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THE SYNTHESIS OF 3,6-DI-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-D-GALACTOSE, A BRANCHED TRISACCHARIDE REPORTED AS A HYDROLYSIS PRODUCT OF BLOOD-GROUP SUBSTANCES*†

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ABSTRACT

Protected disaccharides were the only products that could be isolated after condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoramido-α-D-glucopyranosyl bromide or 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride with benzyl 2,4-di-*O*-benzyl-β-D-galactopyranoside. On the other hand, reaction of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline (6 moles) with the same galactopyranoside (1 mole) gave benzyl 3,6-di-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-β-D-galactopyranoside, which was converted, by alkaline methanolysis followed by hydrogenolysis, to the title compound. This appears identical with an oligosaccharide previously obtained through degradation of a blood-group A glycoprotein from hog gastric mucin.

INTRODUCTION

In 1961, Yosizawa[3] isolated by hydrazinolysis, acidic hydrolysis, and *N*-acetylation of a blood-group A glycoprotein from hog gastric mucin a compound, named "oligosaccharide IV", which he considered to be a trisaccharide having structure **9**. Later, a related, reduced pentasaccharide "Lewis R_L 0.17" was isolated by Lloyd *et al.*[4] from Le^a-active, human, ovarian-cyst material. Further hydrolysis with β-galactosidase gave a reduced trisaccharide which had the same monosaccharide composition and showed, on paper chromatography in one solvent system, the same properties as a borohydride-reduced sample of "oligosaccharide IV". The two compounds were thought to be identical, and the structure *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-β-D-

*Dedicated to Professor Michael Heidelberger, in honor of his 87th birthday.

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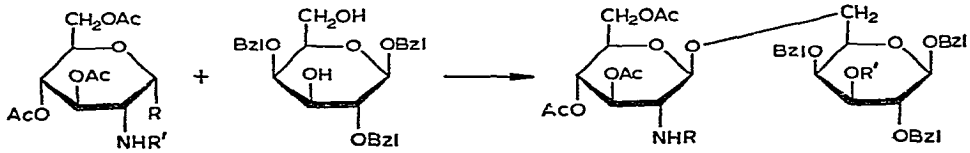
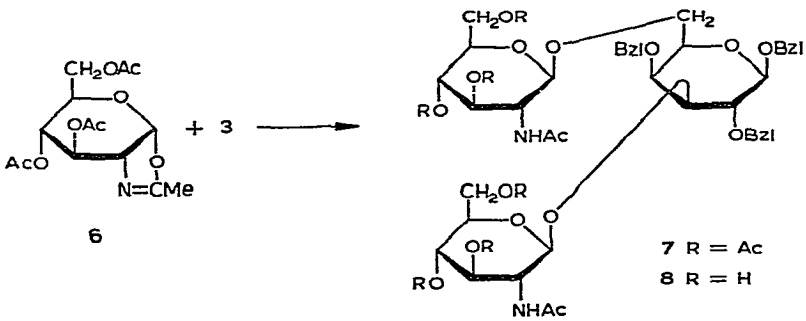
galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-D-galactitol was proposed for "Lewis R_L 0.17". This monosaccharide sequence was incorporated in the often quoted, composite A-, B-, H-, Le^a-, or Le^b-specific oligosaccharide, which incorporates a trisaccharide **9** residue as the main branch-point. For these reasons, a synthetic route to trisaccharide **9** seemed advisable.



1 R = Br, R' = PO(OPh)$_2$
2 R = Cl , R' = Ac

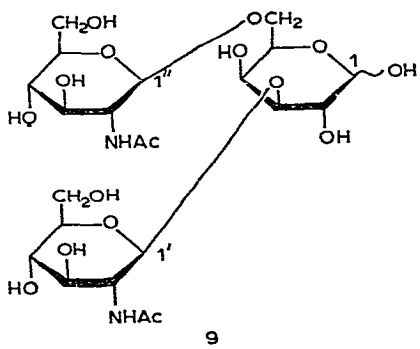
3

4 R = PO(OPh)$_2$, R' = H
5 R = R' = Ac



6

7 R = Ac
8 R = H



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RESULTS AND DISCUSSION

Koenigs–Knorr condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoramido-α-D-glucopyranosyl bromide (**1**) with the primary hydroxyl group of suitably protected derivatives[5–7] gave several β-(1→6)-linked disaccharides in 40–60% yield. The reaction of an equimolecular amount of this bromide **1** with benzyl 2,4-di-

