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Conformational Differences between Linear $\alpha(2\rightarrow8)$-Linked Homosialooligosaccharides and the Epitope of the Group B Meningococcal Polysaccharide†

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ABSTRACT: The α-(2→8)-linked sialic acid oligosaccharides $(NeuAc)_n$ exhibit an unusual degree of heterogeneity in the conformation of their linkages. This was diagnosed by observation in their ^{13}C NMR spectra of an equivalent and unique heterogeneity in the chemical shifts of their anomeric carbons and subsequently confirmed by more comprehensive ^1H and ^{13}C NMR studies. In these studies both one-dimensional and two-dimensional experiments were carried out on the trisaccharide $(NeuAc)_3$ and colominic acid. In addition to the unambiguous assignment of the signals in the spectra, these experiments demonstrated that both linkages of $(NeuAc)_3$ differed in conformation from each other and from the inner linkages of colominic acid. The NMR data indicate that these conformational differences extend to both terminal disaccharides of oligosaccharides larger than $(NeuAc)_5$, a result that has considerable physical and biological significance. In the context of the group B meningococcal polysaccharide, it provides an explanation for the conformational epitope of the group B meningococcal polysaccharide, which was proposed on the evidence that $(NeuAc)_{10}$, larger than the optimum size of an antibody site, was the smallest oligosaccharide able to bind to group B polysaccharide specific antibodies. Because the two terminal disaccharides of $(NeuAc)_{10}$ differ in conformation to its inner residues, the immunologically functional part of $(NeuAc)_{10}$ resides in its inner six residues. This number of residues is now consistent with the maximum size of an antibody site.

The group B meningococcal polysaccharide is a homopolymer of α-(2→8)-linked sialic acid residues and is structurally identical with both colominic acid (Bhattacharjee et al., 1975) and the capsular polysaccharide of *Escherichia coli* K1 (Jennings, 1983). It is poorly immunogenic (Wyle et al., 1972), and although group B meningococcal organisms are able to produce low levels of group B polysaccharide specific antibodies in animals and humans, these antibodies are almost exclusively IgM and of low affinity (Mandrell & Zollinger, 1982). The poor immunogenicity of the group B polysaccharide is probably attributable to tolerance due to cross-reactive tissue components, and this hypothesis is strengthened by the identification of α-(2→8)-linked oligomers of sialic acid common to both the group B meningococcal polysaccharide (Jennings et al., 1985) and the glycopeptides of human and animal tissue (Finne et al., 1983a). Group B polysaccharide specific antibodies are induced by a conformationally controlled epitope. This was unambiguously confirmed by the fact that an unusually large α-(2→8)-linked sialic acid oligomer (decasaccharide) was required either to function as an inhibitor for (Jennings et al., 1985) or to bind to (Finne & Mäkelä, 1985) these antibodies. The glycopeptides of human and animal fetal brain also contain the same epitope and in consequence bind to group B polysaccharide specific antibodies (Finne et al., 1983b; Finne & Mäkelä, 1985). In this paper the conformational nature of the epitope associated with the formation of group B meningococcal polysaccharide specific antibodies has been confirmed by high-resolution NMR studies on colominic acid and some of its constituent oligosaccharides. These studies indicate that at least five residues are required before a linkage with a similar orientation to that of the internal linkages of colominic acid is generated.

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EXPERIMENTAL PROCEDURES

Materials. Colominic acid (*E. coli*) was obtained from Sigma Chemical Co., St. Louis, MO, and a high molecular weight fraction was used in the NMR experiments. This was obtained by passing colominic acid (Na^+ salt) through a Sephadex G-100 column with phosphate-buffered saline (PBS) at pH 7.0 as eluant and isolating the fraction that eluted at $K_D = 0.1$ (20–25 sialic acid residues). Oligosaccharides of DP = 2–6 were obtained by the partial hydrolysis of colominic acid and fractionation of the depolymerized fragments in order of ascending molecular size on an ion-exchange column as described by Jennings et al. (1985).

NMR Methods. ^1H and ^{13}C NMR spectra were recorded on Bruker AM500 and AM200 spectrometers at 300 K with acetone as the internal chemical shift reference (2.225 ppm for ^1H NMR and 31.07 ppm for ^{13}C NMR). Internal acetone is referenced to external tetramethylsilane. Polysaccharides and oligosaccharides were exchanged twice with 99.7% D_2O and then run in 0.4 mL of 99.99% D_2O (5-mm tubes) at concentrations of 50–100 mg/mL.

