Synthesis of Hepta- and Penta-saccharides, Part of the Complex-type Carbohydrate Portion of Glycoproteins

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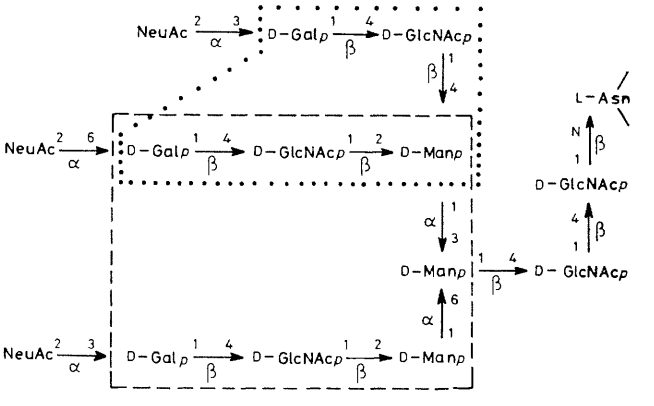
Summary Silver trifluoromethanesulphonate-promoted condensation of 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide with benzyl-3,6-di-*O*-benzyl-α-D-mannopyranoside and benzyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside gave a protected pentasaccharide and a protected heptasaccharide in 56% and 43% yield, respectively, after deblocking the free penta- and heptasaccharides were obtained

THE oligosaccharides that are *N*-glycosidically linked to L-asparagine residues in glycoproteins are of two major types, the 'high-mannose type' which contain D-mannosyl- and *N*-acetyl-D-glucosaminyl-residues and the 'complex type' which contain D-mannosyl-, D-galactosyl-, *N*-acetyl-D-glucosaminyl-, and sialyl-residues [1] The 'complex type' has been found with different degrees of branching The *N*-glycosidically linked carbohydrate portion of fetuin (the predominant glycoprotein of fetal calf serum), which has the structure (**1**),[2] is a representative example

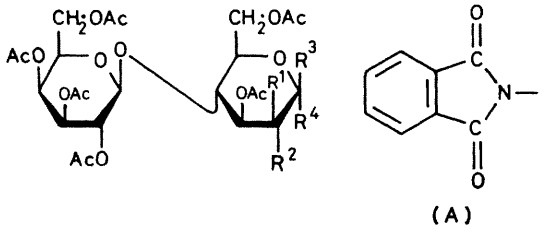
These oligosaccharides are assumed to be involved in different biological functions[1] and the synthesis of oligosaccharides which form parts of these structures is a matter of some interest We now report the synthesis of the reducing heptasaccharide (**2**) [indicated by the dashed line in structure (**1**)] and the reducing pentasaccharide (**3**) [indicated by the dotted line in structure (**1**)]



Hexa-*O*-acetyl-D-lactal[3] (20 g) was subjected to azidonitration[4] by treatment with ceric ammonium nitrate and sodium azide in acetonitrile to give, after work-up, a crystalline mixture (16 5 g) † According to the ^{1}H n m r spectrum

† Yields are not optimized

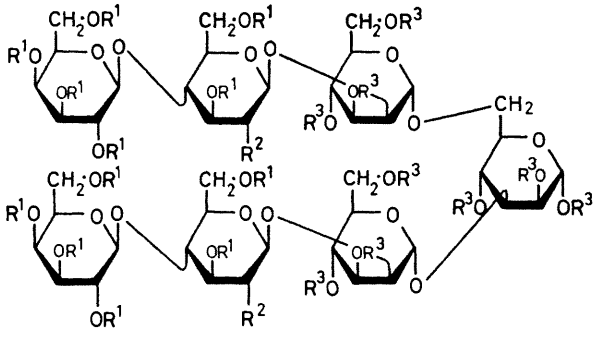
and to the sugar analysis[5] of a sample that had been subjected to hydrogenation over palladium–charcoal, this mixture contained mainly compounds (**4**), (**5**), and (**6**) in the approximate proportions 1:4:8. The mixture was treated



(A)

	R^1	R^2	R^3	R^4
(**4**)	N_3	H	H	ONO_2
(**5**)	H	N_3	H	ONO_2
(**6**)	H	N_3	ONO_2	H
(**7**)	H	A	H	OAc
(**8**)	H	A	OAc	H
(**9**)	H	A	Br	H

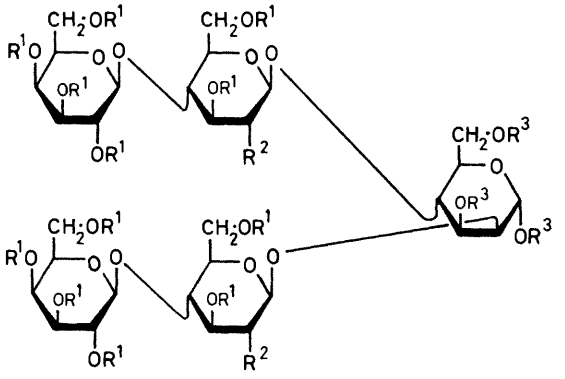
sequentially with hydrogen over palladium–charcoal in ethyl acetate, phthalic anhydride (6 equiv.) in 90% aqueous ethanol adjusted to pH *ca.* 9 (r.t., 2 h), and acetic anhydride–pyridine (r.t., 12 h; 100 °C, 1 h) to give, after silica gel chromatography and crystallization, (**7**)‡ (m.p. 229—230 °C) and (**8**) (m.p. 263—265 °C). In pilot experiments compounds (**7**) and (**8**) were separately transformed into the same crystalline bromide (**9**) (m.p. 109—110 °C) by treatment with hydrogen bromide in methylene chloride (r.t., 4 h). For preparative purposes the crude mixture of (**7**) and (**8**) was used to give (**9**) [34% overall yield from a mixture of (**4**), (**5**) and (**6**)]. Sugar analysis of (**9**) showed D-galactose and D-glucosamine, but no D-mannosamine. The ^{1}H n.m.r. spectrum of (**9**) showed, *inter alia*, a signal for the anomeric proton of the D-glucosaminyl residue at δ 6·39 (1 H, d, *J* 10 Hz) indicating that the β-bromide was obtained. Glycosidation with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide[6] in the presence of silver trifluoromethanesulphonate is known[6,7] to give a high yield of β-glucoside and (**9**) could be expected to behave analogously. For the synthesis of compound (**2**), benzyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside[8] (0·22 mmol) was condensed with (**9**)



