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2-BROMOETHYL GLYCOSIDES IN GLYCOSIDE SYNTHESIS: PREPARATION OF GLYCOPROTEINS CONTAINING α-L-Fuc-(1→2)-D-Gal AND β-D-Gal-(1→4)-D-GlcNAc*

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ABSTRACT

The applicability of 2-bromoethyl glycosides in carbohydrate synthesis is demonstrated by the synthesis of glycosides of α-L-Fuc-(1→2)-D-Gal and β-D-Gal-(1→4)-D-GlcNAc. The bromoethyl aglycon was transformed into the methoxycarbonylethylthioethyl spacer, which allowed coupling of the sugars to proteins (BSA and KLH).

INTRODUCTION

We have described the preparation of 2-bromoethyl glycosides of some mono- and di-saccharides and their use for the preparation of glycoconjugates[1,2]. Being primary aliphatic bromides, the 2-bromoethyl glycosides could conceivably give side reactions with reagents used in standard carbohydrate syntheses. We now demonstrate the compatibility of the 2-bromoethyl group with common methods used in glycoside synthesis. Thus, glycosides of the blood-group H disaccharide [α-L-Fuc-(1→2)-D-Gal] and *N*-acetyl-lactosamine have been synthesised and then utilised to transform proteins into glycoproteins.

RESULTS AND DISCUSSION

2-Bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[2] (**1**) was deacetylated and the product **2** was immediately treated with zinc chloride and benzaldehyde in tetrahydrofuran to give the 4,6-*O*-benzylidene derivative **3** (49%). The deacetylated derivative **2** was prone to decomposition and it was important to perform the acetalation in a solvent in which all reactants were soluble. Selective benzoylation[3] of **3** gave the 3-benzoate **4** (59%), which was α-glycosylated by reaction with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[4], using bromide ion catalysis[5], to give the disaccharide derivative **5** (40%). Removal of the protecting

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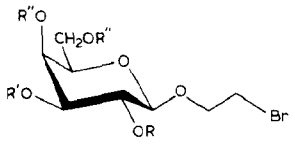
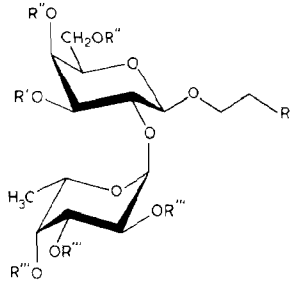
groups and acetylation of the product furnished 2-bromoethyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**7**, 49% from **5**), which would be difficult to prepare from the corresponding acetylated disaccharide because of the non-participating group at position 2. Reaction[6] of **7** with methyl 3-mercaptopropionate in *N,N*-dimethylformamide–Cs_2CO_3 and deacetylation of the product gave 2-(2-methoxycarbonylethylthio)ethyl 2-*O*-α-L-fucopyranosyl-β-D-galactopyranoside (**9**, 62%) suitable for coupling to proteins.



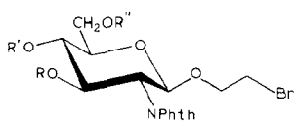
1 R = R′ = R″ = Ac
2 R = R′ = R″ = H
3 R = R′ = H, R″, R″ = CHPh
4 R = H, R′ = Bz, R″, R″ = CHPh



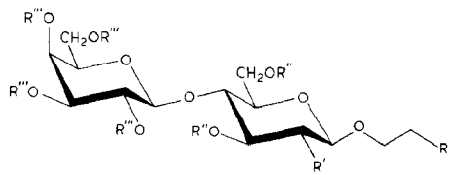
5 R = Br, R′ = Bz, R″, R″ = CHPh, R‴ = Bzl
6 R = Br, R′ = H, R″, R″ = CHPh, R‴ = Bzl
7 R = Br, R′ = R″ = R‴ = Ac
8 R = S⟨ COOMe, R′ = R″ = R‴ = Ac
9 R = S⟨ COOMe, R′ = R″ = R‴ = H
10 R = S⟨ CO-protein, R′ = R″ = R‴ = H

The synthesis of 2-bromoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**11**) from 2-bromoethanol, BF_3-etherate, and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose[7] has been reported[2]. An improved synthesis of the latter compound has been developed which consistently gave 50% of a ~1:4 αβ-mixture. Deacetylation of **11** gave **12**, which, in turn, was converted into the 4,6-*O*-benzylidene derivative **13** (63%).

