Approach to the Synthesis of the Repeating Units of Immunodeterminant Bacterial Polysaccharides: Synthesis and ^{13}C N.M.R. Analysis of β-D-Galactopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-L-rhamnopyranose

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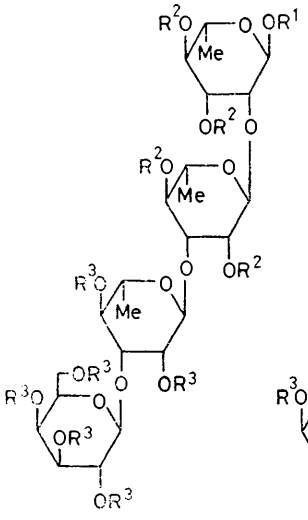
Summary The title tetrasaccharide was obtained by reaction of the α-acetobromo derivative (**6**) of 3-*O*-β-D-galactopyranosyl-L-rhamnose (**4**) and benzyl 3,4-di-*O*-benzyl-2-*O*-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**11**), followed by removal of the protecting groups.

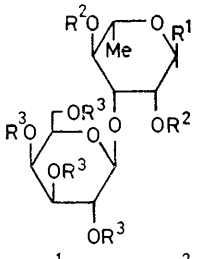
RECENT structural studies[1] of the bacterial extracellular polysaccharides and the *O*-antigenic side chains of cell-wall lipopolysaccharides of bacteria, which act as the immunological determinants of these micro-organisms, revealed that they are composed of repeating units usually consisting of less than seven monosaccharides. The importance of the chemical synthesis of the repeating units or portions thereof, from the chemical, spectroscopic, biosynthetic, and immunological points of view, has been emphasized[2] and the synthesis of several component disaccharides has been achieved. Some component trisaccharides, *e.g.*, β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal*p*,[3] α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-L-Rha*p*,[4] α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-L-Rah*p* (**14**),[5] and a protected derivative of β-D-Man*p*-(1→4)-α-D-Gal*p*-(1→4)-L-Rha*p*[6] have also been synthesized whereas the chemical synthesis of only one tetrasaccharide [β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal*p*-(6←1)-α-D-Glc*p*[7]] which is part of such bacterial polysaccharides has been reported.

As higher oligosaccharides are very difficult to isolate from these natural polysaccharides by either chemical or

enzymic degradation, we have undertaken the synthesis of some of them by chemical means.[4,5] We now describe the preparation and complete ^{13}C n.m.r. assignment of the title tetrasaccharide (**1**) which forms the main chain of the *Klebsiella* serotype K36 polysaccharide,[8,9] the repeating unit of which contains, in addition to (**1**), a pyruvate-acetalated 4-*O*-β-D-glucopyranosyl-D-glucuronic acid side chain linked to O-2 of the third monosaccharide unit of (**1**).†

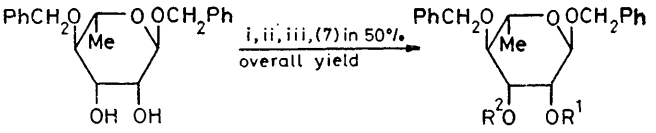


(1) $R^1=R^2=R^3=H$
(12) $R^1=R^2=CH_2Ph$, $R^3=Ac$



(3) $R^1=OCH_2Ph$, $R^2=CH_2Ph$, $R^3=Ac$
(4) $R^1=OH$, $R^2=R^3=H$
(5) $R^1=OAc$, $R^2=R^3=Ac$
(6) $R^1=Br$, $R^2=R^3=Ac$

