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A NEW TYPE OF CARBOHYDRATE-CONTAINING SYNTHETIC ANTIGEN: SYNTHESIS OF CARBOHYDRATE-CONTAINING POLYACRYLAMIDE COPOLYMERS HAVING THE SPECIFICITY OF O:3 AND O:4 FACTORS OF *Salmonella*

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ABSTRACT

The synthesis of a new type of synthetic antigen that contains no protein is described. Two linear polyacrylamide copolymers with carbohydrate branches were obtained *via* radical copolymerisation of the allyl glycosides of the oligosaccharide determinants *O*-β-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-galactopyranose and *O*-3,6-dideoxy-α-D-*xylo*-hexopyranosyl-(1→3)-α-D-mannopyranose with acrylamide. These copolymers, which contained 30% of carbohydrate and had molecular masses exceeding 100 kilodaltons, had the group specificity E and B of *Salmonella*.

INTRODUCTION

Carbohydrate-containing antigens, exposed on the surface of bacterial cells, are responsible for the immunological specificities and pathogenic properties of many micro-organisms. These antigens include capsular polysaccharides[1], lipopolysaccharides[2], and teichoic acids[3]. Artificial antigens that simulate bacterial antigens show great promise for diagnosis[4] and protection[1,5]. The principle for constructing artificial carbohydrate-containing antigens, based on the covalent attachment of the oligosaccharide determinants to a macromolecular carrier (usually a protein), was proposed[6] many years ago and has not changed essentially during the past 50 years. There have been attempts to develop new techniques for attaching the determinants to a carrier protein[7]. Protein-containing carbohydrate antigens, however, cannot be considered as adequate models for polysaccharide antigens, since they simulate the specificity rather than immune response. This is due to the fact that artificial neoglycoproteins are T-dependent, *i.e.*, require T-lymphocyte participation, whereas natural polysaccharide antigens are capable of stimulating B-lymphocytes in the absence of T-cells (T-independent)[8].

Thus, for instance, although synthetic antigens of the neoglycoprotein type stimulate the formation of immunoglobulins having the same specificity as the nat-

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ural polysaccharide, these immunoglobulins do not precipitate microbial cells, whereas the antibodies against polysaccharide possess this capacity[9]. Consequently, there is a need to construct antigens that have the main feature of polysaccharide T-independent antigens, namely, the regularity of chemical structure with multiple repetition of determinant groups along the linear chain.

An approach to this type of synthetic antigen could involve polymerisation of suitable oligosaccharide derivatives or attachment of the carbohydrate units to regular polymers. The "pseudopolysaccharides" obtained in such a way should be characterised by multiple repetition of oligosaccharide determinants, which is typical for bacterial antigenic polysaccharides.

Recently, there has been a growing interest in the synthesis of "pseudopolysaccharides" in relation to the development of polymeric drugs and solid-phase glycosidic synthesis. "Pseudopolysaccharides" have been synthesised, for instance, by reaction of epoxypropyl derivatives of galactose with polyvinyl alcohol[10], formation of malto-oligosaccharide hydrazones with polyacrylic acid hydrazide[11], polymerisation of 1-*O*-(11-methacryloyloxyundecanoyl)-β-D-glucopyranose[12] and D-glucopyranosyl isocyanides[13], and copolymerisation of allyl glycosides of 2-acetamido-2-deoxy-D-galactose, L-fucose, D-mannose, and D-galactose with acrylamide[14].

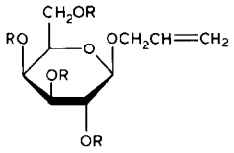
We have been concerned with the synthesis of pseudopolysaccharides containing oligosaccharide determinants of natural antigens, and we now report on the first synthesis of antigens that do not contain a protein carrier.

RESULTS AND DISCUSSION

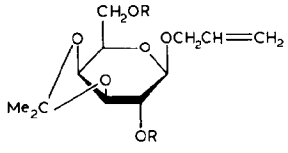
The conversion of oligosaccharide determinants into polymers of high molecular weight *via* copolymerisation reactions is based on the method first used by Hořejší *et al.*[14] and involving allyl glycosides of monosaccharides and acrylamide. Using this method, we have obtained two synthetic antigens which show the group specificity E and B of *Salmonella* (factors O:3 and O:4, respectively); preliminary results have been published[15]. In *Salmonella* serogroups E and B, the group-specific determinants are *O*-β-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-galactopyranose[16] and *O*-α-abequopyranosyl-(1→3)-α-D-mannopyranose[17]. The synthesis of these determinants involved the preparation of the appropriate allyl hexopyranoside with HO-3 unsubstituted, followed by glycosylation with corresponding glycosyl bromides.

The trisaccharide monomer **11** was synthesised as follows. Acetonation of allyl β-D-galactopyranoside (**1**) in the presence of anhydrous cupric sulfate and toluene-*p*-sulfonic acid gave mainly the 3,4-*O*-isopropylidene derivative **2** together with the 4,6-isomer **3**, the structures of which were confirmed by the chemical shifts[18] of the CMe_2 groups in the ^{1}H-n.m.r. spectra of the corresponding acetates **5** and **6**. When **6** was heated with 65% acetic acid, 95% of the 2,6-diacetate **7** was obtained. Treatment of **7** with triethyl orthoacetate in the presence of toluene-*p*-

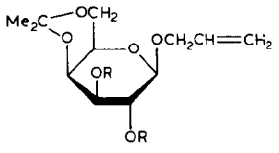
sulfonic acid gave the 3,4-(ethyl orthoacetate), which was not isolated but hydrolysed with 80% acetic acid to give allyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**8**, 80% from **7**). Orthoesters fused to a pyranoid ring are cleaved under these conditions[19] to yield an axial ester.



