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# SYNTHESIS OF SPACER-ARM, LIPID, AND ETHYL GLYCOSIDES OF THE TERMINAL TRISACCHARIDE [α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-GlcNAc] PORTION OF THE BLOOD-GROUP $P_1$ ANTIGEN: PREPARATION OF NEOGLYCOPROTEINS*

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ABSTRACT

The title compounds were prepared *via* the acetylated 2-bromoethyl β-glycoside (**5**) of α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-GlcNAc by displacement of bromide ion with methyl 3-mercaptopropionate, octadecanethiol, and hydrogen, respectively. Silver triflate-promoted glycosylation of 2-bromoethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-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 **5**. The spacer-arm glycoside derived from methyl 3-mercaptopropionate was coupled to bovine serum albumin and key-hole limpet haemocyanin to give neoglycoproteins.

## INTRODUCTION

Carbohydrates of the P blood-group system[2] have been suggested to be specific receptors for uropathogenic *E. coli* bacteria[3a,b] and recently also for *Shigella dysenteriae*[3c]. Studies of the synthesis of these and related compounds have led to the preparation of glycosides of the oligosaccharides α-D-Gal-(1→4)-β-D-Gal[4,8a–c], α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc[4e,5], α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-GlcNAc[6], and β-D-GalNAc-(1→3)-α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc[7].

We now report a synthesis of the terminal trisaccharide portion [α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-GlcNAc] of the $P_1$ antigen, in the form of its 2-bromoethyl glycoside (**5**), and its use to give spacer-arm, lipid, and ethyl glycosides[1,5b,8] and also neoglycoproteins. The synthesis is based on the acetobromo sugar **1**[8b], which is readily available from pectin *via* an enzymic hydrolysis–borohydride reduction sequence[4g].

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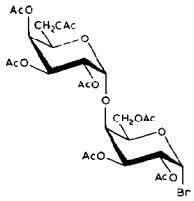

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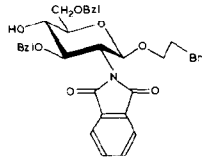

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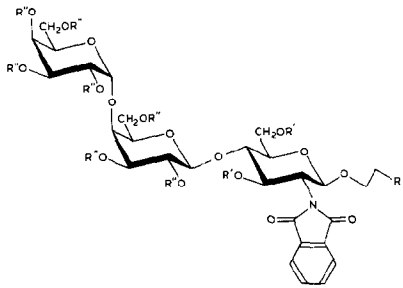
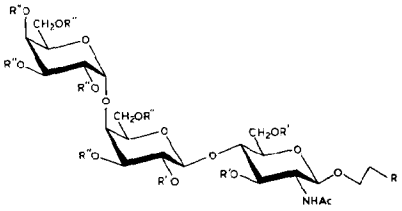


3 R = Br, R' = Bzl, R'' = Ac
4 R = Br, R' = H, R'' = Ac
5 R = Br, R = R'' = Ac
6 R = S⌒COOMe, R' = R'' = Ac
7 R = S⌒⌒⌒⌒⌒⌒⌒⌒⌒⌒, R' = R'' = Ac



8 R = S⌒COOMe, R' = R'' = Ac
9 R = S⌒COOMe, R' = R'' = H
10 R = S⌒⌒⌒⌒⌒⌒⌒⌒⌒⌒, R' = R'' = Ac
11 R = S⌒⌒⌒⌒⌒⌒⌒⌒⌒⌒, R' = R'' = H
12 R = H, R' = Bzl, R'' = Ac
13 R = R' = H, R'' = Ac
14 R = H, R' = R'' = Ac
15 R = R' = R'' = H
16 R = S⌒CONH—BSA, R' = R'' = H
17 R = S⌒CONH—KLH, R' = R'' = H

## RESULTS AND DISCUSSION

Glycosylation of 2-bromoethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**)[8d] with 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl bromide (**1**)[8b], using silver triflate and tetramethylurea in dichloromethane, gave the trisaccharide glycoside **3** (41%). The benzyl groups of **3** were removed by hydrogenolysis in acetic acid[8], to give **4** (92%), acetylation of which gave the key intermediate 2-bromoethyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-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 (**5**, 72%).

