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SYNTHESIS OF THE O-SPECIFIC POLYSACCHARIDE OF *SHIGELLA FLEXNERI*

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Abstract - A regular heteropolysaccharide built of tetrasaccharide repeating units is synthesised by means of regio- and stereospecific polycondensation of a tritylated cyanoethylidene derivative. The polysaccharide obtained is identical with the O-antigenic polysaccharides of *Shigella flexneri* serotypes 3b, 3c, and variant Y. It also represents the basic chain of O-antigenic polysaccharides of all serotypes of this bacterium.

O-Antigenic polysaccharides, which are the components of lipopolysaccharides of gram-negative bacteria, are responsible for biological specificity of the microbial surface, for serological reactions in particular. The characteristic feature of their structure is that they are built of regularly repeating oligosaccharide units and a unique carbohydrate structure is inherent, as a rule, to each species of microorganisms. More than a hundred primary structures of O-antigenic polysaccharides is elucidated to date.[1]

Synthesis of these biopolymers is a very complicated problem. An approach to its solution appeared only after a first general method of synthesis of polysaccharides has been developed in this laboratory.[2] It consists in a triphenylmethylium perchlorate-catalysed strongly stereospecific polycondensation of a tritylated 1,2-O-cyanoethylidene derivative of mono- or oligo-saccharide. The synthesis, using this method, of O-antigenic polysaccharides which are regular block-polymers necessitates preparation of monomers, *i.e.* properly functionalised repeating units of the polymer. Referring to heteropolysaccharides of complex structure, this being the case for majority of O-antigenic polysaccharides, the very synthesis of the corresponding repeating unit with proper functionalisation for subsequent polycondensation and a choice of the most rational strategy to this end is a challenge as well.

The first synthesis of this kind, viz that of O-antigenic polysaccharide of *Salmonella newington*, was performed recently[3] and allowed for the first time to prove chemically the structure, in particular, strict regularity ascribed to the polysaccharide.

Here we describe the synthesis of the linear heteropolysaccharide **1**

$$\rightarrow 3)\text{GlcNAc}(\beta 1\text{-}2)\text{Rha}(\alpha 1\text{-}2)\text{Rha}(\alpha 1\text{-}3)\text{Rha}(\alpha 1\rightarrow$$

which is structurally identical with the carbohydrate chain of O-antigenic polysaccharides of *Shigella flexneri* serotypes 3b, 3c, and variant Y. This polysaccharide is also the basic chain of O-antigenic polysaccharides of the other 10 sero-

types of this bacterium [4] which differ from each other by possessing monosaccharide (α-D-glucopyranose) branching(s) and/or O-acetyl groups. We have synthesised the heteropolysaccharide 1 by way of polycondensation of the tetrasaccharide monomer 34 which contained the glycosylating (1,2-O-cyanoethylidene) function in the terminal, 3-substituted rhamnose unit and the glycosylation site (trityloxy-group) in position 3 of the glucosamine unit.

Polycondensation of monomers with the cyanoethylidene group in a rhamnopyranose residue is known to afford polysaccharides of sufficiently high molecular weight with α-rhamnosidic bond as the only type of intermonomer linkage.[5-8] We have also shown that the tritylated glucosamine derivatives could successfully be employed in the polysaccharide synthesis.[8] It was found thereby that the phthaloyl protection of aminogroup is preferable since polycondensation (and glycosylation [9]) proceeds more effectively than with N-acetyl derivatives. It was thus anticipated that the polycondensation of the monomer 34 is possible and it would proceed with complete stereospecificity. It means that the configuration of the newly formed linkage between rhamnose and glucosamine residues will be identical with that in the natural polysaccharide.

The problem of a synthesis of the monomer became solvable after we have shown that i) the sugar derivatives containing the cyanoethylidene group could be glycosylated themselves without this group being affected [10] thus enabling an assembly of the monomer from a synthon already bearing the cyanoethylidene function; ii) O-acetyl group could selectively be removed from sugar derivatives in the presence of O-benzoyl groups by mild, acid-catalysed methanolysis.[11] Hence, we could differentiate these two most suitable hydroxyl-protecting groups and design a versatile synthetic scheme.

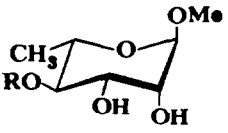
We have explored two strategies for assembling the tetrasaccharide derivative 32, namely, a stepwise (A → BA → CBA → DCBA) and a block-scheme. The latter consisted in a synthesis of two disaccharide blocks BA and DC and their coupling (DC + BA → DCBA). The general principle which we followed throughout the synthesis of the monomer 34 was the creation of synthons in which hydroxyls not to be glycosylated were protected as O-benzoates ('permanent' protective group), while those to serve further as the glycosylation sites were protected as O-acetates and the O-acetyl group was selectively removed prior to glycosylation ('temporary' protective group).

The monosaccharide synthons used were the specifically protected rhamnopyranose derivatives, viz 4-O-benzoyl-1,2-O-(1-cyanoethylidene)-β-L-rhamnopyranose 7, 1,2-di-O-acetyl-3,4-di-O-benzoyl-L-rhamnopyranose 13, and methyl 3,4-di-O-benzoyl-α-L-rhamnopyranoside 14, as well as the glucosamine derivative, 1,3-di-O-acetyl-4,6-di-O-benzoyl-2-deoxy-2-phthalimido-D-glucopyranose 23.

RESULTS AND DISCUSSION

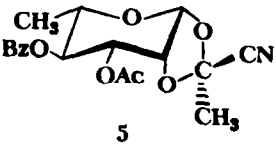
1. Synthesis of 4-O-benzoyl-1,2-O-(1-cyanoethylidene)-β-L-rhamnopyranose 7.
We started from methyl α-L-rhamnopyranoside 2. It was successively acetonated [12], benzoylated, and deacetonated (one-pot procedure) to give 90% of the benzoate 3. Acetolysis of 3 gave an anomeric mixture of 1,2,3-tri-O-acetyl-4-O-benzoyl-L-rhamnopyranoses 4 from which the α-anomer was isolated crystalline in 50% yield. Treatment of 4 with HBr in AcOH afforded the corresponding glycosyl bromide which, in turn, was converted (80%) into 3-O-acetyl-4-O-benzoyl-1,2-O-[1-(exo- and endo-cyano)ethylidene]-β-L-rhamnopyranose 5 and 6 in a ratio of 3.5:1 when treated with NaCN in acetonitrile, *i.e.* under conditions of a general method for synthesis of 1,2-O-cyanoethylidene sugar derivatives.[13] Configuration at C-2 of the dioxolane cycle in the isomers (separated by chromatography) was established on the basis of ^{1}H-NMR data: the CH_3-C-CN group in the major, exo-cyano-isomer 5

is deshielded in comparison with that in the endo-cyano-isomer 6 (cf.[13,14]). Despite both isomeric 1,2-O-cyanoethylidene carbohydrate derivatives exhibit similar glycosylating activity,[15] we employed hereinafter the major product 5 to facilitate TLC and NMR analyses.



2 R = H
3 R = Bz

4



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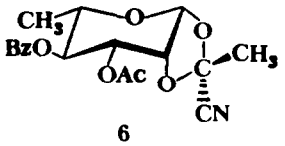

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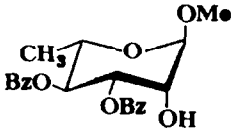

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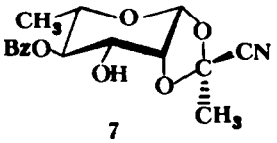

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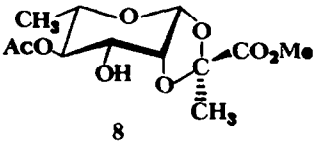
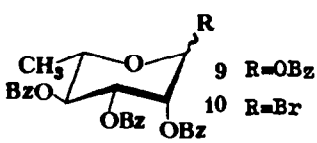


8

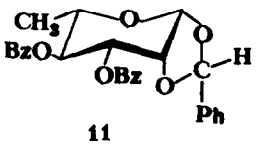
Selective deacetylation of 5 into the hydroxyl-containing cyanoethylidene derivative 7 which served as an aglycon for subsequent glycosylation was accomplished by mild, acid-catalysed methanolysis [11] (0.6 M methanolic hydrogen chloride, 20°, 4 hr). Under these conditions a side-reaction also takes place, viz an addition of methanol to cyanogroup to give imidate and subsequent hydrolysis of the latter into 1-methoxycarbonylethylidene derivative. A compound of this type, 8, was isolated after methanolysis of the 3,4-di-O-acetyl-1,2-O-(1-cyanoethylidene)-β-L-rhamnopyranose and its structure was established on the basis of analytical and spectral data. For 5 the ratio of deacetylation and addition rates was strongly in favour of the former and 7 was isolated in high yield. As for deacetylation of the cyanoethylidene derivatives of oligosaccharides (*vide infra*), this side-reaction could not be neglected, because deacetylation and formation of imidates proceeded in comparable rates.

