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SYNTHESIS OF THE TETRASACCHARIDE *O*-α-L-FUCOPYRANOSYL-(1→2)-[*O*-α-D-GALACTOPYRANOSYL-(1→3)]-*O*-β-D-GALACTOPYRANOSYL-(1→4)-2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE, THE ANTIGENIC DETERMINANT OF HUMAN BLOOD-GROUP B (TYPE 2)

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ABSTRACT

Treatment of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside with benzaldehyde in the presence of zinc chloride, followed by regioselective benzoylation (*N*-benzoylimidazole in dichloromethane) provided crystalline benzyl 2-acetamido-4-*O*-(3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside. Condensation of this disaccharide with 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-methylacetimidyl)-β-L-fucopyranose in nitromethane in the presence of *p*-toluenesulfonic acid and molecular sieves 4 Å gave benzyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside. Debenzoylation, followed by condensation with 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-methylacetimidyl)-β-D-galactopyranose under the same conditions provided a tetrasaccharide derivative that was catalytically hydrogenolyzed into the title compound.

INTRODUCTION

The human blood-group, antigenic determinants associated with the ABO and Lewis systems are oligosaccharides that are biosynthesized by stepwise action of gene-dependent glycosyltransferases, resulting in gene-dependent elongation of the carbohydrate chains. All variants of glycosphingolipid antigens with ABO specificities found in human erythrocytes are produced from Type-2 chains, *i.e.*, chains that are terminated by an *N*-acetyllactosamine [β-D-Gal*p*-(1→4)-GlcNAc] residue. As Type-2 chain is converted into the H and B antigens by the successive action of H and B

*Synthesis of Blood-Group substances, Part 12. A preliminary communication has been published[1]. For Part 11, see ref. 2.

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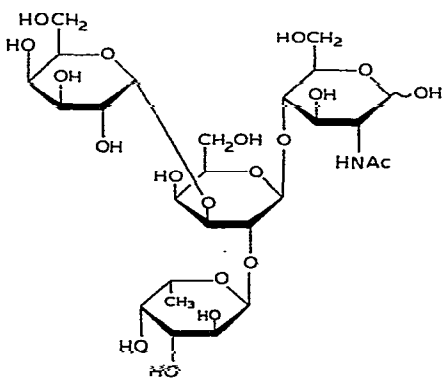
gene-specified glycosyltransferases, the so-called B, Type-2 antigenic determinant is thus the terminal tetrasaccharide **1**.

This compound **1** was isolated by Painter *et al.*[3] from the alkali-degradation products of a blood-group substance isolated from human, ovarian-cyst fluid. In hemagglutination–inhibition tests, **1** was a good inhibitor of the agglutination of B blood-group red-cells by a human anti-B serum[3].

The synthesis of a trisaccharide, derived from **1** by hydrolysis of the L-fucopyranose residue, has been reported in the preceding paper of this series[2]. On the other hand, the B blood-group-active, branched trisaccharide α-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal has been synthesized by various routes[4–6]. We now report the synthesis of the tetrasaccharide **1** using the imidate procedure reported earlier[7].

RESULTS AND DISCUSSION

Condensation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of mercuric bromide and molecular sieves 4 Å and subsequent *O*-deacetylation has been



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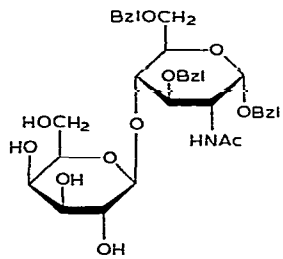

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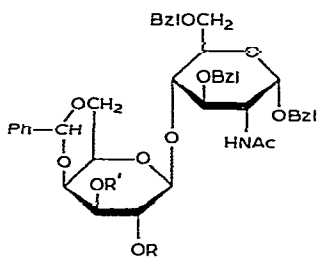
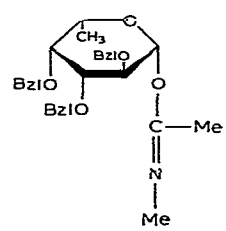


