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Preliminary communication

Synthesis of the basic chain of the O-specific polysaccharides of *Shigella flexneri*

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The basic structure of O-antigens of all serotypes of *Shigella flexneri* is a polysaccharide chain with the biological repeating-unit **1**[1,2].

→2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→

1

Several spacer-arm glycosides of the related di-, tri-, and tetra-saccharides have been synthesised and used to prepare artificial antigens of the neoglycoprotein type[3]. We now report the synthesis of a polysaccharide possessing the structure of the basic chain of *Sh. flexneri* O-antigens.

Synthesis of polysaccharides by the polycondensation method[4] involves a monomer bearing 1,2-*O*-(1-cyanoethylidene) and trityl groups. The monomer we required must have a monosaccharide sequence of only one of the four possible chemical repeating-units, namely, with a 3-substituted rhamnose at the reducing end, thus providing the formation of a polysaccharide differing from the natural one only by its terminal sequences. This difference seems to have little, if any, influence on the immunological properties of the synthetic polysaccharide.

We have shown that (*a*) the cyanoethylidene group survives various glycosylation conditions[5], (*b*) treatment with methanolic 0.6M hydrogen chloride at 20° removes *O*-acetyl groups in the presence of *O*-benzoyl groups[6], (*c*) 2-deoxy-2-phthalimido (or, less successfully, 2-acetamido)glucose derivatives can be used as glycosyl acceptors in $TrClO_4$-catalysed glycosylations and polycondensations[7,8].

Although the deacetylation of cyanoethylidene derivatives is accompanied by a side-reaction (addition of methanol to the cyano group to give imidates)[6], it was thought reasonable to elongate the oligosaccharide chain starting from the rhamnose residue already bearing the cyanoethylidene function.

Synthons for the A–D units of the monomer **2c** and the polysaccharide derived therefrom were the monosaccharide derivatives **3**[6], **4**[5], **5**[6], and **6**[6]. Two approaches were explored, namely, sequential elongation of the oligosaccharide chain starting from the reducing end [(A + B) + C] + D, and synthesis of the disaccharide blocks (BA and DC) and their coupling.

