Synthesis of *N*-acetylglucosaminides of unconjugated and conjugated bile acids

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3-N-Acetylglucosaminides of unconjugated, glycine- and taurine-conjugated bile acids have been synthesized. Bile acids appropriately protected were condensed with acetochloroglucosamine through the 3α-hydroxyl group by means of the Koenigs-Knorr reaction using cadmium carbonate as a catalyst. Subsequent borohydride reduction and/or alkaline hydrolysis provided desired 3-N-acetylglucosaminides of unconjugated bile acids. Glycine-conjugates were obtained from N-acetylglucosaminides of unconjugated bile acids and ethyl glycinate by the carbodiimide method. The preparation of N-acetylglucosaminides of taurine-conjugates was attained by the Koenigs-Knorr reaction of bile acid p-nitrophenyl esters followed by condensation with taurine. 7-N-Acetylglucosaminides of ursodeoxycholates were prepared in a similar fashion. The convenient synthesis of 3-N-acetylglucosaminides of unconjugated bile acids is also described. (*Steroids* **57**:522–529, 1992)

Keywords: bile acid; *N*-acetylglucosaminide; glycine-conjugate; taurine-conjugate; Koenigs-Knorr reaction; *p*-nitrophenyl ester method

Introduction

Over the decades considerable attention has been directed to the metabolism of bile acids in connection with hepatobiliary diseases.[1,2] A particular interest has been focused on the physiological significance of metabolic conjugation at the hydroxyl group on the steroid nucleus. For the purpose of clarifying these problems, we have previously synthesized glucuronides,[3,4] monosulfates,[5] and disulfates[6] of unconjugated, glycine- and taurine-conjugated bile acids as standard specimens, and developed the methods for determination of these bile acids in biological fluids by high-performance liquid chromatography.[7–10]

Recently, the occurrence of ursodeoxycholic acid *N*-acetylglucosaminide, a novel conjugate, in human urine has been reported.[11] However, the complete structures of bile acid *N*-acetylglucosaminides still remain unclear owing to the lack of standard samples. The authentic specimens are therefore required to establish a reliable method for the analysis of bile acid *N*-acetylglucosaminides in biological fluids with regard to metabolic disorders in humans. This article deals with the preparation of 3-*N*-acetylglucosaminides of unconjugated, glycine- and taurine-conjugated bile acids, and 7-*N*-acetylglucosaminides of ursodeoxycholates.

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Experimental

All melting points (mp) were determined on a micro-hot stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX-100 or GX-500 spectrometer at 100 or 500 MHz, respectively, with trimethylsilane as an internal standard (chemical shifts in δ ppm) and the data are collected in Table 1. Unconjugated bile acids were purchased from Sigma Chemicals (St. Louis, MO, USA) and ursodeoxycholic acid was kindly donated by Tokyo Tanabe (Tokyo, Japan).

General procedure for the Koenigs-Knorr reaction

To a solution of bile acid (500 mg) in anhydrous toluene (25 ml) were added freshly prepared $CdCO_3$ (500 mg) and 2-acetamido-1α-chloro-1,2-dideoxy-3,4,6-tri-O-acetyl-D-glucopyranose (acetochloroglucosamine)[12] (500 mg), and the resulting mixture was azeotropically refluxed with stirring. After 3 and 6 hours, additional portions of sugar (250 mg) and $CdCO_3$ (250 mg) were added, and the mixture was further refluxed for 2 hours. The precipitate was removed by filtration and washed with AcOEt. 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 hexane/AcOEt and recrystallization of the eluate gave *N*-acetylglucosaminide triacetate.

Table 1 NMR spectral data for *N*-acetylglucosaminides of bile acids and related compounds

