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SYNTHESIS OF 6-*O*-(6-*O*-β-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSYL)-D-GALACTOPYRANOSE BY USE OF 2,3,4-TRI-*O*-ACETYL-6-*O*-(CHLOROACETYL)-α-D-GALACTOPYRANOSYL BROMIDE, A KEY INTERMEDIATE FOR THE SOLID-PHASE SYNTHESIS OF β-(1→6)-LINKED D-GALACTOPYRANANS

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ABSTRACT

2,3,4-Tri-*O*-acetyl-6-*O*-(chloroacetyl)-α-D-galactopyranosyl bromide (**5**) has been prepared. When condensed with 1,2,3,4-tetra-*O*-acetyl-D-galactopyranose, it yielded 1,2,3,4-tetra-*O*-acetyl-6-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(chloroacetyl)-β-D-galactosyl]-D-galactopyranose (**6**). The *O*-chloroacetyl group could be selectively removed from **6** by treatment with thiourea, and the resulting product was again condensed with **5**, to yield, after deprotection, the trisaccharide title-compound.

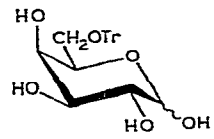
INTRODUCTION

The synthesis of higher oligosaccharides by the Merrifield procedure[1], using an (insoluble) polymer support, has been known for some time[2].

Because of our involvement with extensive studies on monoclonal antibodies having specificity for the (1→6)-β-D-galactopyranosyl linkage, we are interested in the stereoregular formation of a linear, β-(1→6)-linked D-galactopyranan. To the best of our knowledge, no such polysaccharide exists in Nature, the structurally closest one being the galactan from *Prototheca zopfii*[3,4], which has occasional, single D-galactofuranosyl groups as side chains.

RESULTS AND DISCUSSION

Our work on monoclonal immunoglobulins having anti-D-galactan specificity has led to the proposal of a hypothetical, space-filling model for IgA J539, and its coordinates have been determined in detail[5]. In addition, a combining site has been proposed[5]. Future refinement of this model will require experiments for which linear, oligo-D-galactosyl ligands and their derivatives are needed. We have, therefore, devised a method for the continuous synthesis of oligo- and poly-saccharides having β-(1→6)-linked D-galactosyl residues. We were guided by the requirements that



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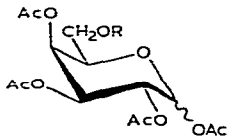
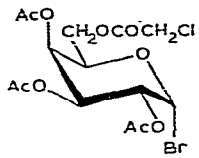


2 R = Ph_3C
3 R = H
4 R = $COCH_2Cl$



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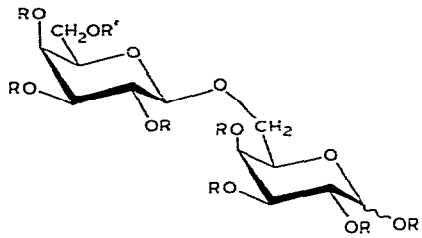
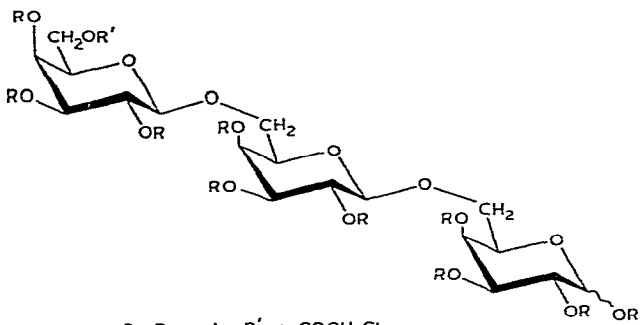


6 R = Ac, R' = $COCH_2Cl$
7 R = R' = H
8 R = Ac, R' = H



9 R = Ac, R' = $COCH_2Cl$
10 R = R' = H

(*i*) the method be relatively simple, and (*ii*) the synthesis of the polysaccharide be such that detachment from a resin support would be facile and without disruption of any synthesized linkages.

In order to assure the formation of a β-intersaccharidic linkage, the key intermediate had to have a 2-*O*-acetyl or similar group, in order to provide neighboring-group participation at the anomeric center. In addition, the known facility of removal of the *O*-chloroacetyl group[6,7] led us to prepare 2,3,4-tri-*O*-acetyl-6-*O*-(chloroacetyl)-α-D-galactopyranosyl bromide as our key intermediate.

