# Studies in the Ganglioside Series. III. Synthesis of 4-*O*-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose[1]

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The Koenigs–Knorr reaction of the stable bromide I with 2-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (II) gave a mixture of the two disaccharides III and VI which led, respectively, to the title compound X and its 1→3 isomer XII. The N-dichloroacetyl derivative XIV was found to be extremely labile to weak alkali and could be converted into X by catalytic hydrogenolysis of the chlorine atoms.

One of the primary objectives of these studies has been the synthesis of the title compound (X). This disaccharide forms a part of the complex ganglioside molecule.[2-4] The synthesis of glycosides of this type has hitherto been hampered by the lack of a stable hexosaminyl bromide necessary for the Koenigs–Knorr reaction. In recent communications[5,6] we described the preparation of a new highly stable and reactive bromide of the type I which enabled us to synthesize several amino sugar disaccharides. We now report the synthesis of N-acetylgalactosaminyl(β 1→4)galactose (X, Scheme I).

Condensation of 2-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (II) with the bromide I gave a mixture of the two isomers III and VI in a ratio of 3:2. After the reaction product was passed successively through a silica gel and a silica gel G column, the isomers were obtained as homogeneous products by crystallization.

For introduction of the N-acetyl group, the isomeric disaccharides III and VI were deacylated catalytically by absolute-methanolic barium methoxide, and the resulting products were treated at room temperature with aqueous barium hydroxide to remove the protective dichloroacetyl group. Subsequent acetylation afforded IV and VII. Their structure was proven by converting them, respectively, into the deacetylated glycosides V and VIII. The former compound consumed 2 mol of periodate, whereas the latter reacted with 1 mol of the reagent.

The disaccharides IV and VII were found to be rather sensitive to the reagents employed for the opening of the anhydro ring, temperature and time being important factors. Thus the latter isomer suffered almost complete cleavage of the glycosidic bond when the reaction was carried out for 2.5 hr at 55° as described for the glucosamine analog.[5] At temperatures ranging from 17 to 25° with a reaction time of 4 hr, satisfactory yields of IX and XI were obtained, although some cleavage could not be prevented. Deacetylation of IX and XI by barium methoxide yielded the disaccharides X and XII, respectively.

Yamakawa and coworkers[7] have assigned the structure XII to a disaccharide which was isolated from hydrolyzates of kidney glycolipids. However, the specific rotation of the synthetic compound (+56°) deviated from that reported for the natural product (+46°). It should be noted that the synthetic disaccharide was homogeneous on tlc in various solvent systems and that the infrared spectrum did not contain an absorption at 11.7 μ characteristic of an α isomer.

In an alternative route, the 1,6-anhydro ring was opened at an earlier stage, namely, in the N-dichloroacetyl derivative III.

The reaction, which was accompanied by acetylation of the free C-3 hydroxyl of the galactosan moiety, proceeded more satisfactorily, since almost no cleavage of the glycosidic linkage had taken place. It gave a high yield of the hepta-*O*-acyl derivative XIII, which was deacylated to XIV. However, an attempt to hydrolyze the dichloroacetyl group in the latter compound led to a complete rupture of the glycosidic linkage. Since N-acetylgalactosaminyl(β 1→4)galactose (Kuhn's ganglio-N-biose II)[3] was reported to be rather stable to alkali, this result must be attributed to the strong electrophilic character of the dichloroacetyl group, which may withdraw electrons from the glycosidic bond. This consideration finds its parallel in the glycoside of N-dichloroacetylsphingosine, $CH_3(CH_2)_{12}$-$CH{=}CHCH(OH)CH(NHCOCHCl_2)CH_2O$ - galactose, which undergoes cleavage by very mild alkaline treatment.[8]

In the preceding paper of this series,[6] we have shown that the dichloroacetyl group can be directly converted into the acetyl group by catalytic hydrogenolysis of the chlorine atoms. Following this procedure, compound XIV gave a satisfactory yield of the final disaccharide X. This behavior, together with other favorable properties described previously,[5,6] make the dichloroacetyl group particularly suitable for the protection of the amine function in the synthesis of alkali labile amino sugar disaccharides.