Reaction of **5** with methyl 3-mercaptopropionate or octadecanethiol in *N,N*-dimethylformamide, using cesium carbonate as promoter[1,5b,8], gave the spacer-arm glycoside **6** (94%) or the neoglycolipid **7** (91%), respectively. Hydrogenolysis of **5** under basic conditions[1,5b,8], followed by *N*-deprotection and reacetylation, gave the ethyl glycoside **14** (28%). Hydrogenolysis of **5** under acid conditions cleaved the benzyl groups but left the 2-bromoethyl group intact (*e.g.*, **3**→**4**), whereas, under basic conditions, the carbon–bromine bond was cleaved but the benzyl groups were not affected (*e.g.*, **3**→**12**).

*N*-Deprotection and reacetylation of **6** and **7** gave **8** (50%) and **10** (52%), respectively. O-Deacetylation of **8**, **10**, and **14** gave spacer-arm glycoside **9** (90%), neoglycolipid **11** (87%), and ethyl glycoside **15** (60%), respectively. The latter was also prepared from **3** *via* **12** and **13**.

The spacer-arm glycoside **9** was coupled[1,5b,8d] to the proteins bovine serum albumin (BSA) and key-hole limpet haemocyanin (KLH), to give the neoglycoproteins **16** and **17** with degrees of binding[8a] of 14 and 250, respectively.

## EXPERIMENTAL

The general methods were as reported[8c]; $Me_4Si$ or sodium 3-(trimethylsilyl)-propionate-$d_4$ (TSP) was used as n.m.r. internal reference.

*2-Bromoethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-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* (**3**). — To a solution of **2**[8d] (4.56 g, 7.65 mmol), tetramethylurea (1.74 g, 15 mmol), and silver trifluoromethanesulfonate (3.5 g, 13.62 mmol) in dichloromethane (30 mL) at 0° was added, with stirring, a solution of **1** (8.25 g, 11.8 mmol) in dichloromethane (13 mL). The mixture was protected from light, stirred at 0° for 2 h and at room temperature for 22 h, and then filtered through Celite. The filtrate was diluted with dichloromethane, washed with M hydrochloric acid and with aqueous sodium hydrogencarbonate, dried ($Na_2SO_4$), and concentrated. The residue (12 g) was subjected to chromatography ($SiO_2$; column, 5 × 18 cm; toluene–ether, 1:2) to give **3** (3.8 g, 41%). Crystallisation from methanol gave material (2.81 g) with m.p. 98–101°, $[\alpha]_D^{21}$ +65° (*c* 0.7, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, δ, *inter alia*, 5.54 (bd, 1 H, *J* 3 Hz, H-4″), 5.26 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.17–5.15 (3 H, H-1,2′,2″), 4.94 (d, 1 H, *J* 3 Hz, H-1″), 4.90 (d, 1 H, *J* 12.5 Hz, PhC$H_2$), 4.80 (d, 1 H, *J* 12 Hz, PhC$H_2$), 4.67 (dd, 1 H, *J* 10.5 and 3 Hz, H-3′), 4.64 (d, 1 H, *J* 8 Hz, H-1′), 4.52 (d, 1 H, *J* 12 Hz, PhC$H_2$), 4.44 (d, 1 H, *J* 12.5 Hz, PhC$H_2$), and 3.29 (bt, 2 H, *J* ~6 Hz, $CH_2Br$); $^{13}C$, δ 100.5 (d, *J* 164 Hz), 99.3 (d, *J* 172 Hz, C-1″), 98.4 (d, *J* 163 Hz), 78.3, 77.1, 76.9, 74.7, 74.5 ($CH_2$), 73.5 ($CH_2$), 72.7, 71.6, 69.2, (2 C, $CH_2$), 68.2, 67.7, 67.4 ($CH_2$), 67.1, 66.9, 61.2 ($CH_2$), 60.2 ($CH_2$), 55.4 (C-2), and 29.9 ($CH_2Br$).

*Anal.* Calc. for $C_{56}H_{65}BrNO_{24}$: C, 55.31; H, 5.39. Found: C, 55.34; H, 5.67.