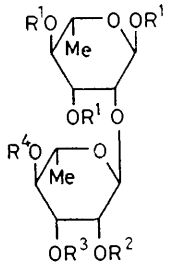
The synthesis involved the preparation of two appropriately functionalized disaccharides (**6**) and (**11**) which were linked together in an $Hg(CN)_2$ catalysed reaction. One of the essential starting compounds in this synthesis, benzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**2**) was recently prepared by us[10] in high yield from the readily available benzyl 4-*O*-benzyl-α-L-rhamnopyranoside. Compound (**2**) gave, with an excess of acetobromogalactose, compound (**3**) (66%, $[\alpha]_D$ −60°, *c* 0·6, $CHCl_3$) which on deprotection (Zemplén, then H_2/Pd–C) gave (**4**),[11]‡ $[\alpha]_D$ + 23° (*c* 1·6 in water). Hydrogenolysis of (**3**) followed by acetylation yielded an anomeric mixture of acetates from which pure (**5**) {m.p. 206—207 °C, $[\alpha]_D$ −31° (*c* 1, $CHCl_3$), δ: 6·02 (H-1, $J_{1,2}$ 2·2 Hz)} was obtained by crystallization from ethanol. Treatment of (**5**) with hydrogen bromide in acetic acid gave (**6**), δ 6·36 (H-1, $J_{1,2}$ 1·6 Hz). Compound (**7**) was prepared from benzyl 4-*O*-benzyl-α-L-rhamnopyranoside (see Scheme), in 50% overall yield, and was transformed to (**8**) with an excess of acetobromo-rhamnose.



(7) $R^1=H$, $R^2=CH_2Ph$
(2) $R^1=CH_2Ph$, $R^2=H$

SCHEME. i, Allyl bromide–Bu_4N^+; ii, $PhCH_2Cl$–KOH; iii, Pd/C–EtOH–AcOH–H_2O.

Removal of the protecting groups as for (**3**) yielded (**9**)‡ {$[\alpha]_D$ −24° (H_2O), lit.[12] $[\alpha]_D$ −28·7° (H_2O)}. Compound (**8**) gave (**10**) upon successive deacetylation (Zemplén), isopropylidenation, benzylation, and sulphuric acid removal of the isopropylidene group. It has now been shown that a carbohydrate diol having unsubstituted OH groups at C-2 and C-3 can be monobenzylated at OH-2 in a regioselective manner even if this diol is part of a disaccharide.



(8) $R^1=CH_2Ph$, $R^2=R^3=R^4=Ac$
(9) (α) $R^1=R^2=R^3=R^4=H$
(10) $R^1=R^4=CH_2Ph$, $R^2=R^3=H$
(11) $R^1=R^2=R^4=CH_2Ph$, $R^3=H$

Thus, benzylation of (**10**) using the tetrabutylammonium ion as catalyst mainly gave (**11**), $[\alpha]_D$ −23·5° (*c* 1, $CHCl_3$), with less than 20% (t.l.c. estimation) of the corresponding 3′,4′-di-*O*-benzyl ether being formed.

Reaction of (**6**) and (**11**) gave the protected tetrasaccharide (**12**), $[\alpha]_D$ −20° (*c* 0·8, $CHCl_3$), from which (**1**) was obtained {$[\alpha]_D$ −30° (*c* 0·5, H_2O)}, by deacetylation and hydrogenolysis.¶

† Numbering begins at the reducing end.

‡ A previous preparation (see ref. 11) of (**4**) *via* a non-obvious synthesis (see S. Turner, 'The Design of Organic Synthesis,' Elsevier, Oxford, 1976) gave a low yield and necessitated structural proof. An ambiguous synthesis of (**9**) was proposed recently (see ref. 12) starting from benzyl 4-*O*-benzyl-β-L-rhamnopyranoside, which is not readily available.

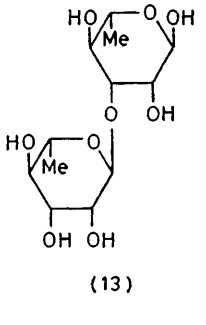
¶ Satisfactory elemental analyses were obtained for all new compounds.

TABLE ^{13}C N.m.r. spectral data (δ/p.p.m.) for compounds (**1**) and (**12**) and their component structures[a]

