The Allyl Group for Protection in Carbohydrate Chemistry. Part 19.[1] The Coupling of Allyl 2,3-Di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside to Bovine Serum Albumin. Preparation of a Diagnostic Reagent for Antibodies to the Major Glycolipid of *Mycobacterium leprae* (the Leprosy Bacillus) in Human Sera†

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Epoxidation of allyl 4-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranoside and subsequent alkaline hydrolysis of the epoxide and hydrogenolysis of the benzyl groups gave 2′,3′-dihydroxypropyl 2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside which was cleaved with sodium metaperiodate to give the corresponding formylmethyl glycoside. Two other routes to the latter compound, *via* allyl 2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, were also developed. The formylmethyl glycoside was coupled to bovine serum albumin using 'reductive amination' in the presence of sodium cyanoborohydride to give a glycoconjugate useful for the serodiagnosis of antibodies to the major glycolipid of *Mycobacterium leprae* in the sera of leprosy patients. 5′,6′-Dihydroxyhexyl and 10′,11′-dihydroxyundecyl 3,6-di-*O*-methyl-β-D-glucopyranosides were also prepared as intermediates for the synthesis of the 4-formylbutyl and 9-formylnonyl glucosides respectively which are also suitable for coupling to bovine serum albumin by the 'reductive amination' technique.

The discovery[2] and structural elucidation[3–5] of a major glycolipid ('phenolic glycolipid-1') in *Mycobacterium leprae*, grown in the nine-banded armadillo (*Dasypus novemcinctus* Linn), with a unique carbohydrate sequence: 3,6-di-*O*-methyl-β-glucopyranose-(1→4)-2,3-di-*O*-methyl-α-rhamnopyranose-(1→2)-3-*O*-methyl-α-rhamnopyranose (the absolute configurations of the sugars have not been determined) and the demonstration[6] of antibodies to this glycolipid in the sera of leprosy patients suggested its use in the serodiagnosis of leprosy (for a review see ref. 7).

In the glycolipid the trisaccharide portion is joined glycosidically *via* a phenolic residue to a large lipidic group, characteristic of mycobacteria. This renders the molecule very hydrophobic and thus gives rise to difficulties in aqueous serodiagnostic assays.[7] It was, therefore, expected that a conjugate of the oligosaccharide portion with a protein would prove a more useful reagent for serological studies.[8]

Our previous synthetic studies[9–11] and serological tests[12,13] have shown that, in competitive inhibition studies with the glycolipid in the enzyme-linked immunosorbent assays (ELISA), the terminal disaccharide 3,6-di-*O*-methyl-β-D-glucopyranose-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranose is a haptenic portion of the molecule and this has been confirmed by studies of Brennan and his associates (for a review see ref. 7).

For coupling of the disaccharide to protein we decided to use the allyl aglycone which was already present in the synthetic intermediate (**11**) which we had used previously[9] for the synthesis of the terminal disaccharide of the glycolipid which was used in the ELISA test.[12] Modifications of the allyl group to provide a reactive site for coupling to macromolecules have been described previously.[14]

Results

A different method of preparing the disaccharide derivative (**11**) using silver trifluoromethanesulphonate[15] instead of mercury(II) cyanide as the condensing agent, which was used in the previous preparation,[9] is described. Compound (**11**) was converted into the benzyl ether (**1**) as described previously[9] and this was converted into the epoxide (**2**) using 3-chloroperbenzoic acid. Initial experiments on the epoxidation conditions were carried out with allyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside[16] which gave the corresponding epoxide (**30**). Hydrolysis of the epoxide (**2**) with tetrabutylammonium hydroxide in aqueous dioxane gave the diol (**3**) from which the benzyl groups were removed by hydrogenolysis to give the glycerol glycoside (**4**). Two other routes to compound (**4**) *via* the allyl glycoside (**7**) were also developed.

Compound (**11**) was converted into the *p*-methoxybenzyl ether (**6**) by way of compounds (**12**)—(**14**) in the same way[9] as the corresponding benzyl ether (**1**) was prepared from (**11**). The *p*-methoxybenzyl group can be removed with dichlorodicyanoquinone[17] or with cerium(IV) ammonium nitrate.[18] In investigative experiments of the action of these reagents on a model compound containing an allyl group it was found that allyl 4-*O*-*p*-methoxybenzyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**16**) was rapidly converted into allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**15**) by dichlorodicyanoquinone and was not further affected by the reagent. However with cerium(IV) ammonium nitrate, compound (**15**) was further degraded at about the same rate as it was formed and dichlorodicyanoquinone was therefore used for subsequent deprotection experiments. Thus, treatment of the *p*-methoxybenzyl ether (**6**) with dichlorodicyanoquinone gave the allyl disaccharide (**7**). Co-polymers of allyl glycosides and acrylamide have been used[19] for the preparation of useful immunochemical reagents and the unprotected allyl glycoside (**7**) should be suitable for this purpose also.