*2-Bromoethyl 2-deoxy-2-phthalimido-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* (**4**). — Compound **3** (5.0 g, 4.1 mmol) was hydrogenolysed (10% Pd/C, 1.0 g, 2 atm.) in acetic acid (50 mL) for 17 h. The mixture was then filtered, concentrated, and dried (oil pump), to give **4** (3.9 g, 92%) as a colourless, amorphous solid, $[\alpha]_D^{21}$ +49° (*c* 1.5, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, δ, *inter alia*, 5.58 (dd, 1 H, *J* 3 and 1 Hz, H-4″), 5.39 (d, 1 H, *J* 8.5 Hz, H-1), 5.34 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.25 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 5.19 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 4.93 (d, 1 H, *J* 3.5 Hz, H-1″), 4.79 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.68 (d, 1 H, *J* 8 Hz, H-1′), and 3.34 (t, 2 H, *J* 6 Hz, $CH_2Br$); $^{13}C$, δ 101.9 (d, *J* ~167 Hz, C-1 or C-1′), 99.8 (d, *J* 174 Hz, C-1″), 98.4 (d, *J* ~163 Hz, C-1 or C-1′), 82.2, 77.3, 74.2, 72.7, 72.5, 69.7, 69.6, 68.43, 68.38, 67.7, 67.4, 67.2, 62.5 ($CH_2$), 60.8 ($CH_2$), 60.4 ($CH_2$), 55.6 (C-2), and 29.9 ($CH_2Br$).

*2-Bromoethyl 3,6-di-O-acetyl-2-deoxy-2-phthalimido-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* (**5**). — Compound **4** (3.76 g, 3.6 mmol) was acetylated (acetic anhydride–pyridine, 1:1; 50 mL) at room temperature for 18 h. Crystallisation of the product from ethanol gave **5** (3.64 g, 90%), m.p. 227–229°, $[\alpha]_D^{21}$ +50° (*c* 0.8, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, $\delta$ 5.76 (dd, 1 H, *J* 10.5 and 8 Hz, H-3), 5.59 (bdd, 1 H, *J* 3 and 1 Hz, H-4″), 5.44 (d, 1 H, *J* 8.5 Hz, H-1), 5.38 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.17 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.13 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.98 (d, 1 H, *J* 3.5 Hz, H-1″), 4.74 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.58 (d, 1 H, *J* 8 Hz, H-1′), 4.64–3.70 (15 H, *inter alia* H-1′), and 3.34 (bt, 2 H, *J* ~6 Hz, $CH_2Br$); $^{13}C$, $\delta$ 101.0 (d, *J* 160 Hz, C-1′), 99.6 (d, *J* 171 Hz, C-1″), 98.1 (d, *J* 166 Hz, C-1), 76.9, 76.8, 72.7, 72.6, 71.6, 71.4, 69.8 ($CH_2CH_2Br$), 69.0, 68.8, 67.9, 67.1 (2 C), 62.2 ($CH_2$), 61.1 ($CH_2$), 60.2 ($CH_2$), 54.7 (C-2), and 29.8 ($CH_2Br$).

*Anal.* Calc. for $C_{46}H_{56}BrNO_{26}$: C, 49.38; H, 5.05. Found: C, 50.16; H, 5.15.

*2-(2-Methoxycarbonylethylthio)ethyl 3,6-di-O-acetyl-2-deoxy-2-phthalimido-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* (**6**). — A mixture of **5** (3.91 g, 3.5 mmol), methyl 3-mercaptopropionate[9] (0.84 g, 7.0 mmol), cesium carbonate (1.30 g, 4.0 mmol), and *N,N*-dimethylformamide (15 mL) was stirred at room temperature for 2.5 h. The reaction was monitored by t.l.c. (ethyl acetate–iso-octane, 3:1). Dichloromethane (100 mL) was added and the mixture was washed with water (50 mL). The aqueous phase was extracted with dichloromethane (25 mL), and the combined organic phases were washed with water (20 mL), dried ($Na_2SO_4$), and concentrated. The residue was crystallised from methanol to give **6** (3.80 g, 94%), m.p. 151–153° (phase transition starting at 118°), $[\alpha]_D^{21}$ +52° (*c* 0.7, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, $\delta$, *inter alia*, 5.74 (m, 1 H, H-3), 5.58 (dd, 1 H, *J* 3 and 1 Hz, H-4″), 5.42 (d, 1 H, *J* 8.5 Hz, H-1), 5.38 (dd, 1 H, *J* 11 and 3.5 Hz, H-3″), 5.17 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.13 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.98 (d, 1 H, *J* 3.5 Hz, H-1″), 4.74 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.59 (d, 1 H, *J* 8 Hz, H-1′), 3.67 (s, 3 H, $CH_3O$), 2.70–2.56 (4 H, $CH_2S$), and 2.42 (bt, 2 H, *J* ~7 Hz, $CH_2S$); $^{13}C$, $\delta$ 101.0 (C-1′ or C-1), 99.6 (C-1″), 98.0 (C-1 or C-1′), 77.0, 76.8, 72.7, 72.6, 71.6, 71.5, 69.5, ($OCH_2CH_2$), 69.0, 68.8, 67.9, 67.0, 62.3 ($CH_2$), 61.1 ($CH_2$), 60.2 ($CH_2$), 54.8 (C-2), 51.8 ($OCH_3$), 34.5 ($CH_2$), 31.3 ($CH_2$), and 27.1 ($CH_2$).

