the product was loaded on an Amberlite XAD-2 (4 ml) column. Elution with MeOH gave the desired salt **3e** which was recrystallized from EtOH/Et_2O as colorless prisms; yield, 140 mg (57%), mp, 176–178°C. IR ν_{max} cm^{-1}: 3422, 1636, 1213, 1048. Analysis calculated for $C_{32}H_{54}O_{11}NNaS \cdot 11/2H_2O$: C, 54.07; H, 8.08; N, 1.97. Found: C, 54.24; H, 8.05; N, 1.84.

RESULTS AND DISCUSSION

In principal, the desired 3-glucosides of nonamidated (**1a–e**) and amidated (**2a–e** and **3a–e**) bile acids could be prepared by a combined use of glycosidation (with α-acetobromoglucose) and amidation (with glycinate ester and taurine) of appropriate unconjugated bile acids, which have been used successfully in earlier studies of analogous steroid conjugates (10–14).

Our initial effort was directed to the synthesis of the nonamidated bile acid 3-glucosides (**1a–c**), except for **1d** and **1e** (see below). As summarized in **Scheme 1,** 3α-hydroxylated bile acid methyl ester-formates (**5f–h**) were chosen as the starting compounds, because the formyloxy derivatives are readily hydrolyzed to the corresponding free acids and crystallized more easily compared with the corresponding acetates (15). **5f–h** were readily obtained in three steps from the parent compounds (**4a–c**); the procedures involve *1)* methyl esterification, *2)* performylation with 99% formic acid, and then *3)* selective deformylation at C-3 with saturated methanolic ammonia (15).

The formation of 3-O-glucosidic linkage was carried out via the Koenigs-Knorr condensation reaction. Thus,



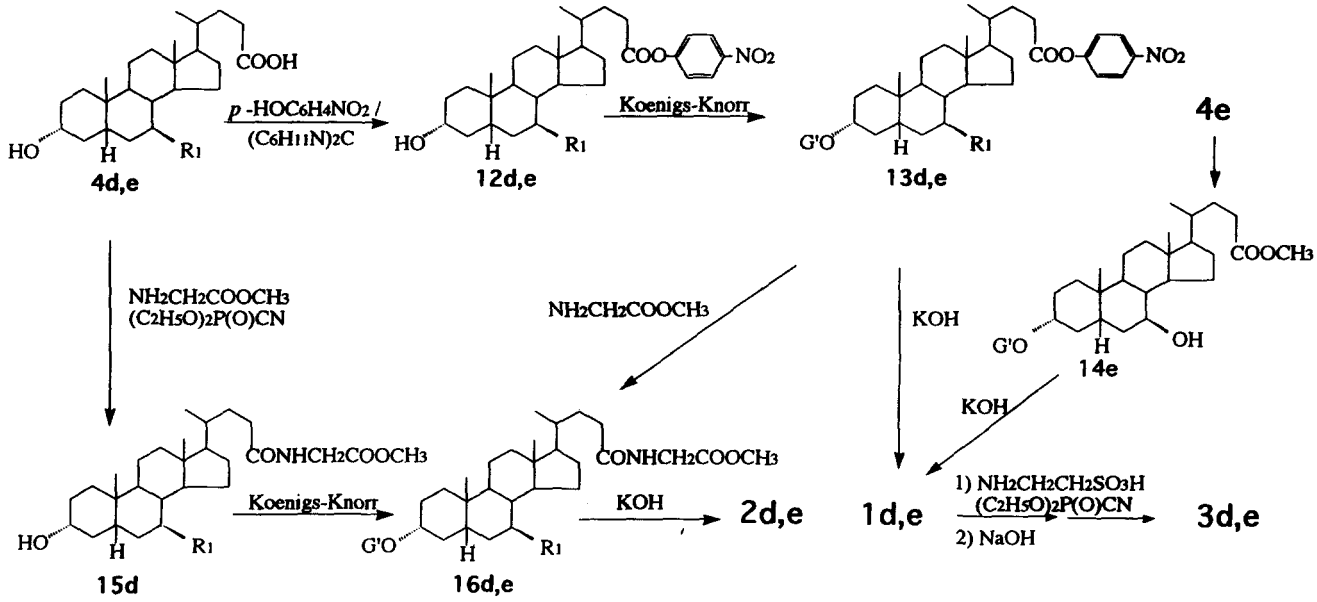
Scheme 1.

