Carbohydrate Research, 118 (1983) 292–301
Elsevier Science Publishers B.V Amsterdam – Printed in The Netherlands

Note

2-Bromoethyl glycosides*: applications in the synthesis of spacer-arm glycosides

JAN DAHMÉN, TORBJORN FREJD, GUNNAR GRONBERG, THOMAS LAVE, GORAN MAGNUSSON, AND GHAZI NOORI

Swedish Sugar Co Ltd., Research and Development, Box 6, S-232 00 Arlöv (Sweden)

(Received November 8th, 1982, accepted for publication, February 1st, 1983)

We have described the preparation[1] of 2-bromoethyl glycosides and now report on their use for the synthesis of spacer-arm glycosides. A preliminary account of this work has been published[2].

Spacer-arm glycosides have been variously prepared[3] and used for coupling to proteins and other carriers. In this way, semi-artificial glycoconjugates have been prepared for the production of carbohydrate-specific antibodies[4] and for the purification of antibodies and lectins on synthetic affinity-chromatography matrixes[5]. Furthermore, artificial glycolipids have been prepared for incorporation into liposomes[6]. In all these instances, there is a need for stereospecific methods that permit the preparation of pure α- or β-glycosides. Usually, the spacer-arm has been connected to the carbohydrate moiety at an early stage of the synthetic route. This approach has drawbacks in that the spacer-arm cannot be varied after the completion of the synthesis and, with long spacer-arms, the difference in chromatographic mobility between α- and β-glycosides is often too small to permit an easy separation. These problems can be avoided by a two-step procedure using easily purified (often crystalline) 2-bromoethyl glycosides to alkylate appropriate thiols.

2-Bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1], 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α- and -β-D-galactopyranosyl)-β-D-galactopyranoside[1], and 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[1] were variously reacted with methyl 3-mercaptopropionate, *p*-aminothiophenol, octanethiol, octadecanethiol, and hexane-1,6-dithiol. The methods and product yields are shown in Table I.

The desirable alkylation reaction should be clean and high-yielding, since it constitutes a late stage in the synthesis sequence. It should also be possible to per-

*Part 3. For Part 2, see ref. 1

0008-6215/83/$ 03.00

TABLE I

PRODUCTS OF REACTION OF THIOLS AND HYDROGEN WITH 2-BROMOETHYL GLYCOSIDES

Product[a]		*Method*[b]	*Yield (%)*[c]
1	β-D-Gal*p*-O∧S∧CO_2Me	A	73
2	α-D-Gal*p*-(1→4)-β-D-Gal*p*-O-∧S∧CO_2Me	B	80
3	β-D-Gal*p*-(1→4)-β-D-Glc*p*-NPhth-O-∧S∧CO_2Me	C	81
4	β-D-Gal*p*-O-∧S-⬡-NH_2	A, E	71, 58
5	β-D-Gal*p*-(1→4)-β-D-Gal*p*-O-∧S-⬡-NH_2	D	99
6a	α-D-Gal*p*-(1→4)-β-D-Gal*p*-O-∧S-⬡-NH_2	A	88
6b		D	97
7	β-D-Gal*p*-O-∧S-∧∧∧	A	70
8	α-D-Gal*p*-(1→4)-β-D-Gal*p*-O-∧S-∧∧∧	B	71
9	α-D-Gal*p*-(1→4)-β-D-Gal*p*-O-∧S-∧∧∧∧∧∧	C	69
10	β-D-Gal*p*-O-∧S-⬡-NO_2	D	62
11	(β-D-Gal*p*-O-∧S)$_2(CH_2)_6$	A	21
12	[α-D-Gal*p*-(1→4)-β-D-Gal*p*-O-∧S]$_2(CH_2)_9$	A	21
13	β-D-Gal*p*-O-CH_2-CH_3	F	68
14	α-D-Gal*p*-(1→4)-β-D-Gal*p*-O-CH_2-CH_3	F	98
15	β-D-Gal*p*-(1→4)-β-D-Glc*p*-NPhth-O-CH_2-CH_3	F	48

[a]Compounds **1–4, 6a,** and **7–15** were isolated in the acetylated form. [b]A, benzene–water, $(C_8H_{17})_3N^+MeBr^-$, NaOH; B, benzene–water, $(C_8H_{17})_3N^+MeBr^-$, Cs_2CO_3; C, *N*,*N*-dimethylformamide, Cs_2CO_3; D, *N*,*N*-Dimethylformamide, NaH; E, **10** with methanol, H_2, Pd/C; F, methanol–water, NaOH, H_2, Pd/C, followed by acetic anhydride–pyridine. [c]Isolated compounds.

form the reaction with protected (*e.g.*, acetylated) sugars, since this would add flexibility in the choice of chromatographic methods for final purification.