Compound	Solvent	18-CH_3	19-CH_3	21-CH_3	3β-H	7β-H	7α-H	12β-H	C_1-H[a]	NCH_2CO	NCH_2CH_2S	$COCH_3$
2	C	0.72	1.17	0.80 d, 6 Hz	3.55			5.05	4.90			1.91, 2.02, 2.07, 2.10
3	C	0.73	0.88	0.81 d, 5 Hz	3.58	3.83		5.04	4.88			1.92, 2.03, 2.09
4	C	0.69	0.90	0.98 d, 5 Hz	3.53	3.80		3.95	4.96			1.95, 2.03, 2.04, 2.08
6	M	0.71	0.92	1.00 d, 5 Hz	3.50	3.80		3.92	4.56			1.96
7	M/C	0.69	0.90	1.00 d, 5 Hz	3.50	3.80		3.94	4.54	3.76		2.00
8	M/C	0.70	0.91	1.02 d, 6 Hz	3.55	3.83		3.96	4.54		2.99, 3.58 t, 6 Hz	2.00
10	C	0.64	1.18	0.91 d, 6 Hz	3.60				4.86			1.94, 2.02, 2.03, 2.10
11	C	0.64	0.87	0.90 d, 6 Hz	3.58	3.80			4.92			1.92, 2.00, 2.06
13	M	0.68	0.92	0.96 d, 6 Hz	3.60	3.80			4.55			1.97
14	M/C	0.67	0.91	0.92 d, 6 Hz	3.55	3.80			4.52	3.73		2.00
15	M/C	0.67	0.91	0.95 d, 6 Hz	3.55	3.82			4.58		2.98, 3.62 t, 6 Hz	2.00
17	C	0.71	0.88	0.79 d, 6 Hz	3.65			5.03	4.82			1.92, 2.03, 2.04, 2.08
18	C	0.67	0.90	0.96 d, 6 Hz	3.60			3.95	4.94			1.94, 2.03, 2.04, 2.08
20	M	0.70	0.90	1.00 d, 6 Hz	3.59			3.92	4.54			1.96
21	M/C	0.70	0.92	1.01 d, 6 Hz	3.55			3.94	4.55	3.76		1.99
22	M/C	0.69	0.91	1.09 d, 6 Hz	3.57			3.95	4.59		2.97, 3.62 t, 6 Hz	2.00
24	C	0.66	0.94	0.91 d, 6 Hz	3.60		4.65		4.94			1.95, 1.97, 2.02, 2.04, 2.09
25	C	0.67	0.93	0.93 d, 5 Hz	3.60		3.60		4.86			1.94, 2.04, 2.08
27	M	0.70	0.95	0.95 d, 5 Hz	3.59		3.59		4.52			1.98
28	M/C	0.69	0.95	0.95 d, 5 Hz	3.55		3.55		4.56	3.77		2.01
29	M/C	0.68	0.94	0.95 d, 5 Hz	3.54		3.54		4.59		2.97, 3.61 t, 6 Hz	2.01
30	C	0.67	1.03	0.92 d, 6 Hz			3.65		4.74			1.92, 2.03, 2.08
32	C	0.62	0.92	0.92 d, 6 Hz	3.64		3.64		4.72			1.91, 2.02, 2.08
34	M/C	0.65	0.93	0.93 d, 6 Hz	3.61		3.61		4.50			1.98
35	M/C	0.65	0.93	0.94 d, 6 Hz	3.60		3.60		4.51	3.80		1.99
36	M/C	0.65	0.94	0.95 d, 5 Hz	3.57		3.57		4.51		2.98, 3.61 t, 6 Hz	1.98
37	C	0.63	0.90	0.90 d, 6 Hz	3.63				4.88			1.96, 2.03, 2.04, 2.08
39	M	0.68	0.93	0.95 d, 6 Hz	3.60				4.52			1.96
40	M/C	0.68	0.94	0.98 d, 6 Hz	3.60				4.54	3.76		1.99
41	M/C	0.65	0.92	0.93 d, 6 Hz	3.61				4.59		2.98, 3.59 t, 7 Hz	2.01

C, $CDCl_3$; M, CD_3OD;
d, doublet; t, triplet.
[a] Doublet, coupling constant J = 8 Hz.

*Methyl 12α-acetoxy-7-oxolithocholate 3-*N*-acetylglucosaminide triacetate (2)*

Methyl 12α-acetoxy-7-oxolithocholate[4] **(1)** (700 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from ether gave **2** (435 mg) as colorless needles; mp 170–172 C, $[\alpha]_D^{27}$ +20.4° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{41}H_{61}NO_{14}$: C, 62.18; H, 7.76; N, 1.77. Found: C, 61.99; H, 7.76; N, 1.82.

*Methyl 12α-acetoxychenodeoxycholate 3-*N*-acetylglucosaminide triacetate (3)*

To a solution of **2** (160 mg) in MeOH (8 ml) was added $NaBH_4$ (40 mg) under ice-cooling and the resulting solution was stirred for 30 minutes. The reaction mixture was diluted with water, acidified with 5% HCl, and then extracted with AcOEt. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated down to dryness under reduced pressure. Recrystallization of the crude product from ether gave **3** (109 mg) as colorless needles; mp 139–141 C, $[\alpha]_D^{27}$ +35.1° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{41}H_{63}NO_{14}$: C, 62.03; H, 8.00; N, 1.76. Found: C, 62.12; H, 8.08; N, 1.53.

*Methyl cholate 3-*N*-acetylglucosaminide triacetate (4)*

Methyl cholate (900 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from ether gave **4** (350 mg) as colorless crystals; mp 209–210 C, $[\alpha]_D^{27}$ −40.1° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{39}H_{61}NO_{13}$: C, 62.30; H, 8.18; N, 1.86. Found: C, 62.11; H, 8.15; N, 2.03.

*Cholic acid 3-*N*-acetylglucosaminide (6)*

To a solution of **3** (200 mg) in MeOH (2 ml) was added 20% KOH in 70% MeOH (1.2 ml) and the mixture was refluxed for 3 hours. The reaction mixture was diluted with water, neutralized with 5% HCl and then concentrated under reduced pressure. The residue obtained was redissolved in water (3 ml) and the aqueous solution was passed through a column [25 × 23 mm internal diameter (ID)] of PrepPAK-500/C18 (3 g, Waters Chromatography Division, Millipore Co., Milford, MA, USA). After successive washing with water (20 ml) and 30% ethanol (20 ml), *N*-acetylglucosaminide was eluted with 60% ethanol (20 ml). Recrystallization of the eluate from MeOH/ether gave **6** (95 mg) as colorless crystals; mp 240–242 C (dec), $[\alpha]_D^{26}$ + 4.4° (c = 0.10, MeOH). Analysis calculated for $C_{32}H_{53}NO_{10} \cdot 3H_2O$: C, 57.72; H, 8.93; N, 2.10. Found: C, 57.38; H, 8.89; N, 2.03.

Treatment of **4** (350 mg) with methanolic KOH in the manner described above followed by recrystallization of the crude product from MeOH/ether gave **6** (256 mg); mp 238–241 C decomposed (dec). Mixed melting point on admixture with the sample obtained above showed no depression.

