Synthesis of *O*-β-D-Galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-D-mannose, a Postulated Trisaccharide of Human Erythrocyte Membrane Sialoglycoprotein

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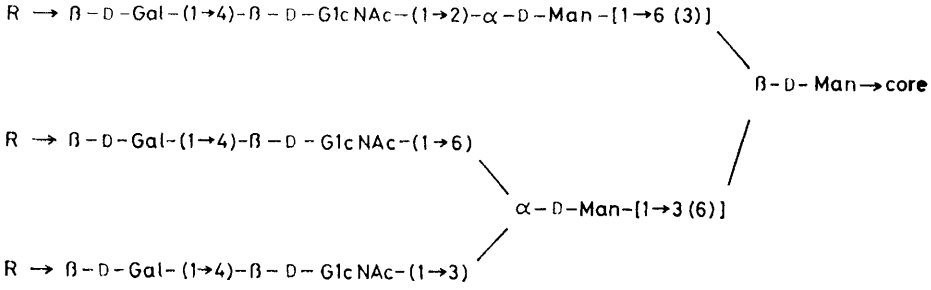
Monoallylation of tributylstannylated benzyl 6-*O*-trityl-α-D-mannopyranoside (3) was efficiently catalysed by tetrabutylammonium bromide to give the 3-*O*- (4) and 2-*O*- (5) allyl ethers in 62 and 15% yields, respectively. Compound (4) was converted into crystalline benzyl 3-*O*-allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (10), which was then isomerized, allylated, and hydrolysed to give benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (11). This compound was condensed with the oxazoline (12), derived from D-lactosamine, to give the protected trisaccharide (13) in 9% yield. Removal of the protecting groups gave the free trisaccharide β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-D-Man (15). The carbohydrate sequence of this trisaccharide has been postulated in some membrane glycoproteins and will be tested for its Ii blood group activity.

RECENT studies [1] of A, B, and H blood group antigens have shown that they can be associated with a glycopeptide or glycoprotein in the erythrocyte membrane; Childs *et al.*[2] have assigned the major intrinsic membrane glycoprotein of human erythrocyte ('Band III' glycoprotein) as a carrier for ABH antigens as well as Ii antigens. Besides the usual carbohydrates found in A-, B-, H-, or Ii-active water-soluble glycoproteins, the D-Gal have inhibitory activity towards the majority of anti-I and anti-i antibodies; but the amounts of these oligosaccharides required to give 50% inhibition were quite high. A possible explanation is that the antigens recognized by those anti-Ii antibodies involve oligosaccharide chains with a repeating [β-D-Gal-(1 ⟶ 4)-β-D-GlcNAc-(1 ⟶ 3)]$_n$ structure. But another possibility is the requirement of a D-mannose unit substituted at



(1) R = β-D-Gal-(1→3), or α-NeuNAc-[2→3 (6)], or nothing

'Band III' glycoprotein contains D-mannose, and its carbohydrate moiety shows a hybrid structure, consisting of blood-group-active side-chains [β-D-Gal-(1 ⟶ 4)-β-D-GlcNAc-(1 ⟶ 3)-repeating structure] and an inner core portion composed of D-mannose and *N*-acetyl-D-glucosamine, the branching points occurring probably at some D-mannose units. A similar structure (1) has been recently proposed by Kornfeld [3] for the carbohydrate moiety of glycopeptides isolated from calf thymocyte plasma membranes: two outer oligosaccharide chains are linked to C-3 and C-6 of an outer D-mannose residue.

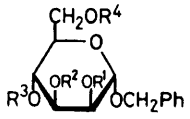
It has been shown [4] that oligosaccharides containing the sequence β-D-Gal-(1 ⟶ 4)-β-D-GlcNAc-(1 ⟶ 3)-C-3 and/or C-6 by those sequences, in the structure proposed by Kornfeld.