Homonuclear shift-correlated 2-D NMR (COSY) experiments and homonuclear *J*-resolved 2-D NMR (JRES) experiments were carried out according to Aue et al. (1976) and Nagayama et al. (1980). COSY experiments with two- and three-step relayed coherence transfer were done according to Wagner (1983) and Bax and Drobny (1985). The heteronuclear shift-correlated 2-D NMR experiment was carried out according to Bax et al. (1981), and the CHORTLE experiment was done with the pulse sequence of Pearson et al. (1985). All phase cyclings were according to the standard software provided by Bruker (DISB86), and all experiments were done in the magnitude mode. Nuclear Overhauser enhancements (NOE) were obtained by difference experiments with multiple irradiation of each line of a multiplet (Neuhaus, 1983; Kinns & Saunders, 1984). Transient NOE values were obtained with

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Table I: Chemical Shifts[a] of Signals in the ^{13}C NMR Spectrum of α-(2→8)-Linked **1** [(NeuAc)$_3$]

carbon	residues of **1**[b] c	b	a
1	174.19	173.10	175.59
2	101.36	102.87	97.35
3	41.22	41.70	40.06
4	69.27	74.48	68.28
5	52.55	53.19	53.19
6	73.46	68.81	71.50
7	68.99	69.99	68.49
8	72.55	79.05	76.50
9	63.44	62.05	61.89
C=O	175.73	175.73	177.76
CH_3	23.07[c]	23.16[c]	22.88

[a] In ppm from internal acetone. Assignments confirmed by 2-D NMR (C,H) COSY experiments. [b] Contiguous residues from the reducing residue a. [c] Tentative assignments.

Table II: Chemical Shifts[a] of Signals in the ^{13}C NMR Spectrum of α-(2→8)-Linked (NeuAc)$_4$

carbon	residues of (NeuAc)$_4$[b] d	c	b	a
1	174.30	173.89	173.15	175.60
2	101.19	101.90	102.81	97.35
3	41.20	40.89	41.61	40.08
4	69.33	68.97	74.56	68.26
5	52.56	53.20	53.20	53.20
6	73.43	74.01	68.97	71.57
7	68.97	70.24[c]	69.69[c]	68.61
8	72.55	78.51	79.16	76.73
9	63.44	62.29[c]	61.88[c]	61.88
C=O	175.41	175.71	175.71	177.72
CH_3	23.08[c]	23.08[c]	23.15[c]	22.88

[a] In ppm from internal acetone. [b] Contiguous residues from the reducing residue a. [c] Tentative assignments.

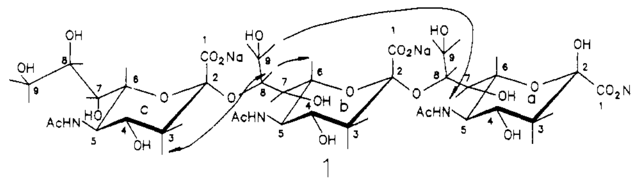

FIGURE 1: Structure of trisaccharide **1**. Arrows depict NOEs pertinent to the conformation of **1**.

a 180° selective pulse (Morris & Freeman, 1978). Proton spin simulation with a line width of 0.5 Hz was performed with the Bruker program PANIC.

RESULTS AND DISCUSSION

Both ^{13}C and ^{1}H NMR spectroscopic studies on colominic acid and some of its component oligosaccharides confirm that the interresidue linkage conformation of the terminally located sialic acid residues of this α-(2→8)-linked sialic acid homopolymer differs from those of its inner residues. Colominic acid was used in this NMR study because it was easily obtainable in a molecular size small enough to yield well-resolved spectra. However, because they are structurally identical with colominic acid, the capsular polysaccharides of group B *Neisseria meningitidis* and *E. coli* K1 must also exhibit this phenomenon.

Conformational differences were first diagnosed by observation in the ^{13}C NMR spectra of a series of α-(2→8)-linked sialooligosaccharides, (NeuAc)$_n$, in which n = 2–6, extensive heterogeneity particularly in the chemical shifts of their linkage carbons (C2 and C8). The chemical shifts of the carbons of the sialic residues of the oligosaccharides and colominic acid are listed in Tables I–IV, the latter being listed in Table III. Assignments made on the trisaccharide (**1**) shown in Figure 1 and on colominic acid were obtained from 2-D (H,H) and

Table III: Chemical Shifts[a] of Signals in the ^{13}C NMR Spectrum of α-(2→8)-Linked (NeuAc)$_5$ and Colominic Acid[b]

carbon	residues of (NeuNAc)$_5$[c] e	d	c	b	a	colominic acid
1	174.32	173.88	173.88	173.07	175.60	174.00
2	101.18	101.90	101.72	102.92	97.35	101.78
3	41.12	40.78	40.78	41.60	40.02	40.76
4	69.23	68.94	68.94	74.46	68.19	69.13
5	52.53	53.15	53.15	53.15	53.15	53.23
6	73.39	74.04	74.04	68.94	71.40	74.01
7	68.94	70.23[d]	69.80[d]	69.71[d]	68.55	69.86
8	72.55	78.56	78.56	79.06	76.43	78.72
9	63.41	62.28	62.05	61.80	61.80	62.06
C=O	175.70	175.70	175.70	175.70	177.75	175.76
CH_3	23.10[d]	23.24[d]	23.24[d]	23.10[d]	22.89	23.30

[a] In ppm from internal acetone. [b] Assignments obtained from CHORTLE experiment. [c] Contiguous residues from the reducing residue a. [d] Tentative assignments.

