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Studies of Oligosaccharides. IX.[1] Synthesis of Gentio-oligosaccharides by Block Condensation

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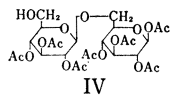
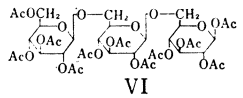
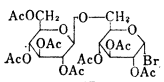
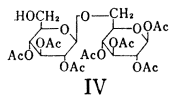
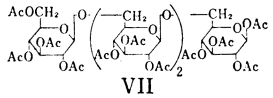
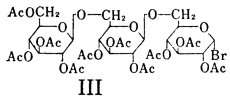
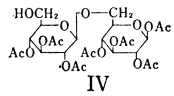
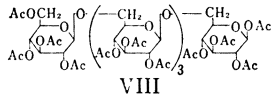
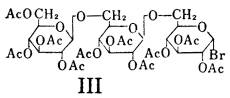
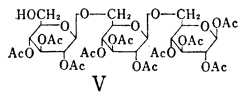
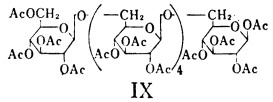
Gentio-triose, -tetraose, -pentaose and -hexaose were synthesized by Königs–Knorr condensation between acetobromo sugars and preformed oligosaccharide blocks bearing unprotected primary hydroxyl groups at non-reducing terminals. Incidental acetyl migration in the latter reactants is also discussed.

An unequivocal chemical synthesis of gentiotriose and gentiotetraose was achieved by application of Königs–Knorr condensation of acetylated gentiobiosyl and gentiotriosyl bromides with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose,[3] respectively, followed by deacetylation of the products. 1,2,3,4-Tetra-O-acetyl-6-O-trityl-β-D-glucopyranose[4] was also used in the synthesis of the triose using a catalyst of silver perchlorate in nitromethane. However, stepwise extension of a sugar chain by adding a monosaccharide unit to the reducing terminal, employed in these syntheses, is considered to be disadvantageous, when the synthesis of higher oligosaccharides is under contemplation. It should be supported by a key reaction coupling two large molecules prepared by the stepwise method. This article describes a detailed study

TABLE I. Synthesis of Gentio-oligosaccharides by Block Condensation

Reactant: Bromide	Reactant: Alcohol	Product	Yield(%)
 I	 IV	 VI	43 (52[a])
 II	 IV	 VII	31 (17[b])
 III	 IV	 VIII	31[c]
 III	 V	 IX	19[c]

a) comparative yield, II+1,2,3,4-tetra-O-acetyl-β-D-glucopyranose
b) comparative yield, III+1,2,3,4-tetra-O-acetyl-β-D-glucopyranose
c) calculated from the yield of the deacetylated products

1) Part VIII: K. Takiura and S. Honda, *Yakugaku Zasshi*, **87**, 1052 (1967).
2) Location: *6-1-1, Toneyama, Toyonaka, Osaka.*
3) B. Helferich and T. Gootz, *Ber.*, **64**, 109 (1931).
4) H. Bredereck, A. Wagner, H. Kuhn, and H. Ott, *Chem. Ber.*, **93**, 1201 (1960).

concerning the synthesis of gentio-oligosaccharides up to hexaose by condensation of preformed oligosaccharide blocks, as a model of block condensation in oligosaccharide synthesis.

The Königs–Knorr condensation between three kinds of acetobromo sugars, I, II and III, and two kinds of oligosaccharide acetates, IV and V, bearing unprotected primary hydroxyl groups at non-reducing terminals, is summarized in Table I.

The acetobromo sugars were prepared by interaction of the corresponding peracetates with hydrogen bromide. Strongly dextrorotatory optical rotation and low $J_{1,2}$ values around 3 Hz are indicative of the α-configuration. The latter reactants were obtained by detritylation of the corresponding trityl derivatives in acids. Incidental cleavage of glycosidic bonds was minimized by warming in 70—80% acetic acid. The C-1 configuration was determined from high $J_{1,2}$ values (7.8 Hz for IV, 8.0 Hz for V) as β for both compounds. Combination of appropriate reactants afforded the acetates of gentio-triose, -tetraose, -pentaose and -hexaose in considerable yields. Subsequent deacetylation led to the corresponding oligosaccharides in amorphous state. Although the yield of the triose acetate obatined by this method (43%) was a little lower than that by the stepwise method (52%), the advantage of block condensation was obvious in the case of the tetraose acetate. The pentaose and the hexaose seem to be the highest oligosaccharides, hitherto synthesized by an unequivocal method.