3 R = R′ = H
4 R = R′ = Ac
5 R = H, R′ = Bz
6 R = R′ = Bz



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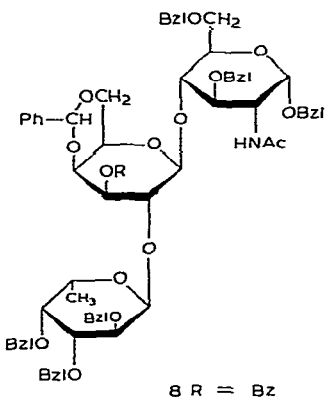
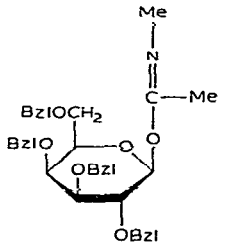


8 R = Bz
9 R = H



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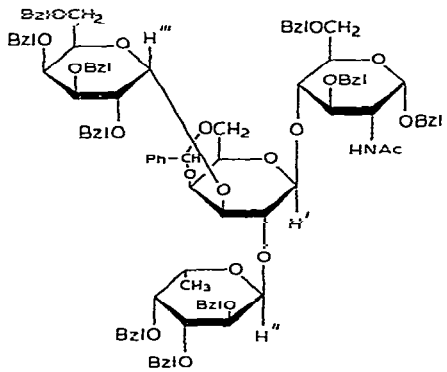

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reported[2,8] to afford the crystalline disaccharide **2**, which is a key starting-material in the present work. Reaction of **2** with benzaldehyde in the presence of zinc chloride gave the crystalline benzylidene compound **3**, which was converted for additional characterization into the crystalline acetate **4**. Disaccharide **3** was then regioselectively benzoylated in high yield (91%) with *N*-benzoylimidazole in dichloromethane to give the crystalline benzoate **5**. H-3′ of **5** appeared in the ^{1}H-n.m.r. spectrum as a deshielded signal (δ 5.0, dd, $J_{2',3'}$ 10, $J_{3',4'}$ 4 Hz). For ^{1}H-n.m.r. spectra comparison, a sample of **3** was benzoylated (benzoyl chloride in pyridine) to give the dibenzoate **6**. In addition to the expected deshielded signal of H-3′ (δ 5.15, dd, $J_{2',3'}$ 10, $J_{3',4'}$ 4 Hz), a highly deshielded signal, attributed to H-2′, was present (δ 5.8, dd, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz). This signal was absent from the n.m.r. spectrum of benzoate **5**.

The imidate procedure[7] was applied to the stereospecific α-L-fucosylation of the alcohol **5**. 2,3,4-Tri-*O*-benzyl-1-*O*-(*N*-methylacetimidyl)-β-L-fucopyranose[10] (**7**) was condensed with the alcohol **5** in nitromethane in the presence of anhydrous *p*-toluenesulfonic acid and activated, powdered molecular-sieves 4 Å for 3 days at room

