Tetrahedron, Vol 37, Supplement No 1, pp 149 to 156, 1981
Printed in Great Britain

0040-4020/81 Suppl 1 0149-08 $02.00/0

SYNTHESIS OF THE O-ANTIGENIC POLYSACCHARIDE OF *SALMONELLA NEWINGTON* AND OF ITS ANALOGUE DIFFERING IN CONFIGURATION AT THE ONLY GLYCOSIDIC CENTRE

N. K. KOCHETKOV,* V. I. BETANELI, M. V. OVCHINNIKOV and L. V. BACKINOWSKY

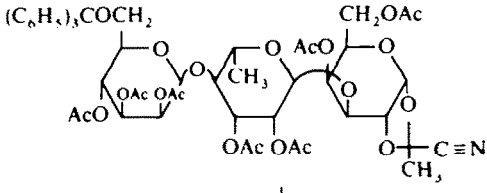
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.

(*Received in U.K. 22 April 1980*)

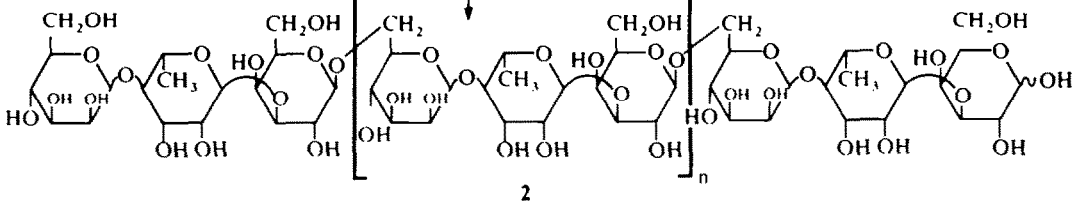
Abstract- Stereospecific polycondensation of trityl ethers of trisaccharide 1,2-O-cyanoethylidene derivatives (**1** and **3**) followed by deprotection afforded regular heteropolysaccharides (**2** and **4**) built up of repeating trisaccharide units, *viz.* →6)-β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→ and →6)-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*-(1→, respectively. The synthetic polysaccharide **2** exhibited the high biological specificity of the natural one, whilst its analogue **4** was found to be practically inactive. The necessary monomers for polycondensation, **1** and **3**, were obtained from the respective trisaccharide α-decaacetates (**5** and **7**) by successively converting them into glycosyl bromides and 1,2-O-cyanoethylidene derivatives which, in turn, were subjected to deacylation followed by tritylation.

Chemical synthesis of complex heteropolysaccharides is a difficult problem due to the necessity to ensure strict regio- and, especially, stereospecificity of all the intermonomeric glycosidic linkages in the polysaccharide to be synthesized. Such a synthesis has not been accomplished yet despite numerous polysaccharides (e.g. microbial capsular and outer-membrane polysaccharides, connective tissue polysaccharides, carbohydrate chains of different glycoproteins, etc.) belong to this important class of biopolymers.

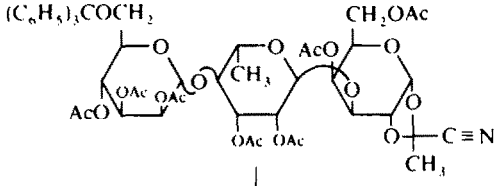
Here we describe the first synthesis of a biologically specific heteropolysaccharide **2** (preliminary communication see Ref. 1) and of its analogue **4**, which



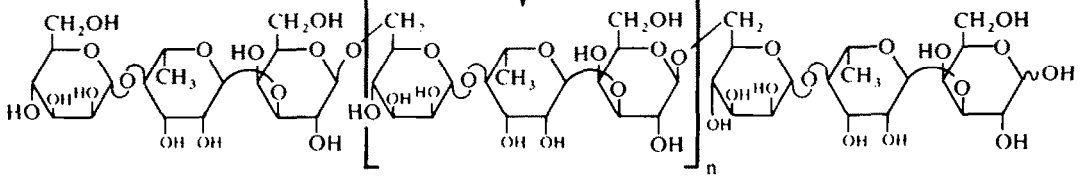
1



2



3



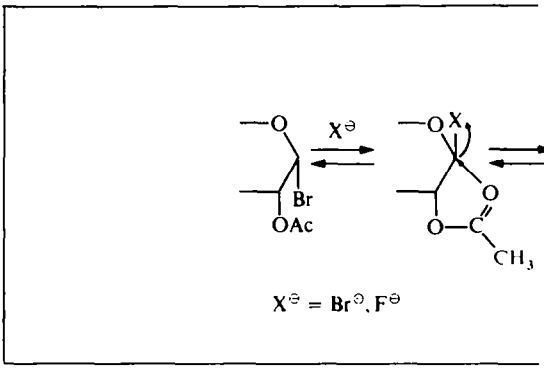
4

is based on (a) the use of tritylated 1,2-O-cyanoethylidene sugar derivatives for the synthesis of regular polysaccharides[2,3] and (b) general method of synthesis of sugar 1,2-O-cyanoethylidene derivatives.[4]

Immunological specificity of bacterial O-antigens is well known[5] to be determined by definite chemical structure of polysaccharide chains of respective lipopolysaccharides attached to the outer cell membrane. According to the literature data[5,6] O-specific chains of the lipopolysaccharide of gram-negative bacterium *Salmonella newington* are the linear heteropolysaccharides represented by formula **2**, containing from 2 to 29 repeating trisaccharide units[7] (n = 0–27). The chemical synthesis of this polysaccharide and its analogue containing α-mannopyranosyl residues is the subject of this communication.