The linearity of log $R_g/1\text{-}R_g$ versus n[5] (Fig. 1) and $[M]_n/n$ versus $n\text{-}1/n$[6] (Fig. 2) demonstrated that the products belong to a homologous series, and the declivity in the latter slope indicates the β-linkage. Complete hydrolysis to D-glucose by β-glucosidase is a proof that the products were not contaminated with α-linked isomers.

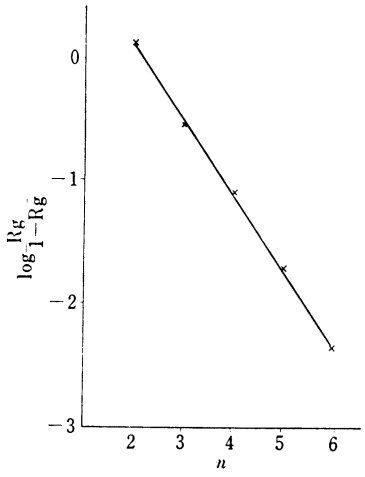


Fig. 1

n: number of D-glucose unit

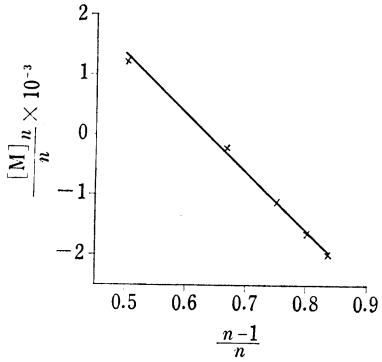


Fig. 2

n: number of D-glucose unit

Careful observation of the reaction mixtures of Königs–Knorr condensation monitoring by thin-layer chromatography revealed the presence of by-products with slightly higher *Rf* values than those of the alcoholic reactants. Since these by-products were also formed in considerable amount without acetobromo sugars, they are presumed to be derived from the alcoholic reactants. The by-product from the reaction between I and IV was isolated and purified by repeated recrystallization to give a compound X with mp 204—205°; $[\alpha]_D$ −17° (IV: mp 182—185°; $[\alpha]_D$ −9.1°). The infrared (IR) spectrum of X was identical with that of IV,

5) D. French and G.M. Wild, *J. Amr. Chem. Soc.*, **75**, 2612 (1953).
6) K. Freudenberg and G. Blomqvist, *Ber.*, **68**, 2070 (1935).

while the nuclear magnetic resonance (NMR) spectrum gave an OH proton at 6.72, comparable with an OH signal of IV at 6.5. Analytical data conformed to the calculated values of hepta-O-acetyl-gentiobiose. Acetylation of X gave octa-O-acetyl-β-gentiobiose, and deacetylation gave gentiobiose. Accordingly this compound is considered to be such an isomer of IV as one of the acetyl groups in IV has migrated to the primary hydroxyl group of the non-reducing terminal. Chromatographic and electrophoretic examination of the hydrolysate of the methylation product of X indicated that it was a mixture of D-glucose and 4-O-methyl-D-glucose. The methylation product was saponified, and reduced with sodium borohydride. The hydrolysate of the reduction product proved to be a mixture of D-glucitol and 4-O-methyl-D-glucose. In consequence, the structure of X was determined as 1,2,3,4,2′,3′,6′-hepta-O-acetyl-β-gentiobiose.

In spite of undesirable acetyl migration, possible contamination with 1—4 linked oligosaccharides was excluded from the result of methylation analysis of the products. They gave 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose, but no trace of 2,3,6-tri-O-methyl-D-glucose. This may be attributed to the greater reaction velocity, in Königs–Knorr condensation, of the primary hydroxyl groups in alcoholic reactants than that of the secondary ones in by-products, though acetyl migration seems to proceed competitively with the condensation, and may decrease the yield of oligosaccharide acetates to some extent.