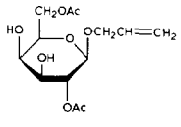
1 R = H
4 R = Ac



2 R = H
5 R = Ac



3 R = H
6 R = Ac



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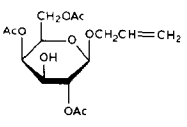

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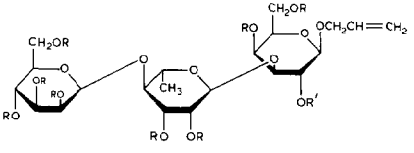


9 R = R′ = Ac
10 R = H, R′ = Ac
11 R = R′ = H

The positions of the hydroxyl groups in **7** and **8** were confirmed by methylation analysis. Treatment of **7** and **8** with diazomethane in the presence of boron trifluoride etherate[20], followed by acid hydrolysis, reduction with sodium borodeuteride, and acetylation, gave 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl[1-^{2}H]-galactitol and 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-D-[1-^{2}H]galactitol, respectively, which were identified by g.l.c.–m.s.[21]. The position of the free hydroxyl

TABLE I

^{13}C-N.M.R. DATA[a] FOR MONOSACCHARIDE DERIVATIVES OF D-GALACTOSE AND D-MANNOSE

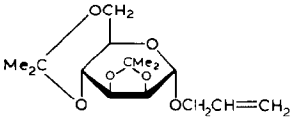
Compound	*Chemical shifts (p.p.m.)*								
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*$OCH_2CH{=}CH_2$*		
							a	*b*	*c*
1	103.3	72.1	74.4	69.7	75.9	61.9	70.6	135.4	117.5
4	101.1	70.2	71.6	68.6	72.1	62.5	70.7	135.0	117.5
7	101.6	73.5	72.8	70.2	73.9	64.5	70.6	135.4	117.0
8	101.6	73.4	71.0	71.5	72.3	63.2	70.8	135.3	117.2
15	99.0	70.8	70.1	68.2	68.2	62.8	69.6	133.5	117.7
16	96.7	72.4	68.7	68.4	68.4	62.8	69.5	133.3	118.1

[a]Compounds **1**, **4**, **7**, and **8**, solutions in CD_3OD; compounds **15** and **16**, solutions in $CDCl_3$; other resonances: CH_3CO, 20.5–21.0; CH_3CO, 170.5–172.3.

group in **8** was also evident from the ^{13}C-n.m.r. data for **1**, **4**, **7**, and **8** (see Table I). On the basis of the data for methyl β-D-galactopyranoside[22] and as expected in comparison with **1**, the resonances for C-2 and C-6 of **7** were shifted to lower field by 1–2 p.p.m. (α-effect of acetylation) and those of C-1 and C-5 were shifted by 1–2 p.p.m. to higher field (β-effect of acetylation). Comparison of the data for **7** and **8** reveals that only the signal for C-4 is shifted to lower field, and therefore HO-3 is unsubstituted.

In acetonitrile in the presence of mercury(II) cyanide, glycosylation of **8** by the glycosyl bromide obtained from 1,2,3-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose[23] yielded the crystalline nona-acetate of **9** (25%). Reaction of **9** with methanolic 3% triethylamine gave a crystalline product **10** which contained one acetyl group (^{13}C- and ^{1}H-n.m.r. data). Removal of this acetyl group required methanolic 0.06M sodium methoxide and gave the desired allyl glycoside **11**. Acid hydrolysis of **11** gave rhamnose, mannose, and galactose in the ratios 1:1:1. The ^{13}C-n.m.r. spectrum (see Table II) of **11** was interpreted on the basis of data for **1**, methyl β-D-mannopyranoside[22], and oligosaccharides containing (1→4)-linked α-L-rhamnopyranosyl and (1→3)-linked β-D-galactopyranosyl residues[24,25]. Moreover, comparison of the data for **10** and **11** indicated the acetyl group in **10** to be at O-2. The resistance of AcO-2 in **10** to saponification is probably related to a steric shielding effect.

The allyl disaccharide-glycoside **21**, corresponding to the O:4 factor of the O-specific polysaccharides of *Salmonella*, was prepared *via* glycosylation of **16** with 3,6-dideoxy-2,4-di-*O*-(*p*-nitrobenzoyl)-α-D-*xylo*-hexopyranosyl bromide (**19**) obtained from methyl 2,4-di-*O*-(*p*-nitrobenzoyl)-α-abequoside (**18**)[26,27].



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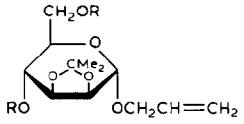
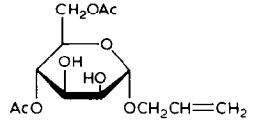


13 R = H
14 R = Ac



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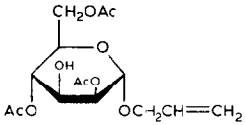

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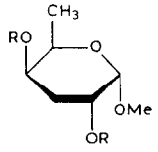
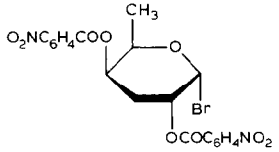