Table IV: Chemical Shifts[a] of Signals in the ^{13}C NMR Spectrum of α-(2→8)-Linked (NeuAc)$_6$

carbon	residues of (NeuAc)$_6$[b] f	e	d	c	b	a
1	174.31	173.94	173.94	173.94	173.15	175.61
2	101.17	101.85	101.74	101.74	102.80	97.35
3	41.21	40.74	40.74	40.74	41.60	40.08
4	69.20	69.07	69.07	69.07	74.55	68.29
5	52.56	53.21	53.21	53.21	53.21	53.21
6	73.41	74.02	74.02	74.02	69.07	71.58
7	69.07	70.27	69.84	69.84	69.33	68.66
8	72.54	78.57	78.65	78.65	79.22	76.75
9	63.44	62.29	62.05	62.05	61.83	61.83
C=O	175.71	175.71	175.71	175.71	175.71	177.71
CH_3	23.10[c]	23.29[c]	23.29[c]	23.29[c]	23.10[c]	22.87

[a] In ppm from internal acetone. [b] Contiguous residues from the reducing residue a. [c] Tentative assignments.

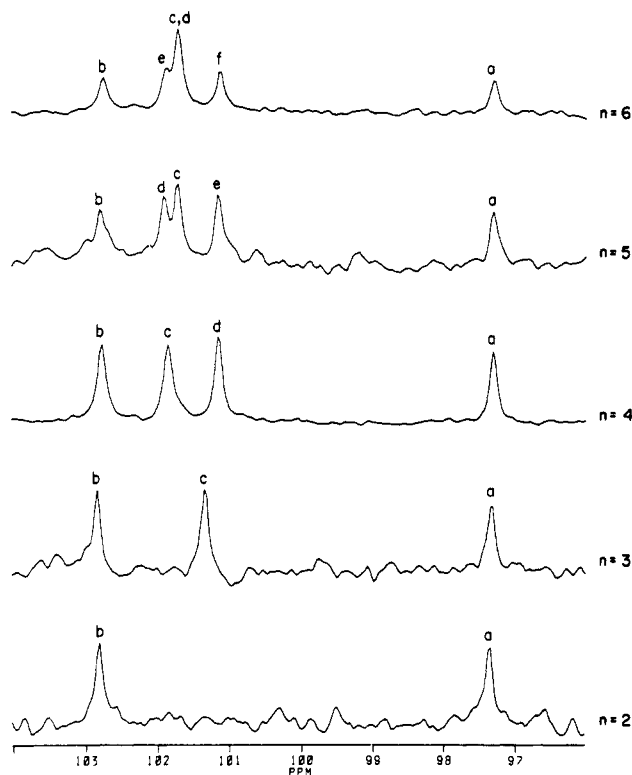

FIGURE 2: ^{13}C resonances of the anomeric carbons of α-(2→8)-linked homosialooligosaccharides (NeuNAc)$_n$ where n = 2–6.

(^{13}C,H) COSY experiments and the assignments made on the other oligosaccharides were made with oligosaccharide **1** as a model. While the assignments made on colominic acid are in substantial agreement with those reported by Bhattacharjee et al. (1975), they confirm that the latter assignments made on the closely spaced C4 and C7 signals should have been reversed.

The signals of the anomeric carbons of the oligosaccharides are shown in Figure 2 and reveal the heterogeneity in their chemical shifts. Except for the signals of the anomeric carbons of the reducing sialic acid residues, which remain constant at 97.35 ppm in the spectra of all the oligosaccharides, coincident signals do not occur until the hexasaccharide is reached, and these signals at 101.74 ppm also coincide with that of the anomeric carbon signal of colominic acid. A similar pattern of chemical shift heterogeneity is also exhibited by the other linkage carbons at C8 on the aglyconic sialic acid residues of the oligosaccharides (Tables I–IV). From these results it can be inferred that heterogeneity in linkage conformation in the hexasaccharide is present in both its terminal disaccharides and that by analogy this is also true for colominic acid. The conformational dependence of ^{13}C NMR chemical shifts of linkage carbons was first proposed by Colson et al. (1974) and was later used empirically to locate conformationally controlled determinants in complex polysaccharides (Jennings et al., 1984). However, only recently has this correlation between glycosidic torsion angle (ψ) and chemical shift been validated with HSEA calculations on a series of oligosaccharides having either α-D-glucopyranosyl or α-D-galactopyranosyl residues at the nonreducing end (Bock et al., 1986). Our 1-D and 2-D ^{1}H NMR studies on trisaccharide **1** and colominic acid also provide further evidence for the validity of this correlation.

In order to compare the differences in the linkage conformations between the oligosaccharides and colominic acid, a 1-D and 2-D ^{1}H NMR study was carried out on **1** and colominic acid. Trisaccharide **1**, shown in Figure 1, was chosen as the oligosaccharide to study because it approximates to the smallest structure incorporating both terminal disaccharides of colominic acid. Strictly speaking, the tetrasaccharide would have been a more precise model but the analysis of its ^{1}H NMR spectrum would have been prohibitive.

