Studies in Oligosaccharide Chemistry. Part 8.[1] Synthesis of Lacto-*N*-triose I, a Core Chain Trisaccharide of Human Blood-group Substances

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Condensation of 4,6-di-*O*-acetyl-1,2-dideoxy-2'-methyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoso[2,1-*d*]-$\Delta^{2'}$-oxazoline (4) with benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-α-D-galactopyranoside (5) in the presence of toluene-*p*-sulphonic acid gave a protected trisaccharide (6) in 84% yield. Removal of the protecting groups gave the free trisaccharide β-D-Gal-(1 → 3)-β-D-GlcNAc-(1 → 3)-D-Gal (10), identical in properties with ' Lacto-*N*-triose-I,' a product of acidic hydrolysis of carbohydrates from human milk and blood-group substances. The carbohydrate sequence of the trisaccharide (10) is representative of the 'core ' portion of blood-group substances.

BLOOD-GROUP substances from ovarian cysts are water-soluble glycoproteins in which two types of carbohydrate chain endings may be present: type 1 chains, β-D-Gal-(1 → 3)-D-GlcNAc, and type 2 chains, β-D-Gal-(1 → 4)-D-GlcNAc. A-, B-, and H-determinants can be based on either type 1 or type 2 chains † but Le-active structures, in which L-fucose is substituted on the 4-position of *N*-acetylglucosamine, are based only on type 1 chains.[2] Alkaline hydrolysis[3] of these substances in the presence of sodium borohydride gives the reduced pentasaccharide (1). This indicates that type 1 and type 2 chains may be linked to the same D-galactose residue. The same structural unit is also present in two oligosaccharides from human milk, ' lacto-*N*-hexaose '[4] and ' lacto-*N*-fucoheptaose.'[5] It appears that in nature type 1 chains are always linked to the 3-position of D-galactose, whereas type 2 chains are substituted on either the 3- or the 6-position of D-galactose. On the other hand, only type 2 chains have been found in A-, B-, or H-active glycosphingolipids from human erythrocyte membranes.[6] Specificities

† According to a recent paper (K. Yamashita, Y. Tachibana, S. Takasaki, and A. Kobata, *Nature*, 1976, **262**, 702), the H-determinant of branched milk oligosaccharides is located only on type 1 chains.

[1] Part 7, C. Augé and A. Veyrières, *Carbohydrate Res.*, in the press.

[2] W. M. Watkins, in ' Glycoproteins,' ed. A. Gottschalk, Elsevier, Amsterdam, 1972, p. 830.

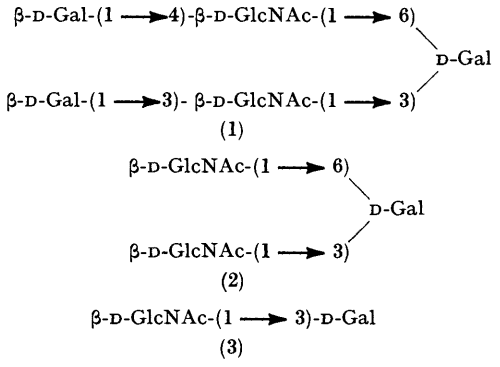
[3] K. O. Lloyd, E. A. Kabat, and E. Licerio, *Biochemistry*, 1968, **7**, 2976.

[4] A. Kobata and V. Ginsburg, *J. Biol. Chem.*, 1972, **247**, 1525.

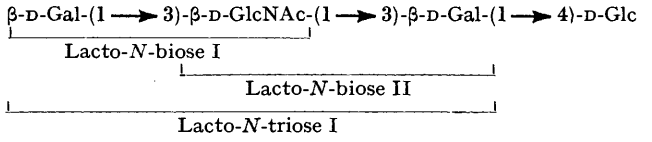
[5] L. Grimmonprez, M. Delautre, S. Bouquelet, and J. Montreuil, *F.E.B.S. Letters*, 1975, **54**, 221.

