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Preliminary communication

Preparation and applications of 2-bromoethyl glycosides: synthesis of spacer-arm glycosides and agglutination inhibitors

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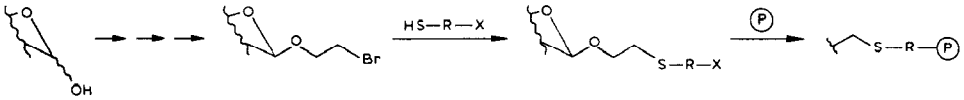
The role of glycoconjugates in biological receptor interactions is now widely appreciated[1]. Chemical synthesis and modification of glycoconjugates has led to artificial immunogens for the preparation of carbohydrate-specific antibodies[2] and artificial glycolipids for incorporation into liposomes[3]. Functionalised glycosides have been used for the preparation of solid phases for affinity chromatography[4]. In all these cases, there is a need for stereospecific syntheses of pure α- or β-glycosides having spacer-arm aglycons for coupling to carrier molecules or particles.

Although *stepwise* construction of spacer-arm glycosides has been described[5], there is still need for general methods, especially those that would allow variation of the spacer arm *after* glycoside synthesis.

We now report a synthesis of spacer-arm glycosides, based on the initial preparation of an anomerically pure 2-bromoethyl glycoside followed by reaction with a suitably functionalised thiol and then coupling to the carrier:



X = NH_2 or CO_2Me

Ⓟ = Protein or particle

The 2-bromoethyl group is stable under the conditions of many of the reactions used in the synthesis of oligosaccharides. 2-Bromoethyl glycosides can be prepared expediently from monosaccharides and some disaccharides by reaction of the acetylated derivative with 2-bromoethanol in dichloromethane containing boron trifluoride etherate[6–8]. Pure, crystalline 1,2-*trans* glycosides can usually be obtained directly from the crude reaction mixtures. Because of the difficulty of separating anomeric glycosides of higher oligosaccharides, other methods of glycoside synthesis were investigated. Thus, 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**1**) and 2-bromoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**2**) were prepared by reacting the corresponding acetylated

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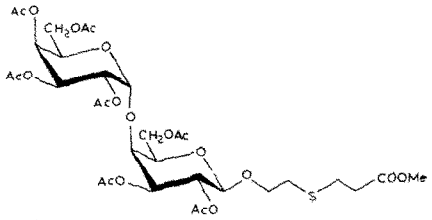
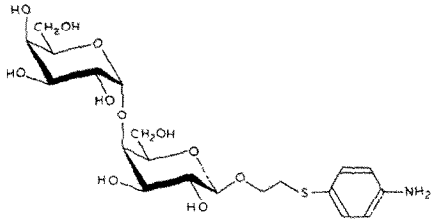
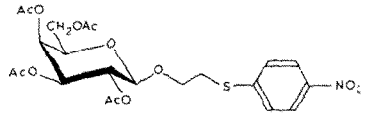
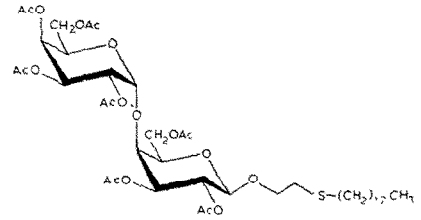
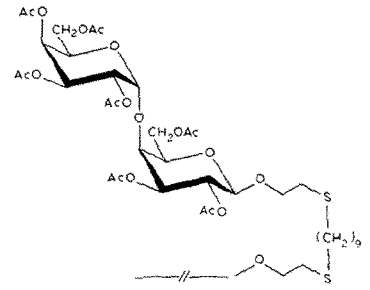
TABLE I

TRANSFORMATION PRODUCTS[a] OF 2-BROMOETHYL GLYCOSIDES

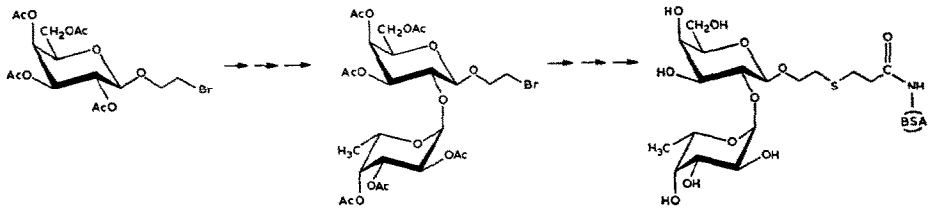
		Method[b]	*Yield (%)*[c]	$[\alpha]_D$ *(degrees)*
	5	*A*	80	+70 (*c* 0.6, $CHCl_3$)
	6	*B*	97	+71 (*c* 0.6, H_2O)
	7	*C*	62	−15 (*c* 1.1, $CDCl_3$)
	8	*D*	69	+55 (*c* 0.5, $CHCl_3$)
	9	*E*	30	+62 (*c* 0.9, $CHCl_3$)

