ELSEVIER

Carbohydrate Research 256 (1994) 289–301

CARBOHYDRATE RESEARCH

# *Escherichia coli* K48 capsular polysaccharide: a glycan containing a novel diacetamido sugar †

Darryl V. Whittaker, Lesley A.S. Parolis, Haralambos Parolis *

*School of Pharmaceutical Sciences, Rhodes University, Grahamstown, 6140, South Africa*

(Received May 19th, 1993; accepted September 30th, 1993)

**Abstract**

The structure of the exocellular capsular polysaccharide expressed by *Escherichia coli* O8:K48:H9 has been investigated by hydrolysis and methylation analysis, and by NMR spectroscopic studies of the polysaccharide and of the oligosaccharides generated by solvolysis with anhydrous HF. The capsular polysaccharide was shown to have the repeating unit:

$$\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow$$
$$\begin{array}{c} 3 \\ \uparrow \\ 1 \\ \beta\text{-L-Sug}p \end{array}$$

where $\beta$-L-Sug$p$ represents 2,3-diacetamido-2,3,6-trideoxy-$\beta$-L-mannopyranose.

## 1. Introduction

Bacteria of the genus *Escherichia coli*, for which the structures of the capsular antigens of some 55 of the 74 encapsulated strains identified [1] are known, show a high degree of heterogeneity in the chemistry of their capsular (K) antigens. Several different amino sugars have been reported as constituents of these polysaccharides, of which 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and 2-acetamido-2-deoxy-D-galactopyranose (GalNAc) are the most common. *N*-Acetylneuraminic acid (Neu5Ac) is less frequently encountered whilst 2-acetamido-2-deoxy-L-fucopyranose has only been found in the K87 polysaccharide

* Corresponding author.

† Presented in part at the XVIth International Carbohydrate Symposium, Paris, France, 5–10 July 1992.


*SSDI* 0008-6215(93)E0382-B

[2]. 2-Acetamido-2-deoxy-D-mannopyranose has only recently been found to occur in a capsular polysaccharide in this genus [3], although the unusual aminouronic acid, 2-acetamido-2-deoxy-D-mannopyranuronic acid (ManNAcA), is the acidic component of the K7 antigen [4]. The capsular polysaccharide from *E. coli* K48, which is another example of a K-antigen that contains no simple hexose residues, contains the first diamino sugar to be encountered in a capsular polysaccharide in this genus.

## 2. Results and discussion

*Isolation and purification.*—The bacteria (*E. coli* O8:K48:H9, culture no. A290a) were grown on Mueller–Hinton agar and the acidic capsular polysaccharide (**PS**) was isolated by selective precipitation using cetyltrimethylammonium bromide in the conventional manner [5]. Further purification of **PS** was effected by dialysis and ion-exchange chromatography on DEAE-Sepharose Cl-6B. The purified material showed a broad distribution of molecular weights on Sephacryl S500 with an average $M_r$ of $5 \times 10^5$.

*Sugar and methylation analysis.*—The polymer was resistant to acid hydrolysis (4 M $CF_3CO_2H$) and the constituent sugars were not released in molar proportions. GlcA and GlcN were identified by analytical GLC–MS of the derived alditol acetates following methanolysis, carboxyl-reduction, and hydrolysis of a sample of **PS**. Analysis of the derived acetylated (−)-2-octyl glycosides [6] by GLC showed that GlcA and GlcN had the D configuration. Methylation of **PS** followed by GLC–MS analysis of the derived, methylated alditol acetates revealed the presence of 4-linked GlcA, 3-linked GlcN, and a very small amount of 3,4-linked GlcN, indicative of a branch point. No methylated alditol acetate of a terminal residue was detected.

*NMR spectroscopy.*—The $^1H$ and $^{13}C$ NMR spectra of the polysaccharide were consistent with a tetrasaccharide repeating unit, thus indicating the presence of a fourth sugar residue. The $^1H$ NMR spectrum of **PS** showed, *inter alia*, a doublet at $\delta$ 1.36 for a 6-deoxyhexosyl residue, three signals [$\delta$ 1.97 (3 H), 2.02 (3 H), 2.05 (6 H)] for methyl protons of four NAc groups, and, in the anomeric region, a signal with a small coupling constant at $\delta$ 5.07 and a complex of overlapping peaks integrating for four protons at $\delta$ 4.5–4.6.

The $^{13}C$ NMR spectrum of **PS** (Fig. 1) showed the presence of four anomeric carbons (99.83, 101.20, 101.95, and 103.46 ppm), five signals for carbonyl carbons (171.74, 174.87, 175.02, 175.27, and 175.61 ppm), four signals for methyl carbons (22.68, 22.94, 23.02, and 23.42 ppm), four signals attributable to C–N carbons of aminodeoxy sugars (52.19, 54.61, 54.89, and 55.83 ppm), and one signal for the methyl carbon of a 6-deoxyhexosyl sugar (18.00 ppm). These data indicated the presence of four acetamido functions in the repeating unit. This, in conjunction with the methylation data, indicated that the fourth residue was a diacetamidotrideoxyhexose.