temperature to give the trisaccharide **8**. Because of identical migration rates of the condensation product **8** and the alcohol **5** in t.l.c., purification was achieved after debenzoylation (sodium methoxide in methanol) to provide the trisaccharide **9** in high yield (89%). This was then easily separable from a small proportion of diol **3** (10%). It was observed that the debenzoylation of disaccharide **5** is notably faster than that of trisaccharide **8**, so that controlled debenzoylation of the condensation mixture was achieved on one occasion to isolate the trisaccharide **8**. In ^{1}H-n.m.r. spectroscopy, H-1″ of **9** appeared as a low-field doublet with a small coupling-constant (δ 5.20, $J_{1'',2''}$ 4 Hz). On the other hand, no low-field H-2′ signal could be observed for this derivative, indicating that α-L-fucosylation occurred at the OH-2′ group, and that no benzoyl group migrated during the condensation. As an additional proof of structure, catalytic hydrogenolysis of **9** gave the previously synthesized[11] blood-group H, Type-2 trisaccharide. The trisaccharide **9** was then condensed with 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-methylacetimidyl)-β-D-galactopyranose (**10**) under the aforementioned conditions (2 days at room temperature) to provide the protected tetrasaccharide **11** in 90% yield. The anomeric proton of the newly introduced D-galactopyranose residue appeared as a low-field doublet with a small coupling constant (δ 5.75, $J_{1''',2'''}$ 4 Hz). This product was deprotected to give the title tetrasaccharide **1** by hydrogenolysis in acetic acid in the presence of palladium-on-carbon. Partial acid hydrolysis afforded L-fucose and the trisaccharide *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, identified by comparison with the derivative reported in the preceding article[2] of this series. After this work had been completed[1], another synthesis of the title tetrasaccharide was reported by Paulsen and Kolář[12].

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi apparatus. Optical rotations were determined at 22–24° with a Perkin–Elmer model 141 polarimeter. I.r. spectra were recorded with a Jouan–Jasco IRA-1 spectrometer. ^{1}H-N.m.r. spectra were recorded for solutions in deuteriochloroform (tetramethylsilane as internal standard) unless otherwise stated. Gas–liquid chromatography of the per-*O*-(trimethylsilyl) derivatives was performed with a Girdel 3000 apparatus provided with a flame-ionization detector and a 3.40-m Pyrex column (4% OV 17 on Gas-Chrom Q, 80–100 mesh), programmed for a rise of 10°/min from 150 to 300°; T_R is given relative to that of hexa-*O*-(trimethylsilyl)-*myo*-inositol. Purity of products was determined by t.l.c. on silica gel 60 F 254 (E. Merck). Components were located by spraying the plates with a 50% solution of sulfuric acid in ethanol, and charring. Column chromatography was performed on silica gel Merck 60 (0.063–0.200 mm), which was used without pretreatment. Microanalyses were performed by the Service Central de Microanalyse du Centre National de la Recherche Scientifique.

Benzyl 2-acetamido-3,6-di-O-benzyl-4-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (**3**). — A suspension of benzyl 2-acetamido-3,6-

di-*O*-2-benzyl-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside[2] (**2**) (1.3 g) in benzaldehyde (5 mL) containing anhydrous, powdered zinc chloride (300 mg) was shaken overnight at room temperature. The mixture was diluted with diisopropyl ether (100 mL), and the resulting precipitate was filtered off and crystallized from ethyl acetate to give the disaccharide **3** (1.37 g, 92%), m.p. 175°, $[\alpha]_D^{20}$ +76° (*c* 1.1, chloroform); ^{1}H-n.m.r.: δ 7.30–7.25 (m, 20 H, 4 Ph), 5.48 (d, 1 H, 9 Hz, NH), 4.90 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 1.72 (s, 3 H, Ac); lit.[12] m.p. 175°, $[\alpha]_D^{22}$ +76.5° (*c* 1.05, chloroform).

Anal. Calc. for $C_{42}H_{47}NO_{11}$: C, 67.99; H, 6.38; N, 1.88. Found: C, 67.84; H, 6.41; N, 1.73.

Benzyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-4*-O-(*2,3-di*-O-*acetyl-4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-α-D-*glucopyranoside* (**4**). — Disaccharide **3** (70 mg) was acetylated (acetic anhydride–pyridine) to give **4** (68 mg, 87%), m.p. 199–200° (from ethyl acetate–hexane), $[\alpha]_D^{22}$ +104° (*c* 1, in chloroform); ^{1}H-n.m.r.: δ 7.40–7.30 (2 s, 20 H, Ph), 5.41 (s, 1 H, C*H*Ph), 5.35 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2'), 5.25 (d, 1 H, *J* 9 Hz, NH), 4.95 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 2.00 and 1.95 (2 s, 6 H, 2 OAc), and 1.72 (s, 3 H, NAc).