Table V: Chemical Shifts[a] of Signals in the ^{1}H NMR Spectrum of α-(2→8)-Linked **1** [(NeuAc)$_3$] and Colominic Acid

proton	residues of 1: c	b	a	colominic acid
H-3a	1.729	1.639	1.748	1.737
H-3e	2.757	2.691	2.201	2.673
H-4	3.668	3.547	3.983	3.600
H-5	3.821	3.782	3.863	3.819
H-6	3.615	3.555	3.877	3.626
H-7	3.576	3.839	3.760	3.896
H-8	3.897	4.113	3.996	4.102
H-9	3.884	4.132	3.940	4.188
H-9′[b]	3.633	3.666	3.733	3.665
CH_3	2.028	2.064[c]	2.060[c]	2.083

[a] Spin simulated parameters. In ppm from internal acetone. [b] Primary geminal protons are distinguished by means of a prime for the proton with the largest vicinal coupling constant. [c] Assignments may be reversed.

Table VI: Vicinal and Geminal Coupling Constants (Hz) of α-(2→8)-Linked **1** [(NeuAc)$_3$][a] and Colominic Acid[b]

$J_{H,H}$	residues of 1: c	b	a	colominic acid
3a,3e	−12.2	−12.2	−12.9	−12
3a,4	12.0	12.1	11.5	12
3e,4	4.4	4.4	5.0	5
4,5	10.0	10.2	10.3	10
5,6	10.3	10.3	10.3	10
6,7	1.9	1.0	0.5	<3
7,8	9.1	2.3	6.9	<3
8,9	2.6	4.7	2.8	5
8,9′	6.4	6.9	3.9	6
9,9′	−12.1	−12.3	−12.3	−12

[a] Spin simulated parameters. [b] Taken from resolution-enhanced spectrum.

The ^{1}H NMR spectrum of the trisaccharide (**1**) is shown in Figure 3 and was difficult to assign directly due to its complexity; therefore, a proton homonuclear correlated 2-D NMR [(H,H) COSY] experiment with two- and three-step relay coherence transfer was performed, and the spectrum is also shown in Figure 3. As a result of this experiment, it was possible to assign all the protons of each sialic acid residue in **1**. Improved resolution of the ^{1}H NMR spectrum of **1** was also obtained when signals were further separated along the J axis by two-dimensional J-resolved spectroscopy. Measurement of chemical shifts and coupling constants from the contour plot of the two-dimensional J-resolved spectrum permitted rapid and unambiguous assignment of individual protons within the unresolved multiplets observed in the one-dimensional spectrum. The chemical shifts and coupling constants of the protons in **1** are listed in Tables V and VI, respectively. Additional confirmation of the assignments was also obtained by a proton simulation experiment. In this experiment the coupling constants and chemical shifts obtained from the J-resolved spectrum were fed into a program, a nine-spin system for each sialic acid residue of **1**. The signals associated with each residue are shown in Figure 4, and the spectrum obtained by the addition of all these signals is identical with that of the resolution-enhanced one-dimensional ^{1}H NMR spectrum of **1**.

Having unambiguously assigned all the proton resonances, NOE experiments could then be carried out. Selected NOE difference spectra for **1** are shown in Figure 5, and the relative

Table VII: Nuclear Overhauser Enhancements for α-(2→8)-Linked **1** [$(NeuAc)_3$]

	observed NOE (negative)								
saturated signal[a]	H_{3a}	H_{3e}	H_4	H_5	H_6	H_7	H_8	H_9	$H_{9'}$
H_{3e}(a)	(a) 12		(a) 4						
H_{3e}(c)	(c) 11		(c) 6	(c) 2					
H_{3e}(b)	(b) 13		(b) 9						
H_{3a}(c)		(c) 14		(c) 7			(b) 1		
H_{3a}(a)		(a) 10		(a) 2					
H_{3a}(b)		(b) 17	(b) 4	(b) 10					
H_8(b)	(c) 1				(b) 4	(b) 5		(b) 17	(b) +
{ H_8(b)	(c) 2				(b) 5	(b) 5			
{ H_9(b)						(a) 4			(b) 6

[a] a is the reducing residue, b is the middle residue, and c is the nonreducing residue of **1**.