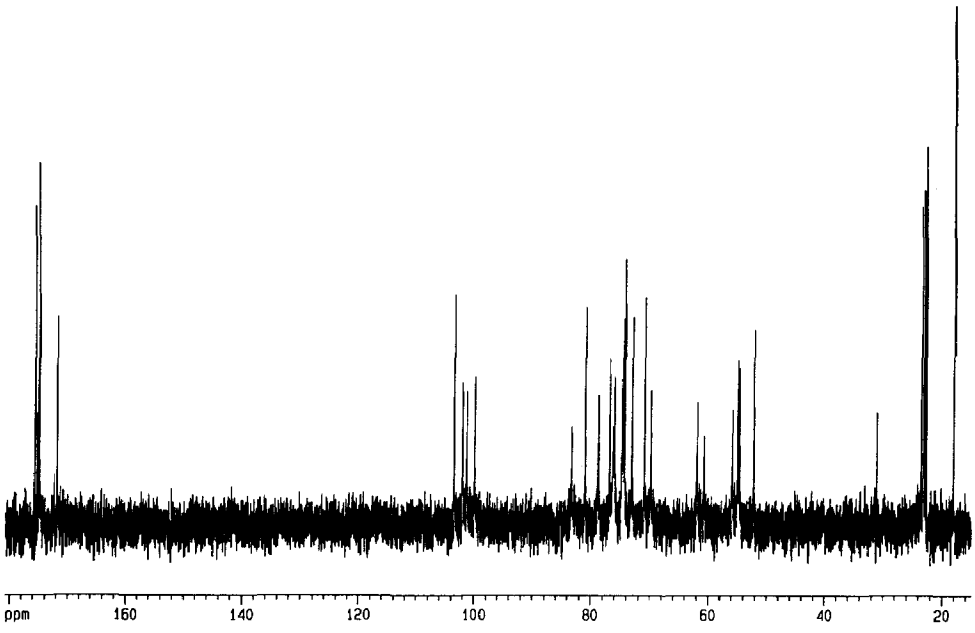



Fig. 1. $^{13}C$ NMR spectrum of **PS** at 328 K for the spectral region 18–170 ppm.

The chemical shifts for the $^{1}H$ and $^{13}C$ resonances of the residues in the repeating unit of **PS** were assigned from COSY [7], HOHAHA [8], HETCOR [9], and NOESY [10] experiments. The four residues in the repeating unit were labelled **a–d** in order of decreasing chemical shift of their H-1 resonances (Table 1). The $^{1}H$-chemical shifts for residues **a**, **b**, and **d** could readily be traced from the COSY (Fig. 2) and HOHAHA spectra of **PS**; however, for unit **c**, the connectivity pattern could not be traced with certainty beyond H-2 because of poorly defined

Table 1
NMR data [a] (400 MHz) for **PS**

| Residue | | 1 | 2 | 3 | 4 | 5 | 6a | 6b |
|---|---|---|---|---|---|---|---|---|
| **a** | H | 5.07 | 4.55 | 3.96 | 3.28 | 3.52 | 1.36 | |
| β-Sug*p* | $^{3}J$ [b] | 1.2 | 4.1 | 10.0 | 9.6 | 5.7 | | |
| | C | 99.83 | 52.19 | 54.61 | 70.87 | 74.46 | 18.00 | |
| **b** | H | 4.60 | 3.40 | 3.66 | 3.81 | 4.04 | | |
| → 4)-β-GlcA | C | 103.46 | 73.01 | 74.60 | 80.91 [c] | 74.25 | 171.74 | |
| **c** | H | 4.60 | 3.68 | 3.87 | 3.88 | 3.47 | 3.73 | 3.88 |
| → 3,4)-β-GlcNAc | C | 101.95 | 54.89 | 78.64 | 76.73 | 75.91 | 60.70 | |
| **d** | H | 4.52 | 3.88 | 3.78 | 3.59 | 3.42 | 3.85 | 3.91 |
| → 3)-β-GlcNAc | C | 101.20 | 55.83 | 83.28 | 69.76 | 76.73 | 61.88 | |

[a] Chemical shifts with acetone as internal reference, δ 2.23 and 31.07 ppm, respectively, for $^{1}H$ and $^{13}C$.
[b] $^{1}H$–$^{1}H$ coupling constants in Hz. [c] Linkage carbons underlined.