when the reaction with α-acetobromoglucose as a coupling reagent and $CdCO_3$ as a catalyst in refluxing benzene was applied to **5f–h** for 4–6 h, the compounds were converted to the bile acid 3-glucoside acetate-methyl esters (**6f–h**) in resonable yields (48–54%) after column chromatographic purification on silica gel. Subsequent elimination of the protecting groups of **6f–h** by treatment with 5% methanolic KOH (refluxing for 1 h), followed by neutralization with 5% H_2SO_4 resulted in simultaneous deformylation and deacetylation at both the sugar and aglycone moieties, together with hydrolysis of the methyl ester at C-24, providing the nonamidated bile acid 3-glucosides (**1a–c**).

Synthesis of the 3-glucosides of glycine- and taurine-amidated bile acids (**2a–c** and **3a–c,** respectively) was accomplished by the activated *p*-nitrophenyl ester method (11, 12). The key intermediates, 3-glucoside acetate-*p*-nitrophenyl esters (**9f–h**), were prepared from **4a–c** in four steps via the compounds **7f–h** through **8f–h;** the procedures consist of *1)* performylation (16), *2)* selective deformylation (**7f–h**) (16), *3) p*-nitrophenyl esterification with *p*-nitrophenol in the presence of DCC (**8f–h;** 72–83% yields) (11, 12), and *4)* glucosidation by the Koenigs-Knorr reaction (**9f–h;** 44–56% yields).

By carrying out the amidation at C-24 of **9f–h** with ethyl glycinate in pyridine at room temperature, analytically pure samples of the glycoconjugate ester-glucoside acetates (**10f–h**) were obtained in good isolated yields (75–87%) after chromatographic purification on silica gel. Alkaline hydrolysis of **10f–h** with 5% methanolic KOH, followed by neutralization with 5% H_2SO_4 to give the desired glycine-amidated bile acid 3-glucosides (**2a–c**) was straightforward. In a similar manner, treatment of **9f–h** with taurine in aqueous pyridine led to the amide formation of the tauroconjugate-glucoside acetates (**11f–h**) in isolated yield of 79–89%. Subsequent alkaline hydrolysis of **11f–h** afforded the corresponding taurine-amidated bile acid 3-glucosides as oily products. Because the hydrolyzed products resisted crystallization attempts, they were passed through the ion-exchange chromatography on a Dowex-50W-X8 (Na^+ form) column to yield the sodium salt (**3a–c**) as crystalline products.

In contrast with the clean reactions for CDCA, DCA, and CA derivatives mentioned above, when the methyl esters of LCA and protected UDCA 7-acetate [prepared in four steps from **4e** (11)] were subjected to the Koenigs-Knorr reaction, the target glucosidated derivatives were isolated in only poor yields (<8%) after careful chromatographic purification. This unsatisfactory result was ascribable to difficulty in the separation of the glucosidated products from the reaction mixtures. Preparation of the desired 3-glucosides of nonamidated and amidated LCA and UDCA (**1d, 1e, 2d, 2e, 3d,** and **3e**) was finally achieved by several routes as shown in **Scheme 2.**



Scheme 2.