Treatment of **13** with sodium hydride–benzyl bromide–tetrahydrofuran gave the 3-*O*-benzyl derivative **14** (62%). The use of *N,N*-dimethylformamide or methyl



11 R = R′ = R″ = Ac
12 R = R′ = R″ = H
13 R = H, R′, R″ = CHPh
14 R = Bzl, R′, R″ = CHPh
15 R = R″ = Bzl, R′ = H



16 R = Br, R′ = NPhth, R″ = Bzl, R‴ = Ac
17 R = Br, R′ = NPhth, R″ = R‴ = Ac
18 R = S⟨ COOMe, R′ = NPhth, R″ = R‴ = Ac
19 R = S⟨ COOMe, R′ = NHAc, R″ = R‴ = H
20 R = S⟨ CO-protein, R′ = NHAc, R″ = R‴ = H

sulphoxide as reaction solvent resulted in extensive dehydrobromination of the bromoethyl group. Benzylation of **13** was also effected with benzyl trichloroacetimidate in dichloromethane and a catalytic amount of triflic acid[8], but the trichloroacetamide formed in this reaction was difficult to remove. The 4,6-*O*-benzylidene group of **14** was selectively opened with sodium cyanoborohydride[9] to give 2-bromoethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**15**, 93%).

Glycosylation of **15** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and silver triflate afforded 84% of the disaccharide derivative **16**. The benzyl groups were removed from **16** by hydrogenolysis (Pd/C), and the crude product was acetylated to give **17**[2] (60%). Chain extension, dephthaloylation[10], and reacetylation gave **18**, which was deacetylated to give 2-(2-methoxycarbonylethylthio)ethyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**19**) as described previously[6].

The spacer-arm glycosides **9** and **19** were coupled to BSA and KLH by a modification of the methods reported by Inman[11] and Lemieux[12]. The solubility of the spacer-arm glycosides was higher in methyl sulfoxide than in the originally used[12] *N,N*-dimethylformamide. The degrees of binding to BSA and KLH were 47 and 395 for **9**, and 25 and 312 for **19**, respectively.

EXPERIMENTAL

General methods. — N.m.r. spectra were recorded with a Varian XL-200 spectrometer for solutions in $CDCl_3$ (internal Me_4Si unless otherwise stated). Only selected, appropriate n.m.r. data are given. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. Evaporations were performed under reduced pressure at <50°. T.l.c. was performed on silica gel (Merck, Kieselgel 60, F254, 0.25-mm layers) with detection by u.v. light or charring with sulfuric acid. Column chromatography was performed on Kieselgel 60 (Merck 230–400 mesh). Duolite C-6 (H^+) resin (methanol-washed and dried over P_2O_5) was used for neutralisations. Organic solutions were dried over Na_2SO_4. Elemental analyses are given for crystalline products. The homogeneity of each non-crystalline product was established by chromatography.

2-Bromoethyl 4,6-O-benzylidene-β-D-galactopyranoside (**3**). — 2-Bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[2] (**1**; 30 g, 66 mmol) was conventionally deacetylated with methanolic 0.1M sodium methoxide (600 mL). The mixture was neutralised with Duolite (H^+) resin, filtered, and concentrated under reduced pressure (<40°), to give crude **2** (18 g, 95%) as an unstable syrup that was used immediately. A solution of dry zinc chloride (14.4 g, 106 mmol) in tetrahydrofuran (80 mL) and benzaldehyde (11.2 mL, 106 mmol) was stirred for 15 min at room temperature. A solution of crude **2** (18 g, ~63 mmol) in tetrahydrofuran (90 mL) was then added and the mixture was stirred overnight in the dark. Evaporation of the solvent gave a residue that was washed with iso-octane, and a solution

in dichloromethane (200 mL) was washed with water (2 × 100 mL), dried, and concentrated *in vacuo*. A solution of the crude product in warm methanol (55 mL) was diluted with ether (200 mL) to give crystalline **3** (12 g). The mother liquor was concentrated, and the residue was subjected to column chromatography (ethyl acetate–methanol, 85:15) to give more **3** (1.9 g; total 13.9 g, 49%), m.p. 138–139°, $[\alpha]_D^{24}$ −30° (*c* 1.2, chloroform-*d*). N.m.r. data: ^{1}H, δ 5.56 (s, 1 H, PhC*H*), 4.35 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1), 4.32 (dd, $J_{6,6}$ 12.7, $J_{5,6}$ 1.5 Hz, H-6), 4.27 (t, O-CH_2CH$_2$), and 4.08 (dd, 1 H, $J_{5,6}$ 1.8 Hz, H-6); ^{13}C, δ 103.1, 101.5 (C-1, PhCH), and 30.4 (CH_2Br).