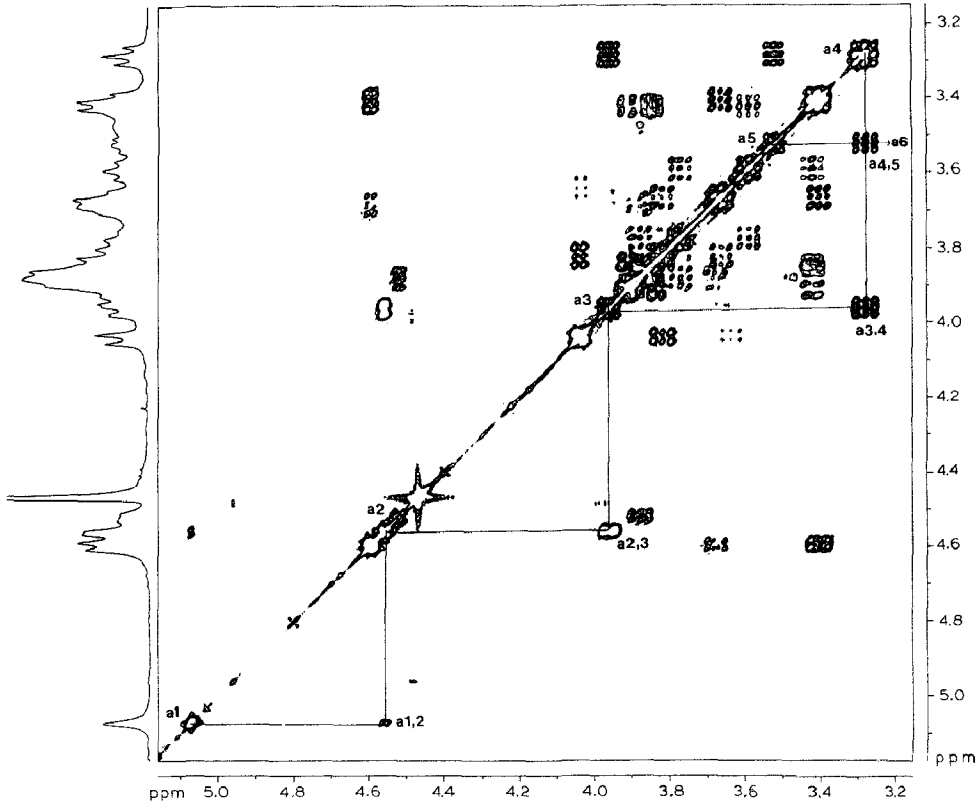



Fig. 2. COSY contour plot of **PS** for the spectral region $\delta$ 3.3–5.3. The $^{1}H$ resonances of the *J*-coupled spin systems for Sug are illustrated on the spectrum. **a**1 connotes H-1 of the residue, and **a**1,2 connotes the cross-peak between H-1 and H-2 of the residue, etc. The 1-D spectrum is displayed along the $f_1$-axis.

cross-peaks for this residue. These $^{1}H$ resonances were compared with $^{13}C$ chemical shift values obtained from a $^{1}H-^{13}C$ shift correlation (HETCOR) experiment (Fig. 3) and, once all known $^{1}H$ chemical shifts had been correlated with their respective $^{13}C$ chemical shifts, four unassigned sets of $^{1}H-^{13}C$ chemical shifts remained. The set of low intensity at $\delta$ 3.73,3.88/60.70 clearly belonged to H-6a,H-6b/C-6 for residue **c**; of the remaining three sets ($\delta$ 3.47/75.91, $\delta$ 3.87/78.64, and $\delta$ 3.88/76.73), the first two were assigned to H-5/C-5 and H-3/C-3 of **c** from observed NOE cross-peaks in the NOESY spectrum between H-1 and H-5 and between H-3 and H-5. The remaining set therefore belonged to H-4/C-4 of **c**. Comparison of the $^{1}H$ and $^{13}C$ NMR data for residues **b**, **c**, and **d** with literature values for methyl glycosides [11–13] permitted the residues in the repeating unit to be identified as indicated in Table 1, and their linkage positions were confirmed by the observed $^{13}C$ glycosylation shifts [14] for C-4 of **b**, C-3 and C-4 of **c**, and C-3 of **d**. The NMR data therefore accord with the presence of 3-linked $\beta$-GlcNAc, 3,4-linked $\beta$-GlcNAc, 4-linked $\beta$-GlcA, and a terminal 2,3-diacetamido-2,3,6-trideoxyhexose (Sug). The fact that unit **c** gave such poorly defined

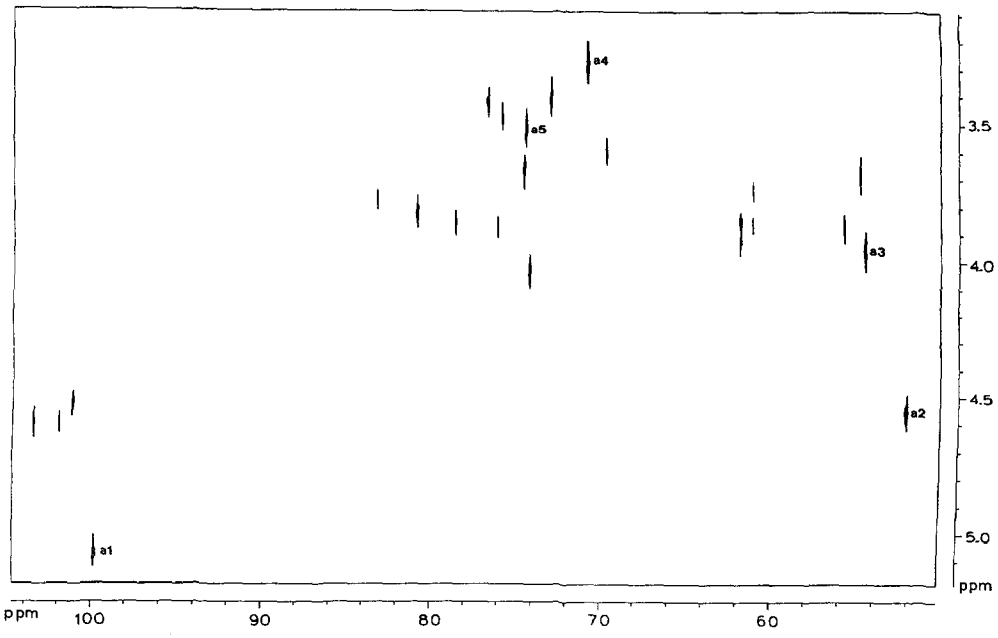



Fig. 3. $^{1}H-^{13}C$ shift-correlation map of the spectral region $f_2$ (120–10 ppm) and $f_1$ (3.1–5.2 ppm) for **PS**. Correlated cross-peaks assigned to Sug are illustrated on the spectrum. **a**1 represents H-1/C-1, etc.

cross-peaks could be ascribed to short $T_2$ values for this residue, resulting from reduced mobility at the branch point [15].

