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A NEW SYNTHESIS OF 4-*O*-α-D-GALACTOPYRANOSYL-D-GALACTOPYRANOSE*

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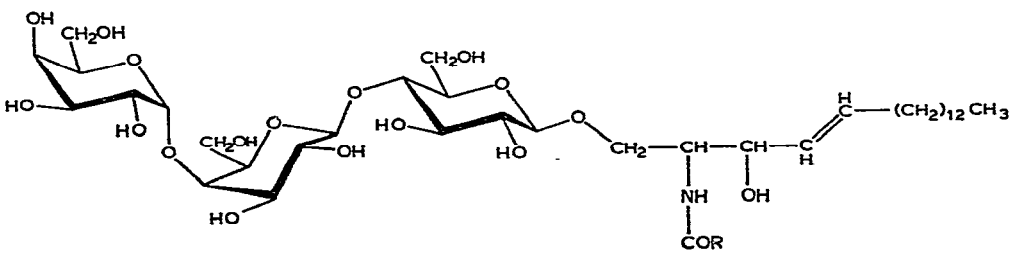
ABSTRACT

The bromide-catalyzed condensation of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide (**11**) with methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3**) gave methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-galactopyranoside (**12**) in 83% yield. The yield of this glycosidation reaction was high, despite the axial orientation of the 4-hydroxyl group of **3**. Stepwise deprotection of **12** afforded methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (**15**). Acetylation of **15**, followed by acetolysis, gave the known α-octaacetate **17**. This scheme constituted a total synthesis of 4-*O*-α-D-galactopyranosyl-D-galactopyranose (**2**) in 25% yield from **3**. The disaccharide **2** is the terminal disaccharide of the ceramide trisaccharide related to Fabry's disease.

INTRODUCTION

Fabry's disease is a member of a family of hereditary, lipid-storage diseases characterized by an enzyme deficiency that results in the accumulation of glycosylsphingolipids. Specifically, Fabry's disease is an α-galactosidase deficiency that causes an accumulation of 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosylceramide[2] (**1**).



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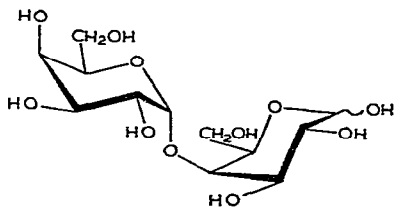
In order to study some of the facets of this disease, we plan a synthesis of the ceramide trisaccharide that is amenable to labelling with ^{14}C in the terminal α-D-galactopyranosyl moiety. The synthesis of the labelled trisaccharide presents a number of challenges, not the least of which is the formation of the 1,2-*cis* linkage of the terminal α-D-galactopyranoside. To obtain a synthetic model, we chose to prepare **2**, the terminal disaccharide of **1**, by condensation of a suitably blocked D-galactopyranosyl halide with methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3**).



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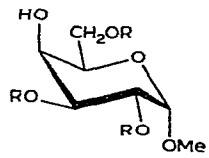
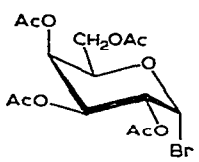


3 R = Bz
8 R = H

Disaccharide **2** had been synthesized previously by Chacon-Fuertes and Martin-Lomas[3] and by Gent, Gigg, and Penglis[4]. Martin-Lomas[3] utilized the Koenigs–Knorr condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**) with 2,3-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**5**) to form the disaccharide. Gigg's synthesis[4] of **2** relied on the chloride-catalyzed condensation of 1,6-anhydro-2,3-di-*O*-benzyl-β-D-galactopyranose (**6**) with 2,3,4-tri-*O*-benzyl-6-*O*-2-butenyl)-D-galactopyranosyl chloride. Each of the 1,6-anhydro-β-D-galactopyranoses, **5** and **6**, contained the more-reactive (see later) 4-hydroxyl group that acted as a nucleophile in the condensation reaction. However, neither previous synthesis could be adapted conveniently for preparation of the trisaccharide **1**.