RESULTS AND DISCUSSION

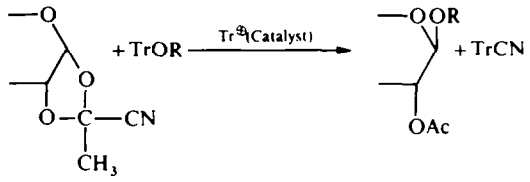
The chemical synthesis of polysaccharides **2** and **4** has been accomplished by polycondensation of respective bifunctional monomers **1** and **3** according to the following scheme. Peracetylated trisaccharides were converted into glycosyl bromides which were treated with sodium cyanide to yield 1,2-O-cyanoethylidene derivatives. The latter were deacetylated, tritylated and acetylated to afford the necessary monomers - peractylated trityl ethers of the trisaccharide 1,2-O-cyanoethylidene derivatives.



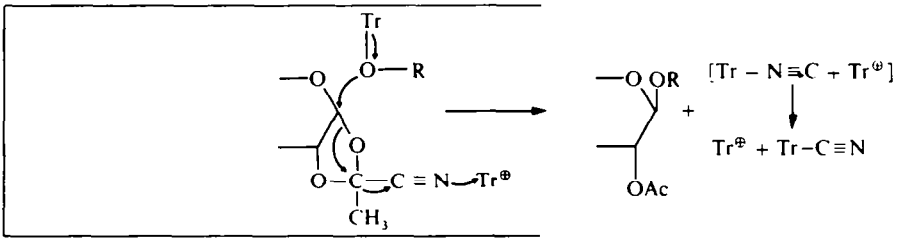
Polycondensation of these monomers in dichloromethane in the presence of catalytic amount of triphenylmethylium perchlorate gives rise to free polysaccharides following deprotection.

Treatment of the trisaccharide α-decaacetates **5**, **7** and **9**, whose practical synthesis has been reported recently,[8] with hydrogen bromide in glacial acetic acid gave the chromatographically homogeneous acetylglycosyl bromides quantitatively; they exhibited higher mobilities in tlc than the starting decaacetates. These glycosyl bromides without additional purification were treated with sodium cyanide in acetonitrile in the presence of tetra-n-butylammonium iodide at room temperature, as described earlier for the synthesis of 1,2-O-cyanoethylidene derivatives of neutral mono- and disaccharides with gluco-, galacto- and manno-configurations.[4] This reaction afford 1,2-O-cyanoethylidene derivatives as the mixtures of diastereomeric pairs differing in configuration at C-2 of the dioxolane ring (CN-*exo*-and CN-*endo*-isomers). Their formation was rationalized[4] as follows:

It is noteworthy that both individual CN-*exo*- and -*endo*-isomers and their mixtures which are formed under the above conditions proved to be highly effective and stereospecific glycosylating agents.[9] When reacted with trityl ethers in the presence of triphenylmethylium perchlorate as a catalyst they afford stereospecifically 1,2-*trans*-glycosides according to the following overall scheme:



Extremely high stereospecificity of this glycosylation reaction may be accounted for by the following tentative push-pull process of substitution at C-1:



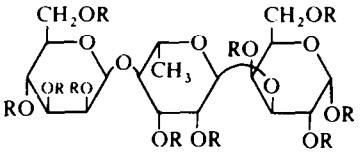
Octaacetates of 1,2-O-cyanoethylidene derivatives of trisaccharides, **10**, **12** and **14**, obtained under the aforementioned conditions, were isolated as chromatographically homogeneous mixtures of CN-*exo*- and -*endo*-isomers. Data on their PMR and Raman spectra are listed in Table 1 [those of 1,2-O-(1-cyanoethylidene)-3,4,6-tri-O-acetyl-α-D-galactopyranose[4] are given for comparison].

The presence of 1,2-dioxolane ring at galactopyranose residues was evident from characteristic singlet signals of C-CH_3 groups (δ 1.76 1.87 ppm) and

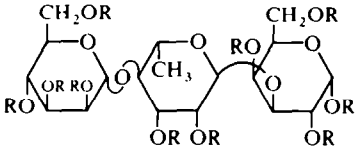
doublets of H-1 (δ 5.68–5.86 ppm), and that of CN-group -from Raman spectra, the latter method proved to be a reliable one for identification of the cyano-group in compounds of this type.[4] Data presented in Table 1 together with analytical ones prove the structures of compounds **10**, **12** and **14**. Thus the previously proposed method[4] is really a general synthetic route to 1,2-O-cyanoethylidene derivatives of mono- and oligosaccharides.

These trisaccharide 1,2-O-cyanoethylidene derivatives had to be deacetylated. It has been shown earlier[10] that treatment of 1,2-O-[1-(*exo*-cyano)-ethylidene]-3,4,6-tri-O-acetyl-α-D-glucopyranose with excess of 0.1M sodium methoxide in abs. methanol for 16 hr at 6° afforded an imino-ester derivative, whose formation can be formally regarded as a result of addition of methanol to the nitrile group of the cyanoethylidene derivative. We succeeded in avoiding this undesirable side reaction by performing the deacetylation with 0.01M sodium methoxide (0.02 equivalents) in abs. methanol containing *ca* 10% of abs. chloroform; complete deacetylation proceeded within 30 min at room temperature.