Anal. Calc. for $C_{15}H_{19}BrO_6$: C, 48.02; H, 5.10. Found: C, 48.14; H, 5.15.

*2-Bromoethyl 3-O-benzoyl-4,6-O-benzylidene-β-*D*-galactopyranoside* (**4**). — To a solution of **3** (10.0 g, 26.7 mmol) in dichloromethane (50 mL) and pyridine (20 mL) at −30° was added, dropwise[3], a solution of benzoyl chloride (5.2 g, 37 mmol) in dichloromethane (60 mL). The temperature was allowed to rise to 0° during ~1 h, the mixture was then washed with water, and the aqueous phase was extracted several times with dichloromethane. The combined extracts were dried, and co-concentrated with toluene, and the residue was subjected to column chromatography (ethyl acetate–iso-octane, 2:1), to give crude **4** which crystallised from 2-propanol. The contents of the mother liquor were rechromatographed. Recrystallisation from 2-propanol gave **4** (7.6 g, 59%), m.p. 141°, $[\alpha]_D^{23}$ +100° (*c* 0.78, chloroform). N.m.r. data: ^{1}H, δ 5.53 (s, 1 H, PhC*H*), 5.17 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-3), 4.51 (d, $J_{1,2}$ 7.7 Hz, H-1), 4.24 (dd, $J_{2,3}$ 10.2 Hz, H-2), 4.10 (dd, 1 H, $J_{6,6}$ 12.7, $J_{5,6}$ 1.8 Hz, H-6), and 3.55 (CH_2Br); ^{13}C, δ 103.3, 100.7 (C-1, PhCH), and 30.5 (CH_2Br).

Anal. Calc. for $C_{22}H_{23}BrO_8$: C, 55.13; H, 4.84. Found: C, 55.02; H, 4.98.

*2-Bromoethyl 3-O-benzoyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranoside* (**5**). — A dichloromethane solution (30 mL) of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide, prepared from 2,3,4-tri-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-β-L-fucopyranose[4] (4.0 g, 6.86 mmol), was added to a mixture of **4** (4.0 g, 8.35 mmol), tetraethylammonium bromide (1.8 g, 8.5 mmol), and 4Å molecular sieves (5 g) in dichloromethane (20 mL) at room temperature under nitrogen. Two additional portions of the fucosyl bromide (from 2.0 g of the precursor) were added after 2 and 5 days, respectively. After 8 days, **4** was almost consumed (t.l.c.; toluene–ethyl acetate, 5:1). The mixture was then filtered through Celite, washed with aqueous sodium hydrogencarbonate (2 × 50 mL) and water (50 mL), dried, and concentrated. The residue was subjected to column chromatography (toluene–ethyl acetate, 5:1) to give **5** (3.0 g, 40%), which, after recrystallisation from iso-octane–ethyl acetate, had m.p. 172°, $[\alpha]_D^{24}$ +26° (*c* 0.7, chloroform). N.m.r. data: ^{1}H, δ 5.50 (s, 1 H, PhC*H*), 5.46 (d, $J_{1',2'}$ 3.0 Hz, H-1'), 5.40 (dd, $J_{2,3}$ 9.7, $J_{3,4}$ 3.8 Hz, H-3), 4.68 (d, $J_{1,2}$ 7.7 Hz, H-1), 4.10 (bd, 1 H, $J_{6,6}$ 13.6 Hz, H-6), and 1.14 (d, 3 H, $J_{5',6'}$ 6.6 Hz, H-6'); ^{13}C, δ 101.7, 100.6, 96.9 (C-1, C-1', PhCH), 30.6 (CH_2Br), and 16.4 (C-6').

Anal. Calc. for $C_{49}H_{51}BrO_{11}$: C, 65.7; H, 5.74. Found: C, 65.1; H, 5.63.