*Glycocholic acid 3-*N*-acetylglucosaminide (7)*

To a solution of **6** (150 mg) in anhydrous dioxane (8 ml) were added ethyl glycinate (200 mg) in anhydrous dioxane (0.4 ml) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide · HCl (150 mg), and the resulting solution was stirred at room temperature overnight. After evaporation of the solvent, the residue obtained was purified by passing through a column of PrepPAK-500/C18 (25 × 23 mm ID) in the manner described for **6** to give ethyl glycocholate 3-*N*-acetylglucosaminide (150 mg), which in turn was dissolved in 3% methanolic KOH (10 ml). The solution was

refluxed for 2 hours, neutralized with 5% HCl, and then subjected to the purification on PrepPAK-500/C18 in the manner described for **6**. Recrystallization from MeOH/ether gave **7** (47 mg) as a colorless amorphous substance; mp 169–171 C (dec); $[\alpha]_D^{25}$ +17.1° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{56}N_2O_{11} \cdot H_2O$: C, 59.46; H, 8.51; N, 4.08. Found: C, 59.55; H, 8.22; N, 4.12.

*Taurocholic acid 3-*N*-acetylglucosaminide* **(8)**

p-Nitrophenyl cholate[5] (320 mg) was subjected to the Koenigs-Knorr reaction to give corresponding 3-*N*-acetylglucosaminide triacetate (160 mg), which in turn was dissolved in pyridine (18 ml). After addition of taurine (48 mg) in H_2O (4.5 ml), the solution was stirred at room temperature overnight. The reaction mixture was concentrated and subjected to methylation with diazomethane in the usual manner. The crude product obtained was dissolved in H_2O and extracted with AcOEt to remove **6** formed as a by-product. The aqueous layer was passed through a column of PrepPAK-500/C18 in the manner described for **6** and taurocholic acid 3-*N*-acetylglucosaminide triacetate **(5)** (83 mg) obtained was subjected to alkaline hydrolysis. Recrystallization of the crude product from MeOH/ether gave **8** (59 mg) as a colorless amorphous substance; mp 197–200 C (dec), $[\alpha]_D^{28}$ +28.9° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{58}N_2O_{12}S \cdot 3H_2O$: C, 52.83; H, 8.35; N, 3.62. Found: C, 52.99; H, 8.36; N, 3.69.

*Methyl 7-oxolithocholate 3-*N*-acetylglucosaminide triacetate* **(10)**

Methyl 7-oxolithocholate[4] **(9)** (500 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from acetone/hexane gave **10** (536 mg) as colorless needles; mp 236–238 C, $[\alpha]_D^{26}$ −11.5° (c = 0.09, $CHCl_3$). Analysis calculated for $C_{39}H_{59}NO_{12} \cdot 1/2H_2O$: C, 63.05; H, 8.14; N, 1.89. Found: C, 62.71; H, 8.10; N, 2.06.

*Methyl chenodeoxycholate 3-*N*-acetylglucosaminide triacetate* **(11)**

Treatment of **10** (540 mg) in the manner described for **3** followed by recrystallization from ether gave **11** (350 mg) as colorless needles; mp 130–132 C, $[\alpha]_D^{26}$ 0.0° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{39}H_{61}NO_{12}$: C, 63.65; H, 8.35; N, 1.90. Found: C, 63.61; H, 8.28; N, 1.88.

Methyl chenodeoxycholate (540 mg) was subjected to the Koenigs-Knorr reaction and an oily product obtained was chromatographed on silica gel (50 g). Elution with AcOEt/ether (1 : 20–1 : 15) and recrystallization of the eluate from ether gave **11** (376 mg) as colorless needles; mp 129–132 C. Mixed melting point on admixture with the sample obtained above showed no depression.

*Chenodeoxycholic acid 3-*N*-acetylglucosaminide* **(13)**

Treatment of **11** (200 mg) with methanolic KOH in the manner described for **6** followed by recrystallization from MeOH/ether gave **13** (76 mg) as colorless needles; mp 166–169 C, $[\alpha]_D^{27}$ −9.3° (c = 0.10, MeOH). Analysis calculated for $C_{32}H_{53}NO_9 \cdot H_2O$: C, 62.62; H, 9.03; N, 2.28. Found: C, 62.53; H, 8.71; N, 2.21.

*Glycochenodeoxycholic acid 3-*N*-acetylglucosaminide* **(14)**

Treatment of **13** (160 mg) in the manner described for **7** followed by recrystallization from MeOH/ether gave **14** (107 mg) as a colorless amorphous substance; mp 155–157 C, $[\alpha]_D^{25}$ −0.4° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{56}N_2O_{10} \cdot H_2O$: C, 60.88; H, 8.71; N, 4.18. Found: C, 60.83; H, 8.36; N, 4.18.

*Taurochenodeoxycholic acid 3-*N*-acetylglucosaminide* **(15)**

Treatment of *p*-nitrophenyl chenodeoxycholate (310 mg) in the manner described for **8** followed by recrystallization from MeOH/ether gave **15** (26 mg) as a colorless amorphous substance; mp 171–173 C, $[\alpha]_D^{28}$ −6.5° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{58}N_2O_{11}S \cdot 2H_2O$: C, 55.27; H, 8.46; N, 3.79. Found: C, 55.41; H, 8.51; N, 4.06.

*Methyl 12α-acetoxylithocholate 3-*N*-acetylglucosaminide triacetate* **(17)**

Methyl 12α-acetoxylithocholate[13] **(16)** (600 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from acetone/hexane gave **17** (425 mg) as colorless needles; mp 170–173 C, $[\alpha]_D^{27}$ +45.1° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{41}H_{63}NO_{13}$: C, 63.30; H, 8.16; N, 1.80. Found: C, 63.01; H, 8.16; N, 1.83.