The $^{1}H-^{1}H$ coupling constants measured for Sug (residue **a**) indicated that it had the *manno* configuration (Tables 1 and 2). A coupled HMQC spectrum [16] of **PS** gave a $J_{C\text{-}1,H\text{-}1}$ value of 166 Hz for Sug, identical to the $J_{C\text{-}1,H\text{-}1}$ value recently reported for a terminal β-ManNAc [17]. A value of 175 Hz was recorded for methyl 2,3-diacetamido-2,3,6-trideoxy-α-D-mannopyranoside synthesised in our laboratory, suggesting a β-linkage for Sug. This was conclusively proved from the strong interresidue NOEs observed between H-1 and H-3, H-1 and H-5, and H-3 and H-5 in the NOESY spectrum of **PS**.

The sequence of the residues **a**–**d** in the repeating unit was established from the 2D-NOESY experiment and was confirmed by an HMBC [18] experiment. The relevant inter- and intra-residue NOE contacts are presented in Table 3. The observed interresidue NOE contacts between anomeric protons and the relevant protons of the adjacent glycosidically linked residues are consistent with the following structure for the repeating unit:

$$\begin{array}{ccc} \mathbf{d} & \mathbf{c} & \mathbf{b} \\ \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1 \rightarrow & & \\ & 3 & \\ & \uparrow & \\ & 1 & \\ & \beta\text{-Sug}p \quad \mathbf{a} & \end{array}$$

where Sug is 2,3-diacetamido-2,3,6-trideoxy-β-mannopyranose.

Table 2
$^1$H and $^{13}$C NMR data [a] for the oligosaccharides **TS** and **DS**

| $^1$H | | TS | DS | $^{13}$C | TS | DS |
|---|---|---|---|---|---|---|
| **a** | H-1 | 5.10/5.14 [b] (1.5) [c] | 4.97/4.99 (1.8) | C-1 | 99.81/99.91 | 100.42/100.55 |
| β-Sug*p* | H-2 | 4.60/4.62 (4.1) | 4.48/4.51 (4.1) | C-2 | 52.06/52.07 | 52.28/52.29 |
| | H-3 | 3.96/3.97 (10.5) | 3.96/3.98 (10.2) | C-3 | 54.68/54.71 | 54.38/54.42 |
| | H-4 | 3.29/3.31 (9.5) | 3.30/3.31 (9.4) | C-4 | 71.15/71.16 | 70.56/70.57 |
| | H-5 | 3.54/3.56 (6.2) | 3.49/3.52 (6.1) | C-5 | 74.50/74.53 | 74.53/74.64 |
| | H-6 | 1.36/1.38 | 1.35/1.38 | C-6 | 17.92/18.01 | 17.97/18.03 |
| **c** | H-1 | 5.23 (3.6) | 5.24 (3.4) | C-1 | 91.39 | 91.76 |
| α-GlcNAc | H-2 | 3.86 (9.3) | 3.85 (9.9) | C-2 | 53.26 | 53.41 |
| | H-3 | 4.02 (10.0) | 3.93 (9.7) | C-3 | 76.39 [d] | 79.96 |
| | H-4 | 3.86 | 3.55 (9.4) | C-4 | 76.45 | 70.29 |
| | H-5 | 3.86 | 3.87 (5.5)(1.8) | C-5 | 71.69 | 72.29 |
| | H-6a | 3.83 | 3.82 (12.0) | C-6 | 60.61 | 61.27 |
| | H-6b | 3.72 | 3.77 | | | |
| **c** | H-1 | 4.73 (8.5) | 4.74 (8.6) | C-1 | 95.50 | 95.59 |
| β-GlcNAc | H-2 | 3.64 (10.4) | 3.64 (9.9) | C-2 | 56.02 | 56.03 |
| | H-3 | 3.85 | 3.73 (9.7) | C-3 | 79.11 | 82.36 |
| | H-4 | 3.85 | 3.52 (9.1) | C-4 | 76.42 | 70.35 |
| | H-5 | 3.44 | 3.45 (5.8)(2.1) | C-5 | 76.03 | 76.52 |
| | H-6a | 3.87 | 3.90 (12.2) | C-6 | 60.70 | 61.45 |
| | H-6b | 3.68 | 3.74 | | | |
| **d** | H-1 | 4.47/4.49 (8.1) | | C-1 | 101.69 | |
| β-GlcNAc | H-2 | 3.77/3.78 (10.0) | | C-2 | 56.95/56.98 | |
| | H-3 | 3.54/3.55 (9.1) | | C-3 | 74.3 | |
| | H-4 | 3.48/3.49 (9.1) | | C-4 | 71.18 | |
| | H-5 | 3.40 | | C-5 | 77.16/77.17 | |
| | H-6a | 3.89 | | C-6 | 61.78 | |
| | H-6b | 3.91 | | | | |

[a] Chemical shifts with acetone as internal standard, δ 2.23 and 31.07 ppm, respectively, for $^1$H and $^{13}$C. [b] Signals doubled due to the reducing GlcNAc residue. [c] $^1$H–$^1$H coupling constants in Hz. [d] Linkage carbons underlined.

Long-range heteronuclear correlation data from the HMBC experiment accorded with this structure and in addition served to confirm the assignments for H-3/C-3 and H-4/C-4 of the disubstituted GlcNAc residue. Three-bond correlations were observed from H-1 of **b** to C-3 of **d** (83.28 ppm), H-1 of **c** to C-4 of **b** (80.91 ppm), H-1 of **a** to C-3 of **c** (78.64 ppm), and H-1 of **d** to C-4 of **c** (76.73 ppm).

