Lipopolysaccharide Solution Conformation: Antigen Shape inferred from High Resolution ^1H and ^{13}C Nuclear Magnetic Resonance Spectroscopy and Hard-sphere Calculations

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High resolution ^1H and ^{13}C n.m.r. measurements have been used to assess the solution conformations of synthetic oligosaccharides which are related to a *Shigella flexneri* O-antigen. Unequivocal assignments of all ^1H and ^{13}C resonances have been made for the model oligosaccharides and the polysaccharide antigen. N.m.r. parameters that aid the conformational interpretations are: chemical shifts, coupling constants, relaxation measurements, and N.O.E. experiments. The conformation of the repeating unit of the O-antigen has also been estimated by hard-sphere calculations, which take into account the *exo*-anomeric effect. The experimental results are in close agreement with the calculated conformations and permit the extrapolation of the oligosaccharide conformation to the polysaccharide chains, which constitute the O-antigen. N.m.r. measurements of this polysaccharide isolated from bacteria support these conclusions. Ordered conformations, such as those discovered for the *Shigella flexneri* O-antigen, are important for antibody–polysaccharide interactions; these are discussed with particular reference to the recognition, by antibodies, of internal oligosaccharide sequences. Space-filling projections of these conformations are presented and used to facilitate the interpretation of serological data in molecular terms. Limitations of the hapten inhibition technique for mapping antibody combining sites are discussed in stereochemical terms. The possible role of sugar units as ' spacers ' between contact sugars, defining the polysaccharide binding surface, is illustrated and the manner in which linear sugar arrays may generate multiple binding surfaces is related to conformation.

THE work of Rees and his co-workers [1] has demonstrated the intimate relationship of polysaccharide shape and biological function. Hard-sphere [2-4] and recently more sohpisticated calculations [5] were found to predict ordered conformations for simple homoglucans [4] or periodic diheteroglycans,[3] such as agarose, hyaluronic acid, and chondroitin 6-sulphate. The biological properties of these important algal polymers, and the polysaccharides of connective tissue, have been rationalized [6,7] in terms of the ordered or random coil conformations predicted from computer model building studies. Little if any work has described solution conformations of more elaborate polysaccharides with complex periodic sequences such as bacterial O-antigens and capsular polysaccharides.[8]

Numerous fibre *X*-ray studies of acidic polysaccharides such as those of Klebsiella capsules have been made,[9] but the resolution afforded by this type of analysis necessitates assumptions of repeating unit geometry. Moreover, since such cell wall polysaccharides perform their function in an aqueous environment, an appreciation of the most likely solution conformation is a prerequisite of a complete understanding in molecular terms of recognition processes such as polysaccharide–antibody binding.

We report here studies designed to elucidate the solution conformation of a complex, linear, periodic polysaccharide, the *Shigella flexneri* sero-group Y,O-antigen. This polysaccharide (Y-PS) contains α-L-rhamnopyranose (α-L-Rham) and 2-acetamido-2-deoxy-β-D-glucopyranose (β-D-GlcNAc) residues in a basic structure [10] ⟨2)-α-L-Rham-(1→2)-α-L-Rham-(→3)-α-L-Rham-(1→3)-β-D-GlcNAc-(1⟩$_n$ (see also Figure 1), upon which α-D-glucopyranosyl and *O*-acetyl substituents elaborate increasingly complex polysaccharide chains, all of which define the different *S. flexneri* sero-groups.[10,11] Ten overlapping di-, tri-, and tetra-saccharide sequences of Y-PS have been chemically synthesized [12-16] and are used here as the basis for assessing the Y-PS conformation. The approach adopted has been to obtain high resolution ^1H and ^{13}C n.m.r. parameters for the synthetic oligosaccharides (1)—(10) and the Y-PS, O-antigen. These parameters exhibit strong conformational dependences and can be interpreted in terms of preferred oligosaccharide conformations. Comparison of these results with those predicted by hard-sphere calculations [17] provides strong evidence in favour of conformations dictated by the *exo*-anomeric effect [18] and steric factors. The conclusions reached for the oligosaccharide conformations are extended to that of the O-antigen in aqueous solution by comparing the n.m.r. parameters for the Y-PS with those of the model oligosaccharides. Finally the conformation extrapolated for the O-antigen is discussed both in terms of current knowledge of polysaccharide solution properties [19,20] and the size and shape of antigenic determinants.[8,21,22]

The hard-sphere *exo*-anomeric (HSEA) approach to model building developed by Lemieux and his co-workers has been successfully applied [17] to the determinants of the human ABO and Lewis blood groups. However, the work we present here is the first report of its extension to a polysaccharide. For linear, complex periodic, polysaccharides such as the Y-PS, conformational rigidity, similar to that discovered by Lemieux *et al.*[17] for highly branched aperiodic structures like the

blood group oligosaccharides, is not necessarily to be expected. High resolution n.m.r. experiments in conjunction with computer assisted model building based on HSEA calculations appear to offer a promising, and to date the first, detailed approach to the description of conformations of complex periodic polysaccharides in aqueous environments.

RESULTS

The chemical structure of the Y-PS repeating unit is depicted in Figure 1 whilst the structures for the model oligosaccharides (1)—(10) are set out in the caption of Figure 1 and in Tables 1 and 2. In addition the caption (Figure 1) establishes the designation of the four pyranose units as residues a—d. These are used to identify the units of the ball and stick projection (Figure 2). This figure represents a hypothetical pentasaccharide, containing all four glycosidic linkages of the chemical repeating unit. The conformation of each linkage is the minimum energy conformation computed by the HSEA method.[17]

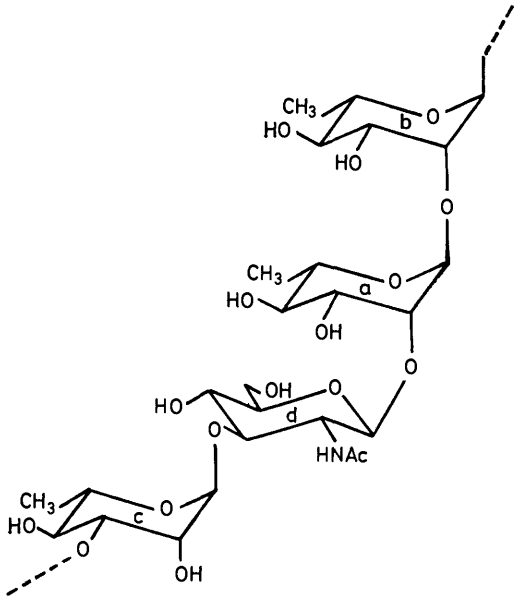

FIGURE 1 A chemical repeating unit of the *Shigella flexneri* sero-group Y,O-antigen. The α-L-rhamnopyranose units are shown with the designations a—c used in the text, Figure 2, and Tables 1—4 to identify the respective units. All rhamnopyranose residues are depicted in the $^1C_4$(L) chair conformation. The 2-acetamido-2-deoxy-β-D-glucopyranose residue, designated unit d, exists as the $^4C_1$(D) chair conformation. The structures of the model compounds (with R = $[C_2H_2]_8CO_2CH_3$]) which represent 10 of the 12 possible combinations of di-, tri-, and tetra-saccharide are as follows:

Structures		Sequence of pyranose units
α-L-Rham(1→2)-α-L-Rham(1→3)-α-L-Rham(1→3)-β-D-GlcNAc-OR	(1)	a b c
β-D-GlcNAc(1→2)-α-L-Rham(1→2)-α-L-Rham(1→3)-α-L-Rham-OR	(2)	d a b c
α-L-Rham(1→3)-α-L-Rham(1→3)-β-D-GlcNAc-OR	(3)	b c d
α-L-Rham(1→2)-α-L-Rham(1→3)-α-L-Rham-OR	(4)	a b c
β-D-GlcNAc(1→2)-α-L-Rham(1→2)-α-L-Rham-OR	(5)	d a b
α-L-Rham(1→3)-β-D-GlcNAc(1→2)-α-L-Rham-OR	(6)	c d a
α-L-Rham(1→3)-α-L-Rham-OR	(7)	b c
α-L-Rham(1→2)-α-L-Rham-OR	(8)	a b
β-GlcNAc(1→2)-α-L-Rham-OR	(9)	d a
α-L-Rham(1→3)-β-D-GlcNAc-OR	(10)	c d