Two kinds of alkylation reaction fulfil the above criteria, namely, anhydrous reaction-conditions with pre-formation of the thiolate ion, and aqueous, phase-transfer conditions of catalysis with simultaneous formation of the thiolate ion. The former resulted in some deacetylation, so that reacetylation was necessary. However, where unwanted acetylation can take place (*e.g.*, the aniline spacer-arm glycosides **4–6**), only deacetylation before purification was practical.

Protecting groups were removed from **1–4, 9, 12, 14,** and **15** under standard conditions which gave **18–20** and **22–26**, respectively, in good yield. Deprotection of **3** was complicated by a partial attack of hydrazine (used for dephthaloylation) on the methyl ester group. *N*-Acetylation of the sugar amino group resulted in simultaneous acetylation of the hydrazide function, which gave **21**.

It is evident from the literature[3] that almost all of the examples of covalent attachment of a glycoside to a carrier molecule involves terminal amino and carboxyl groups and the formation of amide bonds. Hence, *p*-aminothiophenol and methyl 3-mercaptopropionate were selected for the preparation of the complete spacer-arm glycosides (**1–6** in Table I). The aniline derivatives can also be prepared by catalytic hydrogenation of the corresponding nitro compound (*e.g.*, **10→4**).

Methyl and ethyl glycosides have been used as agglutination inhibitors for specificity studies of carbohydrates towards antibodies[7] and lectins[8]. Hydro-

genolysis of the bromine atom in the 2-bromoethyl glycosides gave the corresponding ethyl glycosides in good yield (**13**–**15** in Table I). The basic reaction-conditions used leave benzyl protecting-groups intact, whereas the use of acidic conditions permits the removal of such protecting groups without affecting the 2-bromoethyl aglycon[2,9].

The use of **24**, **25**, and **26** as agglutination inhibitors, **5**, **19**, and **20** for coupling to proteins and particles, and **23** for coating of cell membranes will be reported elsewhere.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded with a Varian XL-200 spectrometer, with Me_4Si or sodium 3-(trimethylsilyl)propionate-d_4 (TSP) as internal reference. N.m.r. assignments were based on double-resonance and INEPT experiments. Solvents were removed with a rotary evaporator and then at <0.1 Torr.

2-(2-Methoxycarbonylethylthio)ethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**1**). — A mixture of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1] (1.76 g, 3.90 mmol), methyl 3-mercaptopropionate[10] (1.0 mL, 9.2 mmol), methyltrioctylammonium chloride (~10 mg), sodium hydroxide (0.36 g, 9.0 mmol), benzene (5 mL), and water (5 mL) was stirred vigorously at room temperature. The reaction, which was 50% complete after ~40 min (t.l.c., SiO_2; ethyl acetate–iso-octane, 2:1), was continued overnight; no deacetylation occurred. The aqueous phase was extracted with benzene, and the combined benzene solutions were washed once with water, dried (Na_2SO_4), and concentrated. Chromatography (SiO_2; ethyl acetate–iso-octane, 1:1) gave **1** (1.41 g, 73%), and recrystallisation from ethanol gave material having m.p. 81–82°, $[\alpha]_D^{24}$ −10.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.42 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.24 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.5 Hz, H-2), 5.05 (dd, 1 H, H-3), 4.54 (d, 1 H, H-1), 4.26–3.65 (m, 5 H, H-5,6,6′ and O-CH_2-CH_2), 3.72 (s, 3 H, Me), 2.88–2.59 (m, 6 H, CH_2-S-CH_2, CH_2-CO), 2.17, 2.09, 2.06, and 2.00 (4 s, each 3 H, 4 AcO).

Anal. Calc. for $C_{20}H_{30}O_{12}S$: C, 48.58; H, 6.11. Found: C, 48.83; H, 6.22.

2-(2-Methoxycarbonylethylthio)ethyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**2**). — A mixture of 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside[1] (251 mg, 0.338 mmol), methyl 3-mercaptopropionate[10] (38 μL, 0.35 mmol), methyltrioctylammonium chloride (~10 mg), cesium carbonate (114 mg, 0.35 mmol), benzene (0.5 mL), and water (0.5 mL) was stirred overnight. T.l.c. (SiO_2; ethyl acetate–iso-octane, 4:1) then revealed reaction to be 60–70% complete. More methyl 3-mercaptopropionate (~20 μL) was added and the mixture was stirred for 24 h; reaction was then complete (t.l.c.). The aqueous phase was extracted twice with dichloromethane, and the combined organic phases were dried (Na_2SO_4) and concentrated. The resulting syrup was subjected to chromatog-