In the second route to the allyl glycoside (**7**), allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**15**)[9] was converted by

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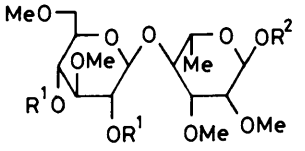
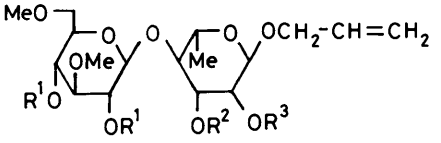
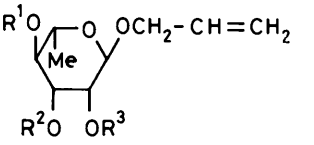
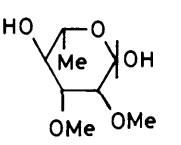


(1) $R^1 = CH_2Ph$, $R^2 = CH_2CH{=}CH_2$
(2) $R^1 = CH_2Ph$, $R^2 = CH_2\overline{CHCH_2O}$
(3) $R^1 = CH_2Ph$, $R^2 = CH_2CH(OH)CH_2OH$
(4) $R^1 = H$, $R^2 = CH_2CH(OH)CH_2OH$
(5) $R^1 = H$, $R^2 = CH_2CHO$
(6) $R^1 = p\text{-}MeOC_6H_4CH_2$, $R^2 = CH_2CH{=}CH_2$
(7) $R^1 = H$, $R^2 = CH_2CH{=}CH_2$
(8) $R^1 = Ac$, $R^2 = CH_2CH{=}CH_2$
(9) $R^1 = H$, $R^2 = CH_2\overline{CHCH_2O}$
(10) $R^1 = H$, $R^2 = CH_2CH(OCOC_{15}H_{31})CH_2OCOC_{15}H_{31}$



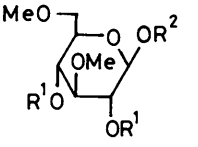
(11) $R^1 = Ac$, R^2, $R^3 = {>}CMe_2$
(12) $R^1 = H$, R^2, $R^3 = {>}CMe_2$
(13) $R^1 = p\text{-}MeOC_6H_4CH_2$, R^2, $R^3 = {>}CMe_2$
(14) $R^1 = p\text{-}MeOC_6H_4CH_2$, $R^2 = R^3 = H$

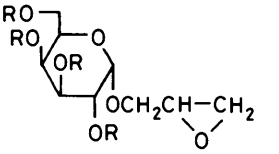




(15) $R^1 = H$, R^2, $R^3 = {>}CMe_2$
(16) $R^1 = p\text{-}MeOC_6H_4CH_2$, R^2, $R^3 = {>}CMe_2$
(17) $R^1 = p\text{-}MeOC_6H_4CH_2$, $R^2 = R^3 = H$
(18) $R^1 = p\text{-}MeOC_6H_4CH_2$, $R^2 = R^3 = Me$
(19) $R^1 = H$, $R^2 = R^3 = Me$

(20)





(21) $R^1 = Ac$, $R^2 = CH_2(CH_2)_8CH{=}CH_2$
(22) $R^1 = H$, $R^2 = CH_2(CH_2)_8CH{=}CH_2$
(23) $R^1 = H$, $R^2 = CH_2(CH_2)_8\overline{CHCH_2O}$
(24) $R^1 = H$, $R^2 = CH_2(CH_2)_8CH(OH)CH_2OH$
(25) $R^1 = H$, $R^2 = CH_2(CH_2)_8CHO$
(26) $R^1 = Ac$, $R^2 = CH_2(CH_2)_3\overline{CHCH_2OCMe_2O}$
(27) $R^1 = H$, $R^2 = CH_2(CH_2)_3\overline{CHCH_2OCMe_2O}$
(28) $R^1 = H$, $R^2 = CH_2(CH_2)_3CH(OH)CH_2OH$
(29) $R^1 = H$, $R^2 = CH_2(CH_2)_3CHO$

(30) $R = CH_2Ph$

way of compound (**16**) into the crystalline *p*-methoxybenzyl ether (**17**) which was then methylated to give (**18**); the *p*-methoxybenzyl group of the latter was removed by the action of dichlorodicyanoquinone to give allyl 2,3-di-*O*-methyl-α-L-rhamnopyranoside (**19**).

The enantiomer of compound (**17**), allyl 4-*O*-*p*-methoxybenzyl-α-D-rhamnopyranoside, was also prepared from allyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside.[10] Acidic hydrolysis of allyl 4-*O*-*p*-methoxybenzyl-2,3-di-*O*-methyl-α-L-rhamnopyranoside (**18**) removed both the *p*-methoxybenzyl group (which is known[20] to be acid labile) and the allyl group to give the known[21,22] 2,3-di-*O*-methyl-L-rhamnopyranose (**20**).

Condensation of allyl 2,3-di-*O*-methyl-α-L-rhamnopyranoside (**19**) with 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl chloride[9] in the presence of mercury(II) cyanide gave the crude disaccharide (**8**) (containing a small amount of the corresponding α-linked disaccharide); this was hydrolysed by base to give crude (**7**) which was purified by chromatography on silica gel.

The allyl glycoside (**7**) was converted into the epoxide (**9**), by the action of 3-chloroperbenzoic acid, and the latter was hydrolysed with aqueous sodium hydroxide solution to give the glycerol glycoside (**4**). The epoxide (**9**) should also be useful for direct coupling to the amino groups of proteins or polymers containing amino groups in order to give an affinity column suitable for purifying the antibody to 'phenolic glycolipid-1'.

