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SYNTHESIS OF SPACER-ARM, LIPID, AND ETHYL GLYCOSIDES OF THE TRISACCHARIDE PORTION [α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc] OF THE BLOOD-GROUP P^k ANTIGEN: PREPARATION OF NEO-GLYCOPROTEINS*

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ABSTRACT

The title compounds were prepared *via* the acetylated 2-bromoethyl glycoside **11** of α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc by displacement of bromide ion with methyl 3-mercaptopropionate, octadecanethiol, and hydrogen, respectively. Silver triflate-promoted glycosylation of 2-bromoethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside with 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl bromide gave **11**. A tetradeuterated analogue of **11** was prepared by essentially the same route. The spacer-arm glycoside formed from methyl 3-mercaptopropionate was coupled to bovine serum albumin and keyhole limpet haemocyanin.

INTRODUCTION

Globotriaosylceramide (the P^k-antigen[2]) and globotetraosylceramide (the P-antigen[2]) have been suggested to function as receptors for pathogenic *E. coli* bacteria in the human urinary tract[3]. Recently, globotriaosylceramide was identified as an antigen associated with the Burkitt lymphoma[4]. We recently reported an improved synthesis[5] of the α-D-Gal-(1→4)-D-Gal unit (considered to be the important part of the *E. coli* receptor) of these ceramides, as well as a series of derivatives[6–8] of value for the biological evaluation of the receptor phenomenon.

Methyl and *p*-nitrophenyl glycosides of the trisaccharide α-D-Gal-(1→4)-β-D-Gal-(1→4)-D-Glc of the P^k-antigen have been prepared[9] *via* suitably protected lactose and galactose derivatives. We now report an alternative route, starting from 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl bromide (**1**) and 2-bromoethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**), leading to the 2-bromoethyl glycoside **11** that was used for the preparation of spacer-arm, lipid, and ethyl glycosides of the P^k-antigen.

*2-Bromoethyl Glycosides, Part 5. For Part 4, see ref. 1.
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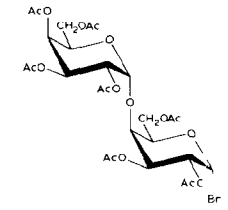

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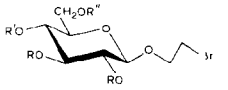

2 R = R′ = R″ = Ac
3 R = H, R′, R″ = CHPh
4 R = Bzl, R′, R″ = CHPh
6 R = R″ = Bzl, R′ = H
7 R = Bz, R′, R″ = CHPh
8 R = Bz, R′ = H, R″ = Bzl

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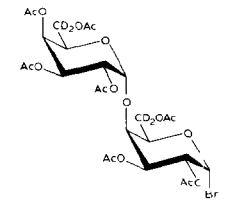

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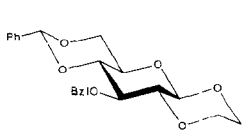

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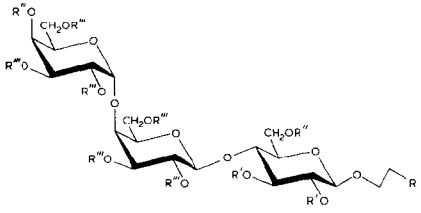
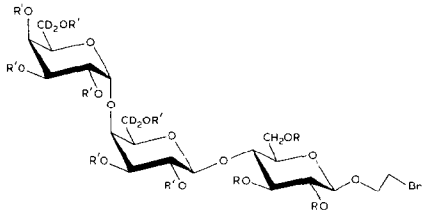
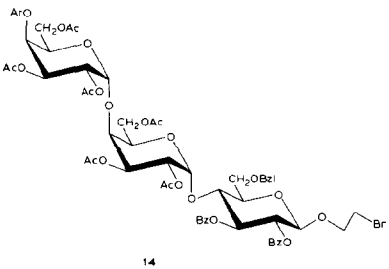


9 R = Br, R′ = R″ = Bzl, R‴ = Ac
10 R = Br, R′ = R″ = H, R‴ = Ac
11 R = Br, R′ = R″ = R‴ = Ac
12 R = Br, R′ = Bz, R″ = Bzl, R‴ = Ac
13 R = Br, R′ = Bz, R″ = H, R‴ = Ac
15 R = S-COOMe, R′ = R″ = R‴ = Ac
16 R = S-COOMe, R′ = R″ = R‴ = H
17 R = S-, R′ = R″ = R‴ = Ac
18 R = S-, R′ = R″ = R‴ = H
19 R = H, R′ = R″ = R‴ = Ac
20 R = H, R′ = R″ = R‴ = H
21 R = S-CONH—BSA, R′ = R″ = R‴ = H
22 R = S-CONH—KLH, R′ = R″ = R‴ = H



24 R = Bzl, R′ = Ac
25 R = H, R′ = Ac
26 R = R′ = Ac



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Specifically deuterated derivatives of carbohydrates can be used to simplify interpretations of mass and ^{1}H-n.m.r. spectra, and as labelled compounds for biochemical and biological studies. A tetradeuterated analogue (**26**) of **11** has also been prepared.