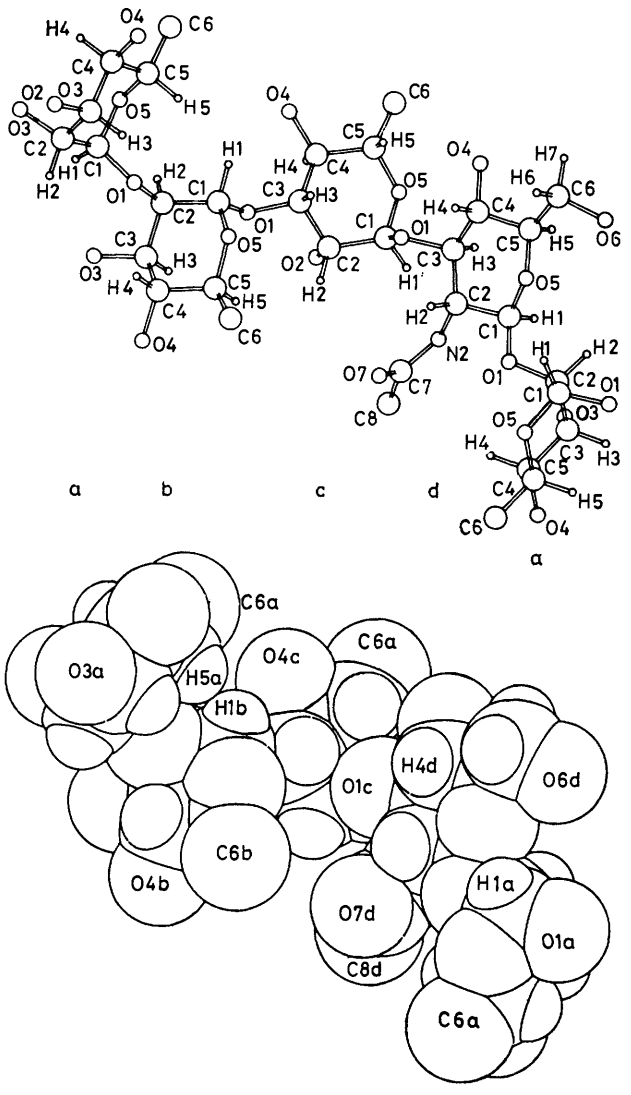

FIGURE 2 A hypothetical pentasaccharide sequence depicted as stick-and-ball, and space filling projections of the minimum energy conformation (Table 5). The illustrated projection is the unit sequence a b c d a, which corresponds to the chemical sequence α-L-Rham(1→2)-α-L-Rham(1→3)-α-L-Rham(1→3)-β-D-GlcNAc(1→2)-α-L-Rham. The identity of the model oligosaccharides (1)—(10) within this chemical unit may be found in Figure 1. Methyl groups of α-L-Rham and β-D-GlcNAc units are represented by single spheres devoid of hydrogen atoms. Similarly the hydrogen atoms of hydroxy-groups are omitted. Selected atoms are labelled to facilitate identification of the space filling projection

The n.m.r. parameters which were measured systematically are as follows: (i) ^{13}C chemical shifts, (ii) ^{13}C–^1H, one- and three-bond coupling constants, (iii) ^1H chemical shifts, (iv) ^1H–^1H coupling constants, (v) ^1H–^1H T_1 values and nuclear Overhauser enhancement (N.O.E.) measurements.

^{13}C *N.m.r. Data.*—The ^{13}C chemical shifts for oligosaccharides (1)—(10) together with the values for the polysaccharide Y-PS are shown in Table 1. The assignments are based on the following: (a) comparison with model com-

pounds, (b) selective proton irradiation, (c) ^{1}H-coupled ^{13}C spectra, (d) isotope shifts, and (e) ^{13}C T_1 values (for some compounds).

The ^{13}C chemical shifts of 2-*O*-(α-L-rhamnopyranosyl)-α-L-rhampyranose and 3-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranose [similar to compounds (7) and (8)] have been published recently.[23,24] These reported shifts differ from ours only in the relative assignment of C(2a) and C(3b) in the non-reducing sugar unit of compound (8) and in the assignment of resonances C(2b), C(3b), and C(3c) in compound (7) relative to the published assignments for the free sugar.[24] However, the assignments given here agree with those published [23] for the methyl glycoside of (7). Our assignments are based upon selective proton irradiation and on the coupled ^{13}C spectra which readily establishes the identity of the C(2) signals of rhamnopyranose units because C(2) has no significant long-range coupling constants and thus these signals appear almost as sharp doublets. The one-bond ^{1}H–^{13}C coupling constants (given in parentheses in Table 1) for anomeric carbon atoms are all in agreement with the proposed anomeric configurations. The three-bond coupling constants across the glycosidic bond (^{13}C–O–C–^{1}H) are also shown in Table 1 and will be discussed below.

H–D Isotope shifts [25] have been measured in some of the spectra but this technique was not a crucial tool in the present investigation.

Relaxation measurements have been used to identify which carbon signals belong to respective pyranose units (a—c) in compounds with three rhamnose units. This is based on the observation [26] that carbon atoms from terminal units (non-reducing end) have a faster motion (smaller correlation time) than units closer to the reducing end. In this case the reducing end is covalently bound to a non-polar group. Because terminal units have a smaller correlation time the atoms of this unit are expected to have longer T_1 values. Although this holds for compounds (1) and (4) it is not the case for (2) and is therefore not a general method for the assignment of carbon signals in oligosaccharides.

The carbon shifts do not exhibit unusual values except for the C(1) signals of α-L-Rham units. These signals are shielded by 1.2 p.p.m. in a units for compounds (2) and (5) when substituted at the 2-position by β-D-GlcNAc and by 1.5 p.p.m. in b units for compounds (1), (2), and (4) when linked at the 2-position with α-L-rhamnopyranosyl substituents; both values are comparable with the chemical shifts of a and b units when these are terminal. This shielding is not observed for the α-L-Rham c unit which is substituted at the 3-position [compare values for compounds (1) and (3) with values for compounds (6) and (10)]. We believe that this observation originates in the ^{13}C shielding effect, described by Lemieux and his co-workers.[17,18]

It should be noted that in order to achieve the consistency of ^{13}C chemical shifts shown in Table 1 it is important to record spectra at the same temperature.[27] Accordingly throughout this study spectra were recorded at 37 °C.

^{1}H N.m.r. Data.—The ^{1}H chemical shifts of the oligosaccharides (1)—(10) and the polysaccharide Y-PS are shown in Table 2. The assignments are based on the following: (a) comparison with model compounds, (b) ^{1}H–{^{1}H} double-resonance experiments, (c) partially relaxed spectra, eventually associated with double-resonance experiments,[28] and (d) selective, ^{13}C–{^{1}H} decoupling experiments, based on the above-mentioned assignment of the ^{13}C chemical shifts.