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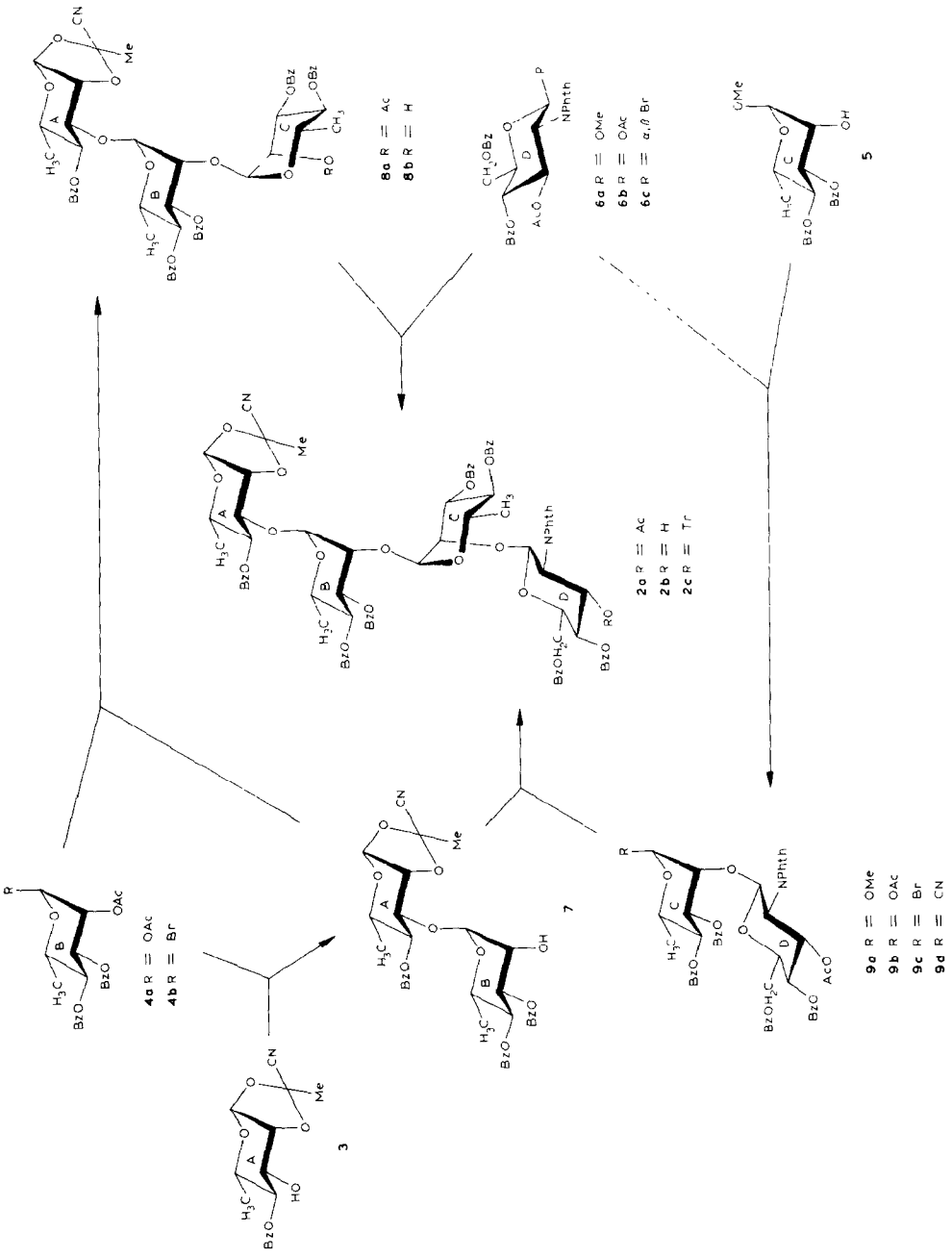

8a R = Ac
8b R = H
6a R = OMe
6b R = OAc
6c R = α,β Br
5
2a R = Ac
2b R = H
2c R = Tr
4a R = OAc
4b R = Br
3
7
9a R = OMe
9b R = OAc
9c R = Br
9d R = CN

TABLE I

PROPERTIES OF COMPOUNDS **2**, **8**, AND **9**

Compound	*M.p. (deg.) (solvent)*	$[\alpha]_D^{22}$ *(deg.) (c, $CHCl_3$)*	*^{13}C-N.m.r. data (chemical shifts in p.p.m.; $CDCl_3$)*									
			C-1 of unit				*C-6 of*		*CH_3--C----CN*			*CH_3COO*
			A	*B*	*C*	*D*	*Rha*	*GlcN*				
2a	154–158 ($CHCl_3$–MeOH)	+105.5 (2.2)	97.1	101.8	101.0	99.0	17.5 17.7 17.9	62.5	26.5	101.8	117.0	20.2
2b	158–161 ($CHCl_3$–MeOH)	+95 (1.1)	97.1	101.8	101.1	99.2	17.5 17.7 17.9	62.7	26.6	101.8	117.0	–
2c [a]	243–245 ($CHCl_3$–MeOH)	+104 (1.3)	97.1	101.9	100.9	98.4	17.7 × 2 17.8	63.4	26.5	101.8	117.0	–
8a	foam	+105 (1.7)	97.0	101.9	99.4	–	17.6 × 2 17.3	–	26.4	101.8	116.9	20.5
8b	foam	+115 (0.7)	97.1	102.0	101.4	–	17.4 17.6 17.8	–	26.5	101.8	117.0	–
9a	174–175 (ethanol)	+8.7 (1.8)	–	–	100.2	99.3	17.6	63.0	–	–	–	20.2
9b	foam	+16 (1.6)	–	–	92.9	99.4	17.7	63.2	–	–	–	20.3 20.9

[a] Also gave a signal at 88.85 p.p.m. for Ph_3CO.