Attempts to isolate Sug using established procedures involving hydrolysis, methanolysis, and acetolysis were unsuccessful. In order to generate smaller fragments for further study, a sample of **PS** was subjected to solvolysis with anhydrous HF. This method is particularly suitable for polysaccharides of this nature as it preserves *N*-acyl substituents and under suitable conditions can be highly selective. Amino sugars and uronic acid residues are known to show greater stability than hexoses and deoxyhexoses, and the presence of a second *N*-acyl

Table 3
Observed NOEs for **PS**

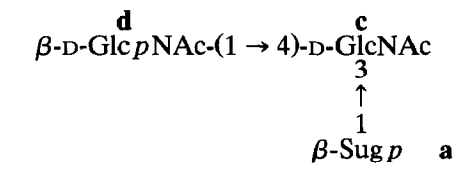
| Residue | Proton | | NOE at | |
|---|---|---|---|---|
| **a** | H-1 | 5.07 [b] | 4.55 (**a**, H-2); | 3.96 (**a**, H-3) |
| | | | 3.52 (**a**, H-5); | 3.87 [a] (**c**, H-3) |
| | H-2 | 4.55 | 3.96 (**a**, H-3) | |
| | H-3 | 3.96 | 3.52 (**a**, H-5) | |
| | H-6 | 1.36 | 3.28 (**a**, H-4) | |
| **b** | H-1 | 4.60 | 3.66 (**b**, H-3); | 4.04 (**b**, H-5) |
| | | | 3.78 (**d**, H-3) | |
| | H-3 | 3.65 | 4.04 (**b**, H-5) | |
| **c** | H-1 | 4.60 | 3.87 (**c**, H-3); | 3.47 (**c**, H-5) |
| | | | 3.81 (**b**, H-4) | |
| | H-3 | 3.87 | 3.47 (**c**, H-5) | |
| **d** | H-1 | 4.52 | 3.78 (**d**, H-3); | 3.42 (**d**, H-5) |
| | | | 3.88 (**c**, H-4) | |
| | H-3 | 3.78 | 3.42 (**d**, H-5) | |

[a] Interresidue (linkage) NOEs are underlined.
[b] Chemical shift in ppm.

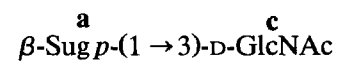
group is known to cause additional stabilisation of the glycosidic linkages of amino sugars [19]. Accordingly, **PS** was expected to be highly resistant. HF solvolysis of **PS** afforded two principal oligosaccharides (**TS** and **DS**) which were isolated by GPC. GlcNAc was the only monosaccharide isolated.

*NMR analysis of TS and DS.*—A detailed study of **TS** and **DS** using 1D $^{1}H$ and $^{13}C$ NMR spectroscopy (COSY, HMQC, HMQC-TOCSY [20], and HMBC) gave the data listed in Table 2. These data show that **TS** and **DS** have the following structures:

**TS**




**DS**




The chemical shifts for the $^{1}H$ resonances of residues **a** and **d** could be readily traced from the connectivities in a COSY spectrum of **TS**. The disubstituted reducing GlcNAc residue, unit **c**, however, could only be fully assigned from HMQC and HMQC-TOCSY spectra (Fig. 4) because of considerable signal overlap. The $^{1}H$ and $^{13}C$ resonances for the residues in **DS** were fully assigned from COSY and HMQC experiments.

Long-range three-bond $^{1}H-^{13}C$ correlations (Fig. 5) in the HMBC experiment performed on **TS** enabled it to be sequenced as indicated. Furthermore, it served

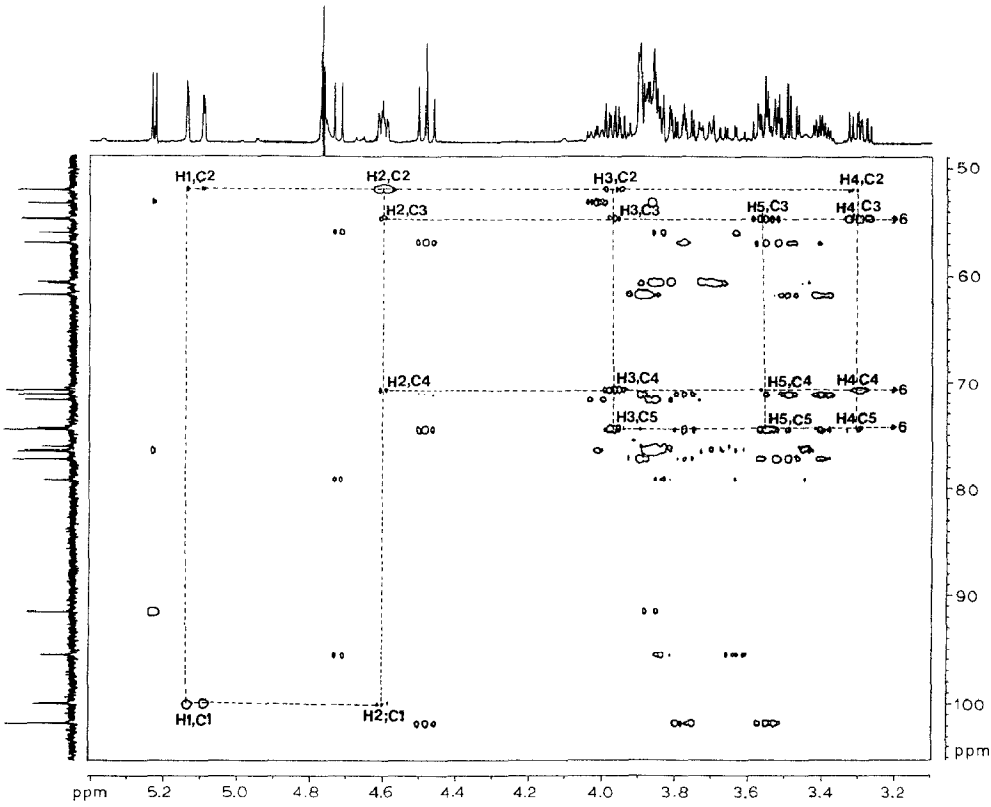



Fig. 4. HMQC-TOCSY $^{1}H-^{13}C$ contour plot of the spectral region $f_1$ 50–105 ppm ($^{13}C$) and $f_2$ 3.2–5.4 ppm ($^{1}H$) for the reducing trisaccharide, **TS**. Heteronuclear connectivities for Sug are connected by broken lines where H1,C2 represents a connectivity from H-1 of Sug to C-2, etc.