[a] Elemental analyses and ^1H- and ^{13}C-n.m.r. data were consistent with the structures assigned. [b] *A*, 3, methyl 3-mercaptopropionate, $Me(n\text{-}C_8H_{17})_3N^+Cl^-$, Cs_2CO_3, benzene, water, room temperature, 16 h; *B*, 3, *p*-aminothiophenol, NaH, $HCONMe_2$, room temperature, 2 h, and then NaOMe/MeOH; *C*, 1, *p*-nitrothiophenol, NaH, $HCONMe_2$, room temperature, 1.5 h; *D*, 3, octadecylthiol, Cs_2CO_3, $HCONMe_2$, room temperature, 24 h; *E*, 3, nonane-1,9-dithiol, $Me(C_8H_{17})_3N^+Cl^-$, NaOH, H_2O, benzene, room temperature, 24 h. [c] Isolated by chromatography.

sugar (5 mmol, αβ-mixture) with 2-bromoethanol (6 mmol) in dry dichloromethane containing boron trifluoride etherate (25 mmol) at room temperature for 5 and 20 h, respectively; **1** (46%) had m.p. 114–116°, $[\alpha]_D^{24}$ −5° (*c* 1.4, deuteriochloroform), and was isolated by crystallisation; **2** (80%) was a syrup, $[\alpha]_D^{23}$ −11° (*c* 0.7, chloroform), isolated by chromatography on silica gel. 2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**3**) was prepared from the disaccharide octa-acetate[9] (29.5 mmol) *via* the acetobromo sugar {m.p. 97–99°, $[\alpha]_D^{21}$ +219° (*c* 0.8, chloroform)}, by reaction under nitrogen with 2-bromoethanol (140 mmol) in dichloromethane and 2,4,6-trimethylpyridine (30 mmol), initially at −78°, using silver triflate (40 mmol). During 23 h, the stirred mixture was allowed to attain room temperature; **3** (77%) had m.p. 174–177° (from methanol), $[\alpha]_D^{23}$ +75° (*c* 2, chloroform). 2-Bromoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**4**) was prepared from the corresponding oxazoline derivative[10] (14 mmol) and 2-bromoethanol (68 mmol) in toluene–nitromethane (1 : 1), using a catalytic amount of toluene-*p*-sulphonic acid, at 110° for 10 min; **4** (39%), isolated by chromatography, had m.p. 174–175°, $[\alpha]_D^{24}$ −7° (*c* 1.1, deuteriochloroform). Elemental analyses and ^{1}H- and ^{13}C-n.m.r. data were consistent with the structures assigned.

It is important that the reactions of the 2-bromoethyl glycosides with functionalised thiols be clean and high-yielding, since they are to be used in late stages of the sequences leading to the spacer-arm glycosides. Also, it should be possible to use protected sugars (*e.g.*, acetates), since this would widen the choice of chromatographic methods for final purification. Table I gives some representative examples, including reaction with a dithiol which leads to a bidentate inhibitor glycoside.

The 2-bromoethyl group is compatible with many of the synthetic reactions used in carbohydrate chemistry, as illustrated by the synthesis of the 2-bromoethyl β-glycoside of H blood-group specific disaccharide **10** {2-bromoethyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside, $[\alpha]_D$ −77° (*c* 0.95, chloroform); ^{1}H-n.m.r. data ($CDCl_3$, internal Me_4Si): δ 5.43 (d, 1 H, *J* 3.7 Hz, H-1′), 4.55 (d, 1 H, *J* 7.9 Hz, H-1), and 1.15 (d, 3 H, *J* 6.7 Hz, H-6′)}. The sequence used, which was essentially that described by Paulsen[11], included, as one stage, hydrogenolysis of benzyl groups; if acetic acid was used as the reaction solvent, the 2-bromoethyl group remained intact[12].



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Much ingenuity has been spent on the problem of attaching spacer-arm glycosides to macromolecules[13]. We have applied well-established reaction conditions to attach **5** and **6** to proteins. Compound **5** was deacetylated and coupled to bovine serum albumin (BSA) and key-hole limpet haemocyanin (KLH) by the method developed by Inman *et al.*[14] and

Lemieux *et al.*[13c]. Assessment of the degree of binding (number of hapten molecules per molecule of protein) by the phenol–sulphuric acid method[15] and by sulphur analysis revealed that ~30 and ~225 hapten molecules were bound to BSA and KLH, respectively.

Compound **6** was coupled, using the thiophosgene method[16], to BSA and KLH. The degrees of binding were 19 and 490, respectively.

Following a route similar to that for **5**, the 2-bromoethyl glycoside **10** was transformed into the corresponding thioether ester {2-(2-methoxycarbonylethylthio)ethyl 2-*O*-α-L-fucopyranosyl-β-D-galactopyranoside, $[\alpha]_D^{20}$ −75° (*c* 0.7, water); ^{1}H-n.m.r. data (D_2O, external Me_4Si): δ 5.29 (d, 1 H, *J* 3.6 Hz, H-1′), 4.54 (d, 1 H, *J* 7.7 Hz, H-1), 3.75 (s, 3 H, OMe), and 1.23 (d, 3 H, *J* 6.4 Hz, H-6′)} and then coupled to BSA.

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