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Note

Synthesis of some cluster glycosides suitable for attachment to proteins or solid matrices*

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Synthetic glycosides suitable for attachment to proteins, or for incorporation into solid matrices, have proved useful in biological research on the potential elucidation of the roles of carbohydrates[1]. In all these studies, simple glycosides of mono- or di-saccharides[2,3] were used, and their distribution in solid matrices or proteins was assumed to be random. However, recognition of carbohydrate groups by cells or binding proteins may require local concentration, or "clustering", of sugar residues. In natural glycoconjugates, such clustering is often provided by branched structures[4].

In order to examine the effect of clustering, a series of glycosides containing more than one sugar residue per point of attachment has been synthesized. The general structure of these glycosides, as shown in the formulas, is that of a tri-glycoside possessing a terminal amino or hydrazido group for attachment to carboxyl or amino groups, respectively, of proteins and solid matrices. These "cluster glycosides" are currently used in a number of biological systems[5].

EXPERIMENTAL

Materials. — 2-Amino-2-(hydroxymethyl)-1,3-propanediol (**1**, Sigma Chem. Co.), 6-(benzyloxycarbonamido)hexanoic acid (Bachem Inc., Marina Del Rey, CA), adipic acid monomethyl ester (methyl hexanedioate), 2-ethoxy-*N*-(ethoxycarbonyl)-1,2-dihydroquinoline (**2**, Aldrich Chem. Co.), and 10% palladium-on-charcoal catalyst (Matheson, Coleman and Bell) were commercially obtained, and used without purification. 6-(Trifluoroacetamido)hexanoic acid[6], 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[7] (**3**), and its D-galactopyranosyl counterpart[7] (**4**), and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[8] (**5**) were prepared by the published methods indicated.

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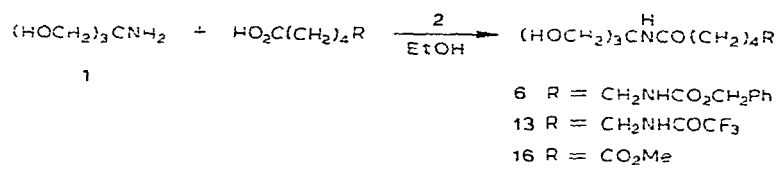
Methods. — Elemental analyses were performed by Galbraith Labs. (Knoxville, TN). Measurements of neutral sugars by the phenol–sulfuric acid method[9], of amino sugars by automated ion-change chromatography[10], and of ester groups by the hydroxamic acid method[11] were conducted by the published procedures.

For measurement of the amino group, the following modification of the TNBS (2,4,6-trinitrobenzenesulfonic acid) method[12] was used. To 0.5 ml of a sample solution (0.02–0.10 μmol of primary amine or hydrazide) were added 0.5 ml each of 0.2M borate buffer (pH 8.7) and 0.2% TNBS, and the mixture was incubated for 30 min at 55°, and then diluted with 0.5M hydrochloric acid (2 ml). The absorbance at 340 nm was read with a suitable spectrophotometer. When this method was used to measure hydrazide, the reaction mixture was kept for 30 min at room temperature, and then diluted with water (2 ml). The absorbance at 500 nm was measured with a Bausch and Lomb colorimeter, Spectronic 20. 6-Aminohexanoic acid and butanoic hydrazide (both from Aldrich Chem. Co.) were used as the respective standard. Proton magnetic resonance (p.m.r.) spectra were recorded with a Jeol NMH-100 spectrometer.

Thin-layer chromatography (t.l.c.) was conducted on silica gel F-254, precoated on alumina (E. Merck Co.), by use of the following solvent systems: (*A*), 4:1 (v/v) ethyl acetate–acetone, and (*B*) 4:1 (v/v) benzene–ethyl acetate. For unprotected glycosides, cellulose precoated on alumina (E. Merck Co.) was used with the following solvent systems: (*1*) 3:2:1 (v/v) ethyl acetate–acetic acid–water, and (*2*) 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water.