2-Bromoethyl 4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**6**). — Compound **5** (4.0 g, 4.46 mmol) was debenzoylated in methanol (250 mL) containing sodium methoxide (4 mmol). The reaction was monitored by t.l.c. (toluene–ethyl acetate, 5:1) and, when complete, the mixture was neutralised with Duolite (H^+) resin and filtered. The resin was washed with methanol, the combined filtrate and washings were concentrated *in vacuo*, and the residue was subjected to column chromatography (toluene–ethyl acetate, 5:1) to give amorphous **6** (2.40 g, 68%), $[\alpha]_D^{23}$ −49° (*c* 0.78, chloroform). N.m.r. data: ^1H, δ 5.55 (s, 1 H, PhC*H*), 5.30 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1'), 4.45 (d, $J_{1,2}$ 8 Hz, H-1), and 1.13 (d, 3 H, $J_{5',6'}$ 6.4 Hz, H-6'); ^{13}C, δ 101.8, 101.4 (C-1, Ph*C*H), 99.1 (C-1'), 30.1 (CH_2Br), and 16.7 (C-6').

2-Bromoethyl 3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**7**). — A solution of **6** (1.70 g, 2.15 mmol) in acetic acid (50 mL) was hydrogenated (10% Pd/C, 0.3 g) at 8–10 p.s.i. overnight, filtered, and co-concentrated *in vacuo* several times with toluene. The residue, which was homogeneous by t.l.c. (chloroform–methanol–water, 65:35:10), was treated conventionally with pyridine (20 mL) and acetic anhydride (10 mL). Column chromatography (iso-octane–ethyl acetate, 3:2) of the product gave amorphous **7** (1.05 g, 72%), $[\alpha]_D^{23}$ −90° (*c* 0.77, chloroform). N.m.r. data: ^1H, δ 5.43 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 5.04 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 4.99 (dd, 1 H, $J_{2',3'}$ 10.9 Hz, H-2'), 4.74 (q, 1 H, $J_{5',6'}$ 6.7 Hz, H-5'), 4.55 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.00 (dd, $J_{2,3}$ 10 Hz, H-2), 3.52 (2 H, CH_2Br), and 1.15 (d, 3 H, H-6'); ^{13}C, δ 101.6 (C-1), 95.5 (C-1'), 29.7 (CH_2Br), and 20.7 ($OCOCH_3$).

2-(2-Methoxycarbonylethylthio)ethyl 3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**8**). — A mixture[6] of **7** (686 mg, 1.0 mmol), methyl 3-mercaptopropionate (0.18 g, 1.5 mmol), and cesium carbonate (489 mg, 1.5 mmol) in *N,N*-dimethylformamide (5.0 mL) was stirred at room temperature for 2 h, poured into water, and extracted with dichloromethane. The extract was washed with water, dried, filtered, and co-concentrated *in vacuo* with toluene several times. The residue was subjected to column chromatography (iso-octane–ethyl acetate, 1:1) to give amorphous **8** 673 mg, 93%), $[\alpha]_D^{24}$ −88° (*c* 1, chloroform). N.m.r. data: ^1H, δ 5.40 (d, H-1'), 5.37 (dd, $J_{2',3'}$ 11, $J_{3',4'}$ 3.4 Hz, H-3'), 5.04 (dd, 1 H, $J_{3,4}$ 3.5, $J_{2,3}$ 10 Hz, H-3), 4.99 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 11 Hz, H-2'), 4.52 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.9 (bt, $J_{5,6}$ 6.0 Hz, H-5), 3.72 (s, OCH_3), and 1.13 (d, 3 H, $J_{5',6'}$ 6.6 Hz, H-6'); ^{13}C, δ 101.5 (C-1), 95.5 (C-1'), 51.8 (OCH_3), 20.7 ($OCOCH_3$), and 15.6 (C-6').

2-(2-Methoxycarbonylethylthio)ethyl 2-O-α-L-fucopyranosyl-β-D-galactopyranoside (**9**). — Deacetylation of **8** (442 mg, 0.61 mmol), as described for **1**, gave amorphous **9** (194 mg, 67%) after column chromatography (chloroform–methanol, 3:1); $[\alpha]_D^{22}$ −79° (*c* 0.9, water). N.m.r. data (D_2O, external Me_4Si): ^1H, δ 5.29 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 4.54 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.75 (s, OCH_3), and 1.23 (d, 3 H, $J_{5',6'}$ 6.4 Hz, H-6'); ^{13}C, δ 104.3, 102.0 (C-1,C-1'), and 18.2 (C-6').