*Methyl deoxycholate 3-*N*-acetylglucosaminide triacetate* **(18)**

Methyl deoxycholate (1g) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product from acetone/hexane gave **18** (863 mg) as colorless prisms; mp 197–200 C, $[\alpha]_D^{24}$ +11.2° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{39}H_{61}NO_{12}$: C, 63.65; H, 8.35; N, 1.90. Found: C, 63.45; H, 8.34; N, 1.93.

*Deoxycholic acid 3-*N*-acetylglucosaminide* **(20)**

Treatment of **17** (200 mg) with methanolic KOH in the manner described for **6** and recrystallization of the crude product from MeOH/ether gave **20** (145 mg) as a colorless amorphous substance; mp 214–216 C (dec), $[\alpha]_D^{28}$ +45.1° (c = 0.10, MeOH). Analysis calculated for $C_{32}H_{53}NO_9 \cdot 3H_2O$: C, 59.15; H, 9.15; N, 2.16. Found: C, 59.38; H, 9.34; N, 2.24.

Treatment of **18** (500 mg) with methanolic KOH in the manner described for **6** and recrystallization of the crude product from MeOH/ether gave **20** (340 mg) as a colorless amorphous substance; mp 214–216 C (dec). Mixed melting point on admixture with the sample obtained above showed no depression.

*Glycodeoxycholic acid 3-*N*-acetylglucosaminide* **(21)**

Treatment of **20** (120 mg) in the manner described for **7** and recrystallization from MeOH/ether gave **21** (30 mg) as a colorless amorphous substance; mp 192–194 C (dec), $[\alpha]_D^{25}$ +23.6° (c = 0.11, MeOH). Analysis calculated for $C_{31}H_{56}N_2O_{10} \cdot 5/2H_2O$: C, 58.51; H, 8.81; N, 4.01. Found: C, 58.66; H, 8.74; N, 4.03.

*Taurodeoxycholic acid 3-*N*-acetylglucosaminide* **(22)**

Treatment of *p*-nitrophenyl deoxycholate in the manner described for **8** gave **22** (45 mg) as a colorless amorphous substance; mp 203–206 C, $[\alpha]_D^{29}$ +24.9° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{58}N_2O_{11}S \cdot 2H_2O$: C, 55.27; H, 8.46; N, 3.79. Found: C, 54.96; H, 8.31; N, 3.90.

Methyl 7β-acetoxylithocholate 3-N-acetylglucosaminide triacetate (24)

Methyl ursodeoxycholate 7-acetate[5] **(23)** (300 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from acetone/hexane gave **24** (188 mg) as colorless needles; mp 216–218 C, $[\alpha]_D^{26}$ +20.6° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{41}H_{63}NO_{13}$: C, 63.30; H, 8.16; N, 1.80. Found: C, 63.46; H, 8.37; N, 1.89.

Methyl ursodeoxycholate 3-N-acetylglucosaminide triacetate (25)

Methyl ursodeoxycholate (560 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from acetone/hexane gave **25** (456 mg) as colorless needles; mp 224–225 C, $[\alpha]_D^{28}$ −6.0° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{39}H_{61}NO_{12}$: C, 63.65; H, 8.35; N, 1.90. Found: C, 63.61; H, 8.29; N, 1.89.

Ursodeoxycholic acid 3-N-acetylglucosaminide (27)

Treatment of **24** (100 mg) with methanolic KOH in the manner described for **6** and recrystallization from MeOH/ether gave **27** (63 mg) as a colorless amorphous substance; mp 200–202 C (dec), $[\alpha]_D^{28}$ +41.2° (c = 0.10, MeOH). Analysis calculated for $C_{32}H_{53}NO_9 \cdot 3/2\ H_2O$: C, 61.71; H, 9.06; N, 2.25. Found: C, 61.41; H, 9.36; N, 2.53.

Treatment of **25** (405 mg) with methanolic KOH in the manner described for **6** and recrystallization from MeOH/ether gave **27** (272 mg) as a colorless amorphous substance; mp 198–200 C (dec). Mixed melting point on admixture with the sample obtained above showed no depression.

Glycoursodeoxycholic acid 3-N-acetylglucosaminide (28)

Treatment of **27** (150 mg) in the manner described for **7** and recrystallization from MeOH/ether gave **28** (67 mg) as a colorless amorphous substance; mp 159–161 C, $[\alpha]_D^{26}$ +28.8° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{56}N_2O_{10}$: C, 62.56; H, 8.65; N, 4.29. Found: C, 62.34; H, 8.71; N, 4.16.

Tauroursodeoxycholic acid 3-N-acetylglucosaminide (29)

Treatment of *p*-nitrophenyl ursodeoxycholate (200 mg) in the manner described for **8** and recrystallization from MeOH/ether gave **29** (12 mg) as a colorless amorphous substance; mp 200–203 C, $[\alpha]_D^{26}$ +24.4° (c = 0.11, MeOH). Analysis calculated for $C_{34}H_{58}N_2O_{11}S \cdot 3H_2O$: C, 53.95; H, 8.52; N, 3.70. Found: C, 54.07; H, 8.37; N, 3.67.

Methyl 7β-hydroxy-3-oxo-5β-cholan-24-oate 7-N-acetylglucosaminide triacetate (30)

Methyl 7β-hydroxy-3-oxo-5β-cholan-24-oate[14] (340 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from acetone/hexane gave **30** (80 mg) as colorless needles; mp 207–209 C, $[\alpha]_D^{24}$ +34.8° (c = 0.09, $CHCl_3$). Analysis calculated for $C_{39}H_{59}NO_{12} \cdot 1/2H_2O$: C, 63.05; H, 8.14; N, 1.89. Found: C, 62.89; H, 8.00; N, 1.91.