Treatment of the glycerol glycoside (**4**) with sodium metaperiodate gave the aldehyde (**5**) which was coupled to the ε-amino groups of the lysine residues of bovine serum albumin (which contains[23] 59 lysine residues in a total of 581 amino acids) by 'reductive amination' with sodium cyanoborohydride in 0.1M phosphate buffer at pH 7.5 following the general procedure developed by Gray and his co-workers.[24] Amino acid analyses of the conjugate showed that *ca.* 50 of the lysine residues had been substituted.

The conjugate performed well in the ELISA tests[13] for antibody to 'phenolic glycolipid-1' in the sera of leprosy patients and was much more convenient to use, because of its water solubility and ease of adhesion to the plastic microtitre ELISA test plates, than the natural glycolipid for this purpose. A batch of conjugate (900 mg, sufficient for 1.8 million test doses) has been prepared and put into ampoules for serodiagnostic tests by World Health Organisation—Immunology of Leprosy (WHO/IMMLEP) supported investigators.[25]

Acylation of the benzyl ether (**3**) with hexadecanoyl chloride in pyridine and subsequent hydrogenolysis of the benzyl groups gave the synthetic glycolipid (**10**) containing the epitope of the natural 'phenolic glycolipid-1'.

The early serological tests with the synthetic sugars had indicated that most of the immunological activity of the natural glycolipid resided in the 3,6-di-*O*-methyl-β-D-glucopyranose part of the molecule and therefore some simple glycosides of this sugar with spacer-arms suitable for conjugation to protein were also prepared. For this purpose the commercially available alcohols hexane-1,5,6-triol and undec-10-enol were used.

Condensation of 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl chloride[9] with the 5,6,-*O*-isopropylidene derivative[26] of hexane-1,5,6-triol gave the crude glycoside (**26**) which on basic hydrolysis gave the diol (**27**); this on acidic hydrolysis then gave the crystalline glycoside (**28**). Compound (**28**) was cleaved with sodium metaperiodate to give the

aldehyde (**29**) which was condensed with bovine serum albumin using the 'reductive amination' technique but the conjugate was less efficient[13] than the disaccharide conjugate in the ELISA assays.

Condensation of undec-10-enol with 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl chloride[9] gave the crude glycoside (**21**) which with base gave the diol (**22**). Epoxidation of compound (**22**) with 3-chloroperbenzoic acid gave the crystalline epoxide (**23**) which on basic hydrolysis gave the crystalline tetrol (**24**) suitable for coupling to protein *via* the aldehyde (**25**).

Experimental

T.l.c. was carried out on microscope slides coated with silica gel G. Solvents were evaporated off under reduced pressure. Optical rotations were measured with a Bendix automatic polarimeter. The light petroleum used had b.p. 40—60 °C unless otherwise stated.

Allyl 4-O-p-*Methoxybenzyl*-α-L-*rhamnopyranoside* (**17**) *and the Corresponding* α-D-*Enantiomer*.—Allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**15**)[9] (5 g) in dry *N,N*-dimethylformamide (10 ml) was added dropwise with stirring to a mixture of sodium hydride (0.6 g) and *p*-methoxybenzyl chloride (3.5 g) in dry *N,N*-dimethylformamide (50 ml). The mixture was stirred for a further 1 h after which time t.l.c. (ether–light petroleum, 1:2) showed complete conversion of compound (**15**) (R_F 0.25) into a product (R_F 0.8). Methanol was added to decompose the excess of sodium hydride and the solution was diluted with water and extracted with ether. The crude product (**16**) was chromatographed on basic alumina (ether) to remove benzylation by-products and was then kept at 40 °C in methanol–6M hydrochloric acid (58:1; 100 ml) for 25 min. An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. Toluene was evaporated from the residue and the product was extracted with dichloromethane and the extract dried (K_2CO_3) and evaporated. The crude product was chromatographed on silica gel (ether) to give the *p-methoxybenzyl ether* (**17**) (4.3 g), m.p. 75—76 °C [from ethyl acetate–light petroleum (b.p. 60—80 °C)], $[\alpha]_D^{26}$ −58.6° (*c* 1 in $CHCl_3$) (Found: C, 63.0; H, 7.7. $C_{17}H_{24}O_6$ requires C, 62.9; H, 7.5%). The experiment was repeated starting with allyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside[10] to give *allyl* 4-O-p-*methoxybenzyl*-α-D-*rhamnopyranoside*, m.p. 76—77 °C, $[\alpha]_D^{26}$ +59.6° (*c* 1 in $CHCl_3$) (Found: C, 63.0; H, 7.5%).

Action of Dichlorodicyanoquinone and Cerium(IV) *Ammonium Nitrate on Allyl* 2,3-O-*Isopropylidene*-4-O-p-*methoxybenzyl*-α-L-*rhamnopyranoside* (**16**).—A mixture of compound (**16**) (230 mg) and cerium(IV) ammonium nitrate (700 mg) in acetonitrile–water (9:1; 4 ml) was stirred at 20 °C. T.l.c. (ether–light petroleum, 1:1) showed a rapid conversion of compound (**16**) (R_F 0.8) into compound (**15**) (R_F 0.3) and *p*-methoxybenzaldehyde (R_F 0.5). The former, however, was rapidly degraded to a product (R_F 0) which, although not further investigated, after 1 h constitutes the major product; some starting material (**16**) however still remained.