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose. — Meth-

anolic sodium methoxide, prepared from sodium (11.5 g, 0.5 mol) and methanol (600 mL), was added during 30 min to a solution of 2-amino-2-deoxy-D-glucose hydrochloride (108 g, 0.5 mol) in water (450 mL). The mixture was stirred for 30 min, and phthalic anhydride (37 g, 0.25 mol) was added; after a further 30 min, triethylamine (69 mL, 0.5 mol) and more phthalic anhydride (40 g, 0.27 mol) were added. The mixture was stirred for 1 h at 50°, the solvents were removed by repeated co-evaporation with toluene, and the residue was dried *in vacuo*. The resulting foam was treated with acetic anhydride (500 mL) at 100° for 1 h. Pyridine (500 mL) was added and, after 1 h, the reagents were removed *in vacuo*. The dark-brown residue was subjected to column chromatography (iso-octane–ethyl acetate, 3:2) to give the title compound (93.6 g, 39%), m.p. 88–92°, $[\alpha]_D^{24}$ +60° (*c* 0.9, chloroform); lit.[7] m.p. 90–94°, $[\alpha]_D^{22}$ +65.5° (*c* 1, chloroform). An $\alpha\beta$-mixture (25.5 g, 11%) was also obtained.

Compound **11** was prepared[2] from the β-acetate.

2-Bromoethyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**). — A solution of **11**[2] (352 mg, 0.65 mmol), conc. HCl (2.1 mL), water (5.5 mL), and acetone (11 mL) was stirred at 70° for 3.5 h, and then concentrated *in vacuo*. The yellow residue was extracted with ethyl acetate (3 × 15 mL), the extract was washed with water, saturated aqueous sodium hydrogencarbonate, and water, dried, and concentrated *in vacuo*. The foamy residue crystallised from ethyl acetate–light petroleum (b.p. 40–60°) to give **12** (198 mg, 73%), m.p. 169–171°, $[\alpha]_D^{20}$ +61° (*c* 1, chloroform). N.m.r. data (acetone-d_6): ^{1}H, δ 5.24 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1); ^{13}C, δ 99.0 (C-1), 57.7 (C-2), and 31.7 (CH_2Br).

Anal. Calc. for $C_{16}H_{18}BrNO_7$: C, 46.17; H, 4.36. Found: C, 46.39; H, 4.34.

2-Bromoethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**13**). — A mixture of **12** (14.8 g, 35.5 mmol), α,α-dimethoxytoluene (22.3 g, 146 mmol), toluene-*p*-sulfonic acid monohydrate (318 mg, 1.7 mmol), and dry acetonitrile (300 mL) was stirred at room temperature for 1 h, neutralised with triethylamine, and concentrated *in vacuo*. The syrupy residue crystallised on trituration with di-isopropyl ether. The crystals were collected, and washed with di-isopropyl ether, and a solution in chloroform was washed with aqueous sodium hydrogencarbonate and water, dried, filtered, and concentrated, to give a crystalline product (12.8 g, 71%). More (2.5 g) **13** was obtained after column chromatography (iso-octane–ethyl acetate, 2:1) of the material in the mother liquor (total yield 15.3 g, 86%). This product was pure by t.l.c. Recrystallisation from ethyl acetate–iso-octane gave **13**, m.p. 188–189°, $[\alpha]_D^{20}$ −38° (*c* 1, chloroform). N.m.r. data: ^{1}H, δ 5.56 (s, PhC*H*), 5.30 (d, $J_{1,2}$ 8.3 Hz, H-1), 4.38 (dd, $J_{5,6}$ 4.2, $J_{6,6}$ 9.8 Hz, H-6), 4.25 (dd, $J_{2,3}$ 10.5 Hz, H-2), and 2.53 (d, 1 H, $J_{OH,H-3}$ 3.2 Hz, HO-3); ^{13}C, δ 102.0 Ph*C*H), 99.1 (C-1), 56.3 (C-2), and 30.0 (CH_2Br).

Anal. Calc. for $C_{23}H_{22}BrNO_7$: C, 54.78; H, 4.40. Found: C, 55.16; H, 4.22.

