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Carbohydrates with relevance to the structure of glycosaminoglycans: The crystal structures of 2-deoxy-2-(sulfoamino)-α-D-glucopyranose sodium salt dihydrate, 2-amino-2-deoxy-α,β-D-glucopyranose 3-(hydrogen sulfate) monohydrate, and 2-amino-2-deoxy-α-D-glucopyranose 6-(hydrogen sulfate) monohydrate

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Abstract

The X-ray crystal structures of the glycosaminoglycan-related monosaccharides 2-deoxy-2-(sulfoamino)-α-D-glucopyranose sodium salt dihydrate, 2-amino-2-deoxy-α,β-D-glucopyranose 3-(hydrogen sulfate) monohydrate, and 2-amino-2-deoxy-α-D-glucopyranose 6-(hydrogen sulfate) monohydrate have been determined at 173 K. In the solid state, the 3-sulfated and 6-sulfated compounds assume the expected zwitterionic form. The 2(N)- and 6-sulfate crystal structures contain only the α-anomers, but the 3-sulfate crystal structure includes both the α- and β-anomers, as well as two different orientations of the hydroxymethyl group. The sodium ion in the 2(N)-sulfate structure is sixfold coordinated, with two water molecules, a sulfate oxygen, a

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ring oxygen, and two hydroxyl oxygens serving as electron donors. The 2(N)-sulfate group makes direct contact with the sodium ion and also makes indirect contact with it through mediating water molecules. In the 3-sulfate structure, the sulfate group participates in a close intramolecular contact with the protonated amino group at C-2. In all three structures the sulfate oxygens engage in close intermolecular interactions with $-NH_3^+$ and $-OH$ groups and with water molecules, although the sole sulfate–water approach in the 6-sulfate structure is exceptional in not being very close (> 3.0 Å). Selected IR and NMR data are presented.

Keywords: Glycosaminoglycans; X-ray crystal structures

1. Introduction

The glycosaminoglycans or GAGs comprise a family of unbranched, (usually) sulfated polysaccharides that includes keratan sulfate, heparan sulfate, heparin, chondroitin sulfate, dermatan sulfate, and hyaluronic acid. These polysaccharides play a wide range of physiological roles, from lending mechanical support in connective tissue to participating in processes such as blood coagulation, wound healing, and embryonic development. Determining the structures and physiological activity of these biologically important polysaccharides and mimicking their activity using GAG analogues prepared in the laboratory are currently areas of intense biomedical interest [1,2]. With the exception of hyaluronic acid (which is not sulfated), a GAG molecule typically consists of a repeating disaccharide unit in which one of the components is a sulfated amino sugar and the other is a uronic acid, so it is not surprising that sulfated monosaccharides have become the focus of attention as simple models for the much larger GAGs. An indication of the importance of GAG-related sulfated carbohydrates is the impressive array of X-ray crystal structure determinations of sulfated (or sulfonated) monosaccharides that have recently appeared in the carbohydrate literature [3–9]. GAG–protein interactions are also of vital interest; the binding of another potential GAG mimic, sucrose octasulfate (SOS) [10], to acidic fibroblast growth factor (aFGF) is the subject of a recent study in which the interactions of the SOS sulfate groups with specific basic amino acid side chains of aFGF are examined [11].

The manner in which glycosaminoglycans interact with peptides and proteins in a specific way has important physiological consequences, and we are investigating these interactions using both crystallographic and synthetic approaches. In our crystallographic investigation we are using high-resolution X-ray crystal structures of sulfated amino sugars as models for the structural features found in GAGs. In this report we describe the crystal structures of three of these sugars: the 2(N)-sulfate, the 3-sulfate, and the 6-sulfate of 2-amino-2-deoxy-D-glucose. The 2(N)-sulfated glucosamine is of particular interest because it is, to our knowledge, the first N-sulfated sugar whose structure has been determined by X-ray crystallography. Our synthetic studies are guided by structural studies and currently involve methods for preparing synthetic materials that mimic GAGs. As an aid and reference for establishing the sulfation pattern produced on GAG model compounds during synthesis, we include in this report selected IR and NMR spectroscopic data for these three sulfated glucosamines.