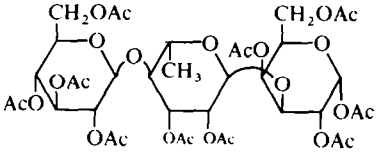
Free 1,2-O-cyanoethylidene derivatives obtained after deacetylation of **10** and **12** were subjected to alkylation with triphenylchloromethane in abs. pyridine. The reaction mixtures revealed in tlc (following peracetylation) a zone of highest mobility,



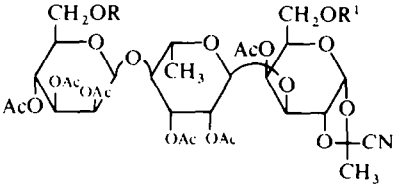
5 R = Ac
6 R = H



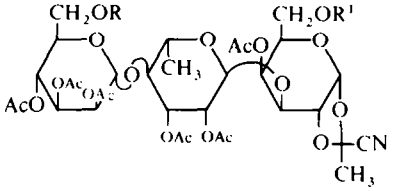
7 R = Ac
8 R = H



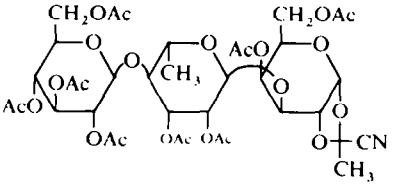
9



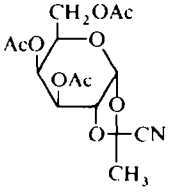
10 R = R′ = Ac
1 R = Tr; R′ = Ac
11 R = R′ = Tr



12 R = R′ = Ac
3 R = Tr, R′ = Ac
13 R = R′ = Tr



14



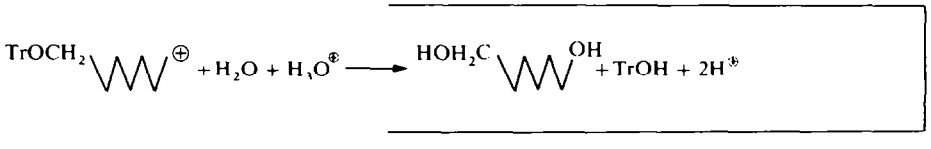
15

containing di-O-trityl derivatives, a less mobile broad zone of mono-O-trityl derivatives, and a zone corresponding to the starting octaacetates **10** and **12**. The use of 1.0 equivalent of triphenylchloromethane resulted in insufficiently effective tritylation and recovery of considerable amount of the starting products. Alkylation with 2–3 equivalents gives rise to di-O-trityl ethers **11** and **13** as the main products. The best results were obtained when the reaction was performed with 1.2–1.5 equivalents of triphenylchloromethane at room temperature for 48–72 hr followed by acetylation.

The necessary monomers **1** and **3** were isolated by column chromatography in 15 and 27% yields, respectively. Their structures followed from the 2:1 ratio of integral intensities of signals of all the Me-groups (including those of rhamnose moiety, cyanoethylidene and acetyl groups) to those of aromatic protons in their PMR spectra, as well as from data on methylation analysis.[11] Mono-O-trityl derivatives **1** and **3** afforded acetates of 2,3-di-O-methyl-rhamnitol, 2,3,4-tri-O-methyl-mannitol and 4,6-di-O-methyl-galactitol in the ratio of 1:1:1 identified by glc-MS. In the case of **3** these main components were accompanied by no more than 5% of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylmannitol and 1,2,3,5,6-penta-O-acetyl-4-O-methyl-galactitol due to possible admixture of the mono-tritylated at the galactose moiety product. The structures of di-O-trityl derivatives **11** and **13** was elucidated analogously. Thus the PMR spectra allowed to estimate the amount of trityl residues in the molecules whereas methylation analysis – to localize them. The persistence of the nitrile group in the product obtained evidenced from the presence of a band at 2240 cm^{-1} in the Raman spectrum of **13** (*cf* Table 1).

The polycondensation of monomers **1** and **3** was performed in dichloromethane in the presence of 0.1 equivalent of triphenylmethylium perchlorate at room temperature for 65 hr using vacuum technique. The reaction mixtures obtained did not contain even traces of the starting monomers (tlc). All the carbohydrate products remained at the application point (development in 1:1 benzene–ethyl acetate) and contained triphenylmethyl group (spraying with sulphuric acid produced bright-yellow colouration darkening upon heating); development in 3:7 acetone–chloroform solvent system revealed a mixture of products with R_f from 0 to 0.5 with the same colouration pattern, the main portion remaining at the application point.

The yellow reaction mixtures were treated with excess of 90% aqueous trifluoroacetic acid to neutralize cations present at the reducing termini of the growing chains and to detritylate the nonreducing termini:



Thereafter tlc revealed a mixture of products (R_f from 0 to 0.4 in 1:1 acetone–chloroform), darkening upon spraying with sulphuric acid followed by heating, the main portion remaining at the application point.

The yellow-greenish reaction mixtures were neutralized by addition of excess of pyridine, which was accompanied by decolouration, deacetylated with sodium methoxide, and gave after neutralisation the polysaccharides **2** and **4**. In the PMR spectra (14% solution in D_2O) were absent signals of the cyanoethylidene and acetyl groups at δ 1.5–2.5 ppm (an intensive doublet of the CH_3 group of rhamnose moiety was present at δ 1.2 ppm, J = 6 Hz), as well as those of trityl group (δ 6–8 ppm). According to analytical gel-chromatography data these polysaccharides were eluted as broad zones, whose beginning coincided with the void volume for Bio-Gel P-4 and retarded from that for Bio-Gel P-6. This indicates to the polymeric nature of **2** and **4**, whose maximum molecular mass lies in the range of 4,000–6,000.

Polysaccharides **2** and **4** were subjected to fractionation on Bio-Gel P-4 into three fractions **2a**, **2b**, **2c** and **4a**, **4b** and **4c** (Table 2).

The products of fractions **2c** and **4c** were eluted earlier than trisaccharides **6** and **8** upon gel-chromatography on Sephadex G-15 as well and thus they are at least hexasaccharides (n ≥ 0, cf. formulae **2** and **4**).

Total acid hydrolysis of all the fractions afforded only rhamnose, mannose and galactose in the ratio of 1:1:1, thus proving their monosaccharide composition.