2-Bromoethyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**14**). — (*a*) A mixture of **13** (20 g, 40 mmol), benzyl bromide (100

mL, 0.842 mol), tetrahydrofuran (20 mL), and sodium hydride (50% in oil; 3 g, 60 mmol) was stirred at room temperature for 24 h and then poured into a cold (0°) mixture of acetic acid (10 mL, 175 mmol) and tetrahydrofuran (490 mL). Stirring was continued for 30 min, the solvent was evaporated, and the residue was partitioned between dichloromethane (400 mL) and water (100 mL). The organic phase was washed with water (100 mL), dried, filtered, and concentrated *in vacuo*, and the residue was subjected to column chromatography. Elution first with toluene removed the excess of benzyl bromide, and then with toluene–ethyl acetate (9:1) gave **14** (17.9 g, 76%). Recrystallisation from methanol gave material (14.7 g, 62%) with m.p. 114–116°, $[\alpha]_D^{21}$ +39° (*c* 1.1, chloroform). N.m.r. data: ^{1}H, δ 5.63 (s, 1 H, PhC*H*), 5.26 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.81 and 4.51 (ABq, 2 H, *J* 12 Hz, PhCH_2), 4.24 (dd, 1 H, *J* 10 and 8 Hz, H-2), 4.07 (dt, 1 H, *J* 11, $J_{2,3}$ 6 Hz, OCH_2CH_2Br), and 3.29 (bt, 2 H, *J* ~6 Hz, CH_2Br); ^{13}C, δ 101.3 and 99.0 (Ph*C*H and C-1), 55.5 (C-2), and 29.9 (CH_2Br).

Anal. Calc. for $C_{30}H_{28}BrNO_7$: C, 60.62; H, 4.75. Found: C, 61.00; H, 4.76.

(*b*) A mixture[8] of **13** (504 mg, 1.00 mmol), benzyl trichloroacetimidate (505 mg, 2.00 mmol), trifluoromethanesulfonic acid (1 drop), and cyclohexane–dichloromethane (2:1, 10 mL) was stirred at room temperature for 2.5 h. T.l.c. (toluene–ethyl acetate, 5:1) then showed complete consumption of **13**. The mixture was poured into aqueous sodium hydrogencarbonate and extracted with dichloromethane, the extract was dried and concentrated, and the residue was subjected to column chromatography (iso-octane–ethyl acetate, 1:1) and then recrystallised as in (*a*) to give **14** (412 mg, 69%).

*2-Bromoethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**15**). — A saturated solution of hydrogen chloride in ether was added to a mixture of **14** (17.1 g, 27.8 mmol), sodium cyanoborohydride (9.5 g, 151 mmol), molecular sieves (3Å, 35 g), and tetrahydrofuran (250 mL)[9]. When gas evolution ceased (25 mL of the hydrogen chloride solution had been added), more (15 mL) hydrogen chloride solution was added. The mixture was stirred for 30 min and then partitioned between dichloromethane (600 mL) and ice–water (100 mL). The organic phase was washed with cold aqueous sodium hydrogencarbonate (100 mL), dried, and concentrated *in vacuo*. The residue was subjected to column chromatography (iso-octane–ethyl acetate, 1:1) to give **15** (10.5 g), as a colourless gum, and a second fraction of slightly impure material (5.8 g) that was rechromatographed (toluene–ethyl acetate, 9:1 and then 4:1) to give more (5.5 g) **15** (total yield, 16 g, 93%), $[\alpha]_D^{21}$ +23° (*c* 0.7, chloroform). N.m.r. data: ^{1}H, δ 5.19 (d, 1 H, *J* 8 Hz, H-1), 4.75 and 4.54 (ABq, 2 H, *J* 12 Hz, PhCH_2), 4.67 and 4.58 (ABq, 2 H, *J* 12 Hz, PhCH_2), 4.26 (dd, 1 H, *J* 11 and 8 Hz, H-3), 4.17 (dd, 1 H, *J* 11 and 8 Hz, H-2), and 3.29 (bt, 2 H, *J* ~6 Hz, CH_2Br): ^{13}C, δ 98.4 (C-1), 55.2 (C-2), and 30.1 (CH_2Br).