Nonamidated UDCA 3-glucoside **1e** was prepared by two routes. First, UDCA *p*-nitrophenyl ester **12e,** without protection of the hydroxyl group at C-7, condensed with α-acetobromoglucose and then hydrolyzed with KOH, provided **1e** (18% from **12e**) (method b). In this route the intermediary glucoside acetate-*p*-nitrophenyl ester **13e** was used for subsequent hydrolysis without isolation, and the hydrolyzed product **1e** was purified by passing through a Cosmosil 140C_{18}-OPN reversed-phase column. LCA 3-glucoside **1d** was also synthesized by the sequence of reactions, via the *p*-nitrophenyl ester **12d** and its 3-glucosidated product **13d** (26% from **12d**). A second route (method a) is the glucosidation at C-3 of unprotected UDCA methyl ester, followed by alkaline hydrolysis of the resulting glucoside acetate-methyl ester **14e** (total yield from **4e;** 38%). The intermediate **14e** was easily purified on a column of silica gel. In both the routes, a direct glucosidation of the unprotected UDCA ester derivatives having two equatorially oriented hydroxyl groups at C-3 and C-7 yielded exclusively the C-3 glucosidated products, as expected from the work in the previous preparation of the corresponding UDCA 3-glucuronide and 3-*N*-acetylglucosamide reported by Goto et al. (11, 12). The result implies that the C-3 hydroxyl group is sterically less hindered and, hence, reacts with the bulky sugar reagent more easily than that at C-7. Of the two routes for the preparation of **1e,** we felt that the second route (method a) was the one of choice because of its simplicity and the ready availability of **14e.**

The synthesis of amidated LCA and UDCA 3-glucosides (**2d, e** and **3d, e**) was undertaken as follows. After the Koenigs-Knorr reaction of the *p*-nitrophenyl esters (**12d** and **12e**), the resulting crude glucoside acetate-*p*-nitrophenyl esters (**13d** and **13e**), which on the exchange reaction with methyl glycinate followed by alkaline hydrolysis, just as was **2a–c,** were converted to the 3-glucosides of glycine-amidated LCA (**2d**) and UDCA (**2e**), respectively. The intermediary glycoconjugate ester-glucoside acetates (**16d** and **16e**) were obtained in isolated yields of 22–28% after chromatographic purification on silica gel. **16d** was much more easily obtained by a direct coupling reaction of **4d** with methyl glycinate in DMF using DEPC and triethylamine as condensing agents (DEPC method) (18) and subsequent glucosidation of the resulting glycine-amidated LCA methyl ester **15d** (44% from **4d**).

Unexpectedly, when the crude activated *p*-nitrophenyl esters (**13d** and **13e**) were treated with taurine and processed as described for the preparation of **11f–h,** the desired tauroconjugate-glucoside acetates were obtained in only low isolated yields of less than 5%. Hence, the nonamidated LCA and UDCA 3-glucosides **1d** and **1e** were coupled directly with taurine by the DEPC method to give the corresponding tauroconjugates, which on treatment with NaOH were led to the desired 3-glucosides of taurine-amidated LCA and UDCA sodium salts (**3d** and **3e**) in resonable isolated yields (63 and 57%, respectively). To obtain pure **3d** and **3e** from the reaction mixtures, Amberlite XAD-2 resin was used as column chromatographic adsorbent.

The ^{1}HNMR spectra provided the confirmatory evidence for the structures of the desired 3-glucosides (**1a–e**). The results are compiled in **Table 1.** Proton signals arising from the β-glucosidic and amidic linkages are particu-

TABLE 1. ^{1}H-NMR spectral data for the 3-glucosides of nonamidated and amidated bile acid derivatives