## Experimental Section[9]

**2-*O*-Acetyl-1,6-anhydro-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-dichloroacetamido-β-D-galactopyranosyl)-β-D-galactopyranose (III).**—The freshly prepared crude bromide I[6] (15 mmol) was dissolved in dry ethylene chloride (100 ml), 2-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (II, 22 mmol) and mercuric cyanide (15 mmol) were added, and the mixture was stirred at 40° for 7 days with protection from light. The cooled, slightly turbid solution was then poured into a mixture of ice–water and chloroform, and the organic layer was shaken thoroughly with 5% sodium hydrogen carbonate and washed with water. The residue obtained after evaporation of the solvent showed on tlc (benzene–methanol, 185:15) the presence of four products, in addition to the starting materials. Two of them were separated by a silica gel column and

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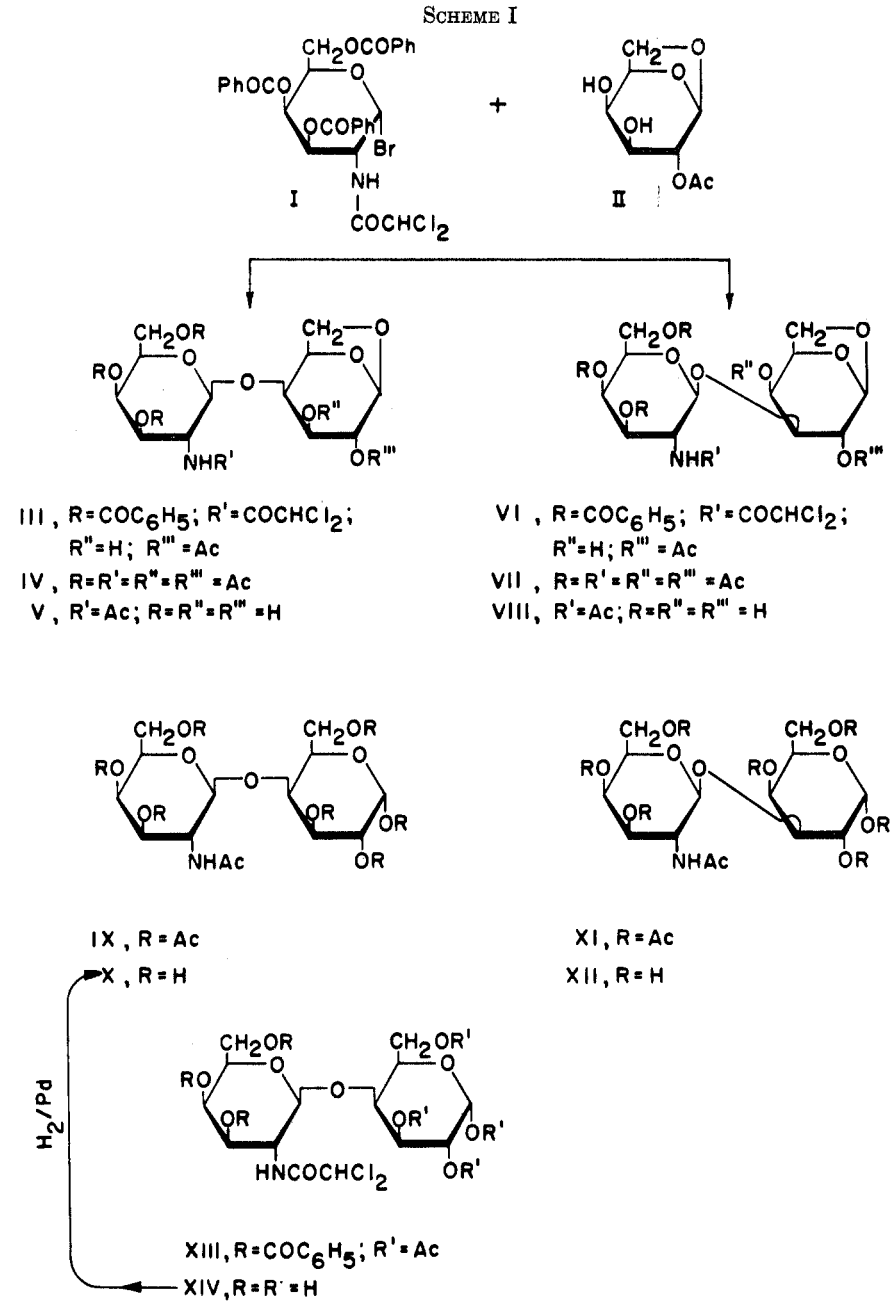
(9) Details concerning specification of chemicals and the type of apparatus used for physical measurements are given in paper I of this series. Optical rotations were determined in 1% chloroform solutions, unless stated otherwise.

SCHEME I




were identified as the debromo derivative of I and the 1→1 digalactosamine derivative. Methylene chloride–ether (4:1) eluted 5.2 g (45%) of a mixture of two compounds, whose nmr spectra agreed with the disaccharides III and VI. Crystallization from ether and recrystallization from 2-propanol yielded 2.3 g of III. A second crop was obtained after the separation of VI as described below: mp 194–195°; $[\alpha]^{20}D$ −2.3°; tlc $R_{II}$ = 1.6. The nmr spectrum showed signals at $\tau$ 1.85–2.75 (15 aromatic H), 4.10, and 7.95 (1-dichloroacetyl and 3-acetyl H).