A mixture of compound (**16**) (100 mg) and dichlorodicyanoquinone (90 mg) in dichloromethane (5 ml) and water (0.3 ml) was stirred at 20 °C. T.l.c. (as above) showed complete conversion of compound (**16**) into compound (**15**) and *p*-methoxybenzaldehyde after 2 h without further breakdown of compound (**15**).

Allyl 4-O-p-*Methoxybenzyl*-2,3,*di*-O-*methyl*-α-L-*rhamnopyranoside* (**18**).—Methyl iodide (3 ml) was added to a stirred mixture of the diol (**17**) (4.25 g) and sodium hydride (1 g) in dry *N,N*-dimethylformamide (40 ml) cooled in ice. The mixture was stirred at 20 °C for 30 min after which time t.l.c. (ether–light petroleum, 1:1) showed complete conversion of (**17**) (R_F 0) into a product (R_F 0.5). Methanol was added to destroy the excess of sodium hydride after which the solution was diluted with water and extracted with ether. The extract was washed with saturated aqueous potassium chloride, dried (K_2CO_3), and evaporated to give the *methyl ether* (**18**) (4.3 g), m.p. 81—83 °C (from light petroleum), $[\alpha]_D^{26}$ −62.3° (*c* 1 in $CHCl_3$) (Found: C, 64.6; H, 8.4. $C_{19}H_{28}O_6$ requires C, 64.75; H, 8.0%).

Allyl 2,3-*Di*-O-*methyl*-α-L-*rhamnopyranoside* (**19**).—A mixture of the *p*-methoxybenzyl ether (**18**) (1.78 g), dichloromethane (80 ml), water (5 ml), and dichlorodicyanoquinone (1.45 g) was stirred at 20 °C for 45 min after which time t.l.c. (ether–light petroleum, 1:1) showed complete conversion of (**18**) (R_F 0.5) into a product (R_F 0.15) and *p*-methoxybenzaldehyde (R_F 0.55). The mixture was diluted with dichloromethane (50 ml), washed with aqueous sodium hydrogen carbonate, dried (K_2CO_3), and evaporated to give the crude product which was chromatographed on silica gel (ether). After elution of *p*-methoxybenzaldehyde, the *alcohol* (**19**) was obtained as an oil (1 g), $[\alpha]_D^{27}$ −41.6° (*c* 1 in $CHCl_3$) (Found: C, 57.3, H, 9.0. $C_{11}H_{20}O_5$ requires C, 56.9; H, 8.7%).

2,3-*Di*-O-*methyl*-L-*rhamnopyranose* (**20**).[21,22]—Allyl 2,3-di-*O*-methyl-4-*O*-*p*-methoxybenzyl-α-L-rhamnopyranoside (**18**) (500 mg) in M-hydrochloric acid (5 ml) and acetone (10 ml) was heated under reflux for 9 h. T.l.c. (ether) then showed conversion of compound (**18**) (R_F 0.95) mainly into allyl 2,3-di-*O*-methyl-α-L-rhamnopyranoside (**19**) (R_F 0.5) and *p*-methoxybenzaldehyde (R_F 0.85) with only a small amount of 2,3-di-*O*-methyl-L-rhamnopyranose (R_F 0). The acetone was evaporated and M-hydrochloric acid (10 ml) was added and refluxing was continued for 3 h after which time t.l.c. showed conversion of compound (**19**) into the required product. An excess of sodium acetate was added and the mixture was evaporated to dryness. Toluene was evaporated from the residue which was then extracted with dichloromethane. Chromatography of the product on silica gel gave 2,3-di-*O*-methyl-L-rhamnopyranose (**20**) as a syrup, $[\alpha]_D^{26}$ +43.5° (*c* 1 in water) {lit.,[21] $[\alpha]_D$ +42.2° (*c* 1.5 in water); lit.,[22] $[\alpha]_D$ +40° (*c* 0.7 in water)}.

Allyl 4-O-(2,4-*Di*-O-*acetyl*-3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-2,3-O-*isopropylidene*-α-L-*rhamnopyranoside* (**11**).[9]—Allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**15**)[9] (1.02 g, 4.2 mmol), 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl chloride[9] (976 mg, 3.1 mmol), tetramethylurea (962 mg, 8.3 mmol), and silver trifluoromethanesulphonate (1.59 g, 6.2 mmol) in dry dichloromethane (30 ml) was stirred at 20 °C for 2 h. The mixture was filtered through Celite and the filtrate was washed with saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated and the crude product was chromatographed on silica gel. The disaccharides and the excess of alcohol (**15**) were eluted together with ether–light petroleum (1:1) and were kept with sodium hydroxide (1 g) in methanol (50 ml) at 50 °C for 1 h. Solid carbon dioxide was added and the solution was evaporated to dryness and the products extracted from the residue with ether. T.l.c. (toluene–acetone, 2:1) showed the alcohol (**15**) (R_F 0.7), the disaccharide (**12**) (R_F 0.4), and a small amount of the α-linked disaccharide (R_F 0.35) (compare ref. 9; the disaccharides being present in a ratio of *ca.* 10:1 respectively). The disaccharides (1.1 g) were separated from the alcohol (**15**) by chromatography on silica gel (ether–light petroleum, 1:2) and were converted into the acetates by the action of acetic anhydride–pyridine in the usual way. Recrystallisation from light petroleum gave the acetate (**11**) (930 mg), m.p. 109—110 °C, $[\alpha]_D^{26}$ −48.9° (*c* 1 in $CHCl_3$) with the

^{1}H n.m.r. spectrum identical with that reported in ref. 9 {lit.,[9] $[\alpha]_D^{23}$ −49.4° (*c* 1 in $CHCl_3$), m.p. 84—85 °C; all subsequent preparations of this compound have shown m.p. 109—110 °C and the m.p. recorded in ref. 9 must be regarded as a misprint or as the m.p. of an unstable crystalline form which has not subsequently been obtained}.