2. Experimental

X-ray crystal structure determination.—D-Glucosamine-2(N)-sulfate (sodium salt), α-D-glucosamine-3-sulfate, and D-glucosamine-6-sulfate are commercially available (Sigma). Crystals of the 2(N)-sulfate were grown by slow evaporation of an aqueous solution. Crystals of the 3-sulfate were grown from aq 2-propanol. A crystal of the 6-sulfate suitable for data collection was taken directly from the bottle of commercially-obtained material. The structure determinations showed that the 2(N)-sulfate had crystallized as a dihydrate and that the 3- and 6-sulfates had crystallized as monohydrates. The 3- and 6-sulfates were found to assume the expected zwitterionic form in the solid state. Further experimental details are given in Table 1.

In the 2(N)-sulfate and 6-sulfate, all the non-hydrogen atoms were refined anisotropi-

Table 1
Crystal data, data collection parameters, and refinement results

	2 (N)-Sulfate	3-Sulfate	6-Sulfate
Formula	$C_6H_{12}NO_8SNa \cdot 2H_2O$	$C_6H_{13}NO_8S \cdot H_2O$	$C_6H_{13}NO_8S \cdot H_2O$
Formula weight	317.24	277.25	277.25
Crystal dimensions	0.35×0.25×0.10	0.30×0.10×0.05	0.40×0.10×0.10
Crystal system	orthorhombic	monoclinic	orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a (Å)	9.330(2)	7.300(2)	9.937(2)
b (Å)	17.151(2)	7.767(1)	20.549(3)
c (Å)	7.695(2)	9.287(2)	5.186(2)
β (°)		98.04(2)	
V (Å^3)	1231(1)	521.4(3)	1059(1)
Z	4	2	4
D_c (g cm^{-1})	1.711	1.766	1.739
$F(000)$	664	292	584
μ (Cu $K\alpha$) (cm^{-1})	31.5	31.6	31.1
$2\theta_{max}$ (°)	140.3	140.2	140.0
No. of measured refl.	2058	1767	1658
No. of unique refl.; R_{int}	1843; 0.011	1527; 0.025	1493; 0.020
No. of observed refl. ($I > 3\sigma(I)$)	1684	1382	1364
No. of variables	220	162	199
Trans. coefficients	0.69–1.00	0.64–1.00	0.89–1.00
Secondary extinction [12]	—	1.4221×10^{-5}	—
Min., max. in final difference map (e$^-$Å^{-3})	−0.28, 0.24	−0.25, 0.28	−0.37, 0.28
R; wR	0.025; 0.027	0.035; 0.039	0.031; 0.040

For all three structures: $T = 173$ K; Diffractometer: Rigaku AFC6S; Radiation: Cu $K\alpha$, $\lambda = 1.54178$ Å; Cell determination: 25 reflections, $40° < 2\theta < 50°$; Data collection: MSC/AFC control software [13]; Scan mode: $\omega/2\theta$; Structure solution: *SHELXS*86 [14]; Structure refinement: *TEXSAN* software package [15]; Decay correction: not required; Absorption correction: psi scans [16]; Figures: *ORTEP*II [17] and *PLUTO* [18]; $R = \Sigma(|F_{obs}| - |F_{calc}|)/\Sigma|F_{obs}|$; $wR = [\Sigma w(|F_{obs}| - |F_{calc}|)^2 / \Sigma w|F_{obs}|^2]^{1/2}$; $w = 4F_{obs}^2 / \sigma^2(F_{obs}^2)$; $R_{int} = \Sigma\Sigma| < F_i^2 > - F_{ij}^2|/\Sigma m < F_i^2 >$.

Table 2
Fractional atomic coordinates and equivalent isotropic displacement parameters (Å^2) for the non-hydrogen atoms of the 2(N)-sulfate