For these reasons we were interested in the synthesis of oligosaccharides related to structure (1), making them available for inhibition studies. Our general synthetic scheme involves: (*a*) the preparation of benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (11), a protected D-mannose derivative with a free OH at position 3 and a 'temporary' allyl group at position 6; (*b*) the introduction of the β-D-Gal-(1 ⟶ 4)-β-D-GlcNAc (β-D-*N*-acetyllactosamine) sequence at position 3 of the reducing D-mannose unit; (*c*) after selective removal of the allyl ether function, the second coupling of a β-D-*N*-

acetyllactosamine unit at position 6. We now report parts (*a*) and (*b*) of that scheme, leading to the title trisaccharide.

RESULTS AND DISCUSSION

Benzyl α-D-mannopyranoside [5] (2) was prepared from D-mannose in 50% yield by the boron trifluoride–ether method, and conveniently separated from unchanged D-mannose by continuous extraction. Benzyl 6-*O*-trityl-α-D-mannopyranoside (3) was then prepared as described by Gorin and Perlin.[6] A recent report by Ogawa and Matsui [7] showed that methyl α-D-mannopyranoside could be selectively alkylated at positions 3 and 6 *via* a tributylstannylated intermediate. When compound (3) was treated with bis(tributylstannyl) oxide, then with allyl bromide, as described for methyl α-D-mannopyranoside, the reaction was very slow and



(2) $R^1 = R^2 = R^3 = R^4 = H$
(3) $R^1 = R^2 = R^3 = H$, $R^4 = CPh_3$
(4) $R^1 = R^3 = H$, $R^2 =$ allyl, $R^4 = CPh_3$
(5) $R^1 =$ allyl, $R^2 = R^3 = H$, $R^4 = CPh_3$
(6) $R^1 = R^3 =$ Ac, $R^2 =$ allyl, $R^4 = CPh_3$
(7) $R^1 =$ allyl, $R^2 = R^3 =$ PhCO, $R^4 = CPh_3$
(8) $R^1 = R^3 = H$, $R^2 = R^4 =$ allyl
(9) $R^1 = R^3 = CH_2Ph$, $R^2 = R^4 = H$
(10) $R^1 = R^3 = CH_2Ph$, $R^2 =$ allyl, $R^4 = H$
(11) $R^1 = R^3 = CH_2Ph$, $R^2 = H$, $R^4 =$ allyl

after seven days at 80 °C, only 31% of the 3-*O*-allyl ether (4) could be isolated from the reaction mixture, which still contained 38% of the starting triol (3).

We found that the reaction of allyl bromide in moderate excess with the stannylated derivative of benzyl 6-*O*-trityl-α-D-mannopyranoside (3) in toluene was strongly accelerated by the addition of tetrabutylammonium bromide (0.1—0.3 equiv.); after 48 h at 80 °C, 62% of the 3-*O*-allyl ether (4) and 15% of the 2-*O*-allyl isomer (5), were isolated from the reaction mixture, no more starting material being recovered. Tetrabutylammonium iodide was found a more efficient catalyst, but was not used since the presence of free iodine made the isolation of the reaction products more difficult.

Pentaco-ordination around tin has been frequently demonstrated,[8] and it is probable that the addition of bromide ions to a tributylstannyl ether reversibly gives an anionic pentaco-ordinate tin species; such co-ordination has been found between various organotin chlorides and tetraethylammonium chloride or bromide.[9] We can postulate that the pentaco-ordinate complex can reversibly dissociate into a highly reactive quaternary ammonium alkoxide and tributyltin bromide.

This mechanism is reminiscent of the known effect of tetra-alkylammonium fluorides upon *O*-trialkylsilyl enol ethers leading to quaternary ammonium enolates.[10]

$$Bu_3SnOR \overset{Br^-}{\rightleftharpoons} Bu_3\bar{S}nBrOR \rightleftharpoons Bu_3SnBr + RO^-$$

The enhanced reactivity of the alkoxide can be attributed to the formation of an ion pair $RO^- \overset{+}{N}Bu_4$ with a large cation–anion separation; such species have been commonly postulated in phase-transfer-catalysed alkoxide displacement [11] reactions and in homogeneous anhydrous systems involving sodium alkoxides [12] or phenoxides.[13]

