Tetrahedron Vol. 43, No. 13, pp. 3109 to 3121, 1987
Printed in Great Britain.

0040-4020/87 $3.00 + .00
Pergamon Journals Ltd.

SYNTHESIS OF THE CAPSULAR POLYSACCHARIDE OF *STREPTOCOCCUS PNEUMONIAE* TYPE 14

N.K.Kochetkov*, N.E.Nifant'ev 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.

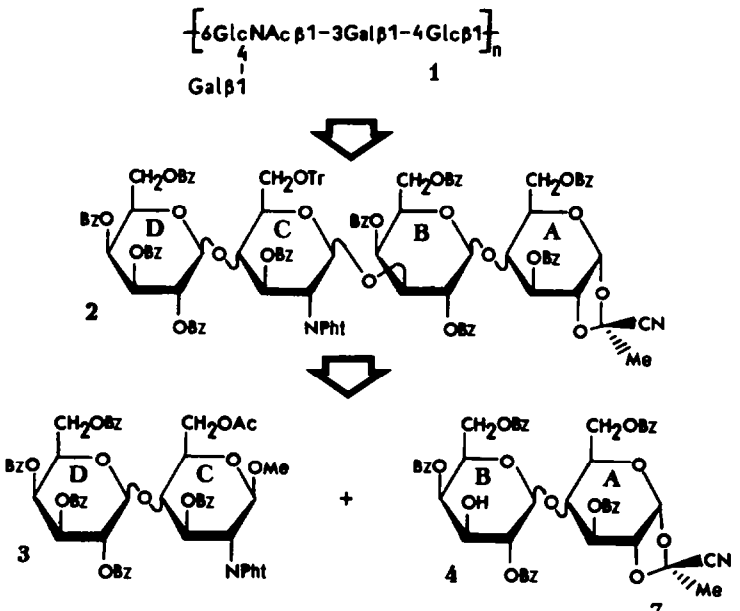
(*Received in UK* 18 *May* 1987)

Abstract - A regular, branched polysaccharide is synthesized whose structure $\{6(Gal\beta\ 1\text{-}4)GlcNAc\beta\ 1\text{-}3Gal\beta\ 1\text{-}4Glc\beta\ 1\}_n$ corresponds to that of the capsular polysaccharide of *Streptococcus pneumoniae* type 14. Synthesis is accomplished by using a stereo- and regiospecific polycondensation of a tritylated 1,2-O-cyanoethylidene derivative of a tetrasaccharide repeating unit. The latter is assembled from lactosamine and lactose precursors protected in such a way as to enable selective O-6-tritylation of the glucosamine moiety.

Bacterial polysaccharides (capsular polysaccharides, O-antigenic chains of lipopolysaccharides, teichoic acids) constitute an important class of biopolymers which exhibit broad-range biological activity and specificity. Studies on bacterial polysaccharides have acquired, in addition to traditional aspects, structural elucidation and synthesis of fragments, a new direction, *viz*. chemical synthesis[1]. The latter is based on a regio- and stereo-specific polycondensation of bifunctional monomers, the properly tritylated 1,2-O-(1-cyanoethylidene) derivatives of mono- or oligosaccharides, the latter corresponding to the repeating unit of a polysaccharide. It is with this method that successful syntheses of O-antigenic, linear heteropolysaccharides of *Salmonella newington*[2] and *Shigella flexneri* [3] have been carried out. As an extension of this method we have accomplished the first synthesis of a regular branched polysaccharide whose structure corresponds to that of the capsular polysaccharide of *Streptococcus pneumoniae* type 14.

The natural polysaccharide is built of the tetrasaccharide repeating units **1** [4]. Retrosynthetic analysis has revealed a posibility to synthesize **1** from the monomer **2** (Scheme 1). The latter , in turn, could be assembled from two disaccharide blocks, the O-benzoylated lactosamine derivative **3** with the temporary 6-O-acetyl protective group and the O-benzoylated lactose 1,2-O-(1-cyanoethylidene) derivative **4** with 3'-hydroxy group free. The encouraging prerequisites taken into account in the analysis shown were i) the possibility to employ sugar 1,2-O-(1-cyanoethylidene) derivatives with a free hydroxy group as glycosyl-acceptors [5]; ii) the preference of N-phthaloyl over N-acetyl protection of amino group in triphenylmethylium perchlorate-catalyzed glycosylation of aminosugars with 1,2-O-(1-cyanoethylidene) derivatives [6], and iii) the possibility to remove

Scheme 1.



selectively O-acetyl group in the presence of O-benzoyl groups [7]. The acyl protective groups are preferable over O-benzyl ones not only in glycosylation [8] but they all can be removed in one step by hydrazinolysis to prepare an unsubstituted polysaccharide.

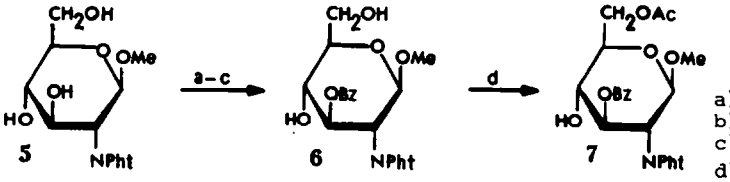
Here we describe the first synthesis of a regular, branched heteropolysaccharide based on the above synthetic strategy.

RESULTS AND DISCUSSION

1. Synthesis of the lactosamine block 3. Two approaches are conceivable to prepare the lactosaminide 3. The first one implied the use of the lactosamine itself and its functionalization. The second one envisaged the galactosylation, with benzobromogalactose 8 or other suitable glycosyl-donors, of methyl 6-O-acetyl-3-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 7 or its trityl ether 10. The choice in favour of the latter route was made since the chemical synthesis of lactosamine according to, e.g. [9-11], and its subsequent functionalization seemed to require larger number of steps.

The alcohol 7 was synthesized from the known methyl 2-deoxy-2-phthalimido-β-D-glucopyranoside 5 [6,12]. It was converted into the diol 6 in 76% overall yield by successive benzylidenation, benzoylation, and deacetalation, followed by selective acetylation into the monoacetate 7 (Scheme 2). The structure of the glucosaminides 6 and 7 and, in particular, the location of acyl substituents, was proved by ^{1}H-NMR spectroscopy (Table 1).

Scheme 2.