The structure of 7 evidenced from its ^{1}H-NMR spectrum: the acetyl signal was absent, the signal for H-3 was upfield shifted in comparison with its position in the spectrum of 5; both cyanoethylidene and benzoyl groups remained intact.

2. *Synthesis of 1,2-di-O-acetyl-3,4-di-O-benzoyl-L-rhamnopyranose 13.* The starting material was L-rhamnopyranose tetrabenzoate 9. It was converted into benzobromorhamnose 10 under standard conditions and the latter was treated with $NaBH_4$ according to a general method for synthesis of 1,2-O-benzylidene and 1,2-O-ethylidene sugar derivatives developed in this laboratory.[16] The 3,4-di-O-benzoyl-1,2-O-benzylidene-β-L-rhamnopyranose 11 thus obtained was debenzylidenated with CF_3COOHaq to give the crystalline diol 12 in 80% yield (from 9). Conventional acetylation of 12 gave the diacetate 13 which is the synthon for the unit B.



9 R=OBz
10 R=Br



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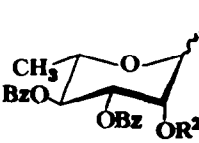
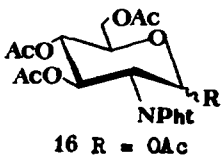


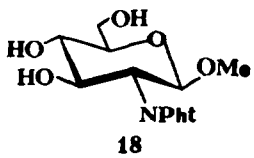
12 R^1=OH, R^2=H
13 R^1=OAc, R^2=Ac
15 R^1=Br, R^2=Ac

3. Synthesis of methyl 3,4-di-O-benzoyl-α-L-rhamnopyranoside 14. This compound was prepared by selective monobenzoylation of the diol 3 in 66% yield. The accompanying side-products, methyl 2,4-di-O- and 2,3,4-tri-O-benzoyl-α-L-rhamnopyranosides (6 and 7%), were easily removed by chromatography. The ^{1}H-NMR spectrum of 14 (δ 5.56-5.68 for H-3,4 and 4.31 for H-2) proved the location of benzoyl groups. Independent synthesis of 14 by deacetylation [11] of methyl 2-O-acetyl-3,4-di-O-benzoyl-α-L-rhamnopyranoside which was prepared either by Helferich methyl glycosidation of 15 or by $HgBr_2$-catalysed isomerisation [17] of 3,4-di-O-benzoyl-1,2-O-(1-methoxyethylidene)-β-L-rhamnopyranose is to be published elsewhere.

4. Synthesis of 1,3-di-O-acetyl-4,6-di-O-benzoyl-2-deoxy-2-phthalimido-D-glucopyranose 23. According to the general concept of synthesis of the polysaccharide 1, the glucosamine unit of the monomer ought to be tritylated at position 3 with aminogroup phthaloylated. The synthon for unit D, compound 23, was obtained as follows. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-D-glucopyranose 16 was quantitatively converted, via the corresponding glycosyl bromide 17, into methyl glycoside 18. It was benzylidenated ($PhCH(OEt)_2$ + TsOH) and 19 was acetylated to give the acetate 20. Treatment of 20 with 90% CF_3COOHaq resulted in its deacetalation (TLC), however, this process was reversed upon concentration of the reaction mixture and the product thus obtained was the starting 20. This difficulty was overcome by performing deacetalation under conditions of deacetonation and detritylation of sugar derivatives [18], i.e. by the action of methanol in boiling nitromethane in the presence of pyridinium perchlorate. Benzoylation of the thus obtained diol 21 gave 22, acetolysis of which afforded the required synthon 23 without affecting the phthalimido group. The overall yield of crystalline 23 was ca. 60% from 16. It is of note that the transformations 16 → 17 → 18 → 19 → 20 → 21 → 22 could be carried out without recourse to special purification of the products at separate steps.



16 R = OAc
17 R = Br



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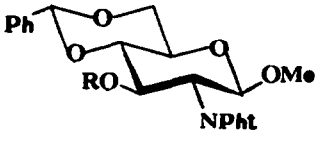
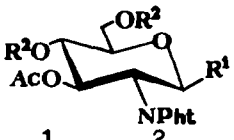


19 R = H
20 R = Ac



21 R^1=OMe, R^2=H
22 R^1=OMe, R^2=Bz
23 R^1=OAc, R^2=Bz
26 R^1=Br , R^2=Bz

Pht = phthaloyl

The set of the ordinary protective groups enabled the simplicity of all synthetic procedures and ease of isolation of intermediate and final products, the majority of which being crystalline. Column chromatography was needed only for isolation of 5 and 14. This made possible large-scale syntheses providing the key intermediates for assembling the tetrasaccharide derivative 32 readily accessible.

5. Synthesis of the BA-block. The disaccharide derivative 24 could be prepared by several routes, the most efficient one being Helferich glycosylation ($Hg(CN)_2$, MeCN) of the alcohol 7 by the bromide 15 prepared from the diacetate 13. The disaccharide 24 was obtained in 84% yield by direct crystallisation. Other synthetic routes involved the $TrClO_4$-catalysed glycosylation [10,19] of 4-O-benzoyl-3-O-trityl-1,2-O-(1-cyanoethylidene)-β-L-rhamnopyranose by the thioorthoester, 3,4-di-O-benzoyl-1,2-O-(1-p-tolylthioethylidene)-β-L-rhamnopyranose, and the glycosylation of 1,2-O-(1-cyanoethylidene)-β-L-rhamnopyranose by the bromide 15 followed by separation of 1 → 3- and 1 → 4-linked disaccharides.[10]

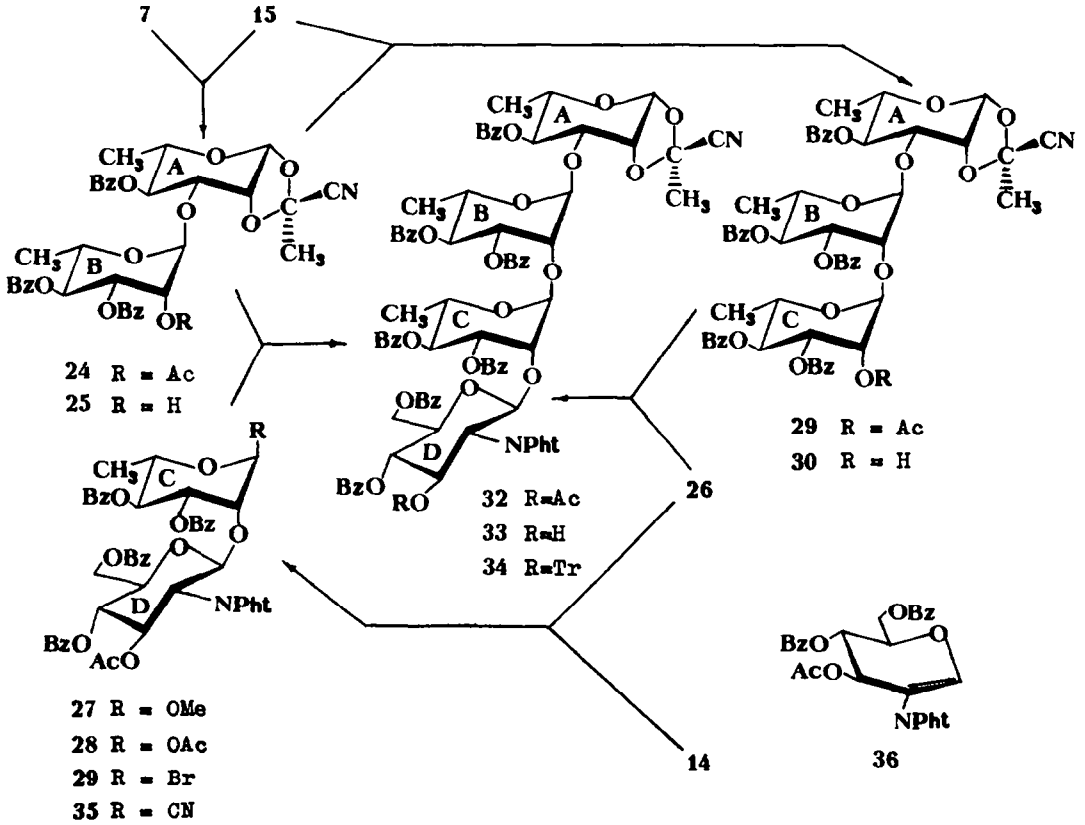
The structure of 24 was confirmed by NMR data. In particular, the presence of

1,2-O-(1-cyanoethylidene) group evidenced from the characteristic signals at δ 26.5, 101.8, and 117.05 (CH_3-C-CN) in the ^{13}C-NMR spectrum. The $^{1}J_{C1,H1}$ value was equal to 176 Hz, that for the monosaccharide derivative 5 being 177.6 Hz.