The ^{1}H n.m.r. parameters (δ and J values) have been published for the model monosaccharides methyl α-L-rhamnopyranoside [29] and 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[17] Spectra of the simple glycosides methyl α- and β-L-rhamnopyranosides and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside were recorded at 270 MHz in order to establish typical shifts and coupling constants. Values for the reducing disaccharide 3-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranose have also appeared in the literature recently [29] and they agree with our results for compound (7). The coupling constants observed for protons in compounds (1)—(10) and the polysaccharide Y-PS do not differ substantially (*i.e.* ±0.3 Hz) from the values published for the model monosaccharides and indicate that no severe distortion of the conformation of the pyranose rings in the component oligosaccharides has taken place. Double-resonance experiments which are facilitated by the dispersion achieved at high field strength served to confirm the assignment of proton resonances, which were also identified by characteristic ^{3}J ^{1}H–^{1}H coupling constants. Furthermore once the ^{13}C spectrum of a given compound was assigned, selective ^{13}C–{^{1}H} coupling could be used in a reverse sense to identify corresponding signals. Shifts assigned in this manner are expressed to three significant figures (Table 2). Residual coupling, measured when the decoupler is stepped through the appropriate ring-proton frequencies, can be used to calculate the chemical shifts of coupled protons.[30] Finally, partially relaxed spectra yielded in some instances simplified spectra of the crowded ring-proton region, since certain resonances relax at different rates. Once identified such assignments may be confirmed by simultaneous spin-decoupling experiments.[28]

An examination of the values given in Table 2 gives the following results. 5-H in α-L-Rham c units are deshielded by 0.25 p.p.m. when linked to the 3-position of β-D-GlcNAc d units. 2-H in α-L-Rham a and b units are deshielded by 0.15 p.p.m. when this unit is linked further to another α-L-Rham unit. This is not observed when the α-L-Rham unit is at the reducing end or linked to d units (β-D-GlcNAc). 1-H of α-L-Rham a and b units are deshielded 0.20 p.p.m. when linked further to α-L-Rham units b and c, respectively. This deshielding is increased by 0.20 p.p.m. when the units a and b are themselves glycosylated by β-D-GlcNAc or α-L-Rham units [compare the ^{1}H chemical shift of 1a-H of compound (2) and (5) with 1a-H of compound (1) and (4) and the shift of 1b-H of compound (1), (2), and (4) with 1b-H of compound (3) and (7)]. Rhamnose 3-H in a and b units are deshielded by 0.10 p.p.m. when these units are located between two other units. This effect is not observed for the α-L-Rham c unit when it is surrounded by two other pyranose units. 1-H of β-D-GlcNAc d units are deshielded 0.20 p.p.m. when this residue is linked to an α-L-Rham a unit.

The proton relaxation data for anomeric protons in oligosaccharides (1)—(10) and for the polysaccharide Y-PS are shown in Table 3. The values can only be compared qualitatively from compound to compound because the correlation times of the different oligosaccharides are not necessarily the same although they are measured at the same temperature (310 K) in water and at approximately the same concentration (0.1M). The results show that relaxation rates of rhamnose anomeric protons in a, b, and

Table 1

Carbon-13 chemical shifts for oligosaccharides (1)—(10) and polysaccharide Y-PS

	Unit a (1→2)-α-L-Rham-(1→2)						Unit b (1→2)-α-L-Rham-(1→3)						Unit c (1→3)-α-L-Rham-(1→3)						Unit d (1→3)-α-D-GlcNAc-(1→2)					
Compound	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
(1)	*103.02* (172:3.6)	*70.96*	*71.19*	*72.88*	*69.95*	*17.52* †	101.62 (170:3.6)	78.81	70.89	73.01	69.30	17.47 †	102.07 (169:3.6)	70.89	78.21	72.45	69.95	17.23	101.46 * (160)	56.13	82.47	69.76	76.80	61.71
(2)	101.83 (172:3.0)	79.65	70.66	73.14 †	70.03	17.54	101.55 (172:3.6)	79.07	70.66	73.03 †	69.54	17.54	100.42 * (170)	69.86	78.30	72.56	68.77	17.45	*103.45* (161)	*56.70*	*74.49*	*70.78*	*76.59*	*61.52*
(3)							*103.13* (170:3.6)	*71.01*	*71.01*	*72.88*	*69.45*	*17.56*	102.09 (169:3.6)	71.01	79.00	72.09	69.81	17.38	101.46 * (160)	56.05	82.59	69.81	76.77	61.74
(4)	*103.10* (170:3.6)	*70.92*	*71.03*	*72.85*	*69.96*	*17.55* †	101.59 (170:3.6)	79.18	70.86	73.08	69.58	17.50 †	100.57 * (169)	69.80	78.29	72.56	68.63	17.68 †						
(5)	*101.91* (172:3.5)	*79.41*	*70.66*	*73.10*	*69.97*	*17.59* †	99.37 * (170)	79.70	69.50	73.10	68.61	17.68 †							*103.46* (161)	*56.72*	*74.56*	*70.73*	*76.62*	*61.61*
(6)	99.55 * (171)	79.58	69.21	73.18	68.69	17.58							*102.06* (169:3.0)	*71.05*	*71.05*	*72.76*	*69.51*	*17.35*	102.75 (162)	56.46	82.03	69.76	76.70	61.55
(7)							*103.10* (170:3.0)	*71.09*	*71.14*	*72.96*	*69.56*	*17.74*	100.69 * (169)	69.71	78.85	72.31	68.50	17.74						
(8)	*103.17* (170:3.5)	*70.95*	*71.05*	*72.84*	*69.89*	*17.66*	99.34 *	79.52	69.52	73.09	68.83	17.66												
(9)	99.49 * (170)	79.80	69.50	73.22	68.72	17.52													*103.45* (160)	*56.70*	*74.51*	*70.64*	*76.62*	*61.53*
(10)													*102.18* (169:3.5)	*70.94*	*71.09*	*72.06*	*69.49*	*17.36*	101.35 * (160)	56.22	82.43	69.74	76.77	61.75
Y-PS	101.85 (172:3.6)	79.52	70.58	73.10	69.94	17.47 †	101.62 (171:3.6)	78.91	70.82	72.96	69.14	17.47 †	102.03 (169:3.5)	71.41	78.10	72.45	69.81	17.22	102.95 (162)	56.46	82.29	69.94	76.68	61.56

* Carbons attached to aglycone. Terminal units are in italics. † These assignments may be reversed between vertical columns of the pyranose units a—d.

Table 2

Proton chemical shift data for oligosaccharides (1)—(10) and polysaccharide Y-PS

	Unit a (1→2)-α-L-Rham-(1→2)						Unit b (1→2)-α-L-Rham-(1→3)						Unit c (1→3)-α-L-Rham-(1→3)						Unit d (1→3)-β-α-GlcNAc-(1→2)						
Compound	1-H	2-H	3-H	4-H	5-H	6-H	1-H	2-H	3-H	4-H	5-H	6-H	1-H	2-H	3-H	4-H	5-H	6-H	1-H	2-H	3-H	4-H	5-H	6-H	6'-H
(1)	*4.855*	*3.963*	*3.685*	*3.370*	*3.64*	*1.161*	5.048	3.941	3.811	3.341	3.604	1.206	4.726	3.733	3.678	3.437	3.911	1.137	4.407 *	3.66	3.500	3.407	3.359	3.811	3.629
(2)	5.053	4.027	3.753	3.242	3.62	1.161	5.067	3.953	3.83	3.353	3.62	1.182	4.653 *	3.871	3.690	3.445	3.62	1.140	*4.595*	*3.62*	*3.45*	*3.36*	*3.334*	*3.827*	*3.634*
(3)							*4.909*	*3.950*	*3.716*	*3.360*	*3.501*	*1.202*	4.659	3.775	3.716	3.383	3.920	1.140	4.412 *	3.701	3.501	3.438	3.360	3.820	3.646
(4)	4.853	3.967	3.690	3.267	3.59	1.157	5.074	3.949	3.832	3.375	3.59	1.190	4.643 *	3.862	3.690	3.451	3.59	1.190							
(5)	5.030	4.012	3.753	3.245	3.62	1.155	4.738 *	3.793	3.719	3.334	3.62	1.181							*4.590*	*3.642*	*3.618*	*3.345*	*3.319*	*3.790*	*3.616*
(6)	4.827 *	3.875	3.679	3.216	3.54	1.156							*4.767*	*3.712*	*3.660*	*3.660*	*3.864*	*1.132*	4.632	3.712	3.530	3.416	3.356	3.795	3.642
(7)							*4.925*	*3.966*	*3.727*	*3.347*	*3.59*	*1.180*	4.628 *	3.862	3.662	3.427	3.59	1.176							
(8)	*4.837*	*3.961*	*3.689*	*3.350*	*3.54*	*1.162*	4.735 *	3.778	3.705	3.346	3.54	1.182													
(9)	4.804 *	3.814	3.662	3.201	3.508	1.161													*4.558*	*3.586*	*3.404*	*3.334*	*3.301*	*3.753*	*3.619*
(10)													*4.742*	*3.689*	*3.594*	*3.320*	*3.865*	*1.131*	4.429 *	3.644	3.504	3.389	3.333	3.813	3.604
Y-PS	5.054	4.057	3.776	3.238	3.602	1.156	5.047	3.972	3.839	3.391	3.650	1.226	4.769	3.780	3.676	3.457	3.929	1.156	4.643	3.750	3.576	3.424	3.361	3.828	3.676

* Unit attached to aglycone. Terminal units are in italics. † Measured at 310 K in D_2O with acetone (1%) as internal reference (2.120 p.p.m.). ^3J Values in all α-L-Rham units and the β-D-GlcNAc unit were similar to the values in the reference monosaccharides.