Glycosylation of 7 aimed at preparing the lactosaminide 3 was performed with benzobromogalactose 8 and silver triflate or mercuric cyanide - mercuric bromide as promotors (Scheme 3), or with 1-O-acetyl-2,3,4,6-tetra-O-benzoyl-β-D-galactopyranose 11 in the presence of trimethylsilyltriflate; the 4-trityl

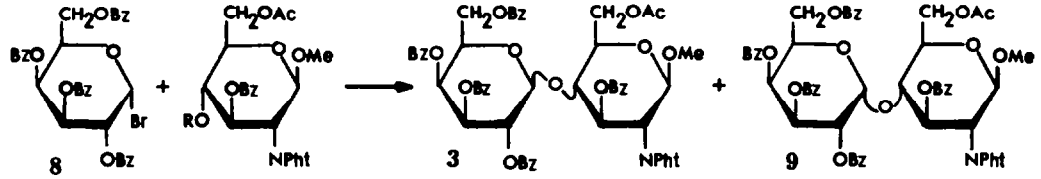
Table 1. Characteristic signals in the ^{1}H- and ^{13}C-NMR spectra of selected synthetic compounds* (δ_H, δ_C)

Compound	Spectral data**
2	C-3B (74.6), C-5B (72.5), C-4C (73.1), C-5C (74.6), C-1D (98.6)
3	H-6C (4.21dd), H-6'C (4.46dd), H-1D (4.87d), C-1C (99.2), C-4C (76.9), C-5C (72.9), C-6C (62.0), C-1D (101.2)
4	H-3B (4.14dd), H-4B (5.74d), C-2B (73.5), C-3B (71.9), C-4B (70.8), C-5B (72.2)
6	H-3 (5.87dd); H-4, H-6 and H-6' (3.90-4.07)
7	H-3 (5.89dd); H-4 and H-5 (3.80-3.87); H-6 and H-6' (4.47-4.57)
9	H-1D (5.75 d), C-4C (74.7), C-1D (97.2)
16	$C(CH_3)_2$ (1.38s and 1.68s), C-3B (77.1), C-4B (73.6)
17	H-3B(3.88dd), H-4B(4.11d), C-2B(73.7), C-3B(72.7), C-4B(69.1),C-5B(73.1)
21	C-2B (71.2), C-3B(78.1), C-4 (70.4), C-5B (70.8), C-1C (98.6), C-4C (76.2), C-5C (72.8), C-1D (101.0)
25	H-3B(5.64dd),H-4B(5.95dd),C-2B(69.8),C-3B(71.6),C-4B(68.3),C-5B(71.8)
26	C-4C (73.3), C-5C (74.1), C-1D (99.0)
27	C-1A (101.0), C-5C (74.2), C-6C (68.3)
28	H-6C, H-6'C and H-6'D (3.78-3.87); C-5C (75.1), C-6C (60.6)

*Complete spectral data will be published elsewhere.**For A-D series cf. Schemes 1, 6, and 7.

ether 10 served as the glycosyl-acceptor in reactions with benzobromogalactose 8 (and silver triflate as promotor) (Scheme 3) and in triphenylmethylium salt-catalyzed condensations with 1,2-O-(α-p-tolylthiobenzylidene)- and 1,2-O-(α-cyanobenzylidene)-3,4,6-tri-O-benzoyl-α-D-galactopyranoses (12 and 13).

Scheme 3.



In all cases a mixture of β- and α-linked disaccharides 3 and 9 was obtained (Table 2). Their structure was established on the basis of ^{1}H- and ^{13}C-NMR spectra (Table 1). Thus, in particular, the low-field shift for C-4 in the ^{13}C-NMR spectra demonstrates the galactose moiety to be linked to O-4 in both disaccharides. The coupling constants $J_{H-1',H-2'}$ and $J_{C-1',H-1'}$ (7.8 and 158.7 Hz for 3 and 3.7 and 172.0 Hz for 9) unequivocally prove the 1,2-trans- and 1,2-cis-configuration of the galactosidic bond in 3 and 9 respectively. As can be seen, the most effective synthesis of the target disaccharide 3 is that under conditions of the run 4 (Table 2) and these were used for its preparation. The 5-mmol-scale galactosylation of 7 afforded the disaccharides 3 and 9 in 44 and 40% yield (Scheme 3).

2. Synthesis of lactose block 4. The starting material was the acetylated lactose 1,2-O-(1-cyanoethylidene) derivative 14 (Scheme 4) whose preparation from acetobromolactose and sodium cyanide according to the general procedure [13] will be published elsewhere. The peracetate 14 was deesterified by sodium methoxide-

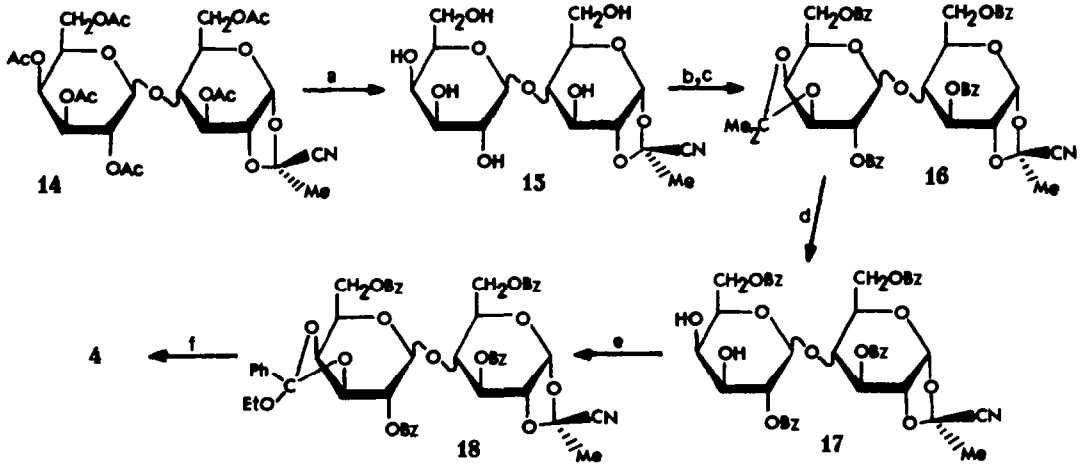
Table 2. Results of galactosylation of 7 and 10 (0.25-0.53 mmol-scale)*

Run №	Glycosyl-donor	Glycosyl-acceptor	Promotor	Solvent	Temperature (°C)	Yield 3+9 (%)	Ratio 3:9
1	8	7	$Hg(CN)_2$-$HgBr_2$	MeCN	60-70	53	1:3.8
2	8	7	$AgOSO_2CF_3$	Toluene	-25 - -30	98	1:2.2
3	8	7	$AgOSO_2CF_3$	CH_2Cl_2	-25 - -30	82	1.05:1
4	8	7	$AgOSO_2CF_3$	$MeNO_2$	-25 - -30	93	1.3:1
5	8	7	$AgOSO_2CF_3$	MeCN	-25 - -30	57	1:2.8
6	8	10	$AgOSO_2CF_3$	CH_2Cl_2	-25 - -30	95	1:1.2
7	11	7	$Me_3SiOSO_2CF_3$	CH_2Cl_2	20	23**	6.7:1
8	12	10	$TrClO_4$	CH_2Cl_2	20	25	2.1:1
9	13	10	$TrClO_4$	CH_2Cl_2	20	45	1.4:1
10	13	10	$TrBF_4$	CH_2Cl_2	20	19	8.5:1

*The ratio of glycosyl-donor:glycosyl-acceptor:promotor was 2:1:2 for runs 1-6, 1:1:0.1 for runs 7-9, and 1:1:0.8 for run 10.**The main product was methyl 4-O-acetyl-3-O-benzoyl-6-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (yield 29%)

catalyzed methanolysis and the product 15 was acetonated with 2,2-dimethoxypropane in DMF in the presence of TsOH at heating to give, after exhaustive benzoylation, the protected acetonide 16. Mild acid hydrolysis gave the 3',4'-diol 17. It was converted into the target alcohol 4 using the traditional procedure for an access to galactopyranose derivatives with 3-hydroxy group free, viz. a regioselective acid-catalysed ring-opening of cyclic 3,4-orthoesters (cf.[14]). In our case it was the orthobenzoate 18 obtained by transesterification of triethyl orthobenzoate with the diol 17 in the presence of TsOH. The structure of the lactose derivatives 16, 17, and 4 was established by ^{1}H- and ^{13}C-NMR spectroscopy using the data for the hexabenzoate 25 (see below). The glucopyranose moieties bearing the 1,2-O-(1-cyanoethylidene) group of compounds 4, 16, 17 and 25 exhibit spectral characteristics which coincide satisfactorily. Negative β-effects of benzoylation are apparent on comparing ^{13}C-NMR spectra of the two pairs, 17 vs. 4 and 4 vs. 25 ($\Delta\delta$ from -0.8 to -3.7 ppm) thus confirming the location of the free hydroxy groups in 17 and 4.