Selective deacetylation of 24 with liberation of a C-2' hydroxyl was necessary to prepare an aglycon for subsequent glycosylation. The aforementioned side-reaction under conditions of acid-catalysed methanolysis made the process rather complex. The reaction mixture contained besides the starting 24 and the desired product 25 two other 8-like, disaccharide products, whose amount increased with time (TLC). It was found reasonable therefore not to bring the deacetylation to completion, but to stop it after 3-3.5 hr, to isolate 24 and 25 by chromatography (the yield of the latter being 35-40%), and to deacetylate the recovered 24 (30-40%) again. The structure of 25 which is the common aglycon for both the stepwise and block synthesis of the tetrasaccharide monomer was confirmed by the ^{13}C-NMR spectral data. The location of a free hydroxyl at C-2' evidenced from comparison of positions of C-1' signals in 25 and 24. In the former it is shifted downfield by 2.5 p.p.m.

6. Synthesis of the DC-block. The disaccharide derivative 28 which is the synthon for the DC-block was prepared from the rhamnoside 14 and the bromide 26. The latter was obtained as an anomeric mixture (α:β ca. 1:3, ^{1}H- and ^{13}C-NMR data) upon HBr-AcOH treatment of the diacetate 23. Helferich glycosylation of 14 proceeded smoothly and gave 80% of 27. This yield could be attained under rigorously anhydrous conditions and the vacuum technique was employed to this end.

Acetolysis of the methyl bioside 27 yielded quantitatively 28 which was needed for the block-synthesis of 32. Spectral data for 27 and 28 were in full agreement with the structures assigned.



7. Stepwise synthesis of the tetrasaccharide derivative 32. The stepwise scheme implied the coupling of the C-unit with the BA-block, i.e. the synthesis of the trisaccharide derivative 29, its deacetylation into the alcohol 30, and, finally, a condensation with the D-unit.

The trisaccharide 29 was synthesised from the aglycon 25 and the bromide 15, the latter being already employed for the synthesis of the disaccharide 24. Helferich glycosylation of 25 afforded 66% of 29. Deprotection of the C-2" hydroxyl was performed by acid-catalysed methanolysis under conditions similar to those used in preparation of 25. Due to appreciable formation of the side-products (vide supra) the deacetylation was interrupted after 4 hr and the target trisaccharide alcohol 30 (ca. 50%) was isolated by chromatography together with ca.20% of the starting 29. Comparison of the ^{13}C-NMR spectra of 30 and 29 proved unambiguously the structure of the latter: signals due to CH_3CO group were absent and the C-1" signal was shifted downfield by 2.9 p.p.m.

Condensation of the bromide 26 with the aglycon 30 was carried out under Helferich conditions (MeCN, $Hg(CN)_2$, $HgBr_2$) and gave 32 in 48% yield after chromatography and crystallisation. Rigorously anhydrous conditions were required for the reaction. With the other combination of promoter and acid acceptor ($AgOSO_2CF_3$ - 2,4,6-collidine) the yield of 32 was much lower (14%), 30 was recovered in 74% yield, and 2-deoxy-2-phthalimido-glucal 36 (44%) was isolated.

8. Block-synthesis of the tetrasaccharide derivative 32. This scheme, i.e. a coupling of two disaccharide derivatives, turned out to be more effective.

The peracylated disaccharide 28 was converted into the chromatographically homogeneous bromide 31 by the action of HBr in $CHCl_3$. It was condensed with the aglycon 25 in MeCN in the presence of $Hg(CN)_2$ and $HgBr_2$ (in molar ratio of 2:1) using the vacuum technique. The tetrasaccharide derivative 32 was isolated by crystallisation in 45% yield. This is almost 3 times as high as that in the stepwise procedure. A side-product, biosyl cyanide 35 (ca.20%) was isolated from the mother liquor by chromatography. When the glycosylation was performed in the absence of $HgBr_2$, the yield of 32 was 32%, while increase in molar proportion of this promoter did not raise the yield of the tetrasaccharide.

Crystalline specimens of 32 obtained by both routes were identical (m.ps, $[\alpha]_D$ values, and ^{13}C-NMR spectra). The presence of characteristic signals for the cyanoethylidene group, four anomeric carbon atoms, and others in the ^{13}C-NMR spectrum of 32 supported its structure.

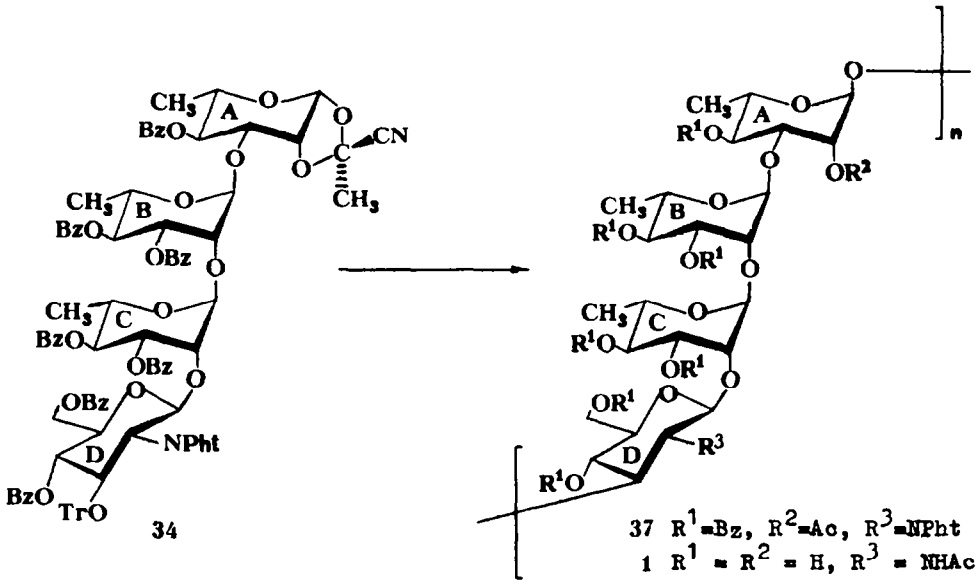
9. Synthesis of the monomer 34. Removal of the only acetyl group from O-3 of the glucosamine moiety in 32 preceded the preparation of the very monomer 34. This was also effected by mild, acid-catalysed methanolysis. In this case the side formation of imidates also hampered seriously the access to the alcohol 33 and, as before, short-term methanolysis gave ca.30% of 33 and 40-45% of the starting 32 was recovered. The downfield shift for C-2 of the glucosamine residue in the ^{13}C-NMR spectrum of 33 as compared with that of 32 (δ 57.2 vs. 54.9) indicated the free hydroxyl to be located at C-3"'.

To convert the cyanoethylidene derivative 33 into a monomer for a $TrClO_4$-catalysed polycondensation a tritylation of the free hydroxyl had to be carried out. A method for tritylation of secondary hydroxyls in carbohydrate derivatives based on action of $TrClO_4$ in the presence of a sterically hindered pyridine base was elaborated in this laboratory.[15,19,20] It was successfully applied for the synthesis of a series of trityl ethers of sugar cyanoethylidene derivatives [5,6,21] and in none of these cases was reported that the cyanoethylidene group has been affected. Unexpectedly, we faced difficulties when performing tritylation of 33 ($TrClO_4$, 2,4,6-collidine, CH_2Cl_2): already after 10-20 min the reaction mixture

contained besides 33 the trityl ether 34 and two unidentified components devoid of the cyanoethylidene group (^{13}C-NMR data) whose amount was increased with time. Therefore the tritylation was stopped after 30-50 min and the reaction products were isolated by chromatography. The yield of the desired monomer 34 was 20±10% with 60±10% of 33 recovered. After six cycles of tritylation-chromatography the overall yield of 34 was 40%. Its ^{13}C-NMR spectrum confirmed the presence of, inter alia, a trityl group ($Ph_3\underline{C}O$, δ 88.85) and 1,2-O-(1-cyanoethylidene) group (unit A, $^1J_{C1,H1}$ 173.9 Hz). Other $^1J_{C1,H1}$ values (172.0, 172.0, and 162.8 Hz for units B, C, and D) proved the presence of two α-L-rhamnopyranosyl and one β-D-glucosaminyl residues.