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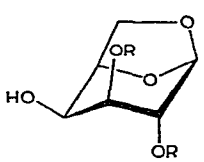
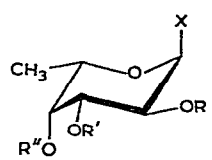


5 R = Ac
6 R = CH_2Ph
7 R = H



9 X = OMe; R = R′ = CH_2Ph; R″ = H
10 X = Br ; R = CH_2Ph; R′ = R″ = $COC_6H_4NO_2$—(p)
21 X = Br ; R = R′ = R″ = Ac

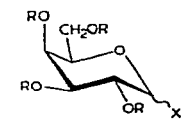
Martin-Lomas[5] found the axial 3-hydroxyl group of 1,6-anhydro-β-D-galactopyranose (**7**) to be less reactive than the equatorial 4-hydroxyl group of **7** towards acetylation with acetic anhydride in pyridine. Similarly, the axial 4-hydroxyl group had been reported to be less reactive than the equatorial 3-hydroxyl group of methyl α-D-galactopyranoside (**8**) towards benzoylation with benzoyl chloride in pyridine[6,7]. However, Flowers[8] recently reported that the Koenigs–Knorr condensation of the axial 4-hydroxyl group of methyl 2,3-di-*O*-benzyl-6-deoxy-α-L-galactopyranoside (**9**) with 2-*O*-benzyl-6-deoxy-3,4-di-*O*-*p*-nitrobenzoyl-α-L-galactopyranosyl bromide (**10**)

gave the α-L-linked, fully protected disaccharide in 85% yield. Significantly, the axial 4-hydroxyl group of **9** was not unreactive in the glycosidation reaction.

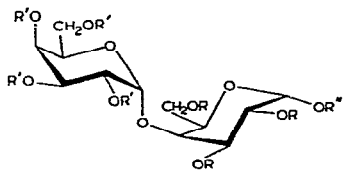
We have synthesized disaccharide **2** by utilizing the bromide-catalyzed condensation of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide (**11**) with methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3**) as the key synthetic step. The nucleophilic 4-hydroxyl group of **3** was axial rather than equatorial, and yet the glycosidation reaction gave the desired α-linked, fully protected disaccharide **12** in 83% yield.

RESULTS AND DISCUSSION

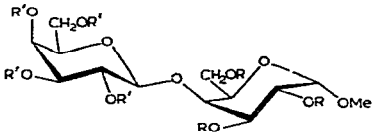
Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3**) was prepared in 65% yield[6] by selective tribenzoylation of commercial (Pfanstiehl Laboratories, Inc., Waukegan, Illinois) methyl α-D-galactopyranoside (**8**). Methyl β-D-galactopyranoside (Pfanstiehl Laboratories) was benzylated with α-chlorotoluene and sodium hydride



11 R = CH_2Ph; X = Br
13 R = CH_2Ph; X = OCONHPh



12 R = Bz; R′ = CH_2Ph; R″ = Me
14 R = H; R′ = CH_2Ph; R″ = Me
15 R = R′ = H; R″ = Me
16 R = R′ = Ac; R″ = Me
17 R = R′ = R″ = Ac
18 R = Bz; R′ = Ac; R″ = Me



19 R = Bz; R′ = Ac
20 R = R′ = H

in *N*,*N*-dimethylformamide[9] to give methyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside. The latter was converted into 2,3,4,6-tetra-*O*-benzyl-1-*O*-*N*-phenylcarbamoyl-D-galactopyranose (**13**) by the method[10] of Kronzer and Schuerch. Treatment of **13** with anhydrous hydrogen bromide gave oily 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide (**11**), which was used immediately in the glycosidation reactions. The anomeric purity of the bromide reactant was unimportant, as the bromide-catalyzed glycosidation conditions allowed equilibration of the anomeric bromides[11,12].

The two coupling components **3** and **11** (2.7 equiv.) were dissolved in a dichloromethane solution of tetraethylammonium bromide containing a few drops of *N*,*N*-dimethylformamide. The solution was refluxed for 4 days over molecular sieves, which scavenged the hydrogen bromide produced in the glycosidation reaction[11]. Column chromatography gave the fully protected disaccharide, methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-galactopyranoside (**12**) as a syrup. That the ^{13}C-n.m.r. spectrum of this syrup revealed only two lines attributable to anomeric carbon atoms implied, within the limitations of ^{13}C-n.m.r. analysis, a high degree of stereoselectivity in the coupling of **3** and **11**. The yield of **12** was 83% from **3** (the limiting reactant) and 31% from **11**.

