SYNTHESIS OF THE PRINCIPAL CHAIN OF THE O-ANTIGENIC POLYSACCHARIDES OF *Shigella Flexneri*. COMMUNICATION 2.* SYNTHESIS OF 4-O-BENZOYL-1,2-O-(1-(CYANOETHYLIDENE)-β-L-RHAMNOPYRANOSE

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Studies of the structure of the O-antigens of the Gram-negative bacterium *Shigella flexneri*, which is a causative agent of dysentery, have shown that the O-antigenic polysaccharides of all serotypes of these bacteria contain the same principal chain [2], the biologically repeating unit of which is [3] the tetrasaccharide →2)α-L-Rhap-(1 → 2)-α-L-Rhap-(1 → 3)-α-L-Rhap-(1 → 3)-β-D-GlcpNAc-(1→. The polysaccharides of *Sh. flexneri* have been studied intensively over the past decade. A number of oligosaccharide fragments of the principal chain and their conjugates with proteins have been synthesized [4] for use as artificial antigens.

We have undertaken the synthesis of the polysaccharide chain itself by regiospecific and stereospecific polycondensation [5], involving the $TrClO_4$-catalyzed polycondensation of mono- or oligosaccharides containing 1,2-O-cyanoethylidene and trityl groups. This method was employed to synthesize a number of regular polysaccharides, including the first reported synthesis of a natural heteropolysaccharide, the O-antigenic polysaccharde [6] of *Salmoella newington* with a trisaccharide repeating unit.

Examination of the structure of the principal polysaccharide chain of *Sh. flexneri* shows that the sequence of only one of the four possible chemically repeating units (which does not coincide with the sequence of the biologically repeating unit), namely with a 3-substituted rhamnose at the reducing terminal, permits the construction of a monomer for polycondensation, since only in this case is it possible to generate the 1,2-O-cyanoethylidene function, which is the glycosating terminal of the monomer. Consequently, the glycosating terminal of the monomer must be a glycosamine with a trityl group in the O^3 position. In developing a synthetic strategy, preliminary experiments showed that: 1) the 1,2-O-cyanoethylidene group is stable under a variety of glycosylating conditions [7, 8]; 2) it is possible to remove selectively O-acetyl groups in the presence of O-benzoyl groups by acidic methanolysis [1, 9]; and 3) glucosamines with N-phthaloyl (and, less successfully, N-acetyl) groups undergo $TrClO_4$-catalyzed polycondensation [10]. With these considerations in mind, the requirement arose to synthesize the tetrasaccharide (I) as the monomer (see Scheme 1).

It was proposed to construct the 1,2-O-cyanoethylidene function in stages preceded strictly speaking by the synthesis of the oligosaccharide chain (the preparation of the monomer in [6] utilized the inverse sequence, starting with the synthesis of the trisaccharide, followed by the introduction of the cyanoethylidene function). The protecting groups chosen were a combination of benzoyl ("permanent") and acetyl ("temporary"). In addition to its convenience during the synthesis (the ability to carry out glycosylation and acetolysis under standard conditions, and the tendency of the acyl derivatives to crystallize), this combination permitted the one-step removal of the protecting groups in the eventual polymer.

We here report the preparation of a synthon for unit A, the "reducing" terminal of the monomer. The cyanoethylidene derivative (CED) (VI), which is the starting point for the growth of the tetrasaccharide chain, was synthesized from methyl-4-O-benzoly-α-L-rhamnopyranoside (II) [1]. Acetolysis of the diol (II) gave an anomeric mixture of 1,2,3-tri-O-acetyl-4-O-benzoly-L-rhamnopyranoses (III), from which the crystalline α-anomer was isolated in 50% yield. Using a general method for the preparation of CED of saccharides [11], the bromide

*For Communication 1, cf. [1].