10. Synthesis and characterisation of the polysaccharide 1. The monomer 34 was subjected to a $TrClO_4$-catalysed polycondensation under standard conditions (10 mol.% of $TrClO_4$, CH_2Cl_2, room temp, vacuum technique, see, e.g., ref. 3) which are also suitable for polycondensation of hexosamine-containing oligosaccharides.[8]

After 16 hr TLC revealed complete disappearance of the starting monomer. The main carbohydrate product (R_f 0.0-0.2, benzene-ethyl acetate, 8:2) was detected along with non-carbohydrate products (TrCN, TrOH) and two minor, faster migrating carbohydrate derivatives, both these and the major product being devoid of a trityl group. The reaction mixture was treated with methanol-pyridine and worked-up conventionally. Column chromatography then gave high yield of the protected polysaccharide 37 as a white powder, readily soluble in ordinary organic solvents.



The ^{13}C-NMR spectrum of 37 exhibited, inter alia, easily attributable, characteristic signals for $\underline{CH_3}CO$ group (δ 20.50), C-5 and C-6 of three α-L-rhamnopyranosyl units (67.49, 67.60, 67.80, cf. [22] and 17.00, 17.41, 17.53), C-2 and C-6 of the glucosamine moiety (56.36 and 62.71), and four anomeric carbon atoms (98.19, 99.28, 100.33, 100.67). No signals for the cyanogroup (δ 110-120) were detected.

The polysaccharide derivative obtained containing only acyl protective groups was deprotected in a single step by hydrazinolysis.[23] After N-acetylation the polysaccharide 1 was isolated by gel-chromatography on Bio-Gel P-4 and lyophilisation in 90% yield. Four signals in its ^{13}C-NMR spectrum corresponded to the anomeric carbon atoms of the units A, B, C, and D (δ 102.21, 101.88, 102.10, and

103.24, $^{1}J_{C,H}$ 170.2, 172.0, 171.1, and 162.7 Hz). The $^{1}J_{C1,H1}$ values indicated all the rhamnosidic bonds, including the newly formed one upon polycondensation, to possess α-configuration. Thus the polycondensation was completely stereospecific. The low-field position of C-3 signal of the glucosamine moiety (δ 82.68) demonstrated this residue to be glycosylated at O-3, hence, the regiospecificity of polycondensation. Minor signals in the spectrum (δ 57.2, 75.0, and 103.7) of ca. 10% intensity compared to that of the main, one-carbon signals, were ascribed to C-2, C-3, and C-1 of the nonsubstituted glucosamine residue at the nonreducing end of the polysaccharide chain.

The structure of the synthetic polysaccharide 1 is completely identical with that of the O-antigenic polysaccharides of *Shigella flexneri* serotypes 3b, 3c, and variant Y, as well as with that of the basic chain of all the rest O-antigenic polysaccharides of this bacterium. This is evident from practical identity of its ^{13}C-NMR spectrum with that of the natural polysaccharide of *Sh. flexneri* variant Y.[24] (Table). The regularity of the polysaccharide structure is well illustrated by its ^{13}C-NMR spectrum (Fig.).

Table. ^{13}C-NMR spectral data for $\frac{\text{synthetic}}{\text{natural}}$ polysaccharides

Monosaccharide unit	C-1	C-2	C-3	C-4	C-5	C-6	$^{1}J_{C1,H1}$
Rha A	102.21 102.03	71.80 71.41	78.64 78.10	72.79 72.45	70.35 69.81	17.63 17.22	170.2 169
Rha B	101.88 101.62	79.26 78.91	71.32 70.82	73.53 72.96	69.81 69.14	17.78 17.47	172.0 171
Rha C	102.10 101.85	79.89 79.52	71.13 70.58	73.65 73.10	70.23 69.94	17.86 17.47	171.1 172
GlcNAc D	103.24 102.95	56.73 56.46	82.68 82.29	70.23 69.94	77.05 76.68	62.09 61.56	162.7 162

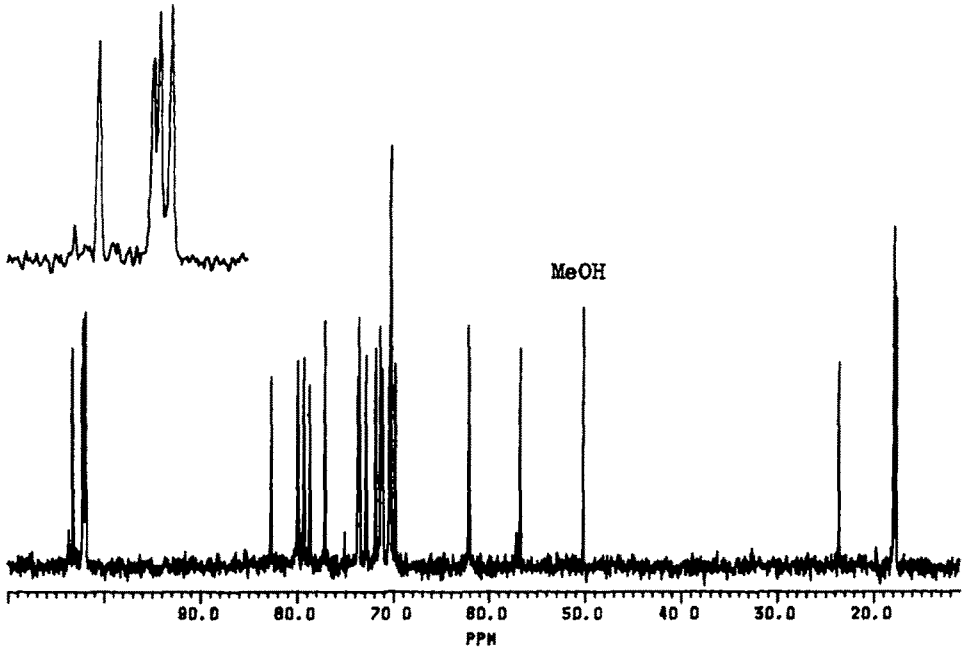


Fig. The ^{13}C-NMR spectrum of the synthetic polysaccharide 1 (D_2O, 60°C, internal MeOH, δ_{TMS} 50.15). Insert refers to the anomeric carbons' region.

The molecular mass of the polysaccharide 1 (ca.6000) was established by gel-permeation chromatography using SynChropack column GPC-100 and Dextrans T-10, T-20, and T-40 as standards. The $\overline{DP}$ value (ca.10) coincided well with that deduced from the ^{13}C-NMR spectrum. Similar $\overline{DP}$ value was reported for the natural polysaccharide,[25] hence, the synthetic polysaccharide corresponds to the natural one by this parameter as well.

The synthesis of a complex heteropolysaccharide presented here is an additional evidence for high effectiveness and wide possibilities of the method of synthesis of regular polysaccharides by way of polycondensation of tritylated cyanoethylidene derivatives of carbohydrates.

EXPERIMENTAL

Acetonitrile was refluxed over $KMnO_4$+K_2CO_3, distilled from P_2O_5 twice and from CaH_2. Nitromethane was distilled from urea at 100 Torr and then from CaH_2. 2,4,6-Collidine was distilled from KOH and then from CaH_2. Dichloromethane was washed with conc H_2SO_4 and water, dried ($CaCl_2$), and distilled from CaH_2. Triphenylmethylium perchlorate was prepared as in ref. 26 and, when used as a catalyst, reprecipitated with dry ether from dry nitromethane as in ref. 3. M.ps were determined with a Kofler apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter at 20±2° for solns in $CHCl_3$. NMR spectra were recorded witha Bruker WM-250 spectrometer in $CDCl_3$, δ scale, internal TMS. Column chromatography (CC) was performed on Silicagel L 40/100 μm (CSSR) with gradient elution benzene → ethyl acetate; TLC - on Silicagel L 5/40 μm (CSSR), components were detected by spraying with 70% H_2SO_4aq followed by charring. The solns were dried by filtration through cotton and concentrated *in vacuo* at 40°.