Methyl ursodeoxycholate 7-N-acetylglucosaminide triacetate (32)

Treatment of **30** (75 mg) in the manner described for **3** followed by recrystallization from acetone/hexane gave **32** (32 mg) as colorless needles; mp 140–141 C, $[\alpha]_D^{25}$ +18.0° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{39}H_{61}NO_{12}$: C, 63.65; H, 8.35; N, 1.90. Found: C, 63.52; H, 8.36; N, 1.95.

Ursodeoxycholic acid 7-N-acetylglucosaminide (34)

Treatment of **32** (130 mg) with methanolic KOH in the manner described for **6** followed by recrystallization from MeOH/ether gave **34** (80 mg) as a colorless amorphous substance; mp 180–181 C (dec), $[\alpha]_D^{26}$ +50.2° (c = 0.10, MeOH). Analysis calculated for $C_{32}H_{53}NO_9 \cdot 1/2H_2O$: C, 63.55; H, 9.00; N, 2.32. Found: C, 63.53; H, 8.97; N, 2.31.

Glycoursodeoxycholic acid 7-N-acetylglucosaminide (35)

Treatment of **34** (35 mg) in the manner described for **7** and recrystallization of the crude product from MeOH/ether gave **35** (14 mg) as a colorless amorphous substance; mp 163–165 C, $[\alpha]_D^{20}$ +44.4° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{56}N_2O_{10} \cdot H_2O$: C, 60.88; H, 8.71; N, 4.18. Found: C, 60.89; H, 8.64; N, 4.22.

Tauroursodeoxycholic acid 7-N-acetylglucosaminide (36)

Treatment of *p*-nitrophenyl 7β-hydroxy-3-oxo-5β-cholan-24-oate[14] (270 mg) in the manner described for **8** followed by reduction with $NaBH_4$, and recrystallization of the crude product from MeOH/ether gave **36** (10 mg) as a colorless amorphous substance; mp 162–164 C (dec), $[\alpha]_D^{20}$ +39.5° (c = 0.11, MeOH). Analysis calculated for $C_{34}H_{58}N_2O_{11}S \cdot H_2O$: C, 56.64; H, 8.39; N, 3.89. Found: C, 56.71; H, 8.37; N, 3.79.

Methyl lithocholate 3-N-acetylglucosaminide triacetate (37)

Methyl lithocholate (200 mg) was subjected to the Koenigs-Knorr reaction and recrystallization of the crude product obtained from acetone/hexane gave **37** (293 mg) as colorless needles; mp 211–214 C, $[\alpha]_D^{27}$ +21.5° (c = 0.10, $CHCl_3$). Analysis calculated for $C_{39}H_{61}NO_{11} \cdot H_2O$: C, 63.48; H, 8.60; N, 1.90. Found: C, 63.66; H, 8.59; N, 1.90.

Lithocholic acid 3-N-acetylglucosaminide (39)

Treatment of **37** (60 mg) with methanolic KOH in the manner described for **6** and recrystallization from AcOEt gave **39** (30 mg) as colorless crystals; mp 223–225 C (dec), $[\alpha]_D^{28}$ +10.9° (c = 0.10, MeOH). Analysis calculated for $C_{32}H_{53}NO_8 \cdot 1/2H_2O$: C, 65.28; H, 9.24; N, 2.38. Found: C, 65.48; H, 9.17; N, 2.38.

Glycolithocholic acid 3-N-acetylglucosaminide (40)

Treatment of **39** (50 mg) in the manner described for **7** and recrystallization of the crude product from MeOH/ether gave **40** (18 mg) as a colorless amorphous substance; mp 232–233 C, $[\alpha]_D^{26}$ +10.3° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{56}N_2O_9 \cdot 3H_2O$: C, 59.11; H, 9.05; N, 4.05. Found: C, 59.07; H, 8.84; N, 3.79.

Taurolithocholic acid 3-N-acetylglucosaminide (41)

Treatment of *p*-nitrophenyl lithocholate (200 mg) in the manner described for **8** and recrystallization of the crude product from