	18-CH_3	19-CH_3	21-CH_3	3β-H	7β-H	7α-H	12β-H	C'$_1$-H	$OCH_2\underline{CH_3}$	$O\underline{CH_2}CH_3$	$>NCH_2CO-$	$>NCH_2CH_2S-$	-NH-	-CHO	$-COCH_3$	-COOCH$_3$	*p*-Substituted Phenyl
1a	0.67	0.92	0.95 (*d*, 6.5)	3.56	3.83			4.40 (*d*, 7.5)									
1b	0.61	0.83	0.91 (*d*, 6.5)	3.61			3.87	4.31 (*d*, 7.5)									
1c	0.70	0.91	1.00 (*d*, 6.5)	3.55	3.84		3.96	4.39 (*d*, 7.5)									
1d	0.65	0.93	0.93 (*d*, 5.0)					4.42 (*d*, 7.5)									
1e[a]	0.70	0.96	0.96 (*d*, 7.0)	3.50		3.71		4.40 (*d*, 8.0)									
2a	0.68	0.93	0.97 (*d*, 6.0)	3.57	3.83			4.41 (*d*, 7.5)			3.75						
2b	0.70	0.93	1.01 (*d*, 6.0)	3.52			3.97	4.40 (*d*, 7.5)			3.77						
2c	0.71	0.92	1.03 (*d*, 6.5)	3.57	3.83		3.97	4.40 (*d*, 7.5)			3.78						
2d[a]	0.67	0.94	0.96 (*d*, 6.5)	3.57				4.42 (*d*, 8.0)			3.75						
2e[a]	0.70	0.97	0.97 (*d*, 5.0)	3.50		3.68		4.40 (*d*, 7.5)			3.76						
3a	0.68	0.93	0.96 (*d*, 6.5)	3.57	3.83			4.40 (*d*, 7.7)				2.98 3.61 (*t*, 6.5)					
3b	0.70	0.93	1.00 (*d*, 6.0)				3.97	4.40 (*d*, 8.0)				2.98 3.62 (*t*, 6.5)					
3c	0.71	0.92	1.02 (*d*, 6.5)		3.82		3.96	4.39 (*d*, 8.0)				2.98 3.62 (*t*, 6.5)					
3d	0.67	0.94	0.95 (*d*, 5.0)					4.42 (*d*, 8.0)				2.98 3.62 (*t*, 6.5)					
3e[a]	0.69	0.96	0.96 (*d*, 7.0)	3.50		3.63		4.40 (*d*, 7.5)				2.98 3.63 (*t*, 6.5)					
6f	0.65	0.92	0.90 (*d*, 6.3)	3.59	5.04			4.57 (*d*, 7.2)						8.07	2.00 2.02 2.04 2.07	3.66	
6g	0.65	0.90	0.81 (*d*, 6.3)	3.57			5.22	4.56 (*d*, 8.1)						8.09	2.00 2.02 2.07	3.66	
6h	0.74	0.92	0.84 (*d*, 6.3)	3.40	5.03		5.23	4.55 (*d*, 8.1)						8.10 8.14	2.00 2.02 2.03 2.07		
8f	0.68	0.94	1.00 (*d*, 5.4)	3.47	5.03									8.08			7.27 8.26 (*dd*, 9.9)
8g	0.77	0.91	0.89 (*d*, 5.4)	3.60			5.26							8.13			7.27 8.27 (*dd*, 9.9)
8h	0.78	0.93	0.91 (*d*, 5.4)	3.58	5.06		5.28							8.10 8.16			7.27 8.27 (*dd*, 9.9)
9f	0.67	0.93	1.00 (*d*, 6.3)	3.60	5.04			4.57 (*d*, 7.2)						8.07	2.00 2.02 2.05 2.07		7.27 8.27 (*dd*, 9.0)
9g	0.76	0.90	0.88 (*d*, 6.3)	3.65			5.22	4.57 (*d*, 7.2)						8.11	2.00 2.03 2.07		7.27 8.27 (*dd*, 9.0)
9h	0.77	0.93	0.91 (*d*, 6.3)	3.65	5.03		5.26	4.55 (*d*, 7.2)						8.10 8.15	2.00 2.03 2.07		7.27 8.26 (*dd*, 9.0)
10f	0.65	0.92	0.94 (*d*, 6.4)	3.66	5.04			4.57 (*d*, 7.2)	1.29 (*t*, 7.2)	4.22 (*q*, 7.2)	4.02 (*d*, 5.4)		5.94 (*t*, 5.4)	8.07	2.00 2.02 2.04 2.07		
10g	0.72	0.89	0.87 (*d*, 6.4)	3.65			5.22	4.57 (*d*, 7.2)	1.28 (*t*, 7.2)	4.22 (*q*, 7.2)	4.03 (*d*, 5.4)		6.15 (*t*, 5.4)	8.08	2.02 2.07		
10h	0.74	0.91	0.92 (*d*, 6.4)	3.68	5.03		5.22	4.54 (*d*, 7.2)	1.28 (*t*, 7.2)	4.21 (*q*, 7.2)	4.01 (*d*, 4.5)		5.90 (*t*, 5.4)	8.10 8.13	2.00 2.02 2.03 2.07		
11f	0.67	0.96	0.95 (*d*, 6.5)	3.50	5.02			4.66 (*d*, 8.0)				2.99 3.61 (*t*, 6.5)		8.10	2.02 2.05 2.07 2.09		
11g	0.76	0.92	0.84 (*d*, 6.5)				5.24	4.64 (*d*, 8.0)				2.98 3.61 (*t*, 6.0)		8.13	2.02 2.04 2.05 2.09		