Allyl 4-O-(2,4-*Di*-O-p-*methoxybenzyl*-3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-2,3-O-*isopropylidene*-α-L-*rhamnopyranoside* (**13**).—*p*-Methoxybenzyl chloride (1.4 g) in dry *N*,*N*-dimethylformamide (5 ml) was added to a mixture of the diol (**12**)[9] (1.7 g), sodium hydride (1 g) and dry *N*,*N*-dimethylformamide (30 ml) and the mixture was stirred at 20 °C for 3 h. T.l.c. (ether–light petroleum, 1:1) then showed complete conversion of compound (**12**) (R_F 0) into a product (R_F 0.5). Methanol was added to destroy the excess of sodium hydride and the solution was diluted with water and extracted with ether. The extract was washed with saturatured aqueous potassium chloride, dried (K_2CO_3), and evaporated. The crude product was chromatographed on silica gel (ether–light petroleum, 1:1) to give the p-*methoxybenzyl ether* (**13**) (2.5 g) as a syrup, $[\alpha]_D^{27}$ −33.0° (*c* 1.5 in $CHCl_3$) (Found: C, 64.1; H, 7.4. $C_{36}H_{50}O_{12}$ requires C, 64.1; H, 7.5%).

Allyl 4-O-(2,4-*Di*-O-p-*methoxybenzyl*-3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**14**).—The isopropylidene derivative (**13**) (2.2 g) in methanol (48 ml) and 6M-hydrochloric acid (0.8 ml) was kept at 50 °C for 20 min after which time t.l.c. (toluene–acetone, 2:1) showed almost complete conversion of compound (**13**) (R_F 0.9) into a major product (R_F 0.5) and traces of more polar products (due to removal of the *p*-methoxybenzyl groups). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off and the product was extracted from the residue with ether. The crude product was chromatographed on silica gel and the trace of compound (**13**) was eluted with ether and then the *diol* (**14**) (1.5 g) was eluted with ether–acetone (10:1) and obtained as a syrup, $[\alpha]_D^{27}$ −48.7° (*c* 1 in $CHCl_3$) (Found: C, 62.3; H, 6.9. $C_{33}H_{46}O_{12}$ requires C, 62.4; H, 7.3%).

Allyl 4-O-(2,4-*Di*-O-p-*methoxybenzyl*-3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-2,3-*di*-O-*methyl*-α-L-*rhamnopyranoside* (**6**).—The diol (**14**) (1.48 g) was converted into the methyl ether (**6**) as described above under the preparation of compound (**18**). The crude product was chromatographed on silica gel (ether) to give the *methyl ether* (**6**) (1.5 g) as a syrup, $[\alpha]_D^{27}$ −34.2° (*c* 1 in $CHCl_3$) (Found: C, 63.0; H, 7.6. $C_{35}H_{50}O_{12}$ requires C, 63.4; H, 7.6%).

Allyl 2,3-*Di*-O-*methyl*-4-O-(3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**7**).—(*a*) A mixture of the *p*-methoxybenzyl ether (**6**) (1.4 g), dichlorodicyanoquinone (1.2 g), dichloromethane (75 ml), and water (4 ml) was stirred at 20 °C. A precipitate of the hydroquinone soon separated from the olive-green solution and after 30 min t.l.c. (ether) showed conversion of compound (**6**) (R_F 0.65) into two products (R_F 0.45 and 0.1). Dichlorodicyanoquinone (200 mg) was added and after 30 min the mixture was diluted with dichloromethane (50 ml) and filtered through Celite. The filtrate was washed with saturated aqueous sodium hydrogen carbonate and dried (K_2CO_3), and evaporated. The crude product was chromatographed on silica gel elution with ether removing the *p*-methoxybenzaldehyde and elution with ether–acetone (2:1) giving the *diol* (**7**) (0.8 g) as a syrup, $[\alpha]_D^{25}$ −58.4° (*c* 1 in $CHCl_3$) (Found: C, 53.6; H, 8.0. $C_{19}H_{34}O_{10}$ requires C, 54.0; H, 8.1%).