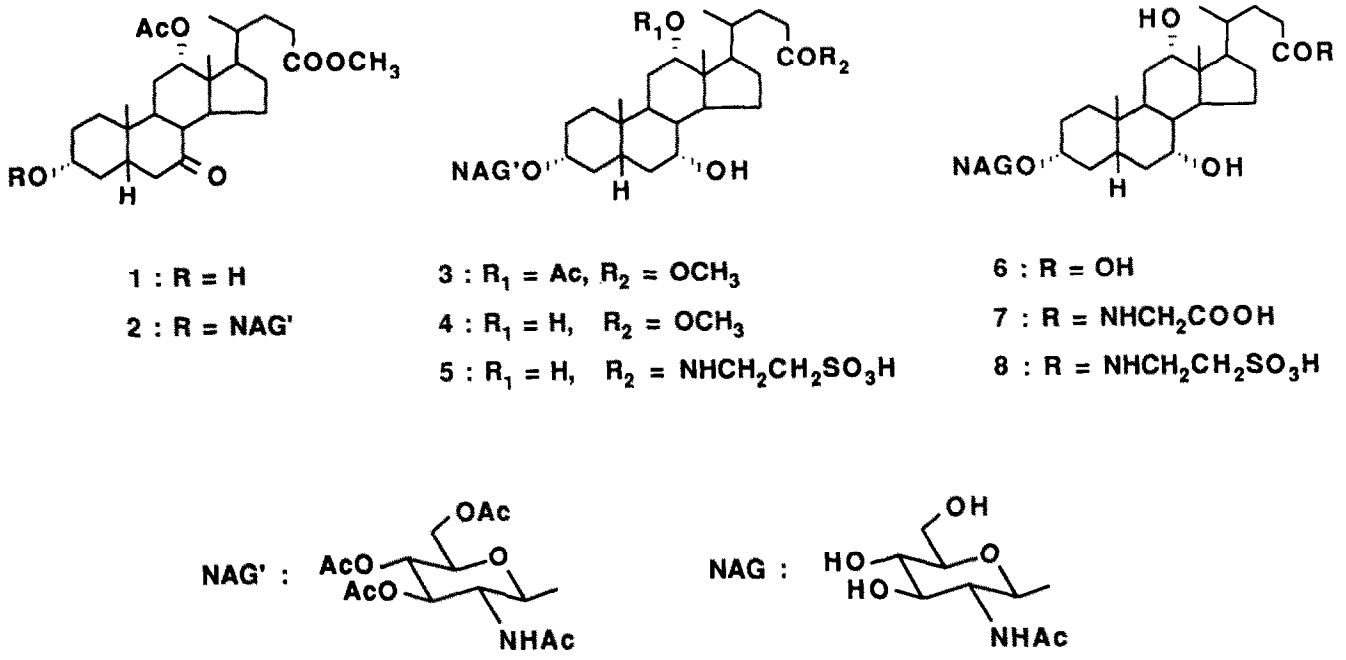


Figure 1 Cholate 3-*N*-acetylglucosaminides and related compounds.

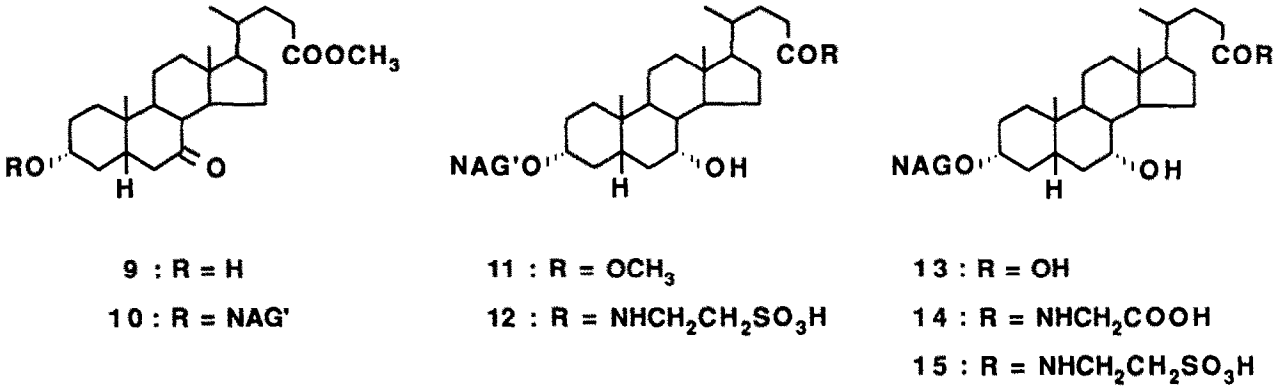
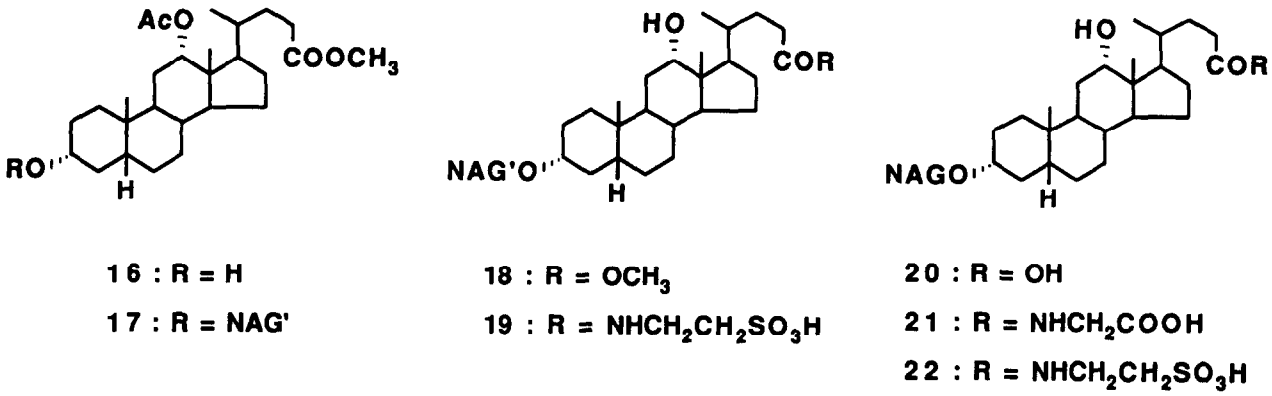


Figure 2 Chenodeoxycholate 3-*N*-acetylglucosaminides and related compounds.

MeOH/ether gave **41** (42 mg) as a colorless amorphous substance; mp 195–198 C, $[\alpha]_D^{29}$ +6.0° (c = 0.10, MeOH). Analysis calculated for $C_{34}H_{58}N_2O_{10}S$: C, 59.45; H, 8.51; N, 4.08. Found: C, 59.33; H, 8.56; N, 3.92.