(**10**) R^1 = Ac, R^2 = A, R^3 = Bn
(**11**) R^1 = Ac, R^2 = NHAc, R^3 = Bn
(**2**) R^1 = H, R^2 = NHAc, R^3 = H

‡ Satisfactorily n m.r. data were obtained for all compounds.

(0·7 mmol) in methylene chloride, using silver trifluoromethanesulphonate–*s*-collidine (0·7 mmol) as a promotor[7] (−50—+20 °C for 16 h). Compound (**10**) {$[\alpha]_{589}$ + 10° ($CHCl_3$)}, isolated as a syrup in 43% yield after silica gel chromatography, was treated subsequently with sodium methoxide in methanol (r.t., 16 h), hydrazine hydrate[7] (10 equiv. in boiling ethanol, 16 h), and acetic anhydride–pyridine (r.t., 16 h) to give, after silica gel chromatography, compound (**11**) {$[\alpha]_{589}$ + 11° ($CHCl_3$)} in 55% yield. Compound (**11**) was finally subjected to de-*O*-acetylation and de-*O*-benzylation by catalytic hydrogenation (Pd–C catalyst) to give, after gel-filtration (Sephadex G-25), compound (**2**) {$[\alpha]_{589}$ + 14° (H_2O)} in 46% yield. The ^{1}H n.m.r. spectrum (200 MHz, D_2O, 85 °C) of (**2**) showed, *inter alia*, signals for anomeric protons at δ 5·14 (1·7 H, *J* small; α-D-mannose-residue and 1,2-linked α-D-mannosyl-residue), 4·89 (1·3 H, *J* small; β-D-mannose-residue and 1,2-linked α-D-mannosyl-residue), 4·60 br (2 H, d; *N*-acetyl-β-D-glucosaminyl-residues), and 4·46 (2 H, d, *J* 7·5 Hz; β-D-galactosyl-residues). Methylation analysis[9] of the alditol of (**2**) showed 2,3,4,6-tetra-*O*-methyl-D-galactose, 3,4,6-tri-*O*-methyl-D-mannose, 3,6-di-*O*-methyl-*N*-methyl-*N*-acetyl-D-glucosamine, and 1,2,4,5-tetra-*O*-methyl-D-mannitol.



(**13**) R^1 = Ac, R^2 = A, R^3 = Bn
(**14**) R^1 = Ac, R^2 = NHAc, R^3 = Bn
(**3**) R^1 = H, R^2 = NHAc, R^3 = H

Bn = benzyl

Benzyl 3,6-di-*O*-benzyl-α-D-mannopyranoside (**12**) {$[\alpha]_{589}$ + 42° ($CHCl_3$)} was prepared in 40% yield from benzyl α-D-mannopyranoside by tributylstannylation[10] and benzylation. Compound (**12**) (0·38 mmol) and (**9**) (1·1 mmol) were condensed, as described above, to give (**13**) {$[\alpha]_{589}$ + 12° ($CHCl_3$)} in 56% yield after silica gel chromatography. Compound (**13**) was then transformed into (**14**) {$[\alpha]_{589}$ −1° ($CHCl_3$)} in 74% yield, as described for the corresponding transformation (**10**) to (**11**). Removal of the *O*-acetyl- and *O*-benzyl- groups of (**14**) gave (**3**) {$[\alpha]_{589}$ −13° (H_2O)} in 61% yield. The ^{1}H n.m.r. spectrum (200 MHz, D_2O, 85 °C) of (**3**) showed, *inter alia*, signals for anomeric protons at δ 5·16 (0·8 H, 4d, *J* 1·5 Hz; α-D-mannose-residue), 4·90 (0·2 H, 4d, $J < 1$ Hz; β-D-mannose-residue), 4·58 br (2 H, d; *N*-acetyl-β-D-glucosaminyl-residues), and 4·47 (2 H, d, *J* 7·5 Hz; β-D-galactosyl-residues). Methylation analysis[9] of the alditol of (**3**) showed 2,3,4,6-tetra-*O*-methyl-D-galactose,

3,6-di-*O*-methyl-*N*-methyl-*N*-acetyl-D-glucosamine, and 1,3,5,6-tetra-*O*-methyl-D-mannitol

The ^{1}H n.m.r. data given above for (**2**) and (**3**) are in good agreement with those reported for related natural oligosaccharides [11]

We thank Professors Per J. Garegg and Bengt Lindberg for their interest and the Swedish Natural Science Research Council for financial support

(*Received*, 21*st July* 1980, *Com.* 788)

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