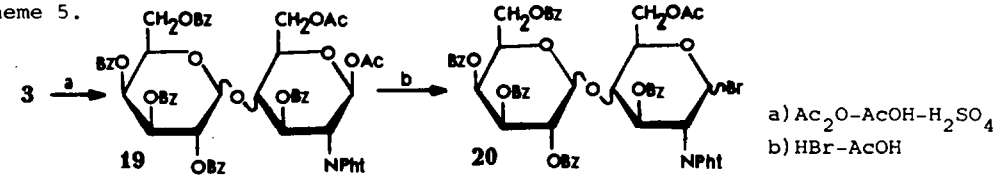
Scheme 4.



a) MeONa-MeOH; b) $Me_2C(OMe)_2$, TsOH; c) BzCl, Py; d) CF_3COOH-H_2O; e) $PhC(OEt)_3$, TsOH; f) AcOH

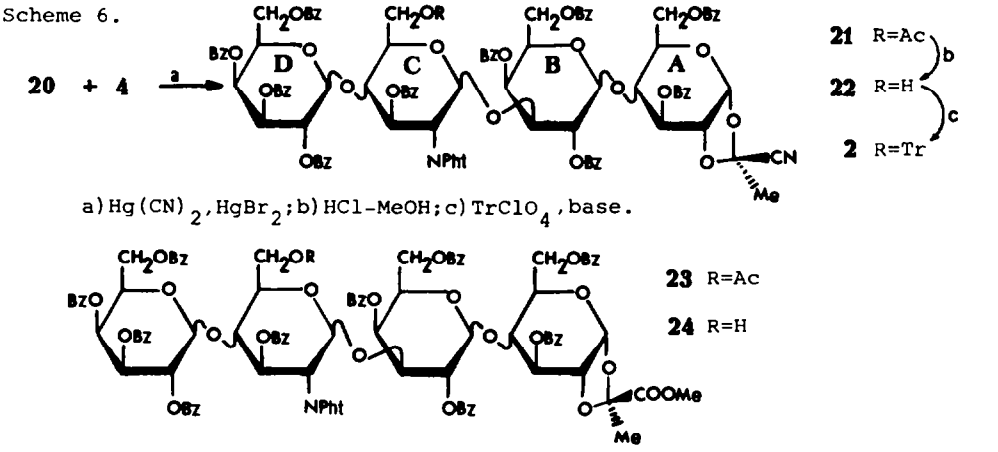
3. Synthesis of the tetrasaccharide monomer 2. Preparation of the monomer itself required, first of all, the coupling of the glycosyl-acceptor 4 with a glycosyl-donor derived from 3 to be carried out. The necessary donor could easily be obtained from 3 in two stages (Scheme 5): acetolysis of the methyl lactosaminide gave the 1-O-acetate 19 which on treatment with hydrogen bromide in glacial acetic acid yielded the glycosyl bromide 20 as a mixture of anomers (cf.[12]), it was used for coupling without additional purification.

Scheme 5.



Condensation of this glycosyl bromide 20 with the acceptor 4 in acetonitrile in the presence of mercuric cyanide and mercuric bromide (Scheme 6) yielded 61% of the tetrasaccharide 21. Spectral (^{1}H- and ^{13}C-NMR) data (Table 1) evidenced to the regioselectivity of glycosylation (i.e. to formation of glucosaminyl-(1-3)-galactose bond) with β-configuration of this newly formed linkage. This conclusion was made from comparison of the unit "B" resonances in ^{13}C-NMR spectra of 4 and 21 which displayed a downfield shift for C-3B in the latter and the characteristic negative β-effects of glycosylation ($\Delta\delta$ C-2B - -2.3 ppm, $\Delta\delta$ C-4B - -0.4 ppm) as well as from $J_{H\text{-}1C,H\text{-}2C}$ value (8.2 Hz) and C-1C chemical shift.

Scheme 6.



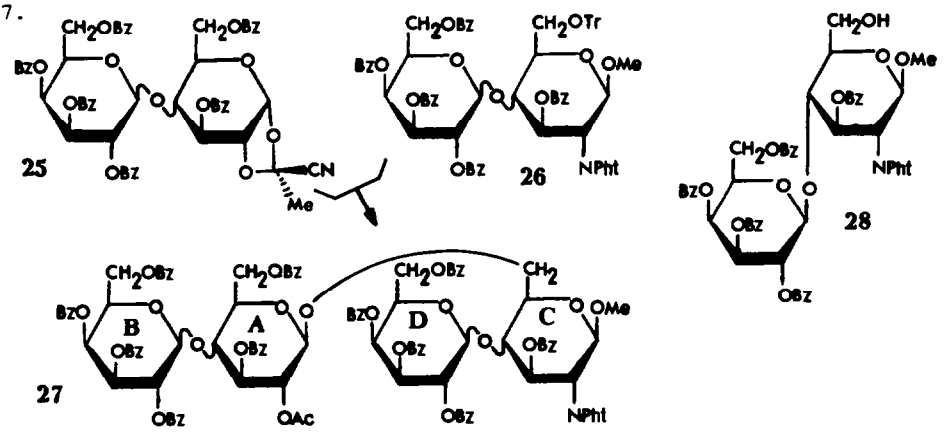
Deacetylation of the tetrasaccharide derivative 21 using mild, acid-catalyzed methanolysis [7] into the alcohol 22 was accompanied with formation of side-products, the methoxycarbonyl derivatives 23 and 24 (from cyanides 21 and 22, cf. [3]) which were identified with the help of ^{1}H-NMR spectral data (δ 1.7 and 3.7 for $CH_3CCOOCH_3$ fragment). These products accumulated with time (TLC) and it was rational to stop the reaction after 5-6 h when the extent of conversion of 21 into 22 was of about 30%. The similar chromatographic mobilities of 21 and 22 precluded their separation and the alcohol 22 was isolated from the reaction mixture together with the starting acetate 21. Treatment with triphenylmethylium perchlorate in the presence of 2,4,6-trimethylpyridine [15] converted the alcohol 22 into the desired monomer 2 while the acetate 21 remained unaffected and could be recovered by chromatography and reprocessed. Repetition of these procedures three more times gave the monomer 2 in an overall yield of 49%.