TABLE 1. Continued

	18-CH_3	19-CH_3	21-CH_3	3β-H	7β-H	7α-H	12β-H	C'$_1$-H	$OCH_2\underline{CH_3}$	$O\underline{CH_2}CH_3$	$>NCH_2CO-$	$>NCH_2CH_2S-$	-NH-	-CHO	$-COCH_3$	$-COOCH_3$	*p*-Substituted Phenyl
11h	0.77	1.00	0.85 (*d*, 6.5)	3.45	5.04		5.25	4.61 (*d*, 8.0)				2.98 3.61 (*t*, 6.5)		8.12 8.16	2.02 2.04 2.05 2.09		
12d	0.67	0.92	0.98 (*d*, 6.3)	3.64											2.03 2.08		7.27 8.27 (*dd*, 9.0)
12e	0.70	0.95	1.00 (*d*, 6.3)	3.56		3.56											7.27 8.26 (*dd*, 9.0)
14e	0.67	0.94	0.92 (*d*, 6.3)	3.55		3.55		4.58 (*d*, 7.2)							2.03 2.08	3.66	
15d	0.64	0.92	0.90 (*d*, 6.3)	3.62							4.04 (*d*, 5.4)		6.01			3.76	
16d	0.64	0.91	0.89 (*d*, 6.3)	3.63				4.59 (*d*, 8.1)			4.05 (*d*, 5.4)		5.96		2.03 2.08	3.77	
16e	0.68	0.94	0.92 (*d*, 6.3)	3.59		3.59		4.57 (*d*, 7.2)			4.05 (*d*, 5.4)		5.99		2.04 2.08	3.76	

Values in parentheses refer to signal multiplicity and coupling constant (Hz): *d*, doublet; *t*, triplet; *dd*, double doublet; *q*, quartet.
[a]Solvent, CD_3OD.

larly useful for their characterization. The anomeric C'$_1$-H signal of the sugar moiety resonated at 4.31 ~ 4.42 ppm as a doublet (J = 7.5–8.0 Hz), indicating a *trans*-diaxial relationship to the vicinal C'$_2$-H. A signal appearing at 3.75 ~ 3.78 ppm as a singlet in the spectra of glycine-amidated compounds (**2a–e**) is assigned to the methylene protons at C-25 in the side chain. On the other hand, two pairs of a triplet signal (J = 6.5 Hz) occurred at 2.98 and 3.61 ~ 3.63 ppm in the spectra of taurine-amidated compounds (**3a–e**) are assigned to the methylene protons at C-25 and C-26.

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