We now report its condensation with 1,2,3,4-tetra-*O*-acetyl-D-galactopyranose to yield the protected disaccharide 1,2,3,4-tetra-*O*-acetyl-6-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(chloroacetyl)-β-D-galactopyranosyl]-D-galactopyranose (**6**), from which the 6-*O*-(chloroacetyl) group could be readily removed by treatment with thiourea in methanol[6]. The product, 1,2,3,4-tetra-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-β-D-galactosyl)-D-

galactopyranose (**8**), was again condensed with **5**, to yield the intermediate 1,2,3,4-tetra-*O*-acetyl-6-*O*-{2,3,4-tri-*O*-acetyl-6-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(chloroacetyl)-*β*-D-galactopyranosyl]-*β*-D-galactopyranosyl}-D-galactopyranose (**9**), which was deprotected to yield the title compound, **10**. Thus, we have shown the general approach for the synthesis of galacto-oligo- and -poly-saccharides having the *β*-(1→6)-linkage. It is a general method that can be successfully applied to the preparation of any (1→6)-hexo-pyranan or -furanan whose sugar residue has an equatorial O-2 when in the $^4C_1$ (D) or $^1C_4$ (L) conformation.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined with a Perkin–Elmer 141 polarimeter at 20 ± 1°. Preparatory chromatography was performed with silica gel 60 (70–270 mesh). Solvents were of Analytical Grade. Sephadex G-15 (Pharmacia Fine Chemicals, Piscataway, NJ) was used for gel filtration. Thin-layer chromatography was performed on precoated plates of silica gel GF (250 μm; Analtech), and compounds were detected by spraying with 5% sulfuric acid in methanol, followed by charring. Gas–liquid chromatography (g.l.c.) was performed in a Finnigan 9500 gas chromatograph equipped with a flame-ionization detector. Gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) was performed in a V.G. micromass 7070F spectrometer (70 eV) connected to a Perkin–Elmer Sigma-3 gas chromatograph having a column of 3% of SP-2340 on Gas Chrom Q (100–120 mesh). ^{1}H-N.m.r. spectra were recorded with a Varian EM-360A spectrometer at 60 MHz. Chemical shifts are given relative to internal tetramethylsilane. The 100-MHz, p.m.r. experiments were performed with a Jeol JNM-FX 100 spectrometer.

6-O-Trityl-D-galactose (**1**). — This compound was prepared by a method similar to that described by Kam and Oppenheimer[8]. A suspension of D-galactose (40 g) in dry pyridine (600 mL) was stirred, and chlorotriphenylmethane (a total of 70 g) was added during two days. The resulting, clear solution was evaporated, and the residue was freed of pyridine by several additions and evaporations of toluene. A solution of the resulting syrup in dichloromethane (800 mL) was washed with water, dried (sodium sulfate), and filtered, and the filtrate was evaporated to a syrup which was added to the top of a column of silica gel. The impurities were eluted with dichloromethane, and the desired product was obtained by elution with acetone. The latter eluate was evaporated to a syrup which crystallized from 95% ethanol (50 mL), to yield crystalline **1** (59.5 g, 63%), m.p. 90–95°, $[\alpha]_{589}^{20}$ +6.2→+12.5° (*c* 1.0, H_2O).

Anal. Calc. for $C_{25}H_{26}O_6$: C, 71.07; H, 6.20. Found: C, 71.06; H, 6.10.

1,2,3,4-Tetra-O-acetyl-6-O-trityl-D-galactose (**2**). — Compound **1** (50 g) was dissolved in a mixture of pyridine (350 mL) and acetic anhydride (175 mL), and the solution was kept overnight at room temperature, and then poured onto crushed ice. The precipitate was collected by filtration, washed thoroughly with cold water,

and dried, to yield 67.8 g (97%) of **2**. A sample (100 mg) of the product was purified by preparative t.l.c., using 1:12 ethyl acetate–benzene. Thus obtained, **2** had $[\alpha]_{589}^{20}$ −8.2° (*c* 0.6, $CHCl_3$)*.

Anal. Calc. for $C_{33}H_{34}O_{10}$: C, 67.11; H, 5.80. Found: C, 67.39; H, 6.11.