Its structure was confirmed by NMR spectra (Table 1). Thus, e.g., the pre-

servation of the 1,2-O-(1-cyanoethylidene) function evidenced from the presence, in the ^{13}C-NMR spectrum, of the characteristic signals for CH_3-C-CN at 24.1, 99.5, and 116.5 ppm and, in the ^{1}H-NMR spectrum, of signals for $\underline{CH_3}CCN$ (s, δ 1.84), H-1A (d, δ 5.71, $J_{H-1,H-2}$ 5.0 Hz), and H-2A (δ 4.20). That the trityl group was at O-6C was established from ^{13}C-NMR data with the use of tritylation effects found upon comparing spectra of 3 and 26 (see below). Thus, e.g., the signal for C-5C is shifted downfield ($\Delta\delta$ +1.8 ppm) and those for C-4C and C-1D are shifted upfield ($\Delta\delta$ -3.1 and -2.4 ppm) when passing from the acetate 21 to the trityl ether 2. Comparison of spectra of these two compounds reveals also a well pronounced long-range effects of acetyl-for-trityl exchange on C-3B and C-5B ($\Delta\delta$ -3.5 and +1.7 ppm).

4. Synthesis of a branched tetrasaccharide 27 as a modelling of elementary act of glycosidic bond formation upon polycondensation of the monomer 2. Glucosylation (and lactosylation) of O-6 tritylated glucosamine (and lactosamine) derivatives has not been performed hitherto. Therefore it seemed reasonable to carry out a glycosylation of the trityl ether 26 with the cyanoethylidene derivative 25 prior to the very polycondensation of the monomer 2. This coupling of lactose and lactosamine building blocks should result in (1-6)-bond formation, which is β in the natural polysaccharide, and thus may be regarded as a model of elementary glycosylation act of the polycondensation process. The cyanoethylidene derivative 25 represents the glycosylating function of the monomer 2 and the trityl ether 26 its glycosyl-acceptor site (Scheme 7).

Scheme 7.



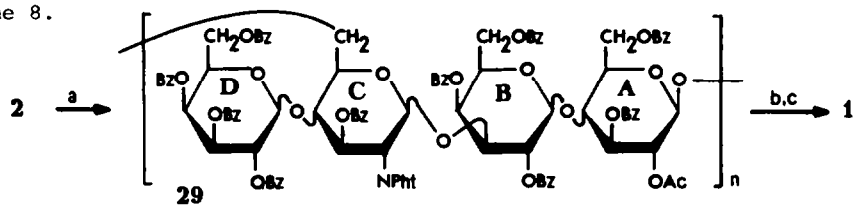
The hexabenzoate 25 was obtained from 14 by Zemplén deacetylation followed by benzoylation. The lactosaminide 3 served as a starting material for preparation of 26. Selective O-deacetylation of 3 using mild, acid-catalyzed methanolysis [7] gave the alcohol 28 whose structure followed from ^{1}H-NMR (upfield shift of H-6C signals for 28 in comparing with 3) and ^{13}C-NMR spectral data (upfield shift of C-6C and downfield shift of C-5C in 28 as compared to 3). Action of triphenylmethylium perchlorate on 28 in the presence of 2,4,6-trimethylpyridine [15] gave rise to the trityl ether 26. Its detritylation with 90% trifluoroacetic acid regenerated 28 thus proving the location of the trityl group in 26 at O-6C. Replacement of O-acetyl group in 3 by O-trityl group in 26 results in significant changes in chemical shifts for C-4C ($\Delta\delta$ -3.6 ppm), C-5C ($\Delta\delta$ +1.2 ppm), and C-1D ($\Delta\delta$ -2.2 ppm). This feature was employed for interpretation of the ^{13}C-NMR spectrum of 2 (see above).

Glycosylation of the trityl ether 26 with the 1,2-O-(1-cyanoethylidene) derivative 25 was performed in the presence of triphenylmethylium perchlorate (0.2

equiv.) using vacuum technique[16] and afforded the branched tetrasaccharide 27 in high yield (84%). This tetrasaccharide represents a derivative of the repeating unit of the polysaccharide 1. Spectral data support its structure. Signals for C-6C and C-5C are downfield (by 7.7 ppm) and upfield (by 0.9 ppm) shifted as compared with the respective signals in the ^{13}C-NMR spectrum of 28, thus proving O-6-lactosylation of the latter. That the newly formed glycosidic bond is β (as well as others) followed from $^{1}J_{C,H}$ coupling constants (163.6 Hz) for anomeric centres, from $^{3}J_{H-1A,H-2A}$(8.5 Hz), and from chemical shift for C-1A (101.0 ppm). Thus the condensation of the cyanoethylidene derivative 25 with the trityl ether 26 was both effective and stereospecific and this was a favourable premise for successful synthesis of the polysaccharide 1 by polycondensation of the monomer 2.

5. Polycondensation of the monomer 2 and characterization of the polysaccharide 1. Polycondensation of the bifunctional monomer 2 was performed under standard conditions [1-3], i.e. in dichloromethane in the presence of triphenylmethylium perchlorate (0.1 equiv.) at room temperature. Trial experiment has shown the monomer to be absent after 18 h and the product formed to be devoid of the trityl group (TLC). Therefore in a preparative run the reaction mixture was also kept for 18 h and thereafter the catalyst was destroyed by addition of aqueous pyridine. The protected polysaccharide 29 (Scheme 8) was separated from the noncarbohydrate products (TrCN, TrOH) by column chromatography on silica gel.

Scheme 8.



a) $TrClO_4$; b) H_2NNH_2-H_2O; c) Ac_2O-H_2O-MeOH

The ^{13}C-NMR spectrum of 29 contained, inter alia, signals for the anomeric carbons (δ 98.7 and 100.6-102.2), C-2C (δ 54.9), and acetyl group. No signals at δ 24, 99.5, 117, and 87, characteristic for $\underline{C}H_3$-$\underline{C}$-$\underline{C}N$ and the $Ph_3\underline{C}$ carbons were present thus demonstrating the absence in the polycondensation product of the cyanoethylidene and trityl groups and is in keeping with previous data [17,18].

Deprotection of 29 was accomplished by hydrazinolysis [3], subsequent N-acetylation then gave the polysaccharide 1 which was isolated by gel chromatography on Bio-Gel P-4 with concomitant removal of aceto-, phthalo-, and benzohydrazides. The polysaccharide 1 was eluted from a TSK 40 column as two poorly resolved peaks (fractions 1a and 1b), the yield of the former, possessing higher molecular weight, being 59% and that of the latter 26%.

The structure of the polysaccharide 1a was elucidated by means of methylation analysis [19-21] and ^{13}C-NMR spectroscopy. The Hakomori methylation [22] of the polysaccharide followed by acid hydrolysis, borohydride reduction, and acetylation afforded acetates of only 2,3,4,6-tetra-, 2,3,6-tri, and 2,4,6-tri-O-methylhexitols as well as 1,4,5,6-tetra-O-acetyl-3-O-methyl-2-(N-methyl)acetamido-2-deoxyglucitol identified by GLS-MS and by direct comparison with the authentic sample for the latter. It thus shows that the repeating unit of the polysaccharide contains a nonsubstituted,side hexopyranosyl residue, two monosubstituted (at O-3 and O-4) hexopyranose residues, and the disubstituted (O-4, O-6) glucosamine residue (branching point) that is in complete agreement with the structure anticipated.