TABLE 3

Relaxation rates (s^{-1}) for anomeric protons [a] and *c* values [b]

Compound	Unit a (1→2)-α-L-Rham(1→2)	Unit b (1→2)-α-L-Rham(1→3)	Unit c (1→3)-α-L-Rham(1→3)	Unit d (1→3)-β-D-GlcNAc*C* (1→2)
(1)	*1.28* (124)	2.17 (140)	1.72 (157)	2.22 *
(2)	1.80 (175)	2.17 (140)	1.30 *	*2.08* (138)
(3)		*1.25* (116)	1.54 (141)	189 *
(4)	*1.37* (133)	1.82 (117)	1.47 *	
(5)	1.82 (180)	1.92 *		*2.38* (150)
(6)	1.49 *		1.32 (121)	2.22 (147)
(7)		*1.30* (137)	1.37 *	
(8)	*1.33* (129)	1.96 *		
(9)	1.41 *			*1.96* (124)
(10)			*1.28* (117)	2.22 *
Y-PS	1.45 [c] (117)	1.45 [c] (117)	1.24 (113)	1.61 (107)

[a] Measured at 310 K as 0.1M solutions in D_2O using the inversion–recovery technique. [b] *C* Values calculated using the interproton distances of the minimum energy conformations (see text). [c] Overlapping resonances.

* Indicates unit at the reducing end. Non-reducing end, in italics.

c units are 1.38 ($\pm$0.11) s^{-1} when the unit is terminal either at the non-reducing or reducing end [except for 1b-H in compounds (3), (5), and (8)]. When the units are surrounded by two other units the relaxation rate is enhanced to 1.98 $\pm$ 0.18 s^{-1} for units a and b and to 1.63 $\pm$ 0.09 s^{-1} for unit c. For the d unit the rates are found at a value of 2.13 $\pm$ 0.25 s^{-1} independent of the arrangement in the oligosaccharide. The results from N.O.E. measurements performed using the difference technique [31] are shown in Table 4 and will be discussed below.

of that carbon atom. The values of the ϕ^H and ψ^H angles which correspond to the minimum energy conformation are shown in Table 5 together with some distances which are important to the following discussion. The convention used to define the glycosidic torsion angles ϕ^H and ψ^H is that adopted by Lemieux and Koto.[18] The calculations have been performed for all four disaccharides and for two different pentasaccharides with the calculations starting from both ends of these oligomers. The results in this linear structure are consistent within a ϕ^H/ψ^H variation of $\pm5°$

TABLE 4

Observed [a] and calculated [b] nuclear Overhauser enhancements for oligosaccharides (1)—(5) and polysaccharide Y-PS

Compound	Proton saturated	Observed [a] and relative NOE				Calculated [b] relative NOE			
(1)	1b-H	2b-H	3c-H		5a-H	2b-H	3c-H		5a-H
		13% (0.33)	13% (0.33)		13% (0.33)	0.32	0.35		0.32
	1a-H	2b-H + 2a-H				2b-H + 2a-H			
		25%				1.0			
(2)	1a-H + 1b-H	2a-H	2b-H	3c-H	5a-H	2a-H	2b-H	3c-H	5a-H
		4% (0.17)	8% (0.25)	6% (0.26)	5% (0.22)	0.26	0.41	0.18	0.16
(3)	1b-H	2b-H	3c-H			2b-H	3c-H		
		11% (0.48)	12% (0.52)			0.59	0.41		
	1c-H	2c-H	3d-H			2c-H	3d-H		
		7% (0.44)	9% (0.56)			0.51	0.49		
(4)	1a-H	2a-H + 2b-H				2a-H + 2b-H			
		24%				1.0			
	1b-H	2b-H	3c-H		5a-H	2b-H	3c-H		5a-H
		14% (0.26)	20% (0.38)		18% (0.35)	0.32	0.35		0.32
(5)	1a-H	2a-H	2b-H			2a-H	2b-H		
		8% (0.3)	19% (0.7)			0.53	0.47		
	1d-H	2a-H	3d-H		5d-H	2a-H	3d-H		5d-H
		17% (0.29)	19% (0.32)		23% (0.39)	0.32	0.38		0.30
Y-PS	1a-H + 1b-H	2a-H	2b-H	3c-H	5a-H	2a-H	2b-H	3c-H	5a-H
		−5% (0.14)	−15% (0.43)	−10% (0.29)	−5% (1.04)	0.26	0.41	0.18	0.07

[a] Performed in the difference mode.[31] Accuracy considered $\pm$20%. [b] Calculated from equation (2) using the interproton distances of the minimum energy conformations.

Hard-sphere Calculations.—The hard-sphere calculations which make allowance for energy contributions due to the *exo*-anomeric effect (HSEA calculations) have been performed as described previously [17,32] using neutron diffraction data for the co-ordinates of the α-L-Rham units [33] and *X*-ray data [34] for the co-ordinates of carbon and oxygen atoms of the β-D-GlcNAc unit. The hydrogen co-ordinates for this compound have been generated by a computer program,[32] which positions the hydrogens at a 1.10 Å distance from the carbon atom to which it is bound along a bond-vector defined by the other C–C and C–O bond vectors independent of the method used for the calculation of the minimum energy conformation of the molecule.

A hypothetical pentasaccharide is illustrated in Figure 2 by computer drawn ball and stick and space filling projections. These are constructed using the ϕ^H and ψ^H values given in Table 5 and the co-ordinates of the minimum energy molecule. Addition of successive tetrasaccharide repeating units generates co-ordinates for hypothetical octa- and dodeca-saccharides in the most ordered, minimum energy conformations. Space filling plots of these (Figures 3a and b) offer a better impression of the overall shape of the

TABLE 5

Hard-sphere *exo*-anomeric effect calculations

	Unit a (1→2)-α-L-Rham-(1→2)		Unit b (1→2)-α-L-Rham-(1→3)		Unit c (1→3)-α-L-Rham-(1→3)		Unit d (1→3)-β-D-GlcNAc-(1→2)	
ϕ^H, ψ^H values (°) for minimum energy conformation (±5°)	45, 15		50, 15		40, 15		50, 10	
Selected interatomic distances (in Å) for minimum energy conformation	1a-H-2b-H	2.35	1b-H-3c-H	2.42	1c-H-3d-H	2.29	1d-H-2a-H	2.36
	5a-H-1b-H	2.36	1b-H-5a-H	2.36	3c-H-5b-H	2.54	1d-H-3a-H	2.74
	1a-H-3b-O	2.63	5b-H-2c-H	2.31	5c-H-4d-O	2.64	3d-H-5c-O	2.70
	2a-H-5d-O	2.58	1b-H-4c-O	2.74	1c-H-2c-H	2.55	1d-H-3d-H	2.57
	1a-H-2a-H	2.55	1b-H-2b-H	2.55			1d-H-5d-H	2.47
			2b-H-5a-O	2.61				