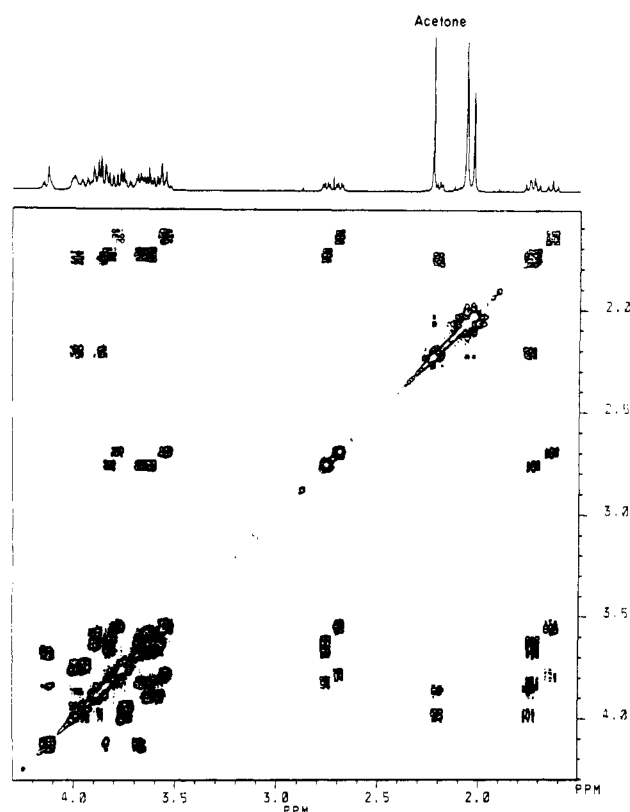

FIGURE 3: Homonuclear 2-D shift correlated H,H COSY spectrum, with three-step relay, of **1** in D_2O (310 K) with the 1-D spectrum above. The initial (t_1, t_2) matrix of 256 × 2048 points was zero-filled to 1024 × 2048 points and processed with unshifted sine bell window functions, a magnitude calculation, and symmetrization about the diagonal. The final resolution in both dimensions was 1 Hz/point.

NOEs obtained from these spectra are listed in Table VII. An examination of the coupling constants and NOEs of the protons associated with the two different linkages in **1** indicates a striking difference in conformation between these linkages. That conformational differences are confined to the linkage regions of **1** is confirmed by the fact that the coupling constants and NOEs of the ring protons of **1** indicate that the conformations of the individual sialic acid rings remain essentially in the same conformation ($_5C^2$) as described for their respective monomeric units (Joachims et al., 1967). This is also true for the exocyclic chain of the terminal residue (c) of **1**, the preferred conformation of which is similar to that proposed by others (Brown et al., 1975; Sabesan et al., 1983; Lindon et al., 1984).

Despite the fact that the linkage regions between the residues of **1** are complex, both are composed of four bonds with potential for unrestricted rotational freedom, the coupling constants (Table VI) and NOE data (Table VII) indicate that the linkage carbons adopt a rather precise conformation. For

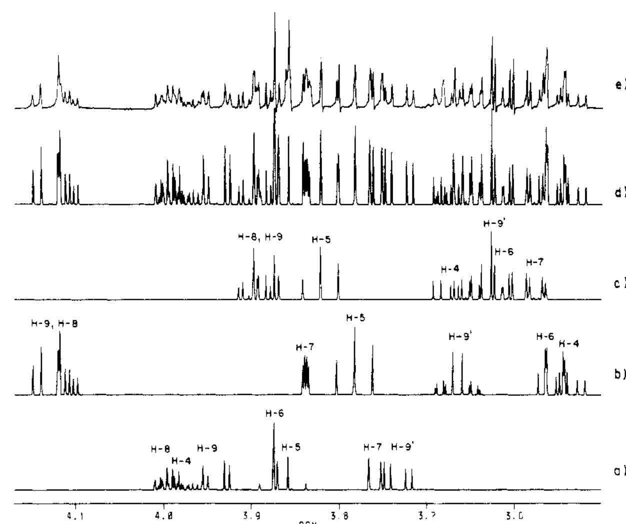

FIGURE 4: Comparison of the simulated and experimental spectrum of **1**. Simulation for each individual residue (a, b, and c) is shown in (a), (b), and (c) and their sum in (d). The resolution-enhanced experimental spectrum is shown in (e).

the exocyclic chain of residue b of **1**, a strong NOE between H_6^b and H_8^b (Figure 5) and the small vicinal coupling constants (Table VI) associated with these protons indicate that both sets of rotamers about the C_8–C_7 bond and the C_7–C_6 bond are confined to a preferred rotamer with ϕ (H_8, C_8, C_7, H_7) = +60° for the C_8–C_7 bond and ϕ (H_7, C_7, C_6, H_6) = −60° for the C_7–C_6 bond. In addition the orientation of the nonreducing terminal sialic acid residue (c) in relation to residue b can be determined from the NOE between H_{3a}^c and H_8^b, which puts these two protons in close proximity.

The conformation of the linkage between residues b and a of **1** (Figure 1) is completely different to the linkage between residues c and b. Residue a, which is in its preferred β-D form as determined by ^1H and ^{13}C chemical shift data (Tables I and V) (Brown et al., 1975; Jennings & Bhattacharjee, 1977), is bent back toward the middle residue (b). This can be deduced by the enhancement of the H_7^a signal when H_9^b is irradiated, indicating the proximity of these two protons. The coupling constants are also in agreement with a large change in the linkage conformation between residues b and a. In this case the coupling constant between H_6^c and H_7^c is also small, indicating that they are in gauche orientation, while the coupling constant between H_7^{1c} and H_8^{1c} is large, showing that these two protons are trans to each other.

The chemical shifts and coupling constants associated with the protons of the sialic acid repeating unit of colominic acid were derived from its resolution-enhanced ^1H NMR spectrum and are also listed in Tables V and VI. Similar values for these parameters were also reported for some of these protons by Lindon et al. (1984). NOE experiments were carried out

Table VIII: Nuclear Overhauser Enhancements for Colominic Acid

saturated signal	observed NOE (negative)[a]								
	H_{3a}	H_{3e}	H_4	H_6	$H_{9'}$	H_5	H_7	H_8	H_9
H_{3a}		32	9			7	$\underline{1}$	$\underline{4}$	
H_{3e}	32		15			3	$\underline{1}$	$\underline{2}$	
H_5	5	2					8	1	
H_7	<1			8		8		8	3
H_8	$\underline{3}$	$\underline{1}$		9	6		9		14
$H_{9'}$							4	17	23

[a] Interresidue NOE intensity values are underlined.