O-benzyl-β-D-galactopyranoside[8] (**3**) in benzene in the presence of mercuric cyanide gave **4** in 40% yield. The β-D configuration of the disaccharide linkage was strongly suggested by the comparison of the molecular rotation of **4** with those of the parent monosaccharides (see Table I). Attempts to condense a second molecule of bromide **1** with the secondary hydroxyl group at C-3 of the D-galactose unit led, however, to complex mixtures containing large amounts of the disaccharide **4** and of products which had lost phenol molecules, and it was not possible to isolate a trisaccharide.

TABLE I

MOLECULAR ROTATIONS[a] OF COMPOUNDS **4** AND **7** COMPARED TO THE SUM OF THE MOLECULAR ROTATIONS OF THE CONSTITUENTS

Compound	$[M]_D$ (*degrees*) × 10^{-2}
Benzyl 2,4-di-*O*-benzyl-β-D-galactopyranoside[8] (**3**) + methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoramido-β-D-glucopyranoside[5]	−134
Compound **3** + benzyl 2,3,4-tri-*O*-acetyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoramido-β-D-glucopyranosyl)-β-D-glucopyranoside[7] − benzyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside[7]	−128
Compound **4**	−158
Compound **3** + two methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[9] (**10**)	−309
Compound **3** + compound **10** + methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside[10] (**11**)	+158
Compound **3** + two compounds **11**	+625
Compound **7**	−391

[a]Optical rotations determined in chloroform.

Since the diphenoxyphosphoramido group does not always favor formation of β-D linkages when the condensation is effected with a secondary hydroxyl group[11–13], 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**2**), where the presence of the *N*-acetyl participating group should ensure the exclusive β-D configuration of the glycoside bond formed, was condensed (1.5 mol) with **3** to give the β-D-(1→6)-linked disaccharide **5**, isolated as the derivative acetylated at C-3 of the D-galactose moiety in only 15% yield. This low yield can be accounted for by the lack of reactivity of the chloride and the strong tendency to decomposition.

Finally, when 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline (**6**) was condensed with **3** (molar ratio of **6** to **3**, 6:1) in the presence of *p*-toluenesulfonic acid, the formation of a disaccharide identical to that obtained previously in the reaction with the chloride was observed by t.l.c. This product was almost not observed on t.l.c. as the trisaccharide **7** was formed, and the latter compound was isolated pure in 50% yield by direct crystallisation from the reaction mixture. The reaction was accompanied by an isomerisation of the oxazoline **6** to 2-acetamido-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol[14] which, like **6**, is very soluble in ether, whereas the condensation products are practically insoluble. Also

the presence of benzyl groups on the D-galactose moiety facilitated the detection of condensation products when t.l.c. plates were observed under u.v. light.

The convenient *O*-deacetylation of **7** with methanolic sodium methoxide gave the trisaccharide **8** as a monohydrate, in 80% yield. For the hydrogenolysis of the benzyl groups at atmospheric pressure and ambient temperature, repeated additions of the catalyst (10% palladium-on-charcoal) were necessary, and even after 48 h partially debenzylated products were still present. The free trisaccharide **9** was nevertheless obtained pure, as a crystalline dihydrate, by chromatography on silica gel, but in low yield (20%) due to poor resolution of the mixture.

The β-D configuration at both glycosidic linkages in trisaccharide **9** is strongly suggested by the comparison of the molecular rotation of **7** with those of the parent monosaccharides (see Table I). The 240 MHz p.m.r. spectrum in dimethyl sulfoxide-d_6 can be interpreted in a manner consistent with structure **9**, allowing for the presence of pyranose, and most probably furanose tautomers of the D-galactose moiety. Thus, each anomeric proton of the 2-acetamido-2-deoxy-D-glucopyranosyl residues appears as two closely overlapping doublets of unequal intensity, with J 7.6 Hz. The physical properties of the trisaccharide **9** were identical in all respects with those of the natural product isolated by Yosizawa[3].