*Anal.* Calcd for $C_{37}H_{35}Cl_2NO_{14}$: C, 56.35; H, 4.47; Cl, 8.99. Found: C, 56.45; H, 4.60; Cl, 8.70.

**2-*O*-Acetyl-1,6-anhydro-3-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-dichloroacetamido-β-D-galactopyranosyl)-β-D-galactopyranose (VI).**—The residue obtained from the evaporation of the combined mother liquors of III was dissolved in methylene chloride and the solution was passed through a column of silica gel G. Methylene chloride–ether (94:6) eluted 1.8 g of VI, 0.6 g of a mixture of both isomers, and finally 0.7 g of III. Crystallization of VI from ethanol–hexane (19:1) gave 1.6 g: mp 139–140°; $[\alpha]^{20}D$ +5.0°; tlc [benzene–methanol (185:15)] $R_{III}$ = 1.36. In the nmr spectrum a shift of the acetyl protons was observed which appeared at $\tau$ 8.01 as compared with $\tau$ 7.95 found in III.

*Anal.* Calcd for $C_{37}H_{35}Cl_2NO_{14}$: C, 56.35; H, 4.47; Cl, 8.99. Found: C, 56.34; H, 4.33; Cl, 8.93.

**2,3-Di-*O*-acetyl-1,6-anhydro-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranose (IV).**—To a solution of III (1.54 g) in absolute methanol (25 ml) was added, at −10°, 1 *N* methanolic barium methoxide (2 ml). After 3 hr at 2° the deacylation was complete. For the hydrolysis of the dichloroacetyl group, more barium methoxide (6 ml) and water (3 ml) were added, and the solution was allowed to stand at room temperature for 20 hr. The resulting suspension was neutralized with methanolic hydrogen chloride, and the solvent was evaporated *in vacuo*. The thoroughly dried residue was acetylated with acetic anhydride (10 ml) and pyridine (15 ml), and the resulting product was purified by a silica gel G column. Elution with methylene chloride–ether (3:1) gave 1 g (90%) of IV. After crystallization from ethyl acetate–isopropyl ether (8:2), the product had mp 122–123°; $[\alpha]^{22}D$ −27.2°; tlc [benzene–methanol (8:2)] $R_{III}$ = 0.6.

*Anal.* Calcd for $C_{24}H_{33}NO_{15}$: C, 50.08; H, 5.78. Found: C, 49.81; H, 6.00.

**2,4-Di-*O*-acetyl-1,6-anhydro-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranose (VII).**—The 1→3 isomer was converted into the N-acetyl derivative by the sequence of reactions described above. The product eluted from the column with methylene chloride–ether (7:3) was crystallized from ether and recrystallized from ethanol–hexane (19:1): yield 70%; mp 208°; $[\alpha]^{21}D$ −66.1°; tlc [benzene–

methanol (9:1)] $R_{VI}$ = 0.65, $R_{IV}$ = 1.2. The nmr spectrum showed signals of six 3-acetyl protons at $\tau$ 7.90, 7.94, 7.94, 8.01, 8.01, and 8.03.

*Anal.* Calcd for $C_{24}H_{33}NO_{15}$: C, 50.08; H, 5.78. Found: C, 50.11; H, 5.64.

**Periodate Oxidation of V and VIII.**—After deacetylation of the ester groups of IV and VII with barium methoxide, the resulting products were subjected to periodate oxidation as described previously for the glucosamine analogs.[5] Compound IV consumed 2.2 mol, whereas its 3-*O* isomer reacted with 1.05 mol of the reagent.

**1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranose (IX).**—Opening of the anhydro ring in IV was effected by treating 0.57 g with acetic anhydride (15 ml), glacial acetic acid (6 ml), and concentrated sulfuric acid (0.10 ml) at 25–26° for 4 hr. Anhydrous sodium acetate (1 g) was then added, and the suspension was taken to dryness. The residue was extracted with chloroform; the extract was washed with water and evaporated *in vacuo*. Methylene chloride–ether (6:4) eluted from a silica gel G column 0.46 g (70%) of a homogenous substance. After crystallization from ethanol–water (8:2) it had mp 104–106°; $[\alpha]^{22}D$ +42.7°; tlc [benzene–methanol (9:1)] $R_{IV}$ = 0.79. The nmr spectrum showed signals at $\tau$ 7.84, 7.90, 7.90, 7.94, 7.94, 8.01, 8.01, and 8.04 (eight acetyl groups).

*Anal.* Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80. Found: C, 49.54; H, 5.65.