Atom	x	y	z	B(eq) [a]
S1	0.43716 (7)	0.80236 (4)	0.97494 (8)	1.25 (2)
NA1	0.2033 (1)	0.88281 (6)	0.6623 (1)	1.34 (4)
O1	0.4489 (2)	0.5696 (1)	0.8180 (3)	2.2 (1)
O1W	0.3694 (2)	0.8904 (1)	0.4389 (3)	1.92 (9)
O2W	0.2533 (2)	1.0192 (1)	0.6690 (3)	1.85 (9)
O3	0.1576 (2)	0.7471 (1)	0.6367 (3)	1.41 (8)
O4	0.1296 (2)	0.6193 (1)	0.3932 (3)	1.65 (8)
O5	0.5037 (2)	0.6035 (1)	0.5325 (2)	1.22 (7)
O6	0.4886 (2)	0.6021 (1)	0.1884 (3)	2.1 (1)
O7	0.5853 (2)	0.8031 (1)	0.9151 (2)	1.77 (8)
O8	0.4233 (2)	0.7966 (1)	1.1627 (2)	1.67 (8)
O9	0.3564 (2)	0.8677 (1)	0.9038 (3)	1.93 (9)
N1	0.3719 (2)	0.7191 (1)	0.9078 (3)	1.3 (1)
C1	0.4933 (3)	0.6295 (2)	0.7086 (3)	1.4 (1)
C2	0.3876 (3)	0.6978 (2)	0.7228 (3)	1.1 (1)
C3	0.2427 (3)	0.6783 (1)	0.6417 (4)	1.1 (1)
C4	0.2643 (3)	0.6472 (2)	0.4582 (4)	1.1 (1)
C5	0.3693 (3)	0.5788 (1)	0.4600 (4)	1.3 (1)
C6	0.4018 (3)	0.5476 (2)	0.2808 (4)	1.7 (1)

[a] $B(\text{eq}) = (8\pi^2/3)\Sigma_i\Sigma_j U_{ij}\mathbf{a}_i^*\mathbf{a}_j^*\mathbf{a}_i\mathbf{a}_j$.

cally. In the 3-sulfate, all the non-hydrogen atoms were refined anisotropically in a straightforward manner except for two oxygen atoms, O-1 and O-6, each of which was found to be disordered over two different positions. For both O-1 and O-6, oxygen atoms were placed in the two disordered positions and their occupancies and isotropic thermal parameters were refined. Upon convergence, the occupancies were fixed so that they summed to 1.0, and other affected atoms (such as the hydrogens bonded to these oxygens) were assigned corresponding occupancies. The more heavily populated components (O-1 and O-6) were refined anisotropically while the less populated components (O-1A and O-6A) were refined isotropically. Hydrogen atom positions in both the 2(N)-sulfate and 6-sulfate were refined. An attempt to refine hydrogen atom positions in the 3-sulfate led to unreasonable bond lengths, so hydrogens were placed in calculated positions (hydrogens bonded to carbons) or left in difference map positions (hydrogens bonded to oxygens or nitrogen) and were not refined.

Fractional atomic coordinates and equivalent isotropic displacement parameters for the non-hydrogen atoms of the three structures are given in Tables 2–4. *ORTEP*II drawings showing the atom numbering scheme for each compound are presented as Figs 1–3.

Infrared and NMR spectroscopy.—Infrared spectra of the three glucosamine sulfates were taken in KBr pellets using a Nicolet 5DXB-FTIR spectrometer. Samples used were of the commercial, non-recrystallized material. Band positions associated specifically with the sulfate group are listed in Table 5. Proton and ^{13}C NMR spectra were taken in Me_2SO-d_6 at room temperature using an IBM-Bruker 500-MHz spectrometer for the

Table 3
Fractional atomic coordinates and equivalent isotropic displacement parameters (Å^2) for the non-hydrogen atoms of the 3-sulfate

Atom	x	y	z	B(eq) [a]
S1	0.6804 (1)	0.5361	0.9687 (1)	1.49 (3)
O1	0.0687 (5)	0.7876 (6)	0.7281 (4)	1.4 (2)
O1W	0.7369 (4)	0.6218 (5)	1.3585 (3)	2.6 (1)
O1A	−0.103 (1)	0.509 (1)	0.708 (1)	2.7 (2)
O3	0.5460 (4)	0.4814 (4)	0.8249 (3)	1.6 (1)
O4	0.5719 (4)	0.7010 (5)	0.5822 (3)	2.7 (1)
O5	0.0807 (4)	0.5841 (4)	0.5442 (3)	1.7 (1)
O6	0.2338 (5)	0.4888 (5)	0.2916 (4)	2.0 (2)
O6A	0.091 (2)	0.656 (1)	0.253 (1)	1.5 (2)
O7	0.8238 (4)	0.4053 (4)	0.9728 (4)	2.6 (1)
O8	0.5694 (4)	0.5204 (6)	1.0859 (3)	2.9 (1)
O9	0.7476 (4)	0.7066 (4)	0.9474 (3)	2.1 (1)
N1	0.1868 (4)	0.5483 (6)	0.9447 (3)	1.4 (1)
C1	0.0559 (6)	0.6146 (6)	0.6915 (4)	1.5 (2)
C2	0.2135 (5)	0.5234 (6)	0.7893 (4)	1.3 (1)
C3	0.3997 (5)	0.5951 (5)	0.7611 (4)	1.3 (1)
C4	0.4148 (5)	0.5962 (6)	0.5986 (4)	1.6 (2)
C5	0.2429 (6)	0.6687 (6)	0.5069 (4)	1.7 (2)
C6	0.2451 (7)	0.6482 (9)	0.3442 (5)	3.5 (3)