Preparation of Acylglycosyl Bromides. To a soln of peracylated mono- or di-saccharide derivative (10 mmol) in $CHCl_3$ (30 ml) and AcOH (5-10 ml) was added acetyl bromide (50-55 mmol) and then water (50-55 mmol) in AcOH (5-10 ml) was added at 0° dropwise. The soln was left at 20° and after completion of the reaction (TLC; bromides exhibit higher mobilities than the starting acyl derivatives) the reaction mixture was diluted with $CHCl_3$ (120-150 ml) and poured into ice-water (200-250 ml). The aqueous layer was extracted with $CHCl_3$ (20-25 ml), the combined organic solns were washed with ice-water and cold, satd $NaHCO_3$aq (2x200 ml), filtered through cotton, evaporated, and the residue was dried *in vacuo*. The chromatographically homogeneous glycosyl bromides thus obtained in almost quantitative yield were directly used in glycosylations.

4-O-Benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-L-rhamnopyranose (7). A mixture of crystalline 2 (17.8 g, 0.1 mol), acetone dimethylacetal (50 ml) and TsOH·H_2O (0.2 g) was kept at 20° until dissolution (1.5-2 hr), diluted with $CHCl_3$ (100 ml), washed with $NaHCO_3$aq (3x100 ml) and water, and evaporated to give 20g of a chromatographically homogeneous syrup. It was dissolved in pyridine (50 ml), benzoyl chloride (17.4 ml, 0.15 mol) was added with cooling, and the soln was kept at 20° for 20 min. It was treated with water (10-12 ml) upon cooling, then poured into $NaHCO_3$aq (1.5 l), and stirred for 0.5 hr. The oily product was dissolved in $CHCl_3$ (350 ml), washed with M HCl (3x200 ml) and water, and 90% CF_3COOHaq (40 ml) was added to the soln. After 10-15 min at 20° the soln was washed with water, satd $NaHCO_3$aq, and water, dried, and evaporated to give 3 (25.4 g, 90%) as a syrup, $[\alpha]_D$ -82.3°(c 2.9).

Acetic anhydride (100 ml) was mixed with 3 (28 g) and a soln of conc H_2SO_4 (1.5 ml) in Ac_2O (50 ml) was added at 0°. After 2 hr at 20° the soln was poured onto 600 g of ice, stirred for 5-6 hr at 0°, and the crystalline precipitate was filtered off. It was dissolved in $CHCl_3$ (350 ml) and the soln was washed with water, $NaHCO_3$aq, and water. Concentration of the dried soln gave 4 (27.55 g, 70%) which could directly be used in the next step. Crystallisation from ether-pentane gave the α-anomer (14.2 g, 51.5%), m.p. 115-117°, $[\alpha]_D$ -28°(c 1.8) (Found: C,57.95; H, 5.12. Calc for $C_{19}H_{22}O_9$: C, 57.86; H, 5.62%).

To a soln of a glycosyl bromide, prepared from 11 g (28 mmol) of 4, in MeCN (50 ml) was added powdered NaCN (6.9 g, 140 mmol; dried *in vacuo* over P_2O_5 at 100°) and the mixture was stirred for 2 d at 20°. It was diluted with $CHCl_3$-hexane (1:2 v/v, 400 ml) and washed with water. The bottom, aqueous layer was extracted with the same solvent system (100 ml), the combined organic solns were washed with water, dried, and evaporated. CC of the residue gave 5 (5.07 g, 50%) and 6 (1.41 g, 14%) together with mixed fractions (1.39 g). *Compound 5*: m.p. 115-117° (MeOH), $[\alpha]_D$ +47.5°(c 1.8) (Found: C, 59.87; H, 5.28; N, 4.28. Calc for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88%). ^1H-NMR: 5.50d (1H, J 2 Hz, H-1), 4.65dd (1H, J 2, 4 Hz, H-2), 5.50dd (1H, J 4, 10 Hz, H-3), 5.34t (1H, J 10 Hz, H-4), 3.75dq (1H, H-5), 1.30d (3H, J 6.2 Hz, H-6), 1.97s (3H, CH_3CCN), 2.03s (3H, CH_3CO); ^{13}C-NMR: 97.0 ($^1J_{C,H}$ 177.6 Hz, C-1), 78.6 (C-2), 69.3, 70.3, 70.8 (C-3,4,5), 17.6 (C-6), 26.55, 101.7, 116.7 (CH_3-C-CN), 20.5, 170.1 (CH_3CO), 165.5 (PhCO). *Compound 6*: m.p. 180°(EtOAc-petroleum ether), $[\alpha]_D$ +116.7°(c 1.8) (Found: C, 59.85;H, 5.21; N, 3.88%). ^1H-NMR: 5.65d (1H, J 2 Hz, H-1), 4.54t (1H, H-2), 5.38-5.48m (2H, H-3, 4), 3.82dq (1H, H-5), 1.38d (3H, J 6 Hz, H-6), 1.85s (3H, CH_3CCN), 2.03s(3H,AcO).

To a soln of 5 (2.97 g, 8.2 mmol) in 8 ml of $CHCl_3$ and 40 ml of MeOH was added

acetyl chloride (1.6 ml) at 0°, the soln was kept at 20° for 4 hr, treated with excess of $KHCO_3$aq , and concentrated. The residue was partitioned between $CHCl_3$ and water (150 ml of each), the aqueous layer was extracted with $CHCl_3$ (10 ml) and the combined extracts were washed with water (2x30 ml), dried, and evaporated. Crystallisation of the residue from EtOAc-petroleum ether gave 7 (2.38 g, 91%), m.p. 155-157°, $[\alpha]_D$ -13.2°(c 1.4) (Found: C, 60.37; H, 5.39; N, 4.42. Calc for $C_{16}H_{17}NO_6$: C, 60.18; H, 5.37; N, 4.39%). ^{1}H-NMR: 5.43d (1H, J 2 Hz, H-1), 4.58dd (1H, J 2, 4.2 Hz, H-2), 4.11br.d (1H,H-3), 5.10t (1H, J 9.5 Hz, H-4), 3.65dq (1H, H-5), 1.27d (3H, J 6.2 Hz, H-6), 1.93s (3H, CH_3CCN).

1,2-Di-O-acetyl-3,4-di-O-benzoyl-L-rhamnopyranose (13). The rhamnose tetrabenzoate 9 (0.1 mol) was converted into bromide 10 and then treated with $NaBH_4$ (6 g, 0.15 mol) in MeCN (200 ml) as in [16]. After completion of the reaction a soln of AcOH (12 ml) in water (20 ml) was carefully added, the soln was evaporated, and the residue was partitioned between water and $CHCl_3$ (350 ml of each). The chloroform soln of 11 was washed with water (3x250 ml) and treated with 90% CF_3COOHaq (40 ml). Following debenzylidenation (10-15 min) the reaction mixture was washed with water (3x250 ml), $NaHCO_3$aq, dried, and evaporated. The residue was triturated with hexane (3-4x250 ml) to give 12 (29.8g, 80%), m.p. 137-145°($CHCl_3$-hexane), $[\alpha]_D$ +88°(c 1.58, 16 hr) (Found: C, 64.39; H, 5.62. Calc for $C_{20}H_{20}O_7$: C, 64.50; H, 5.41%). Conventional acetylation of 12 afforded 95% of the diacetate 13 as a foam, $[\alpha]_D$ +35.7°(c 2.35).

Methyl 3,4-di-O-benzoyl-α-L-rhamnopyranoside (14). To a chilled (0°) soln of 3 (1.4 g, 5 mmol) in pyridine (6 ml) was added a soln of benzoyl chloride (0.6 ml, 5.2 mmol) in dry benzene (2 ml). After 20 min at 0° the mixture was conventionally worked-up, CC then gave 14 (1.28 g, 66%), m.p. 155-160°(MeOH), $[\alpha]_D$ +26°(c 0.79) (Found: C, 65.33; H, 5.92. Calc for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74%). ^{1}H-NMR: 4.80d (1H, J 2 Hz, H-1), 4.31t (1H, H-2), 5.68-5.56m (2H, H-3,4), 4.10m (1H, H-5), 1.33d (3H, J 6 Hz, H-6), 3.46s (3H, OCH_3).