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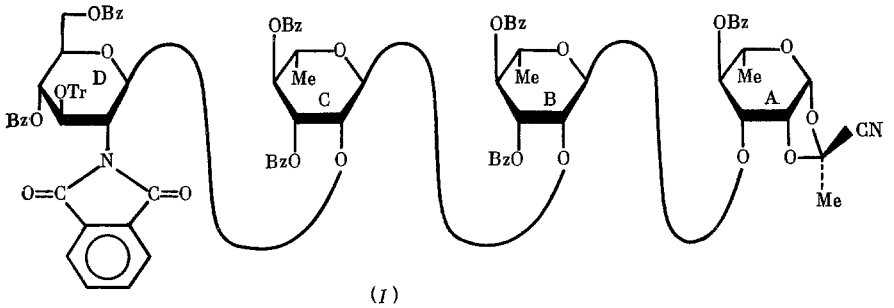
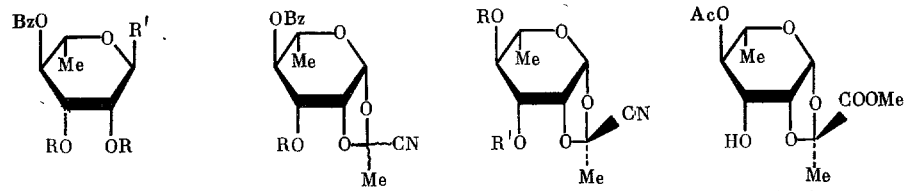
Scheme 1



(I)



(II)-(IV) (V)-(VI) (VII),(VIII),(X)-(XII) (IX)

R = H, R′ = OMe (II); R = Ac, R′ = OAc (III); R = Ac, R′ = Br (IV); R = Ac, exo-CN (V); R = Ac, endo-CN (Va); R = H, exo-CN (VI); R = H, endo-CN (VIa); R = R′ = Ac (VII); R = Ac, R′ = H (VIII); R = R′ = H (X); R = H, R′ = $ClCH_2CO$ (XI); R = Bz; R′ = $ClCH_2CO$ (XII).

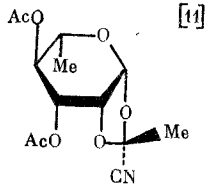
(VI), obtained from acetate (III), was reacted with an excess of NaCN in MeCN to give a mixture of exo and endo-cyano isomers (V) and (Va) in a ratio of 3.5:1 and an overall yield of 80%. Isomers (V) and (Va) were separated by column chromatography (CC), isomer (V) being the one normally used in the subsequent syntheses. The structures of the CED (V) and (Va) were confirmed analytically and spectrally, in particular by the low-field chemical shift of the MeC group signal in the principal isomer (V) as compared with the minor endo-cyano isomer (Va) in the PMR spectra (Table 1, cf. [11, 12]).

The strategy of any oligosaccharide synthesis is based on an alternation of liberation of the required hydroxyl group, and glycosylation. In the present case, this meant that the cyanoethylidene protection had to be conserved during the workup procedures associated with acidic methanolysis. We have previously reported briefly [9] that during the selective deacetylation of CED by 0.6 M HCl in methanol a side reaction occurs, namely, addition of MeOH to the nitrile group to give unstable imidates which undergo hydrolysis to compounds such as (IX).* Compound (IX),† in addition to the principal CED product (VIII) was obtained by treating the diacetate (VII) with 0.6 M HCl in MeOH. The structure of (IX) was confirmed by analysis (absence of nitrogen) and by its spectral data (presence of a signal for MeOOC in the PMR spectrum). The formation of by-products was significant after 2-3 h reaction under these conditions, and hence the outcome of the reaction was dependent on the ratio of the rate of deacetylation to that of the side reaction. Deacetylation of the CED isomers (V) and (Va) takes place smoothly over 3-4 h to give high yields of the CED (VI) and (VIa), respectively, containing a free hydroxyl group at C^3. Compounds such as (IX) (Scheme 1) are formed only in insignificant amounts (TLC). The selectivity of the deacetylation of the CED (VII), (V), and (Va) is confirmed unambiguously by comparing their PMR spectra with those of the hydroxycompounds (VI), (VIa), and (VIII) obtained therefrom, the spectra of the latter containing no signal for a single acetyl group, and displaying a high-field shift of the methine proton when HCOAc → HCOH (see Table 1).