17 R = H
18 R = *p*-$NO_2C_6H_4CO$



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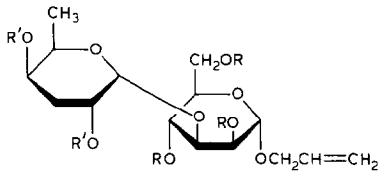
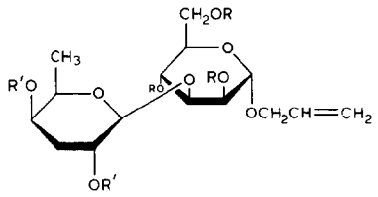


20 R = Ac, R′ = *p*-$NO_2C_6H_4CO$
21 R = R′ = H



22 R = Ac, R′ = *p*-$NO_2C_6H_4CO$
23 R = R′ = H

Allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**16**) was synthesised as follows. Fischer glycosidation of D-mannose with allyl alcohol and acetonation of the product mixture with 2,2-dimethoxypropane and toluene-*p*-sulfonic acid yielded 60% of allyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside (**12**). Graded, acid hydrolysis[28] of **12** gave the 2,3-*O*-isopropylidene-α-D-mannopyranoside (**13**, 68.5%), the structure of which was confirmed by the ^{1}H-n.m.r. data of its diacetate **14**. Hydrolysis of syrupy **14** with dilute acetic acid gave allyl 4,6-di-*O*-acetyl-α-D-mannopyranoside (**15**, 90%). The position of the *O*-acetyl groups in **15** followed from ^{13}C-n.m.r. data (see Table I). Compared to their positions in methyl α-D-mannopyranoside[22], the C-3 and C-5 signals were shifted to higher field (β-effect of acetylation). The diacetate **15** was transformed into the 2,3-(ethyl orthoacetate), which was then hydrolysed with 80% acetic acid to give allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**16**, 90%). Comparison of the ^{13}C-n.m.r. data (Table I) for **15** and **16** indicates the latter to be acetylated at O-2 (α-effect of acetylation for the C-2 signal and β-effects for the C-1 and C-3 signals)*. Methylation analysis of **16** gave 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-D-[1-^{2}H]mannitol, which was identified by g.l.c.–m.s.[21] (characteristic series of ions with *m/z* 190, 130, 100, 88), and confirmed that HO-3 in **16** was unsubstituted.

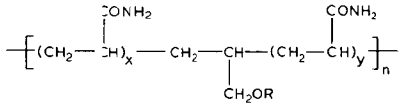
Treatment of the methyl glycoside **18** with hydrogen bromide in dichloromethane gave the glycosyl bromide **19**, which was used immediately to glycosylate **16** in acetonitrile in the presence of mercury(II) cyanide. The major (**20**, 75%) and minor (**22**, 19%) products were isolated by chromatography. The α configuration of the interglycosidic bond in **20** was confirmed by the ^{1}H-n.m.r. doublet for H-1′ ($J_{1',2'}$ 3.8 Hz). Treatment of **20** with barium oxide in boiling methanol gave crystalline allyl 3-*O*-α-abequopyranosyl-α-D-mannopyranoside (**21**, 89%). Acid hydrolysis of **21** gave mannose and abequose in the ratio 1:1 (sugar analyser), and methylation analysis of **21** gave 1,5-di-*O*-acetyl-2,4-di-*O*-methyl-3,6-dideoxy-D-*xylo*-[1-^{2}H]hexitol (primary m.s. fragments at *m/z* 176, 131, and 118) and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-[1-^{2}H]mannitol (primary fragments at *m/z* 161, 118, and 45).

The possibility that migration of AcO-2 in **16** had occurred during glycosyla-

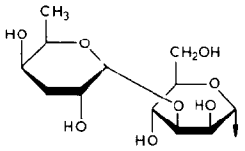
*In the anomeric region, there was a signal at 98.8 p.p.m. with an intensity <5% of that at 96.7 p.p.m., indicating the presence of allyl 3,4,6-tri-*O*-acetyl-α-D-mannopyranoside.

tion, with the formation of a (1→2)-linked disaccharide derivative, was ruled out by the ^{13}C-n.m.r. data for acetylated **21**. In the anomeric region, there was one signal (96.8 p.p.m.)* with double integrated intensity, indicating that the C-1 resonances of the mannose and abequose residues were shifted due to the β-effects of acetylation of HO-2 and HO-2'. The ^{1}H-n.m.r. signal for H-1' in the minor product **22** and its de-esterified derivative was a doublet with $J_{1',2'}$ 7.5 Hz, indicating the interglycosidic bond to be β. Methylation analyses of **21** and **23** yielded the same result and thereby confirmed the abequose residue in **23** to be pyranoid. A disaccharide derivative with β-abequofuranosidic linkage was obtained[26] as a minor product on glycosylation of *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside by the glycosyl bromide prepared from **18**.

The incorporation of the oligosaccharide determinants **11** and **21** into polymers **24** and **25** was carried out *via* radical copolymerisation with acrylamide in water in the presence of ammonium persulphate and *N,N,N',N'*-tetramethylethylenediamine[14].



24 R =



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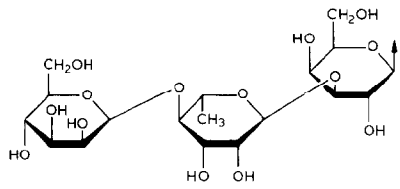

The polymers **24** and **25** contained 30–34% of carbohydrate and were isolated in yields of 24–28% (based on allyl glycoside) by gel filtration on Sephadex G-50**. The relatively low extent of incorporation of allyl glycosides in the copolymerisation is probably due to their lower reactivity as compared to that of acrylamide. Unreacted allyl glycosides were recovered from the fraction of low molecular weight (eluted from Sephadex G-50) by subsequent chromatography on silica gel or by gel filtration on Sephadex G-15.