Disaccharide **12** was debenzoylated catalytically with sodium methoxide in

methanol to **14**. Catalytic hydrogenolysis (palladium-on-charcoal) of **14** gave methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (**15**) as a hygroscopic syrup, in 80% yield from **12**. The structure of **15** was evident from ^{13}C-n.m.r. spectral analysis, a complete description[13] of which will appear shortly.

Treatment of **15** with acetic anhydride and pyridine gave methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranoside (**16**). Acetolysis of syrupy **16** with sulfuric acid, acetic acid, and acetic anhydride gave the known[3,4], crystalline 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (**17**). The overall yield of **17** from **3** was 29%. The reducing disaccharide, 4-*O*-α-D-galactopyranosyl-D-galactopyranose (**2**), had been prepared previously by Martin-Lomas[3] in 85% yield from **17**. Thus our synthesis constituted a total synthesis of **2** from **3** in 25% yield.

Gigg[4] synthesized **2** from **6** in 7% yield, and Martin-Lomas[3] prepared **2** from **5** in 34% yield. Both our synthesis and that of Martin-Lomas[3] provide practical syntheses of **2** from readily available monosaccharide precursors.

A synthesis of **2** by the Koenigs–Knorr reaction was also attempted. For 6 days an excess of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**) was treated with **3** in nitromethane containing mercuric cyanide. Chromatographic purification of the crude product gave a 30% yield of methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-α-D-galactopyranoside as a mixture of anomers at C-1′. Treatment of this mixture of **18** and **19** with sodium methoxide in methanol gave a mixture of methyl 4-*O*-β-D-galactopyranosyl-α-D-galactopyranoside (**20**) and **15**. ^{13}C-N.m.r. analysis[13] of this mixture revealed that **20** predominated over **15** by ~7:6.

The bromide-catalyzed condensation of **3** and **11** gave a high yield of α-linked disaccharide. Gigg[4] reported that t.l.c. analysis of his crude preparation of **17** indicated one major product and that this fact was because of a "a high degree of stereoselectivity in the glycosidation reaction". In general, halide-catalyzed condensations that involve glycopyranosyl halides bearing non-participating groups at C-2 have given high yields of α-linked glycosides[11,12]. The mercuric cyanide-catalyzed condensation reported by Flowers[8] gave a high yield of α-linked disaccharide from **10**, which also contains a non-participating O-substituent at C-2.

On the other hand, Flowers[8] reported that the mercuric cyanide-catalyzed condensation of **9** with 2,3,4-tri-*O*-acetyl-6-deoxy-α-L-galactopyranosyl bromide (**21**) gave a mixture of α- and β-linked disaccharides, in which the β-linked isomer predominated. The mercuric cyanide-catalyzed condensation of **3** and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**) also gave a mixture of α- and β-linked disaccharides that favored the β-linked isomer. The mercuric cyanide-catalyzed condensation of **4** with **5**, reported by Martin-Lomas[3], gave yields of 40% for the α-linked disaccharide and 30% for the β-linked disaccharide. The electrophile (**4** or **21**) in each of these condensations that was less selective towards formation of

α-linked disaccharides was a glycopyranosylhalide having a participating 2-substituent (acetoxyl)*.