RESULTS AND DISCUSSION

2-Bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[7] (**2**) was deacetyl-

ated with methanolic sodium methoxide, and a solution of the product in *N,N*-dimethylformamide was immediately added to a mixture of zinc chloride and benzaldehyde[10] to give 73% of the 4,6-*O*-benzylidene derivative **3**. Treatment of **3** with benzyl bromide, under conditions of phase-transfer catalysis, gave crystalline 2-bromoethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**4**) and 3-*O*-benzyl-4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-glucopyranose (**5**), in yields of 80 and 20%, respectively. Treatment[11] of **4** with sodium cyanoborohydride gave 59% of 2-bromoethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**). Reaction of **3** with benzoyl chloride in pyridine gave 86% of the 2,3-dibenzoate **7**. Reduction of the benzylidene group in **7**, as described above[11], then gave 65% of 2-bromoethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (**8**).

The partly protected 2-bromoethyl glycosides **6** and **8** were subjected to silver triflate-promoted glycosylations with 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl bromide[7] (**1**). With **6**, 55% of the β-glycoside **9** was formed together with a small proportion of α-glycoside (see below), whereas, with **8**, a ~3:1 αβ-mixture (**14** and **12**) was obtained. Catalytic hydrogenolysis[6,8] of **12** gave a quantitative yield of **13** which, on treatment with methanolic sodium methoxide and then reacetylation, gave the trisaccharide derivative **11**.

Catalytic hydrogenation of 2-bromoethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**9**) in acetic acid (conditions under which the bromoethyl group does not react[8]) gave a quantitative yield of **10**. Acetylation of **10** then gave 69% of the 2-bromoethyl glycoside **11**. The tetradeuterated compound **26** was prepared by the same method from **23** and **6** *via* **24** and **25**.

Nucleophilic displacement of bromide ion from **11**, using methyl 3-mercaptopropionate[12] or octadecanethiol in *N,N*-dimethylformamide containing cesium carbonate, gave the spacer-arm glycoside **15** (87%) and the neoglycolipid **17** (87%). Deacetylation of **15** and **17** gave 2-(2-methoxycarbonylethylthio)ethyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**16**, 97%) and 2-(octadecylthio)ethyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**18**, 88%). The glycoside **16** was coupled to bovine serum albumin and keyhole limpet haemocyanin, using a modification[1] of the Inman–Lemieux procedure[6,13,14] with methyl sulfoxide as solvent instead of *N,N*-dimethylformamide, thus furnishing the glycoproteins **21** and **22**, respectively.

Catalytic hydrogenation of **11** under basic conditions[6], with acetylation of the product, gave 62% of the ethyl glycoside **19**. Deacetylation of **19** gave a quantitative yield of ethyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**20**). The use of crude **11** in the latter reaction sequence gave a small proportion (<5%) of the α-glycoside (t.l.c. and ^{1}H-n.m.r.) that had been formed in the reaction of **6** with **1**. The structure of **20** was confirmed by sugar[15] and methylation[16] analysis.

EXPERIMENTAL

General methods were as reported[8]. Me_4Si and sodium 3-(trimethylsilyl)propionate-d_4 (TSP) were used as n.m.r. references.

2-Bromoethyl 4,6-O-benzylidene-β-D-glucopyranoside (**3**). — A solution of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[7] (**2**; 30 g, 66 mmol) in warm methanol (300 mL) was rapidly cooled and treated with methanolic 0.1M sodium methoxide (30 mL) at room temperature for 3 h. The reaction was monitored by t.l.c. (SiO_2; chloroform–methanol–water, 65:35:10, lower phase). The reaction mixture was filtered through a column (5 × 4 cm) of Duolite C-26 (H^+) resin and the solvent was removed. A solution of the residue (18.6 g) in *N,N*-dimethylformamide (20 mL) was added dropwise (10 min) with stirring to a mixture of zinc chloride (44 g, 103 mmol) and benzaldehyde (48 g, 471 mmol). After 21 h, ether (500 mL) and ice–water (300 mL) were added and the aqueous phase was extracted with ether (2 × 100 mL). The combined extracts were washed with aqueous sodium hydrogencarbonate (2 × 100 mL) and water (50 mL). Dichloromethane was added to prevent crystallisation. The solution was dried (Na_2SO_4) and concentrated, and the residue (18.1 g, 73%) was recrystallised from ethanol to give **3**, m.p. 157–159°, $[\alpha]_D^{21}$ −43° (*c* 2, chloroform). N.m.r. data: ^1H ($CDCl_3$ + D_2O, Me_4Si), δ 5.52 (s, 1 H, PhC*H*), 4.43 (d, 1 H, *J* 8 Hz, H-1), 4.32 (dd, 1 H, *J* 11 and 5 Hz), and 4.14 (td, 1 H, *J* 6 and 11 Hz); ^{13}C ($CDCl_3$, Me_4Si), δ 103.3, 101.9 (C-1), and 30.1 (CH_2Br)

Anal. Calc. for $C_{15}H_{19}BrO_6$: C, 48.01; H, 5.10. Found: C, 48.18; H, 5.04.