Results and discussion

Our initial effort was directed to the synthesis of 3-*N*-acetylglucosaminides of unconjugated bile acids by an unequivocal route. For this purpose, bile acids appropriately protected at C-7 and/or C-12 were needed as key compounds. Thus methyl 12α-acetoxy-7-oxo- **(1)**, 7-oxo- **(9)**, and 12α-acetoxylithocholate **(16)**, obtainable from methyl cholate, chenodeoxycholate, and deoxycholate by oxidation with *N*-bromosuccinimide and/or acetylation followed by partial hydrolysis, respectively, were taken as pertinent starting materials. Methyl lithocholate and methyl ursodeoxycholate 7-acetate **(23)**, derivable from methyl ursodeoxycholate by selective oxidation with the Fetizon's reagent,[15] acetylation, and then borohydride reduction were also used. Introduction of the *N*-acetylglucosamine residue into the 3α-hydroxyl group was achieved by the use of the Koenigs-Knorr reaction[16] with acetochloroglucosamine in anhydrous toluene using cadmium carbonate as a catalyst, yielding the corresponding 3-*N*-acetylglucosaminide triacetates **(2, 10, 17, 24, 37)**. Subsequent reduction of the 7-oxo group with sodium borohydride and/or removal of protecting groups in both steroid and sugar moieties with methanolic potassium hydroxide gave desired 3-*N*-acetylglucosaminides of unconjugated bile acids **(6, 13, 20, 27, 39)** (Figures 1–5). Similarly, methyl 7β-hydroxy-3-oxo-5β-cholan-24-oate was subjected to the Koenigs-Knorr reaction to provide the 7-*N*-acetylglucosaminide triace-

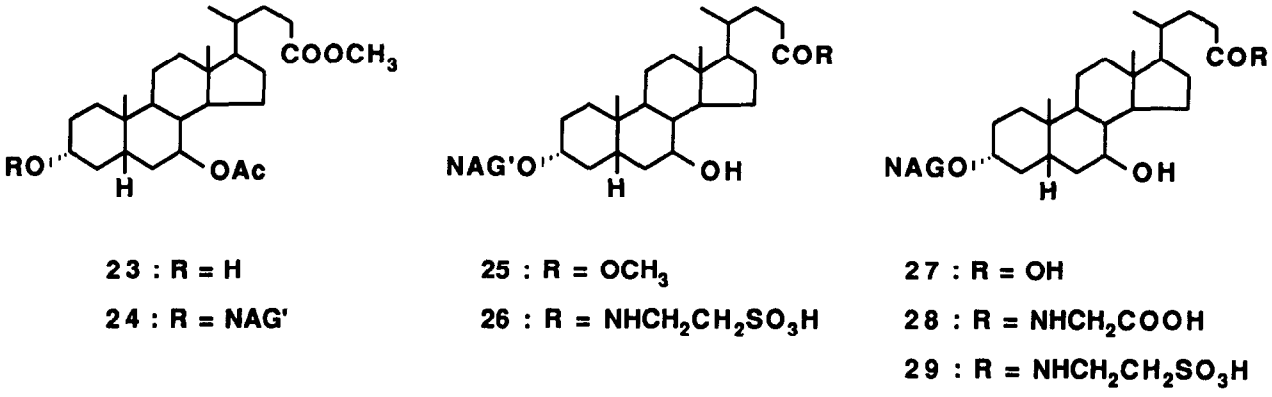


16 : R = H
17 : R = NAG'

18 : R = OCH_3
19 : R = $NHCH_2CH_2SO_3H$

20 : R = OH
21 : R = $NHCH_2COOH$
22 : R = $NHCH_2CH_2SO_3H$

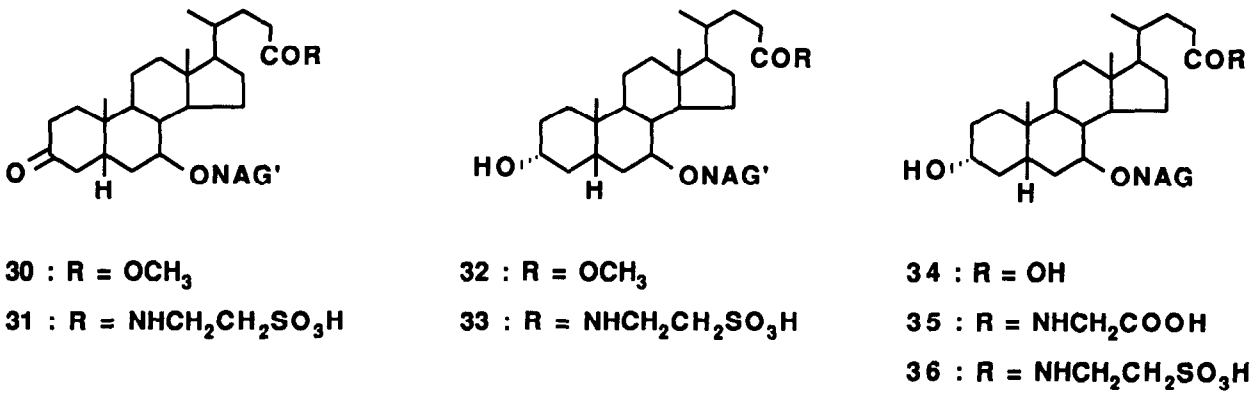
Figure 3 Deoxycholate 3-*N*-acetylglucosaminides and related compounds.