The polymers **24** and **25** passed freely through an Amicon Diaflo XM 300 membrane, but were almost completely retained by an XM 100 membrane, which corresponds to a mass of 100–300 kilodaltons. This finding accords with the data obtained by analytical ultracentrifugation (35,000 r.p.m.) of aqueous 0.5 and 0.7% solutions of **25**. A sedimentation coefficient s°_{20,H_2O} 3.9S was obtained, relative

*C-1 of methyl 2,4-di-*O*-acetyl-α-abequopyranoside resonates at 96.5 p.p.m.

**The copolymers were eluted from Sephadex G-100 and G-200 and Sepharose 2B within the void volumes, which emphasises a difference in the properties of the copolymers as compared to those of polysaccharides and proteins.

TABLE II

^{13}C-N.M.R. DATA[a] FOR COMPOUNDS CONTAINING THE O:3 DETERMINANT AND FOR AUXILIARY COMPOUNDS

Compound	*Unit*	*Chemical shifts (p.p.m.)*								
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*$OCH_2CH{=}CH_2$*		
								a	*b*	*c*
1	Gal	103.1	72.0	74.1	69.9	76.3	62.2	71.8	134.8	119.8
	Man	101.9	71.9	74.4	68.05	77.5	62.3[b]			
10[c]	Rha	103.3	71.65[b]	71.3[b]	80.75	68.3	18.2			
	Gal	101.0	72.7	79.95	69.6	76.5	62.0[b]	71.9	134.7	119.6
	Man	101.9	71.8	74.4	68.05	77.4	62.1			
11	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	71.8	134.8	120.0
	Man	101.8	71.8	74.4	68.2	77.5	62.4[b]			
25	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]			
d	Man	101.75	71.8	74.25	67.95	77.3	62.2			
	Rha	95.05	72.2	71.2	80.8	68.2	18.3			
e	Rha	103.4	71.3	71.3	82.25	68.9	18.15			
	Gal	104.4	71.3	81.8	68.6	76.3	62.1			

[a]Solutions in D_2O. [b]Assignments may be reversed. [c]Spectrum contains a signal for CH_3CO at 21.6 p.p.m. [d]β-D-Man*p*-(1→4)-α-L-Rha*p*[24]. [e]β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→6)-D-Glc*p*[25].

TABLE III

^{13}C-N.M.R. DATA[a] FOR COMPOUNDS CONTAINING THE O:4 DETERMINANT AND FOR AUXILIARY COMPOUNDS

Compound	*Unit*	*Chemical shifts (p.p.m.)*								
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*$OCH_2CH{=}CH_2$*		
								a	*b*	*c*
15	Abe	100.35	64.2	34.3	69.5	67.4	16.6			
b	Man	101.9	71.2	71.8	68.0	73.7	62.1			
20	Abe	101.35	64.7	34.2	69.5	67.9	16.5			
	Man	100.6	71.3	79.6	67.2	74.1	62.0	69.25	119.2	134.5
24	Abe	100.3	64.8	34.3	69.6	68.0	16.8			
	Man	100.9	71.5	79.5	67.4	74.4	62.6			
23	Abe	104.3	66.5	38.1	69.4	75.6	16.9			
	Man	100.0	69.3	79.9	66.5	73.9	62.2	69.4	119.8	134.5

[a]Solutions in D_2O. [b]Methyl α-D-mannopyranoside[22].

homogeneity was indicated, and a molecular mass of 160 kilodaltons[29] was estimated.

The structures of **24** and **25** were confirmed by the results of sugar analysis and by ^{13}C-n.m.r. data (see Tables II and III). The chemical shifts of the ^{13}C signals belonging to the carbohydrate residues of **24** and **25** practically coincided with those of the respective monomers **11** and **21**. A slight shift of the C-1 resonance for the galactosyl residue in **25** is caused by a change in the aglycon structure after incorporation of the monomer into the copolymer. Thus, there was no alteration of the carbohydrate moieties during the copolymerisation. The copolymers **24** and **25** also gave ^{13}C signals for $CONH_2$ (181.2 and 180.6 p.p.m.), CH (43.5–42.8 p.p.m.), and CH_2 groups (37.2–35.2 p.p.m.). Taking into account the spin–lattice relaxation times and enhancements of the signal intensities due to the n.O.e. for different carbon atoms in polymers[30], an approximate estimate of the relative content of $CH_2CH(CH_2OR)$ fragments of the main chain in the copolymers (where R is a carbohydrate substituent) with respect to $CH_2CH(CONH_2)$ fragments can be made. Thus, the ratios of the integrated intensities of the signals for C-6 in the hexose residue and those for total non-carbohydrate methine groups were 1:10 for **24** and 1:13 for **25**. These estimates accorded with the carbohydrate contents revealed by sugar analysis.

Data on the serological specificity of **25** have been presented[31]. In two serological reactions (double radial immunodiffusion according to Ouchterlony and passive hemagglutination inhibition) with homologous and heterologous antisera, **25** exhibited clear-cut monofactor specificity O:3 of *Salmonella*. Moreover, in the double diffusion test, **25** was precipitated by homologous antisera at concentrations ~100-fold lower than those of lipopolysaccharides from *Salmonella* serogroup E. In the passive hemagglutination test, **25** was 10 times more effective an inhibitor than the corresponding lipopolysaccharide. Copolymer **24** had monofactor specificity O:4 of *Salmonella* and, in the same serological reactions, was, respectively, 50 and 4 times more active than the lipopolysaccharide from *S. typhimurium* (serogroup B). The enhanced activity of both copolymers **24** and **25** in the serological reactions is probably attributed to a relatively high content of determinant groups.