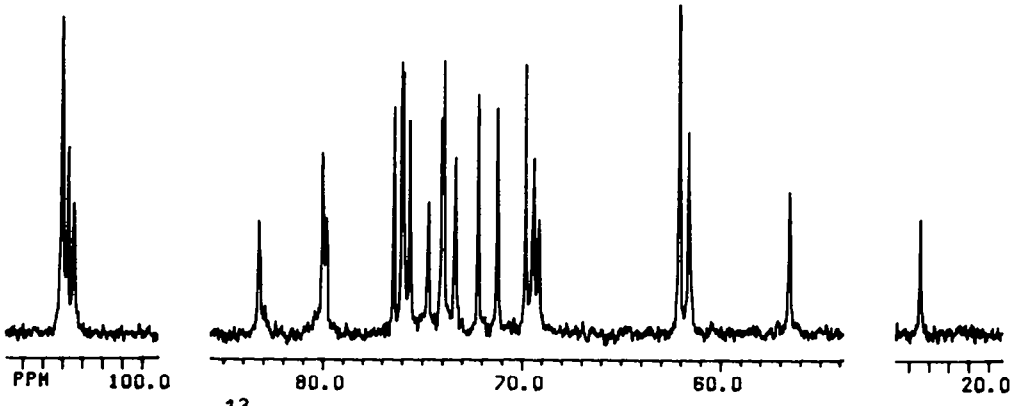


Fig. ^{13}C-NMR spectrum of polysaccharide 1a (D_2O, internal standard MeOH, δ 50.15 with respect to tetramethylsilane).

Table 3. ^{13}C-NMR spectral data for compounds 1a, 30, and 31 (δ, D_2O).

Compound	Unit*	C-1	C-2	C-3	C-4	C-5	C-6	C(O)---CH_3		OCH_3
1a	Glc-A	103.8	73.4	75.7**	79.8	76.0**	61.6			
	Gal-B	103.6	71.3	83.3	69.5	76.1	62.2			
	GlcN-C	104.1	56.5	74.0	80.0	74.7	69.1	175.9	23.4	
	Gal-D	104.15	72.2	73.9	69.9	76.5	62.2			
30	GlcN	103.0	56.3	73.7	80.2	76.0	61.5	175.7	23.4	58.1
	Gal	104.1	72.2	73.9	69.8	76.5	62.1			
31	Glc	103.6	73.6	75.6**	79.8	75.9**	61.5			
	GlcN	103.1	56.2	74.0	80.0	74.8	68.9	175.6	23.4	58.3
	Gal***	104.1	72.2	74.0	69.8	76.5	62.1			

*For A-D series cf. Scheme 8. **Assignment may be reversed. ***All signals of double intensity, that at δ 74.0 of triple intensity.

It can be seen (Fig.) that the polysaccharide 1a is a regular one with a tetrasaccharide repeating unit. That the configurations of all glycosidic bonds including the newly formed one are β followed from $^1J_{C,H}$ values for anomeric centres (162-164 Hz). Three low-field signals (δ 79.8, 80.0, 80.3) correspond to ring atoms bearing glycosyloxy substituents, of four C-6's three are nonsubstituted (δ 61.6 and 62.2x2). Thus, one primary and three secondary carbon atoms are involved in glycosidic bonds. Interpretation of the ^{13}C-NMR spectrum of 1a (Table 3) could be done with the use of spectral data for Galβ 1-4GlcNAcβ-OMe 30, Galβ 1-4Glcβ 1-6(Galβ 1-4)GlcNAcβ-OMe 31, Galβ 1-4Glcβ-OMe 32[23], GlcNAcβ-$O(CH_2)_6NH_2$ 33[24], Galβ 1-6GlcNAcβ-$O(CH_2)_6NH_2$ 34[24], Galβ 1-3Galβ 1-4Glc [25], and Galβ 1-4GlcNAcβ 1-3Galβ-OMe [26]. The two former oligosaccharides (30 and 31, Table 3) were obtained by deprotection (hydrazinolysis) followed by N-acetylation of 3 and 27 respectively; the spectrum of 30 prepared in this work coincided satisfactorily with that reported [27]. Advantage was taken of spectral data for the lactoside 32 and lactosaminide 30 to interpret the spectrum of the tetrasaccharide 31 using α- and β-effects of glycosylation at O-6 (+7.5 and -1.0 ppm) found from comparison of spectra of compounds 33 and 34.

Thus, methylation and spectral data for 1a show quite definitely that the polycondensation of the monomer 2 was regio- and stereospecific and the structure of the synthetic polysaccharide corresponds to that of the capsular polysaccharide of Streptococcus pneumoniae type 14. Optical rotation values for 1a and 1b (+8.4° and +12.0°) coincide well with that of the natural polysaccharide (+5°)[4]. The fraction 1a was eluted from the TSK HW-40(S) column with the void volume and thus its molecular weight can be estimated as ca. 6000 and the number-average

degree of polymerisation as ca. 10.

The compounds 1a, 1b, and 31 were tested for inhibitory activity in S. pneumoniae type 14 - anti-14 system (ELISA). The tetrasaccharide 31 exhibited low activity whereas 1b and especially 1a proved to be effective inhibitors. The concentrations required for 50% inhibition were >100, 0.8, 0.2, and 0.08 μg/ml for 31, 1b, 1a, and the natural polysaccharide respectively.

The synthesis of the polysaccharide 1a together with other syntheses reported [1-3] clearly demonstrate broad possibilities of the method of polycondensation of tritylated 1,2-O-(1-cyanoethylidene) derivatives for an acces to regular homo- and heteropolysaccharides.

EXPERIMENTAL

The reagents and solvents as well as the instruments used are described elsewhere [3,18]. TLC was performed on Kieselgel 60 (Merck) plates in solvent systems EtOAc-toluene 1:4 (A), 1:2 (B), and 1:1 (C) followed by spraying with 50-70% H_2SO_4 and charring. Column chromatography was performed on silica gel L 40/100 μm (CSSR) using gradient elution from benzene to EtOAc.

Methyl 3-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6). To a soln of 5 (4.5 g, 13.9 mmol) [6,12] in abs. MeCN (20 ml) benzaldehyde dimethylacetal (13 ml, ca. 70 mmol) and $TsOH \cdot H_2O$ (ca. 20 mg) were added. The reaction mixture was kept at 20° for 17 hr, then pyridine (6 ml) was added followed by dropwise addition, at 0-5°, of benzoyl chloride (5.8 ml). After completion of benzoylation (ca. 3 hr at 20°) the excess of benzoyl chloride was destroyed by treatment with MeOH (5 ml), then the reaction mixture was taken to dryness and the residue was dried by addition and distillation of toluene in vacuo. It was then dissolved in 90% aq CF_3COOH (20 ml), the soln was kept for ca. 1 hr at 20° and poured into ice-water (200 ml). The $CHCl_3$-extracts (150 ml plus 2x50 ml) were combined, washed with satd aq $NaHCO_3$ and water, filtered through cotton, and evaporated. The residue was crystallized from $CHCl_3$-hexane and the crystalline product was purified by chromatography ($CHCl_3 \rightarrow CHCl_3$-MeOH 93:7) to give 6 (4.5 g, 76%), m.p. 168-169°($CHCl_3$-hexane), $[\alpha]_D$+111.8°(c 0.94, $CHCl_3$), R_f 0.15 (C). (Found: C, 62.03; H, 5.24; N, 3.34. Calc for $C_{22}H_{21}NO_8$: C, 61.82; H, 4.95; N, 3.28%)