Methylation analysis of fractions **2a** and **2b** gave only acetates of 2,3-di-O-methyl-rhamnitol, 2,3,4,6-tetra-O-methyl-mannitol, 2,3,4-tri-O-methyl-mannitol and 2,4,6-tri-O-methyl-galactitol, thus indicating the regiospecificity of linkages in the polysaccharide **2**, whose structure corresponds to that of the natural polysaccharide from *Salmonella newington*. In the case of the polysaccharide **4** the aforementioned methylated sugar derivatives were accompanied by small amount of 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl-galactitol, originating probably due to the admixture present in the starting monomer.

The most critical point of the synthesis – the stereospecificity of the galactosyl-mannose linkages formed – was established from the Smith degradation (i.e. periodate oxidation, borohydride reduction and mild acid hydrolysis) of fractions **2c** and **4c**. Glc-analysis of the product obtained following acetylation revealed the presence of but 1-O-β-D-galactopyranosyl-glycerol, the amount of α-anomer found to be less than 1% (authentic 1-O-β- and -α-D-galactopyranosyl-glycerols, obtained as described in,[12] served as reference compounds). It thus follows that the anomeric purity of the β-(1→6)-galactopyranosyl-mannose linkages between the trisaccharide repeating units in **2** and **4** is better than 99%, and the polycondensation process can be considered as a reaction with practically absolute stereospecificity.

The molecular mass of the polysaccharides **2** and **4**

was additionally established by two more methods. Borohydride reduction of fractions **2a**, **2b** and **4a** under mild conditions excluding β-elimination from the 3-O-substituted reducing galactopyranose terminus[13] afforded the reduced polysaccharides bearing galactitol at the reducing extremity. Glc-analysis of the products obtained upon methanolysis and acetylation (with authentic samples as reference compounds) allowed to evaluate the molecular mass from the ratio of the peak area of methyl rhamnosides to that of galactitol.

Secondly, the reducing power of the fractions **2a**, **2b**, **4a** and **4b** was measured (with trisaccharide **8** as a reference compound) according to the Park–Johnson method.[14] The data obtained by both methods were very close to each other (Table 2). The structure of the synthetic polysaccharide **2** was confirmed by high-resolution ^{13}C NMR spectroscopy. Spectra of **2** prior to fractionation and of fractions **2a** and **2b** were qualitatively very similar. The spectrum of **2a** exhibited three signals of approximately equal intensities in the region of anomeric carbons resonance at δ 104.3, 103.4 and 101.8 ppm, which correspond to C-1 of β-D-galactopyranose, α-L-rhamnopyranose and β-D-mannopyranose, respectively.[15–17] The comparable intensities of these signals and the absence of signals due to reducing galactose moiety serve as an additional proof of the polymeric nature of **2a**. The presence of signals of C-6 of D-galactopyranose (δ 62.1 ppm) and of L-rhamnopyranose (δ 18.5 ppm) of equal intensities evidenced to the regularity of the polysaccharide obtained. The positions of substitution of the monosaccharide residues followed unequivocally from the down-field chemical shifts of C-3 of D-galactopyranose (δ 81.8 ppm), of C-4 of L-rhamnopyranose (δ 80.8 ppm) and of C-6 of D-mannopyranose (δ 70.25 ppm). Analysis of the interpreted spectrum justifies the high regio- and stereospecificity of the synthetic polysacchride **2a** and is in full agreement with the expected structure.

These arguments hold true for polysacchride **2b** as well, though this fraction possessed lesser molecular mass, and, consequently, signals were present at δ 97.5 and 93.5 ppm, which correspond to C–1 of the reducing galactose moiety. Complete interpretation of spectra of fractions **2a** and **2b** (Table 3) has been performed by comparison with the spectra of model compounds discussed earlier.[16,17]

Preliminary serological assays of the products obtained were carried out as described recently.[18] Polysaccharide **2a** possessed high inhibitory properties in the passive haemagglutination reaction in the *Salmonella* O-factor 3 anti-3 system, it was 8-fold as active as the β-mannose trisaccharide **6**. This is thus indicative of the high biological activity of the synthetic natural polysaccharide **2**. As to trisaccharide **8** and polysaccharide **4a**, both containing α-D-mannopyranose residues, they appeared to be practically inactive in the system mentioned (their activities were by 2 orders less). It demonstrates high biological specificity of the synthesized natural polysaccharide **2**. The detailed data of these serological data will be published elsewhere.

Table 1. Data of PMR ($CDCl_3$) and Raman spectra

Compound	Yield, % [a]	exo-CN to endo-CN ratio [b]	Chemical shifts, δ, ppm (J, Hz)				ν_{CN}, cm^{-1}
			CH_3- of Rha		CH_3CO	H-1 of Gal	Raman spectrum
10	63	3.5:1	1.36 (6)	1.85 endo 1.78 exo	1.98; 2.02x2; 2.08x2; 2.14; 2.18x2	5.85(5) exo-CN 5.68(5) endo-CN	2240
12	62	3.7:1	1.39 (6)	1.87 endo 1.76 exo	1.96; 1.98; 2.00; 2.06x2; 2.10; 2.14x2	5.86(5) exo-CN 5.72(5) endo-CN	2240
14	43	3.2:1	1.30 (6)	1.82 endo 1.76 exo	1.96x2; 1.98; 2.06x3; 2.12x2	5.82(5) exo-CN 5.70(5) endo-CN	2240
15 [c]	95	2.8:1	-	1.85 endo	2.06x2; 2.11 exo-CN	5.88(5) exo-CN	2235
			-	1.76 exo	2.06x2; 2.09 endo-CN	5.72(5) endo-CN	2238

[a] After column chromatography on silica gel

[b] Determined from the ratio of integral intensities of signals of CH_3-groups of the dioxolane ring

[c] Data from Ref. 4

Table 2. Products of fractionation of the polysaccharides **2** and **4** on Bio-Gel P-4