*Anal.* Calc. for $C_{50}H_{63}NO_{28}S$: C, 51.85; H, 5.48. Found: C, 51.69; H, 5.54.

*2-(Octadecylthio)ethyl 3,6-di-O-acetyl-2-deoxy-2-phthalimido-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* (**7**). — A mixture of **5** (1.68 g, 1.5 mmol), octadecanethiol (0.81 g, 2.8 mmol), cesium carbonate (0.50 g, 1.53 mmol), and *N,N*-dimethylformamide (11 mL) was stirred at room temperature for 7.5 h and worked-up as described for **6**. The resulting residue was subjected to chromatography ($SiO_2$; column, 5 × 18 cm; ethyl acetate–iso-octane, 2:1 followed by 4:1, and finally ethyl acetate), to give **7** (1.80 g, 91%). Crystallisation from methanol gave material with

m.p. 150–152°, $[\alpha]_D^{21}$ +47° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, $\delta$ 5.75 (m, 1 H, H-3), 5.58 (bd, 1 H, *J* ~3 Hz, H-4″), 5.42 (d, 1 H, *J* 8 Hz, H-1), 5.38 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.16 (dd, 1 H, *J* 11 and 3.5 Hz, H-3″), 5.13 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.98 (d, 1 H, *J* 3.5 Hz, H-1″), 4.74 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.63–4.34 (4 H, *inter alia* H-1′), 4.58 (d, 1 H, *J* 8 Hz, H-1′), 2.55 (t, 2 H, *J* 7 Hz, $CH_2S$), 2.31 (t, 2 H, *J* 7 Hz, $CH_2S$), 1.40–1.15 (bs), and 0.88 (t, 3 H, *J* 6.5 Hz, $CH_3$); $^{13}C$, $\delta$ 101.0 (d, *J* 160 Hz, C-1′ or C-1), 99.6 (d, *J* 173 Hz, C-1″), 98.1 (d, *J* 160 Hz, C-1 or C-1′), 77.8, 72.7, 72.6, 71.62, 71.56, 69.7 ($OCH_2CH_2$), 69.0, 68.8, 67.9, 67.1 (2 C), 62.4 ($CH_2$), 61.1 ($CH_2$), 60.2 ($CH_2$), and 54.2 (C-2).

*Anal.* Calc. for $C_{64}H_{93}O_{26}S$: C, 58.03; H, 7.08. Found: C, 58.18; H, 7.18.

*2-(2-Methoxycarbonylethylthio)ethyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-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* (**8**). — Compound **6** (700 mg, 0.6 mmol) was treated as described below for **7** in the preparation of **10**. The resulting, crude **8** was subjected to chromatography ($SiO_2$; ethyl acetate–iso-octane, 4:1, and ethyl acetate) to give pure material (387 mg, 60%), $[\alpha]_D$ +35° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, $\delta$, *inter alia*, 5.86 (d, 1 H, *J* 9 Hz, NH), 5.58 (dd, 1 H, *J* 3 and <1 Hz, H-4″), 5.39 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.19 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.12 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 5.11 (t, 1 H, *J* 8.5 Hz, H-3), 5.00 (d, 1 H, *J* 3.5 Hz, H-1″), 4.76 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.60–4.38 (5 H, *inter alia* H-1,1′), 3.71 (s, $CH_3O$), 2.82 (bt, 2 H, *J* 7 Hz, $SCH_2CH_2CO$), 2.72 (t, 2 H, *J* 6.5 Hz, $OCH_2CH_2$), and 2.62 (bt, 2 H, *J* 7 Hz, $SCH_2CH_2CO$); $^{13}C$, $\delta$ 101.0 (2 C, C-1,1′), 99.6 (C-1″), 77.0, 76.1, 72.9, 72.6, 71.9, 69.2 ($OCH_2CH_2$), 69.0, 68.7, 67.9, 67.1 (2 C), 62.4 ($CH_2$), 61.4 ($CH_2$), 60.4 ($CH_2$), 53.4 (C-2), 51.9 ($CH_3O$), 34.6 ($CH_2$), 31.5 ($CH_2$), and 27.3 ($CH_2$).