to confirm the acyl substitution pattern of Sug and the two GlcNAc residues, i.e., connectivities were observed between the ring protons linked to the acyl-substituted carbons and the carbonyl carbons of the acyl substituents.

*Absolute configuration of Sug.*— Due to the resistance of Sug to hydrolysis, its absolute configuration could not be determined using conventional methods. However, a tentative assignment was made on the basis of $^{13}C$ chemical shift differences, as described by Lipkind et al. [21], from the disaccharide $\beta$-Sug*p*-(1 → 3)-D-GlcNAc.

In a disaccharide, the formation of the glycosidic linkage between the two units leads to predictable changes in the $^{13}C$ NMR spectra of both the glycon and the "aglycon" (Sug and GlcNAc, respectively). These effects depend on the position and configuration of the glycosidic linkage, the general configuration of the aglycon, and the relative absolute configurations of both residues [21]. If the absolute stereochemistry of the aglycon is known, the absolute stereochemistry of the glycon can be deduced from the influence it has on the $^{13}C$ chemical shifts of the aglycon.

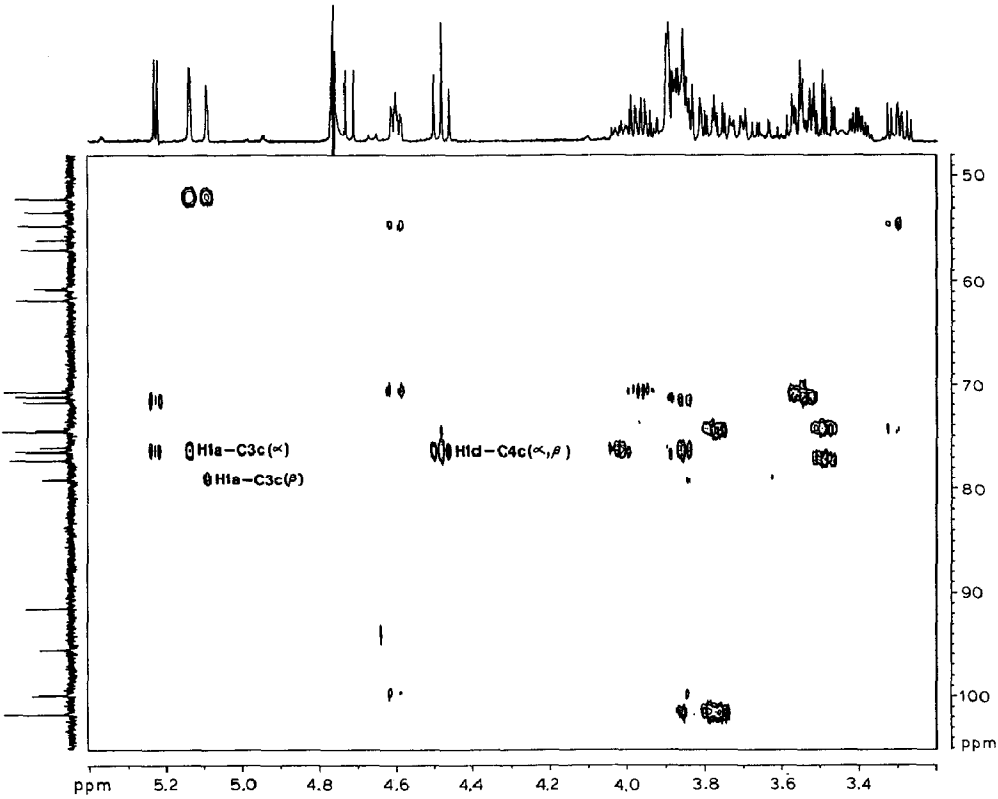



Fig. 5. HMBC spectrum of **TS** for the spectral region $f_1$ 48–105 ppm ($^{13}$C) and $f_2$ 3.2–5.4 ppm ($^{1}$H). Only the three-bond interresidue connectivities are indicated. H1**a**–C3**c**($\alpha$) connotes the through-bond connectivity from H-1 of residue **a** to C-3 of residue **c** when **c** exists as the $\alpha$ anomer, etc.

The largest differences in chemical shift are observed for those carbons involved in the glycosidic linkage (the $\alpha$-effect, with a large downfield shift) and the carbons immediately adjacent to the substituted carbon on the aglycon, which experience smaller upfield shifts (the $\beta$-effect) [22]. $^{13}$C Chemical shifts can also be affected by inductive effects and by spatial proton–proton interactions ($\gamma$ gauche effects) which polarize C–H bonds, resulting in unique changes in the shielding of the carbon nuclei involved [22]. The latter effect only occurs when the aglycon carries at least one equatorial proton at a $\beta$-carbon. In the present study, since the aglycon has the *gluco* configuration, any changes in the $^{13}$C chemical shifts at the $\beta$-carbons can be attributed to inductive effects which occur when one of these carbons is in close proximity to the ring oxygen of the glycon [22].