Experimental

Melting points were determined on a hot stage using a Yanagimoto micro melting point apparatus and are uncorrected. Specific rotations were measured in a 1 dm tube. IR spectra were obtained with a Hitachi EPI G-2 infrared spectrophotometer. NMR spectra were observed on a Hitachi-Perkin Elmer H-60 instrument, and chemical shifts are expressed on the τ-scale in ppm for deuteriochroloform solutions with tetramethylsilane as internal standard. Thin-layer chromatography (TLC) was conducted on plates coated with Wakogel B-5 using a solvent system, benzene–ethyl acetate (7: 3), otherwise mentioned. Spots were visualized by spraying with concentrated sulfuric acid. Ascending paper chromatography (PC) was carried out, except for methylation analysis, on Whatmann No. 1 filter paper using a solvent system, *n*-butanol–pyridine–water (6: 4: 3), otherwise stated. For methylation analysis descending chromatography was performed using *n*-butanol–ethanol–water–ammonia (40: 10: 49: 1) at 25°. R_g and R_{tmg} represent relative mobility to D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose, respectively. Detection was effected with alkaline silver nitrate[7] and with aniline hydrogen phthalate.[8] Paper electrophoresis (PE) was carried out on Whatmann No. 1 filter paper, using borate buffer (pH 9.8) and applying a potential 12 V/cm. Components were detected with aniline hydrogen phthalate. M_g represents relative mobility to D-glucose. Chloroform used as a solvent for Königs–Knorr condensation was treated with calcium chloride before distillation to remove contaminant ethanol. All evaporations were performed in a diminished pressure below 40°.

Hepta-O-acetyl-α-gentiobiosyl Bromide (II)——This compound was prepared by a slight modification of the Zemplén's method.[9] Octa-O-acetyl-β-gentiobiose (100 g) was dissolved in chloroform (1 liter). After cooling to 0°, a cold acetic acid solution (250 ml) saturated with hydrogen bromide was added, and the mixture was kept at 0° for 2 hr. The reaction mixture was poured into crashed ice (3 kg), and the mixture was extracted three times with chloroform (500 ml). Combined extracts were washed three times with cold water (2 liter), followed by a cold saturated aqueous solution (2 liter) of sodium hydrogen carbonate, dried over calcium chloride, and evaporated to dryness. The residual syrup was taken into ether (1 liter) and the solution was allowed to stand. Crystallization initiated immediately and completed after storage in a refregirator. Yield, 72 g (70%). Recrystallization from ether afforded needles, mp 143—144.5°; $[\alpha]_D^{20}+110°$ ($c=1.0$, chloroform). Lit.[9]: mp 131—133.5°; $[\alpha]_D^{15}+111.8°$ (chloroform). *Anal.* Calcd. for $C_{26}H_{53}O_{17}Br$: C, 44.60; H, 5.11; Br, 11.43. Found: C, 44.75; H, 5.01; Br, 11.14. NMR: 3.41 (H_1, doublet, $J_{1,2}=3.0$ Hz).

Deca-O-acetyl-α-gentiotriosyl Bromide (III)——Of the compound III (8.5 g, 56%) was obtained by bromination VI (15 g) at 0° for 1 hr in the similar manner as for II. Prolonged reaction caused degradation of the product. The crude product was purified on a Wakogel C-200 column (5×40 cm) using benzene–ethyl acetate (4: 1) as an eluant, followed by recrystallization from ether to give cubic crystals, mp 194—195°;

7) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).
8) S.M. Partridge, *Nature*, **164**, 443 (1949).
9) G. Zemplén, *Ber.*, **57**, 702 (1924).

$[\alpha]_D^{20}+60.0°$ ($c=1.1$, chloroform). Lit.[3]: mp 178—183°; $[\alpha]_D^{17}+63.3°$ (chloroform). *Anal.* Calcd. for $C_{38}H_{51}O_{25}Br$: C, 46.21; H, 5.21; Br, 8.09. Found: C, 46.12; H, 4.94; Br, 7.89. NMR: 3.3 (H_1, doublet, $J_{1,2}=3.3$ Hz).

1,2,3,4,2′,3′,4′-Hepta-O-acetyl-β-gentiobiose (IV)——Octa-O-acetyl-β-gentiobiose (100 g, 0.15 mole) was stirred in 0.05N methanolic sodium methoxide (1 liter). Immediately after dissolution, gentiobiose separated as amorphous precipitate, which was dissolved, after standing for 40 min, by addition of a small amount of water. The solution was deionized by stirring with Amberlite IR-120 (free acid form, 30 g), followed by Amberlite IRA-410 (carbonate form, 30 g) and evaporated to dryness. The remaining water was removed as much as possible by repeating the procedure, dissolution of the residue in dehydrated pyridine followed by azeotropic distillation of the solvent. The resulting syrup was dissolved, without purification, in dehydrated pyridine, and trityl chloride (42 g, 0.15 mole) was added. Since TLC showed the presence of the starting materials even after reaction for 80 hr, trityl chloride (5.6 g, 0.02 mole) was supplemented, and the reaction mixture was allowed to stand overnight. Acetic anhydride (400 ml) was added, and after 3 days the mixture was poured into ice water (10 liter) with stirring. The trityl ether[10] of IV separated as amorphous powder. Yield, 140 g.