1,2,3,4-Tetra-O-acetyl-6-O-(chloroacetyl)-D-galactose (**4**). — A solution of compound **2** (25 g) in glacial acetic acid (200 mL) was treated for 3 min at room temperature with 6% hydrogen bromide in glacial acetic acid (40 mL). The solution was poured into ice–water, and the precipitate was removed by filtration. The filtrate was diluted with dichloromethane, successively washed with aqueous sodium hydrogencarbonate and water, dried (sodium sulfate), and concentrated to a syrup **3**. The ^{1}H-n.m.r. spectra showed the absence of the trityl group. Compound **3** was dissolved in dichloromethane (90 ml), pyridine (8.5 mL) was added, and the solution was cooled in ice–salt. Chloroacetyl chloride (8 mL) in dichloromethane (25 mL) was added dropwise, with stirring, during 1 h. After a further 45 min, water was added, and stirring was continued for 1 h. The aqueous layer was then separated, and extracted with dichloromethane, and the extract was added to the organic phase. This organic phase was thrice washed with water, dried (sodium sulfate), and evaporated to a syrup, which was purified by chromatography on a column of silica gel; impurities were eluted with dichloromethane, and then **4** was eluted with 99:1 dichloromethane–methanol. Crystallization from ethyl acetate–hexane yielded 6.9 g (38%) of **4**. In subsequent experiments, the material could be crystallized directly from the crude syrup, using 95% ethanol and nucleating crystals. Recrystallization from 95% ethanol gave prismatic needles, m.p. 129–131°, $[\alpha]_{589}^{20}$ +20° (*c* 1.5, $CHCl_3$): ^{1}H-n.m.r. data ($CDCl_3$): δ 2.0 (s, 3 H, AcO), 2.03 (s, 3 H, AcO), 2.11 (s, 3 H, AcO), 2.18 (s, 3 H, AcO), 4.05 (s, 2 H, $ClCH_2CO$), 4.1–5.53 (m, 6 H), and 5.7 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1).

Anal. Calc. for $C_{16}H_{21}ClO_{11}$: C, 45.24; H, 4.98; Cl, 8.35. Found: C, 45.43; H, 5.06; Cl, 8.18.

2,3,4-Tri-O-acetyl-6-O-(chloroacetyl)-D-galactopyranosyl bromide (**5**). — Compound **4** (5 g) was dissolved in glacial acetic acid (12.5 mL) containing 30% of hydrogen bromide. The reaction was monitored by t.l.c., and, after 3 h, dichloromethane (50 mL) and ice water (60 mL) were added. The organic phase was separated, successively washed with aqueous sodium hydrogencarbonate solution and water, dried (sodium sulfate), and evaporated to a syrup which was purified by chromatography on a column of silica gel, using 2:1 ethyl acetate–hexane. The purified **5** (4.8 g, 81% yield) gave a single spot in t.l.c., and had $[\alpha]_{589}^{20}$ +181° (*c* 5.0, $CHCl_3$); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.0 (s, 3 H, AcO), 2.03 (s, 3 H, AcO), 2.16 (s, 3 H, AcO), 4.06 (s, 2 H, $ClCH_2CO$), 4.16–5.66 (m, 6 H), and 6.65 (d, 1 H, $J_{1,2}$ 4 Hz, H-1).

1,2,3,4-Tetra-O-acetyl-6-O-[2,3,4-tri-O-acetyl-6-O-(chloroacetyl)-β-D-galacto-

*The ^{1}H-n.m.r. spectrum of **2** showed it to be composed of ~90% of 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-D-galactopyranose and ~10% of the corresponding furanose. The derivative **4** (see later in this paper) and, hence, **5**, were found to be the pyranose only. In any case, deacetylation of the product of condensation of **5** and **3** removes any possibility for isomerism in the reducing moiety of either the di- or tri-saccharide.

syl]-D-*galactopyranose* (**6**). — To a solution of compound **3** (3.9 g) in chloroform (10 mL) were added silver carbonate (5.0 g) and powdered Drierite (5.0 g), and the suspension was stirred for 1 h at room temperature. The flask was wrapped in aluminum foil, and a solution of compound **5** (5.8 g) in chloroform (10 mL) was added dropwise. After 1 h, iodine (1.5 g) was added, and the mixture was stirred for 24 h, and processed in the usual way. The resulting products were purified by chromatography on a column of silica gel, with 1:1 ethyl acetate–hexane as the eluant. Appropriate fractions were pooled, and evaporated, to yield a foam (4.27 g, 54%). Compound **6** had $[\alpha]_{589}^{20}$ +14° (*c* 0.9, $CHCl_3$): ^{1}H-n.m.r. data ($CDCl_3$): δ 1.98 (s, 6 H, 2 AcO), 2.04 (s, 3 H, AcO), 2.06 (s, 3 H, AcO), 2.12 (s, 3 H, AcO), 2.16 (s, 6 H, 2 AcO), 4.06 (s, 2 H, $ClCH_2CO$), 4.5 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), and 5.68 (d, 1 H, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{28}H_{37}ClO_{19}$: C, 47.12; H, 5.18; Cl, 4.97. Found: C, 46.72; H, 5.01; Cl 4.92.