raphy (SiO_2; ethyl acetate–iso-octane, 2:1), to give **2** as a syrup (211 mg, 80%), $[\alpha]_D^{23}$ +70° (*c* 0.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.57 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4'), 5.39 (dd, 1 H, $J_{2',3'}$ 11.0 Hz, H-2'), 5.19 (dd, 1 H, H-3'), 5.17 (dd, 1 H, $J_{2,3}$ 11 Hz, H-2), 5.00 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.80 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.51 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.70 (s, 3 H, MeO), 2.82, 2.75, 2.63 (3 t, each 2 H, *J* 7 Hz, CH_2-S-CH_2, CH_2-CO), 2.13, 2.11, 2.08, 2.08, 2.06, 2.04, and 1.98 (7 s, each 3 H, 7 AcO).

*2-(2-Methoxycarbonylethylthio)ethyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**3**). — A suspension of 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[1] (2.5 g, 3.01 mmol), methyl 3-mercaptopropionate[10] (1.96 g, 16.3 mmol), and cesium carbonate (1.96 g, 6.02 mmol) in dry *N*,*N*-dimethylformamide (20 mL) was stirred at room temperature for 2 h, and then partitioned between ether and water. The aqueous phase was extracted with ether, and the combined ether solutions were dried (Na_2SO_4), filtered, and concentrated. The residue was subjected to chromatography (SiO_2; toluene–ethyl acetate, 1:1), to give **3** (2.13 g, 81%) as an amorphous solid, $[\alpha]_D^{20}$ +13° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 7.78 (m, 4 H, aromatic), 5.75 (dd, 1 H, *J* 7.8 and 10.6 Hz, H-3), 5.40 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.34 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4'), 5.13 (dd, 1 H, $J_{1',2'}$ 7.7, $J_{2',3'}$ 10.4 Hz, H-2'), 4.97 (dd, 1 H, H-3'), 4.55 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 3.62 (s, 3 H, MeO), 2.60 (m, 4 H, CH_2-S-CH_2), 2.44 (m, 2 H, CH_2CO), 2.16, 2.14, 2.07, 2.04, 1.97, and 1.91 (6 s, each 3 H, 6 AcO).

*2-(p-Aminophenylthio)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranoside* (**4**). — (*a*) A mixture of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1] (2.05 g, 4.50 mmol), *p*-aminothiophenol (1.2 g, 9.6 mmol), methyltrioctylammonium chloride (20 mg), sodium hydroxide (0.3 g, 7.5 mmol), benzene (5 mL), and water (5 mL) was stirred vigorously for 9 h and then worked-up as described above. Chromatography (SiO_2; ethyl acetate–iso-octane, 2:1) gave **4** (1.6 g, 71%) as an amorphous solid, $[\alpha]_D^{21}$ +8° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 7.25, 6.63 (ABq, 4 H, J_{AB} 8.6 Hz, aromatic), 5.39 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.22 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.4 Hz, H-2), 5.01 (dd, 1 H, H-3), 4.45 (d, 1 H, H-1), 2.92 (t, 1 H, *J* 6.8 Hz, S-CH_2), 2.15, 2.08, 2.05, and 2.00 (4 s, each 3 H, 4 AcO).

(*b*) 2-(*p*-Nitrophenylthio)ethyl 2,3,4,6-tetra-*O*-acetyl β-D-galactopyranoside (**10**; 90 mg, 0.18 mmol) was hydrogenated overnight (4 atm. H_2, 10% Pd/C, 90 mg) in methanol (75 mL), to give **4** (58%, after chromatography).

2-(p-Aminophenylthio)ethyl 4-O-β-D-galactopyranosyl-β-D-galactopyranoside (**5**). — To a solution of *p*-aminothiophenol (205 mg, 1.64 mmol) in *N*,*N*-dimethylformamide (4 mL) was added sodium hydride (70 mg, 2.9 mmol), and the mixture was stirred under nitrogen for 20 min at room temperature. A solution of 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside[1] (640 mg, 0.86 mmol) in *N*,*N*-dimethylformamide (7

mL) was then added, and the mixture was stirred under nitrogen for 2 h and poured into cold water (40 mL). The aqueous solution was extracted with ether (4 × 20 mL), and the combined ether solutions were washed with water (10 mL), dried (Na_2SO_4), and concentrated. The residue contained partly deacetylated compounds. Concentration of the aqueous solution gave more partly deacetylated material. The combined residues were deacetylated (methanolic sodium methoxide) and the product was filtered through a column (SiO_2; ethyl acetate–methanol, 2:3), to give **5** (425 mg, 99%). Recrystallisation from methanol gave material having m.p. 104–106°, $[\alpha]_D^{23}$ −1° (*c* 1.1, water). ^{1}H-N.m.r. data [$(CD_3)_2SO$ plus D_2O, 50°, TSP]: δ 7.13, 6.58 (2 d, each 2 H, *J* 8.5 Hz, aromatic), 4.28 (d, 1 H, *J* 7.0 Hz, H-1′), 4.28 (d, 1 H, *J* 7.5 Hz, H-1), and 2.90 (t, 2 H, *J* 7.0 Hz, CH_2-S).