EXPERIMENTAL

General methods. — The general methods were as described previously[2]. The p.m.r. spectrum of **9** was recorded at 240 MHz for a solution in dimethyl sulfoxide-d_6 at 37°, with a spectrometer constructed in this University[15]. Chemical shifts are given in p.p.m. from the tetramethylsilane peak ($\delta = 0$). To exchange labile protons with deuterons, the sample was previously dissolved in deuterium oxide, the resulting solution was freeze-dried, and this process was repeated three times.

Benzyl 2,4-di-O-benzyl-6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-diphenoxyphosphoramido-β-D-glucopyranosyl)-β-D-galactopyranoside (**4**). — A solution of benzyl 2,4-di-*O*-benzyl-β-D-galactopyranoside[8] (**3**, 0.90 g, 2 mmol) and mercuric cyanide (0.90 g, 3,4 mmol) in dry benzene (20 ml) was boiled until a few ml of the solvent had distilled. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoramido-α-D-glucopyranosyl bromide[5] (**1**, 1.2 g, 2 mmol) was added, and the mixture was boiled under reflux for 3 h. Whilst still hot, the suspension was filtered, and the residues washed with benzene (200 ml). Chloroform (100 ml) was added, and the solution was washed twice with ice-cold, aqueous sodium chloride, and then three times with water, and finally dried (sodium sulfate). Evaporation of the solvents yielded a light-brown foam (1.8 g) which was chromatographed on a column of silica gel (200 g) with 1:1 (v/v) benzene–ethyl acetate as eluent; **4** (0.78 g, 40%) was crystallized from 2-propanol, m.p. 200–201°, $[\alpha]_D^{20}$ −16.3° (*c* 1.17, chloroform); i.r. data: ν_{max}^{KBr} 3510 (OH), 3250 (NH), 1740 (OAc), 770, 730, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{51}H_{56}NO_{16}P$: C, 63.15; H, 5.82; N, 1.44; P, 3.19. Found: C, 63.29; H, 5.79; N, 1.51; P, 3.23.

Benzyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-2,4-di-O-benzyl-β-D-galactopyranoside (**5**)*. — A solution of **3** (0.50 g, 1.1 mmol) and mercuric cyanide (0.56 g, 2.2 mmol) in dry 1,2-dichloroethane (40 ml) was boiled until 20 ml of the solvent had distilled. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[16] (**2**, 0.80 g, 2.2 mmol) was added, and the mixture was boiled under reflux for 4 h, a further addition of **2** (0.40 g, 1.1 mmol) and mercuric cyanide (0.28 g, 1.1 mmol) being made after 2 h. The reaction mixture was diluted with dichloromethane (200 ml) and washed with a saturated solution of sodium hydrogencarbonate and water, dried (sodium sulfate), and evaporated *in vacuo*. T.l.c. (1:1, v/v, ether–ethyl acetate) showed the presence of a main component, together with unreacted **3** and decomposition products. The mixture was acetylated with pyridine (5 ml) and acetic anhydride (2 ml) overnight at room temperature. The resulting solution was evaporated *in vacuo* to a syrup (0.52 g), which was chromatographed on a column of silica gel with 9:1 (v/v) ethyl acetate–ether as eluent; **5** (0.10 g, 15%) was recrystallized from ether, m.p. 170°, $[\alpha]_D^{20}$ −2.3° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 3290 (NH), 3085, 3060 and 3030 (Ph), 1740 (OAc), 1660 (Amide I),1550 (Amide II), 740, and 700 cm^{-1} (Ph).

Anal. Calc. for $C_{43}H_{51}NO_{15}$: C, 62.84; H, 6.26; N, 1.70; O, 29.20. Found: C, 62.88; H, 6.36; N, 1.66; O, 28.96.