**4-*O*-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (X).**—A solution of IX (0.180 g) in absolute methanol (20 ml) was treated with 1 *N* barium methoxide (0.1 ml) during 4 hr at 2°. The solution was neutralized by stirring with Dowex 50W-X, filtered, and taken to dryness. Crystallization from methanol–ether (1:1) yielded 85 mg (83.5%) of a hygroscopic powder: mp 148–150°; $[\alpha]^{28}D$ +55.5° (*c* 1, water); tlc [benzene–methanol (1:2)] $R_{lactose}$ = 0.76, $R_{IX}$ = 0.45.

*Anal.* Calcd for $C_{14}H_{25}NO_{11}\cdot{}^1/_2H_2O$: C, 42.86; H, 6.68; N, 3.57. Found: C, 42.70; H, 6.72; N, 3.70.

This disaccharide was also obtained by hydrogenolysis of XIV.

**1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranose (XI).**—The ring opening in VII was carried out at 17–19°. Methylene chloride–ether (7:3) eluted 60% of the product, which was crystallized from ether–hexane: mp 96–97°; $[\alpha]^{22}D$ +59.4°; tlc [benzene–methanol (185:15)] $R_{VII}$ = 0.85. The nmr spectrum was identical with that of IX.

*Anal.* Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.48; H, 5.84; N, 2.20.

**3-*O*-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (XII).**—The free disaccharide was obtained in 65% yield as described for X, after precipitation from methanol–ether and crystallization from 2-propanol: mp 163–165°; $[\alpha]^{23}D$ +56° (*c* 1, water); tlc [benzene–methanol (4:6)] $R_{lactose}$ = 0.75, $R_X$ = 0.95.

*Anal.* Calcd for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57. Found: C, 43.31; H, 7.07.

**1,2,3,6-Tetra-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-dichloroacetamido-β-D-galactopyranosyl)-α-D-galactopyranose (XIII).**—The glycoside III (200 mg) was dissolved in a mixture of acetic anhydride (7 ml), acetic acid (3 ml), and concentrated sulfuric acid (0.05 ml) and kept for 3 hr at 50°. Sodium acetate (200 mg) was added and the mixture was evaporated. The residue was taken up in chloroform (200 ml) which was washed several times with water. The chloroform solution was evaporated, and the residue was crystallized from alcohol–water (9:1): yield 200 mg (84.5%); mp 112°; $[\alpha]^{30}D$ +46.0°; tlc [benzene–methanol (8:2)] $R_{III}$ = 1.26. The nmr spectrum showed signals of 15 aromatic protons, 12 acetoxy protons, and one N-dichloroacetyl proton.

Compound XIV, resulting from the catalytic deacetylation of XIII, was not isolated, but was converted into X by hydrogenolysis as described previously.[6]

**Registry No.**—III, 22176-21-2; IV, 22176-22-3; VI, 22176-23-4; VII, 22176-24-5; IX, 22176-25-6; X, 22176-26-7; XI, 22212-29-9; XII, 22176-27-8; XIII, 22176-28-9.

# Nucleosides. LXIII. Synthetic Studies on Nucleoside Antibiotics. 3. Total Synthesis of 1-(4-Amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine, the Nucleoside Moiety of Gougerotin[1]

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The total synthesis of C-substance (the nucleoside product obtained from the antibiotic Gougerotin) from methyl 2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-galactoside (**1**) is described. 1-(4-Azido-4-deoxy-β-D-glucopyranosyl)-cytosine (**6**) was prepared by condensation of 4-*O*-mesyl-tri-*O*-benzoyl-β-D-galactosyl bromide (**2**, obtained from **1**) with $N^4$-acetylcytosine in the presence of mercuric cyanide in nitromethane followed by treatment with sodium azide and subsequent deacylation. Reduction of the azido derivative (**6**) afforded 1-(4-amino-4-deoxy-β-D-glucosyl)cytosine (**7**) which was selectively 4′-N-acetylated to **9** and peracetylated to the pentaacetate (**8**). Tritylation of **6** followed by benzoylation and detritylation gave 1-(4-azido-2,3-di-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl)-$N^4$-benzoylcytosine (**13**). Oxidation of **13** with chromic anhydride in wet pyridine–acetic acid afforded, after debenzoylation, 1-(4-azido-4-deoxy-β-D-glycopyranosyluronic acid)cytosine (**15**). Reduction of **15** yielded 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine, identical with C-substance obtained by acid hydrolysis of Gougerotin. Gougerotin-derived C-substance was converted to nucleosides **8** and **9**, which were identical with those obtained by chemical synthesis from **1**.

The nucleoside antibiotic Gougerotin, isolated by Kanzaki, *et al.*,[2] from *Streptomyces gougerotii*, inhibits protein biosynthesis by preventing the transfer of amino acids from amino acyl-tRNA to protein.[3] Details of the mechanism of this inhibition have been studied,[4] and in a recent report[5] it was shown that Gougerotin acts on the 50S ribosomal subunit and

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