*2-Bromoethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**16**). — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.72 g, 6.60 mmol) in di-

chloromethane (50 mL) was added with stirring during 45 min to a mixture of **15** (2.0 g, 3.3 mmol), silver triflate (1.7 g, 6.6 mmol), 4 Å molecular sieves (2 g), tetramethylurea (0.77 mL, 6.6 mmol), and dichloromethane (70 mL) at −70°. The mixture was allowed to reach room temperature overnight, filtered through Celite, washed with aqueous sodium hydrogencarbonate and water, dried, and concentrated *in vacuo*, to give a crude product (4.4 g) that was subjected to column chromatography (iso-octane–ethyl acetate, 1:1) to give **16** (2.3 g, 75%), $[\alpha]_D^{25}$ +22° (*c* 1.2, chloroform). N.m.r. data: ^{1}H, δ 5.28 (bd, 1 H, $J_{4',3'}$ 3.5 Hz, H-4'), 5.15 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{2',1'}$ 8.0 Hz, H-2'), 5.15 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.84 (d, 1 H, *J* 4.9 Hz, PhCH_2), 4.78 (d, 1 H, *J* 4.3 Hz, PhCH_2), 4.57 (d, 1 H, H-1'), 4.47 (t, 2 H, *J* ~12 Hz, PhCH_2), 4.26 (dd, 1 H, *J* 8.2, *J* 10.5 Hz, H-3), and 3.29 (2 H, CH_2Br); ^{13}C, δ 100.2, 98.4 (C-1, C-1'), 55.3 (C-2), and 29.9 (CH_2Br).

*2-Bromoethyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**17**). — A solution of **16** (2.18 g, 2.35 mmol) in acetic acid (50 mL) was hydrogenated (Pd/C, 0.45 g) at 7–8 p.s.i. for 3.5 h, filtered, and concentrated *in vacuo*, to give crude 2-bromoethyl 2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (1.70 g, 87%), $[\alpha]_D^{25}$ −1.3° (*c* 2.7, chloroform). N.m.r. data: ^{1}H, δ 5.38 (d, 1 H, $J_{4',3'}$ 3.3 Hz, H-4'), 5.34 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 5.26 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{1',2'}$ 7.9 Hz, H-2'), 4.68 (d, 1 H, H-1'), and 3.33 (t, 2 H, CH_2Br); ^{13}C, δ 101.9 (C-1'), 98.4 (C-1), 55.6 (C-2), and 29.9 (CH_2Br).

The crude product was conventionally acetylated with pyridine (25 mL) and acetic anhydride (10 mL) at room temperature, to give **17**[2] (1.17 g, 60%), after column chromatography (iso-octane–ethyl acetate, 1:2).

Compound **17** was transformed into **19** *via* **18**, as described previously[6].

Glycoproteins. — (*a*) A solution of **9** (97 mg, 0.21 mmol) and hydrazine hydrate (85%, 0.80 mL) in ethanol (5 mL) was left overnight and then concentrated, and an aqueous solution of the residue was lyophilised. The resulting hydrazide was pure by t.l.c. (chloroform–methanol–water, 65:35:10), and a solution of a part (32 mg, 0.070 mmol) in methyl sulfoxide (1 mL) was treated with 4M hydrogen chloride in 1,4-dioxane (205 μL, 0.15 mmol) and *tert*-butyl nitrite (18 μL, 0.15 mmol) in methyl sulfoxide (0.1 mL). The mixture was stirred at room temperature for 30 min, a solution of sulfamic acid (10 mg, 0.11 mmol) in methyl sulfoxide (0.1 mL) was added, and, after 15 min, the mixture was added dropwise with stirring to a solution of bovine serum albumin (BSA, 32 mg) in sodium tetraborate–potassium hydrogencarbonate buffer (2.0 mL, 0.08M $Na_2B_4O_7$ and 0.35M $KHCO_3$). The pH was maintained at 9.0–9.3 by the addition of M sodium hydroxide. The mixture was stirred for 16 h at room temperature, dialysed (H_2O, 72 h), and lyophilised, to give **10**-BSA. The number of hapten molecules per molecule of protein (the degree of binding) was 47, as determined by the phenol–sulfuric acid method[13].

(*b*) The procedure in (*a*) was followed using the hydrazide of **9** (55 mg, 0.12 mmol) and key-hole limpet haemocyanin (KLH, 25 mg), to give **10**-KLH. The degree of binding was 325.

(*c*) The procedure in (*a*) was followed using the hydrazide of **19** (38 mg, 0.07 mmol; and 51 mg, 0.094 mmol; respectively) with BSA (32 mg) and KLH (20 mg). The degree of binding of the resulting **20**-BSA was 25, and that of **20**-KLH was 312.

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