*2-(*p*-Aminophenylthio)ethyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**6a**). — 2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside[1] (3.72 g, 5 mmol) was treated as in (*a*) above. The crude product was subjected to chromatography (SiO_2; ethyl acetate–iso-octane, 3:1), to give **6a** (3.46 g, 88%), $[\alpha]_D^{21}$ +75° (*c* 0.55, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 7.26 (d, 2 H, *J* 8.5 Hz, aromatic), 6.63 (d, 2 H, *J* 8.5 Hz, aromatic), 5.57 (bd, 1 H, *J* 3 Hz, H-4′), 5.38 (dd, 1 H, *J* 11 and 3 Hz, H-3′), 5.20 (dd, 1 H, *J* 11 and 3.5 Hz, H-2′), 5.17 (dd, 1 H, *J* 10.5 and 7.7 Hz, H-2), 5.00 (d, 1 H, *J* 3.4 Hz, H-1′), 4.80 (dd, 1 H, *J* 10.5 and 3 Hz, H-3), 4.45 (d, 1 H, *J* 7.7 Hz, H-1), 4.60–4.38 (m, 3 H), 4.26–3.54 (m, 9 H), 2.95 (t, 2 H, *J* 7.5 Hz, CH_2S), 2.13, 2.10, 2.08, 2.06, 2.06, 2.04, and 1.99 (7 s, each 3 H, 7 AcO).

*2-(*p*-Aminophenylthio)ethyl 4-O-α-D-galactopyranosyl-β-D-galactopyranoside* (**6b**). — (*a*) 2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside[1] (170 mg, 0.23 mmol) was treated as described above for **5**. The crude, deacetylated product was filtered through a column (SiO_2; ethyl acetate–methanol, 2:3), to give **6b** (110 mg, 97%) as an amorphous solid.

(*b*) A solution of **6a** (3.2 g) in methanol (32 mL) and methanolic sodium methoxide (2 mL, 0.1M) was stored for 6 h. A trace of **6b** was added and the mixture was left overnight. The precipitate **6b** (1.5 g, 75%) was collected and had m.p. 100–103°, $[\alpha]_D^{22}$ +68° (*c* 1.7, water). ^{1}H-N.m.r. data [$(CD_3)_2SO$ plus D_2O, 50°, TSP]: δ 7.13, 6.56 (2 d, each 2 H, *J* 8.4 Hz, aromatic), 4.83 (d, 1 H, *J* 3.3 Hz, H-1′), 4.14 (d, 1 H, *J* 7.6 Hz, H-1), and 2.89 (t, 2 H, *J* 7.5 Hz, CH_2-S).

Anal. Calc. for $C_{20}H_{31}NO_{11}S$: C, 48.67; H, 6.33. Found: C, 48.31; H, 6.62.

2-(Octylthio)ethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**7**). — A mixture of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1] (2.0 g, 4.4 mmol), octanethiol (0.7 g, 4.8 mmol), methyltrioctylammonium chloride (20 mg), sodium hydroxide (0.3 g, 7.5 mmol), benzene (9 mL), and water (8 mL) was stirred vigorously for 3 h and then worked-up as described for **1**. Chromatography (SiO_2; ethyl acetate–iso-octane, 2:1) of the product gave **7** (1.6 g, 70%) as a colourless oil, $[\alpha]_D^{21}$ −9° (*c* 1.2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.40 (dd, 1 H, $J_{4,5}$

1.0 Hz, H-4), 5.23 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.03 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 4.52 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 2.71, 2.53 (2 t, each 2 H, J 7.0 Hz, CH_2-S-CH_2), 2.16, 2.08, 2.06, 1.99 (4 s, each 3 H, 4 AcO), and 0.88 (t, 3 H, J 7.0 Hz, CH_3-CH_2).