23 : R = H
24 : R = NAG'

25 : R = OCH_3
26 : R = $NHCH_2CH_2SO_3H$

27 : R = OH
28 : R = $NHCH_2COOH$
29 : R = $NHCH_2CH_2SO_3H$



30 : R = OCH_3
31 : R = $NHCH_2CH_2SO_3H$

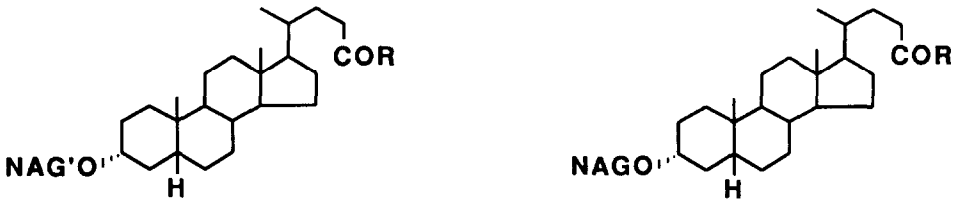
32 : R = OCH_3
33 : R = $NHCH_2CH_2SO_3H$

34 : R = OH
35 : R = $NHCH_2COOH$
36 : R = $NHCH_2CH_2SO_3H$

Figure 4 Ursodeoxycholate 3- and 7-*N*-acetylglucosaminides and related compounds.

tate **(30)**, which on borohydride reduction followed by alkaline hydrolysis was readily transformed into ursodeoxycholic acid 7-*N*-acetylglucosaminide **(34)**.

It is well known that the equatorial hydroxyl group at C-3 is sterically less hindered and, hence, reacts with a bulky reagent more easily than those at C-7 and C-12. Accordingly, the convenient synthesis of unconjugated bile acid 3-*N*-acetylglucosaminides was then performed. Upon completion of the Koenigs-Knorr reaction, methyl cholate, chenodeoxycholate, and deoxycholate gave 3-*N*-acetylglucosaminide triacetates **(4, 11, 18)** as sole products in the yields of 50–60%. As for methyl ursodeoxycholate having two hydroxyl groups of equatorial nature at C-3 and C-7, the sugar moiety was predominantly introduced into the C-3 position **(25)**. Besides, a trace amount of the isomeric C-7 conjugate **(32)** was also formed as judged by TLC. After elimination of protecting groups by alkaline hydrolysis, the structures of these compounds were confirmed by direct comparison with authentic specimens **(6, 13, 20, 27)** on TLC. This facile synthetic route is useful for obtaining the desired 3-*N*-acetylglucosaminide.



37 : R = OCH_3

38 : R = $NHCH_2CH_2SO_3H$

39 : R = OH

40 : R = $NHCH_2COOH$

41 : R = $NHCH_2CH_2SO_3H$

Figure 5 Lithocholate 3-*N*-acetylglucosaminides and related compounds.

Next, the synthesis of 3- and 7-*N*-acetylglucosaminides of glycine- and taurine-conjugated bile acids was undertaken. *N*-acetylglucosaminides of unconjugated bile acids **(6, 13, 20, 27, 34, 39)** were subjected to the coupling reaction with ethyl glycinate in dioxane using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as a condensing agent to give ethyl esters of glycine-conjugates, which on hydrolysis with methanolic potassium hydroxide were led to desired glycine-conjugated bile acid *N*-acetylglucosaminides **(7, 14, 21, 28, 35, 40)**.

On the contrary, the preparation of taurine-conjugates in a similar manner could not be effected due to the lack of suitable solvents for dissolving both bile acids and taurine. As described above, the Koenigs-Knorr reaction of bile acid methyl esters provided solely 3-*N*-acetylglucosaminide triacetates. Accordingly, an alternative synthetic route using the activated ester method was then taken. The Koenigs-Knorr reaction of *p*-nitrophenyl esters of bile acids occurred readily at the 3α-hydroxyl group, yielding *N*-acetylglucosaminide triacetates, which on the exchange reaction with taurine **(5, 12, 19, 26, 38)** followed by alkaline hydrolysis were converted to desired taurine-conjugated bile acid 3-*N*-acetylglucosaminides **(8, 15, 22, 29, 41)**. Similarly, tauroursodeoxycholate 7-*N*-acetylglucosaminide **(36)** was synthesized from *p*-nitrophenyl 7β-hydroxy-3-oxo-5β-cholan-24-oate by the Koenigs-Knorr reaction and subsequent treatment with taurine **(31)** and then with sodium borohydride.

The NMR spectral properties of *N*-acetylglucosaminides and related compounds are listed in Table 1. The data are indicative of the formation of the β-D-glucopyranoside structure. The anomeric proton signal of the sugar moiety appeared at 4.52–4.95 ppm as a doublet (J = 8 Hz), indicating a *trans*-diaxial relationship to the vicinal 2′-proton. The β-glucoside linkage in these *N*-acetylglucosaminides was further confirmed by characterizing bile acids liberated by incubation with a β-*N*-acetylglucosaminidase preparation.

The chromatographic separation of *N*-acetylglucosaminides of unconjugated, and glycine- and taurine-conjugated bile acids by high-performance liquid chromatography is being conducted in these laboratories, and the details will be reported elsewhere.

Acknowledgments

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Names and abbreviations

Cholic acid	3α,7α,12α-trihydroxy-5β-cholan-24-oic acid
Chenodeoxycholic acid	3α,7α-dihydroxy-5β-cholan-24-oic acid
Deoxycholic acid	3α,12α-dihydroxy-5β-cholan-24-oic acid
Ursodeoxycholic acid	3α,7β-dihydroxy-5β-cholan-24-oic acid
Lithocholic acid	3α-hydroxy-5β-cholan-24-oic acid

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