2-Bromoethyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside (**4**) and *3-O-benzyl-4,6-O-benzylidene-1,2-O-ethylidene-β-D-glucopyranose* (**5**). — A mixture of **3** (4.4 g, 11.7 mmol), benzyl bromide (20 mL, 77 mmol), tetrabutylammonium hydrogensulfate (0.5 g), and aqueous 10% sodium hydroxide (44 mL) was stirred at room temperature for 17 h, diluted with toluene, washed with water, dried (Na_2SO_4), and concentrated. Column (5 × 18 cm) chromatography (SiO_2; toluene, then 19:1 toluene–ethyl acetate, and finally ethyl acetate) gave **4** (5.2 g, 80%) and **5** (0.9 g, 20%).

Compound **4** had m.p. 97–98° (from ethanol), $[\alpha]_D^{21}$ −30° (*c* 2, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.58 (s, 1 H, PhC*H*), 4.98, 4.77 (ABq, 2 H, J_{AB} 11 Hz, PhCH_2), 4.92, 4.81 (ABq, 2 H, J_{AB} 11.5 Hz, PhCH_2), 4.56 (d, 1 H, *J* 7.5 Hz, H-1), 4.35 (dd, 1 H, *J* 10.5 and 5 Hz), 4.22 (dt, 1 H, *J* 11 and 5.5 Hz, CH_2CH$_2$Br), and 3.52 (bt, *J* 6 Hz, CH_2Br); ^{13}C, δ 104.1, 101.1 (C-1), and 30.0 (CH_2Br).

Anal. Calc. for $C_{29}H_{31}BrO_6$: C, 62.70; H, 5.63. Found: C, 62.94; H, 5.62.

Compound **5** had m.p. 160.5–162.5° (from ethanol–ethyl acetate), $[\alpha]_D^{21}$ −21° (*c* 1.5, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.57 (s, 1 H, PhC*H*), 4.86 (s, 2 H, PhCH_2), 4.42 (d, 1 H, *J* 8 Hz, H-1), 4.38 (dd, 1 H, *J* 10 and 4.5 Hz), and 3.31 (bt, 1 H, *J* 7.5 Hz); ^{13}C, δ 101.5 and 99.1 (C-1 and PhCH).

Anal. Calc. for $C_{22}H_{24}O_6$: C, 68.73; H, 6.29. Found: C, 68.58; H, 6.33.

2-Bromoethyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (**6**). — A mixture of **4** (29.5 g, 53 mmol), sodium cyanoborohydride (9 g, 143 mmol), molecular sieves (3 Å, 30 g), and tetrahydrofuran (175 mL) was stirred while hydrogen chloride-saturated ether (225 mL) was added dropwise[11]. Additional amounts of cyanoborohydride (1 g) and ethereal acid (25 mL) were then added. T.l.c. (SiO_2; toluene–ethyl acetate, 19:1) showed that **4** had been consumed. Toluene (500 mL) was added, and the mixture was washed with ice–water (250 mL) and cold aqueous sodium hydrogencarbonate (250 mL), dried (Na_2SO_4), and concentrated to give a semi-crystalline residue (32 g). Column (10 × 18 cm) chromatography (SiO_2; ethyl acetate–iso-octane, 1:2) and recrystallisation of the residue (17.5 g, 59%) from toluene–iso-octane gave **6**, m.p. 63–64°, $[\alpha]_D^{21}$ −18° (*c* 1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.03, 4.72 (ABq, 2 H, J_{AB} 10.5 Hz, PhCH_2), 4.95, 4.72 (ABq, 2 H, J_{AB} 11.5 Hz, PhCH_2), 4.60, 4.57 (ABq, 2 H, J_{AB} 12.0 Hz, PhCH_2), 4.47 (d, with virtual coupling[17], 1 H, $J_{1,2}$ 7.5 Hz, H-1), and 4.23 (dt, 1 H, *J* 11 and 5.5 Hz, CH_2CH$_2$Br); ^{13}C, δ 103.8 (C-1) and 30.3 (CH_2Br).

Anal. Calc. for $C_{29}H_{33}BrO_6$: C, 62.48; H, 5.97. Found: C, 62.64; H, 5.97.