Helferich glycosylation of the "disaccharide aglycon" 7[6], common to both schemes, by the rhamnosyl bromide **4b**[5] (2 mol) gave 66% of the trisaccharide derivative **8a*** (Table I). Deacetylation[6] of **8a** during 5.5 h gave the "trisaccharide aglycon" **8b** (35–40%, 25–30% recovery of **8a**).

The glucosyl bromide **6c** was obtained from the methyl glycoside **6a**[6], which was acetolysed (1% of H_2SO_4 in acetic anhydride, 20°, 5 h) to give the β-acetate **6b** (77%), m.p. 118–120° (from ethanol), $[\alpha]_D^{22}$ +65.5° (*c* 1.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 6.67 (d, $J_{1,2}$ 8.5 Hz, H-1), 2.01, 1.80 (2 s, 2 OAc). Treatment of **6b** with hydrogen bromide in acetic acid gave **6c** as an ~1:3 α,β-mixture. ^{1}H-N.m.r. data ($CDCl_3$): δ 6.58 (d, $J_{1,2}$ 9 Hz, H-1β), 6.68 (d, $J_{1,2}$ 3.5 Hz, H-1α), 6.93 (dd, H-3α), 6.05 (dd, H-3β); two sets of signals due to the α and β anomers were observed in the ^{13}C-n.m.r. spectrum.

Glycosylation of **8b** with **6c** (1.8 mol) in acetonitrile in the presence of $Hg(CN)_2$ (1.8 mol) and $HgBr_2$ (1.4 mol) gave 48% of the tetrasaccharide derivative **2a** after column chromatography and crystallisation. All glycosylations were performed under argon and required rigorous anhydrous conditions.

The disaccharide derivative **9a**, which was the precursor for the block synthesis of the tetrasaccharide, was prepared (80%) by glycosylation of **5**[6] with **6c** (1.2–1.3 mol) in acetonitrile in the presence of $Hg(CN)_2$, or $Hg(CN)_2$ and $HgBr_2$, followed by column chromatography and crystallisation. Acetolysis of **9a** gave **9b** which, with HBr in chloroform, gave chromatographically homogeneous glycosyl bromide **9c**. Coupling of the "disaccharide aglycon" **7** with **9c** (1.2 mol) in acetonitrile in the presence of $Hg(CN)_2$ (1.2 mol) and $HgBr_2$ (0.6 mol) yielded 45% of **2a** by crystallisation, and subsequent column chromatography gave more **2a**, **7** (~20%), and the biosyl cyanide **9d** (20–25%), m.p. 134–135° (from ethanol), $[\alpha]_D^{22}$ +0.57° (*c* 2.1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 114.6 (CN), 99.75 (C-1′). Crystalline specimens of **2a** obtained by both routes of synthesis were identical, thus indicating stereoselective glycosylation by bromide **9c** having a non-participating group at C-2. The block scheme is preferable since it avoids one deacetylation and the overall yield of **2a** from **7** by this route is 2–3 times as high as that obtained in the stepwise scheme.

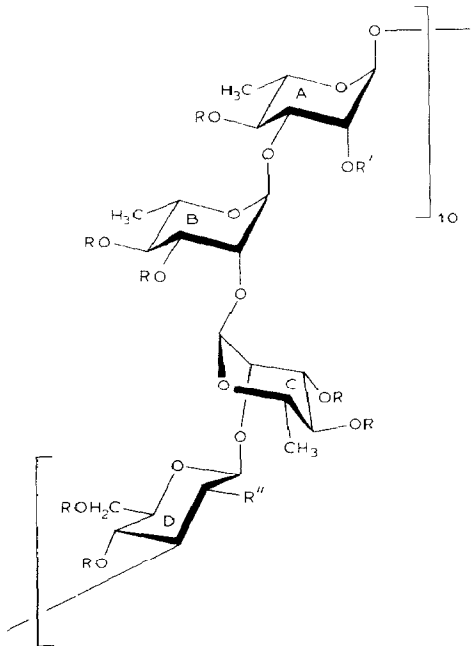
Deacetylation of **2a** gave 25–30% of the desired alcohol **2b**, and 35–40% of **2a** was recovered. The downfield shift of the signals for C-2‴ (57.2 p.p.m.) of **2b** as compared with that (54.9 p.p.m.) of the corresponding signal for **2a** confirms the location of free hydroxyl at C-3‴ in **2b**.