Methyl 6-O-acetyl-3-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7). To a stirred soln of 6 (1.4 g, 3.3 mmol) and pyridine (0.8 ml) in dry $CHCl_3$ (10 ml) a soln of acetyl chloride (0.225 ml, 3.6 mmol) in dry $CHCl_3$ (5 ml) was added dropwise in 1 hr at 20°, stirring was continued for 20 min, the soln was concentrated, the residue was coevaporated with toluene, and crystallized from $CHCl_3$-hexane to give 7. Column chromatography of the mother liquor (benzene-acetone) gave additional portion of 7, total yield 1.33 g (86%), m.p. 181-184° ($CHCl_3$-hexane), $[\alpha]_D$+98.6°(c 0.73, $CHCl_3$), R_f 0.42(C) (Found: C, 61.31; H, 4.57; N, 3.11. Calc for $C_{24}H_{23}NO_9$: C, 61.40; H, 4.94; N, 2.98%).

Methyl 6-O-acetyl-3-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β- and -α-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (3 and 9). To a stirred soln of 7 (2.35 g, 5.0 mmol), silver triflate (2.57 g, 10.0 mmol), and tetramethylurea (0.9 ml, 10 mmol) in abs. $MeNO_2$ (50 ml) at -25 → -30°, under argon, was added in 1 hr a soln of 8 (6.32 g, 9.6 mmol) in abs. $MeNO_2$. Stirring was continued at this temperature for 3 hr, $CHCl_3$ (300 ml) was then added, solids were filtered off, the soln was washed with 5% aq $Na_2S_2O_3$ (2x200 ml) and water (2x200 ml), concentrated, and the residue was chromatographed to give 9 (2.09 g, 40%), foam, $[\alpha]_D$+121.3°(c 0.38, $CHCl_3$), R_f 0.48(A), and 3 (2.30 g, 44 %), foam, $[\alpha]_D$+70.0°(c 0.5, $CHCl_3$), R_f 0.40(A)(Found (for 3): C, 66.14; H, 4.77; N, 1.30; (for 9): C, 66.81; H, 4.55; N, 1.53. Calc for $C_{58}H_{49}NO_{18}$: C, 66.47; H,

4.77; N, 1.34%).

3,6-Di-O-benzoyl-4-O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-1,2-O-[1-(exo-cyano)ethylidene]-α-D-glucopyranose (16). To a soln of 14 (3.23 g, 5.0 mmol) in abs. MeOH (100 ml) was added 0.5 M methanolic MeONa (1 ml) and the soln was kept at 20° until complete conversion of 14 into 15 (R_f 0.15, $CHCl_3$–MeOH 3:1) occured (ca. 1 hr). It was neutralized with a cation-exchange resin KU-2 (H^+), filtered, and taken to dryness. The hexaol 15 obtained (ca. 2 g) was dissolved in dry DMF (5 ml), then acetone dimethylacetal (1.5 ml, 11.3 mmol) and $TsOH \cdot H_2O$ (20 mg) were added and the soln was heated at 70–80° for 10 hr. The reaction mixture was diluted with pyridine (10 ml), benzoyl chloride (4.6 ml, 40 mmol) was added dropwise with cooling, and the mixture was left at 20° overnight. Methanol (5 ml) was added to destroy the excess of benzoyl chloride, the soln was diluted with $CHCl_3$ (100 ml), washed with water, filtered through cotton, evaporated, and evaporated with toluene. Column chromatography of the residue gave 16 (2.66 g, 63%), foam, $[\alpha]_D$+20.6°(c 0.78, $CHCl_3$), R_f 0.72 (A) (Found: C, 65.47; H, 5.16; N, 1.49. Calc for $C_{46}H_{43}NO_{15}$: C, 65.01; H, 5.10; N, 1.65%).

3,6-Di-O-benzoyl-4-O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-1,2-O-[1-(exo-cyano)ethylidene]-α-D-glucopyranose (17). Deacetonation of 16 (1.97 g, 2.32 mmol) was performed by treatment with 90% aq CF_3COOH (20 ml) for 30 min at 20°. The reaction mixture was poured in water (100 ml), the product was extracted with $CHCl_3$ (50 ml plus 2x20 ml) and isolated from the combined extracts by chromatography; the diol 17 (1.51 g, 81%) was obtained as a foam, $[\alpha]_D$–9.1°(c 0.35, $CHCl_3$), R_f 0.35(C) (Found: C, 63.56; H, 4.92; N, 1.81. Calc for $C_{43}H_{39}NO_{15}$: C, 63.78; H, 4.85; N, 1.73%).

3,6-Di-O-benzoyl-4-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-1,2-O-[1-(exo-cyano)ethylidene]-α-D-glucopyranose (4). A soln of 17 (1.0 g, 1.26 mmol), triethyl orthobenzoate (2.9 ml, 13 mmol), and $TsOH \cdot H_2O$ (20 mg) in abs. benzene (15 ml) was kept at 20° for 2 hr to afford the 3',4'-orthobenzoate 18 (TLC). To the reaction mixture was added 80% aq AcOH (30 ml) and after 20 min the soln was poured in water (200 ml). Extraction with $CHCl_3$ (3x50 ml) and column chromatography gave 4 (1.08 g, 96%) as a foam, $[\alpha]_D$+6.1°(c 0.33, $CHCl_3$), R_f 0.25(A) (Found: C, 65.32; H, 4.70; N, 1.38. Calc for $C_{50}H_{43}NO_{16}$: C, 65.71; H, 4.74; N, 1.53%).

1,6-Di-O-acetyl-3-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranose (19). The methyl lactosaminide 3 (2.0 g, 1.9 mmol) was dissolved in a mixture of Ac_2O (24.3 ml), AcOH (25.2 ml), and conc. H_2SO_4 (1.98 ml) and left overnight at +5°. The soln was poured onto ice (500 ml), stirred for 2 hr, and extracted with $CHCl_3$ (100 ml plus 3x50 ml). The combined extracts were processed conventionally, crystallisation from $CHCl_3$–hexane gave the diacetate 19 (1.55 g, 75%), m.p. 139–141°, $[\alpha]_D$+80.0°(c 0.5, $CHCl_3$), R_f 0.34(A) (Found: C, 66.15; H, 4.73; N, 1.32. Calc for $C_{59}H_{49}NO_{19}$: C, 65.86; H, 4.59; N, 1.30%).