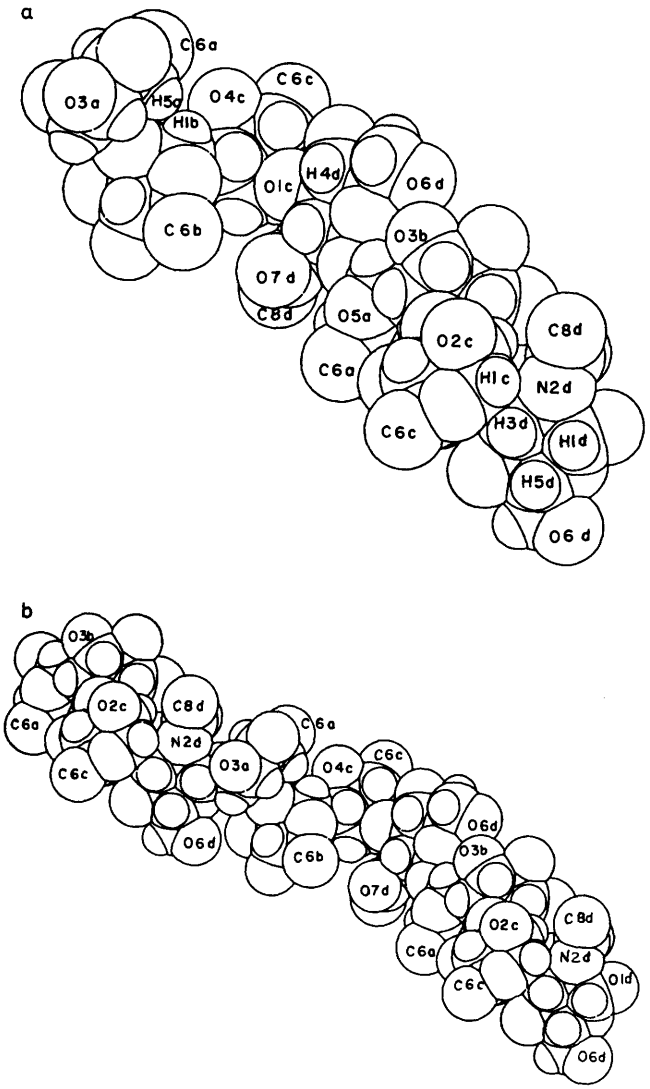

FIGURE 3 a, An octasaccharide sequence of Y-PS presented as a hard-sphere projection. Two repeating units are shown with the sequence of pyranose units $a_1b_1c_1d_1a_2b_2c_2d_2$. b, A dodecasaccharide sequence of Y-PS viewed from the opposite side to that of a. The sequence of the three repeating units is $a_1b_1c_1d_1a_2b_2c_2d_2a_3b_3c_3d_3$

surface presented by the polysaccharide, in its most ordered conformation.

DISCUSSION

The chemical syntheses[12-16] of the oligosaccharides (1)—(10) were carried out to provide haptens and artificial antigens, to probe the antibody binding sites specific for a linear unbranched and electrostatically neutral antigen, the sero-group Y,O-antigen of *S. flexneri*. Binding studies of the O-antigen to antibodies generated by synthetic antigens, and inhibition of the precipitation of Y-PS with its homologous anti-sera (using synthetic haptens), should aid the elucidation of the nature of binding sites with specificity for 'internal' oligosaccharide sequences of polysaccharide antigens. The manner in which internal sequences of a polysaccharide, devoid of branching or charged groups, bind in the antibody combining site is not well understood in molecular terms.[21,22,35,36] Monoclonal antibodies which bind the Y-PS have been produced in this laboratory[37] by the hybridoma technique,[38] and their availability in quantity will permit antibody interaction with chemically defined antigens and haptens to be studied by spectroscopic methods, including n.m.r. In order to systematically approach the molecular basis of this recognition, an appreciation of the solution conformation of the antigen, Y-PS, is essential.

High resolution Fourier transform ^1H and ^{13}C n.m.r. spectrometers provide the methodologies with which to explore the features of oligo-[17,39] and poly-saccharide conformation. In combination with computer-assisted model building this approach has led to a detailed appreciation of ABO and Lewis blood group geometries.[17,39] But unlike these highly branched, globular structures, linear oligosaccharides, such as structures (1)—(10), might be expected to exhibit far less rigidity. Although the energetically favoured conformations of the glycosidic linkages present in Y-PS are not so severely constrained as those of the terminal trisaccharides of the human blood group determinant, hard-sphere (HSEA)[17,32] calculations suggest that these minima will represent the polysaccharide solution conformation to a substantial degree. In this regard it must be understood that, although in the subsequent discussion glycoside linkages are expressed in terms of a

single preferred conformer, the intention is simply to indicate that conformer which may be the most highly populated within a range of conformers in the well of the potential energy surface. The n.m.r. parameters will reflect a weighted average of these conformers. The conformer predicted by calculation is expected to coincide with this time-averaged conformer.

The work of Lemieux *et al.*[18] which attributes preferred conformations of glycosidic linkages to the *exo*-anomeric effect and minimised non-bonding interactions may be summarised as follows. The *exo*-anomeric effect directs the anomeric torsional angle, ϕ *ca.* |55—50°|, and the torsional angle, ψ *ca.* |0—15°|, in the absence of significant attractive or repulsive interactions. In these energetically favoured conformations the protons of anomeric and algyconic carbon atoms will be held in virtual van der Waals contact. The hard-sphere *exo*-anomeric (HSEA) method has been developed in order to predict conformational preference about glycosidic linkages for oligosaccharides in aqueous solution. Although not rigorous when compared to force field[40] or *ab initio* molecular orbital calculations,[41] this approach is conceptually simple and to date has provided excellent agreement between observed and anticipated conformations.[17,32,39] The concept of these calculations is as follows: a glycosidic linkage is constructed between two pyranose residues, the atomic co-ordinates of which are obtained from published *X*-ray data, and non-bonded interactions are calculated for pairs of ϕ, ψ values in a range about the anticipated energy well (or through 360° but this is generally unnecessary). The energetically favoured conformation is obtained in terms of a set of atomic co-ordinates and the ϕ, ψ values. Additional sugars may be added to the resultant disaccharide and the construction of tri- through to oligo-saccharides proceeds in stepwise fashion. At each step a distance matrix correlating all inter-atomic distances is generated for the minimum energy conformer. A distance matrix may also be obtained for any pair of ϕ, ψ values. Such matrices are of particular value in determining proton–proton and oxygen–proton distances. The former may be directly measured through T_1 [41-44] or N.O.E. experiments [17,39,44] and the latter inferred from specific shielding effects. In this way HSEA calculations provide a semiquantitative rationale for the interpretation of certain n.m.r. parameters.

Assignment of ^{13}C and ^{1}H resonances (Tables 1 and 2) for compounds (1)—(10) was essential prior to assignment of the polysaccharide spectra. Analyses of disaccharides (7)—(10) provide ^{13}C shifts typical of both terminal units (' reducing ' and ' non-reducing '). The identification of internal units of the trisaccharides (3)—(6) was then possible, and since the four overlapping trisaccharide sequences were available, assignment of the two tetrasaccharide compounds (1) and (2) was greatly simplified. These assignments may be seen to be internally consistent to within 0.2 p.p.m. for each pyranose unit when it occupies similar terminal or internal positions within a sequence. Despite this consistency these assignments were corroborated by coupled and selectively decoupled spectra. If unit a of compound (2), units b and c of compound (1), and unit d of compound (6) are then used as comparative models for assignment of the ^{13}C spectrum of Y-PS, very small shift differences are observed between the units of the polymer and those of the oligomers. In fact 23 resonances correlate to within 0.3 p.p.m. whilst the remaining signal differs by 0.5 p.p.m. (Table 1). The close correlation of shifts for overlapping sequences (1)—(6) and the polymer Y-PS is suggestive of similar conformations for both model compounds and the polysaccharide. This is substantiated by coupling constants, proton chemical shift differences, T_1, and N.O.E. values.

Proton chemical shift changes may be checked against the HSEA method and thereby provide a test of the inter-residue geometry. Protons close to oxygen atoms (<2.8 Å) are expected to be deshielded, the extent of which is related to the proton–oxygen distance and to the number of proximal oxygen atoms.[17] For the energetically favoured conformation presented here (Figure 2 and Table 5), observed deshieldings correlate with the distance matrix of the pentasaccharide (Figure 2). For example, it is seen that 5c-H is deshielded by 0.25 p.p.m. when a c unit is linked to a d unit and in agreement with this HSEA calculations indicate a 5c-H to 4d-O distance of 2.64 Å (Table 5). All deshielding of proton chemical shifts, discussed in the Results section, can be explained in these terms, except for 3c-H which should be deshielded due to a 3c-H to 5b-O distance of 2.54 Å. We have no explanation for the absence of this anticipated deshielding, except that the chemical shift of 3-H in rhamnose rings may be sensitive to small changes in the pyranose ring conformation. This is indicated by the small downfield shift of 3-H in methyl α-L-rhamnopyranoside (0.12 p.p.m.) compared to the value of 3-H in methyl β-L-rhamnopyranoside (see Experimental section), whereas 5-H is deshielded (0.27 p.p.m.) as expected in the α-compound relative to the β-compound.