Tritylation of secondary hydroxyl groups in cyanoethylidene derivatives, using triphenylmethylium perchlorate, gives[4,9–12] yields in the range of 20–90% with no side-products. However, tritylation of **2b** (1.1 mol of $TrClO_4$, 1.13 mol of 2,4,6-trimethylpyridine in dichloromethane, 30–50 min, room temperature) afforded the desired ether **2c** (~20%) together with two unidentified products, one of which was tritylated and both of which were devoid of a cyanoethylidene group; prolonged treatment increased the proportion of the side-products.

Polycondensation of **2c** was performed under standard conditions[13] (0.1 mol

*Correct C, H, and N analyses were obtained for all crystalline compounds, and the ^{1}H- and ^{13}C-n.m.r. spectra were in agreement with the structures assigned.

of $TrClO_4$ in dichloromethane, 20°) for 16 h. T.l.c. then revealed the absence of **2c** and the polysaccharide derivative **10a** was isolated by column chromatography on silica gel (benzene → ethyl acetate) as a white powder (80% yield), $[\alpha]_D^{22}$ +117° (*c* 1, chloroform), R_F 0.5 (benzene–ethyl acetate, 7:3) and 0.9 (benzene–ethyl acetate, 1:1). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.00, 17.41, 17.53 (C-6 of 3 Rha), 20.50 (CH_3COO), 56.36, 62.71 (C-2 and C-6 of GlcN), 67.49, 67.60, 67.80 (C-5 of 3 α-L-Rha, *cf.* ref. 14), 98.19, 99.28, 100.33, and 100.67 (anomeric carbons).



10a R = Bz, R' = Ac, R'' = NPhth
10b R = R' = H, R'' = NHAc

Treatment[15] of **10a** with 99% hydrazine hydrate in boiling ethanol for 24 h, followed by *N*-acetylation with Ac_2O in aqueous methanol, gel chromatography on Bio-Gel P-4, and lyophilisation gave **10b** (90%), $[\alpha]_D^{22}$ −50° (*c* 0.8, water). The ^{13}C-n.m.r. spectrum of **10b** contained four signals (δ 102.21, 101.88, 102.10, and 103.24; $^1J_{C,H}$ 170.2, 172.0, 171.1, and 162.7 Hz) corresponding to C-1 of units A–D, respectively, indicating all the rhamnose units to be α and confirming the stereospecificity of the polycondensation. 3-*O*-Glycosylation of the GlcN unit was indicated by the downfield chemical shift for C-3 (δ 82.68). Minor signals (δ 57.2, 75.0, and 103.67 p.p.m.) were assigned to C-2, C-3, and C-1 of the unsubstituted GlcNAc unit at the non-reducing end of the polysaccharide chain. The ^{13}C-n.m.r. spectra of **10b** and the O-antigenic polysaccharide[16] of *Sh. flexneri* were identical.

The molecular mass of **10b** (~6,000), determined by gel-permeation chromatography on a SynChropak GPC-100 column (using Dextrans T-10, T-20, and T-40 as standards), indicates a d.p. of ~10.

This synthesis (together with the published synthesis of the O-specific polysaccharide of *Salmonella newington*[13]) demonstrates the applicability of polycondensation as a route to microbial heteropolysaccharides of regular structure.

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