1,3-Di-O-acetyl-4,6-di-O-benzoyl-2-deoxy-2-phthalimido-D-glucopyranose (23). To a stirred soln of D-glucosamine hydrochloride (20 g, 93 mmol) in water (100 ml) were added $NaHCO_3$ (7.8 g, 92.8 mmol) and powdered phthalic anhydride (13.6 g, 92 mmol). More $NaHCO_3$ (7.8 g) was added to the suspension portionwise for 3-4 hr. The homogeneous soln was acidified with 2M HCl (60-70 ml) and concentrated. The precipitate was filtered off, washed with small amount of cold water, and dried over P_2O_5. The product obtained (20-22 g, 65-72%) was treated with Ac_2O (50 ml) in pyridine (100 ml) for 16 hr at 20°, conventional work-up gave crystalline 16 (23.8 g, 53%) as a mixture of α- and β-anomers (R_f 0.48 and 0.56, EtOAc-benzene, 2:3). Crystallisation from 200 ml of EtOH gave 13.4 g of the α-anomer,m.p. 128-131°, $[\alpha]_D$+112° (c 0.9);lit. /27/ m.p. 131°(MeOH), $[\alpha]_D$ +98°(c 0.5); lit. /28/ m.p. 126-127°(MeOH), $[\alpha]_D$ +119.2°(c 1). Additional crop of 16 (6.9 g) was isolated from the mother liquor, and the rest (3.5 g) despite the presence of slowly migrating admixtures was also suitable for preparation of the bromide 17.

The tetraacetate 16 (10.75 g, 22.5 mmol) was converted into the bromide 17, the latter was dissolved in MeOH (80 ml) and left overnight at 20°. The soln was neutralised with Dowex 1 (HCO_3^-), the filtrate was decolourised with Darco charcoal and evaporated to give 7.3 g (100%) of 18 , m.p. 195-197°(EtOAc-heptane), $[\alpha]_D$ -10° (c 1, MeOH); lit. /9/ m.p. 198-199°, $[\alpha]_D$ -10.2° (MeOH).

To a soln of 18 (7.3 g, 22.5 mmol) in MeCN (30 ml) were added TsOH·H_2O (30 mg) and $PhCH(OEt)_2$ (16 ml), after 16 hr at 20° $CHCl_3$ (150 ml) was added, the soln was washed with $NaHCO_3$aq, water, and evaporated. The residue was triturated with heptane (4x30 ml), addition of EtOH gave the crystalline 19 (9.2 g, 100%), m.p. 192-192.5°(EtOH), $[\alpha]_D$ -32°(c 1.7) (Found: C, 64.26; H, 4.99; N, 3.63. Calc for $C_{22}H_{21}NO_7$: C, 64.22; H, 5.14; N, 3.40%). Conventional acetylation of 19 gave, quantitatively,the acetate 20, m.p. 255-258°(sinters at 247°; $CHCl_3$-ether-hexane), $[\alpha]_D$ -6.5°(c 1.4). ^{1}H-NMR: 5.38d (1H, J 8.5 Hz, H-1), 4.29dd (1H, J 8.5, 10.5 Hz, H-2), 5.90dd (1H, J 10.5, 9 Hz, H-3), 3.87t (1H,H-4), 4.45m (1H, H-5), 3.83-3.70m (2H, H-6,6'), 5.56s (1H, PhCH), 3.45s (3H, OCH_3).

A mixture of 20 (9.25 g, 20 mmol) and pyridinium perchlorate (3.6 g,20 mmol) in $MeNO_2$ (15 ml) and MeOH (100 ml) was heated to boiling, $MeNO_2$ (20-30 ml) was added to homogeneity and the soln was refluxed for 2-3 hr. Pyridine (0.2 ml) was added at 20°, the soln was evaporated, and the residue was triturated with petroleum ether to give 21. Conventional benzoylation then gave 9.5 g (83%) of 22, m.p. 169.5-170.5°(EtOH), $[\alpha]_D$ +50°(c 1.8) (Found: C, 65.28; H, 4.65; N, 3.45. Calc for $C_{31}H_{27}NO_{10}$: C, 64.91; H, 4.74; N, 2.44%). ^{1}H-NMR: 5.43d (1H, J 8.4 Hz, H-1), 4.42dd (1H, J 8.4, 10.7 Hz, H-2), 6.05dd (1H, J 10.7, 9 Hz, H-3), 5.57dd (1H, J 9, 10 Hz, H-4), 4.18ddd (1H, J 10, 3.5, 6.5 Hz, H-5), 4.49, 4.63 two dd (2x1H, J 12 Hz, H-6,6'), 3.48s (3H, OCH_3), 1.79s (3H, CH_3CO). ^{13}C-NMR: 99.7 (C-1), 57.0 (C-2), 70.3, 70.4 (C-3,4), 72.1 (C-5), 63.2 (C-6), 54.85 (OCH_3), 20.4, 170.1 (CH_3CO), 165.2 (PhCO).

A soln of conc H_2SO_4 (0.2 ml) in Ac_2O (20 ml) was added at 0° to a suspension of 22 (5.7 g, 10 mmol) in Ac_2O (20 ml), after 3.5-4 hr at 20° the homogeneous soln was poured onto ice (200 g), and stirred for 3-4 hr at 0°. Conventional work-up (*cf.* preparation of 4) gave after crystallisation from EtOH 4.64 g (77%) of 23, m.p. 118-120°, $[\alpha]_D$ +65.5°(c 1.6). ^{1}H-NMR: 6.67d (1H, J 8.5 Hz, H-1), 4.60dd (1H, J 8.5, 10.5 Hz, H-2), 6.16dd (1H, J 10.5, 9.5 Hz, H-3), 5.62t (1H, H-4), 4.35ddd (1H, J 9.5, 4.5, 3 Hz, H-5), 4.45, 4.62 two dd (2x1H, J 12 Hz, H-6, 6'), 1.80, 2.01 two s (2x3H, CH_3CO). ^{13}C-NMR: 90.05 (C-1), 53.9 (C-2), 70.6 (C-3), 69.3 (C-4), 72.9 (C-5), 62.8 (C-6), 20.7, 20.2 ($\underline{C}H_3CO$).

4-O-Benzoyl-3-O-(3,4-di-O-benzoyl-α-L-rhamnopyranosyl)-1,2-O-[1-(exo-cyano)ethylidene]-β-L-rhamnopyranose (25). To a suspension of 7 (1.72 g, 5.4 mmol) and $Hg(CN)_2$ (1.76 g, 7 mmol) in MeCN (5 ml) was added, with stirring, a soln of the bromide 15 (prepared from 7 mmol of the acetate 13) in 10 ml of MeCN. After 1 hr the reaction mixture was conventionally worked-up to give, after crystallisation from MeOH, 3.24 g (84%) of 24. Additional crop of 24 (0.35 g) was obtained from the mother liquor (total yield 93%). 24 had m.p. 195-197° and $[\alpha]_D$ +105°(c 1) (Found: C, 63.83; H, 5.21; N, 1.88. Calc for $C_{38}H_{37}NO_{13}$: C, 63.77; H, 5.21; N, 1.95%). ^{13}C-NMR: 96.9 ($^1J_{C,H}$ 176 Hz, C-1A), 100.3 ($^1J_{C,H}$ 172.1 Hz, C-1B), 80.3 (C-2A), 70.2 (C-2B,3B), 78.3 (C-3A), 71.5 (C-4A,4B), 69.3 (C-5A), 67.8 (C-5B), 17.5, 17.6 (C-6A,6B), 20.6, 169.6 (CH_3CO), 26.5, 101.8, 117.1 (CH_3-C-CN), 165.6, 165.9 (PhCO).

Methanolic hydrogen chloride (prepared by addition, at 0°, of 1.2 ml of acetyl chloride to 10 ml of methanol) was added to a soln of 24 (1.2 g, 1.68 mmol) in $CHCl_3$ (10 ml). After 4 hr at 20° the reaction mixture was neutralised with $KHCO_3$ aq following addition of $CHCl_3$ (150 ml), evaporated, the residue was partitioned between water and $CHCl_3$, the organic layer was washed with water, dried, and evaporated. The residue was subjected to CC to give 24 (470 mg, 39%), R_f 0.59 (EtOAc-benzene, 1:2), and 25 (420 mg, 37%), R_f 0.45, m.p. 207-209°(MeOH), $[\alpha]_D$ +107°(c 0.9) (Found: C, 63.98; H, 5.24; N, 2.27. Calc for $C_{36}H_{35}NO_{12}$: C, 64.18; H, 5.24; N, 2.08%). ^{1}H-NMR: 5.46d (1H, J 2 Hz, H-1A), 5.09s (1H, H-1B), 4.72dd (1H, J 2, 4 Hz, H-2A), 4.04 br.s (1H, H-2B), 4.20dd (1H, J 4, 9.5 Hz, H-3A), 5.57-5.67m (2H, H-3B,4B), 5.38t (1H, H-4A), 3.69dq (1H, H-5A), 4.38m (1H, H-5B), 1.29, 1.36 two d (2x3H, J 6 Hz, H-6A,6B), 2.00s (3H, CH_3CCN). ^{13}C-NMR: 97.0 (C-1A), 102.8 (C-1B), 80.5 (C-2A), 70.3 (C-2B), 78.1 (C-3A), 72.2 (C-3b), 71.4, 71.9 (C-4A,4B), 69.7 (C-5A), 67.9 (C-5B), 17.5, 17.7 (C-6A,6B), 26.6, 101.8, 117.0 (CH_3-C-CN).