The catalytic effect of quaternary ammonium halides upon the alkylation of tributylstannyl ethers was extended to other substrates with various alkylating reagents (benzyl bromide, chloromethyl methyl ether, *etc.*), and also to the selective alkylation of various dibutylstannylene derivatives of vicinal diols.[14]

Recently,[15,16] monoalkylation of monosaccharide diols has been made possible by the phase-transfer technique. When the triol (3) was allowed to react with allyl bromide (1.7 equiv.) and tetrabutylammonium bromide (0.25 equiv.) in aqueous sodium hydroxide (1.5 equiv.) and dichloromethane under reflux for 4 h, the 3-*O*-allyl (4) and 2-*O*-allyl (5) ethers, and the starting material, were obtained in yields of 36, 23, and 24%, respectively. The absence of a protective group at the 4-position might be responsible for these results, since benzyl 4-*O*-benzyl-α-L-rhamnopyranoside could be allylated or benzylated at the 2-position in 75% yield.[16]

While our work was in progress, Srivastava and Schuerch [17] reported that methyl 6-*O*-trityl-α-D-mannopyranoside could be alkylated at the 3-position in 75% yield *via* a 2,3-dibutylstannylene derivative.

The structural assignments for compounds (4) and (5) were based on analysis of the ^1H n.m.r. spectra of their acetylated (6) and benzoylated (7) derivatives. In the spectrum of the 2,4-di-*O*-acetyl compound (6), the 2-H signal appeared as a doublet of doublet at δ 5.32 ($J_{1,2}$ 1.5, $J_{2,3}$ 3.5 Hz), downfield from the signal given by the anomeric proton at δ 4.92 (d, $J_{1,2}$ 1.5 Hz). The 3-H signal could not be identified in the spectrum of the 3,4-di-*O*-acetyl isomer, but appeared as a doublet of doublet at δ 5.60 ($J_{2,3}$ 3.0, $J_{3,4}$ 10 Hz) also downfield from the 1-H signal [δ 5.04 (s)] in the spectrum of the 3,4-di-*O*-benzoyl compound. (7). In both spectra, 4-H appeared as a triplet ($J_{3,4} = J_{4,5}$ 10 Hz) [δ *ca.* 5.1 for compound (6) and δ 5.72 for compound (7)].

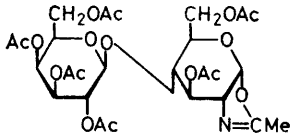
Arnap and Lönngren [18] have reported the preparation of benzyl 3,6-di-*O*-allyl-α-D-mannopyranoside (8) by selective *O*-allylation of benzyl α-D-mannopyranoside under the conditions given by Ogawa and Matsui.[7] Under our conditions of catalysis with tetrabutylammonium bromide, we observed a much faster reaction leading to comparable yields (57%). Benzylation, then de-*O*-allylation, gave the crystalline diol (9), identical to the product previously described.[18]

Compound (4) was *O*-benzylated, then subjected to

acidic hydrolysis to give the crystalline benzyl 3-*O*-allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (10) in 85% yield. A sample of (10) was de-*O*-allylated to give the known diol (9), thus confirming the structure of the 3-*O*-allyl ether (4).

Compound (10) was isomerized into the 3-*O*-prop-1-enyl ether, then allylated at position 6. Acidic hydrolysis afforded the desired benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (11) isolated as a syrup in 80% yield.

Condensation of the oxazoline [19] (12) derived from D-lactosamine (100% molar excess) with compound (11) in the presence of toluene-*p*-sulphonic acid proceeded with difficulty, and gave, after seven days at 60 °C, a 9% yield [based on (11)] of the pure, crystalline, protected



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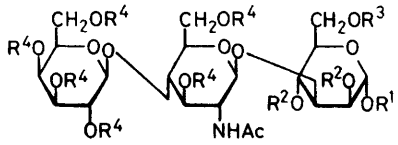

(13) $R^1 = R^2 = CH_2Ph$, R^3 = allyl, R^4 = Ac
(14) $R^1 = R^2 = CH_2Ph$, R^3 = H, R^4 = Ac
(15) $R^1 = R^2 = R^3 = R^4$ = H

trisaccharide (13). Such a low yield was unexpected since the same oxazoline (12) had been condensed in 47% yield onto benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyranoside,[20] and other derivatives of D-mannopyranose had been glycosidated at the 3-position with reasonable yields.[21]

Isomerization of compound (13) to the 6-prop-1-enyl ether, followed by acidic hydrolysis, gave the amorphous alcohol (14), a useful starting material for a further condensation at the 6-position of the reducing D-mannose unit.