6-O-β-D-Galactopyranosyl-D-galactose (**7**). — Compound **6** was deacetylated by treatment with 0.02M sodium methoxide in methanol (20 mL) for 3 h. Water (5 mL) was added, and the solution was de-ionized and lyophilized. The resulting material was purified on a column of Sephadex G-15, using water as the eluant, to yield **7** (0.62 g), $[\alpha]_{589}^{20}$ +34° (*c* 1, H_2O); (lit.[9] +34°): ^{1}H-n.m.r. data (D_2O, 90°): δ 4.26 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 4.4 (d, 0.75 H, $J_{1,2}$ 7.1 Hz, H-1), 5.06 (d, 0.25 H, $J_{1,2}$ 2.5 Hz, H-1), and 3.2–4.0 (m, 12 H).

Anal. Calc. for $C_{12}H_{22}O_{11} \cdot H_2O$: C, 40.04; H, 6.72. Found: C, 40.64; H, 7.06.

The large coupling-constant (6.5 Hz) for one of the anomeric protons indicates a β-glycosidic linkage. The other doublets for anomeric protons presumably represent those of the reducing moiety, and the values indicate the presence of 75% of the β and 25% of the α anomer[10].

The structure of the disaccharide was confirmed by methylation analysis. A sample of **7** (10 mg) was methylated by the Haworth[11] and Hakomori[12] procedures. The methylated disaccharide was hydrolyzed, and the products converted into the corresponding alditol acetates. Analysis[13,14] showed the presence of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol in equimolar proportions.

6-O-(6-O-β-D-Galactopyranosyl-β-D-galactopyranosyl)-D-galactopyranose (**10**). — A solution of compound **6** (3.2 g) in methanol (160 mL) containing thiourea (750 mg), was stirred for 68 h. The solvent was evaporated, the residue was taken up in chloroform, and the insoluble material was removed by filtration. The filtrate was evaporated, and the residue purified by chromatography on a column of silica gel with 4:1 ethyl acetate–hexane. The product (**8**; 1.7 g, 60%) showed a single spot in t.l.c., and had $[\alpha]_{589}^{20}$ +26° (*c* 1.7, $CHCl_3$). The ^{1}H-n.m.r. spectrum showed the absence of the chloroacetyl group.

A suspension of compound **8** (0.85 g), Drierite (4 g), and silver carbonate (1.5 g) in benzene (6 mL) was stirred for 3 h. Then, a solution of bromide **5** (0.8 g) in benzene (5 mL) was added, followed by crystalline iodine (0.3 g). After being stirred for 3 days, the mixture was filtered, and the filtrate was added to the top of a

column of silica gel, and eluted with 4:1 ethyl acetate–hexane. Compound **9** thus obtained was amorphous (0.32 g, 25%), and had $[\alpha]_{589}^{20}$ +12° (*c* 1.5, $CHCl_3$).

Anal. Calc. for $C_{40}H_{53}ClO_{27}$: C, 47.97; H, 5.22; Cl, 3.62. Found: C, 47.82; H, 5.22; Cl, 3.62.

Compound **9** was *O*-deacetylated by treatment with 25mM sodium methoxide in methanol. De-ionization, and purification by chromatography on a column of Sephadex G-15, gave pure **10** (92 mg), $[\alpha]_{589}^{20}$ +20° (*c* 1.2, H_2O).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot H_2O$: C, 41.34; H, 6.55. Found: C, 40.95; H, 7.02.

Quantitative estimation of galactose by the phenol–sulfuric acid method, before and after reduction of compound **10** with sodium borohydride, gave a ratio of 1.6:1, which is consistent with **10** being a trisaccharide.

The reduced oligosaccharide was methylated by the Hakomori method[12], the product hydrolyzed, and the sugars converted into the alditol acetates. Analysis by g.l.c. and g.l.c.–m.s. showed the presence of 5,6-di-*O*-acetyl-1,2,3,4-tetra-*O*-methylgalactitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol in the molar ratios of 1:1.09:1.1.

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