The results on the immunogenicity of the synthetic antigens of this type, as well as their protective properties, will be published elsewhere.

EXPERIMENTAL

T.l.c. was performed on "Silufol" plates (Czechoslovakia) with chloroform–acetone mixtures 97:3 (*A*) and 87:13 (*B*), chloroform–ethanol mixtures 5:5 (*C*) and 95:5 (*D*), benzene–acetone mixtures 5:5 (*E*) and 8:2 (*F*), and toluene–ethyl acetate mixtures 4:1 (*G*) and 7:3 (*H*). Silica gel L 40/100 (Czechoslovakia) was used for column chromatography. Descending p.c. was performed on Filtrak FN 11 paper (GDR) with 1-butanol–pyridine–water (6:4:3). ^{1}H-N.m.r. spectra were recorded with Varian DA-60-IL (60 MHz), Tesla BS-467 (100 MHz), and Bruker

WM-250 (250 MHz) instruments, and ^{13}C spectra with Bruker WP-60 (15.08 MHz) and Bruker WM-250 (62.89 MHz) instruments for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal MeOH, δ 50.15). The spectra of **24** and **25** were recorded at 70°. G.l.c.–m.s. was performed with a Varian MAT-111 instrument (FRG) equipped with a stainless-steel column (150 × 0.4 cm), packed with 3% of SE-30 on Varaport-30 (100–120 mesh) at 170°, using helium as carrier gas at 15 mL/min. G.l.c. was performed with a Pye-Unicam 104 (Model 64) chromatograph with a flame-ionisation detector and a stainless-steel column (150 × 0.4 cm), packed with 3% of SE-30 on Diatomite Q (100–120 mesh) at 185°. Carbohydrate analysis was conducted after hydrolysis (2M HCl, 100°, 4 h) with a Technicon analyser (U.S.A.), using a column (25 × 0.6 cm) packed with Durrum DAx4 resin, 0.5M borate buffer (pH 9) at 55°, and an elution rate of 60 mL/h. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter. Melting points were determined with a Kofler apparatus and are uncorrected. Chemicals from a disc-electrophoresis kit (Reanal, Hungary) were used for copolymerisation. Acrylamide was twice recrystallised from chloroform.

*Acetonation of allyl β-*D*-galactopyranoside* (**1**). — A mixture of **1**[32] (1.5 g), anhydrous cupric sulphate (6 g), toluene-*p*-sulfonic acid (2–3 mg), and dry acetone (75 mL) was stirred for 24 h at room temperature, neutralised with triethylamine, and concentrated. T.l.c. (solvent *E*) of the residue revealed components with R_F 0.45 (major) and 0.25 (minor). Column chromatography (acetone gradient, 0→60% in benzene) gave, first, allyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**2**; 1.33 g, 75%), m.p. 91–92° (from acetone–light petroleum), $[\alpha]_D^{22}$ +10° (*c* 2, chloroform) (Found: C, 55.12; H, 7.68. $C_{12}H_{20}O_6$ calc.: C, 55.37; H, 7.75%). The 2,6-diacetate **5** had m.p. 61.5–63° (from ethanol–light petroleum), $[\alpha]_D^{23}$ +24° (*c* 2, chloroform). ^{1}H-N.m.r. data (CCl_4): δ 1.31 (s, 3 H, Me^{α}), 1.54 (s, 3 H, Me^{γ}), 2.05 (s, 6 H, 2 Ac), and 5.84 (m, 1 H, $OCH_2CH{=}CH_2$) (Found: C, 55.87; H, 6.94. $C_{16}H_{24}O_8$ calc.: C, 55.80; H, 7.03%).

Eluted second was allyl 4,6-*O*-isopropylidene-β-D-galactopyranoside (**3**; 0.31 g, 17.5%), m.p. 88.5–90° (from ether–light petroleum) (Found: C, 55.23; H, 7.49. $C_{12}H_{20}O_6$ calc.: C, 55.37; H, 7.75%). The 2,3-diacetate **6** was a syrup, $[\alpha]_D^{20}$ +31° (*c* 1.9, chloroform). ^{1}H-N.m.r. data (CCl_4): δ 1.38 (s, 3 H, Me), 1.44 (s, 3 H, Me), 2.00 and 2.03 (2 s, 6 H, 2 Ac), and 5.84 (m, 1 H, $OCH_2CH{=}CH_2$).

*Allyl 2,6-di-*O*-acetyl-β-*D*-galactopyranoside* (**7**). — To a solution of **5** (1.43 g) in acetic acid (16 mL) was added water (8 mL), and the mixture was heated at 100° for 35 min and then concentrated *in vacuo*. Water and ethanol were distilled from the residue. T.l.c. (solvent *E*) revealed a main component with R_F 0.35 and a contaminant with R_F 0.1. Column chromatography (acetone gradient, 0→35% in chloroform) gave **7** (1.26 g, 95%), m.p. 75–76.5° (from ether–light petroleum), $[\alpha]_D^{20}$ −9.5° (*c* 2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.10 and 2.13 (2 s, 6 H, 2 Ac), and 5.85 (m, 1 H, $OCH_2CH{=}CH$) (Found: C, 51.09; H, 6.74. $C_{13}H_{20}O_8$ calc.: C, 51.31; H, 6.63%).