Benzyl 3,6-di-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4-di-O-benzyl-β-D-galactopyranoside (**7**). — A solution of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline[17] (**6**, 1.32 g, 4 mmol) in dry toluene (15 ml) was added to a solution of **3** (0.90 g, 2 mmol) in dry nitromethane (15 ml). The mixture was boiled until 10 ml of the solvent had distilled, and *p*-toluenesulfonic acid (13 mg) was added. The solvent was slowly distilled for a further 10 min, then a further amount of **6** (1.32 g, 4 mmol) in dry toluene (15 ml) and *p*-toluenesulfonic acid (13 mg) was added. After distillation of a few ml of solvent, the mixture was gently boiled under reflux for 2 h, a further addition of **6** (1.32 g, 4 mmol) in dry toluene (15 ml) being made after 1 h. The resulting brown solution was cooled to room temperature, diluted with dichloromethane (50 ml) containing pyridine (1 ml), and evaporated *in vacuo*. The residue was dissolved in the minimal volume of ethanol, ether was added, and the solution was kept overnight at +5°. It afforded a gelatinous product, which was thoroughly washed with ether and dried (0.96 g, 43%). A second crop (0.16 g; total yield, 50%) was obtained by the addition of more ether to the mother liquor. Examination by t.l.c. (7:7:1, v/v, benzene–ether–methanol) showed the compound to be pure. The mother liquor contained large amounts of 2-acetamido-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol. The trisaccharide **7** was crystallized from methanol, m.p. 228° (dec.), $[\alpha]_D^{20}$ −35.3° (*c* 1.02, chloroform); i.r. data: ν_{max}^{KBr} 3300 (NH), 3080, 3060 and 3030 (Ph), 1740 (OAc), 1660 (Amide I), 1530 (Amide II), 740, and 700 cm^{-1} (Ph); n.m.r. data: δ 1.55 (3 H, OAc), 1.85 (3 H, OAc), 2.00 (18 H, 2 NAc, and 4 OAc), and 7.28 (15 H, 3 Ph).

*This compound was prepared by Dr. C. A. Johnson.

Anal. Calc. for $C_{55}H_{68}N_2O_{22}$: C, 59.56; H, 6.18; N, 2.53; O, 31.74. Found: C, 59.37; H, 6.08; N, 2.58; O, 32.04.

Benzyl 3,6-di-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,4-di-O-benzyl-β-D-galactopyranoside (**8**). — A solution of **7** (1.50 g) in 1:1 (v/v) chloroform–methanol (50 ml) was treated with 0.5M sodium methoxide in methanol (7 ml) overnight at room temperature. The chloroform was evaporated at room temperature, and the resulting methanol solution was treated first with Dowex 50 (X-8, H^+) ion-exchange resin, and then with silver carbonate. The filtrate was evaporated to give a syrup which was recrystallized from ethanol–ether to give **8** (0.93 g, 80%), m.p. 208–210° (dec.), $[\alpha]_D^{20}$ −33.8° (*c* 1.065, methanol); t.l.c. (5:3:2, v/v, butanol–pyridine–water): R_F 0.64.

Anal. Calc. for $C_{43}H_{56}N_2O_{16}\cdot H_2O$: C, 59.03; H, 6.68; N, 3.20; O, 31.09. Found: C, 59.08; H, 6.43; N, 3.34; O, 30.93.

3,6-Di-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose (**9**). — A solution of **8** (0.70 g) in 95% ethanol (70 ml) was hydrogenated for 48 h at room temperature and atmospheric pressure in the presence of 10% palladium-on-charcoal (0.70 g). The catalyst was removed and the filtrate was evaporated. The residue, which contained some incompletely hydrogenolyzed compounds, was chromatographed on a column of silica gel (100 g) with 5:3:2 (v/v) butanol–pyridine–water as eluent to yield the pure trisaccharide **9** (0.10 g, 20%), crystallized from methanol–ether, m.p. 142–144° (dec.), $[\alpha]_D^{20}$ +6.5° (*c* 1.01, water: no mutarotation); paper chromatography (5:3:2, v/v, butanol–pyridine–water): R_{Glc} 0.28, and $R_{Lactose}$ 0.64; i.r. data: ν_{max}^{KBr} 3400 (OH and NH), 1640 (Amide I), 1550 (Amide II), 940, and 890 cm^{-1} (these frequencies are not readily interpretable and are mentioned here for comparison with data from Yosizawa[3]); n.m.r. data (240 MHz, dimethyl sulfoxide-d_6): δ 4.95 (d, $J_{1,2}$ 3.2 Hz, equatorial H-1), 4.94 (broad, ring proton ?), 4.60 (d, 0.6 H, $J_{1,2}$ 7.6 Hz, axial H-1), 4.50, 4.48 (2 d, 1 H, $J = J' = 7.6$ Hz, H-1′ or H-1″), 4.39, 4.37 (2 d, 1 H, $J = J' = 7.6$ Hz, H-1″ or H-1′), 2.50 (residual H of dimethyl sulfoxide-d_6), 1.83, 1.84 (2 s, 0.9 × 6 H, 2 *N*Ac), and 1.68 (s, 0.1 × 6 H, 2 *N*Ac of minor tautomer).