Product	Elution time, min [a]	Yield, mg (%)	$[\alpha]_D^{20}$ (H_2O, equil.)	Molecular mass (n) determined from	
				Glc analysis	Reducing power
2a	42	40(34)	-23.9°	5.2×10^3(9)	5.8×10^3(10)
2b	75	30(25.5)	-21.8°	2.4×10^3(3)	2.4×10^3(3)
2c	93	40(34)	+1.5°	-	-
4a	42	30(33)	+27.8°	5.6×10^3(10)	6.3×10^3(11)
4b	70	20(22)	+50.3°	-	3.4×10^3(5)
4c	95	30(33)	+29.2°	-	-

[a] Upon analytical gel-chromatography on a Bio-Gel P-4 column, elution times of dextrane T-40, trisaccharide **8** and D-mannose were 42, 103 and 115 min, respectively

Table 3. Data of ^{13}C NMR spectra of the polysaccharides **2a** and **2b** (δ, ppm)

	2a			**2b**		
	Man [a]	Rha	Gal	Man [a]	Rha	Gal
C-1	101.8	103.4	104.3	101.8	103.45	104.3
C-2	71.6	71.4	71.4	71.6	71.4	71.4
C-3	74.3	71.6	81.8	74.2	71.8	81.7
C-4	68.0	80.8	69.7	68.0	80.8	69.6
C-5	76.25	69.1	76.45	76.25	69.1	76.6
C-6	70.25	18.5	62.1	70.3	18.25	62.1

[a] C-5 and C-6 of the terminal mannopyranose residue resonate at 77.5 and 62.25 ppm, respectively

CONCLUSION

As has been already mentioned, the synthesis of the O-antigenic polysaccharide of *Salmonella newington* presented here, is the first synthesis of a biologically active and highly specific polysaccharide. It demonstrates the possibilities of the method of synthesis of polysaccharides developed in our laboratory for solution of complex problems in this field.

EXPERIMENTAL

Optical rotations were determined with a Perkin–Elmer 141 polarimeter at 20°, m.ps. with a Kofler apparatus. PMR spectra were recorded with a Tesla BS-497 spectrometer (100 MHz, CSSR) with Me_4Si as the internal standard. ^{13}C-NMR spectra were obtained with a Bruker HX-90E-WH-360 spectrometer (90.5 MHz) having a 16/8 K memory. The number of transients was *ca* 30,000, the pulse width 17 μsec and the aquisition time 0.7 sec. Spectra were recorded at 20° for 13% solns of free polysaccharides in D_2O with MeOH as an internal standard (δ 50.15 ppm relative to Me_4Si). All chemical shifts are expressed in δ. Glc-MS was carried out on a Varian MAT-111 (GNOM) instrument on a steel 1 m-long column packed with 5% of SE-30 on Chromaton N-AW-DMCS (A) using helium as a carrier gas. The same packing in a glass 1m-long column fitted in Pye Series 105 Chromatograph and 6% of NPGS + 4% of Apiezon L on Chromaton N-AW (B) in a steel 2m-long column fitted in LChM-8MD instrument with nitrogen as a carrier gas were used for glc. Raman spectra were recorded on a "Spex Ramalog-6" spectrometer, excitation wavelength being 514.5 nm, excitation power ~100 200 mW. Column chromatography was performed on silica gel L 100/250 μ (Chemapol) (50 g per 1 g of a mixture) with gradient elution from benzene to EtOAc, tlc on silica gel LS 5/40 μ (Chemapol) with detection by spraying with 25% HSO_4 aq followed by heating at *ca* 150°. PC was carried out by descending method on Filtrak FN-11 paper, visualization of spots by KIO_4 $AgNO_3$–KOH reagent.[19] The following solvent systems were used for chromatography: benzene–ethyl acetate 1:1 (C), toluene EtOAc 1:1 (D), BuOH pyridine–H_2O 6:4:3 (E). Ion-exchange chromatography of neutral sugars was carried out with the Technicon SC-II system on a column (25 × 0.6 cm) packed with anion-exchange resin DA × 4 (Durrum, U.S.A.) in 0.5 M sodium borate buffer (pH 9.0) at 55°. The elution rate was 1 ml/min. Analytical gel chromatography was performed on columns with Bio-Gel P-6, 200-400 mesh (40 × 1.5 cm) and Bio-Gel P-4, 100 200 mesh (57 × 1.0 cm). The orcinol sulphuric acid reagent was used to monitor separations. Solutions were concetrated *in vacuo* at 40°.

Acetonitrile was dried with $CaCl_2$ and distilled from $CaCl_2$ and then from CaH_2. Nitromethane was distilled over urea at 100 mm Hg and then from CaH_2. CH_2Cl_2 and $CHCl_3$ were washed with conc. H_2SO_4 and water, dried with $CaCl_2$ and distilled from CaH_2. Pyridine was distilled from KOH and then from metallic Na. Acetic anhydride was distilled from P_2O_5.

Triphenylchloromethane was recrystallised twice from a toluene heptane mixture containing *ca* 5% of acetyl chloride, the crystals were separated by decantation and dried *in vacuo* at room temp.

Triphenylmethylium perchlorate was obtained as described in[20] and purified by reprecipitation before use as follows: 1.2 g of the product were dissolved in nitromethane (10 ml) at room temp and precipitated by addition of abs. ether (50 ml). The soln was removed by decantation and the bright-yellow crystals (0.7 g) were dried *in vacuo*.