In disaccharides of this nature, it is known that the aglycon tends to orientate itself away from the side of the glycon which carries substituents, i.e., at C-2, C-3, and C-4, and towards the side of the ring where the ring oxygen is situated which is less sterically hindered [22]. Accordingly, the $\beta$-carbon, for which a relatively larger (in absolute value) negative $\beta$-effect of glycosylation is observed, should be closer

Table 4
$^{13}C$ Chemical shift glycosylation effects for **DS** at 303 K

| D-GlcNAc | | β-Sug*p*-(1 → 3)-D-GlcNAc | Chemical shift difference |
|---|---|---|---|
| α Anomer | | | |
| C-1 | 91.67 | 91.76 | 0.09 |
| C-2 | 54.91 | 53.41 | −1.50 |
| C-3 | 71.52 | 79.96 [a] | **8.44** |
| C-4 | 70.91 | 70.29 | −0.62 |
| C-5 | 72.40 | 72.29 | −0.11 |
| C-6 | 61.43 | 61.27 | −0.16 |
| β Anomer | | | |
| C-1 | 95.76 | 95.59 | −0.17 |
| C-2 | 57.55 | 56.03 | −1.52 |
| C-3 | 74.73 | 82.36 [a] | **7.63** |
| C-4 | 70.69 | 70.35 | −0.34 |
| C-5 | 76.78 | 76.52 | −0.26 |
| C-6 | 61.59 | 61.45 | −0.14 |

[a] Linkage carbons underlined.

to O-5 of the glycon ring. It is therefore possible to determine the absolute stereochemistry of the glycon in this class of disaccharides, if the absolute stereochemistry of the aglycon is known, by observing which β-carbon experiences greater deshielding.

A $^{13}C$ spectrum of GlcNAc was run under conditions identical to those for the spectrum acquired for β-Sug*p*-(1 → 3)-D-GlcNAc, and the chemical shift differences (Table 4) between the GlcNAc's were compared. The results show that C-2 of the reducing GlcNAc experiences greater deshielding than C-4. This deshielding is attributed to the closer proximity of C-2 to O-5 of the glycon than C-4. The minimum energy conformations established for the disaccharides β-L-Sug*p*-(1 → 3)-D-GlcNAc and β-D-Sug*p*-(1 → 3)-D-GlcNAc show that this is only possible when Sug has the L configuration. This evidence suggests that Sug has the L configuration.

## 3. Conclusion

The structure of the repeating-unit oligosaccharide of the capsular polysaccharide of *E. coli* K48 is as shown in the abstract. Although several diaminouronic acids, diaminodideoxy sugars, and diaminotrideoxy sugars have been encountered in Nature [23–26], this is the first report of the occurrence of 2,3-diacetamido-2,3,6-trideoxy-β-L-mannopyranose. Recently, Anderson et al. [27] reported the presence of 2-acetamido-3-formamido-2,3,6-trideoxy-D-mannopyranose in the O-antigen of *E. coli* O119.

## 4. Experimental

*General methods.*—Analytical GLC was performed with a Hewlett–Packard 5890A gas chromatograph, fitted with a flame-ionisation detector and a 3392A recording integrator, with helium as carrier gas. A J&W Scientific fused-silica DB-17 bonded-phase capillary column (30 m × 0.25 mm; having a film thickness of 0.25 $\mu$m), with a head pressure of 100 kPa, was used for separating alditol acetates and partially methylated alditol acetates. The temperature programme used was: 180°C for 2 min then 3°C $min^{-1}$ to 240°C. A Hewlett–Packard 5988A GLC–MS instrument, with the same column, was used to confirm the identities of derivatised sugars. Acetylated octyl glycosides were prepared according to the procedure of Leontein et al. [6] and were separated by GLC on the above column, using the same programme but with a column pressure of 140 kPa. **PS** (in the acid form) was methylated according to the Hakomori procedure as modified by Phillips and Fraser [28], and methanolyses were carried out with refluxing 3% methanolic HCl at 80°C for 16 h. Samples were carboxyl-reduced with $NaBH_4$ in anhyd MeOH and hydrolyses were performed with 4 M $CF_3CO_2H$ at 125°C for 1 h. The molecular weight determination of **PS** was performed on a dextran-calibrated column of Sephacryl S500 (70 × 1.6 cm), using 0.1 M NaOAc buffer (pH 5.00) as eluent. Material was detected by refractive index.

*Isolation and purification of the K48 polysaccharide.*—An authentic culture of *E. coli* O8 : K48 : H9 (A290a) was obtained from Dr. I. Ørskov (Copenhagen), and the bacteria were propagated on Mueller–Hinton agar at 37°C for 18 h. The harvested bacteria were suspended in aq 1% phenol and stirred at 4°C for 48 h after which the cells were removed by ultracentrifugation and the polysaccharide was isolated by precipitation of the supernatant solution into ETOH. Acidic polysaccharide was purified by selective precipitation using cetyltrimethylammonim bromide and then further purified by dialysis (12000 MW cut-off) and finally by ion-exchange chromatography on DEAE-Sepharose Cl-6B, using gradient elution with NaCl (0.1–0.5 M).

*Degradation of PS with anhydrous HF.*—**PS** (20 mg) was subjected to solvolysis with anhyd HF (introduced using the apparatus described by Sanger and Lamport [29]) and the sample was left to stir for 3 h at 25°C. Excess of HF was removed under a stream of $N_2$ after which 50% AcOH (2 mL) was added and the mixture stirred for 1 h in order to hydrolyse the resulting glycosyl fluorides. The AcOH was removed at 40°C under reduced pressure and the residue reconstituted in 0.5 mL of water. This was applied to a column of Bio-Gel P-2 (70 × 1.6 cm), using water as eluent, and the resulting fractions were isolated and freeze-dried.