The crude trityl ether (140 g) was dissolved in 70% acetic acid (900 ml) at 60°, and the solution was kept at this temperature for 3 hr. Triphenyl carbinol separated nearly quantitatively on cooling, which was filtered off. Water (6 liter) and chloroform (2 liter) were added to the filtrate and the mixture was shaken vigorously. The organic layer was repeatedly washed with water, followed by a saturated aqueous solution (2 liter) of sodium hydrogen carbonate, dried over calcium chloride, decolorized with charcoal, and evaporated to dryness. The residual syrup was crystallized from ethanol–ether to give crystalline IV (43 g). The second crop (25 g) was obtained from the mother liquor. The total yield amounted to 72%, based on octa-O-acetyl-β-gentiobiose. Recrystallization from ethanol afforded needles, mp 182—185°; $[\alpha]_D^{20}-9.1°$ ($c=0.9$, chloroform). Lit.[10]: mp 162—167°; $[\alpha]_D^{20}+24.7°$ ($c=1.0$, chloroform). *Anal.* Calcd. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 49.04; H, 5.86. NMR: 4.3 (H_1, doublet, $J_{1,2}=7.8$ Hz), 6.5 (OH, broad singlet). IR ν_{max}^{Nujol} cm^{-1}: 3520 (OH), 1740 (C=O of acetyl groups), 1040 (C-O-C).

1,2,3,4,2′,3′,4′,2″,3″,4″-Deca-O-acetyl-β-gentiotriose (V)——The compound VI (20 g, 0.021 mole) was dissolved in 0.05N methanolic sodium methoxide (300 ml). After standing for 40 min, the separated gentiotriose was dissolved by addition of a small amount of water, and the solution was deionized and evaporated to dryness in a similar manner as for IV or as mentioned above. After azeotropic distillation with pyridine, the residual syrup was tritylated with trityl chloride (7.3 g, 0.026 mole) in pyridine (100 ml) at 50°, stirring for 60 hr. Acetic anhydride (30 ml) was added and the mixture was allowed to stand overnight. Upon addition of water (3 liter) to the reaction mixture, solid material precipitated, which was collected and washed with water. Purification on a Wakogel C-200 column (5×40 cm) using benzene–ethyl acetate (1:1) as an eluant, followed by crystallization from ethanol afforded the trityl ether of V (11 g, 46%) as needles, mp 128—129.5°; $[\alpha]_D^{20}+13.6°$ ($c=2.5$, chloroform). *Anal.* Calcd. for $C_{57}H_{66}O_{26}$: C, 58.66; H, 5.70. Found: C, 58.31; H, 5.60. NMR: 4.3 (H_1, doublet, $J_{1,2}=8.0$ Hz).

The trityl ether (11 g) was suspended in 80% acetic acid (260 ml) and kept at 60° for 45 min. The separated triphenyl carbinol was filtered off, and the filtrate was extracted three times with chloroform (100 ml). Combined extracts were dried over calcium chloride, and evaporated to dryness. The residual syrup was purified on a Wakogel C-200 column (5×40 cm) using benzene–ethyl acetate (1:2) as an eluant, and crystallized from ethanol to give cubic crystalls of V (3.8 g, 44%), mp 123—125°; $[\alpha]_D^{20}-5.2°$ ($c=1.0$, chloroform). *Anal.* Calcd. for $C_{38}H_{52}O_{26}$: C, 49.35; H, 5.67. Found: C, 49.15; H, 5.65. NMR: 4.2 (H_1, doublet, $J_{1,2}=7.9$ H_3). IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1725 (C=O of acetyl groups), 1050 (C-O-C).