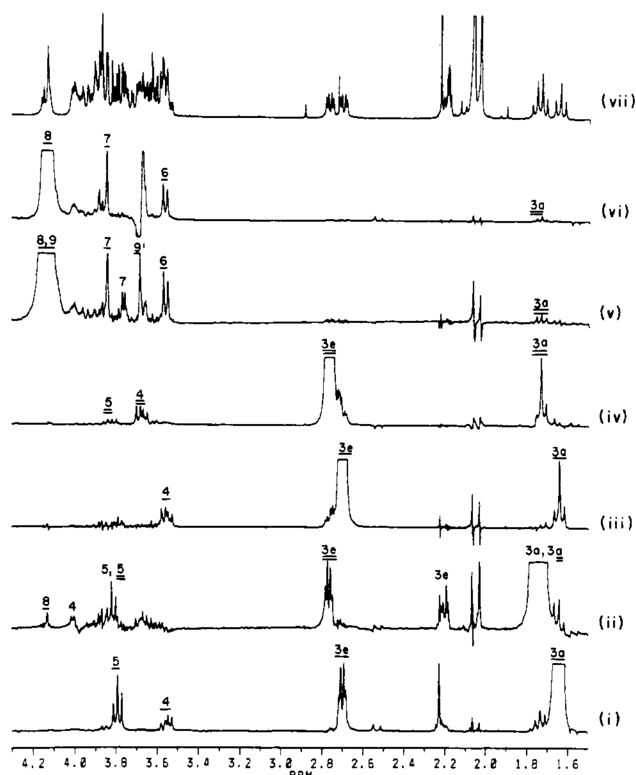

FIGURE 5: NOE difference spectra of **1**. Resonances of residue a are not underlined, those of residue b are underlined once, and those of residue c are underlined twice. The spectra represent the irradiation of (i) H_{3a} of b, (ii) H_{3a} of a and H_{3a} of c, (iii) H_{3e} of b, (iv) H_{3e} of c, (v) H_8 and H_9 of b, and (vi) H_8 (mostly) of b. The spectrum of **1** is shown in (vii).

directly on colominic acid. Selected NOE difference spectra are shown in Figure 6, and the relative NOEs obtained from these spectra are listed in Table VIII. From this information it can be determined that the conformation of the linkage between two consecutive sialic acid residues (a and b of **2** as shown in Figure 7) located internally in colominic acid is different from that of both linkages in **1**. Although the linkage conformation of **2** is more similar to that between residues c and b of **1** than that between residues b and a of **1**, the differences in the former are still striking and show, despite the possibility of averaging, a considerable difference in the orientation of the sialic acid residues. That the orientation of residues a and b of **2** is different to that of residues c and b of **1** can be determined from the NOE data of **2** (Table VIII) where an enhancement on H_8^b was not only generated by irradiating H_{3a}^a, as was the case with the equivalent signals (H_8^b, H_{3a}^c) in **1** (Table VII), but also by irradiating H_{3e}^a. Therefore for **2**, H_8^b must be situated midway between, and in close proximity to, both geminal H_3 protons of residue a. On the basis of coupling constants (Table VI) and NOEs (Table VIII) assigned to H_6^b, H_7^b, and H_8^b, the remainder of the linkage region of **2** appears to be similar to that between residues c

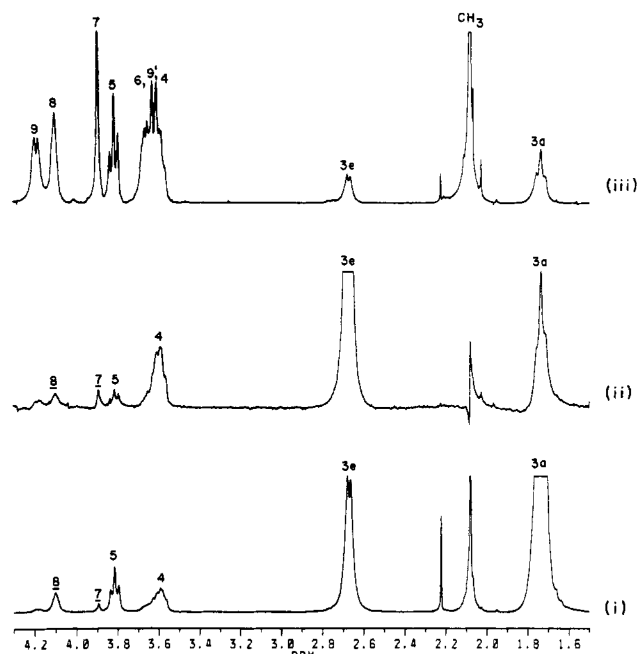

FIGURE 6: Transient NOE difference spectra of colominic acid with 50-ms delay after selective inversion of (i) the H_{3a} resonance and (ii) the H_{3e} resonance. The reference spectrum is shown in (iii), and interresidue enhancements are underlined.