4,6-*Di*-O-*acetyl*-3-O-[2,3-*di*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*mannopyranosyl*)-α-L-*rhamnopyranosyl*]-1,2-O-(1-*cyanoethylidene*)-α-D-*galactopyranose* **10**. Decaacetate **5** (3.3 g, 3.6 mmol) was treated with a soln of HBr (40% w/w) and Ac_2O (1% w/w) in glacial AcOH as described earlier.[8] The chromatographically homogeneous glycosyl bromide obtained (its mobility in tlc relative to **5** in solvent system C is equal to 1.13) was treated with NaCN (890 mg, 18.2 mmol) and tetra-n-butylammonium iodide (1.3 g, 3.5 mmol) in acetonitrile (10 ml) at room temp for 15 hr with stirring. The mixture was worked-up as described earlier[4] for synthesis of **15** to give after chromatography **10** (2.0 g, 63%) as a white solid, mobility in tlc relative to **5** is equal to 1.21 (C), $[\alpha]_D$ − 8.5 (*c* 2.30, $CHCl_3$). (Found: C, 50.52; H, 5.77; N, 1.62%. Calc. for $C_{37}H_{49}O_{23}N$: C, 50.74; H, 5.64; N, 1.60%.) Data of PMR and Raman spectra are given in Table 1.

4,6-*Di*-O-*acetyl*-3-O-[2,3-*di*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-α-L-*rhamnopyranosyl*]-1,2-O-(1-*cyanoethylidene*)-α-D-*galactopyranose* **12**. This compound was obtained analogously, starting from **7** (6.5 g, 7.15 mmol), in a yield of 3.88 g (62%), white solid, mobility in tlc relative to **7** is equal to 1.35 (C), $[\alpha]_D$ + 26.9 (*c* 2.65, $CHCl_3$). (Found: C, 50.79; H, 6.12; N, 1.57%). Calc. for $C_{37}H_{49}O_{23}N$: C, 50.74; H, 5.64; N, 1.60%). Data of PMR and Raman spectra see Table 1. Crystallisation from abs. MeOH at − 5 afforded crystalline sample of **12**, m.p. 180–182°, $[\alpha]_D$ + 26.2 (*c* 1.45; $CHCl_3$), whose PMR spectrum was superimposable with that of an amorphous sample.

4,6-*Di*-O-*acetyl*-3-O-[2,3-*di*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-α-L-*rhamnopyranosyl*]-1,2-O-(1-*cyanoethylidene*)-α-D-*galactopyranose* **14**. Decaacetate **9** (2.9 g, 3.2 mmol) was converted into glycosyl bromide as described above and then treated with NaCN (780 mg, 15.9 mmol) and tetra-n-butyl-ammonium iodide (1.2 g, 2.7 mmol) in acetonitrile (20 ml) to yield **14** (1.2 g, 43%) as a white solid, mobility in tlc relative to **9** is equal to 1.28 (C), $[\alpha]_D$ − 6.2° (*c* 3.36, $CHCl_3$). (Found: C, 50.67; H, 5.83; N, 1.83. Calc. for $C_{37}H_{49}O_{23}N$: C, 50.74; H, 5.64; N, 1.60%). Data of PMR and Raman spectra see Table 1.

4,6-*Di*-O-*acetyl*-3-O-[2,3-*di*-O-*acetyl*-4-O-(2,3,4-*tri*-O-*acetyl*-6-O-*triphenylmethyl*-β-D-*mannopyransyl*)-α-L-*rhamnopyranosyl*]-1,2-O-(1-*cyanoethylidene*)-α-D-*galactopyranose* **1**. To a soln of **10** (1.64 g, 1.8 mmol) in $CHCl_3$ (2 ml) were added abs. MeOH (10 ml) and 0.1 M methanolic NaOMe (2 ml) and the soln was kept for 30 min at ambient temp. The mixture was neutralized with 0.1 M methanolic AcOH (0.22 ml) and concentrated to a thick syrup. The residue was coevaporated with pyridine (2 × 5 ml), dissolved in pyridine (9 ml) and triphenylchloromethane (775 mg, 2.7 mmol) was added. After 48 hr at room temp the mixture was chilled to 0°, Ac_2O (4 ml) was added and the mixture was left at room temp for 15 hr. To the chilled (0°) soln MeOH (3 ml) was added and the mixture was left at room temp. After 1 hr the mixture was diluted with 1:2 $CHCl_3$–hexane (150 ml) and washed with water (4 × 100 ml). The upper, organic layer was separated, concentrated and coevaporated with heptane to dryness. The residue was dried *in vacuo* and applied onto a column with silica gel (silica gel slurry for the column packing was made in benzene containing 1% of pyridine). Chromatography gave the starting **10** (270 mg, 17%), a mixture of mono-O-trityl derivatives (590 mg) and **11** (530 mg, 23%), white solid, mobility in tlc relative to **10** is equal to 1.78 (D), $[\alpha]_D$ − 13.5 (*c* 0.74, $CHCl_3$). PMR data (CCl_4): 1.4–2.2 (24 H, CH_3 of rhamnopyranose, cyanoethylidene and acetyl groups), 7.0–7.4 (30 H, aromatic). Methylation analysis revealed the presence of acetates of 2,3-di-O-methyl rhamnitol, 2,3,4-tri-O-methyl–mannitol and 4-O-methyl-galactitol 1:1:1 (glc–MS).

The repeated column chromatography of a mixture of monotrityl ethers gave **1** (290 mg, 15%), white solid, mobility in tlc relative to **10** is equal to 1.59 (D), $[\alpha]_D$ − 9.8 (*c* 2.60; $CHCl_3$). PMR data (CCl_4): 1.4–2.2 (27 H, CH_3 of rhamnopyranose, cyanoethylidene and acetyl groups), 7.0–7.4 (15 H, aromatic). Methylation analysis revealed the presence only of acetates of 2,3-di-O-methyl-rhamnitol, 2,3,4-tri-O-methyl mannitol and 4,6-di-O-methyl-galactitol in the ratio 1:1:1 (glc–MS).