2-Bromoethyl 2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside (**7**). — Benzoyl chloride (20 mL, 240 mmol) was added (7 min) dropwise with stirring to a solution at 0° of **3** (17 g, 45.3 mmol) in pyridine (85 mL). Ice–water (125 mL) was added after 1 h and the mixture was extracted with ether–dichloromethane (2:1, 750 mL). The extract was washed with aqueous sodium hydrogencarbonate (100 mL) and aqueous sodium chloride (100 mL), dried (Na_2SO_4), and concentrated. Recrystallisation of the residue (27.7 g, 82%) from toluene–ethanol gave **7**, m.p. 176–178°, $[\alpha]_D^{21}$ −0.1° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.80 (t, 1 H, *J* 9 Hz, H-3), 5.55 (s, 1 H, PhC*H*), 5.50 (dd, 1 H, *J* 9 and 8 Hz, H-2), 4.88 (d, 1 H, *J* 8 Hz, H-1), 4.44 (dd, 1 H, *J* 10 and 5 Hz, H-5), 4.16 (dt, 1 H, *J* 11 and 6 Hz, CH_2CH$_2$Br), and 3.39 (t, 2 H, *J* 6 Hz, CH_2Br); ^{13}C, δ 101.8 and 101.5 (C-1 and PhCH), and 29.5 (CH_2Br).

Anal. Calc. for $C_{29}H_{27}BrO_8$: C, 59.70; H, 4.66. Found: C, 60.08; H, 4.80.

2-Bromoethyl 2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside (**8**). — A mixture of **7** (26.1 g, 45 mmol), sodium cyanoborohydride (7 g, 110 mmol), molecular sieves (4 Å, 20 g), and tetrahydrofuran (150 mL) was treated essentially as in the preparation of **6**, to give **8** (17.1 g, 65%). Recrystallisation from iso-octane–toluene gave material with m.p. 78–80°, $[\alpha]_D^{21}$ +53° (*c* 1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 4.76 (d with virtual coupling[17], 1 H, $J_{1,2}$ 8 Hz, H-1), 4.65, 4.61 (ABq, 2 H, J_{AB} 12 Hz, PhCH_2), 4.14 (dt, 1 H, *J* 11 and 6 Hz, CH_2CH$_2$Br), and 3.40 (bt, 2 H, *J* ~7 Hz, CH_2Br); ^{13}C, δ 101.1 (C-1) and 29.65 (CH_2Br).

Anal. Calc. for $C_{29}H_{29}BrO_8$: C, 59.49; H, 4.99. Found: C, 59.19; H, 5.11.

2-Bromoethyl 2,3,6-tri-O-benzyl-4-O-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**9**). — A solution of **1**[7] (21 g, 30 mmol) in dichloromethane (70 mL) was added (45 min) dropwise with stirring to a solution (−78°, N_2) of **6** (14 g, 25 mmol), silver tri-

fluoromethanesulfonate (9 g, 35 mmol), and tetramethylurea (4.6 g, 39.6 mmol) in dichloromethane (130 mL). After 5 h, the mixture, then at room temperature, was filtered through Celite, diluted with dichloromethane, washed with M hydrochloric acid and aqueous sodium hydrogencarbonate, dried (Na_2SO_4), and concentrated. The residue (35 g) was subjected to chromatography (SiO_2; ethyl acetate–iso-octane gradient, 1:1→3:2) to give **1** (2 g) and **9** (16.5 g). Further chromatography of a fraction containing impure **9** gave additional material (1.3 g; total yield, 17.8 g, 70% based on reacted **6**). Recrystallisation from ethanol gave material with m.p. 155–158°, $[\alpha]_D^{21}$ −58° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.58 (dd, 1 H, *J* 3 and <1 Hz, H-4″), 5.30 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.17 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.11 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 5.03 (d, 1 H, *J* 11.5 Hz, PhCH_2), 4.94 (d, 1 H, *J* 3.5 Hz, H-1″), 4.94 (d, 1 H, *J* 11 Hz, PhCH_2), 4.83 (d, 1 H, *J* 11.5 Hz, PhCH_2), 4.74 (d, 1 H, *J* 12 Hz, PhCH_2), 4.69 (d, 1 H, *J* 11 Hz, PhCH_2), 4.67 (d, 1 H, *J* 8 Hz, H-1′), 4.58 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.50 (d, 1 H, *J* 12 Hz, PhCH_2), 4.43 (d, 1 H, *J* 8 Hz, H-1), and 4.59–4.41 (3 H, *inter alia* H-1 and PhCH_2); ^{13}C, δ 103.6 (d, *J* 158 Hz, C-1), 100.6 (d, *J* 156 Hz, C-1′), 99.8 (d, *J* 172 Hz, C-1″), and 30.2 (CH_2Br).

Anal. Calc. for $C_{55}H_{67}BrO_{23}$: C, 56.16; H, 5.74. Found: C, 55.90; H, 5.72.

2-Bromoethyl 4-O-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**10**). — Catalytic hydrogenation (1 atm., 10% Pd/C, 0.2 g) of **9** (1.05 g, 0.89 mmol) in acetic acid (50 mL) for 2 h, followed by filtration and removal of the solvent, gave **10** (0.81 g, 100%). Recrystallisation from methanol gave material with m.p. 178–180°, $[\alpha]_D^{21}$ +77° (*c* 1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.59 (bd, 1 H, *J* ~3 Hz, H-4″), 5.38 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.24 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 5.22 (dd, 1 H, *J* 11 and ~3 Hz, H-2″), 4.97 (d, 1 H, *J* 3.5 Hz, H-1″), 4.81 (dd, 1 H, *J* 11 and 3.5 Hz, H-3′), 4.67 (d, *J* 8 Hz, H-1′), 4.44 (d, 1 H, *J* 8 Hz, H-1), and 4.57–4.34 (3 H, *inter alia* H-1); ^{13}C, δ 102.6, 101.8 (C-1 and C-1′), 99.6 (C-1″), and 30.0 (CH_2Br).

