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Preliminary communication

A new type of carbohydrate-containing synthetic antigen: synthesis of carbohydrate-containing polysaccharide copolymers with the specificity of O:3 and O:4 factors of *Salmonella*

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Capsular and somatic antigens are responsible for the pathogenic properties of a number of bacteria and are widely used in the serological classification of these microorganisms. Hence, artificial antigens that simulate bacterial antigens have promise for diagnosis[1] and protection[2]. The principle for constructing artificial carbohydrate-containing antigens, proposed more than 50 years ago[3], and which involves covalent attachment[4] of the oligosaccharide units to a carrier protein, remains unchanged.

We now report on the synthesis (based on another principle) of carbohydrate antigens containing no protein by converting oligosaccharide determinants into polymers of high molecular weight *via* the copolymerisation reaction. The same principle was used in the preparation, from certain allyl glycosides and acrylamide, of "pseudopolysaccharides", *i.e.*, linear polyacrylamide polymers with carbohydrate branches[5]. Using this method, we have obtained two synthetic antigens **2a** and **2b** which manifest the group specificity E and B of *Salmonella* (factors O:3 and O:4, respectively).

The key stage in the synthesis of trisaccharide monomer **1a** was the glycosylation of allyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**7**) with 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-L-rhamnopyranosyl bromide[6]. Derivative **7** was obtained as follows. Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with allyl alcohol in chloroform in the presence of silver oxide, followed by Zemplén deacetylation, gave allyl β-D-galactopyranoside {**3**, m.p. 101–102°, $[\alpha]_D^{20}$ −11° (*c* 2, water)}, acetonation of which gave allyl 3,4-*O*-isopropylidene-β-D-galactopyranoside {**4**, m.p. 91–92°, $[\alpha]_D^{22}$ +10° (*c* 2, chloroform)}. Acetylation of **4** yielded allyl 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside {**5**, m.p. 61.5–63°, $[\alpha]_D^{23}$ +24° (*c* 2, chloroform)}. Treatment of **5** with dilute acetic acid gave allyl 2,6-di-*O*-acetyl-β-D-galactopyranoside {**6**, m.p. 75–76.5°, $[\alpha]_D^{20}$ −9.5° (*c* 2, chloroform)}. The reaction of **6** with triethyl orthoacetate in the presence of *p*-toluenesulfonic acid gave the 3,4-(ethyl orthoacetate), hydrolysis of which with 80% acetic acid gave a high yield of **7** {m.p. 81–83°, $[\alpha]_D$ −12.5° (*c* 2.2, chloroform)}. The positions of the hydroxyl groups in **6** and **7** were proved by methylation analysis[7] (methylation with diazomethane–boron trifluoride etherate and reduction with sodium borodeuteride), which gave 1,2,5,6-tetra-*O*-acetyl-

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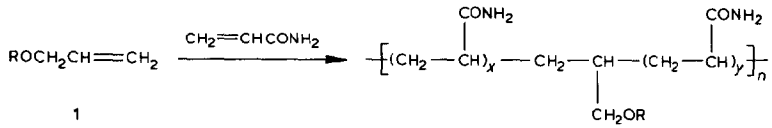

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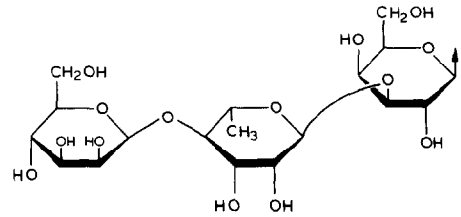

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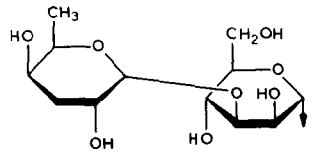

3,4-di-*O*-methyl[1-^{2}H]galactitol and 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl[1-^{2}H]galactitol, respectively, identified by g.l.c.–m.s. The position of HO-3 in **7** followed independently from the ^{13}C-n.m.r. data for **6** and **7** (Table I); the C-4 signal of **7** was shifted to a higher field (α-effect of acetylation), the C-3 and C-5 signals were shifted to lower field (β-effect of acetylation), and the chemical shift of the C-2 signal was almost unchanged.

TABLE I

^{13}C-N.M.R. DATA[a] FOR MONOSACCHARIDE DERIVATIVES

Compound	*Chemical shifts (p.p.m.)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
3	103.3	72.1	74.4	69.7	75.9	61.9
6	101.6	73.5	72.8	70.2	73.9	64.5
7	101.6	73.4	71.0	71.5	72.3	63.2
11	99.0	70.8	70.1	68.2	68.2	62.8
12	96.7	72.4	68.7	68.4	68.4	62.8

[a] Bruker WP-60 instrument: **3**, **6**, and **7**, solutions in CD_3OD; **11** and **12**, solutions in $CDCl_3$; allyl group signals are not reported.

Glycosylation of **7** was performed in acetonitrile in the presence of mercury(II) cyanide to give a moderate yield of the nona-acetate of **1a**, deacetylation of which with methanolic 0.06M sodium methoxide afforded allyl 3-*O*-(4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranosyl)-β-D-galactoside (**1a**), m.p. 232–235°, $[\alpha]_D^{20}$ −51.5° (*c* 1.7, water). The structure of **1a** was confirmed by sugar analysis (rhamnose, mannose, and galactose in the ratios 1:1:1 were detected in a hydrolysate by using a sugar analyser) and by the ^{13}C-n.m.r. spectrum (Table II), the signals of which were assigned on the basis of published data for disaccharides[8].