The synthon (VI) was also obtained by an alternative method which avoided acidic methanolysis, starting from the diol (X) [15], obtained by deacetylating the diacetate (VII) by treatment with MeONa in a methanol—pyridine mixture. We have previously shown that the deacetylation of 1,2-O-(arylthio)orthoesters of sugars under these conditions, in contrast to the reaction in methanol alone, avoids the replacement of the arylthio groups by methoxy. In the case of CED, as a result of the very high rate of deacetylation in the presence of pyridine,

*Compounds of this type are formed in the base-catalyzed deacetylation of CED [13, 14].
†This compound was erroneously represented in [9] as 1,2-O-(1-methoxycarbonylethylidene)-β-L-rhamnopyranose.

TABLE 1. PMR Spectral Data for CED (V)-(VIII) and (IX)

Compound	Chemical shifts (δ, ppm) and coupling constants (J, Hz)												
	H^1	H^2	H^3	H^4	H^5	H^6	MeC	MeCOO	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
 [11]	5,52 d	4,44 d.d.	5,10–5,20 m		3,62 m	1,32 d	1,80	2,08 2,13	2	3,8	–	–	6
(VII)	5,47 d	4,60 d.d.	5,27 d.d	5,03 t	3,63 d.q.	1,26 d	1,97	2,09 2,15	2	3,8	9,5	9,2	6,2
(VIII)	5,39 d.	4,45 d.d.	3,49 d.d.	4,85 t	3,94 d.q.	1,25 d	1,95	2,15	2	4	9	9	6
(IX) *	5,48 d	4,55 d.d.	3,86 d.d.d.	4,90 t	3,48 d.q.	1,22 d	1,75	2,14	2,5	4,25	9,5	9,5	6
(Va)	5,65 d	4,54 t	5,38–5,48 m		3,82 d.q.	1,38 d	1,85	2,03	2	2,5	—	–	6
(VIa)	5,58 d	4,47 d.d.	4,05 d.d.d.	5,18 t	3,72 d.q.	1,35 d	1,84	–	2	4	9,25	9,25	6
(V)	5,50 d	4,65 d.d.	5,50 d.d.	5,34 t	5,75 d.q.	1,30 d	1,97	2,03	2	4	10	9,5	6,2
(VI)	5,43 d	4,58 d.d.	4,11 br.d.	5,10 t.	3,65 d.q.	1,27 d	1,93	–	2	4,2	9,5	9,5	6,2

*COOMe signal, δ 3.77 ppm.

the formation of compounds such as (IX) is suppressed. Selective mono- (or monochloro-)-acetylation of the diol (X) (efficient 3-O-acetylation and 3-O-benzoylation of this diol have been reported [15]), followed by benzoylation and removal of the chloroacetyl protection by treatment with thiourea in MeCN gave the crystalline CED (VI) in 40% yield (all operations were carried out without isolation of the pure products at each step). The properties of the CED (VI), obatined by the two routes, were identical. Preparatively speaking, however, the first route is prefereed, and it has therefore been used to prepare large amounts of the CED (VI).

EXPERIMENTAL

The methods and apparatus employed have been described in the preceding communication [1].