[6-(Benzyloxycarbonamido)hexanamido]tris(hydroxymethyl)methane (**6**). — A mixture of **1** (50 mmol, 6.06 g), 6-(benzyloxycarbonamido)hexanoic acid (55 mmol,



14.6 g), and **2** (60 mmol, 14.8 g) in absolute ethanol (500 ml) was boiled under reflux for 5 h, allowed to cool to room temperature, and evaporated to a syrup. Addition of anhydrous ether (250 ml) yielded crystals of **6**. Recrystallization from ethyl acetate (250 ml) gave 11.7 g of pure **6** (88.5% yield), m.p. 82–83°; p.m.r. data (Me_2SO-d_6): δ 1.1–1.7 (m, 6, C–CH_2), 2.20 (t, 2, CO–CH_2), 3.06 (m, 2, N–CH_2), 3.64 (d, 6, O–CH_2), 4.94 (t, 3, OH), 5.94 (s, 2, Ph–CH_2), 7.34 (m, 1, CO–NH), and 7.54 (s, 5, Ph). After deuterium exchange, the signals at δ 4.94 and 7.34 disappeared.

Anal. Calc. for $C_{18}H_{28}N_2O_6$ (368.52): C, 58.69; H, 7.66; N, 7.60. Found: C, 58.72; H, 7.67; N, 7.65.

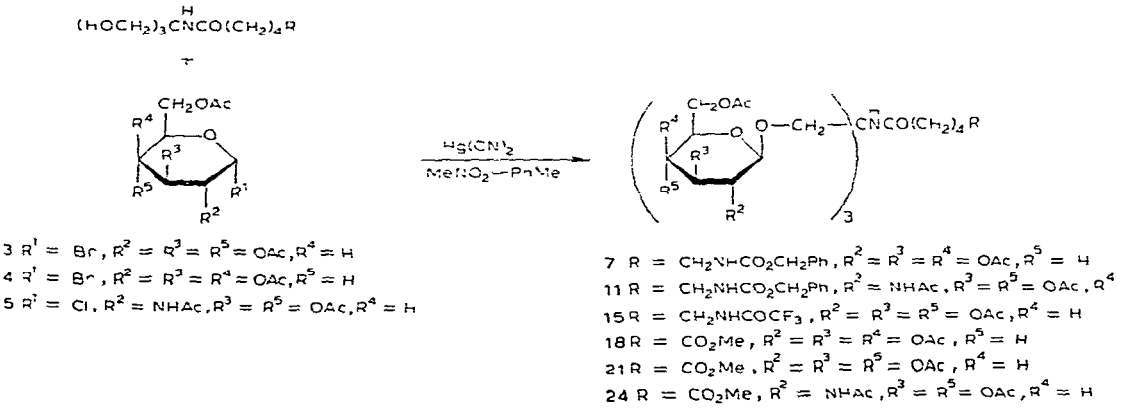
*[6-(Benzyloxycarbonamido)hexanamido]tris[(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)oxymethyl]methane* (**7**). — A mixture of **6** (10 mmol, 3.68 g), **4** (30 mmol, 12.3 g), and mercuric cyanide (30 mmol, 7.57 g) in 1:1 (v/v) benzene–nitromethane

(200 ml) was stirred at room temperature. After ~24 h, 10 mmol each of **4** and mercuric cyanide were added, and the mixture was stirred for 2 more days. The mixture was evaporated to a syrup, which was dissolved in chloroform (150 ml); the solution was washed four times with M sodium chloride, dried (sodium sulfate), and evaporated to a syrup. This was purified in 3 batches on a column (4 × 190 cm) of Sephadex LH-20 with 95% ethanol as the eluant. The fractions containing triglycoside **7** were combined, and evaporated to a syrup (9.0 g, 66.2% yield), which was homogeneous by t.l.c. in solvent *A*.

6-(Aminohexanamido)tris(β-D-galactopyranosyloxymethyl)methane (**8**). — Deacetylation of **7** (9.0 g, 6.6 mmol) with barium methoxide (1.5 mmol) in dry methanol (60 ml) overnight at room temperature, followed by de-ionization, evaporation, and repeated co-evaporation with toluene, yielded hygroscopic crystals (5.1 g, 5.9 mmol; 90%) of **9**, m.p. 135–138°.

Hydrogenolysis of **9** (3.0 g) in 60% acetic acid (50 ml) in the presence of 10% palladium-on-charcoal (0.35 g) was conducted in a Brown hydrogenator[13]. After 6–7 h, the reaction was complete; the catalyst was then filtered off, the filtrate was concentrated, and the concentrate fractionated on a column (5 × 195 cm) of Sephadex G-25 (fine) equilibrated in 0.1M acetic acid. Fractions 151–162 (20 ml per fraction) contained pure **8** (t.l.c., solvents *1* and *2*); they were pooled, and evaporated, to yield amorphous **8** (2.2 g, 87%).