[6] S. Hakomori and A. Kobata, in ' The Antigens,' ed. M. Sela, vol. 2, 1974, p. 79.

associated with the ABO system, such as Ii might be related to this ' core ' portion of the blood-group substances, thus underlying the now well known A, B, H, and Le determinants.[7]



For these reasons we were interested in the synthesis of oligosaccharides related to compound (1), making them readily available for chemical and inhibition studies, as it is difficult to isolate them from natural sources in sufficient quantity. We have already reported syntheses of the oligosaccharides (2) [8] and (3) [1] (' lacto-*N*-biose II ') (Figure), and we now report the synthesis of the trisaccharide (10). This was first described [9] as a product of partial, acidic hydrolysis of ' lacto-*N*-tetraose ' (Figure), a sugar from human milk, and

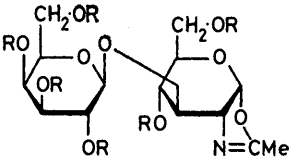


The oligosaccharides obtained in the hydrolysis of ' lacto-*N*-tetraose,' with the names given to them by Kuhn *et al.*[9]

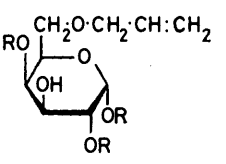
named ' lacto-*N*-triose I.' Later Morgan *et al.*[10] found the same trisaccharide among the products of acidic hydrolysis of blood-group substances.

We have recently reported [11] a convenient preparation of the protected disaccharide oxazoline (4) derived from ' lacto-*N*-biose I.' Glycosidation with carbohydrate-derived oxazolines generally gives excellent yields of 1,2-*trans*-glycosides, so the oxazoline (4) seemed to be an attractive reagent for the introduction of a type 1 chain with a β-anomeric linkage in a single step. This oxazoline was treated with the protected D-galactose derivative (5),[1] with a free OH at position 3 and a ' temporary ' allyl group at position 6. Condensation of the oxazoline (4) (100% molar excess) with compound (5) in the presence of toluene-*p*-sulphonic acid, followed by chromatography on silica gel, gave 84% yield [based on (5)] of the pure, amorphous, protected trisaccharide (6). Isomerisation[12] of compound (6) to the 6-prop-1-enyl ether (7) with the use of chloro(tristriphenylphosphine)-rhodium(I) led to a 1 : 2 mixture of the 6-prop-1-enyl ether (7) with the starting allyl ether (6). It was necessary to add more catalyst to achieve a better conversion, which was, however, still incomplete. Nevertheless samples of catalyst from the same batch were normally efficient in other isomerisation reactions,[1] giving the corresponding prop-1-enyl ethers in more than 80% yield.

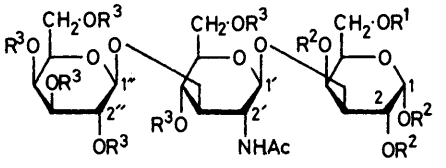
O-Deacetylation of compound (8) [the product of hydrolysis of the ether (7)] followed by hydrogenolysis of the benzyl ether functions gave the free trisaccharide ' lacto-*N*-triose I ' (10), obtained as the crystalline



(4) R = Ac



(5) R = CH_2Ph



(6) $R^1 = CH_2{\cdot}CH{:}CH_2$, $R^2 = CH_2Ph$, $R^3 = Ac$
(7) $R^1 = CH{:}CH{\cdot}CH_3$, $R^2 = CH_2Ph$, $R^3 = Ac$
(8) $R^1 = H$, $R^2 = CH_2Ph$, $R^3 = Ac$
(9) $R^1 = R^3 = H$, $R^2 = CH_2Ph$
(10) $R^1 = R^2 = R^3 = H$