Anal. Calc. for $C_{34}H_{49}BrO_{23}$: C, 45.09; H, 5.45. Found: C, 45.22; H, 5.22.

2-Bromoethyl 2,3,6-tri-O-acetyl-4-O-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**11**). — (*a*) A solution of **10** (6.65 g, 7.3 mmol) in acetic anhydride–pyridine (1:1, 132 mL) was left at room temperature for 15 h and then co-concentrated with toluene. Crystallisation of the residue from ethanol gave **11** (5.22 g, 69%), m.p. 179–181°, $[\alpha]_D^{21}$ +45° (*c* 1.6, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.59 (bdd, 1 H, *J*~3 and <1 Hz, H-4″), 5.40 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.22 (t, 1 H, *J* ~9 Hz, H-3), 5.18 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.11 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.5 Hz, H-1″), 4.92 (dd, 1 H, *J* 9.5 and 8 Hz, H-2), 4.74 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.56 (d, 1 H, *J* 8 Hz, H-1), 4.53 (d, 1 H, *J* 8 Hz, H-1′), 4.02 (bd, 1 H, *J* ~2.5 Hz, H-4′), 3.65 (q, 1 H, *J* 9.5 and 2 Hz, H-5), and 3.46 (bt, 2 H, *J*~6 Hz, CH_2Br); ^{13}C, δ 101.1 (d, *J* 165 Hz, C-1′), 100.7 (d, *J* 165 Hz, C-1), 99.6 (d, *J* 172 Hz, C-1″), and 29.9 (CH_2Br).

Anal. Calc. for $C_{40}H_{55}BrO_{26}$: C, 46.56; H, 5.37. Found: C, 46.79; H, 5.39.

(*b*) Catalytic hydrogenation (1 atm., 10% Pd/C, 53 mg) of **12** (218 mg, 0.18 mmol) in acetic acid (12 mL) for 1.5 h, followed by filtration and removal of the solvent, gave a quantitative yield of 2-bromoethyl 2,3-di-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**13**). Recrystallisation from methanol gave material with m.p. 218–220°, $[\alpha]_D^{21}$ +69° (*c* 0.7, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.72 (t, 1 H, *J* 9.5 Hz, H-3), 5.59 (bd, 1 H, *J* ~3 Hz, H-4″), 5.35 (dd, 1 H, *J* 9.5 and 8 Hz, H-2), 5.29 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.12 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.98 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 4.87 (d, 1 H, *J* 3.5 Hz, H-1″), 4.80 (d, 1 H, *J* 8 Hz, H-1), 4.66 (dd, 1 H, *J* 11 and 3 Hz, H-3′), 4.66 (d, 1 H, *J* 8 Hz, H-1′), 4.45 (bt, *J* ~6.5 Hz, H-5″), and 3.39 (t, 2 H, *J* 6.5 Hz, CH_2Br).

Anal. Calc. for $C_{48}H_{57}BrO_{25}$: C, 51.76; H, 5.16. Found: C, 51.93; H, 5.14.

Deacetylation of **13** with methanolic 0.02M sodium methoxide (20 mL) for 24 h, followed by neutralisation with Duolite C-26 (H^+) resin and concentration, gave a residue that was acetylated (acetic anhydride–pyridine, 1:1, 50 mL) at room temperature for 22 h. Co-concentration with toluene then gave a residue that was subjected to column (1 × 14 cm) chromatography (SiO_2; ethyl acetate–iso-octane, 2:1) to give **11** (80 mg, 43% from **12**).

*2-Bromoethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α- and -β-D-galactopyranosyl]-β-D-glucopyranoside* (**14** *and* **12**). — A solution of **1**[7] (2.7 g, 4 mmol) in dichloromethane (10 mL) at −78° was added to a solution of **8** (5.85 g, 10 mmol), silver trifluoromethanesulfonate (3.08 g, 12 mmol), and tetramethylurea (14.0 g, 12 mmol) in dichloromethane (15 mL). The mixture was treated as for the preparation of **9**. Chromatography (SiO_2; ethyl acetate–iso-octane, 2:1) gave **14** (1.59 g, 34%) and a later fraction containing **12** (0.52 g, 11%).