The p.m.r. spectra of **9** and **8** confirmed the structures assigned; they showed the appropriate functional groups (benzyl, aminohexanoyl, oxymethylene, and galactose) in correct ratios. Furthermore, colorimetric analysis for the amino group (TNBS method) and galactose (phenol–sulfuric acid method) in **8** indicated the ratio of these groups to be 1.00:3.13.



Tris(2-acetamido-2-deoxy-β-D-glucopyranosyloxymethyl)-(6-aminohexanamido)-methane (**10**). — A solution of **6** (10 mmol) in 1:1 (v/v) nitromethane–benzene (200 ml) was stirred with **5** (30 mmol) and mercuric cyanide (30 mmol) for 2 days.

After addition of a further 20 mmol each of **5** and mercuric cyanide, the mixture was stirred for 2 more days. The insoluble residue was filtered off, and the filtrate was evaporated to a syrup; the residue was dissolved in chloroform (150 ml), and the solution was washed 3 times with M sodium chloride, dried (sodium sulfate), and evaporated.

Fractionation of the products on a column of Sephadex LH-20 (as for **7**) yielded **11**, which was deacetylated as usual, to yield **12** as an amorphous material, the combined yield from the two steps being 73%. Hydrogenolysis of **12**, and subsequent fractionation on a column of Sephadex G-25 as already described, gave pure **10** (t.l.c., solvent *1*) in 85% yield.

Analysis for amino group (TNBS method) and amino sugar (sugar analyzer) showed the ratio of 1.00:2.96. The p.m.r. spectrum of **10** was in agreement with the structure expected, showing *N*-acetyl, aminohexanoyl, and oxymethylene groups in the correct ratios.

2-(Hydroxymethyl)-2-[6-(trifluoroacetamido)hexanamido]-1,3-propanediol (**13**).— Compound **13** was prepared from 6-(trifluoroacetamido)hexanoic acid and **1**, with **2** as the coupling agent, in the same way as for the preparation of **6**. Crystalline **13** was obtained in 87% yield; m.p. 97–99°. Its p.m.r. spectrum was in agreement with the structure given.

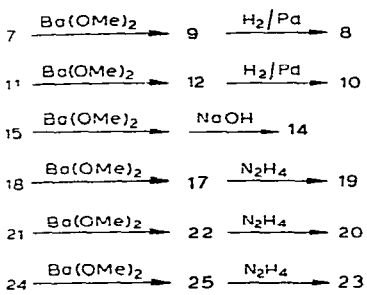
Anal. Calc. for $C_{12}H_{21}F_3N_2O_5$ (330.31): C, 43.63; H, 6.41; N, 8.48. Found: C, 43.68; H, 6.42; N, 8.59.

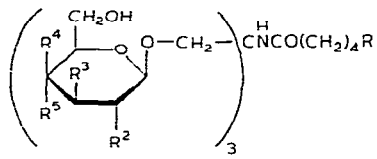
(6-Aminohexanamido)tris(β-D-glucopyranosyloxymethyl)methane (**14**). — A mixture of **13** (0.99 g, 3 mmol), **3** (5.75 g, 14 mmol), and mercuric cyanide (3.54 g, 14 mmol) in 1:1 (v/v) toluene–nitromethane (100 ml) was stirred for 2 h at 60°. The mixture was evaporated, giving a semi-solid mass, which was shaken with a mixture of chloroform (100 ml) and M sodium chloride (100 ml). The chloroform solution was washed twice with M sodium chloride solution, dried, and evaporated to a syrup, which was fractionated on a column of Sephadex LH-20 as already described. The fractions containing **15** were pooled, and evaporated, and the residue was co-evaporated repeatedly with toluene for removal of water. The syrupy **15** was de-acetylated with 0.02M barium methoxide (50 ml) overnight at room temperature, and evaporated to a syrup, which was treated with M sodium hydroxide (20 ml) for 4 h at room temperature for *N*-de(trifluoroacetyl)ation. The mixture was acidified with glacial acetic acid, and applied to a column (5 × 195 cm) of Sephadex G-25 in 0.1M acetic acid for purification as described for **8**. The fractions containing **14** (by analyses for carbohydrate and amino group) were pooled, and evaporated to dryness, to yield **14**, which contained amino group to glucose in the ratio of 1:2.99. The overall yield of **14** from **13** was 45%.