*2-(2-Methoxycarbonylethylthio)ethyl 2-acetamido-2-deoxy-4-*O*-(4-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**9**). — Compound **8** (350 mg, 0.3 mmol) was dissolved in methanol (10 mL) by warming. The solution was rapidly cooled, methanolic sodium methoxide (0.2M, 0.2 mL) was added, and the mixture was stirred at room temperature for 18 h, neutralised with Duolite C-26 ($H^+$) resin, filtered, and concentrated. The residue was dissolved in methanol (7 mL), and methanolic hydrazine hydrate (0.1M, 3 mL) was added. The mixture was heated under reflux for 33 h and more methanolic hydrazine hydrate (2 × 3 mL) was added (after 24 and 30 h). The reaction was monitored by t.l.c. (chloroform–methanol–water, 65:35:10, lower phase). The solvent was removed and the residue was treated with a mixture of acetic anhydride (2.5 mL), ethanol (12 mL), and water (4 mL) at room temperature for 1 h. The ethanol was removed, and the residue was partitioned between water (20 mL) and dichloromethane (5 mL). The aqueous phase was washed with dichloromethane (3 × 5 mL) and concentrated. The residue was eluted from a column (3 × 57 cm) of Sephadex G-10 with water, to give **9** (157 mg, 75%), $[\alpha]_D^{22}$ +34° (*c* 0.7, water). N.m.r. data: $^1H$ [$(CD_3)_2SO$ + $D_2O$, 50°, $Me_4Si$], $\delta$, *inter alia*, 4.80 (d, 1 H, *J* 3.5 Hz, H-1″), 4.40 (d, 1 H, *J* 8 Hz, H-1 or H-1′), 4.28 (d, 1 H, *J* 7 Hz, H-1 or H-1′), 4.08 (dt, 1 H, *J* 6 and 1 Hz), 3.61

(s, $CH_3O$), 2.75–2.70 and 2.61–2.54 (m, each 2 H, $SCH_2CH_2CO$), 2.64 (t, 2 H, *J* 7 Hz, $OCH_2CH_2S$), and 1.80 (s, 3 H, AcN); $^{13}C$ ($D_2O$, TSP), δ 106.1, 103.8, 103.1, 81.6, 80.1, 78.2, 77.7, 75.3, 75.0, 73.7, 73.6, 72.1 ($CH_2$), 72.0, 71.8, 71.4, 63.3 ($CH_2$), 63.2 ($CH_2$), 62.9 ($CH_2$), 58.0 (C-2), 55.2 ($CH_3O$), 37.1 ($CH_2$), 33.8 ($CH_2$), 29.4 ($CH_2$), and 23.2.