O-Deacetylation of compound (14), followed by hydrogenolysis of the benzyl ether functions gave the free trisaccharide (15), in 32% overall yield from (13). The β-configuration of the created glycosidic bond was confirmed by a 250-MHz ^{1}H n.m.r. spectrum (solvent D_2O); the signal of the anomeric proton of the *N*-acetyl-D-glucosamine unit (δ 4.70 and 4.71) corresponds to two closely overlapping doublets ($J_{1',2'}$ 7.5 Hz) with similar intensities. The signal of the anomeric proton of the D-galactose unit appears as a doublet ($J_{1'',2''}$ 7.5 Hz) at the usual location (δ 4.48) for this type of linkage.[20] The free reducing D-mannose unit gives two signals for the anomeric proton corresponding to a 1 : 1 mixture of the two configurations obtained by mutarotation, 1-H_α [δ 4.85 (0.5 H, s)] and 1-H_β [δ 5.17 (0.5 H, s)]. The presence of two configurations at the D-mannose unit is noticeable on the signal of the anomeric proton of the *N*-acetyl-D-glucosamine unit, as we saw above.

EXPERIMENTAL

Solvents were evaporated off under reduced pressure. Ether refers to diethyl ether throughout. Optical rotations were measured at 20 °C with a Roussel–Jouan electronic digital micropolarimeter. N.m.r. spectra were recorded at 250 MHz with a Cameca model STN 250 spectrometer with Fourier-transform unit, with $CDCl_3$ as solvent and tetramethylsilane as internal standard, or with D_2O as solvent and tetramethylsilane (0.2% solution in $CDCl_3$) as external reference. T.l.c. was carried out on plates of silica gel (with fluorescence indicator; layer thickness 0.25 mm, E. Merck, Darmstadt, Germany); ethanolic sulphuric acid (19 : 1, v/v) with charring was used for component detection. Silica gel Merck (70—325 mesh; E. Merck) was used for column chromatography. Paper chromatography was performed on Whatman No. 1 paper. Free sugars were detected with the aniline hydrogenphthalate reagent. Elemental analyses were performed by the Laboratoire Central de Micro-Analyse du C.N.R.S.

Benzyl α-D-*Mannopyranoside* (2).—A mixture of D-mannose (50 g, 0.28 mol), boron trifluoride–ether (7 ml, 55 mmol), and dry benzyl alcohol (500 ml) was heated with stirring at 95 °C for 150 min, then cooled, diluted with ether–hexane (1 : 1) (3 l), and kept overnight at 4 °C. The gummy material which separated was filtered off, washed with ether, and dissolved in water (200 ml); the resulting solution was neutralized by addition of potassium hydrogencarbonate, then continuously extracted overnight with ethyl acetate. The organic layer was concentrated until crystallization occurred then kept for a few hours at 4 °C. As checked by t.l.c. [ethyl acetate–methanol, (4 : 1)], the resulting crystalline material (R_F 0.50) was pure benzyl α-D-mannopyranoside (2) (37.4 g, 50%), free from D-mannose; m.p. 128—130 °C, $[\alpha]_D$ +72° (*c* 1.0 in H_2O) {lit.,[5] m.p. 132—133 °C, $[\alpha]_D^{25}$ +73.5° (*c* 1.5 in H_2O)}.

Benzyl 6-O-*Trityl*-α-D-*mannopyranoside* (3).—A mixture of benzyl α-D-mannopyranoside (2) (6.2 g, 23 mmol) and chlorotriphenylmethane (8.4 g, 30 mmol) in pyridine (40 ml) was heated at 90 °C for 4 h, then cooled, poured into aqueous potassium carbonate, and kept overnight at 4 °C. The gum that was precipitated was dissolved in chloroform. The extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel; elution with chloroform–ethanol (9 : 1) gave compound (3) (8.9 g, 76%) as a foam, slightly contaminated by triphenylmethanol.