O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-(6-O-acetyl-3-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-α-D-glucopyranose (21). To a soln of 19 (1.31 g, 1.22 mmol) in abs. benzene (13 ml) was added 40% HBr in glacial AcOH (20 ml) and the mixture was kept at 20° for 1 hr. Then it was poured into ice-water (300 ml), extracted with $CHCl_3$ (100 ml plus 2x50 ml), the combined extracts were washed with water, aq $NaHCO_3$, and water, filtered through cotton, concentrated, and dried in vacuo to give 20

(1.3 g) as a mixture of anomers (cf. [12]), R_f 0.53(A), major product, and R_f 0.41. Glycosylation of the alcohol 4 with the glycosyl bromide 20 was performed as described before [3,12], reagents being dried by twofold freeze-drying from benzene and MeCN distilled from CaH_2 using vacuum technique ($4x10^{-3}$ Torr). To a stirred soln of 4 (0.80 g, 0.87 mmol), mercuric cyanide (0.31 g, 1.22 mmol), and mercuric bromide (0.22 g, 0.61 mmol) in MeCN (15 ml) was added dropwise, under argon, a soln of 20 (1.30 g, 1.17 mmol) in MeCN (20 ml) in 30 min and stirring was continued for 1 hr at 20°. The reaction mixture was concentrated, the residue was partitioned between $CHCl_3$ (80 ml) and satd aq KBr (100 ml), the organic layer was washed with aq KBr (100 ml) and water (2x100 ml), filtered through cotton, and concentrated. Chromatography of the residue and rechromatography (EtOAc-heptane 1:1) gave 21 (1.03 g, 61%), foam, $[\alpha]_D$+43.7°(c 1.54, $CHCl_3$), R_f 0.32(A).

Conversion of the acetate 21 into the monomer 2. A soln of the acetate 21 (1.19 g, 0.62 mmol) in dry $CHCl_3$ (7 ml) and abs. MeOH (7 ml) was treated with methanolic HCl prepared by addition, at 0°, of acetyl chloride (0.6 ml, 8 mmol) to abs. MeOH (15 ml). The reaction mixture was kept at 20° for 5–6 hr, poured into ice-water (100 ml), and extracted with $CHCl_3$ (3x30 ml). The combined extracts were chromatographed to give a mixture of 21 and 22 (0.98 g), and a mixture of methoxycarbonyl derivatives 23 and 24 (0.13 g). To a soln of the former mixture and 2,4,6-trimethylpyridine (0.3 ml) in dry CH_2Cl_2 (5 ml) was added, portionwise, $TrClO_4$ (60–70 mg) until bright-yellow colour persisted. The excess of the reagent was destroyed by addition of 1:3 methanol-pyridine (0.5 ml), the mixture was diluted with $CHCl_3$ (100 ml), washed with water (100 ml), filtered through cotton, and concentrated. The residue was chromatographed to give the monomer 2 (0.32 g, 24%) and starting acetate 21 (0.48 g). The latter was subjected to deacetylation and tritylation as described above three more times, the monomer 2 was obtained in a total yield of 0.64 g (49%), $[\alpha]_D$+16.7°(c 2.4, $CHCl_3$), R_f 0.52(A); the yield of 23+24 was 0.21 g.

3,6-Di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-1,2-O-[1-(exo-cyano)ethylidene]-α-D-glucopyranose (25). This compound was obtained by conventional benzoylation of the hexaol 15 (prepared from 14 (0.65 g, 1 mmol)), yield 0.83 g (82%), $[\alpha]_D$+89.1°(c 0.55, $CHCl_3$), R_f 0.67(benzene-EtOAc 8:1)(Found C, 67.37; H, 4.61; N, 1.38. Calc for $C_{57}H_{47}NO_{17}$: C, 67.26; H, 4.65; N, 1.38%).

Methyl 3-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (28). To a soln of 3 (0.50 g, 0.48 mmol) in $CHCl_3$ (10 ml) methanolic HCl (cf. synthesis of 2) (9 ml) was added, the mixture was kept at 20° for 17 hr, after conventional work-up and chromatography was obtained the alcohol 28 (0.48 g, 98%), foam, $[\alpha]_D$+44.5°(c 0.75, $CHCl_3$), R_f 0.20(A) (Found:C,66.74;H,4.30;N,1.37. Calc for $C_{56}H_{47}NO_{17}$:C, 66.86; H, 4.71; N, 1.39%).

Methyl 3-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-6-O-trityl-β-D-glucopyranoside (26). Tritylation of the alcohol 28 (0.35 g, 0.35 mmol) in CH_2Cl_2 (10 ml) with $TrClO_4$ (0.14 g, 0.40 mmol) in the presence of 2,4,6-trimethylpyridine (0.1 ml, 0.75 mmol) was performed as described in the preparation of 2, the yield of 26 was 0.41 g (94%), m.p. 176–179° ($CHCl_3$-hexane), $[\alpha]_D$-7.9°(c 0.38, $CHCl_3$), R_f 0.53(A) (Found: C, 72.50; H, 5.29; N, 0.96. Calc for $C_{75}H_{61}NO_{17}$: C, 72.16; H, 4.93; N, 1.12%).

Methyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-(2-O-acetyl-3,6-di-O-benzoyl-β-D-glucopyranosyl)-(1→6)-3-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (27). Glycosylation of the trityl ether 26 (312 mg, 0.25 mmol) with the cyanoethylidene derivative 25 (254 mg, 0.25 mmol) in CH_2Cl_2(2.5 mmol) in the presence of

$TrClO_4$ (17.2 mg, 0.05 mmol) was performed using vacuum technique [16], after 17 hr at 20° the reaction product 27 was isolated by chromatography, yield 420 mg (84%), foam, $[\alpha]_D$+51.7° (c 1.3, $CHCl_3$), R_f 0.27 (A).

Polycondensation of the monomer 2. Polycondensation of 2 (640 mg, 0.3 mmol) under the action of $TrClO_4$ (10.2 mg, 0.03 mmol) in CH_2Cl_2 (6 ml) was carried out using vacuum technique as described [2]. After 18 hr at 20° the bright-yellow reaction mixture was treated with 75% aq pyridine (0.5 ml), the discoloured soln was diluted with $CHCl_3$ (80 ml), washed with water (2x50 ml), the organic layer was concentrated, the residue was coevaporated with $CHCl_3$-toluene (5 ml each, twice) and then chromatographed to give 29 (505 mg, ca. 90%) as a white amorphous powder, $[\alpha]_D$+39.4°(c 1.6, $CHCl_3$), R_f 0-0.3 (A), ca. 0.9 (B).

Preparation of the polysaccharide 1. The polycondensation product 29 was dissolved with heating in EtOH (25 ml) and 99% hydrazine hydrate (2.5 ml) and boiled under reflux for 20 hr with gradual addition of water (5 ml) to dissolve the precipitate formed. The reaction mixture was concentrated, water was added to, and distilled from, the residue (3x5 ml), which was dissolved in 75% aq MeOH (20 ml) and treated with Ac_2O (10 ml) at 20° overnight. The mixture was concentrated, the residue was taken in water (15 ml) and washed with n-BuOH (3x15 ml). The aqueous soln was concentrated and the residue subjected to gel-filtration on Bio-Gel P-4 (55x2.5 cm, -400 mesh, V_o 110 ml) with 0.1M aq AcOH as an eluent. The carbohydrate-containing fractions (monitoring with orcinol-H_2SO_4) were concentrated to give the polysaccharide 1 (140 mg, ca. 90%). It was fractionated on a column with TSK HW-40(S) gel (80x1.6 cm, 25-40 μm, V_o 50 ml) in water. Two fractions were collected, 1a (eluted within 50-63 ml), 92 mg (59%), $[\alpha]_D$+8.4° (c 1.0, water), and 1b (eluted within 64-83 ml), 41 mg (26%), $[\alpha]_D$+12.0°(c 1.0, water.