2-(Hydroxymethyl)-2-(5-methoxycarbonylpentanamido)-1,3-propanediol (**16**). — A mixture of monomethyl adipate (17.6 g, 110 mmol), **1** (12.1 g, 100 mmol), and **2** (29.7 g, 120 mmol) in absolute ethanol (500 ml) was boiled for 5 h under reflux. The mixture was then evaporated to a syrup, which was mixed with ether (400 ml) and kept for 3 h in the cold for crystallization of **16**. The p.m.r. spectrum of the crystals

(33.3 g, 84% yield), m.p. 90–91°, showed the signals for the functional groups in the correct ratios.

Anal. Calc. for $C_{11}H_{21}NO_6$ (263.29): C, 50.18; H, 8.04; N, 5.32. Found: C, 50.33; H, 8.11; N, 5.32.





8 R = CH_2NH_2, $R^2 = R^3 = R^4 = OH$, $R^5 = H$
9 R = $CH_2NH_4CO_2CH_2Ph$, $R^2 = R^3 = R^4 = OH$, $R^5 = H$
10 R = CH_2NH_2, $R^2 = NHAc$, $R^3 = R^5 = OH$, $R^4 = H$
12 R = $CH_2NHCO_2CH_2Ph$, $R^2 = NHAc$, $R^3 = R^5 = OH$, $R^4 = H$
14 R = CH_2NH_2, $R^2 = R^3 = R^5 = OH$, $R^4 = H$
17 R = CO_2Me, $R^2 = R^3 = R^4 = OH$, $R^5 = H$
19 R = $CONHNH_2$, $R^2 = R^3 = R^4 = OH$, $R^5 = H$
22 R = CO_2Me, $R^2 = R^3 = R^5 = OH$, $R^4 = H$
20 R = $CONHNH_2$, $R^2 = R^3 = R^5 = OH$, $R^4 = H$
25 R = CO_2Me, $R^2 = NHAc$, $R^3 = R^5 = OH$, $R^4 = H$
23 R = $CONHNH_2$, $R^2 = NHAc$, $R^3 = R^5 = OH$, $R^4 = H$

Tris(β-D-galactopyranosyloxymethyl)-(5-methoxycarbonylpentanamido)methane (**17**). — A mixture of **16** (1.32 g, 5 mmol), **4** (8.22 g, 20 mmol), and mercuric cyanide (5.06 g, 200 mmol) in 1:1 (v/v) nitromethane–toluene (100 ml) was heated for 2.5 h at 60°. The mixture was evaporated to a syrup, and the syrup was dissolved in chloroform (50 ml). The solution was extracted 3 times with M sodium chloride, dried (sodium sulfate), and evaporated to a syrup, which was fractionated on a column (4 × 150 cm) of Sephadex LH-20 with 95% ethanol. The fractions containing **18** (homogeneous by t.l.c., solvent *A*) were combined, and evaporated to a syrup, which was twice co-evaporated with toluene (to remove water), and deacetylated with barium methoxide as usual. De-ionization, and evaporation of the mixture, yielded amorphous **17** in 75% overall yield; its p.m.r. spectrum was in agreement with the structure expected.

Tris(β-D-galactopyranosyloxymethyl)-(5-hydrazinocarbonylpentanamido)methane (**19**). — Compound **17** (1.5 g, 2.05 mmol) was treated with hydrazine monohydrate (123 mmol; 6 ml) overnight, and then the mixture was repeatedly co-evaporated with toluene. Purification of **19** was accomplished on a column (5 × 195 cm) of Sephadex G-25 with 0.1M acetic acid. Fractions containing pure **19** were pooled, and evaporated, to yield amorphous **19**, 1.29 g (83% yield).

The ratio of hydrazide (TNBS method) to galactose (phenol–sulfuric acid method) in **19** was 1:3.11.

Tris(β-D-glucopyranosyloxymethyl)-(5-hydrazinocarbonylpentanamido)methane (**20**). — Compound **20** was prepared from **16** and **3** *via* **21** and **22**, in essentially the same way as for **19**. The overall yield was 54%, and the ratio of hydrazide group to glucose was 1:3.04.

Tris(2-acetamido-2-deoxy-β-D-glucopyranosyloxymethyl)-(5-hydrazinocarbonylpentanamido)methane (**23**). — Compound **23** was prepared from **16** and **5** as for **19**, *via* intermediates **24** and **25**, the overall yield being 59%. The ratio of hydrazide to 2-amino-2-deoxy-D-glucose in **23** was 1:2.85.

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