*Allyl 2,4,6-tri-*O*-acetyl-β-*D*-galactopyranoside* (**8**). — A mixture of **7** (1.5 g),

triethyl orthoacetate (5 mL), and nitromethane (7 mL) was boiled for 10 h in the presence of toluene-*p*-sulfonic acid (2–3 mg), cooled, neutralised with triethylamine (0.3 mL), and diluted with chloroform followed by ice–water. T.l.c. showed that **7** had been almost completely converted into the 3,4-(ethyl orthoacetate), R_F 0.5 (solvent *F*). The mixture was extracted with chloroform (2 × 70 mL), the extract was concentrated, 80% acetic acid was added to the residue, and the mixture was kept for 40 min at room temperature. T.l.c. (solvent *F*) then showed the absence of orthoester and formation of a product with R_F 0.25. The mixture was concentrated, ethanol and toluene were evaporated from the residue to remove the traces of acetic acid, and the residue was then subjected to column chromatography (acetone gradient, 0→15% in chloroform), to give **8** (1.35 g, 79%), m.p. 81–83°, $[\alpha]_D^{20}$ −12.5° (*c* 2.2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.07, 2.12, and 2.17 (3 s, 9 H, 3 Ac) (Found: C, 51.79; H, 6.25. $C_{15}H_{22}O_9$ calc.: C, 52.02; H, 6.40%).

*Allyl 3-O-(4-O-β-*D*-mannopyranosyl-α-*L*-rhamnopyranosyl)-β-*D*-galactopyranoside* (**11**). — Triacetate **8** (500 mg, 1.53 mmol) was glycosylated by using the glycosyl bromide (950 mg, 1.53 mmol) obtained[23] from 1,2,3-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose, and dry acetonitrile (10 mL) in the presence of mercury(II) cyanide (385 mg, 1.53 mmol). After 12 h, the mixture was concentrated, the residue was extracted with chloroform, the extract was washed with M potassium iodide containing a small amount of sodium hydrogencarbonate and then water, dried ($MgSO_4$), and concentrated. T.l.c. (solvent *B*) then revealed a mixture of products of condensation, a product with R_F 0.42, and a small proportion of **8**. Column chromatography (acetone gradient, 0→11% in chloroform) gave a crude condensation product that was treated with ethanol to give the nona-acetate **9** (350 mg, 25%), m.p. 201–203° (from ethanol), $[\alpha]_D^{20}$ −18° (*c* 1.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.31 (d, 3 H, $J_{5,6}$ 5 Hz, rhamnose Me), 2.03–2.20 (m, 27 H, 9 Ac), and 5.82 (m, 1 H, $OCH_2CH{=}CH_2$) (Found: C, 52.03; H, 6.38. $C_{39}H_{54}O_{24}$ calc.: C, 51.65; H, 6.00%).

A solution of **9** (330 mg) in methanolic 3% triethylamine (300 mL) was kept overnight at room temperature and then concentrated, and methanol was evaporated from the residue to give a quantitative yield of the monoacetate **10**, m.p. 265–268° (from methanol), $[\alpha]_D^{20}$ −52.5° (*c* 0.8, water), R_{Gal} 1.55 (p.c.). ^{1}H-N.m.r. data (D_2O): 2.17 (s, 3 H, Ac), 1.29 (d, 3 H, $J_{5,6}$ 5 Hz, rhamnose Me), and 5.91 (m, 1 H,$OCH_2CH{=}CH_2$) (Found: C, 48.19; H, 6.79. $C_{23}H_{38}O_{16}$ calc.: C, 48.41: H, 6.71%).

To a suspension of **10** (210 mg) in dry methanol (50 mL) was added methanolic M sodium methoxide (3 mL). The mixture was stirred at room temperature for 12 h, neutralised with KU-2 (H^+) resin, and concentrated, to give **11** in quantitative yield, which crystallised on the addition of ethanol; m.p. 232–235° (from aqueous ethanol), $[\alpha]_D^{20}$ −51.5° (*c* 1.7, water); homogeneous in p.c. (R_{Gal} 1) (Found: C, 48.27; H, 7.09; $C_{21}H_{36}O_{15}$ calc.: C, 47.72; H, 6.86%).

*Allyl 2,3:4,6-di-*O*-isopropylidene-α-*D*-mannopyranoside* (**12**). — A mixture of D-mannose (12 g) and allyl alcohol (24 mL) containing 3% of hydrogen chloride was heated at 70° for 5 h, neutralised with conc. ammonia, and co-concentrated with water until the removal of allyl alcohol was complete. The syrupy residue contained mainly allyl mannosides (p.c., R_{Man} 1.56). A solution of the residue in dry acetone (150 mL) was stirred with 2,2-dimethoxypropane (45 mL) and toluene-*p*-sulfonic acid (10–20 mg) for 16 h, neutralised with triethylamine, and concentrated. Column chromatography (acetone gradient, 0→10% in benzene) of the residue gave the major component **12** (11.8 g, 59%), R_F 0.74 (solvent *D*), m.p. 59° (from hexane), $[\alpha]_D^{20}$ +12.5° (*c* 2, chloroform). ^{1}H-N.m.r. data (CCl_4): δ 1.28 (s, 3 H, Me), 1.33 (s, 3 H, Me), 1.48 (s, 6 H, 2 Me), 4.92 (d, 1 H, $J_{1,2}$< 0.5 Hz, H-1), and 5.84 (m, 1 H, $OCH_2CH{=}CH_2$) (Found: C, 59.93; H, 7.99. $C_{15}H_{24}O_6$ calc.: C, 59.98; H, 8.05%).