(*b*) A mixture of 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl chloride[9] (11.6 g), allyl 2,3-di-*O*-methyl-α-L-rhamnopyranoside (**19**) (8.2 g), mercury(II) cyanide (7.25 g), and dry acetonitrile (70 ml) was heated under reflux for 1 h after which time t.l.c. (ether–light petroleum, 2:1) showed conversion of the chloride (R_F 0.8) and alcohol (**19**) (R_F 0.3) into a major product (R_F 0.15). The solvent was evaporated off and the residue was taken up in ether and the solution washed with concentrated aqueous potassium iodide (to remove mercury salts) and dried ($MgSO_4$). Evaporation of the ether gave an oil which was chromatographed on silica gel (ether) to give the major product [crude (**8**), containing some α-linked isomer] (14.5 g). [T.l.c. (ether) R_F 0.5 with a trace of alcohol (**19**) at R_F 0.6.] This was treated with sodium hydroxide (3 g) in methanol (200 ml) at 50 °C for 1 h after which time t.l.c. (ether–acetone, 2:1) showed a major product [R_F 0.8, which co-chromatographed with the product from (*a*)] and a minor product (R_F 0.7) presumed to be the α-linked isomer of (**7**). Solid carbon dioxide was added and the solvent was evaporated off and the product extracted from the residue with dichloromethane. The extract was dried (K_2CO_3) and evaporated and chromatography of the crude product on silica gel (ether) eluted first the unchanged alcohol (**19**) and then, with ether–acetone (4:1), the major product (7.5 g), $[\alpha]_D^{25}$ −55° (*c* 1 in $CHCl_3$), identical with the material described in (*a*).

(±)-2′,3′-*Epoxypropyl* 2,3,4,6-*Tetra*-O-*benzyl*-α-D-*galactopyranoside* (**30**).—Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside[14] (2.2 g) and 3-chloroperbenzoic acid (2.1 g) in chloroform (150 ml) was kept at 20 °C for 6 days. The solution was washed with aqueous sodium metabisulphite (5 g in 50 ml of water) and concentrated aqueous potassium carbonate, dried (K_2CO_3), and evaporated. The product was chromatographed on silica gel (ether–light petroleum, 1:1) to give the *epoxide* (**30**) (1.7 g) as a syrup, $[\alpha]_D^{21}$ +29.2° (*c* 1.3 in $CHCl_3$) (Found: C, 74.1; H, 6.9. $C_{37}H_{40}O_7$ requires C, 74.5; H, 6.8%).

(±)-2′,3′-*Epoxypropyl* 4-O-(2,4-*Di*-O-*benzyl*-3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-2,3-*di*-O-*methyl*-α-L-*rhamnopyranoside* (**2**).—A solution of the allyl glycoside (**1**) (5.6 g) and 3-chloroperbenzoic acid (6.1 g) in chloroform (400 ml) was kept at 20 °C for 7 days. T.l.c. (ether–light petroleum, 2:1) showed almost complete conversion of (**1**) (R_F 0.5) into a product (R_F 0.2). The solution was washed with aqueous sodium metabisulphite (as above) and evaporated. The residue was taken up into ether and the solution washed with concentrated aqueous potassium carbonate, dried (K_2CO_3), and evaporated. The crude product was chromatographed on silica gel to give first, with ether–light petroleum (2:1), a trace of compound (**1**) and then, with ether, the *epoxide* (**2**) (4.6 g), $[\alpha]_D^{23}$ −28.6° (*c* 1 in $CHCl_3$) (Found: C, 64.5; H, 7.5. $C_{33}H_{46}O_{11}$ requires C, 64.1; H, 7.5%).

(±)-2′,3′-*Dihydroxypropyl* 4-O-(2,4-*Di*-O-*benzyl*-3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-2,3-*di*-O-*methyl*-α-L-*rhamnopyranoside* (**3**).—A mixture of the epoxide (**2**) (4.5 g), dioxane (80 ml), water (16 ml), and aqueous tetrabutylammonium hydroxide (40%; 8 ml) was kept at 60 °C for 20 h. The solution was diluted with water and the product was extracted with ether. The extract was washed with saturated aqueous potassium chloride, dried (K_2CO_3), and evaporated. T.l.c. (ethyl acetate-acetone, 2:1) showed complete conversion of the epoxide (**2**) (R_F 0.8) into the major product (R_F 0.4). The crude product was chromatographed on silica gel (ether) to give the *diol* (**3**) (4.4 g), $[\alpha]_D^{21}$ −25° (*c* 1 in $CHCl_3$) (Found: C, 62.6; H, 7.7. $C_{33}H_{48}O_{12}$ requires C, 62.25; H, 7.6%).

(±)-2′,3′-*Epoxypropyl* 2,3-*Di*-O-*methyl*-4-O-(3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**9**).—A solution of the allyl glycoside (**7**) (830 mg) and 3-chloroperbenzoic acid (1.3 g) in chloroform (80 ml) was kept at 20 °C

for 5 days after which time t.l.c. (ether–acetone, 2:1) showed almost complete conversion of compound (**7**) (R_F 0.7) into a product (R_F 0.6). The solution was washed with aqueous sodium metabisulphite and aqueous potassium carbonate (as above), dried (K_2CO_3), and evaporated to give the product as an oil which was chromatographed on silica gel. The *epoxide* (**9**) (700 mg), $[\alpha]_D^{22}$ −45° (*c* 1 in $CHCl_3$) was eluted with ether–acetone (2:1) (Found: C, 52.1; H, 8.0. $C_{19}H_{34}O_{11}$ requires C, 52.0; H, 7.8%).