The axial orientation of the 4-hydroxyl group in **3** was no hindrance to disaccharide synthesis with galactopyranosyl halides. Although the bromide-catalyzed glycosidation reaction of **3** and **11** required 4 days in refluxing dichloromethane for completion, the yield of α-linked disaccharide product was excellent.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points were determined in capillary tubes with a Thomas–Hoover apparatus and are uncorrected. Proton-decoupled ^{13}C magnetic resonance spectra were recorded with a Varian XL-100-15 Fourier-transform spectrometer modified for multinuclear operation[14] and equipped with a Varian 620L computer for data handling. Column chromatography was performed on silica gel reagent (90–200 mesh), Accurate Chemical and Scientific Corp. EM Silica gels GF254 and PF254 were used for t.l.c. Solutions were evaporated or concentrated under diminished pressure, unless otherwise indicated.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-galactopyranoside* (**12**). — A 50-ml round bottom flask was charged with methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside[6] (**3**, 507 mg, 1 mmol), tetraethylammonium bromide (215 mg, 1 mmol), and molecular sieves (5Å, 1.5 g). To this mixture was added a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide[11] (**11**, ~2.7 mmol) in dichloromethane (10 ml). The mixture was stirred until all but the sieves had dissolved, and then 15 drops of dry *N,N*-dimethylformamide were added to the mixture. The mixture was protected from light and boiled for 4 days under reflux and under dry nitrogen. The mixture was then diluted with 10 ml of dichloromethane, filtered through fine sintered glass, and the flask was rinsed with two 10-ml portions of dichloromethane. The dichloromethane solution was washed twice with water, once with saturated sodium hydrogencarbonate solution, and then again with water. Each of the aqueous washes was back-extracted with dichloromethane. The dichloromethane layers were pooled, dried (sodium sulfate), filtered, and concentrated to a viscous, light-brown syrup (1.97 g). This syrup was dissolved in 2 ml of benzene and loaded on a column of silica gel (150 g, 210 × 34 mm). The column was developed with increasingly polar benzene–ether mixtures. The disaccharide product was eluted by 20:1 benzene–ether. Evaporation of this solution gave a thick, colorless syrup (860 mg, 83%) of nearly pure **12**. An analytical sample, $[\alpha]_D^{20}$ +88° (*c* 1.0, chloroform), was obtained from the foregoing syrup (84% recovery) by preparative t.l.c.

*The coupling of **3** with **4** occurred in nitromethane, rather than in the nitromethane–benzene mixtures used in the condensations of **9** with **21** (ref. 8) and of **4** with **5** (ref. 3). The anomeric ratio of products may also be affected by the different polarities of the solvent systems.

on silica gel with 1:1 ether–petroleum ether as developer; ^{13}C-n.m.r. in acetone-d_6, internal tetramethylsilane: δ 98.1 (C-1), 101.1 (C-1'),

Anal. Calc. for $C_{62}H_{60}O_{14}$: C, 72.36; H, 5.88. Found: C, 72.79; H, 6.01.

Methyl 4-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-α-D-galactopyranoside (**14**). — Compound **12** (562 mg, 0.545 mmol) was dissolved in anhydrous ether (10 ml) and abs. methanol (10 ml). A small piece of sodium was added, and the resulting solution was kept stoppered in the dark for one day. The solution then was evaporated, and the residue was treated with ether and water. The layers were separated, and the aqueous layer was extracted with ether. The ether layers were pooled, dried (sodium sulfate), filtered, and evaporated to give a viscous oil (513 mg) that contained the desired product and methyl benzoate. This oil was dissolved in 2 ml of ether and 50 ml of petroleum ether was then added to the ether solution. The petroleum ether was decanted from the colorless gum that formed on the walls of the flask. The gum was washed with two 100-ml portions of petroleum ether. The remaining solvent was evaporated from the gum, and compound **14** was recovered as a colorless syrup (309 mg, 79%); $[\alpha]_D^{21}$ +50° (*c* 1.0, chloroform).

Methyl 4-O-(α-D-galactopyranosyl)-α-D-galactopyranoside (**15**). — Compound **14** (309 mg, 0.431 mmol) was dissolved in 180 ml of abs. ethanol in a Parr bottle. To this solution was added 1.0 g of 10% palladium-on-carbon (Matheson, Coleman, & Bell) and 20 ml of distilled water. The resulting mixture was shaken for 64 h on a Parr apparatus under 48 lb.in^{-2} of hydrogen. The mixture was filtered through Celite, and the alcohol solution was concentrated to a hygroscopic colorless glass of compound **15** (160 mg, 104%); $[\alpha]_D^{20}$ +180° (*c* 0.96, water); ^{13}C-n.m.r. in D_2O; δ 67.45 (internal 1,4-dioxane), 100.35 (C-1), 101.35 (C-1').

Methyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-galactopyranoside (**16**). — Compound **15** (52 mg, 0.146 mmol) was dissolved in pyridine (2 ml). To this solution was added acetic anhydride (0.5 ml). The flask was stoppered and kept in the dark for 5 days at room temperature. To the crude syrup obtained upon evaporation was added 3 ml of abs. methanol, and the resulting solution was heated for about 5 min on a steam bath. When the methanolic solution had cooled to room temperature, it was evaporated. The residue was dissolved in toluene (5 ml), and the solution was evaporated again. The toluene treatment was repeated, and evaporation gave a glass (99 mg, 104%) that was purified by preparative t.l.c. on silica gel with 3:1 ether–benzene as developer. The major band, R_F 0.5, was eluted with chloroform. Filtration and evaporation gave **16** as a colorless glass (57 mg, 60%).

1,2,3,6-Tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (**17**). — Compound **16** (47 mg, 0.072 mmol) was treated with a cold (just above freezing) solution of acetic anhydride (3 ml), acetic acid (1 ml), and conc. sulfuric acid (80 μl). The resulting mixture was swirled and allowed to warm to room temperature as the disaccharide dissolved. When the solution had become clear, it was kept for 20 min. The solution was then poured into a stirred mixture of 15 ml chloroform and 15 ml half-saturated sodium hydrogencarbonate solution

in a 250-ml Erlenmeyer flask. When bubbling ceased, the chloroform layer was separated, and the aqueous layer was extracted with chloroform. The chloroform layers were pooled, washed twice with half-saturated sodium hydrogencarbonate solution, and then with water. The chloroform solution was dried (sodium sulfate) and evaporated to give a crude, light-yellow oil, contaminated with acetic anhydride. This oil was dissolved in hot ethanol (5 ml), the solution was filtered hot, and the filtrate was placed in a freezer. Tiny cubic crystals formed slowly. Filtration gave 35 mg (71%) of pure **17**, m.p. 150–151°, $[\alpha]_D^{20}$ +141° (*c* 1.0, chloroform); lit.[3] m.p. 153–154°, $[\alpha]_D^{20}$ +138° (*c* 2, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64. Found: C, 49.58; H, 5.59.

Methyl 4-O-β-D-galactopyranosyl-α-D-galactopyranoside (**20**) *and* **15**. — Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3**, 506 mg, 1.0 mmol), powdered Drierite (1.0 g), and mercuric cyanide (510 mg, 2.0 mmol) were stirred in nitromethane (5 ml), and the mixture was cooled to 0° under nitrogen. The flask was unstoppered briefly, and powdered 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**, 820 mg, 2.0 mmol) was added. When the resulting mixture had reached room temperature under nitrogen, it was stoppered tightly and stirred in the dark for 3 days. Additional mercuric cyanide (510 mg) and **4** (820 mg) were then added to the mixture, which was stirred stoppered in the dark for 3 more days. The mixture was then filtered through fine sintered glass, and the flask was washed with 3 volumes each of chloroform and water. The combined mixture was shaken and separated. The aqueous layer was extracted with chloroform, and the chloroform extracts were pooled. The organic phase was washed with saturated sodium hydrogencarbonate solution, water, and saturated sodium chloride solution. The chloroform solution was dried (sodium sulfate) and evaporated to a foam (1.88 g). Column chromatography (150 g of silica gel) of this foam, with 10:1 benzene–ether as the eluant, gave a foamy fraction (0.69 g) that contained disaccharide products. Preparative t.l.c. gave a mixture of **18** and **19**; yield 0.26 g (31%). This mixture was deacylated for 2 days in 10 ml dilute methanolic sodium methoxide. The solution was neutralized with acetic acid and IRC-50 (H^+) resin. Filtration and evaporation gave a colorless syrup. The syrup was dissolved in water, and the aqueous solution was washed twice with dichloromethane. Filtration and evaporation of the aqueous solution gave a colorless glass (0.18 g) that contained methyl 4-*O*-β-D-galactopyranosyl-α-D-galactopyranoside (**20**), methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (**15**), and some sodium acetate. A portion of this mixture was dissolved in D_2O and analyzed by ^{13}C-n.m.r. spectroscopy in D_2O: δ 67.45 (internal 1,4-dioxane), 105.3, intensity 75 (C-1′ of **20**), 101.4, intensity 64, (C-1′ of **15**), 100.3, intensity 130 (C-1 of **20** and **15**).

ACKNOWLEDGMENTS

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