1,2,3-Tri-O-acetyl-4-O-benzoyl-α-L-rhamnopyranose (III). To 28 g of the chromatographically homogeneous diol (II), obtained from 100 mmoles of methyl-α- L-rhamnopyranoside as described in [1], was added 100 ml of acetic anhydride followed at 0°C by a solution of 1.5 ml of conc. sulfuric acid in 50 ml of acetic anhydride. The mixture was kept for 2 h at 20°C, until the starting material had dissolved completely, and the solution was then poured onto 600 g of ice and stirred for 5-6 h at 0°C. The crystalline solid was isolated, dissolved in 350 ml of chloroform, washed with water, saturated $NaHCO_3$ solution (3 × 300 ml), and water, dried, and evaporated to give 27.55 g (70%) of a crystalline mixture of α- and β-acetates (III) which it was possible to use directly in the next step. Crystallization of the mixture from ether and hexane gave 14.2 g of the α-acetate, mp 115-117°C $[\alpha]_D$ −28° (C 1.8). Found: C 57.95; H 5.12%. $C_{19}H_{22}O_9$. Calculated: C 57.86; H 5.62%. PMR spectrum (δ, ppm; J, Hz): 1.23 d (3H, H^6, J = 6), 1.82 s (3H, MeCO), 2.12 s (6H, 2MeCO), 4.08 d.q. (1H, H^5), 5.22-5.45 m (3H, H^2, H^3, H^4), 6.02 d (1H, H^1, J = 2), 7.42-8.12 m (5H, Ph).

3-O-Acetyl-4-O-benzoyl-1,2-O-[1-(exo- and endocyano)ethylidene]-β-L-rhamnopyranose (V) and (Va). To a solution of the bromide (IV), obtained as described in [1] from 11 g (28 mmoles) of the mixed acetates (III) (after removal of the crystalline α-acetate) in 50 ml of MeCN was added 6.9 g (140 mmoles) of NaCN, and mixture was stirred for 43 h at 20°C. It was then diluted with 400 ml of a 1:2 mixture of chloroform and hexane, washed with water, the lower aqueous layer washed with 100 ml of the same solvent mixture, the combined organic solutions wahsed with water (2 × 400 ml), dried, evaporated, and the residue subjected to CC to give 5.07 g (50%) of (V), 1.39 g (13.75%) of a mixture of (V) and (Va), and 1.41 g (14%) of (Va). (V), mp 115-117°C (MeOH), $[\alpha]_D$ +47.5° (C 1.8). Found: C 59.87; H 5.28; N 4.28%. $C_{18}H_{19}NO_7$. Calculated: C 59.83; H 5.30; N 3.88%. ^{13}C NMR spectrum (δ, ppm): 17.6 (C^6), 20.5 (MeCOO), 26.55 (MeCCN), 69.3, 70.3, 70.8 (C^3 – C^5), 78.6 (C^2), 97.0 (C^1, $^1J_{C,H}$ = 177.6 Hz), 101.7 (MeCCN), 116.7 (CH), 165.5 (PhCO), 170.1 (MeCOO). (Va): mp 180°C (ethyl acetate–light petroleum), $[\alpha]_D$ +116.7° (C 1.8). Found: C 59.85; H 5.21; N 3.88%.

4-O-Benzoyl-1,2-O-[1-(1-(exocyano)ethylidene]-β-L-rhamnopyranose (VI). a) To a solution of 2.97 g (8.2 mmoles) of (V) in 8 ml of chloroform was added 40 ml of MeOH, followed at 0° C by 1.6 ml of acetyl chloride. The mixture was kept for 4 h at 20°C, then 25 ml of water and an excess of solid $KHCO_3$ were added, the mixture evaporated, and the residue partitioned between water and chloroform (150 ml of each), the aqueous layer extracted with 10 ml of chloroform, the combined extracts washed with water (2 × 30 ml), dried, evaporated and the residue crystallized from a mixture of ethyl acetate and hexane to give 2.38 g (91%) of (VI), mp 155-157°C, $[\alpha]_D$ −13.2° (C 1.4). Found : C 60.37; H 5.39; N 4.42%. $C_{16}H_{17}NO_6$. Calculated: C 60.18; H 5.37; N 4.39%.