*Allyl 2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**13**). — To a solution of **12** (2.64 g) in acetone (52 mL) were added water (52 mL) and toluene-*p*-sulfonic acid (520 mg), and the mixture was heated at 40° for 20 min. T.l.c. then revealed a small proportion of **12** and a reaction product with R_F 0.33 (solvent *D*). The mixture was neutralised with M sodium hydrogencarbonate and concentrated, and the residue was extracted several times with chloroform. The extract was concentrated and the residue was subjected to column chromatography (ethanol gradient, 0→20% in chloroform), to give **13** (1.56 g, 68.5%), m.p. 76–78° (from hexane–ether), $[\alpha]_D^{20}$ +37.5° (*c* 2, chloroform) (Found: C, 55.33; H, 7.79. $C_{12}H_{20}O_6$ calc.: C, 55.37; H, 7.75%).

Acetylation of **13** with acetic anhydride in pyridine (20°, 16 h) gave a quantitative yield of syrupy allyl 4,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-D-mannopyranoside (**14**), $[\alpha]_D^{20}$ +2° (*c* 3, chloroform), R_F 0.52 (solvent *D*). ^{1}H-N.m.r. data (CCl_4): δ 1.20 (s, 3 H, Me), 1.50 (s, 3 H, Me), 2.00 and 2.03 (2 s, 6 H, 2 Ac), 4.98 (d, 1 H, $J_{1,2}$ <0.5 Hz, H-1), and 5.86 (m, 1 H, $OCH_2CH{=}CH_2$).

*Allyl 4,6-di-*O*-acetyl-α-*D*-mannopyranoside* (**15**). — A solution of **14** (2.2 g) in 50% acetic acid (20 mL) was heated for 30 min at ~100°, and then concentrated. Ethanol and toluene were evaporated from the residue, which was then subjected to column chromatography (ethanol gradient, 0→20% in benzene), to yield **15** (1.77 g, 91%), m.p. 109–110° (from acetone–light petroleum), $[\alpha]_D^{20}$ +58° (*c* 2, chloroform), R_F 0.42 (solvent *D*). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.08 and 2.14 (2 s, 6 H, 2 Ac), 4.94 (d, 1 H, $J_{1,2}$ <0.5 Hz, H-1), and 5.87 (m, 1 H, $OCH_2CH{=}CH_2$) (Found: C, 51.26; H, 6.79. $C_{13}H_{20}O_8$ calc.: C, 51.31; H, 6.63%).

*Allyl 2,4,6-tri-*O*-acetyl-α-*D*-mannopyranoside* (**16**). — A solution of **15** (3.8 g) in dry benzene (100 mL) was stirred for 16 h with triethyl orthoacetate (30 mL) in the presence of toluene-*p*-sulfonic acid monohydrate (3–5 mg). T.l.c. then showed complete disappearance of **15** (R_F 0.42), and a product (2,3-orthoester) with R_F 0.78 (solvent *D*). The mixture was neutralised with triethylamine, washed with cold water, and concentrated, and a solution of the residue in 80% acetic acid (8 mL) was concentrated after 30 min. Ethanol and toluene were evaporated from

the residue, which was then subjected to column chromatography (acetone gradient, 0→40% in benzene) to yield **16** (3.86 g, 89%), $[\alpha]_D^{20}$ +21.5° (*c* 2, chloroform), R_F 0.38 (solvent *F*). ^{1}H-N.m.r. data (CCl_4): δ 1.98, 2.01, 2.05 (3 s, 9 H, 3 Ac), and 5.84 (m, 1 H, $OCH_2CH{=}CH_2$) (Found: C, 51.92; H, 6.56. $C_{15}H_{22}O_9$ calc.: C, 52.02; H, 6.40%).

Allyl 2,4,6-tri-O-*acetyl-3*-O-*(3,6-dideoxy-2,4-di*-O-p-*nitrobenzoyl-α- and -β-*D-xylo-*hexopyranosyl)-α*-D-*mannopyranoside* (**20** *and* **22**). — A solution of **18**[27] (612 mg, 1.33 mmol) in dichloromethane (30 mL) was saturated with hydrogen bromide at −10° and then stored for 2 h at −10°. T.l.c. showed the disappearance of **18** (R_F 0.68, solvent *G*) and the formation of the glycosyl bromide **19** (R_F 0.81). The mixture was concentrated as quickly as possible, and dichloromethane was twice evaporated from the residue which was then dried *in vacuo* for 5–10 min and immediately used for glycosylation. A solution of **19** in dichloromethane (1 mL) and acetonitrile (4 mL) was added under argon during 10–15 min to a stirred solution of **16** (308 mg, 0.89 mmol) and mercury(II) cyanide (335 mg, 1.33 mmol) in acetonitrile (4 mL). After 16 h, the mixture was diluted to 50 mL with toluene, washed with saturated aqueous sodium hydrogencarbonate and water, dried ($MgSO_4$), and concentrated. Column chromatography (ether gradient, 0→30% in benzene) of the residue gave **20** (530 mg, 75%), R_F 0.48 (solvent *H*), and **22** (130 mg, 18.9%), R_F 0.42.

Compound **20** had $[\alpha]_D^{20}$ +124.5° (*c* 2.2, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.25 (d, 3 H, $J_{5,6}$ 6.5 Hz, abequose Me), 1.8–2.5 (m, 2 H, H-3,3′ of abequose), 1.82, 2.08, and 2.25 (3 s, 9 H, 3 Ac), 4.90 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1 of mannose), 5.18 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1 of abequose), 5.87 (m, 1 H, $OCH_2CH{=}CH_2$), and 8.05–8.42 (8 H, aromatic protons) (Found: C, 53.90; H, 4.71; N, 3.36. $C_{35}H_{38}N_2O_{18}$ calc.: C, 54.25; H, 4.95; N, 3.62%).