*2-(Octylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**8**). — A mixture of 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside[1] (484 mg, 0.65 mmol), octanethiol (110 mg, 0.75 mmol, 130 μL), methyltrioctylammonium chloride (~10 mg), cesium carbonate (245 mg, 0.75 mmol), benzene (1 mL), and water (1 mL) was treated as described for **7**. Chromatography of the product gave **8** (250 mg, 71% based on reacted starting-material), $[\alpha]_D^{23}$ +65° (*c* 1.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.57 (dd, 1 H, $J_{4',5'}$ 1 Hz, H-4'), 5.39 (dd, 1 H, $J_{2',3'}$ 11, $J_{3',4'}$ 3.5 Hz, H-3'), 5.20 (dd, 1 H, $J_{1',2'}$ 3.5 Hz, H-2'), 5.19 (dd, 1 H, $J_{2,3}$ 11, $J_{1,2}$ 7.5 Hz, H-2), 5.01 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.81 (dd, 1 H, $J_{3,4}$ 3 Hz, H-3), 4.51 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 2.73, 2.53 (t, each 2 H, J 7 Hz, CH_2-S-CH_2), 2.13, 2.11, 2.08, 2.08, 2.07, 2.04, and 1.99 (7 s, each 3 H, 7 AcO).

*2-(Octadecylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**9**). — A mixture of 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside[1] (743 mg, 1 mmol), octadecanethiol (315 mg, 1.1 mmol), cesium carbonate (391 mg, 1.2 mmol), and dry *N*,*N*-dimethylformamide (8 mL) was stored at room temperature under nitrogen for 24 h, and then partitioned between water and ether. The ether phase was dried (Na_2SO_4) and concentrated, and the residue was subjected to chromatography (ethyl acetate–iso-octane, 1:1) to give **9** (300 mg, 69%), $[\alpha]_D^{23}$ +55° (*c* 0.5, chloroform). The main part of the spectrum (δ 1.9–5.6) was practically identical with that of **8**.

*2-(*p*-Nitrophenylthio)ethyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranoside* (**10**). — *p*-Nitrothiophenol (50 mg, 0.32 mmol) was stirred with a suspension of sodium hydride (oil-free, 11 mg, 0.46 mmol) in *N*,*N*-dimethylformamide (3 mL) at room temperature. A solution of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1] (110 mg, 0.24 mmol) in *N*,*N*-dimethylformamide (3 mL) was added during 10 min, and the mixture was stirred for another 75 min and then poured into ice–water (20 mL) and ether (40 mL). The aqueous phase was extracted with ether (40 mL), and the combined organic phases were washed with cold water (20 mL), dried (Na_2SO_4), and concentrated. Chromatography (SiO_2; ethyl acetate–iso-octane, 1:1) of the product gave **10** (80 mg, 62%) as a light-yellow oil, $[\alpha]_D^{23}$ −15° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 8.14, 7.36 (ABq, 4 H, J_{AB} 9.2 Hz, aromatic), 5.40 (dd, 1 H, $J_{4,5}$ 1.0 Hz, H-4), 5.22 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.01 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 4.52 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.25 (t, 2 H, J 6.5 Hz, S-CH_2), 2.17, 2.05, 2.04, and 1.99 (4 s, each 3 H, 4 AcO).

*1,12-Bis(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyloxy)-3,10-dithiadodecane* (**11**). — A mixture of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1] (915 mg, 2.0 mmol), hexane-1,6-dithiol (150 mg, 1.0 mmol, 153 μL),

benzene (3 mL), and potassium hydroxide (112 mg, 2 mmol), water (3 mL), and methyltrioctylammonium chloride (~10 mg) was stirred under nitrogen for 24 h. The aqueous phase was extracted with dichloromethane, and the combined organic phases were washed with water, dried (Na_2SO_4), and concentrated. The residue was subjected to chromatography (SiO_2; ethyl acetate–iso-octane, 1:1), to give the starting 2-bromoethyl glycoside (120 mg), **11** (190 mg, 21%), and 2-(6-mercaptohexylthio)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**16**: 280 mg, 27%).

Compound **11** was a syrup, $[\alpha]_D^{23}$ −10° (*c* 1.4, chloroform-*d*). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.40 (d, 2 H, H-4), 5.22 (dd, 2 H, $J_{2,3}$ 10.5 Hz, H-2), 5.03 (dd, 2 H, $J_{3,4}$ 3.5 Hz, H-3), 4.51 (d, 2 H, $J_{1,2}$ 8.0 Hz, H-1), 2.70, 2.53 (t, each 4 H, *J* 7.0 Hz, CH_2-S-CH_2), 2.16, 2.09, 2.06, and 1.99 (4 s, each 6 H, 4 AcO).

Compound **16** was a syrup, $[\alpha]_D^{23}$ −9° (*c* 1.3, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.41 (d, 1 H, H-4), 5.24 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.04 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 4.53 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 2.72 (t, 2 H, *J* 7.0 Hz, S-CH_2), 2.60–2.50 (m, 4 H, CH_2-S and CH_2-SH), 2.16, 2.08, 2.06, and 1.99 (4 s, each 3 H, 4 AcO).