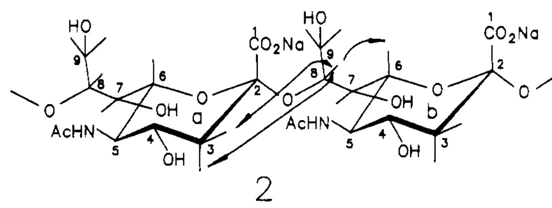

FIGURE 7: Structure of disaccharide **2** located internally in colominic acid. Arrows depict NOEs pertinent to the conformation of **2**.

and b of **1**, although minor changes would still be compatible with the data. The NOE between H_6^b and H_8^b, is also present in **2**, and the coupling constants between H_6^{2b}, H_7^{2b} and H_7^{2b}, H_8^{2b} are of the same order of magnitude as those found for the equivalent protons in **1** (Table VI). A minor difference in the NOE pattern between **1** and **2** was that irradiation of H_{3a}^{2a} and H_{3e}^{2a} also caused a small enhancement on H_7^{2b}; however, this may not be important to the linkage conformation. CPK models of **2** show that the close proximity of both geminal H_3 protons of **2** to H_7^{2b} is unlikely and that therefore these NOEs could be attributed to three spin effects.

The above NMR analyses demonstrate that the linkages in **1** are conformationally different to each other and to the inner linkages of colominic acid. The extent of this difference can be seen by examining CPK models of **1** and an equivalent internally located trisaccharide sequence of colominic acid. The models are shown in Figure 8, and two dominant features indicative of the structural difference between them can be

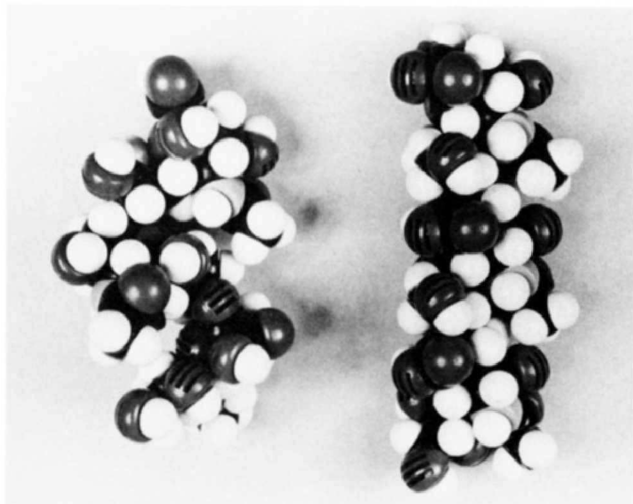

FIGURE 8: Space-filling models (CPK) of trisaccharide **1** (left) and the same trisaccharide located internally in colominic acid (right).

readily visualized. First, due to the folding back of the reducing sialic acid residue of **1**, trisaccharide **1** is much shorter than the internal trisaccharide. Second, the carboxylate groups of **1** appear to be randomly arranged, whereas those of the internal trisaccharide are essentially lined up on one side of the molecule. This is exemplified by comparing the coupling constants of the H_9 protons of **1** and colominic acid (Table VI). For **1** the vicinal coupling constants of the H_9 protons of each residue are all different, whereas those of colominic acid are all the same.

All these observations have important biological implications in that the unusual length requirement of α-(2→8)-linked sialic acid oligosaccharides (decamer) to bind to group B meningococcal polysaccharide specific antibodies (Jennings et al., 1985; Finne & Mäkelä, 1985) can be rationalized. The fact that a decasaccharide is the minimum size required is completely consistent with our NMR studies because we have shown that the two terminal disaccharides of the decasaccharide would differ in conformation from its inner residues. Thus, the immunologically functional moiety of the decamer is the inner six residues. This number is now consistent with the inhibition studies of Kabat and Mayer (1961) using a dextran–antidextran system in which the inhibition power of dextran-derived oligosaccharides maximized at the hexasaccharide. Also consistent with the lack of a conformationally controlled determinant in the dextrans is the fact that linear α- and β-D-(1→4)-linked and α- and β-D-(1→6)-linked oligosaccharides related to dextran exhibit no conformational heterogeneity in their linkages as determined by the absence of anomeric and linkage ^{13}C chemical shift heterogeneity in their ^{13}C NMR spectra (Bock et al., 1984). It is interesting to note that the dependence of the conformation of the group B meningococcal polysaccharide on chain length probably also explains a similar unusually large oligomer (eight sialic acid residues) requirement of a bacteriophage endosialidase, which cleaves colominic acid (Finne & Mäkelä, 1985).

That the group B polysaccharide contains a preferred conformation not found in the smaller oligosaccharides could be due to cooperative stabilization, which is dependent on chain length. The forces involved in this stabilization are not known with certainty but would be a very important factor in any theoretical calculations of the conformation of the determinant. Obviously any potential energy calculations would require an oligosaccharide of at least six residues to adequately describe the preferred conformation of colominic acid. Charge is probably involved, but due to the fact that the isomeric α-(2→9)-linked group C meningococcal polysaccharide forms a conventional determinant (Jennings et al., 1985), additional environmental factors must be important. Perhaps the unique disposition of the carboxylate and acetamido groups in colominic acid, each being lined up on opposite sides of the molecule (Figure 8), could be a factor in the stabilization energy. This alignment of the carboxylate groups leaves them all in close proximity to 9-(hydroxymethyl) groups, which is consistent with the reported (Lifely et al., 1981) ease of lactone formation between these groups in the group B polysaccharide. The close proximity of these groups could also result in hydrogen bonding, possibly with the participation of water molecules, which could also be a factor in stabilizing the conformation of the group B polysaccharide.