Further evidence for preferred conformations in the oligosaccharides (1)—(10) and for the preponderance of similar features in the polymer chain derive from heteronuclear ^{3}J coupling constants across the glycosidic linkage,[45] which determine approximate ψ^{H} values. In principle both ϕ^{H} and ψ^{H} may be correlated with heteronuclear coupling constants, but for practical reasons only the anomeric carbon to aglyconic proton ^{3}J values are readily measured. These values are related to ψ^{H} in a Karplus-type curve.[45] The small 15—10° ψ^{H} angles (Table 5) predicted by HSEA calculations are in good agreement with the observed ^{3}J values across the three α-L-Rham linkages (Table 1). This is true for Y-PS as well as oligosaccharides (1)—(10). It is not possible to determine this ^{3}J value for β-D-GlcNAc (d units) accurately due to additional coupling between C(1) and 2- and 5-H of the same sugar unit.

As mentioned earlier the virtual eclipsing of the anomeric carbon to inter-residue oxygen bond with that of the aglyconic carbon–proton bond (ψ^{H} *ca.* 0°) and the preference for ϕ^{H} *ca.* 55—50°, results in virtual van der

Waal contact of the anomeric and aglyconic protons.[18] That this situation holds is readily demonstrated by both proton T_1 measurements and difference N.O.E. spectra.[17] The latter technique often provides unequivocal confirmation of the spatial geometry of the glycosidic bond by portraying only those proton multiplets receiving N.O.E. from selective saturation of anomeric resonances as shown in Figure 4.

Both the proton relaxation [42,43] (Table 3) and N.O.E.[38,46] measurements (Table 4) show that each anomeric proton receives a relaxation contribution from the proton attached to the aglyconic carbon atom, in addition to contributions from those protons of the glycosidic ring to which the anomeric proton belongs.[17]

The proton T_1 values can be related to the geometry of the molecule through expression (1) assuming intramolecular dipole–dipole relaxation only. r_{ij} Is the distance between nuclei i and j, and τ_c is the motional correlation time. If the molecule is tumbling isotropically in solution it is expected that $R_1 \div \sum_{j \neq i} r_{ij}^{-6} = C$ where C = constant $\times$ τ_c.

$$1/T_1 = R_1 = \text{constant} \times \tau_c \sum_{j \neq i} r_{ij}^{-6} \qquad (1)$$

C Values are calculated using the interproton distances of the minimum energy conformation and are listed in parentheses in Table 3. These data show that experimentally determined R_1 values are in good accord with the molecular model. Similarly assuming dipole–dipole intramolecular relaxation and isotropic motion the N.O.E. effect observed for proton d when proton s is saturated is well approximated for the purpose of estimating relative N.O.E.s by expression (2) [46] where r_{ds} is the distance between protons d and s and r_{dj} is the distance between

$$\text{N.O.E. of } d(s) = r_{ds}^{-6} \div 2 \sum_{d \neq j} r_{dj}^{-6} \qquad (2)$$

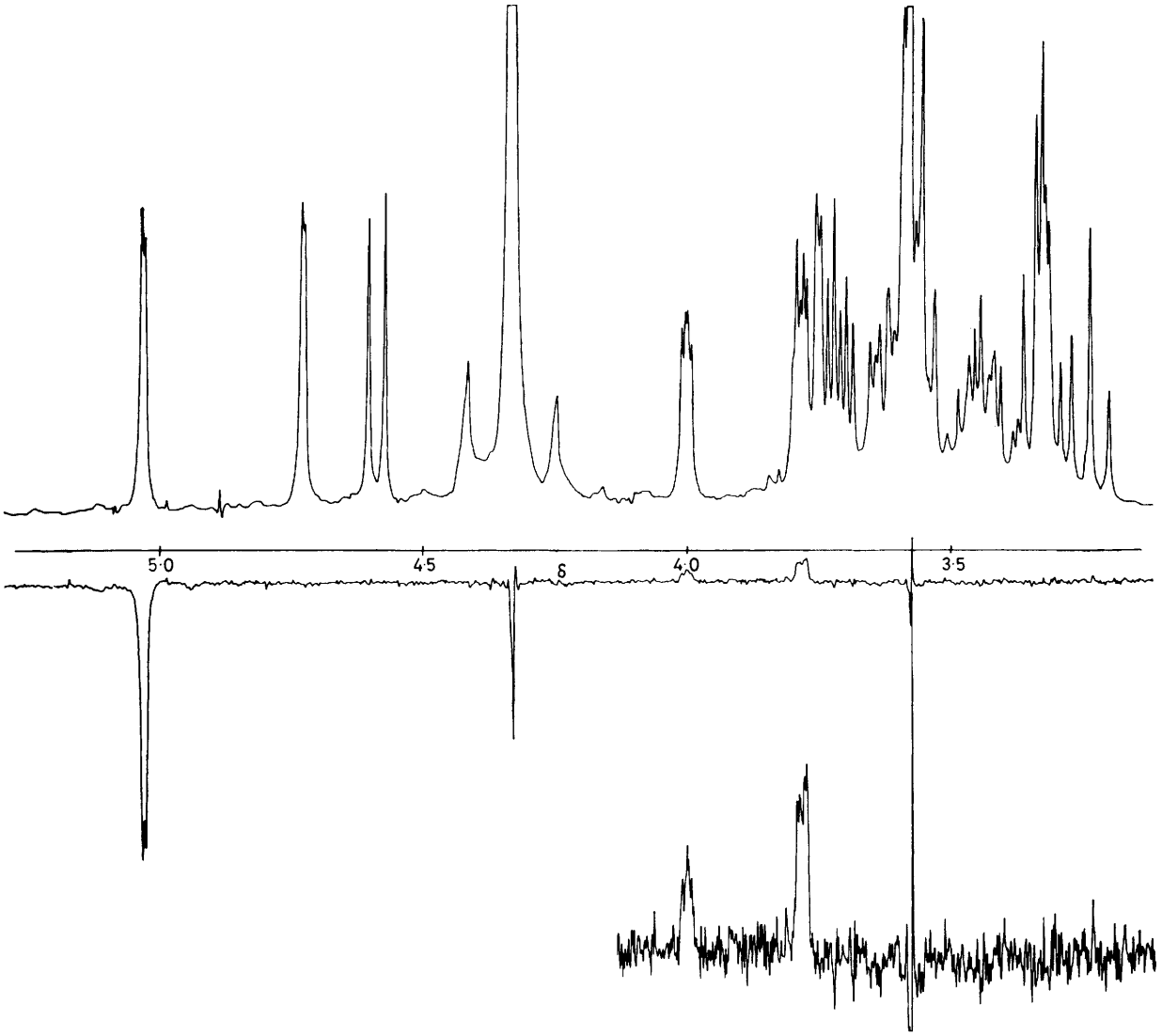

FIGURE 4 The ^{1}H n.m.r. spectrum of trisaccharide (5) and, below, the difference N.O.E. spectrum obtained by saturation of the 1-H resonance of the α-L-Rham a unit. The 2a-H resonance at δ 4.02 and the 2b-H resonance at δ 3.793 are the only protons sufficiently close (Table 5) to 1a-H to receive a significant N.O.E.

proton *d* and all other protons in the molecule. The calculated relative N.O.E.s using the interproton distances of the minimum energy molecule are given in Table 4 and the comparison with experimental values shows excellent agreement. The absolute value of an N.O.E. is not in itself important especially since for the polysaccharide, which has a longer correlation time than the oligosaccharides, N.O.E.s can be negative (Table 4) and field dependent [47,48] as illustrated in Figure 5.