Compound **14** was amorphous and had $[\alpha]_D^{21}$ +122° (*c* 1.3, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^{1}H, δ 5.73 (t, 1 H, *J* 9 Hz, H-3), 5.51 (bd, 1 H, *J* ~2.5 Hz, H-4″), 5.19 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.16 (dd, 1 H, *J* 11 and 3.5 Hz, H-2′), 5.02 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.90 (d, 1 H, *J* 3.5 Hz, H-1″), 4.79 (d, 1 H, *J* 8 Hz, H-1), 4.74, 4.62 (ABq, 2 H, J_{AB} 12.5 Hz, PhCH_2), and 3.42 (bt, 2 H, *J* 7 Hz, CH_2Br); ^{13}C, δ 100.8 (d, *J* 164 Hz, C-1), 99.5 (d, *J* 172 Hz, C-1″), 97.0 (d, *J* 175 Hz, C-1′), and 29.6 (CH_2Br).

Compound **12** had m.p. 189–192° (from ethanol), $[\alpha]_D^{21}$ +65° (*c* 0.5, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^{1}H, δ 5.65 (t, 1 H, *J* 9.5 Hz, H-3), 5.58 (bd, 1 H, *J* 3 Hz, H-4″), 5.41 (dd, 1 H, *J* 9.5 and 8 Hz, H-2), 5.24 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.06 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.04 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.85 (d, *J* 3.5 Hz, H-1″), 4.78, 4.56 (ABq, 2 H, J_{AB} 12 Hz, PhCH_2), 4.73 (d, 1 H, *J* 8 Hz, H-1), 4.52 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.49 (d, 1 H, *J* 8 Hz, H-1′), 4.41 (bt, 1 H, *J* 7 Hz, H-5″), and 3.41 (bt, 2 H, *J* 7 Hz, CH_2Br); ^{13}C, δ 101.2, 100.7 (2 d, *J* 161 and 160 Hz, C-1 and C-1′), 99.4 (d, *J* 172 Hz, C-1″), and 29.6 (CH_2Br).

Anal. Calc. for $C_{55}H_{63}BrO_{25}$: C, 54.86; H, 5.27. Found: C, 54.84; H, 5.28.

*2-(2-Methoxycarbonylethylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**15**). — A mixture of **11** (3.09 g, 3 mmol), methyl 3-mercaptopropionate[12] (0.72 g, 6 mmol), cesium carbonate (1.2 g, 3.7 mmol), and *N,N*-dimethylformamide (15 mL) was stirred at room temperature for 1.5 h, and then partitioned between dichloromethane (100 mL) and water (25 mL). The organic phase was washed with water (20 mL), dried (Na_2SO_4), and concentrated. Chromatography (SiO_2; ethyl acetate–iso-octane, 2:1) gave **15** (2.81 g, 87%) as an amorphous solid, $[\alpha]_D^{21}$ +36° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.59 (dd, 1 H, *J* 3 and 1 Hz, H-4″), 5.40 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.21 (t, 1 H, *J* 9 Hz, H-3), 5.18 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.11 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.5 Hz, H-1″), 4.90 (dd, 1 H, *J* 9.5 and 8 Hz, H-2), 4.73 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.53 (d, 2 H, *J* 8 Hz, H-1 and H-1′), 3.71 (s, 3 H, MeO), 2.81 and 2.62 (2 bt, each 2 H, *J* ~7 Hz, SCH_2CH_2CO), and 2.71 (t, 2 H, *J* 7 Hz, CH_2S); ^{13}C, δ 100.9, 100.4 (2 d, *J* 164 Hz, C-1 and C-1′), 99.4 (d, *J* 174 Hz, C-1″), and 51.6 (MeO).

*2-(2-Methoxycarbonylethylthio)ethyl 4-*O*-(4-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**16**). — A solution of **15** (1.07 g, 1 mmol) in methanolic 3mM sodium methoxide (40 mL) was left at room temperature for 42 h. The reaction was monitored by t.l.c. (SiO_2; chloroform–methanol–water, 65:35:10, lower phase). Crystalline **16** (412 mg) was then collected, washed with methanol, and dried. The filtrate was neutralised with Duolite C-26 (H^+) resin, and concentrated; the residue crystallised from methanol to give a second crop of **16** (182 mg; total yield, 594 mg, 97%) as needles, m.p. 115–118°, $[\alpha]_D^{21}$ +54° (*c* 0.8, water). N.m.r. data: ^1H (Me_2SO-d_6, 50°, D_2O added, Me_4Si), δ 4.82, 4.29, 4.26 (3 d, each 1 H, *J* 3.5, 7, and 8 Hz, H-1″, H-1, H-1′), and 3.62 (s, 3 H, MeO); ^{13}C (D_2O, TSP), δ 178.0, 106.0, 104.8 (2 d, *J* 163 and 160 Hz, C-1, C-1′), 103.0 (d, *J* 170 Hz, C-1″), 81.3, 80.1, 78.2, 77.6, 77.1, 75.6, 74.9, 73.6, 73.5, 71.8, 71.7 (CH_2), 71.66, 71.3, 63.2 (CH_2), 63.1 (CH_2), 62.8 (CH_2), 55.0 (MeO), 36.9 (CH_2), 33.5 (CH_2), and 29.2 (CH_2).

Anal. Calc. for $C_{24}H_{42}O_{18}S$: C, 44.30; H, 6.50. Found: C, 43.41; H, 6.71.