1-O-Acetyl-2-O-(3-O-acetyl-4,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,4-di-O-benzoyl-L-rhamnopyranose (28). The vacuum technique (ref. 29) was used for glycosylation. Solvents were degassed ($4x10^{-3}$ Torr) and distilled twice from CaH_2. An alcohol to be glycosylated and promoter(s) were dried for 6-10 hr. A soln of a glycosyl bromide in dry benzene was placed in a flask connected via an adaptor to the vacuum system and lyophilised, then it was dried for 1-2 hr. MeCN was distilled into the flasks with the reagents, dry argon was introduced, and a soln of a bromide was added with stirring to a soln of an aglycon and promoter(s).

Treatment of 23 with HBr in AcOH afforded the bromide 26 as a mixture of α- and β-anomers which was used for glycosylation as such. ^{1}H-NMR: 6.68d (J 3.5 Hz, H-1α), 6.58d (J 9 Hz, H-1β), 4.80dd (J 3.5, 11 Hz, H-2α), 4.78t (J 9.5 Hz, H-2β), 6.93dd (J 9, 11 Hz, H-3α), 6.05dd (J 9, 9.5 Hz, H-3β), 5.62t (J 9.5 Hz, H-4α), 5.68t (J 9.5 Hz, H-4β), 4.47-4.57m (H-5α, H-6α,6β), 4.32ddd (J 3, 4.5, 9.5 Hz, H-5β), 4.66dd (J 3, 11.5 Hz, H-6'α,6'β), 1.83s (CH_3COα), 1.78s (CH_3COβ). ^{13}C-NMR: α-anomer, 87.4 (C-1), 56.6 (C-2), 67.5, 70.5, 73.0 (C-3,4,5), 62.1 (C-6), 20.6 (CH_3CO); β-anomer, 69.7, 70.7, 77.2, 77.5 (C-1,3,4,5), 58.6 (C-2), 62.9 (C-6), 20.2 (CH_3CO).

A soln of the bromide 26 (prepared from 9.1 mmol of 23) in 15 ml of MeCN was added for 30-40 min to a soln of 14 (2.67 g, 6.9 mmol) and $Hg(CN)_2$ (2.29 g, 9.1 mmol) in 10-12 ml of MeCN and the mixture was stirred for 16 hr at 20°. Conventional work-up and CC gave 5.59 g (87%) of 27 contaminated with 3-5% (TLC) of slowly migrating products. Crystallisation from 10 ml of $CHCl_3$ and 125 ml of EtOH afforded analytically pure 27 (5 g, 78%), m.p. 174-175°, $[\alpha]_D$ +8.7°(c 1.8) (Found: C, 65.91; H, 4.92; N, 1.26. Calc for $C_{51}H_{45}NO_{16}$: C, 66.01; H, 4.89; N, 1.51%). ^{1}H-NMR: 4.96d (1H, J 1.5 Hz, H-1C), 5.63d (1H, J 8.5 Hz, H-1D), 4.46 br.t (1H, J 2.5 Hz, H-2C), 4.58dd (1H, J 8.5, 10.5 Hz, H-2D), 5.25-5.35m (2H, H-3C,4C), 5.98dd (1H, J 10.5, 9 Hz, H-3D), 5.50t (1H, J 9 Hz, H-4D), 3.95m (1H, H-5C), 4.12ddd (1H, H-5D), 1.25d (3H, J 6 Hz, H-6C), 4.45dd (1H, J 6, 12.5 Hz, H-6D), 4.60dd (1H, J 3.5, 12.5 Hz, H-6'D), 3.30s (3H, OCH_3), 1.72s (3H, CH_3CO). ^{13}C-NMR: 100.2 (C-1C), 99.3 (C-1D), 77.1 (C-2C), 54.9, 55.05 (C-2D, OCH_3), 66.45 (C-5C), 17.55 (C-6C), 63.0 (C-6D), 20.2 (CH_3CO).

A soln of conc H_2SO_4 (0.5 ml) in Ac_2O (40 ml) was added at 0° to a suspension of 27 (9.78 g, 10.5 mmol) in Ac_2O (40 ml). After 4-5 hr at 20° the soln was poured onto 700 g of ice, stirred for 4-5 hr at 0°, the precipitate was filtered off, washed with water, and dried over P_2O_5, the yield of 28 was 10g (100%), $[\alpha]_D$ +16.3° (c 1.5)(after CC). ^{1}H-NMR: 6.44d (1H, J 2 Hz, H-1C), 5.70d (1H, J 8.5 Hz, H-1D), 4.47t (1H, J 2.5 Hz, H-2C), 4.60dd (1H, J 8.5, 10.5 Hz, H-2D), 5.33dd (1H, J 2.5, 10 Hz, H-3C), 5.96dd (1H, J 10.5, 9 Hz, H-3D), 5.40dd (1H, J 10, 8.5 Hz, H-4C), 5.54t (1H, J 9.5 Hz, H-4D), 4.07dq (1H, H-5C), 4.16m (1H, H-5D), 1.26d (3H, J 6 Hz, H-6C), 4.50dd (1H, J 5, 12 Hz, H-6D), 4.62dd (1H, J 3, 12 Hz, H-6'D), 1.72, 2.10 two s (2x3H, CH_3CO). ^{13}C-NMR: 92.9 (C-1C), 99.4 (C-1D), 76.2 (C-2C), 55.0 (C-2D), 69.2 (C-5C), 17.65 (C-6C), 63.2 (C-6D), 20.3, 20.85 (CH_3CO).

O-(3,4-Di-O-benzoyl-α-L-rhamnopyranosyl)-(1→2)-O-(3,4-di-O-benzoyl-α-L-rhamnopyranosyl)-(1→3)-4-O-benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-L-rhamnopyranose (30). A soln of the bromide 15 (prepared from 0.6 mmol of 13) in 3 ml of MeCN was added dropwise with stirring to a soln of 25 (0.337 g, 0.5 mmol) and $Hg(CN)_2$ (0.15 g, 0.6 mmol) in 2 ml of MeCN. After 2 hr an additional portion of the bromide 15 (0.6 mmol) and $Hg(CN)_2$ (0.6 mmol) was added. The reaction mixture was conventionally worked-up after 1.5 hr, CC then gave 0.43 g (80%) of the amorphous 29, $[\alpha]_D$ +104.8°(c 1.7). ^{13}C-NMR: 97.0 (C-1A), 101.9 (C-1B), 99.4 (C-1C), 80.3 (C-2A), 76.8 (C-2B), 77.9 (C-3A), 67.6, 68.1 (C-5B,5C), 17.3, 17.6x2 (C-6A,6B,

6C), 20.5, 169.0 (CH_3CO), 26.4, 101.8, 116.9 (CH_3-C-CN). ^{1}H-NMR: 5.57dd (J 1.5, 3.5 Hz, H-2C), 1.03, 1.30, 1.45 three d (J 6 Hz, H-6A,6B,6C), 2.02s (CH_3CO), 2.04s (CH_3CCN).

To a soln of 29 (0.56 g, 0.52 mmol) in 1 ml of $CHCl_3$ and 3 ml of MeOH acetyl chloride (0.12 ml) was added, after 4 hr at 20° the reaction mixture was worked-up as described for 25, CC gave 29 (0.12 g, 21.6%) and 30 (0.29 g, 54%), $[\alpha]_D$ +115°(c 0.68). ^{13}C-NMR: 97.1 (C-1A), 102.0 (C-1B), 101.4 (C-1C), 80.4 (C-2A), 76.8 (C-2B), 78.1 (C-3A), 67.7, 68.1 (C-5B,5C), 17.4, 17.6, 17.8 (C-6A,6B,6C), 26.5, 101.8, 117.0 (CH_3-C-CN). ^{1}H-NMR: 4.32 br.s (H-2C), 1.04, 1.32, 1.42 three d (J 6 Hz, H-6A,6B,6C), 2.04s (CH_3CCN).