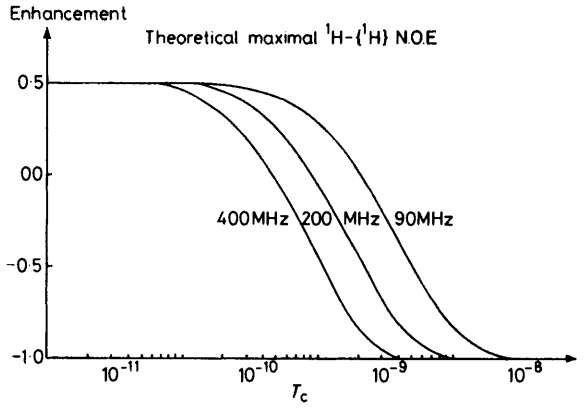

FIGURE 5 The plots show the field dependence of ^{1}H-{^{1}H} N.O.E. values calculated using an equation from the literature [47]

The anomeric protons of β-D-GlcNAc d units relax faster due to two important relaxation contributions (from 3- and 5-H) within the same ring. But a relaxation contribution from 2-H of the rhamnose unit a is observed in agreement with the HSEA calculation, which indicates a distance between 1d- and 2a-H of 2.36 Å. Examination of the data in Table 3 shows that 1b-H relaxes faster when it bears the a unit in the 2-position. N.O.E. measurements [compounds (1), (3), and (4), Table 4] show that this contribution originates from 5a-H, and in agreement with this the HSEA calculations give a distance between 5a- and 1b-H of 2.36 Å. Examination of the minimum energy conformation shows a significant proton–proton distance between 5b- and 2c-H of 2.31 Å. It is not possible to perform an N.O.E. experiment to confirm this (due to off-resonance saturation), because the chemical shift difference between the protons is only 0.15 p.p.m. Furthermore, the relaxation time of the two protons cannot be determined accurately due to other partially overlapping signals. However, it is clear from the relative behaviour of 2c-H in compounds with both the b and c units that 2c-H relaxes much faster than it does in compounds lacking the b unit. Thus N.O.E. and T_1 parameters are shown to provide further experimental evidence for the energetically favoured conformers of each of the four glycosidic linkages in accordance with HSEA calculations.

The correlation of ^{13}C and ^{1}H n.m.r. data between model compounds (1)—(10) and Y-PS provides evidence for the existence of similar conformations in these molecules. Specific shielding effects and T_1 and N.O.E. values in proton spectra relate inter-residue conformations for overlapping oligomer sequences (1)—(6) and these are also observed in the polymer Y-PS. The anomeric ^{13}C shifts are known to be particularly sensitive to changes associated with the conformational requirements of the glycosidic linkage,[18] and the correlation of shift data between the model compounds and Y-PS suggests conformational similarities. These are further supported by heteronuclear ^3J coupling constants across the three α-L-Rham glycosidic linkages (Table 1). Because a single interatomic distance across the glycosidic bridge cannot unambiguously define disaccharide conformation the HSEA distance matrices are crucial in conjunction with n.m.r. for effective conformational assessments. We have used at least four inter-residue distances, proton–proton and oxygen–hydrogen, to confirm the anticipated conformations of each glycosidic linkage present in the oligosaccharides and polysaccharide. In every case these distances were corroborated by proton chemical shift changes and T_1 and N.O.E. values. Although each inter-residue conformation is highly constrained the time averaging inherent to n.m.r. methods together with the approximations of the HSEA method [17,32] require ϕ^H, ψ^H amplitudes of at least $\pm 10°$ (Table 5). However, significant non-bonded interactions are encountered when ϕ^H, ψ^H fluctuations beyond $\pm 15°$ are envisaged. Some of these effects have their origin in compression of certain of the selected interatomic distances cited in Table 5. Other distances are lengthened and it is concluded that the most highly populated conformations of the oligosaccharides (1)—(10), and also the polysaccharide, are those lying in a narrow range about the energetically favoured values (Table 5 and Figure 2).

The early work of Rees and his co-workers (reviewed in ref. 1) on polysaccharide geometry indicates that all polysaccharides, irrespective of composition or linkage, are capable of adopting ordered conformations which of necessity possess helical character. Such ordered conformations would place sugar units and functional groups at regular and predictable spacial dispositions. In contrast to this, statistical mechanical treatments [19] of the solution conformations of amylose [49] and cellulose [50] lead to the conclusion that amylose is wholely disorganised in solution. Despite the earlier conclusions regarding the predominance of the random coil in solution a significant refinement of these conclusions has recently appeared. Monte Carlo calculations were used to generate representative amylose chain conformations,[20] clearly demonstrating that random coil conformations may include significant portions of chain sequences possessing helical character, as was originally proposed by Rao and his co-workers.[51] Indeed the successful correlation of the ' linkage rotation ' parameter for dimer and homopolymer conformations [4] may be considered as supporting evidence for polysaccharide chains, which retain the majority of glycosidic linkages in conformations tending toward the minimum energy conformation.

O-Antigens have molecular weights [52,53] and chain lengths considerably smaller than the high molecular

weight polysaccharides just mentioned, but the conclusions we have reached concerning the likely conformation of O-antigenic chains is in broad agreement with many studies of these larger polymers. Our interpretation of the solution conformation of Y-PS as judged by n.m.r. measurements is consistent with a somewhat restricted random coil concept of polysaccharide chains. Although we anticipate the major proportion of glycosidic linkages to lie within ± 10—$15°$ of the calculated ϕ^H, ψ^H values (Table 5), the amplitude about each torsional angle should permit sufficient flexibility of the polymer chain to qualify as a random coil in the context of Brant's [20] recent refinements of this rather imprecise generalisation. The essence of this model is that despite continuously fluctuating ϕ^H, ψ^H values within the allowed range, a ' snapshot ' at any instant would reveal polysaccharide chains with the largest proportion of linear sequences adopting conformations close to those depicted in Figure 2. The extension of the geometry of Figure 2 to octa- and dodeca-saccharides (Figure 3a and 3b) demonstrates the disposition of topographical features about the polymer axis and is not intended to convey the impression that these are realistic models of chains. The stereochemical consequence of the ordered arrangements (Figure 3a) is that the second repeating unit, $a_2b_2c_2d_2$, is rotated by *ca.* 180° with respect to the sequence $a_1b_1c_1d_1$. That is, the second repeating unit shows the opposite surface to that presented by the first repeating sequence. The dodecasaccharide (Figure 3b) illustrates that the third repeating sequence in the most ordered conformation duplicates the spacial orientation of the first sequence. Thus the octasaccharide could be termed the ' stereochemical ' repeating unit of Y-PS. Although on average the polysaccharide will not be so highly ordered over many repeating units it is evident that identical linear arrays will be disposed at many surfaces of the polysaccharide and not only along one edge of the chain. These and other features of the repeating unit geometry will have particular immunochemical consequences for this polysaccharide antigen.

If it is possible to generate reliable models of polysaccharide conformations as our results suggest it should be possible to apply this knowledge to the existing framework of antibody binding site data. It is known from immunochemical [21,35,54] and *X*-ray [55,56] studies that the dimensions of antibody surface complementary to antigen fall in the range 25—36 × 10—17 Å × 6—12 Å deep. Within such extremes the binding site may approximate a shallow groove [55] or deep cleft.[56] Polysaccharides, particularly their internal sequences, are thought to bind to the former type of site,[35,57] which requires binding along a limited, rather than an extensive all-embracing surface. It seems reasonable to assume that the thermodynamics of antigen–antibody binding will be most favourable for antigen conformations not far removed from the energetically preferred conformers [58] (Figure 5 and Table 5). Using Figures 2, 3a, and 3b as models one may attempt deductions of the extent and nature of antigenic determinants of this polysaccharide antigen Y-PS. The convoluted surface generated by 1,2-linkages in this chain can be compared to branching in a limited sense, and this abrupt topography may satisfy antibody binding within a limited span of three pyranose residues. In the projection viewed in Figure 2 unit c is back and away from the forward surface; binding along this edge of the oligomer sequence would feature possible protein ' contacts ' with residues $b_1d_1a_2$. Inspection of Figure 3a indicates a fourth ' contact ' residue c_2 in this sequence. In this hypothetical four-unit $b_1d_1a_2c_2$ determinant the function of units c_1 and b_2 in a linear hexasaccharide array would be to act as ' non-contact ' chiral spacers which hold and direct the ' contact ' residues in a manner dictated by linkage, configuration, and glycosidic conformation. Kabat *et al.* have reported this interpretation for a ligand-lectin interaction.[57,59] Estimates of antibody binding site dimensions by hapten inhibition could, if the above hypothesis is correct, be at variance with the actual situation. The compact chain geometry of Y-PS would permit more pyranose units to be accommodated in a binding site than the currently accepted upper limit of a hexasaccharide.[8,21,57,60] This value is based upon the classical studies of Kabat and relates to antibodies directed against the highly flexible and extended α1,6-dextrans.[21,57] The work of Rees and Scott [4] suggests that this type of linkage is the least ordered and most flexible of polysaccharide chains. Thus, for compact chain geometries such as Y-PS, octasaccharides rather than hexasaccharides could represent the upper limit of binding site size. Irrespective of the extent of the determinant it can be appreciated that the disposition of identical determinants at opposite faces of the polysaccharide will permit multiple binding points for immunoglobulin molecules of single specificity. Since this binding would occur at opposite sides of the polymer chain the maximum number of bulky antibody molecules could be accommodated along relatively short chain segments. Thus we have found that Y-PS precipitates with monoclonal antibody specific for this O-antigen.[37] Presumably the multivalent antigen provides the multi-point binding essential for antigen–antibody lattice formation and resultant precipitation.