Allylation of Benzyl 6-O-*Trityl*-α-D-*mannopyranoside* (3) — A solution of benzyl 6-*O*-trityl-α-D-mannopyranoside (3) (11.3 g, 22 mmol) in dry toluene (400 ml) was treated with bis(tributylstannyl) oxide (19.8 g, 33 mmol) under reflux for 4 h with continuous removal of water, then concentrated to half-volume and cooled to 80 °C. Allyl bromide (10 ml, 115 mmol) and tetrabutylammonium bromide (1 g, 3 mmol) were added and the mixture was stirred under nitrogen at 80 °C for 24 h; t.l.c. [ether–light petroleum, (7 : 3)] then showed the presence of two new compounds (R_F 0.54 and 0.78) and large amounts of unchanged triol (3) (R_F 0.13)

derived from hydrolysis of its tributylstannylated derivative.* After a further addition of allyl bromide (10 ml, 115 mmol) and tetrabutylammonium bromide (1 g, 3 mmol), heating and stirring were continued for a further 24 h, after which time t.l.c. showed only traces of the triol (3). The toluene solution was cooled and washed with 10% aqueous potassium hydrogencarbonate solution, then with water; the aqueous washings were re-extracted with chloroform, and the combined toluene and chloroform extracts were evaporated together with silica gel to form a powder, which was applied to a column of silica gel. Elution with ether–light petroleum (3 : 2) gave three main fractions.

(*a*) The first-eluted fraction (7.5 g, 62%) contained the slightly impure benzyl 3-*O*-allyl-6-*O*-trityl-α-D-mannopyranoside (4) isolated as a foam, $[\alpha]_D$ +28° (*c* 1.0 in $CHCl_3$); δ($CDCl_3$) 2.40 (1 H, d, OH, exchanges with D_2O), 2.65 (1 H, s, OH, exchanges with D_2O), 4.53 and 4.77 (2 H, 2d, $J_{a,b}$ 12 Hz, $PhCH_aH_bO$), 4.96 (1 H, d, $J_{1,2}$ 1 Hz, 1-H), 5.18—5.34 (2 H, m, $OCH_2CH{=}CH_2$), 5.86—6.00 (1 H, m, $OCH_2CH{=}CH_2$), and 7.21—7.44 (20 H, 4 Ph). A portion was acetylated overnight at room temperature to give compound (6) as a foam; δ($CDCl_3$) 1.80 (3 H, s, 4-OAc), 2.13 (3 H, s, 2-OAc), 4.61 and 4.86 (2 H, 2d, $J_{a,b}$ 12 Hz, $PhCH_aCH_bO$), 4.92 (1 H, d, $J_{1,2}$ 1.5 Hz, 1-H), 5.06—5.23 (3 H, m, 4-H and $OCH_2CH{=}CH_2$), 5.32 (1 H, dd, $J_{1,2}$ 1.5, $J_{2,3}$ 3.5 Hz, 2-H), 5.68—5.82 (1 H, m, $OCH_2CH{=}CH_2$), and 7.20—7.45 (20 H, 4 Ph).

(*b*) The second fraction contained a mixture of the two allyl ethers (4) and (5) (0.6 g, 5%).

(*c*) The third fraction (1.9 g, 15%) contained the slightly impure benzyl 2-*O*-allyl-6-*O*-trityl-α-D-mannopyranoside (5) isolated as a foam, $[\alpha]_D$ +16° (*c* 1.4 in $CHCl_3$); δ($CDCl_3$) 2.34 (1 H, d, OH, exchanges with D_2O), 2.58 (1 H, s, OH, exchanges with D_2O), 4.52 and 4.78 (2 H, 2d, $J_{a,b}$ 12 Hz, $PhCH_aH_bO$), 4.94 (1 H, s, 1-H), 5.14—5.30 (2 H, m, $OCH_2CH{=}CH_2$), 5.78—5.93 (1 H, m, $OCH_2CH{=}CH_2$), and 7.20—7.45 (20 H, 4 Ph). A portion was benzoylated overnight at room temperature to give compound (7) as a foam; δ($CDCl_3$) 4.68 and 4.93 (2 H, 2d, $J_{a,b}$ 12 Hz, $PhCH_aH_bO$), 5.04 (1 H, s, 1-H), 5.04—5.24 (2 H, m, $OCH_2CH{=}CH_2$), 5.60 (1 H, dd, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, 3-H), 5.72 (1 H, t, $J_{4,5}$ 10 Hz, 4-H), 5.70—5.88 (1 H, m, $OCH_2CH{=}CH_2$), 7.04—7.37 (26 H, m, Ar-H), 7.71 (2 H, m, Ar-H), and 7.94 (2 H, m, Ar-H).