Hendeca-O-acetyl-β-gentiotriose (VI)——To a mixture of IV (1.23 g, 1.9 mmole), freshly prepared silver carbonate (0.91 g, 3.3 mmole), pulverized Drierite (4.1 g), iodine (0.07 g) and chloroform (5 ml) was added dropwise a chloroform solution (5 ml) containing 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (I) (0.92 g, 2.2 mmole). Stirring was continued for 2 days under shielding of the light. TLC showed the disappearance of the starting materials and the presence of three spots. The reaction mixture was filtered, and the filtrate was washed with a 10% sodium thiosulfate solution. After evaporation of the solvent, the residual syrup was crystallized from acetone–ethanol to give crystalline VI (0.80 g, 43% based on IV). Recrystallization from the same solvent system afforded needles, mp 221—223°; $[\alpha]_D^{20}-7.4°$ ($c=0.8$, chloroform). Lit.[4]: mp 218°; $[\alpha]_D^{22}-7.2°$ (chloroform). *Anal.* Calcd. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.46; H, 5.70.

From the mother liquor the compound X corresponding to the middle spot was obtained and purified by crystallization from ethanol. Repeated recrystallization from ethanol, acetone–ethanol, and finally ethyl acetate gave needles (0.01 g, 8% based on IV), mp 204—205°; $[\alpha]_D^{25}-17.0°$ ($c=1.0$, chloroform). *Anal.* Calcd. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 49.08; H, 5.52. NMR: 6.72 (OH, broad singlet). The IR spectrum was identical with that of IV. Acetylation of X with acetic anhydride in the presence of

10) N. Roy and C.P.J. Glaudemans, *J. Org. Chem.*, **33**, 1559 (1968).
11) K. Takiura and S. Honda, *Yakugaku Zasshi*, **87**, 1052 (1967).

pyridine afforded needles, mp 195—196°; $[\alpha]_D^{25}$ −6.8° (c=1.0, chloroform). Authentic octa-O-acetyl-β-gentiobiose: mp 196°; $[\alpha]_D^{20}$ −5.0° (c=5.0, chloroform). PC of the saponified product of X in 0.05N methanolic sodium methoxide gave a single spot (R_g 0.56) corresponding to authentic gentiobiose (R_g 0.56). Methylation of X with methyl iodide in N,N-dimethylformamide using a catalyst of silver oxide,[11] followed by subsequent hydrolysis of the product in 5% HCl under reflux for 4 hr, yielded a syrup, which gave two spots (R_{tmg} 0.20, 0.37) on PC. R_{tmg} of authentic D-glucose 0.20, 4-O-methyl-D-glucose 0.37. PE also indicated two spots (M_g 1.00, 0.21). M_g of authentic 4-O-methyl-D-glucose 0.21. A part of the methylation product was dissolved in a small volume of 0.05N methanolic sodium methoxide, and after standing for 2 hr, an excess amount of sodium borohydride was added. Deionization with ion exchangers, followed by evaporation, afforded a syrup of the reduction product, which was hydrolyzed in 5% HCl under reflux for 2 hr. The hydrolysate gave two spots on TLC using ethyl acetate–ethanol (4: 1). The faster moving spot was positive to aniline hydrogen phthalate and was identical with 4-O-methyl-D-glucose. While the slower moving spot was not detected with aniline hydrogen phthalate, the behavior and the *Rf* value corresponded to D-glucitol.

The compound VI was prepared in a hundred fold scale for further use.

Gentiotriose——The compound VI (20 g) was dissolved in 0.05N methanolic sodium methoxide (300 ml). After standing for 3 hr, the separated gentiotriose was dissolved by addition of water (200 ml). The solution was deionized with ion exchangers, concentrated to a small volume, and poured into ethanol (2 liter) with stirring. Gentiotriose precipitated quantitatively as amorphous powder, which was collected and dried on phosphorus pentoxide. mp 143—151°. $[\alpha]_D^{20}$ −1.1° (c=0.93, water, 24 hr). *Anal.* Calcd. for $C_{18}H_{32}O_{16}\cdot 2H_2O$: C, 40.00; H, 6.71. Found: C, 39.40; H, 6.84. R_g 0.22.

Tetradeca-O-acetyl-β-gentiotetraose (VII)——To a mixture of IV (1.27 g, 2.0 mmole), freshly prepared silver carbonate (0.56 g, 2.0 mmole), pulverized Drierite (2.7 g), iodine (0.07 g) and chloroform (5 ml) was added dropwise a chloroform solution (5 ml) containing II (1.40 g, 3.4 mmole). Stirring was continued for 2 days, under shielding of the light. The reaction mixture was filtered, and the filtrate was washed with a 10% sodium thiosulfate solution. After evaporation of the solvent, the residual syrup was crystallized from ethanol to give crystalline VII (0.78 g, 31% based on IV). Recrystallization from isopropanol–isopropyl ether afforded needles, softening point 134—136°; mp 212—213°; $[\alpha]_D^{20}$ −10.7° (c=0.6, chloroform). Lit.[3]: softening point 135°; mp 207—209°; $[\alpha]_D^{20}$ −11.1° (chloroform). *Anal.* Calcd. for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62. Found: C, 49.54; H, 5.66.