Anal. Calc. for $C_{46}H_{56}NO_{13}$: C, 66.89; H, 6.22; N, 1.69. Found: C, 66.42; H, 6.31; N, 1.63.

Benzyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-4*-O-(*2,3-di*-O-*benzoyl-4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-α-D-*glucopyranoside* (**6**). — A small amount of disaccharide **3** was benzoylated (benzoyl chloride in pyridine) to give crystalline **6**, m.p. 194° (from ether), prepared for ^{1}H-n.m.r. spectroscopy: δ 5.80 (dd, 1 H, $J_{1',2'}$ 8 Hz, H-2'), 5.15 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 4 Hz, H-3'), 5.05 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 1.68 (s, 3 H, NAc).

Benzyl 2-acetamido-4-O-(*3*-O-*benzoyl-4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-*3,6-di*-O-*benzyl-2-deoxy*-α-D-*glucopyranoside* (**5**). — A solution of imidazole (61.6 mg) in 1,2-dichloroethane (2 mL) was added to a solution of benzoyl chloride (63.6 mg) in 1,2-dichloroethane (1 mL). The precipitate was filtered off, and the solution of *N*-benzoylimidazole thus obtained was stirred during 3 days under reflux in the presence of **3** (280 mg). The mixture was diluted with chloroform, washed with water, dried (Na_2SO_4), and evaporated. The residue crystallized from ether to give **5** (290 mg, 91%), m.p. 177–179°, $[\alpha]_D^{22}$ +120° (*c* 1.2, chloroform); ^{1}H-n.m.r.: δ 5.55 (d, 1 H, *J* 9 Hz, NH), 5.00 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 4 Hz, H-3'), 4.92 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.10 (1 H, OH), and 1.76 (s, 3 H, Ac); lit.[12] m.p. 177°, $[\alpha]_D^{20}$ +117.8° (*c* 1.05, chloroform).

Anal. Calc. for $C_{49}H_{51}NO_{12}$: C, 69.57; H, 6.08; N, 1.65. Found: C, 69.24; H, 6.09; N, 1.65.

Benzyl O-(*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*2*)-O-(*4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-(*1*→*4*)-*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-α-D-*glucopyranoside* (**9**). — A solution of disaccharide **5** (270 mg) and 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-methylacetimidyl)-β-L-fucopyranose (**7**) (263 mg) in anhydrous nitromethane (2 mL) was stirred for 3 days at room temperature under nitrogen in the presence of powdered

molecular-sieves 4 Å (500 mg) and anhydrous *p*-toluene sulfonic acid (90 mg). The mixture was filtered, and the filtrate washed with aqueous, saturated sodium hydrogencarbonate and then water, dried (Na_2SO_4), and evaporated. The residue was debenzoylated (sodium methoxide in methanol) to give a residue that was chromatographed on silica gel (30 g); elution with 49:1 (v/v) chloroform–methanol gave the trisaccharide **9** (327 mg, 89%), syrup, $[\alpha]_D$ +13° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 5.20 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''), 3.12 (1 H, OH), 1.70 (s, 3 H, Ac), and 1.08 (d, 3 H, *J* 7 Hz, CH_3); lit.[12] $[\alpha]_D^{20}$ +9.06° (*c* 1.06, chloroform).

Anal. Calc. for $C_{69}H_{75}NO_{15}$: C, 71.55; H, 6.53; N, 1.21. Found: C, 71.84; H, 6.45; N, 1.31.

A solution of **9** (40 mg) in acetic acid (2 mL) was hydrogenolyzed in the presence of 10% palladium-on-charcoal (100 mg) for 48 h. The reaction mixture was filtered, and the filtrate evaporated to give a powder. After reduction with sodium borohydride and per-*O*-(trimethylsilyl)ation, the product was homogeneous in g.l.c. (T_R 2.90) and indistinguishable from a previously synthesized sample[11].