b) To a solution of 2.17 g (7.25 mmoles) of the diacetate (VII) [11] in 12 ml of dry pyridine was added 35 ml of 0.1 M MeONa in MeOH. After 7-8 h, the mixture was neutralized with cation-exchange resin KU-2 in the pyridinium form (washed with MeOH), the resin filtered off, washed with 100 ml of MeOH, the filtrates evaporated to dryness, the residue of the diol (X) was dissolved in 12 ml of dry CH_2Cl_2, and 1 ml of pyridine added, followed at −30°C by the dropwise addition of 0.6 ml (8 mmoles) of chloroacetyl chloride in 6 ml of CH_2Cl_2 over 30 min. The mixture was stirred for 1 h at −20 to −30°C (after this time the yellow color which appeared when the acid chloride was added had disappeared), the mixture was treated with a further 1 ml of pyridine followed by a solution of 1.2 ml (10.6 mmoles) of benzoyl chloride in 5 ml of CH_2Cl_2, kept for 16 h at 20°C, the mixture decomposed by adding 0.5 ml of water diluted with 50 ml of chloroform, washed with water, 2 M HCl, water, and saturated $NaHCO_3$ solution, evaporated, and the residue dried *in vacuo* to give 2.86 g of product (a deep yellow foam), which was dissolved in a mixture of 16 ml of MeCN and 6 ml of water. Thiourea (2.75 g; 36 mmoles) was added, the mixture stirred for 16 h at 20°C, part of the solvent evaporated,

the residue diluted with choloroform, washed with 100 ml of water, the aqueous layer extracted with chloroform (3 × 50 ml), the combined extracts washed with water, evaporated, and the residue subjected to CC to give 90 mg (39%) of (VI), mp 155-157°C (ethyl acetate–hexane). The product was identical with that obtained as described above.

4-O-Benzoyl-1,2-O-[1-(endocyano)ethylidene]-β-L-rhamnopyranose (VIa).* To a solution of 2.15 g (5.9 mmoles) of (Va) in 15 ml of chloroform was added 0.6 ml of acetyl chloride, and the resulting solution was treated at 0°C with a solution of 2.2 ml of acetyl chloride in 70 ml of MeOH, kept at 20°C for 3 h, worked up as described above for (VI), method (a) the chloroform solution evaporated, and the residue treated with ethyl acetate–hexane (1:3), whereupon it crystallized. Recrystallization from ethyl acetate and hexane gave 1.64 g (87%) of (VIa), mp 158-159°C $[\alpha]_D$ +52° (C, 1).

4-O-Acetyl-1,2-O-[1-(exocyano)ethylidene]-β-L-rhamnopyranose (VIII) and 4-O-Acetyl-1,2-O-[1-(exomethoxycarbonyl)ethylidene]-β-L-rhamnopyranose (IX). a) To a mixture of 1.2 g (4 mmoles) of (VII) [11] and 20 ml of MeOH was added at 0°C 0.8 ml of acetyl chloride, and the mixture kept for 2.5 h at 20°C. According to TLC (ethyl acetate–benzene, 1:2), the reaction mixture contained three products with R_f 0.56, 0.28, and 0.14. The mixture was diluted with 150 ml of chloroform, washed with water, the aqueous layer extracted with 10 ml of chloroform, the combined chloroform solutions washed with water, dried, evaporated, and the residue subjected to CC to give 0.67 g of (VIII), yield 65%, R_f 0.28, mp 113-114°C (ether–hexane), $[\alpha]_D$ −25° (C 1.1). Found: C 51.57; H 5.93; N 5.35%. $C_{11}H_{15}NO_6$. Calculated: C 51.35; H 5.87; N 5.44%.

b). To a mixture of 1.2 g (4 mmoles) of (VII) in 20 ml of MeOH was added 1 ml of acetyl chloride, kept for 3 h at 20°C, and worked up as described above. CC gave 0.43 g (42%) of (VIII), R_f 0.28, and 0.22 g (21%) of (IX), R_f 0.14, mp 170-172°C (ethyl acetate–methanol–hexane), $[\alpha]_D$ −12.2° (C 1.5) Found: C 49.70; H 6.34; N not found. $C_{12}H_{18}O_8$. Calculated: C 49.65; H 6.25%.

CONCLUSIONS

1,2-O-Cyanoethylidene derivatives of rhamnopyranose with a free hydroxyl group in the 3 position and with readily removable protect at O^3 have been synthesized. These are synthons for oligosaccharide synthesis.

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