4,6-*Di*-O-*acetyl*-3-O-[2,3-*di*-O-*acetyl*-4-O-(2,3,4-*tri*-O-*acetyl*-6-O-*triphenylmethyl*-α-D-*mannopyranosyl*)-α-L-*rhamnopyranosyl*]-1,2-O-(1-*cyanoethylidene*)-α-D-*galactopyranose* **3**. The amorphous **12** (880 mg, 1 mmole) was deacetylated as described above, the product treated with triphenylchloromethane (330 mg, 1.2 mmol) in pyridine (5 ml) for 72 hr at room temp and the mixture was worked-up as above. Column chromatography afforded **12** (220 mg, 25%), a mixture of mono-trityl ethers (300 mg) and **13** (100 mg, 8%), white solid, mobility in tlc relative to **12** is equal to 1.88 (D), $[\alpha]_D$ + 22.6° (*c* 2.00, $CHCl_3$), PMR data (CCl_4): 1.4–2.2 (24 H, CH_3 of rhamnopyranose, cyanoethylidene and acetyl groups), 7.0–7.4 (30 H, aromatic), a band at 2240 cm^{-1} was present in the Raman spectrum. Methylation analysis gave acetates of 2,3-di-O-methyl-rhamnitol, 2,3,4-tril-O-methylmannitol and 4-O-methyl-galactitol 1:1:1 (glc–MS).

The repeated chromatography of a mixture of mono-tritYl ethers gave **3** (290 mg, 27%), white solid, mobility in tlc relative to **12** is equal to 1.57 (D), $[\alpha]_D$ + 33.1° (*c* 1.19, $CHCl_3$), PMR data (CCl_4): 1.4–2.2 (27 H, CH_3 of rhamnopyranose, cyanoethylidene and acetyl groups), 7.0–7.4 (15 H, aromatic). Methylation analysis revealed the presence of acetates of 2,3-di-O-methyl-rhamnitol, 2,3,4-tri-O-methyl-mannitol and 4,6-di-O-methyl-galactitol (1:1:1) and, besides, no more than 5% of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl–mannitol and 1,2,3,5,6-penta-O-acetyl–4-O-methyl–galactitol (glc–MS).

Polycondensation. In one limb of a tuning fork-shaped tube fitted with a ground joint, which enables its connection to a vacuum system, was placed a soln of monomer **1** (260 mg, 0.24 mmol) in abs. benzene (1.5 ml), and in the other − 0.1 M soln of triphenylmethylium perchlorate in nitromethane (0.24 ml) and the solns were freeze-dried (10^{-3} mm Hg). Nitromethane (twice distilled from CaH_2 at 10^{-3} mm Hg) (1.0 ml) was distilled into the first limb, and lyophilisation was repeated followed by drying of the residues for several hr. CH_2Cl_2 (twice distilled from CaH_2 at 10^{-3} mm Hg) (2.0 ml) was distilled into the tube, and the solns of monomer and catalyst were mixed and a yellow transparent soln left at room temp for 65 hr in the darkness. The reaction vessel was connected with atmosphere and the yellow mixture was treated with 90% aqueous trifluoroacetic acid (1.0 ml) for 30 min at room temp. Pyridine (1.0 ml) was then added, the discoloured soln was diluted with $CHCl_3$ (50 ml) and washed with water (3 × 50 ml). The organic layer was separated, taken to dryness and dried *in vacuo*. The residue (250 mg) was dissolved in $CHCl_3$ (6 ml), abs. MeOH (20 ml) and M methanolic NaOMe (2.0 ml) were added. A white floccular ppt appeared in *ca* 20 min and the mixture was stirred at room temp overnight. It was diluted with water (40 ml), whereupon complete dissolution occurs, and neutralized with a cation-exchange resin KU-2 (H^+). The resin was filtered off, washed with water (3 × 10 ml) and the combined aqueous soln was washed with $CHCl_3$ (3 × 50 ml). The aqueous layer was

separated, clarified by centrifugation and evaporated. The residue was dried *in vacuo* to yield the polysaccharide **2** (127 mg), $[\alpha]_D$ −15.2 (*c* 1.27, H_2O, equilibrium).

Analogously, starting from the monomer **3** (200 mg, 0.19 mmol), the polysaccharide **4** (84 mg) was obtained, $[\alpha]_D$ +32.5° (*c* 0.84, H_2O, equilibrium).

Fractionation of polysaccharides. The polysaccharides obtained were fractionated on a Bio-Gel P-4 column (100–200 mesh, 58 × 1.8 cm, V_0 43 ml, elution with 0.1 M AcOH, elution rate 0.8 ml/min; dextrane T-40 and mannose were eluted from this column at 43-rd and 150-th minutes, respectively) and three fractions were collected: (a) 28–63 ml; (b) 64–95 ml, and (c) 96–120 ml. The pooled fractions were concentrated and freeze-dried to yield products **2a**, **2b**, **2c**, **4a**, **4b** and **4c**, whose yields and properties are listed in Table 2.

The material of each fraction (*ca* 0.5 mg) was heated in 0.3 M HCl (2 ml) at 100° for 20 hr, the solns were coevaporated with water (3 × 5 ml) and the hydrolysates were analysed by anion-exchange chromatography. In all cases only rhamnose, mannose and galactose were detected in the ratio of 1:1:1, equimolar mixture of the above authentic monosaccharides serving as a reference.