Compound **22** had $[\alpha]_D^{20}$ +36° (*c* 1, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 1.32 (d, 3 H, $J_{5,6}$ 6.5 Hz, abequose Me), 1.75, 2.10 and 2.11 (3 s, 9 H, 3 Ac), 2.00 (ddd, 1 H, $J_{3a,4}$ 3, $J_{2,3a}$ 11.5, $J_{3e,3a}$ 15 Hz, H-3*a* of abequose), 2.68 (ddd, 1 H, $J_{3e,4}$ 3, $J_{2,3e}$ 5, $J_{3e,3a}$ 15 Hz, H-3*e* of abequose), 4.31 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3 of mannose), 4.85 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1 of mannose), 4.85 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1 of abequose), 5.15 (ddd, 1 H, $J_{2,3a}$ 11.5, $J_{1,2}$ 7.5, $J_{2,3e}$ 5 Hz, H-2 of abequose), 5.23 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, H-2 of mannose), 5.26 (m, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4 of mannose), 5.36 (m, 2 H, $OCH_2CH{=}CH_2$), 5.94 (m, 1 H, $OCH_2CH{=}CH_2$), and 8.15–8.40 (8 H, aromatic protons).

Allyl 3-O-*(3,6-dideoxy-α*-D-xylo-*hexopyranosyl)-α*-D-*mannopyranoside* (**21**). — A mixture of **20** (1.85 g) and barium oxide (1.8 g) in dry methanol (200 mL) was boiled for 1 h. T.l.c. then revealed a product with R_F 0.31 (solvent *C*). The mixture was neutralised with carbon dioxide and concentrated, a suspension of the residue in ethanol was centrifuged, and the supernatant solution was concentrated on to a small amount of silica gel. The residue was applied to the top of a column of silica gel and eluted (ethanol gradient, 0→65% in chloroform), to yield **21** (730 mg, 88.5%), m.p. 180–182° (from ethanol–ether), $[\alpha]_D^{20}$ +131° (*c* 1, water). ^{1}H-N.m.r.

data (250 MHz, D_2O): δ 1.22 (d, 3 H, $J_{5,6}$ 6.5 Hz, abequose Me), 1.98–2.19 (m, 2 H, H-3*a*,3*e* of abequose), 4.05 (ddd, 1 H, $J_{1,2}$ 3.8, $J_{2,3e}$ 5.05, $J_{2,3a}$ 12 Hz, H-2 of abequose), 4.16 (q, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3 Hz, H-2 of mannose), 4.30 (m, 2 H, $OCH_2CH=CH_2$), 4.95 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1 of mannose), 5.14 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1 of abequose), 5.38 (m, 2 H, $OCH_2CH=CH_2$), and 6.04 (m, 1 H, $OCH_2CH=CH_2$) (Found: C, 51.53; H, 7.31. $C_{15}H_{26}O_9$ calc.: C, 51.42; H, 7.48%).

*Allyl 3-O-(3,6-dideoxy-β-*D*-xylo-hexopyranosyl)-α-*D*-mannopyranoside* (**23**). — A mixture of **22** (560 mg) and barium oxide (0.6 g) in dry methanol (70 mL) was boiled for 1 h and then treated as described for **21**. Column chromatography (ethanol gradient, 0→60% in chloroform) gave **23** (237 mg, 94%), $[\alpha]_D^{22.5}$ −2° (*c* 2, water), R_F 0.54 (solvent *C*) as a glass. ^{1}H-N.m.r. data (250 MHz, D_2O): δ 1.28 (d, 3 H, $J_{5,6}$ 6.5 Hz, abequose Me), 1.8 (ddd, 1 H, $J_{3a,4}$ 3, $J_{2,3a}$ 12 $J_{3e,3a}$ 14 Hz, H-3*a* of abequose), 2.29 (ddd, 1 H, $J_{3e,4}$ 3, $J_{2,3e}$ 5, $J_{3e,3a}$ 14 Hz, H-3*e* of abequose), 4.02 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 9 Hz, H-3 of mannose), 4.18 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 9 Hz, H-2 of mannose), 4.31 (m, 2 H, $OCH_2CH=CH_2$), 4.54 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1 of abequose), 5.02 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1 of mannose), 5.40 (m, 2 H, $OCH_2CH=CH_2$), and 6.04 (m, 1 H, $OCH_2CH=CH_2$).

Copolymerisation. — A solution of allyl glycoside **11** (70 mg) and acrylamide (35 mg) in distilled water (0.7 mL) was deaerated by using a water pump for 15–20 min, and then treated with *N,N,N′,N′*-tetramethylethylenediamine (2 μL) and ammonium persulphate (0.8–1 mg). The mixture was kept at 20° for 2 h (an increase in temperature to 40–50° did not affect the yield of copolymer), diluted with 0.2M/0.1M pyridine–acetate buffer (1 mL), and applied to a column (35 × 2 cm) of Sephadex G-50 which was eluted with pyridine–acetate buffer (14 mL/h). Fractions (3.5 mL) were analysed by using a sugar analyser. Fractions 16–25 were combined and concentrated, and water was evaporated from the residue. Lyophilisation and drying of the residue over phosphorus pentaoxide yielded **25** (55.8 mg, 28%), $[\alpha]_D^{20}$ −16.5° (*c* 1, water).

Copolymer **24**, $[\alpha]_D^{25.5}$ +40° (*c* 1, water), was obtained by a similar procedure.

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