Benzyl 2,4-*Di*-O-*benzyl*-α-D-*mannopyranoside* (9).—A mixture of benzyl α-D-mannopyranoside (2) (540 mg, 2 mmol) and bis(tributylstannyl) oxide (1.8 g, 3 mmol) in toluene (50 ml) was refluxed for 4 h with continuous removal of water, then concentrated to half-volume and cooled to 80 °C. Allyl bromide (0.5 ml, 5.8 mmol) and tetrabutylammonium bromide (200 mg, 0.6 mmol) were added and the mixture was stirred under nitrogen at 80 °C for 24 h; t.l.c. [acetone–chloroform (1 : 3)] then showed that no starting material (R_F 0.01) remained, and that there was a major compound (R_F 0.11) and a minor faster-moving compound (R_F 0.55). Further amounts of allyl bromide (0.5 ml, 5.8 mmol) and tetrabutylammonium bromide (100 mg, 0.3 mmol) were added, and the mixture was stirred at 80 °C for a further 24 h. T.l.c. indicated small amounts of the product of R_F 0.11 and a large increase of the faster-moving product (R_F 0.55). The reaction was stopped and processed as described above. The major reaction product (8) was isolated as a crude syrup (400 mg, 57%) by column chromatography on silica gel using chloroform–ethanol (98 : 2) as eluant. It was benzylated by treatment with sodium hydride (130 mg) and benzyl bromide (0.5 ml) in *NN*-dimethylformamide (7 ml) for 20 h at room temperature. After the usual processing, the crude product was de-*O*-allylated as described by Arnap and Lönngren.[18] Column chromatography on silica gel with ethyl acetate–toluene (1 : 9) gave the pure diol (9) (220 mg, 71%), m.p. 83—84 °C, $[\alpha]_D$ +50° (*c* 1.19 in $CHCl_3$) {lit.,[18] m.p. 85—86 °C, $[\alpha]_D^{22}$ +51° (*c* 0.2 in $CHCl_3$)}.

Benzyl 3-O-*Allyl*-2,4-*di*-O-*benzyl*-α-D-*mannopyranoside* (10).—A solution of compound (4) (7 g, 12.7 mmol) in *NN*-dimethylformamide (80 ml) was treated with sodium hydride (1.2 g, 50 mmol) and benzyl bromide (6 ml, 50 mmol) at 0 °C for 1 h, and then overnight at room temperature; t.l.c. [ether–light petroleum (1 : 4)] then showed complete conversion of the starting material into one product. The excess of hydride was decomposed by the addition of methanol to the mixture. The resulting solution was diluted with ether (500 ml), washed with water, and evaporated. The residue was dissolved in acetone (144 ml) and 1M-hydrochloric acid (16 ml); the solution was refluxed until t.l.c. [ether–light petroleum (7 : 3)] showed complete hydrolysis (*ca.* 90 min). An excess of sodium hydrogencarbonate was added and the solvents were evaporated. The residue was extracted with chloroform; the extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel; elution with ether–light petroleum (2 : 3) gave the *alcohol* (10) (5.3 g, 85%) which crystallized by trituration with light petroleum, m.p. 57—58 °C, $[\alpha]_D$ +59° (*c* 1.1 in $CHCl_3$); δ($CDCl_3$) 1.98 (1 H, t, OH, exchanges with D_2O), 4.88 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1), 5.12—5.33 (2 H, m, $OCH_2CH{=}CH_2$), 5.84—6.00 (1 H, m, $OCH_2CH{=}CH_2$), and 7.16—7.28 (15 H, 3 Ph) (Found: C, 73.3; H, 7.0; O, 19.9. $C_{30}H_{34}O_6$ requires C, 73.4; H, 7.0; O, 19.6%).