dihydrate after chromatography on silica gel in 37% overall yield from (6). This was identical with the compound described by Kuhn *et al.*[9] The β-configuration at both internal anomeric centres was confirmed by a 250 MHz ^1H n.m.r. spectrum (solvent D_2O; at 80 °C). Measurement of the intensities of the signals of 1-H_α [δ 4.63 (0.7 H, d, $J_{1,2}$ 7.8 Hz)] and 1-H_β [δ 5.29 (0.3 H)] allowed the estimation of the β : α ratio as 2.3 : 1 for the free reducing D-galactose unit. The chemical shifts of the anomeric protons of the non-reducing D-galactose units in the α- and β-tautomers of the trisaccharide (10) in D_2O are not noticeably different [δ 4.52 (d, $J_{1,2}$ 7.3 Hz)]. On the other hand, the signal of the anomeric proton of the *N*-acetyl-D-glucosamine unit is broad (δ 4.87), and probably corresponds to two closely overlapping poorly resolved doublets ($J_{1,2}$ *ca.* 8 Hz), with different intensities. In the case of ' lacto-*N*-biose II ' (3), we found [1] two signals for the glucosamine anomeric proton at δ 4.76 ($J_{1,2}$ 8.4 Hz) and 4.77 ($J_{1,2}$ 8.4 Hz). There was no evidence from the spectrum for the presence of a contaminant with the α-configuration of the *N*-acetyl-D-glucosamine unit.

Compound (8) may be considered a useful starting

[7] T. Feizi, E. A. Kabat, G. Vicari, B. Anderson, and W. L. Marsh, *J. Exp. Med.*, 1971, **133**, 39.
[8] S. David and A. Veyrières, *Carbohydrate Res.*, 1975, **40**, 23.
[9] R. Kuhn, A. Gauhe, and H. H. Baer, *Chem. Ber.*, 1956, **89**, 1027.
[10] V. P. Rege, T. J. Painter, W. M. Watkins, and W. T. J. Morgan, *Nature*, 1963, **200**, 532.
[11] C. Augé and A. Veyrières, *Carbohydrate Res.*, 1976, **46**, 293.
[12] P. A. Gent and R. Gigg, *J.C.S. Chem. Comm.*, 1974, 277.

material for the synthesis of the pentasaccharide (1). In this respect it is noteworthy that a protected oxazoline derived from D-lactosamine (type 2 chain) has been described.[13]

EXPERIMENTAL

General methods were as described in ref. 1.

Benzyl 3-O-[2-*Acetamido*-4,6-*di*-O-*acetyl*-2-*deoxy*-3-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-6-O-*allyl*-2,4-*di*-O-*benzyl*-α-D-*galactopyranoside* (6).—A mixture of the oxazoline (4) [11] (0.617 g, 1.0 mmol), the galactopyranoside (5) [1] (0.490 g, 1.0 mmol), and toluene-*p*-sulphonic acid monohydrate (0.020 g) in dry nitromethane–toluene (1 : 1; 9 ml) was heated under nitrogen at 60 °C for 16 h. T.l.c. (benzene–ether–methanol, 7 : 7 : 1) then showed the absence of the oxazoline (4) (R_F 0.19) and the presence of a major product (R_F 0.41) and some of compound (5) (R_F 0.68). More oxazoline (4) (0.617 g, 1.0 mmol) in dry nitromethane–toluene (1 : 1; 4 ml) was added, and the mixture was heated at 60 °C for a further 24 h, after which only a trace of compound (5) was present (t.l.c.). The mixture was cooled, neutralized with a few drops of pyridine, and evaporated. The crude product (1.60 g) was chromatographed on silica gel; elution with toluene–ether–methanol (7 : 7 : 1) gave the pure *glycoside* (6) (R_F 0.41) (0.940 g, 84%) as a foam, $[\alpha]_D^{20}$ +18.8° (*c* 1.27 in $CHCl_3$), $\nu_{max.}$ (KBr) 1 765 (OAc), 1 700 (Amide I), 1 555 (Amide II), 1 245 (OAc), and 745 and 700 cm^{-1} (Ph), δ ($CDCl_3$) 1.67 (3 H, s, OAc), 1.93, 1.98, 2.04, and 2.10 (18 H, NAc and 5 OAc), 5.80 (1 H, m, O·CH_2·C*H*:CH_2), and 7.32 (15 H, 3 Ph) (Found: C, 60.5; H, 6.3; N, 1.4; O, 31.6. $C_{56}H_{69}NO_{22}$ requires C, 60.7; H, 6.3; N, 1.3; O, 31.8%).