Determination of configuration of galactosyl-mannose linkages. Polysaccharide **2c** (40 mg) was dissolved in 0.1 M sodium periodate (5 ml) and the soln was left at room temp for 65 hr in the darkness. $NaBH_4$ (30–40 mg) was added and the soln was left at room temp for 15 hr. The soln was acidified with AcOH and desalted by passing through a column of Sephadex G-15. The eluate was taken to dryness and the residue was treated with 0.5 M HCl (2 ml) at room temp for 48 hr. The hydrolysate was coevaporated with water, dried *in vacuo* and acetylated with 1:1 Ac_2O–pyridine (2 ml) at room temp for 15 hr. To the mixture EtOH (2 ml) was added, the soln left at room temp for 1 hr, diluted with $CHCl_3$ (20 ml) and washed with water (3 × 20 ml). The $CHCl_3$ layer was separated and taken to dryness. According to tlc examination, a product with R_f 0.5–0.6 (D), which coincided with authentic samples of anomeric 1-O-D-galactopyranosyl glycerols acetates,[12] constituted no less than 80%. It was isolated by column chromatography and subjected to glc (250°, column A). In comparison with authentic samples 1-O-β-D-galactopyranosyl-glycerol acetate was identified as the only product (retention time 18.0 min); the corresponding α-anomer has a retention time of 13.2 min. Analogous results were obtained upon Smith degradation of the polysaccharide **4c**.

Determination of degree of polymerization of polysaccharides. According to PC (E), the polysaccharides **2a** and **4a** remain at the application point after 7 days. The bulk of polysaccharides **2b** and **4b** behave similarly and faint zones were also detected with mobilities of 0.1–0.2 relative to trisaccharide **6**, which, in turn, had a migration of 36 cm.

Polysaccharides **2a**, **2b** and **4a** (trisaccharide **8** was studied simultaneously) (ca 2–3 mg) were dissolved in 1% aqueous boric acid (2 ml) and portions of $NaBH_4$ (3 × 5 mg) were added within 5 hr. The mixture was left at +5° for 15 hr, deionized with a cation-exchange resin KU-2 (H^+), the latter was filtered off and washed with water (2 × 3 ml). The combined aqueous solns were taken to dryness and coevaporated with MeOH (3 × 5 ml). The residue was dissolved in M methanolic HCl (1 ml) and heated in a sealed tube at 100° for 20 hr. The methanolysate was taken to dryness and then acetylated and worked-up as above. The $CHCl_3$ layer was investigated by glc (column B, 175°, 3°/min). In comparison with authentic samples were found acetates of methyl rhamnosides (retention time 4.8 min), methyl galacto- and mannosides (retention time 11.0 min) (rhamnitol acetate emerges at 9.2 min under the above conditions) and two products with retention times of 10.0 and 15.5 min. The latter coincided with authentic galactitol acetate, while the former is apparently an acetate of anhydrogalactitol(s) (it was found in a control experiment) formed upon methanolysis and acetylation. The degree of polymerization was calculated from the ratio of the peak area of methyl rhamnosides to the sum of peak areas of galactitol and its anhydroderivative(s).

Acknowledgements The authors express their gratitude to Dr. A. S. Shashkov for recording and aid in interpretation of the ^{13}C NMR spectra, to Dr. Yu. Ya. Tendetnick for serological assays and to Dr. V. V. Berdjugin for recording of the Raman spectra.

REFERENCES

[1]V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky and N. K. Kochetkov, *Dokl. Akad. nauk SSSR* **251,** 108 (1980).
[2]A. F. Bochkov, I. V. Obruchnikov, V. M. Kalinevitch and N. K. Kochetkov, *Bioorgan. Chem.* **2,** 1085 (1976); *Tetrahedron Letters* 3403 (1975).
[3]I. V. Obruchnikov and N. K. Kochetkov, *Izv. Akad. nauk SSSR, Ser. Khim.* 2574 (1977); *Tetrahedron Letters* 57 (1977).
[4]V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky and N. K. Kochetkov, *Izv. Akad. nauk SSSR, Ser. Khim.* 2751 (1979); *Carbohydr. Res.* **68,** C11 (1979).
[5]K. Jann and O. Westphal, *Microbial polysaccharides* in *The Antigens* (Edited by M. Sela) Vol. 3, pp. 1–125. Academic Press, New York (1975).
[6]C. G. Hellerqvist, B. Lindberg, J. Lönngren and A. A. Lindberg, *Acta Chem. Scand.* **25,** 939 (1971).
[7]J. M. Ryan and H. E. Conrad, *Arch. Biochem. Biophys.* **162,** 530 (1974).
[8]V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky and N. K. Kochetkov, *Carbohydr. Res.* **84,** 211 (1980).
[9]V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky and N. K. Kochetkov, *Ibid.* **76,** 252 (1979).
[10]B. Helferich and K. L. Bettin, *Chem. Ber.* **104,** 1701, 3356 (1971).
[11]P. E. Jansson, L. Kenne, H. Liendgren, B. Lindberg and J. Lönngren, *A Practical Guide to the Methylation Analysis of Carbohydrates*, No. 8, pp. 1–75. University of Stockholm Chemical Communications (1976).
[12]P. A. Gent and R. Gigg, *J. Chem. Soc.* Perkin Trans I 364 (1975).
[13]H. M. Flowers, *Carbohydr. Res.* **18,** 211 (1971).
[14]Z. Dische, *Methods Carbohydr. Chem.* **1,** 513 (1962).
[15]A. S. Shashkov and O. S. Chizhov, *Bioorgan. Chem.* **2,** 437 (1976).
[16]N. K. Kochetkov, V. I. Torgov, N. N. Malysheva, A. S. Shashkov and E. M. Klimov, *Tetrahedron* **36,** 1227 (1980).
[17]N. K. Kochetkov, V. I. Torgov, N. N. Malysheva and A. S. Shashkov, *Ibid.* **36,** 1099 (1980).
[18]Yu. Ya. Tendetnick, N. M. Ovcharova, A. Ya. Chernyak and B. A. Dmitriev, *Bioorgan. Chem.* **6,** 250 (1980).
[19]A. I. Usov and M. A. Rechter, *Zh. Obshch. Khim.* **39,** 912 (1969).
[20]H. J. Dauben, Jr., L. R. Honnen and K. M. Harmon, *J. Org. Chem.* **25,** 1442 (1960).