*2-(Octadecylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**17**). — A mixture of **11** (650 mg, 0.63 mmol), octadecanethiol (360 mg, 1.26 mmol), cesium carbonate (250 mg, 0.77 mmol), and *N,N*-dimethylformamide (3 mL) was stirred at room temperature for 78 h. The reaction was monitored by t.l.c. (SiO_2; ethyl acetate–iso-octane, 3:1). The mixture was diluted with dichloromethane (50 mL), washed with water (2 ×25 mL), dried (Na_2SO_4), and concentrated. Column (5 × 18 cm) chromatography (SiO_2; toluene and toluene–ethyl acetate, 1:1) gave amorphous **17** (677 mg, 87%), $[\alpha]_D^{21}$ +33° (*c* 1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.59 (dd, 1 H, *J* 3 and 1 Hz, H-4″), 5.40 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.22 (t, *J* 9 Hz, H-3), 5.18 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.11 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.5 Hz, H-1″), 4.90 (dd,

1 H, *J* 9.5 and 8 Hz, H-2), 4.73 (dd, 1 H, *J* 11 and 2.5 Hz, H-3'), 4.52 (d, 2 H, *J* 8 Hz, H-1 and H-1'), 4.58–4.39 (5 H, *inter alia* H-1 and H-1'), 2.68, 2.51 (2 t, each 2 H, *J* 7 Hz, CH_2SCH_2), and 0.88 (t, 3 H, *J* 6.5 Hz, CH_3CH_2); ^{13}C, δ 101.1, 100.6 (2 d, *J* 164 and 162 Hz, C-1, C-1'), 99.6 (d, *J* 175 Hz, C-1''), and 14.1 (CH_3).

2-(Octadecylthio)ethyl 4-O-(4-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**18**). — A solution of **17** (1.0 g, 0.81 mmol) in methanolic 3mM sodium methoxide (100 mL) was left at room temperature for 24 h, neutralised with Duolite C-26 (H^+) resin, filtered, and concentrated, and a solution of the residue in water (300 mL) was lyophilised to give **18** (580 mg, 88%), $[\alpha]_D^{21}$ +42° (*c* 0.7, methyl sulfoxide). N.m.r. data: ^1H (Me_2SO-d_6, 50°, D_2O added, Me_4Si), δ 4.81 (d, 1 H, *J* 3.5 Hz, H-1''), 4.27, 4.24 (2 d, each 1 H, *J* 7 and 8 Hz, H-1 and H-1'), 2.67, 2.52 (2 t, each 2 H, *J* 7.5 Hz, CH_2SCH_2), and 0.86 (t, 3 H, *J* 6.5 Hz, Me); ^{13}C ($CDCl_3$–CD_3OD, 1:5), δ 105.2, 104.1 (2 d, *J* 162 Hz, C-1, C-1'), 102.6 (d, *J* 172 Hz, C-1''), 81.1, 80.0, 76.3 (3 C), 74.6 (2 C), 72.9, 72.5, 71.1, 70.9, 70.4, 70.3 (CH_2), 62.7 (CH_2), 62.0 (CH_2), 61.3 (CH_2), 33.2–29.7 (CH_2), 23.5 (CH_2), and 14.4 (CH_3).

Ethyl 2,3,6-tri-O-acetyl-4-O-[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**19**). — A solution of **11** (0.52 g, 0.5 mmol) in methanolic 7mM sodium methoxide (40 mL) was left at room temperature for 2 h and sodium hydroxide (95 mg) in water (2 mL) was then added. The mixture was hydrogenated (1 atm., Pd/C, 10%, 100 mg) for 100 min, filtered, neutralised with M hydrochloric acid, and concentrated. The residue was treated with acetic anhydride–pyridine (1:1, 100 mL) for 17 h at room temperature and the mixture was then co-concentrated with toluene. The residue was partitioned between dichloromethane (100 mL) and water (25 mL), and the organic phase was dried (Na_2SO_4) and concentrated. Column (5 × 18 cm) chromatography (SiO_2; ethyl acetate–iso-octane, 2:1) gave amorphous **19** (300 mg, 62%), $[\alpha]_D^{21}$ +41° (*c* 1, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^1H, δ 5.91 (d, 1 H, *J* 3 Hz, H-4''), 5.40 (dd, 1 H, *J* 11 and 3 Hz, H-3''), 5.21 (t, 1 H, *J* 9 Hz, H-3), 5.18 (dd, 1 H, *J* 11 and 3.5 Hz, H-2''), 5.11 (dd, 1 H, *J* 11 and 8 Hz, H-2'), 4.99 (d, 1 H, *J* 3.5 Hz, H-1''), 4.88 (dd, 1 H, *J* 9 and 8 Hz, H-2), 4.73 (dd, 1 H, *J* 11 and 2.5 Hz, H-3'), 4.56–4.38 (5 H, *inter alia* H-1 and H-1'), 4.02 (d, 1 H, *J* 2.5 Hz, H-4'), and 1.19 (t, 3 H, *J* 7 Hz, CH_3CH_2); ^{13}C, δ 100.9, 100.1 (2 d, *J* 160 and 164 Hz, C-1, C-1'), 99.4 (d, *J* 172 Hz, C-1''), and 14.9.