In conclusion, it is interesting to speculate as to whether the group B polysaccharide conformational determinant is unique. Certainly there is some preliminary evidence that would indicate that it is not. For instance, there are reports (Mehmet et al., 1986) of a similar size dependence for oligosaccharides obtained from keratan sulfate in binding to keratan-specific monoclonal antibodies. Also, the large molecular size (12–14 residues) of dermatan sulfate oligosaccharides required to bind to heparin cofactor II seems unusually large for the entire oligosaccharide to be included in a binding site (Tollefsen et al., 1986).

Registry No. $(NeuAc)_3$, 76152-09-5; $(NeuAc)_4$, 96425-83-1; $(NeuAc)_5$, 110935-75-6; $(NeuAc)_6$, 96425-82-0; colominic acid, 9013-15-4.

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Biosynthesis of a Specifically Deuterated Diunsaturated Fatty Acid ($18{:}2^{\Delta 6,9}$) for ^{2}H NMR Membrane Studies†

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ABSTRACT: A unique procedure for the biosynthesis and subsequent isolation of a series of specifically deuteriated *cis,cis*-octadeca-6,9-dienoic acids has been developed. An auxotroph of *Tetrahymena*, which lacks Δ9 and Δ12 desaturase activity, is supplemented with specifically deuteriated oleic acid and converts it to the corresponding deuteriated *cis,cis*-octadeca-6,9-dienoic acid, $18{:}2^{\Delta 6,9}$. The deuteriated fatty acid is subsequently isolated by argentation chromatography and HPLC. To demonstrate the utility of the procedure, we describe here the biosynthesis of *cis,cis*-octadeca-6,9-dienoic acid deuteriated at positions 9 and 10. Gas and thin-layer chromatography of the isolated fatty acid showed that it was greater than 99% pure while ^{13}C NMR and mass spectrometry of the *O*-(trimethylsilyl) derivative confirmed that the 18-carbon fatty acid contains two double bonds located at positions 6 and 9. The yield, from an 11-L culture, was typically 100 mg of which 35% was found to be deuteriated at both the 9- and 10-positions. The deuteriated fatty acid was esterified to 1-hexadecanoyl-*sn*-glycero-3-phosphocholine, and aqueous, multilamellar dispersions of the lipid were studied by ^{2}H NMR. Each spectrum consists of two overlapping powder patterns and therefore yields two quadrupolar splittings. Over a temperature range from 0 to 40 °C, one splitting decreases from 6.6 to 1.8 kHz while the other increases from 4.5 to 5.3 kHz. The magnitudes of the two splittings are equivalent between 10 and 15 °C. The values of the splittings, and their response to temperature, differ significantly from those of the corresponding deuteriated oleic acid in microbial membranes [Rance, M., Jeffrey, K. R., Tulloch, A. P., Butler, K. W., & Smith, I. C. P. (1980) *Biochim. Biophys. Acta 600*, 245–262] and in bilayers of 1-hexadecanoyl-2-*cis*-octadec-9-enoyl-*sn*-glycero-3-phosphocholine (POPC) [Seelig, J., & Waespe-Šarčevič, N. (1978) *Biochemistry 17*, 3310–3315]. The results suggest that a fatty acyl chain containing two double bonds has physicochemical properties very different from those of the corresponding acyl chain with a single double bond.

In recent years considerable evidence has accumulated suggesting a unique role for polyunsaturated lipids in eukaryotic membranes. These lipids modulate a variety of membrane-associated processes [for a review see Spector and Yorek (1985)] and are thought to play an essential role in neural tissue (Lamptey & Walker, 1976; Crawford et al., 1984) and in the retina (Neuringer et al., 1984).

The effect of polyunsaturated lipids is generally attributed to their ability to "fluidize" membranes. A high degree of unsaturation in a membrane is thought to correlate with a low gel to liquid crystal transition temperature and a high degree of mobility and disorder of the lipids. Although the double bond itself is a relatively ordered, immobile structure (Seelig & Waespe-Šarčevič, 1978; Rance et al., 1980; Dufourc et al., 1984), this correlation appears to hold for membranes containing saturated and monounsaturated acyl chains (Davis & Keough, 1983; Stubbs et al., 1981; Seelig & Seelig, 1977). The extension to highly unsaturated systems, however, has no rigorous physicochemical basis. For example, differential scanning calorimetry has shown that liposomes containing a variety of polyunsaturated lecithins have similar transition temperatures (Coolbear et al., 1983), and fluorescence depolarization of diphenylhexatriene, in similar bilayers, suggests that the order and rates of motion of the lipid acyl chains are very similar (Stubbs et al., 1981). Both techniques suggest

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