(±)-2′,3′-*Dihydroxypropyl* 2,3-*Di*-O-*methyl*-4-O-(3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**4**).—(*a*) A solution of the epoxide (**9**) (3.13 g) and sodium hydroxide (4 g) in water (50 ml) was heated under reflux for 1.5 h. The solution was cooled, solid carbon dioxide was added, and the solvent was evaporated off. Toluene was evaporated from the residue (3 times), the product was extracted from the residue with dichloromethane and the extract dried (K_2CO_3) and evaporated. T.l.c. (ethyl acetate–acetone, 1:3) showed complete conversion of the epoxide (R_F 0.7) into a product (R_F 0.5). Chromatography of the product on silica gel (ether–acetone, 2:1) gave the *glycerol glycoside* (**4**) (2.6 g), $[\alpha]_D^{21}$ −42° (*c* 1 in $CHCl_3$) (Found: C, 49.9; H, 7.8. $C_{19}H_{36}O_{12}$ requires C, 50.0; H, 7.95%).

(*b*) The dibenzyl ether (**3**) (1.2 g) and Pd–C (10%, Fluka, 450 mg) in ethanol (50 ml) were stirred under hydrogen at atmospheric pressure and temperature for 18 h after which time t.l.c. (as above) showed complete conversion of compound (**3**) (R_F 0.95) into the product (R_F 0.5). The mixture was filtered through Celite and the filtrate evaporated. The crude product was chromatographed on silica gel as in (*a*) to give compound (**4**) (780 mg), $[\alpha]_D^{25}$ −40° (*c* 1 in $CHCl_3$) identical with the material described in (*a*).

(±)-2′,3′-*Dihexadecanoyloxypropyl* 2,3-*Di*-O-*methyl*-4-O-(3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**10**).—A solution of the glycerol glycoside (**3**) (230 mg) and hexadecanoyl chloride (400 mg) in dry pyridine (10 ml) was kept at 20 °C for 2 h after which time t.l.c. (ethyl acetate–acetic acid, 49:1) showed absence of starting material (**3**). Water (0.5 ml) was added to react with the excess of hexadecanoyl chloride and the solution was stirred for 30 min and diluted with ether (100 ml). The solution was washed with 3M-hydrochloric acid (to remove the pyridine) and with saturated aqueous potassium chloride and dried ($MgSO_4$). The product was chromatographed on silica gel in light petroleum–ether–acetic acid (7:2.8:0.2) which eluted the hexadecanoic acid and then further elution with ether–light petroleum (2:1) gave the product. A portion of the product (200 mg) and Pd–C (10%, Fluka, 100 mg) was stirred in ethanol (20 ml) under hydrogen at atmospheric temperature and pressure for 12 h. After filtration and evaporation of the filtrate the crude product was chromatographed on silica gel to give the *ester* (**10**) (130 mg), m.p. 67—69 °C (from methanol), $[\alpha]_D^{24}$ −26° (*c* 1 in $CHCl_3$) (Found: C, 66.0; H, 10.7. $C_{51}H_{96}O_{14}$ requires C, 65.6; H, 10.4%).

Formylmethyl 2,3-*Di*-O-*methyl*-4-O-(3,6-*di*-O-*methyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranoside* (**5**).—A solution of the glycerol glycoside (**4**) (2 g) and sodium metaperiodate (1.45 g) in water (80 ml) was kept at 20 °C for 1.5 h after which time t.l.c. (ethyl acetate–acetone, 1:1) showed complete conversion of compound (**4**) (R_F 0.1) into a product (R_F 0.5). Most of the water was removed by evaporation at 40 °C and dichloromethane (100 ml) and magnesium sulphate (10 g) were added to the residue. The mixture was filtered and evaporated and toluene was evaporated from the residue to give the aldehyde (**5**) as a syrup which was used directly to prepare the conjugate with bovine serum albumin.

Conjugation of the Aldehyde (**5**) *with Bovine Serum Albumin*.—A solution of the aldehyde (**5**) (2 g), bovine serum albumin (1 g, Sigma, Fraction V powder, 96—99% albumin) and sodium cyanoborohydride (1.6 g) in 0.1M-phosphate buffer, pH 7.6 (60 ml) [prepared from 0.2M-disodium hydrogen phosphate solution (43.5 ml) and 0.2M-sodium dihydrogen phosphate solution (6.5 ml) diluted to 100 ml with water] was kept at 37 °C for 5 days. It was then transferred to dialysis tubing and the tubes stirred in distilled water for 3 days the water being changed every 12 h. The combined contents of the dialysis tubes were freeze dried to give the conjugate as a powder (950 mg). For amino acid analyses a portion of the conjugate was hydrolysed with 6M-hydrochloric acid in a sealed tube at 110 °C for 18 h. The mixture was evaporated to dryness and the amino acids were analysed on a Beckman 121 MB analyser. A sample of the bovine serum albumin used for the preparation of the conjugate was treated similarly. From the analytical figures, and using a standard of 61 residues of leucine in bovine serum albumin, the bovine serum albumin showed 60 lysine residue whereas the conjugate showed 12 lysine residues indicating that 48 of the lysine residues had been substituted. A portion of the conjugate was hydrolysed with M-methanolic hydrogen chloride in a sealed tube at 85—90 °C for 6 h and the methyl glycosides of 3,6-di-*O*-methyl-D-glucopyranoside were analysed as the trimethylsilyl derivatives by g.l.c. on an SE 30 column. Comparison with a standard of 3,6-di-*O*-methyl-D-glucose[9] treated in the same way indicated that 11% of the weight of the conjugate was 3,6-di-*O*-methylglucose which was consistent with the results obtained from the amino acid analyses assuming a molecular weight[23] of 66 210 for bovine serum albumin.