Anal. Calc. for $C_{22}H_{38}N_2O_{16}\cdot 2H_2O$: C, 42.44; H, 6.80; N, 4.50; O, 46.26. Found: C, 41.92; H, 6.67; N, 4.60; O, 46.29.

For the natural trisaccharide, Yosizawa[3] reported $[\alpha]_D^{23}$ +6.5° (*c* 0.9, water, no mutarotation); i.r. data: ν_{max}^{KBr}: 895 cm^{-1}, paper chromatography (5:3:2, v/v, butanol–pyridine–water): R_{Glc} 0.29 and $R_{Lactose}$ 0.63; total nitrogen: 4.68 (calc. for $C_{22}H_{38}N_2O_{16}$: 4.78).

Trisaccharide **9** was reduced with sodium borohydride into 3,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactitol; paper chromatography (6:4:3, v/v, butanol–pyridine–water): $R_{Lactose}$ 0.78. For the natural compound, Lloyd *et al.*[4] reported: $R_{Lactose}$ 0.70.

REFERENCES

1 S. DAVID, J. EUSTACHE, AND A. LUBINEAU, *J. Chem. Soc., Perkin I*, (1974) 2274–2278.
2 C. L. BREWER, S. DAVID, AND A. VEYRIÈRES, *Carbohyd. Res.*, 36 (1974) 188–190.
3 Z. YOSIZAWA, *J. Biochem.* (Tokyo), 51 (1962) 145–154.

4 K. O. LLOYD, E. A. KABAT, AND E. LICERIO, *Biochemistry*, 7 (1968) 2976–2990.
5 L. ZERVAS AND S. KONSTAS, *Chem. Ber.*, 93 (1960) 435–446.
6 D. BUNDLE AND N. SHAW, *Carbohyd. Res.*, 21 (1972) 211–217.
7 R. IWAMOTO AND Y. IMANAGA, *Carbohyd. Res.*, 24 (1972) 133–139.
8 S. DAVID, C. A. JOHNSON, AND A. VEYRIÈRES, *Carbohyd. Res.*, 28 (1973) 121–123.
9 K. ONODERA, S. KITAOKA, AND H. OCHIAI, *J. Org. Chem.*, 27 (1962) 156–159.
10 R. KUHN, F. ZILLIKEN, AND A. GAUHE, *Chem. Ber.*, 86 (1953) 466–467.
11 K. HEYNS, K. PROPP, R. HARRISON, AND H. PAULSEN, *Chem. Ber.*, 100 (1967) 2655–2663.
12 K. HEYNS, R. HARRISON, AND H. PAULSEN, *Chem. Ber.*, 100 (1967) 271–279.
13 P. SCHMITT AND P. SINAŸ, *Carbohyd. Res.*, 29 (1973) 99–111.
14 W. L. SALO AND H. G. FLETCHER, JR., *J. Org. Chem.*, 34 (1969) 3189–3190.
15 M. D. SAUZADE AND S. K. KAN, *Advan. Electron. Electron Phys.*, 34 (1973) 1–93.
16 D. HORTON, *Methods Carbohyd. Chem.*, 6 (1972) 282–285.
17 K. L. MATTA AND O. P. BAHL, *Carbohyd. Res.*, 21 (1972) 460–464.