*2-(Octadecylthio)ethyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-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**). — Compound **7** (1.46 g, 1.1 mmol) was dissolved in methanol (75 mL) and tetrahydrofuran (10 mL) by warming. The clear solution was rapidly cooled to ~40° and methanolic sodium methoxide (0.2M, 5 mL) was added. The mixture was stirred at room temperature for 5 h and deacetylation was then complete (t.l.c.; chloroform–methanol–water, 65:35:10, lower phase). The solution was neutralised with Duolite C-26 ($H^+$) resin, filtered, and concentrated. The residue was dissolved in boiling methanol (50 mL), hydrazine hydrate (0.5 g, 10 mmol) was added, and boiling under reflux was continued for 17.5 h. The solvent was then removed and the residue was acetylated conventionally with pyridine and acetic anhydride. Chromatography ($SiO_2$; column, 5 × 18 cm; ethyl acetate–iso-octane gradient: 2:1→4:1→ethyl acetate) of the product gave amorphous **10** (0.72 g, 52%), $[\alpha]_D^{21}$ +32° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, δ 5.67 (d, 1 H, *J* 9.5 Hz, NH), 5.58 (bd, 1 H, *J* 3 Hz, H-4″), 5.39 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.23–5.01 (3 H, H-2′,2″,3), 4.99 (d, 1 H, *J* 3.5 Hz, H-1″), 4.75 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.58–4.37 (5 H, *inter alia* H-1 and H-1′), 4.54 and 4.49 (2 d, each 1 H, *J* 8 Hz each, H-1,1′), 2.69 (t, 2 H, *J* 7 Hz, $OCH_2CH_2S$). 2.51 (t, 2 H, *J* 7 Hz, $SCH_2$), and 0.88 (t, 3 H, *J* 6.5 Hz, $CH_3CH_2$): $^{13}C$, δ 101.1 and 101.0 (d, *J* each 160 Hz, C-1,1′), 99.6 (d, *J* 172 Hz, C-1″), 76.9, 75.9, 72.7, 72.6 (2 C), 71.8, 69.1 ($OCH_2CH_2$), 68.9, 68.7, 67.8, 67.1, 67.0, 62.4 ($CH_2$), 61.3 ($CH_2$), 60.2 ($CH_2$), 53.3 (C-2), 23.3 ($CH_3CONH$), 22.7 ($CH_2$), and 14.1.

*2-(Octadecylthio)ethyl 2-acetamido-2-deoxy-4-*O*-(4-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**11**). — To a solution of **10** (560 mg, 0.45 mmol) in methanol (25 mL) was added methanolic sodium methoxide (0.2M, 1 mL), and the mixture was stirred at room temperature for 14.5 h. The amorphous precipitate was dissolved by adding methanol (50 mL) and warming to ~40°. The solution was cooled to room temperature, neutralised with Duolite C-26 ($H^+$) resin, filtered, and concentrated. A suspension of the residue in water (100 mL) was lyophilised to give amorphous **11** (340 mg, 87%), $[\alpha]_D^{21}$ +35° (*c* 0.6, methyl sulfoxide). N.m.r. data: $^1H$ [$(CD_3)_2SO$ + $D_2O$, 50°, $Me_4Si$], δ, *inter alia*, 4.81 (d, 1 H, *J* 3.5 Hz, H-1″), 4.40 and 4.29 (2 d, each 1 H, *J* 7.5 Hz, H-1,1′), 2.60 (t, 2 H, *J* 7 Hz, $CH_2S$), 2.50 (t, 2 H, *J* 7 Hz, $SCH_2$), 1.81 (s, 3 H, AcN), and 0.86 (t, 3 H, *J* 6.5 Hz, $CH_3CH_2$); $^{13}C$ [$(CD_3)_2SO$, $Me_4Si$], δ 168.7, 103.8 (d, *J* 162 Hz, C-1 or C-1′), 100.7 and 100.5 (2 d, *J* ~162 and 172 Hz, C-1″ and C-1 or C-1′), 81.2, 77.1, 75.0 (2 C), 72.8, 72.1, 71.0, 70.8, 69.1, 68.7, 68.5, 68.5 ($OCH_2CH_2$), 60.3 (2 $CH_2$), 59.3 ($CH_2$), 54.5, 31.4–28.1 ($CH_2$), 22.9, 22.1 ($CH_2$), and 13.9 ($CH_3CH_2$).

*Ethyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-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* (**12**). — Compound **3** (310 mg, 0.26 mmol) was hydrogenolysed (10% Pd/C, 0.3 g, 39 p.s.i.) in methanolic sodium methoxide (0.02M, 33 mL) at room temperature for 22 h. The solution was then filtered, neutralised with Duolite C-26 ($H^+$) resin (the pH was adjusted to 7.5 with solid sodium hydrogencarbonate), filtered, and concentrated (<1 Torr). Methanol (25 mL) and hydrazine hydrate (0.8 g, 16 mmol) were added to the residue, and the mixture was boiled under reflux for 3 h. T.l.c. (chloroform–methanol–water 65:35:10, lower phase) then showed reaction to be complete. The solvent was removed and the residue was acetylated conventionally (acetic anhydride–pyridine, 1:1). Chromatography ($SiO_2$; column, 1.5 × 38 cm; ethyl acetate–iso-octane, 3:1, and finally ethyl acetate) of the product gave **12** (151 mg, 55%). Recrystallisation from 2-propanol–iso-octane gave material with m.p. 97–100°, $[\alpha]_D^{22}$ +40° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, δ 5.93 (d, 1 H, *J* 8 Hz, NH), 5.55 (dd, 1 H, *J* 3 and 1 Hz, H-4″), 5.32 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.19 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.14 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 4.99 (d, 1 H, *J* 3.5 Hz,. H-1″), 4.58–4.44 (3 H, *inter alia* H-1 or H-1′ and PhC*H*), 4.54 (d, 1 H, *J* 8 Hz, H-1 or H-1′), 4.47 (d, 1 H, *J* 12 Hz, PhCH), 4.31 (dd, 1 H, *J* 11 and 6.5 Hz), and 1.18 (t, 3 H, *J* 7 Hz, $CH_3CH_2$); $^{13}C$, δ 100.0 (d, *J* 165 Hz, C-1 or C-1′), 99.7 (d, *J* 163 Hz, C-1 or C-1′), 99.3 (d, *J* 172 Hz, C-1″), 77.2, 76.8, 75.9, 74.3, 73.5 ($CH_2$), 73.1 ($CH_2$), 72.4, 71.9, 69.0, 68.8 ($CH_2$), 68.4, 67.7, 67.2, 67.0, 64.2 ($CH_2$), 61.4 ($CH_2$), 60.4 ($CH_2$), 53.1 (C-2), 23.3 ($CH_3CONH$), and 15.0 ($CH_3CH_2$).

*Anal.* Calc. for $C_{50}H_{65}NO_{23}$: C, 57.30; H, 6.25. Found: C, 57.49; H, 6.30.

*Ethyl 2-acetamido-2-deoxy-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**). — Compound **12** (475 mg, 0.45 mmol) was hydrogenolysed (10% Pd/C, 200 mg, 1 atm.) in acetic acid (10 mL) for 5.5 h at room temperature. The mixture was filtered and the solvent was removed. Recrystallisation of the residue from ethanol gave **13** (291 mg, 74%), m.p. 260–262°, $[\alpha]_D^{22}$ +67° (*c* 0.9, chloroform). N.m.r. data: $^1H$ ($CDCl_3$ + $D_2O$, $Me_4Si$), δ 5.59 (dd, 1 H, *J* 3 and 1 Hz, H-4″), 5.37 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.23 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 5.21 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 4.96 (d, 1 H, *J* 3.5 Hz, H-1″), 4.80 (dd, 1 H, *J* 11 and 3 Hz, H-3′), 4.75 (d, 1 H, *J* 8.5 Hz, H-1), 4.68 (d, 1 H, *J* 8 Hz, H-1′), 4.51 (bt, 1 H, *J* 7 Hz, H-5″), 4.44 (dd, 1 H, *J* 11 and 8.5 Hz), and 1.21 (t, 3 H, *J* 7 Hz, $CH_3CH_2$); $^{13}C$ ($CDCl_3$, $Me_4Si$), δ 101.8 (C-1 or C-1′), 100.2 (C-1 or C-1′), 99.8 (C-1″), 81.8, 77.3, 73.9, 72.7, 72.6, 71.9, 68.5, 68.4, 67.8, 67.4, 67.3, 65.3, 62.5, 61.0, 60.4, 56.6 (C-2), and 15.1.

*Anal.* Calc. for $C_{36}H_{53}NO_{23}$: C, 49.82; H, 6.16. Found: C, 50.57; H, 6.14.

*Ethyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-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* (**14**). — Compound **5** (0.65 g, 0.58 mmol) was hydrogenolysed (10% Pd/C, 0.31 g, 40 p.s.i.) in methanolic sodium methoxide (0.02M, 56 mL) at room temperature for 20 h. The solution was filtered, neutralised with Duolite C-26 ($H^+$)

resin, filtered, and concentrated. A solution of the residue in methanol (20 mL) and hydrazine hydrate (1.7 g, 34 mmol) was boiled under reflux for 23 h and then concentrated, and the residue was acetylated conventionally (acetic anhydride–pyridine, 1:1). Chromatography of the product ($SiO_2$; column, 1.5 × 46 cm; ethyl acetate) gave **14** (158 mg, 28%), m.p. 130–133°, $[\alpha]_D^{22}$ +41° (*c* 0.7, chloroform). N.m.r. data ($CDCl_3$, $Me_4Si$): $^1H$, $\delta$ 5.73 (d, 1 H, *J* 9.5 Hz, NH), 5.58 (bd, 1 H, *J* ~3 Hz, H-4″), 5.39 (dd, 1 H, *J* 11 and 3 Hz, H-3″), 5.19 (dd, 1 H, *J* 11 and 3.5 Hz, H-2″), 5.12 (dd, 1 H, *J* 11 and 8 Hz, H-2′), 5.10 (dd, 1 H, *J* 9.5 and 8 Hz, H-3), 5.00 (d, 1 H, *J* 3.5 Hz, H-1″), 4.76 (dd, 1 H, *J* 11 and 2.5 Hz, H-3′), 4.58–4.37 (5 H, *inter alia* H-1, H-1′), 4.55 (d, 1 H, *J* 8 Hz, H-1 or H-1′), 4.46 (d, 1 H, *J* 7.5 Hz, H-1 or H-1′), and 1.19 (t, 3 H, *J* 7 Hz, $CH_3CH_2$); $^{13}C$, $\delta$ 101.1 (d, *J* 164 Hz, C-1 or C-1′), 100.7 (d, *J* 160 Hz, C-1 or C-1′), 99.6 (d, *J* 174 Hz, C-1″), 77.0, 76.1, 72.8, 72.7, 72.5, 71.9, 69.0, 68.7, 67.9, 67.1 (2 C), 65.0 ($CH_2$), 62.5 ($CH_2$), 61.4 ($CH_2$), 60.3 ($CH_2$), 53.4 (C-2), and 15.1 ($CH_3CH_2$).

*Anal.* Calc. for $C_{40}H_{57}NO_{25}$: C, 50.47; H, 6.04. Found: C, 50.31; H, 6.02.

*Ethyl 2-acetamido-2-deoxy-4-O-(4-O-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**15**). — (*a*) Compound **14** (58 mg, 0.06 mmol) was *O*-deacetylated with methanolic sodium methoxide (0.036M, 5.5 mL) at room temperature for 21 h. The mixture was neutralised with Duolite C-26 ($H^+$) resin, filtered, and concentrated, and a solution of the residue in water was lyophilised to give **15** (21 mg, 60%), $[\alpha]_D^{22}$ +38° (*c* 0.6, water). N.m.r. data: $^1H$ [$(CD_3)_2SO$ + $D_2O$, 50°, $Me_4Si$], $\delta$, *inter alia*, 4.80 (d, 1 H, *J* 3.5 Hz, H-1″), 4.37 (d, 1 H, *J* 8 Hz, H-1 or H-1′), 4.29 (d, 1 H, *J* 7.5 Hz, H-1 or H-1′), 4.07 (bt, 1 H, *J* ~6.5 Hz), 1.81 (s, 3 H, AcN), and 1.08 (t, 3 H, *J* 7 Hz, $CH_3CH_2$); $^{13}C$ ($D_2O$, TSP), $\delta$ 177.4, 106.1, 103.4, 103.1, 81.6, 80.1, 78.2, 77.7, 75.4, 75.0, 73.7, 73.6, 72.0, 71.7, 71.4, 69.1 ($CH_2$), 63.3 ($CH_2$), 63.2 ($CH_2$), 62.9 ($CH_2$), 58.1, 25.0, and 17.1.

(*b*) Compound **13** (213 mg, 0.25 mmol) was *O*-deacetylated as in (*a*), to give **15** (118 mg, 84%).

*Glycoproteins* **16** *and* **17**. — Compound **9** (500 mg, 0.58 mmol) was transformed[5b] into the corresponding hydrazide, and the crude product (170 mg) was coupled[5b] to bovine serum albumin (BSA, 32 mg) to give **16**. The degree of binding was 14. Glycoprotein **17** was prepared[5b] from the hydrazide (330 mg) and key-hole limpet haemocyanin (KLH, 30 mg). The degree of binding was 250.

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