TABLE II

^{13}C-N.M.R. DATA[a] FOR OLIGOSACCHARIDE AND POLYMER DERIVATIVES

Compound	*Unit*	*Chemical shifts (p.p.m.)*					
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
	Man	101.9	71.8	74.4	68.05	77.4	62.1
1a	Rha	103.6	71.45	71.45	80.8	69.15	18.2
	Gal	102.9	71.8	82.0	69.7	76.3	62.2
	Man	101.8	71.8	74.4	68.2	77.5	62.4[b]
2a	Rha	103.2	71.7[b]	71.7	80.9	69.1	18.2
	Gal	104.4	71.5[b]	81.65	69.75	76.1	62.3[b]
	Abe	101.35	64.7	34.2	69.5	67.9	16.5
1b	Man	100.6	71.3	79.6	67.2	74.1	62.0
	Abe	101.3	64.8	34.3	69.6	68.0	16.8
2b	Man	100.9	71.5	79.5	67.4	74.4	62.2

[a] Solutions in D_2O. [b] Assignments may be reversed.

Allyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside (**1b**), corresponding to the O:4 factor of *Salmonella*, was synthesised by the glycosylation of **12** with 3,6-dideoxy-2,4-di-*O*-(*p*-nitrobenzoyl)-α-D-*xylo*-hexopyranosyl bromide, prepared by the procedure of Eklind *et al.*[9].

Allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**12**) was obtained as follows. Treatment of D-mannose with a boiling solution of 3% of hydrogen chloride in allyl alcohol gave a mixture of allyl mannosides, treatment of which with acetone–2,2-dimethoxypropane and toluene-*p*-sulfonic acid yielded allyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside (**8**), m.p. 59°, $[\alpha]_D$ +12.5° (*c* 2, chloroform). Acid hydrolysis of **8** in aqueous acetone[10] selectively removed one isopropylidene group, to give 70% of allyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**9**), m.p. 76–78°, $[\alpha]_D$ +37.5° (*c* 2, chloroform). Acetylation of **9** afforded allyl 4,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-D-mannopyranoside (**10**), the structure of which was confirmed by p.m.r. spectroscopy. Treatment of **10** with dilute acetic acid then gave allyl 4,6-di-*O*-acetyl-α-D-mannopyranoside (**11**), m.p. 109–110°, $[\alpha]_D$ +58° (*c* 1.5, chloroform). Acetylation of HO-2 in **11** was achieved *via* the 2,3-orthoester derivative, as described above for **7**, to give **12**,

$[\alpha]_D$ +21.5° (*c* 2, chloroform). The structure of **12** was confirmed by methylation analysis (which gave 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl[1-^{2}H] mannitol) and the ^{13}C-n.m.r. data (Table I; α- and β-effects of acetylation were observed for the C-2 and the C-1 and C-3 signals, respectively).

Glycosylation of **12** in acetonitrile by 3,6-dideoxy-2,4-di-*O*-(*p*-nitrobenzoyl)-α-D-*xylo*-hexopyranosyl bromide[9] in the presence of mercury(II) cyanide under argon gave 75% of a disaccharide derivative, $[\alpha]_D$ +124.5° (*c* 2.2, chloroform), which was deesterified with barium oxide in boiling methanol to give **1b**, m.p. 180–182°, $[\alpha]_D$ +131° (*c* 1, water). The α-configuration of the abequosyl bond in **1b** was confirmed by the p.m.r. data; the signal of H-1$_{Abe}$ was a doublet at δ 4.87 ($J_{1,2}$ 4 Hz), and there was coincidence of the C-1,2,3,4,5,6 signals of the abequosyl residue in **1b** and those of methyl α-abequoside.

The glycosides **1a** and **1b** were converted into antigens **2a** and **2b** by radical copolymerisation with acrylamide (weight ratio, 2:1) in water in the presence of ammonium persulphate and *N,N,N',N'*-tetramethylethylenediamine. The polymers, which contained 30–34% of carbohydrates, were isolated in yields of 24–28% (based on allyl glycosides) by gel filtration on Sephadex G-50. Aqueous solutions of **2a** and **2b** were ultrafiltered through Amicon Diaflo membranes; the polymers passed freely through an XM 300 membrane and were almost completely retained by an XM 100 membrane, which corresponds to a mass within the range 100–300 kilodaltons. The structures of **2a** and **2b** were confirmed by quantitative sugar analysis after acid hydrolysis, and by the ^{13}C-n.m.r. spectra (Table II) where the signals of the carbons of the carbohydrate residues coincided with those for **1a** and **1b**, respectively. In addition, the spectra of copolymers gave signals for $CONH_2$ (181.2 and 180.5 p.p.m.), CH (43.2 p.p.m.), and CH_2 groups (37.0 and 36.2 p.p.m.). From the ratio of the integrated intensities of the signals for C-6 in the galactose (or mannose) residue and the methine groups, the relative content of $-CH_2CH(OR)$ fragments (where R is the oligosaccharide residue) with respect to $-CH_2CH(CONH_2)$ fragments was estimated as 1:13 for **2a** and 1:10 for **2b**.

The results of the immunogenicity, and the serological and the protective properties, of the synthetic antigens **2a** and **2b** will be published elsewhere.

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