Gentiotetraose——Deacetylation of VII in the similar manner as for the triose afforded quantitatively gentiotetraose as amorphous powder, mp 164—171°; $[\alpha]_D^{20}$ −6.5° (c=1.23, water, 24 hr). *Anal.* Calcd. for $C_{24}H_{42}O_{21}\cdot 2H_2O$: C, 41.03; H, 6.60. Found: C, 41.24; H, 7.01. R_g 0.075.

Gentiopentaose——The Königs–Knorr condensation was undertaken by addition of a chloroform solution (5 ml) containing III (4.0 g, 4.0 mmole) to a mixture of IV (2.6 g, 4.0 mmole), freshly prepared silver carbonate (1.2 g, 4.4 mmole), pulverized Drierite (6.0 g), iodine (0.15 g) and chloroform (4 ml). Syrupy heptadeca-O-acetyl-β-gentiopentaose (VIII) was obtained after treatment of the reaction mixture as for VI, which was deacetylated in 0.05N methanolic sodium methoxide (150 ml). After deionization, followed by evaporation, the residual syrup was fractionated on a charcoal column (5×40 cm) by stepwise elution with water, then 10%, 15%, 20%, 25% and 30% aqueous ethanol. The 25% aqueous ethanolic fraction was concentrated to a small volume, and poured into ethanol (200 ml) with stirring to give homogeneous gentiopentaose (1.1 g, 31% based on IV) as amorphous powder, mp 162—168°; $[\alpha]_D^{20}$ −9.9° (c=2.0, water, 24 hr). *Anal.* Calcd. for $C_{30}H_{52}O_{26}\cdot 5H_2O$; C, 39.22; H, 6.80. Found: C, 39.81; H, 7.01. R_g 0.02.

Gentiohexaose——From V (3.7 g, 4.0 mmole), freshly prepared silver carbonate (1.2 g, 4.4 mmole), pulverized Drierite (6.0 g), iodine (0.15 g), III (4.0 g, 4.0 mmole) and chloroform (10 ml), syrupy eicosa-O-acetyl-β-gentiohexaose (IX) was obtained, which was deacetylated and fractionated on a charcoal column (5×40 cm) in the similar manner as for the pentaose. The 25% and the 30% aqueous ethanolic fractions were combined, concentrated to a small volume, and poured into ethanol (200 ml) with stirring to give gentiohexaose (0.75 g, 19% based on V) as amorphous powder. For analytical purpose a part of the product was further purified by preparative PC on Whatmann No. 3 filter paper. mp 62°; $[\alpha]_D^{20}$ −12.0° (c=2.51, water, 24 hr). *Anal.* Calcd. for $C_{36}H_{62}O_{31}\cdot 6H_2O$: C, 39.35; H, 6.79. Found: C, 39.09; H, 6.78. R_g 0.004.

Homogeneity of the Synthesized Gentio-oligosaccharides——a) Enzymic Hydrolysis with β-Glucosidase: The oligosaccharides (1 mg) and β-glucosidase (1 mg, Sigma Chemicals Co., Ltd., lot 125B-0100, prepared from almond) were dissolved in 0.1 ml of distilled water, and solutions were incubated at 35°. PC of hydrolysates indicated that the biose, the triose, the tetraose, the pentaose and the hexaose were hydrolyzed completely to D-glucose after incubation for 9 hr, 24 hr, 52 hr, and 70 hr, respectively. For reference these oligosaccharides were incubated without the enzyme. They did not yield D-glucose even after 70 hr. Maltose under the identical condition also unchanged after 70 hr.

b) Methylation Analysis: Methylation[12] of the oligosaccharides yielded syrups, each of which, on subsequent hydrolysis in 5% HCl under reflux for 2 hr, gave two spots (R_{tmg} 0.90, 1.00) on PC. R_{tmg} of authentic 2,3,4-tri-O-methyl-D-glucose 0.90, 2,3,6-tri-O-methyl-D-glucose 0.84.

12) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).