Methylation analysis of 1a. The polysaccharide 1a (3 mg) was methylated according to [22], the excess of MeJ was removed by evaporation, the mixture was diluted with water (5 ml) and the soln was slowly (1 drop/s) passed through a SEP-PAK C18 cartridge ("Waters"). The cartridge was washed with water (10 ml) and MeOH (10 ml). The latter eluate was taken to dryness and the residue was hydrolyzed, under argon, with 2M aq CF_3COOH at 121° for 1 hr in a sealed test-tube. The hydrolysate was concentrated, water was added to, and distilled from, the residue (3x5 ml), and $NaBH_4$ (ca. 5 mg) was added to the soln. The reaction mixture was kept overnight at 20°, acidified with AcOH (0.5 ml), and concentrated. Boric acid was removed from the residue by repeated codistillation with MeOH (5x5 ml), then Ac_2O-pyridine (1:1, 1 ml) was added and acetylation, under argon, was conducted in a sealed tube for 1 hr at 100°. The products obtained were isolated conventionally and analyzed by glc-ms on a Varian-MAT 111 ("Gnom") instrument (stainless-steel column (100x0.5 cm) with 5% SE-30 on Chromaton N-AW-DMCS (0.160-0.200 mm), carrier gas helium, temperature programming 180- 250° at 4°/min). Peaks with T_R 1.00, 1.50, 1.67, and 7.73 (relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-galactitol) were observed and identified, from their mass-spectra as acetates of 2,3,4,6-tetra-O-methyl-hexitol, 2,3,6-tri-O-methyl-hexitol, 2,4,6-tri-O-methyl-hexitol, and 2-deoxy-3-O-methyl-2-(N-methyl)acetamido-glucitol [19-21]; the latter was also identified by comparison with the authentic sample.

Methyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside 30. The disaccharide derivative 3 (100 mg) was subjected to hydrazinolysis and N-acetylation as described above, gel-chromatography on TSK HW-40(S) gel gave 30 (31 mg, 82%, eluted within 95-105 ml), m.p. 269-272°(MeOH), $[\alpha]_D$-22.8°(c 1.0,

water). Lit. data: m.p. 283-285°(MeOH-EtOH), $[\alpha]_D$-16.7°(c 0.3, water) [27]; m.p. 243-245° (MeOH), $[\alpha]_D$-23.1° (c 0.86, water) [28].

Methyl O-(β-D-galactopyranosyl)-(1→4)-O-(β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (31). This terasaccharide was obtained by hydrazinolysis of 27 (200 mg) followed by N-acetylation and isolated by gel-chromatography on TSK HW-40(S) gel, elution volume 91-100 ml, yield 55 mg (73%), amorphous powder, $[\alpha]_D$-4.4° (c 2.75, water).

Acknowledgements: The authors express their gratitude to Dr. A.S.Shashkov (Institute of Organic Chemistry, Moscow) for recording NMR spectra, to Dr. A.Weintraub (Department of Clinical Bacteriology, Karolinska Institute, Huddinge Hospital, Sweden) for providing the natural polysaccharide and homologous antiserum, and Dr. A.Ya.Chernyak (Institute of Organic Chemistry, Moscow) for performing the inhibition studies.

REFERENCES

1. N.K.Kochetkov, Sov.Scient.Rev.Sect.B. Chem.Rev. 4, 1 (1982).
2. N.K.Kochetkov, V.I.Betaneli, M.V.Ovchinnikov, L.V.Backinowsky, Tetrahedron 37, suppl. 9, 149 (1981).
3. N.K.Kochetkov, N.E.Byramova, Yu.E.Tsvetkov, L.V.Backinowsky, Tetrahedron 41, 3363 (1985).
4. B.Lindberg, J.Lönngren, D.A.Powell, Carbohydr. Res. 58, 177 (1977).
5. V.I.Betaneli, L.V.Backinowsky, N.E.Byramova, M.V.Ovchinnikov, M.M.Litvak, N.K.Kochetkov, Carbohydr. Res. 113, C1 (1983).
6. M.V.Ovchinnikov, N.E.Byramova, L.V.Backinowsky, N.K.Kochetkov, Bioorg. Khim. 9, 401 (1983).
7. N.E.Byramova, M.V.Ovchinnikov, L.V.Backinowsky, N.K.Kochetkov, Carbohydr. Res. 124, C8 (1983).
8. M.V.Ovchinnikov, N.E.Byramova, L.V.Backinowsky, N.K.Kochetkov, Bioorg. Khim. 9, 391 (1983).
9. J.Alais, A.Veyrieres, Carbohydr. Res. 93, 164 (1981).
10. J.Arnarp, J.Lönngren, J.Chem.Soc. Perkin Trans. 1 2070 (1981).
11. B.A.Dmitriev, Yu.A.Knirel, N.K.Kochetkov, Izv. Akad. Nauk SSSR. Ser. Khim. 2365 (1973).
12. N.E.Byramova, L.V.Backinowsky, N.K.Kochetkov, Izv. Akad. Nauk SSSR. Ser. Khim. 1140 (1985).
13. V.I.Betaneli, M.V.Ovchinnikov, L.V.Backinowsky, N.K.Kochetkov, Carbohydr. Res. 68, C11 (1979).
14. R.U.Lemieux, H.Driguez, J. Amer. Chem. Soc. 97, 4069 (1975).
15. V.I.Betaneli, M.V.Ovchinnikov, L.V.Backinowsky, N.K.Kochetkov, Carbohydr. Res. 76, 252 (1979).
16. L.V.Backinowsky, N.E.Nifant'ev, V.I.Betaneli, M.I.Struchkova, N.K.Kochetkov, Bioorg. Khim. 9, 74 (1983).
17. V.I.Betaneli, M.M.Litvak, L.V.Backinowsky, N.K.Kochetkov, Izv. Akad. Nauk SSSR. Ser. Khim. 1172 (1985).
18. L.V.Backinowsky, N.E.Nifant'ev, N.K.Kochetkov, Bioorg.Khim. 10, 1212 (1984).
19. P.-E.Jansson, L.Kenne, H.Liedgren, B.Lindberg, J.Lönngren, Chem. Commun. Univ. Stockholm 1 (1976).
20. K.Stellner, H.Saito, S.Hakomori, Arch.Biochem.Biophys. 155, 464 (1973).
21. G.O.H.Schwarzmann, R.W.Jeanloz, Carbohydr. Res. 34, 161 (1973).
22. H.E.Conrad, Methods Carbohydr. Chem. 6, 361 (1972).
23. P.E.Pfeffer, K.M.Valentine, F.W.Parrish, J.Amer.Chem.Soc. 101, 1265 (1979).
24. J.Vernon, Y.C.Lee, M.Koreeda, Tetrahedron Lett. 22, 1069 (1981).
25. J.G.Collins, J.H.Bradbury, E.Trifonoff, M.Messer, Carbohydr. Res. 92, 136 (1981).
26. J.Dahmen, G.Gnosspelius, A.-C.Larsson, T.Lave, G.Noori, K.Palsson, T.Frejd, G.Magnusson, Carbohydr. Res. 138, 17 (1985).
27. T.Takamura, T.Chiba, S.Tejima, Chem.Pharm.Bull. 29, 2270 (1981).
28. R.Kuhn, W.Kirschenlohr, Ann. Chem. 600, 135 (1956).