In its role as antigen *in vivo* a linear polysaccharide generates antibodies with specificities for different aspects of the polymer chain.[60,61] The Y-PS generates two such well identified specificities which are designated O-factors 3 and 4.[62] To date the structural elements defining these determinants are not identified.[11] Examination of the linear sequences depicted in Figures 2, 3a, and 3b illustrates the manner in which opposite surfaces of identical sugar sequences could generate dual specificities. This would depend upon which aspect of the oligomer sequence interacts with those lymphocytes which ultimately differentiate to antibody synthesizing cells. The possibility that an oligomer sequence generates antibodies to opposite surfaces has been discussed by others,[8,60] but in the particular case of Y-PS we believe that the O-factors 3 and 4 may well relate to antibody

specificities directed to opposite surfaces of similar or overlapping oligomer sequences. This possibility seems to be well founded in the model of chain conformation we have proposed. One of these O-factors probably includes the 2-acetamido-function of the Y-PS d unit, which upon inspection of Figures 2, 3a, and 3b is seen to be a topographically prominent feature of certain surfaces of the chain. In relation to this it may also be noted, that the acetamido-function is positioned between two *C*-methyl groups of units b, and a_2 (Figure 2). Examination of Figure 3a shows that a third *C*-methyl group of unit c_2 is also projected toward the same surface. Methyl groups of rhamnose residues have been cited as the hydrophobic sites, of a *Streptococcal* polysaccharide, which are largely responsible for the high affinity antibodies produced by vaccination with streptococcal vaccines.[63] In a similar manner the rhamnose *C*-methyl groups of units b_1, a_2, and c_2 could serve as the inital recognition points for antibody binding, with hydrophobic bonding providing the initial attractive forces for the reaction. Subsequently, polar contacts with the acetamido-group could also be established. Hydrophobic bonding has been proposed as the basis of recognition for certain carbohydrate antibody interactions [63,64] and the ubiquitous nature of 6-deoxy-sugars in bacterial polysaccharides [8,60] may indicate a prominant role for this type of binding.

Few if any studies of polysaccharide conformation have focused attention on important periodic heteropolysaccharides. It is shown here that even for a complex heteropolymer it is possible to study solution conformation. This has been rendered possible by the use of high resolution ^{13}C and ^1H n.m.r. spectroscopy in conjunction with hard-sphere calculations. Although synthesis was used here to provide essential oligosaccharide fragments specific degradation techniques may be used to obtain oligomeric fragments and consequently the overall strategy employed here has generality. A linear, unbranched polymer such as Y-PS might be considered a worse case for assessing polymer conformation, since with the exceptions of 1,6-bonds these will be the least constrained. Nevertheless, the HSEA calculations were shown to possess considerable predictive value in this case. In an era of renewed interest in polysaccharide vaccines for human use, the ability to assess antigen shape will facilitate the interpretation of serological data upon sound stereochemical principles.

EXPERIMENTAL

Compounds (1)—(10) were available from synthesis.[12-16] Lipopolysaccharide (LPS) was extracted from *Shigella flexneri* sero-group Y cells and liberation of O-chains from the (LPS) was performed according to published procedures.[10]

^1H N.m.r. spectra were obtained at 310 K in D_2O as *ca.* 0.1M solutions on a Bruker HX-270 instrument operating at 270 MHz. The use of a spectral width of 3 KHz, with a data memory of 64 K, gave a digital resolution of ± 0.1 Hz. The pulse width used was 15 μs (90°). Acetone (1%) was used as internal reference (δ 2.12). Relaxation data were obtained by the inversion recovery method [65] using the initial slope approximation. Samples were not degassed. The T_1 values were determined from a three parameter exponential fit. The nuclear Overhauser enhancement experiments were preformed in the difference mode.[31]

^{13}C N.m.r. spectra were obtained at 310 K in D_2O as *ca.* 5% solutions on the same instrument, mentioned above, operating at 67.89 MHz. The use of a spectral width of 10 KHz with a data memory of 32 K gave a a digital resolution of ± 0.6 Hz. The pulse width used was 12 μS (90°). Dioxan (1%) was used as internal reference (δ 67.40 p.p.m.). The relaxation experiments were done as described above.

^1H N.m.r. data for model compounds are as follows: Methyl α-L-rhamnopyranoside, δ (D_2O) 4.586 (1 H, d, $J_{1,2}$ 1.6 Hz, 1-H), 3.821 (1 H, dd, $J_{2,3}$ 3.5 Hz, 2-H), 3.602 (1 H, dd, $J_{3,4}$ 9.5 Hz, 3-H), 3.325 (1 H, dd, $J_{4,5}$ 9.5 Hz, 4-H), 3.563 (1 H, dq, $J_{5,6}$ 6.2 Hz, 5-H), 3.288 (3 H, s, OCH_3), and 1.193 (3 H, d, 6-H); methyl β-L-rhamnopyranoside, δ (D_2O) 4.432 (1 H, d, $J_{1,2}$ 0.9 Hz, 1-H), 3.874 (1 H, dd, $J_{2,3}$ 3.3 Hz, 2-H), 3.477 (1 H, dd, $J_{3,4}$ 9.2 Hz, 3-H), 3.26 (1 H, dd, 4-H), 3.29 (1 H, m, 5-H), 3.414 (3 H, s, OCH_3), and 1.207 (3 H, d, 6-H); methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, δ (D_2O) 4.342 (1 H, d, $J_{1,2}$ 8.4 Hz, 1-H), 3.576 (1 H, dd, $J_{2,3}$ 9.6 Hz, 2-H), 3.436 (1 H, dd, $J_{3,4}$ 9.2 Hz, 3-H), 3.324 (1 H, dd, $J_{4,5}$ 9.2 Hz, 4-H), 3.353 (1 H, m, $J_{5,6}$ 1.6, $J_{5,6}$ 4.8 Hz, 5-H), 3.824 (1 H, m, $J_{6,6'}$ 12.0 Hz, 6-H), 3.643 (1 H, m, $J_{6,6'}$ 12.0 Hz, 6'-H), 3.400 (s, OCH_3), and 1.933 (3 H, s, $NHCOCH_3$).

The hard-sphere calculations were performed using a modified version [32] of the HSEA program described by Lemieux and his co-workers.[17] The α-L-rhamopyranose co-ordinates used in the calculations were those published for a neutron diffraction study.[33] The carbon and oxygen co-ordinates for 2-acetamido-2-deoxy-β-D-glucopyranose were taken from the *X*-ray data [34] of the terminal non-reducing residue of 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose. The hydrogen co-ordinates for this β-D-GlcNAc residue were generated by a computer program [32] (see Results section for a description of this procedure). The co-ordinates of minimum energy conformations were used with an established plotting routine, PLUTO-78,* to construct the projections (Figures 2, 3a, and 3b).

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* This is a version of PLUTO-78 (modified by B. Clegg, Anorganisch-Chemisches Institut der Universitaet, Goettingen, West Germany) which is adapted for the IBM computer at NEUCC, Technical University of Denmark. Original version of PLUTO-78 by S. Motherwell, University Chemical Laboratories, Cambridge.

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