Benzyl 6-O-*Allyl*-2,4-*di*-O-*benzyl*-α-D-*mannopyranoside* (11).—To a solution of compound (10) (3.4 g, 6.9 mmol) in ethanol–benzene–water (8 : 3 : 1) (100 ml) were added chloro(tristriphenylphosphine)rhodium(I) (0.25 g, 0.27 mmol) and 1,4-diazabicyclo[2.2.2]octane (1.75 g, 15.6 mmol), and the mixture was refluxed for 4 h. T.l.c. [ether–light petroleum (7 : 3)] then showed nearly complete isomerisation of the starting material (R_F 0.62) into a prop-1-enyl ether (R_F 0.68). The solution was cooled and evaporated, the residue dissolved in chloroform, and the extract washed with water until neutral, dried ($MgSO_4$), and then evaporated. The residue was dissolved in *NN*-dimethylformamide (35 ml) and treated with sodium hydride (0.75 g, 31 mmol) and allyl bromide (5 ml, 58 mmol) at 0 °C for 1 h, and then overnight at room temperature. The mixture was processed as described for compound (10). The residue was dissolved in acetone (54 ml) and 1M-hydrochloric acid (6 ml); the solution was refluxed until t.l.c. [ether–light petroleum (2 : 3)] showed complete hydrolysis (*ca.* 20 min). After the usual processing, the crude product was chromatographed on silica gel; elution with ether–light petroleum (3 : 7) gave the *alcohol* (11) as a syrup (2.7 g, 80%), $[\alpha]_D$ +32° (*c* 0.8 in $CHCl_3$); δ($CDCl_3$) 2.32 (1 H, d, OH, exchanges with D_2O), 5.00 (1 H, d, $J_{1,2}$ 1.5 Hz, 1-H), 5.12—5.30 (2 H, m, $OCH_2CH{=}CH_2$), 5.84—6.00 (1 H, m, $OCH_2CH{=}CH_2$), and 7.20—

* Tributylstannyl ethers are immediately hydrolysed when spotted on plates of silica gel, and therefore cannot be detected as such. Tributyltin bromide, arising from the reaction of a tributylstannyl ether with an alkyl bromide, is also rapidly hydrolysed and thus liberates hydrogen bromide. When the reaction mixture is washed with aqueous potassium hydrogencarbonate solution, bis(tributyltin) carbonate is formed, and liberates carbon dioxide when mixed with silica gel.

7.28 (15 H, 3 Ph) (Found: C, 73.6; H, 7.2; O, 19.8. $C_{30}H_{34}O_6$ requires C, 73.4; H, 7.0; O, 19.6%).

Benzyl 3-O-[2-*Acetamido*-3,6-*di*-O-*acetyl*-2-*deoxy*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-6-O-*allyl*-2,4-*di*-O-*benzyl*-α-D-*mannopyranoside* (13).—A mixture of the oxazoline (12) (2.3 g, 3.7 mmol), the alcohol (11) (1.8 g, 3.7 mmol), and anhydrous toluene-*p*-sulphonic acid (37 mg) in dry dichloroethane was heated under nitrogen at 60 °C for 17 h. T.l.c. [benzene–ether–methanol (7 : 7 : 1)] then showed a new compound at R_F 0.41 but also large amounts of unchanged oxazoline (12) (R_F 0.34) and alcohol (11) (R_F 0.95). More toluene-*p*-sulphonic acid (20 mg) was added to readjust the pH of the reaction mixture to 4; heating and stirring were continued for a further 80 h. T.l.c. then showed that the oxazoline (12) was no longer present but that large amounts of the alcohol (11) still remained. More oxazoline (12) (2.3 g, 3.7 mmol) and toluene-*p*-sulphonic acid (40 mg) were added, and the reaction mixture was heated and stirred for a further 65 h. Many decomposition products arising from the oxazoline (12) having appeared, the reaction was then stopped. The mixture was cooled, neutralized with a few drops of pyridine, and evaporated; the residue was applied to a silica gel column. Elution with ethyl acetate–toluene (1 : 1) gave the pure *glycoside* (13) (R_F 0.41) [0.36 g, 9% from (11)], which was crystallized from ether, m.p. 120—121 °C; $[\alpha]_D$ +8° (*c* 1.1 in $CHCl_3$); δ($CDCl_3$) 1.63 (3 H, s, OAc), 1.88, 1.94, 1.98, and 2.12 (18 H, NAc and 5 OAc), 5.80—5.96 (1 H, m, $OCH_2CH{=}CH_2$), and 7.28—7.34 (15 H, 3 Ph) (Found: C, 60.6; H, 6.2; N, 1.2; O, 31.8. $C_{56}H_{69}NO_{22}$ requires C, 60.7; H, 6.3; N, 1.3; O, 31.8).