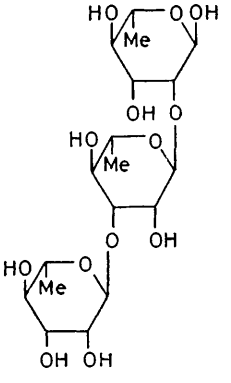
Carbon[b]	Compounds (**9**)[c]	(**13**)[c,d]	(**14**)[c]	(**4**)[c,e]	(**1**)[c]	(**12**)	(**5**)	(**11**)
C-1	93·4		93·4		93·4	93·3		98·2
	93·9[f]		93·9[f]			(168·7)[a]		
2	79·9		79·6		79·8	74·5		75·3
3	70·9		70·8		70·7	80·7		80·2
4	73·2		73·4		73·3	80·3		80·6
5	69·1		69·1		69·2	68·6		68·3
6	17·4		17·6		17·4	18·1		18·0
C-1′	102·8	94·6	102·4		102·6	98·8		98·6
	102·2[g]		101·9[g]			(170)[a]		
2′	70·9	71·1	70·0		70·7	78·2		78·5
3′	70·6	78·5	78·4		78·6	78·2		71·4
4′	72·8	72·4	72·2		72·1	80·8		82·3
5′	69·8	69·2	69·7		70·0	69·0		67·7
6′	17·6	17·5	17·6		17·5	18·1		18·0
C-1″		102·7	102·7	94·4	102·6	98·9	90·8	
				(169·5)[a]		(171)[a]		
2″		72·0	71·0	71·4	70·6	71·6	71·0	
3″		71·0	71·2	80·6	80·7	75·1	74·1	
4″		73·0	73·0	71·9	71·9	72·0	72·4	
5″		69·7	69·7	68·8	69·7	67·1	69·1	
6″		17·7	17·8	17·8	17·7	17·4	17·4	
C-1‴				105·0	105·0	101·3	101·5	
				(161·8)[a]		(160)[a]		
2‴				71·9	72·0	68·4	68·8	
3‴				73·4	73·4	71·0	70·6	
4‴				69·4	69·4	66·9	67·1	
5‴				75·7	75·8	71·0	71·0	
6‴				61·7	61·8	61·2	61·3	

[a] Spectra were measured with a Varian XL-100-FT spectrometer at 25·16 MHz. The spectra of the free oligosaccharides were recorded at 50 °C in D_2O using internal dioxan (δ = 67·3 p.p.m.) as reference, and the spectra of the protected ones were measured in $CDCl_3$, using Me_4Si (δ = 0 p.p.m.), at ambient temperature. The values in parentheses are the $^1J_{C-1,H-1}$ coupling constants in Hz. [b] Numbering begins at the reducing end. The numbering of compounds (**4**), (**5**), and (**13**) begins with C-1″, C-1″, and C-1′, respectively. [c] Chemical shifts are shown for the α-anomer at the reducing end only. [d] Our assignments agree with those published (ref. 14), except for C-2 and C-2′. [e] Our assignments agree with those published (see P. Colson and R. R. King, *Carbohydrate Res.*, 1976, **47**, 105). [f] C-1 of the β-anomer. [g] For the origin of this line see the text.

The ^{13}C n.m.r. spectrum of (**1**) is shown in the Table along with the spectra of its component structures (**4**),[11] (**9**),[12] (**13**),[2,4] and (**14**)[5] and the spectra of the protected derivatives (**5**), (**11**), and (**12**).



(13)



(14)

The assignation of the spectra of the free disaccharides (**4**), (**9**), and (**13**) is based upon the known spectra of their components,[13] and that of the non-reducing end unit is based upon the spectra of the corresponding benzyl glycosides, knowing that the benzyl group at the C-1-OH position causes a shift at C-1 similar to that of a sugar unit. The spectral lines of the reducing unit may be assigned by considering the glycosylation shift increments. The assignation of the spectrum of (**14**) follows from that of (**9**) and (**13**) whereas that of (**1**) is obtained from the spectra of (**4**) and (**14**). The assignment for (**5**) was corroborated by the published ^{13}C n.m.r. spectrum of 1,2,3,4-tetra-*O*-acetyl-α-L-rhamnopyranose,[14] that for (**11**) was assisted by the spectra of (**7**) and benzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside, and that for (**12**) follows from the spectra of (**5**) and (**11**).

In compounds (**1**), (**9**), and (**14**), the α- and β-anomers of the reducing unit are present in a ratio of *ca.* 9:1. This agrees with the published ratio for (**9**), measured by ^1H n.m.r. spectroscopy.[12] The presence of the β-anomer of the reducing end unit of (**9**) and (**14**) was detected by the C-1′ line of the second rhamnose unit which had a second, low intensity peak *ca.* 0·5 p.p.m. higher than its main resonance.

The full ^{13}C n.m.r. spectrum of the *Klebsiella* serotype K36 polysaccharide was published[9] without an indication of the frequencies of the resonance lines. The analysis of the ^{13}C n.m.r. spectrum of (**1**) as given above may be a useful contribution to the complete assignation of the resonance lines of this polysaccharide and other related ones.

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