O-(3-O-Acetyl-4,6-di-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-O-(3,4-di-O-benzoyl-α-L-rhamnopyranosyl)-(1→2)-O-(3,4-di-O-benzoyl-α-L-rhamnopyranosyl)-(1→3)-4-O-benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-L-rhamnpyranose (32). a) A soln of the bromide 26 (prepared from 2.88 mmol of 23) in MeCN (15 ml) was added with stirring to a suspension of 30 (1.65 g, 1.6 mmol), $Hg(CN)_2$ (0.81 g, 3.2 mmol), and $HgBr_2$ (0.86 g, 2.4 mmol) in 6 ml of MeCN (the reagents and solvent were preconditioned using the vacuum technique, *vide supra*) and the mixture was stirred for 16 hr at 20°. After conventional work-up, CC, and crystallisation from $CHCl_3$-MeOH was obtained 32 (1.21 g, 48%), m.p. 154-158°, $[\alpha]_D$ +106° (c 1.1).

Glycosylation of 30 (0.38 g, 0.37 mmol) by the bromide 26 (from 0.75 mmol of 23) in 10 ml of CH_2Cl_2 in the presence of $AgOSO_2CF_3$ (0.19 g, 0.74 mmol) and 0.1 ml of 2,4,6-collidine at -20° - -30° → 20° afforded after conventional work-up and CC 30 (0.28 g, 74%), 32 (0.08 g, 14%), and 36 (0.18 g, 44%), $[\alpha]_D$ -75.2°(c 1.3), syrup; ^{1}H-NMR: 6.89s (1H, H-1), 5,76dd (1H, $J_{3,4}$ 3.5, $J_{3,5}$ 1.5 Hz, H-3), 5.70t (1H, J 3.5 Hz, H-4), 4.93m (1H, H-5), 4.77d (1H, J 1 Hz, H-6), 4.80s (1H, H-6'), 1.96s (3H, CH_3CO); ^{13}C-NMR: 148.5 (C-1), 105.8 (C-2), 67.5, 67.8, 74.6 (C-3,4,5), 61.8 (C-6), 20.6 (CH_3CO), 165.1, 166.0, 167.7, 170.0 (CO).

b) To a suspension of 25 (4.7 g, 7 mmol), $Hg(CN)_2$ (2.16 g, 8.6 mmol),and $HgBr_2$ (1.55 g, 4.3 mmol) in 50 ml of MeCN (vacuum technique) was added with stirring for 30-40 min a soln of 31 (prepared from 8.7 mmol of 28 by treatment with hydrogen bromide in $CHCl_3$) in 30 ml of MeCN. After 16 hr at 20° the mixture was conventionally worked-up, crystallisation from 30 ml of $CHCl_3$ and 100 ml of MeOH then gave 32 (5 g, 45.5%). Recrystallisation afforded 4.7 g (43%) of 32, m.p. 154-157°, $[\alpha]_D$ +105.5°(c 2.1) (Found: C, 65.88; H, 4.92; N, 2.06. Calc for $C_{86}H_{76}N_2O_{27}$: C, 65.81; H, 4.88; N, 1.78%). ^{13}C-NMR: 97.1 (C-1A), 101.8 (C-1B), 101.0 (C-1C), 99.0 (C-1D), 80.4 (C-2A), 54.9 (C-2D), 77.7 (C-3A), 67.6, 68.0 (C-5B,5C), 17.5, 17.7, 17.9 (C-6A,6B,6C), 26.5, 101.8, 117.0 (CH_3-C-CN), 20.2, 169.8 (CH_3CO). From the mother liquor were isolated, by CC, 25 (0.87 g, 18.5%) and 35 (1.76 g, 22%), m.p. 134-135°(EtOH), $[\alpha]_D$ +0.57°(c 2) (Found: C, 66.47; H, 4.64; N, 3.18. Calc for $C_{51}H_{42}N_2O_{15}$: C, 66.37; H, 4.59; N, 3.03%). ^{13}C-NMR: 99.8 (C-1D), 54.7 (C-2D), 67.1 (C-5C), 17.5 (C-6C), 62.7 (C-6D), 20.2 (CH_3CO), 114.6 (CN).

Synthesis of the Monomer (34). To a soln of 32 (4.0 g, 2.55 mmol) in 13 ml of $CHCl_3$ and 40 ml of MeOH was added acetyl chloride (2.12 ml) at 0° and then a soln of AcCl in $CHCl_3$ (2.5% v/v) to make the mixture homogeneous (ca.20 ml). It was kept at 20° for 3.5 hr, worked-up as described in preparation of 25, and chromatographed to give 32 (1.6 g, 40%) and 33 (1.17 g, 30%), m.p. 158-161°($CHCl_3$-MeOH), $[\alpha]_D$ +95.4°(c 1). ^{13}C-NMR: 97.1 (C-1A), 101.8 (C-1B), 101.1 (C-1C), 99.2 (C-1D), 80.4 (C-2A), 57.2 (C-2D), 77.8 (C-3A), 67.6, 67.9 (C-5B,5C), 17.5, 17.7, 17.9 (C-6A,6B,6C), 62.7 (C-6D), 26.6, 101.8, 117.0 (CH_3-C-CN).

Triphenylmethylium perchlorate (0.42 g, 1.22 mmol) was added portionwise for 20-30 min with stirring to a soln of 33 (1.73 g, 1.17 mmol) and 2,4,6-collidine (0.17 ml, 1.29 mmol) in CH_2Cl_2 and stirring was continued for 10-15 min. To a violet soln was added pyridine-MeOH (3:1 v/v, 1-2 ml), then it was diluted with $CHCl_3$, washed with water, dried, and evaporated. After CC were obtained the recovered 33 (1.26 g, 72.8%) and 34 (0.31 g, 15%). Rechromatography and crystallisation from $CHCl_3$-MeOH gave 0.24 g of 34, m.p. 243-245°, $[\alpha]_D$ +104.3°(c 1.33). ^{13}C-NMR: 97.05 ($^1J_{C,H}$ 173.9 Hz, C-1A), 101.90($^1J_{C,H}$ 172.0 Hz, H-1B), 100.89 ($^1J_{C,H}$ 172.0 Hz, C-1C), 98.36 ($^1J_{C,H}$ 162.8 Hz, C-1D), 80.40 (C-2A), 56.81 (C-2D), 67.67, 67.90 (C-5B,5C), 17.67x2, 17.84 (C-6A,6B,6C), 63.44 (C-6D), 26.52, 101.76, 117.00 (CH_3-C-CN), 88.85 (Ph_3CO).

The unreacted 33 was tritylated and fractionated as above. After six cycles 34 (1.66 g,39.8%) was obtained from 3.6 g (2.36 mmol) of 33.

Polycondensation of the Monomer 34 (0.44 g, 0.25 mmol) was effected by the action of $TrClO_4$ (8.5 mg, 0.025 mmol) in 3 ml of CH_2Cl_2 using the vacuum technique as described in ref. 3,8. After 16 hr at 20° to the bright-yellow soln pyridine-MeOH (3:1 v/v, 0.1 ml) was added, the decolourised soln was diluted with $CHCl_3$ (50 ml), washed with water, dried, and evaporated. The residue was chromatographed over 50 g of Silicagel and 37 (265 mg, 70%) was isolated as a white, amorphous powder, R_f 0.5 (benzene-EtOAc, 7:3), 0.9 (benzene-EtOAc, 1:1). Polycondensation of the other, crystalline specimen of 34 (0.63 g) gave 485 mg (90%) of 37, $[\alpha]_D$ +117.4°(c 1).

Preparation of the Polysaccharide 1. A suspension of 37 (200 mg) in 15 ml of EtOH and 1.5 ml of 99% hydrazine hydrate was refluxed for 20-25 hr (the mixture becomes homogeneous after 5-6 hr), the soln was evaporated, 1-butanol was added to, and distilled from, the residue which was then dried *in vacuo*. It was then treated with 4 ml of Ac_2O in 10 ml of MeOH and 2 ml of water overnight. The mixture was taken to dryness, the residue was suspended in water (6 ml), and centrifuged. The supernatant was separated, the residue was again washed with water, the combined

solns were concentrated, and subjected to gel-chromatography on a column with Bio-Gel P-4 (55x2.5 cm, -400 mesh, V_o ca. 110 ml) in 0.1 M AcOH. The eluate emerged over an interval of 90-150 ml was concentrated and lyophilised. Drying at 60° over P_2O_5 for 3 hr gave 80 mg (92%) of 1, $[\alpha]_D$ -46°(c 0.87, water). Starting from 450 mg of the other specimen of 37 was obtained 1, $[\alpha]_D$ -55°(c 0.75, water) in a yield of 180 mg (93.5%).

The polysaccharide 1 was eluted with R_T 4.67 min (SynChropack GPC-100, 5μ, 25x 0.46 cm, eluent water, 0.6 ml/min). Dextranes T-10, T-20, and T-40 were eluted with R_T 4.43, 3.80, and 3.28 min, respectively.

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