3-O-[2-*Acetamido*-2-*deoxy*-4-O-(β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-D-*mannose* (15).—A mixture of the allyl ether (13) (0.28 g, 0.25 mmol), chloro(tristriphenylphosphine)rhodium(I) (14 mg), and 1,4-diazabicyclo[2.2.2]-octane (96 mg) in ethanol–benzene–water (7 : 3 : 1) (22 ml) was refluxed for 3 h. More catalyst (14 mg) was added, and heating was continued for 4 h. T.l.c. [benzene–ethyl acetate (1 : 1)] showed nearly complete isomerization of the allyl ether (13) (R_F 0.36) into the prop-1-enyl ether (R_F 0.40). The solvents were evaporated off and the residue was taken up in 1M-hydrochloric acid–acetone (1 : 4) (15 ml) and kept at room temperature for 6 h. T.l.c. [benzene–ether–methanol (7 : 7 : 1)] showed complete hydrolysis of the prop-1-enyl ether (R_F 0.38) into the alcohol (R_F 0.22). The mixture was processed as usual, then chromatographed on silica gel with acetone–chloroform (1 : 4) to give the alcohol (14) (0.19 g 70%) as a foam, $[\alpha]_D$ +14° (*c* 0.64 in $CHCl_3$). The alcohol (14) (0.19 g) was deacetylated overnight at room temperature in 0.05M-sodium methoxide in methanol (10 ml). T.l.c. [propan-2-ol–ethyl acetate–water (3 : 3 : 1)] then indicated complete conversion of compound (14) into a deacetylated product (R_F 0.83). The solution was neutralized with Amberlite IR 120 (H^+) ion-exchange resin and evaporated. The residue (0.13 g) was hydrogenated in glacial acetic acid (12 ml) over 10% palladium–charcoal (0.15 g) at room temperature and atmospheric pressure for 40 h. The reaction mixture was evaporated to dryness, without removal of the catalyst; the residue was chromatographed on silica gel using propan-2-ol–ethyl acetate–water (3 : 3 : 1) as eluant. The fractions containing the pure trisaccharide (15) (R_F 0.12) were evaporated, the residue was dissolved in water (5 ml) and the aqueous solution was freeze-dried. The amorphous trisaccharide (15) [46 mg, 32% from (13)] showed only one spot (R_F 0.68) on examination by t.l.c. on silica gel with propan-2-ol–ethyl acetate–water (3 : 3 : 2); $[\alpha]_D$ −14° (*c* 1.07 in H_2O, at equilibrium); δ(D_2O) 2.03 (3 H, s, NAc), 4.48 (1 H, d, $J_{1'',2''}$ 7.5 Hz, 1″-H), 4.70 and 4.71 (1 H, 2 d, $J_{1',2'}$ 7.5 Hz, 1′-H), 4.85 (0.5 H, s, 1-H_α), and 5.17 (0.5 H, s, 1-H_β); paper chromatography, R_{Glc} 0.56 and R_{Lact} 0.87 in ethyl acetate–pyridine–water (2 : 1 : 2) (upper layer).

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