3-O-[2-*Acetamido*-2-*deoxy*-3-O-(β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-D-*galactose* (10).—A mixture of the allyl ether (6) (0.225 g, 0.20 mmol), chloro(tristriphenylphosphine)rhodium(I) (3 mg), and 1,4-diazabicyclo[2.2.2]octane (21 mg) in ethanol–benzene–water (7 : 3 : 1; 5.5 ml) was heated under reflux for 4 h. A portion was then hydrolysed for 30 min at room temperature by dilution with acetone containing a drop of N-hydrochloric acid. T.l.c. (chloroform–ethanol, 19 : 1) then distinguished between the remaining allyl ether (6) (R_F 0.35), which was not hydrolysed, and the alcohol (8) (R_F 0.24), obtained by hydrolysis of the prop-1-enyl ether (7). Chloro(tristriphenylphosphine)-rhodium(I) (3 mg) was added and the mixture was heated under reflux for a further 4 h, after which only a small amount of the allyl ether (6) was present (by t.l.c. of a hydrolysed portion). The solvents were evaporated off, and the residue was taken up in N-hydrochloric acid–acetone (1 : 4; 5 ml) and kept at room temperature for 3 h. T.l.c. (chloroform–ethanol, 19 : 1) then showed complete removal of the prop-1-enyl group. An excess of potassium hydrogen carbonate was added, the solids were removed by centrifugation, and the supernatant was evaporated. The residue was extracted with chloroform, and the solution washed with water, then dried ($MgSO_4$). Evaporation gave the alcohol (8) as a foam (0.200 g) which could not be crystallized.

The alcohol (8) (0.200 g) was deacetylated overnight at room temperature in methanol–water–triethylamine (20 : 5 : 2; 6.8 ml). T.l.c. (propan-2-ol–ethyl acetate–water, 3 : 3 : 2) then indicated complete conversion of compound (8) into a deacetylated product (9) (R_F 0.72). The solvents were evaporated off and the residue was taken up in ethanol and water; the solution was evaporated to give (9) as a foam (0.149 g).

Compound (9) (0.149 g) was hydrogenated in glacial acetic acid (10 ml) over 10% palladium–charcoal (0.149 g) at room temperature and atmospheric pressure for 3 days. T.l.c. (propan-2-ol–ethyl acetate–water, 3 : 3 : 2) showed a major product (R_F 0.16) with three minor contaminants of higher R_F value. Removal of the catalyst and evaporation of the filtrate left a residue (0.100 g) which was chromatographed on silica gel with propan-2-ol–ethyl acetate–water (3 : 3 : 2) to give the pure *trisaccharide* (10) [40 mg, 37% from (6)], which was crystallized from methanol; m.p. 185—190°, $[\alpha]_D^{20}$ +19.8° (*c* 0.76 in H_2O; no mutarotation) {lit.,[9] m.p. 183—185°, $[\alpha]_D^{23}$ +21.5 ⟶ +19.3° (1 h; *c* 2 in H_2O)}, $\nu_{max.}$ (KBr) 1 640 (Amide I), 1 575 (Amide II), and 960 and 900 cm^{-1}, δ (D_2O; 80 °C) 2.09 (3 H, NAc), 4.52 (1 H, d, $J_{1'',2''}$ 7.3 Hz, 1''-H), 4.63 (0.7 H, d, $J_{1.2}$ 7.8 Hz, 1-H_α), 4.87br (1 H, 1'-H), and 5.29br (0.3 H, 1-H_β); paper chromatography: R_{Glc} 0.29 and $R_{Lactose}$ 0.52 in ethyl acetate–pyridine–water, 2 : 1 : 2 (upper layer) (lit.,[9] R_{Glc} 0.26 and $R_{Lactose}$ 0.51), R_{Glc} 0.26 and $R_{Lactose}$ 0.53 in butanol–pyridine–water, 5 : 3 : 2; g.l.c. at 270 °C of the per-*O*-(trimethylsilyl) trisaccharide gave a major peak (95%) at 7.25 min and a minor peak (5%) at 5.60 min [*cf.* per-*O*-(trimethylsilyl) raffinose, 3.90 min] (Found: C, 41.1; H, 6.7; N, 2.1; O, 49.5. $C_{20}H_{35}NO_{16}$,$2H_2O$ requires C, 41.3; H, 6.8; N, 2.4; O, 49.5%).

We thank Professor S. David for his help during this work.

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[13] B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 411.