1,15-Bis[2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyloxy]-3,13-dithiapentadecane (**12**). — 2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside[1] (1486 mg, 2.0 mmol) and nonane-1,9-dithiol were treated as described for **11**. Chromatography (SiO_2; ethyl acetate–iso-octane 4:1) of the product gave the starting 2-bromoethyl glycoside (457 mg), **12** (312 mg, 21%), and 2-(9-mercaptononylthio)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**17**: 335 mg, 20%).

Compound **12** was a syrup, $[\alpha]_D^{23}$ +62° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.56 (d, 2 H, $J_{3',4'}$ 3.0 Hz, H-4'), 5.39 (dd, 2 H, $J_{2',3'}$ 11.0 Hz, H-2'), 5.20 (dd, 2 H, $J_{2,3}$ 11.0 Hz, H-2), 5.18 (dd, 2 H, H-3'), 5.00 (d, 2 H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.81 (dd, 2 H, $J_{3,4}$ 10.5 Hz, H-3), 4.50 (d, 2 H, $J_{1,2}$ 8.0 Hz, H-1), 2.72, 2.53 (2 t, each 4 H, *J* 7.0 Hz, CH_2-S-CH_2), 2.13, 2.10, 2.07, 2.06, 2.04, and 1.98 (6 s, each 3 H, 6 AcO).

Compound **17** was a syrup, $[\alpha]_D^{23}$ +60° (*c* 1.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.58 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4'), 5.40 (dd, 1 H, $J_{2',3'}$ 11.0 Hz, H-2'), 5.21 (dd, 1 H, H-3'), 5.19 (dd, 1 H, $J_{2,3}$ 11.0 Hz, H-2), 5.01 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.82 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.52 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 2.74, 2.55, 2.52 (3 t, each 2 H, *J* 7 Hz, CH_2-SH, CH_2-S-CH_2), 2.14, 2.11, 2.08, 2.07, 2.04, and 1.99 (6 s, each 3 H, 6 AcO).

Ethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**13**). — A solution of 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[1] (98 mg, 0.22 mmol) in methanol (5 mL) and 0.3M potassium hydroxide (5 mL) was hydrogenated (10% Pd/C, 47 mg) at atmospheric pressure for 3 h. The mixture (pH 6) was filtered and concentrated, to give a residue that was pure by t.l.c. (SiO_2, chloroform–methanol–water, 65:35:10). The residue was conventionally acetylated (acetic anhydride–pyridine 1:1, 60°, 1.5 h). Crystallisation of the product from ether–iso-

octane gave **13** (55 mg, 68%), m.p. 92–93°, $[\alpha]_D^{22}$ −31° (*c* 1.2, benzene); lit.[11] m.p. 88°, $[\alpha]_D^{20}$ −29.8° (*c* 10, benzene).

*Ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**14**). — To a solution of 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside[1] (740 mg, 1 mmol) in methanol (14 mL) was added methanolic 0.1M sodium methoxide in methanol (2 mL), and the mixture was stirred at room temperature for 2 h. Sodium hydroxide (87 mg, 2.2 mmol) in water (2 mL) was added together with 10% Pd/C (120 mg), and the mixture was hydrogenated (15 h) at atmospheric pressure. Filtration (Celite) and concentration (<0.1 Torr) then gave a colourless, amorphous residue that was conventionally acetylated with acetic anhydride–pyridine. Recrystallisation of the product (650 mg, 98%) gave **14** as needles, m.p. 133–135°, $[\alpha]_D^{22}$ +81° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$, Me_4Si): δ 5.57 (d, 1 H, H-4′), 5.39 (dd, 1 H, $J_{3',4'}$ 3.2 Hz, H-3′), 5.20 (dd, 1 H, $J_{2',3'}$ 11.0 Hz, H-2′), 5.18 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 5.01 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.82 (dd, 1 H, $J_{3,4}$ 2.6 Hz, H-3), 4.48 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.06 (d, 1 H, H-4), 3.92, 3.59 (2 dq, each 1 H, *J* 9.7 and 7.1 Hz, CH_3-CH_2-O), 2.13, 2.11, 2.08, 2.07, 2.06, 2.04, 1.99 (7 s, each 3 H, 7 AcO), and 1.22 (t, 3 H, *J* 7.1 Hz, CH_3-CH_2-O).

Anal. Calc. for $C_{26}H_{35}O_{18}$: C, 50.06; H, 6.07. Found: C, 50.43: H, 6.09.

*Ethyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside*[12] (**15**). — To a solution of 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[1] (138 mg, 0.17 mmol) in methanol (6 mL) was added sodium methoxide (10 mg) and, after 1 h, 0.1M sodium hydroxide (10 mL). The mixture was hydrogenated (1.5 atm., 10% Pd/C), filtered (Celite), and concentrated, and the residue was conventionally acetylated with acetic anhydride–pyridine. Chromatography (SiO_2; toluene–ethyl acetate, 3:2) of the product gave **15** (60 mg, 48%), m.p. 239–240°, $[\alpha]_D^{25}$ +12° (*c* 1, chloroform); lit.[12] m.p. 239–240°, $[\alpha]_D^{27}$ +12° (*c* 0.2, chloroform).

*2-(2-Methoxycarbonylethylthio)ethyl β-*D*-galactopyranoside* (**18**). — To a solution of **1** (1 g, 2.32 mmol) in dry methanol (25 mL) was added methanolic sodium methoxide from sodium (0.05 g) in methanol (10 mL). After 4 h, the mixture was neutralised with Duolite (H^+) resin, filtered, and concentrated, to give **18** (590 mg, 78%), which was pure according to t.l.c. (SiO_2; chloroform–methanol–water, 65:35:10) and had $[\alpha]_D$ −3° (*c* 1, water). ^{1}H-N.m.r. data (D_2O, external Me_4Si): δ 4.44 (d, 1 H, $J_{1,2}$ 7.62 Hz, H-1), 4.08 (m, 1 H, OCH_2-CH_2), 3.72 (s, 3 H, OMe), 3.52 (dd, 1 H, H-2), and 2.92–2.70 (m, 6 H, CH_2-S-CH_2, CH_2CO).

*2-(2-Methoxycarbonylethylthio)ethyl 4-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranoside* (**19**). — By essentially the above procedure, **2** (300 mg) was converted into **19** (140 mg, 75%), $[\alpha]_D^{24}$ +75° (*c* 1.4, water). ^{1}H-N.m.r. data (D_2O, TSP): δ 4.97 (d, 1 H, *J* 3.5 Hz, H-1′), 4.50 (d, 1 H, *J* 7.6 Hz, H-1), and 3.74 (s, 3 H, MeO).

*2-(2-Methoxycarbonylethylthio)ethyl 2-acetamido-2-deoxy-4-*O*-β-*D*-galactopyranosyl-β-D-glucopyranoside* (**20**). — To a solution of **3** (2.01 g, 2.3 mmol) in

methanol (50 mL) was added sodium methoxide (50 mg). The mixture was stirred overnight, neutralised with Duolite (H^+) resin, filtered, and concentrated. The residue was heated (reflux) with hydrazine hydrate (990 mg, 19.8 mmol, 0.95 mL) in ethanol (100 mL) for 3.5 h. Evaporation of the solvent and co-concentration with 1-butanol gave the free amino sugar as a yellow solid, a solution of which in ethanol (100 mL) and water (30 mL) was stirred with acetic anhydride (20 mL) at room temperature for 3 h. T.l.c. (ethyl acetate–methanol–water, 4:2:1) then revealed two products. The solvent was removed and the residue was subjected to chromatography (SiO_2; chloroform–methanol, 2:1), to give **20** (482 mg, 39%) and 2-[2-(2-acetylhydrazino)carbonylethylthio]ethyl 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**21**; 180 mg, 14%).

Compound **20** had $[\alpha]_D^{24}$ −14° (*c* 0.8, water). ^{1}H-N.m.r. data (D_2O, TSP): δ 4.54 (bd, 1 H, *J* 7.0 Hz, H-1), 4.45 (d, 1 H, *J* 7.4 Hz, H-1′), 3.70 (s, 3 H, MeO), 2.85–2.65 (m, 6 H, CH_2-S-CH_2 and CH_2CO), and 2.03 (s, 3 H, AcN).

Compound **21** had $[\alpha]_D^{24}$ −13° (*c* 0.5, water). ^{1}H-N.m.r. data (D_2O, TSP): δ 4.60 (bd, 1 H, *J* 7.5 Hz, H-1), 4.49 (d, 1 H, *J* 7.6 Hz, H-1′), 2.92–2.62 (m, 6 H, CH_2-S-CH_2, CH_2CO), 2.08, and 2.06 (2 s, each 3 H, 2 AcN).

2-(p-Aminophenylthio)ethyl β-D-galactopyranoside (**22**). — Deacetylation of **4** (1 g), essentially as described above, gave **22** (510 mg, 77%) as a glass, $[\alpha]_D^{22}$ −2° (*c* 1.8, water). ^{1}H-N.m.r. data [$(CD_3)_2SO$ plus D_2O, 50°, TSP]: δ 7.14, 6.58 (2 d, each 2 H, *J* 8.4 Hz, aromatic), 4.11 (m, virtual coupling, 1 H, H-1), and 2.91 (t, 2 H, *J* 7.3 Hz, CH_2-S).