*NMR spectroscopy.*—Samples were deuterium-exchanged several times by freeze-drying from $D_2O$, and then examined as solutions in 99.99% $D_2O$ (0.45 mL) containing a trace of acetone as internal standard ($\delta$ 2.23 for $^1H$ and 31.07 for $^{13}C$). Spectra were recorded on a Bruker AMX-400 spectrometer equipped with an X32 computer. Experiments on **PS** were carried out at 328 K and all experiments on **TS** and **DS** at 303 K. COSY-45 experiments were performed on **PS** and **TS** whilst a COSY-90 pulse sequence was used for **DS**. Data matrices were typically

512 × 2048 data points with 64 transients per $t_1$ delay and were zero-filled to 1024 data points in the $t_1$ dimension. Prior to transformation and symmetrisation, a non-shifted sine-bell window function was applied and the digital resolution of the resulting matrices was typically 3.5 Hz per point. The NOESY experiment was performed using a similar data set with a 0.3-s mixing delay, and a phase-shifted sine-squared window function was applied during transformation. For the HOHAHA spectrum of **PS**, a mixing time of 84 ms was used, and for **TS** 89 ms, with shifted sine-squared filtering in $t_1$ and $t_2$. The $^1H-^{13}C$ shift-correlated (HETCOR) experiment was recorded using a spectral width of 12.02 kHz in $t_2$ and 1.8 kHz in $t_1$, and processed with Gaussian functions. Inverse experiments: HMQC, 256 × 4096 data matrix with 72 scans per $t_1$ value, zero-filled to 1024 data points in $t_1$, 1-s recycle delay; HMQC-TOCSY, 512 × 4096 data matrix, 80 scans per $t_1$ value, mixing time 25 ms with a 1-s recycle delay; HMBC, 256 × 2048, 112 scans per $t_1$ value, $\Delta 1$ and $\Delta 2$ durations of 3 and 60 ms, respectively, 1-s recycle delay and a sine-squared filter.

## 5. Acknowledgments

The authors thank Dr. I. Ørskov (Copenhagen) for the test strain of *E. coli* K48, the Foundation for Research Development (Pretoria) for financial support (to H.P.) and a special merit doctoral bursary (to D.W.), and the Loewenstein Educational Trust for financial assistance (to D.W.). The authors are also grateful to Dr. J.-R. Brisson (National Research Council, Ottawa, Canada) for the conformational calculations.

## 6. References

[1] I. Ørskov, F. Ørskov, B. Jann, and K. Jann, *Bacteriol. Rev.*, 41 (1977) 667–710.
[2] L. Tarcsay, B. Jann, and K. Jann, *Eur. J. Biochem.*, 23 (1971) 505–514.
[3] D.V. Whittaker, L.A.S. Parolis, and H. Parolis, in preparation.
[4] F. Tsui, R. Boykins, and W. Egan, *Carbohydr. Res.*, 102 (1982) 263–271.
[5] K. Okutani and G.G.S. Dutton, *Carbohydr. Res.*, 88 (1980) 259–271.
[6] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
[7] W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
[8] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[9] A. Bax and G. Morris, *J. Magn. Reson.*, 42 (1981) 501–505.
[10] R. Baumann, G. Wider, R.R. Ernst, and K. Wuthrich, *J. Magn. Reson.*, 44 (1981) 402–406.
[11] K. Bock and H. Thøgerson, *Annu. Rep. NMR Spectrosc.*, 13 (1982) 1–57.
[12] B. Matsuhiro, A. Zanlungo, and G.G.S. Dutton, *Carbohydr. Res.*, 97 (1981) 11–18.
[13] K. Izumi, *Carbohydr. Res.*, 170 (1987) 19–25.
[14] J.H. Bradbury and G.A. Jenkins, *Carbohydr. Res.*, 126 (1984) 125–156.
[15] J. Dabrowski, *Methods Enzymol.*, 179 (1989) 122–156.
[16] A. Bax, R.H. Griffey, and B.L. Hawkins, *J. Magn. Reson.*, 55 (1983) 301–315.
[17] A. Helander and L. Kenne, *Carbohydr. Res.*, 221 (1991) 245–251.
[18] A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.

[19] Y.A. Knirel, E.V. Vinogradov, and A.J. Mort, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 167–202.
[20] L. Lerner and A. Bax, *J. Magn. Reson.*, 69 (1986) 365–380.
[21] G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 59–75.
[22] A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, and N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.
[23] Y.A. Knirel, E.V. Vinogradov, A.S. Shashkov, B.A. Dmitriev, and N.K. Kochetkov, *Carbohydr. Res.*, 104 (1982) c4–c7.
[24] J. Röppel, H. Mayer, and J. Weckesser, *Carbohydr. Res.*, 40 (1975) 31–40.
[25] T. Chowdhury, P.-E. Jansson, B. Lindberg, J. Lindberg, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, 215 (1991) 303–314.
[26] L. Kenne, B. Lindberg, K. Peterson, E. Katzenellenbogen, and E. Romanowska, *Carbohydr. Res.*, 78 (1980) 119–126.
[27] A.N. Anderson, J.C. Richards, and M.B. Perry, *Carbohydr. Res.*, 237 (1992) 249–262.
[28] L.R. Phillips and B.A. Fraser, *Carbohydr. Res.*, 90 (1981) 149–152.
[29] M.P. Sanger and D. Lamport, *Anal. Biochem.*, 128 (1983) 66–70.