Benzyl O-(*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*2*)-O-(*3*-O-*benzoyl*-*4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-(*1*→*4*)-*2-acetamido*-*3,6-di*-O-*benzyl*-*2-deoxy*-α-D-*glucopyranoside* (**8**). — In one experiment, the aforementioned debenzoylation was quenched after selective debenzoylation of unreacted starting material **5**. Processing gave the trisaccharide **8**, syrup, $[\alpha]_D^{22}$ +36.7° (*c* 1.6, chloroform); ^{1}H-n.m.r.: δ 8.05–7.95 and 7.35–7.27 (m, 4 OH, Ph), 5.45 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''), 5.02 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.70 (s, 3 H, NAc), and 1.1 (d, 3 H, *J* 7 Hz, CH_3); lit.[12] +38.4° (*c* 0.99, chloroform).

Anal. Calc. for $C_{76}H_{79}NO_{16}$: C, 72.33; H, 6.31; N, 1.11. Found: C, 71.99; H, 6.45; N, 0.80.

Benzyl O-(*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1*→*2*)-[O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*galactopyranosyl*)-(*1*→*3*)-O-(*4,6*-*benzylidene*-β-D-*galactopyranosyl*)-(*1*→*4*)-*2-acetamido*-*3,6-di*-O-*benzyl*-*2-deoxy*-α-D-*glucopyranoside* (**11**). — A solution of trisaccharide **9** (112 mg) and 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-methylacetimidyl)-β-D-galactopyranose (**10**; 90 mg) in anhydrous nitromethane (1 mL) was stirred at room temperature under nitrogen for 2 days in the presence of powdered molecular-sieves 4 Å (200 mg) and anhydrous *p*-toluenesulfonic acid (36 mg). The mixture was filtered, and the filtrate washed with aqueous, saturated sodium hydrogencarbonate and water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (18 g); elution with 7:4 (v/v) ethyl acetate–hexane gave the tetrasaccharide **11** (136 mg, 90%), syrup, $[\alpha]_D^{22}$ +33.9° (*c* 1.4, chloroform); ^{1}H-n.m.r.: δ 7.30–7.10 (m, 35 H, 7 Ph), 5.75 (d, 1 H, $J_{1''',2'''}$, H-1'''), 5.40 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''), 1.70 (s, 3 H, Ac), and 1.15 (d, 3 H, *J* 7 Hz, Me).

Anal. Calc. for $C_{103}H_{106}NO_{20}$: C, 73.76; H, 6.37. Found: C, 74.03; H, 6.38.

O-α-L-*Fucopyranosyl*-(*1*→*2*)-[O-α-D-*galactopyranosyl*-(*1*→*3*)]-O-β-D-*galactopyranosyl*-(*1*→*4*)-*2-acetamido*-*2-deoxy*-D-*glucopyranose* (**1**). — Compound **11** (306 mg) in acetic acid (20 mL) was hydrogenolyzed in the presence of 10% palladium-on-carbon (300 mg) for 2 days. The mixture was filtered, and the filtrate evaporated

to give the tetrasaccharide **1** (113 mg, 90%) as an amorphous, hygroscopic powder, $[\alpha]_D^{22}$ +14.1° (*c* 0.30, water); lit.[3]: $[\alpha]_D^{22}$ +12° (*c* 0.5, water); lit.[12] $[\alpha]_D^{20}$ +14.5° (water). This compound was homogeneous in g.l.c. (T_R 5.65) after reduction with sodium borohydride and per-*O*-(trimethylsilyl)ation.

Mild acid-hydrolysis (0.1M hydrochloric acid, 3 h, 85°) gave three derivatives, identified by g.l.c. after the aforementioned treatment: **1** (T_R 5.65), *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose[1] (T_R 3.43), and L-fucopyranose (T_R 0.51).

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