2-(Octadecylthio)ethyl 4-O-α-D-galactopyranosyl-β-D-galactopyranoside (**23**). — Deacetylation of **9** (280 mg), essentially as described above, gave **23** (183 mg, 95%), $[\alpha]_D^{27}$ +33.5° (*c* 0.8, methanol–chloroform, 1:1). ^{1}H-N.m.r. data (CD_3OD–$CDCl_3$ 1:1, Me_4Si): δ 5.01 (d, 1 H, *J* 2.8 Hz, H-1′), 4.31 (d, 1 H, *J* 7.2 Hz, H-1), 2.78 (t, 2 H, *J* 7.2 Hz, CH_2-S), 2.57 (t, 2 H, *J* 7.3 Hz, CH_2-S), and 0.89 (t, 3 H, *J* 6.5 Hz, Me).

1,15-Bis(4-O-α-D-galactopyranosyl-β-D-galactopyranosyloxy)-3,13-dithiapentadecane (**24**). — Deacetylation of **12** (300 mg), essentially as described above, gave **24** (135 mg, 75%), $[\alpha]_D^{27}$ +58° (*c* 0.8, methanol). ^{1}H-N.m.r. data (D_2O, TSP): δ 4.96 (d, 2 H, *J* 3.7 Hz, H-1′), 4.48 (d, 2 H, *J* 7.6 Hz, H-1), 2.81 (t, 4 H, *J* 6.5 Hz, CH_2-S-CH_2), and 2.62 (t, 4 H, *J* 7.3 Hz, CH_2-S-CH_2).

Ethyl 4-O-α-D-galactopyranosyl-β-D-galactopyranoside (**25**). — Deacetylation of **14** (4.80 g), essentially as described above, gave **25** (2.69 g, 60%) as a foam, $[\alpha]_D^{21}$ +106° (*c* 1.1, water). ^{1}H-N.m.r. data [$(CD_3)_2SO$ plus D_2O, TSP]: δ 4.83 (d, 1 H, *J* 3.5 Hz, H-1′), 4.12 (d, 1 H, *J* 7.0 Hz, H-1), and 1.14 (t, 3 H, *J* 7.0 Hz, Me).

Ethyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranoside (**26**). — Deacetylation, dephthaloylation, and *N*-acetylation of **15** (1.87 g), essentially as described for **3**, gave **26** (502 mg, 49%), $[\alpha]_D^{28}$ −22° (*c* 0.3, water). ^{1}H-N.m.r. data (D_2O, TSP): δ 4.54 (bd, 1 H, H-1), 4.46 (d, 1 H, *J* 7.8 Hz, H-1′), 2.16 (s, 3 H, MeCO), and 1.15 (t, 3 H, *J* 7.0 Hz, CH_3CH_2).

REFERENCES

1 J. DAHMÉN, T. FREJD, G. GRONBERG, T. LAVE, G. MAGNUSSON, AND G. NOORI, *Carbohydr. Res.*, 116 (1983) 303-307.
2 J. DAHMÉN, T. FREJD, G. MAGNUSSON, AND G. NOORI, *Carbohydr. Res.*, 111 (1982) c1–c4.
3 C. P. STOWELL AND Y. C. LEE, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 225–281; J. D. APLIN AND J. C. WRISTON, JR., *CRC Crit. Rev. Biochem.*, (1981) 259–306; R. U. LEMIEUX, D. R. BUNDLE, AND D. A. BAKER, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
4 R. U. LEMIEUX, *Chem. Soc. Rev.*, (1978) 423–452.
5 J. H. PAZUR, *Adv. Carbohydr. Chem. Biochem.*, 39 (1981) 405–447.
6 J. C. CHABALA AND T. Y. SHEN, *Carbohydr. Res.*, 67 (1978) 55–63; J. S. SLAMA AND R. R. RANDO, *Biochemistry*, 19 (1980) 4595–4600.
7 E. A. KABAT, *Methods Enzymol.*, 70 (1980) 3–49.
8 N. SHARON AND H. LIS, *Science*, 177 (1972) 949–959.
9 R. L. AUGUSTINE, *Catalytic Hydrogenation*, Dekker, New York, 1965.
10 B. R. BAKER, M. V. QUERRY, S. BERNSTEIN, S. R. SAFIR, AND Y. SUBBAROW, *J. Org. Chem.*, 12 (1947) 167–173.
11 E. FISCHER AND F. ARMSTRONG, *Ber.*, 35 (1902) 3153–3155.
12 J. DAHMÉN, T. FREJD, G. MAGNUSSON, AND G. NOORI, *Carbohydr. Res.*, 114 (1983) 328–330.