(±)-5′,6′-*Dihydroxyhexyl* 3,6-*Di*-O-*methyl*-β-D-*glucopyranoside* (**28**).—A mixture of 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-β-D-glucopyranosyl chloride[9] (1.5 g), the 5,6-*O*-isopropylidene derivative of (±)-hexane-1,5,6-triol[23] (1.02 g). [Prepared from hexane-1,5,6-triol (10 g, Aldrich), acetone (50 ml), 2,2-dimethoxypropane (30 ml), and toluene-*p*-sulphonic acid (50 mg) at 20 °C for 1 h. Triethylamine (2 ml) was added and the solvents were evaporated off and the residue was chromatographed on basic alumina, the product being eluted with ether–methanol (49:1)], mercury(II) cyanide (1.8 g) in dry acetonitrile (25 ml), was heated under reflux for 1 h. The solvent was evaporated off and ether (100 ml) and concentrated aqueous potassium iodide (100 ml) were added to the residue. The ether layer was washed again with aqueous potassium iodide, dried ($MgSO_4$), and evaporated. T.l.c. (toluene–acetone, 2:1) showed conversion of the chloride (R_F 0.8) into a major product (R_F 0.7) together with the excess of alcohol (R_F 0.6). The crude product (**26**) (1.97 g) and sodium hydroxide (600 mg) were heated in methanol (40 ml) at 50 °C for 30 min. Solid carbon dioxide was added and the solvents were evaporated off. The products were extracted with ether and t.l.c. (as above) showed the presence of the excess of alcohol (R_F 0.6) and the crude compound (**27**) (R_F 0.2). The crude compound (**27**) (1.2 g) (containing a little of the α-anomer) was obtained by chromatography on silica gel and was heated under reflux with methanol (59 ml) and 6M-hydrochloric acid (1 ml) for 30 min. The solution was cooled, sodium hydrogen carbonate (1 g) was added, and the solvents were evaporated off. The product was extracted from the residue with dichloromethane and chromatographed on silica gel (acetone) to give the crude glycoside (**28**). Crystallisation from ethyl acetate gave the pure *glycoside* (**28**) (516 mg), m.p. 75—77 °C, $[\alpha]_D^{24}$ −35.9° (*c* 0.84 in $CHCl_3$) (Found: C, 52.0; H, 8.8. $C_{14}H_{28}O_8$ requires C, 51.8; H, 8.7%). This compound was cleaved with sodium metaperiodate and the aldehyde (**29**) was conjugated with bovine serum albumin as described above for the preparation of the disaccharide conjugate.

(±)-10′,11′-*Epoxyundecyl* 3,6-*Di*-O-*methyl*-β-D-*glucopyranoside* (**23**).—A mixture of 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl chloride[9] (5 g), undec-10-enol (3.3 g, Aldrich) and mercury(II) cyanide (2.5 g) in dry acetonitrile (35 ml) was heated under reflux for 2.5 h. The solvent was evaporated off and the product was isolated as described above for the preparation of compound (**26**). T.l.c. (toluene–acetone, 2:1) showed some alcohol (R_F 0.6) and a major product [crude (**21**), R_F 0.7]. The product was kept with sodium hydroxide (2 g) in methanol (100 ml) at 50 °C for 30 min. Solid carbon dioxide was added and the solvent was evaporated off. The residue was extracted with ether and the extract dried (K_2CO_3) and evaporated. T.l.c. (as above) showed the presence of the excess of alcohol (R_F 0.6) and the crude diol (**22**) (R_F 0.4) and these were separated by chromatography on silica gel (ether) to give the crude diol (**22**) (4.9 g, containing some α-glycoside). A solution of the crude diol (**22**) (4.25 g) and 3-chloroperbenzoic acid (6.3 g) in chloroform (200 ml) was kept at 20 °C for 24 h after which time t.l.c. (ether) showed complete conversion of the alkene (**22**) (R_F 0.5) into the epoxide (**23**) (R_F 0.35). The solution was washed with aqueous sodium metabisulphite and aqueous potassium carbonate, dried (K_2CO_3), and evaporated. The crude product was crystallised from ether–light petroleum (1:1) to give the *epoxide* (**23**) (3 g), m.p. 60—61 °C, $[\alpha]_D$ −33.8° (*c* 1 in $CHCl_3$) (Found: C, 60.9; H, 9.85. $C_{19}H_{36}O_7$ requires C, 60.6; H, 9.6%).

(±)-10′,11′-*Dihydroxyundecyl* 3,6-*Di*-O-*methyl*-β-D-*glucopyranoside* (**24**).—A mixture of the epoxide (**23**) (1 g), sodium hydroxide (4 g), and water (50 ml) was heated under reflux for 1.5 h. Solid carbon dioxide was added to the cooled mixture and the solvent was evaporated off. The product was extracted from the residue with dichloromethane and the extract dried (K_2CO_3) and evaporated to give the product as an oil (1.07 g) which was crystallised from ethyl acetate–light petroleum (b.p. 60—80 °C) to give the *glycoside* (**24**), m.p. 77—80 °C (800 mg), $[\alpha]_D^{24}$ −33.7° (*c* 1 in $CHCl_3$) (Found: C, 57.8; H, 9.75. $C_{19}H_{38}O_8$ requires C, 57.8; H, 9.7%).

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