Ethyl 4-O-(4-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**20**). — A solution of **19** (440 mg, 0.46 mmol) in methanolic 5mM sodium methoxide (40 mL) was left at room temperature for 18 h, neutralised with Duolite C-26 (H^+) resin, and concentrated, and a solution of the residue in water was lyophilised to give **20** (245 mg, 100%), $[\alpha]_D^{21}$ +71° (*c* 0.5, water). N.m.r. data: ^1H (Me_2SO-d_6, 50°, D_2O added, Me_4Si), δ 4.81 (d, 1 H, *J* 3.5 Hz, H-1''), 4.28, 4.20 (d, each 1 H, *J* 7 and 8 Hz, H-1 and H-1'), and 1.14 (t, 3 H, *J* 7 Hz, CH_3CH_2); ^{13}C (D_2O, TSP), δ 106.1, 104.5, 103.2 (C-1,1',1''), 81.5, 80.2, 78.3, 77.7, 77.3, 75.8, 75.0, 73.7, 73.6, 72.0, 71.8, 71.4, 69.1 (CH_2), 63.3 (CH_2), 63.2 (CH_2), 62.9 (CH_2), and 17.1.

The results of sugar[15] and methylation[16] analysis were in agreement with the proposed structure.

Glycoprotein synthesis (cf. *ref. 1*). — (*a*) A solution of **16** (44 mg, 0.07 mmol) and hydrazine hydrate (85%, 0.25 mL) in ethanol (2 mL) was left overnight and then concentrated, and a solution of the residue in water was lyophilised. The resulting hydrazide was dissolved in methyl sulfoxide (1 mL), and then 4M hydrogen chloride in 1,4-dioxane (105 μL) and a solution of *tert*-butyl nitrite (18 μL, 0.15 mmol) in methyl sulfoxide (0.1 mL) were added. The mixture was stirred at room temperature for 30 min and a solution of sulfamic acid (10 mg, 0.11 mmol) in methyl sulfoxide (0.1 mL) was added. After 15 min, the mixture was added dropwise, with stirring, to a solution of bovine serum albumin (BSA; 65 mg, 1 μmol) in sodium tetraborate–potassium hydrogencarbonate buffer (2.5 mL, 0.08M $Na_2B_4O_7$ and 0.35M $KHCO_3$). The pH was maintained at 9.0–9.3 by additions of M sodium hydroxide. The mixture was stirred for 16 h at room temperature, and then dialysed (H_2O, 72 h) and lyophilised to give glycoprotein **21**. The degree of binding (number of hapten molecules per molecule of protein) was 14, as determined by the phenol–sulfuric acid method[18]. With 0.35 mmol of **16** and 1 μmol of BSA, the degree of binding was 43.

(*b*) The procedure in (*a*) was followed, using **16** (114 mg, 0.18 mmol) and key-hole limpet haemocyanin (KLH, 30 mg, 0.034 μmol), to give glycoprotein **22** with a degree of binding of 480.

*2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-[6,6-$^2H_2$]galactopyranosyl)-α-*D*-[6,6-$^2H_2$]galactopyranosyl bromide* (**23**). — Compound **23** was prepared from 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-[6,6-$^2H_2$]galactopyranosyl)-α-D-[6,6-$^2H_2$]galactopyranose {m.p. 153–155°, $[\alpha]_D^{21}$ +138° (*c* 0.9, chloroform), prepared from "digalacturonic acid" as described[5] for the protio compound except for the use of sodium borodeuteride in the reduction step; n.m.r. data were as expected} as described for **1**, and used directly in the next step.

*2-Bromoethyl 2,3,6-tri-*O*-benzyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-[6,6-$^2H_2$]galactopyranosyl)-β-*D*-[6,6-$^2H_2$]galactopyranosyl}-β-*D*-glucopyranoside* (**24**). — Compound **24**, prepared from **23** and **6** as described for **9**, had m.p. 155–157°, $[\alpha]_D^{21}$ +58° (*c* 0.7, chloroform). The n.m.r. data were as expected.

*2-Bromoethyl 4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-[6,6-$^2H_2$]galactopyranosyl)-β-*D*-[6,6-$^2H_2$]galactopyranosyl}-β-*D*-glucopyranoside* (**25**). — Compound **25**, prepared from **24** as described for **10**, had m.p. 177–179°, $[\alpha]_D^{21}$ +78° (*c* 0.5, chloroform). The n.m.r. data were as expected.

*2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-[6,6-$^2H_2$]galactopyranosyl)-β-*D*-[6,6-$^2H_2$]galactopyranosyl}-β-*D*-glucopyranoside* (**26**). — Compound **26**, prepared from **25** as described for **11**, had m.p. 181–183°, $[\alpha]_D^{21}$ +44° (*c* 0.9, chloroform). The n.m.r. data were as expected.

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