[a] $B(\text{eq}) = (8\pi^2/3)\Sigma_i\Sigma_j U_{ij}\mathbf{a}_i^*\mathbf{a}_j^*\mathbf{a}_i\mathbf{a}_j$.

proton spectra and an IBM-Bruker 125-MHz spectrometer for the ^{13}C spectra. Selected peak positions are listed in Tables 6 and 7.

3. Discussion

Molecular geometry.—As noted previously, two positions are found in the 3-sulfate structure for the oxygen atom bonded to C-1 and also for the oxygen atom bonded to C-6. The disordered position of O-1/O-1A is the result of both α- and β-anomers of glucosamine 3-sulfate being present in the crystal, anomerization of the starting α-glucosamine 3-sulfate apparently having occurred during recrystallization of the X-ray sample. Based on the occupancies found for the O-1 and O-1A sites, the ratio of the α-anomer to the β-anomer in the crystal is 2:1. The simultaneous presence of both anomers has been observed in several previous carbohydrate crystal structures [19]. Other carbohydrate structures disordered at the primary hydroxyl group have also been reported [19]. In the 3-sulfate structure, the C–O bonds at the primary hydroxyl group are shorter than expected (C-6–O-6 = 1.329(7) Å and C-6–O-6A = 1.31(1) Å compared to 1.426(4) Å in the 2(N)-sulfate), and the C-1–O-1A bond (representing the β-anomer) is longer (1.45(1) Å compared to 1.390(7) Å in potassium β-D-glucopyranose 6-sulfate [3]). These discrepancies are almost certainly a consequence of the disorder.

In the analysis of the molecular geometry of cyclic monosaccharides, the O-5–C-5 bond is generally expected to be longer than the O-5–C-1 bond as a result of the

Table 4
Fractional atomic coordinates and equivalent isotropic displacement parameters (Å^2) for the non-hydrogen atoms of the 6-sulfate

Atom	x	y	z	B(eq) [a]
S1	0.3830 (1)	0.89206 (4)	0.0860 (2)	1.20 (4)
O1W	0.2187 (3)	0.7588 (1)	−0.0691 (7)	2.3 (1)
O1	0.7616 (3)	1.0860 (1)	0.4524 (6)	1.5 (1)
O3	0.6180 (3)	1.2379 (1)	−0.0162 (6)	1.5 (1)
O4	0.3901 (3)	1.1603 (1)	0.0536 (7)	1.8 (1)
O5	0.6624 (2)	1.0396 (1)	0.0862 (6)	1.3 (1)
O6	0.4610 (3)	0.9559 (1)	0.1810 (6)	1.6 (1)
O7	0.4533 (3)	0.8418 (1)	0.2285 (6)	1.8 (1)
O8	0.4022 (3)	0.8885 (1)	−0.1924 (5)	1.7 (1)
O9	0.2432 (3)	0.9005 (1)	0.1586 (6)	1.8 (1)
N1	0.8707 (4)	1.1879 (2)	0.1657 (7)	1.3 (1)
C1	0.7702 (4)	1.0784 (2)	0.1851 (8)	1.3 (2)
C2	0.7624 (4)	1.1455 (2)	0.063 (1)	1.3 (2)
C3	0.6244 (4)	1.1765 (2)	0.1109 (8)	1.1 (1)
C4	0.5164 (4)	1.1310 (2)	0.0052 (8)	1.2 (2)
C5	0.5300 (4)	1.0653 (2)	0.1412 (8)	1.2 (2)
C6	0.4297 (4)	1.0153 (2)	0.044 (1)	1.7 (2)

[a] $B(\text{eq}) = (8\pi^2/3)\Sigma_i\Sigma_j U_{ij}\mathbf{a}_i^*\mathbf{a}_j^*\mathbf{a}_i\mathbf{a}_j$.

anomeric effect [5,20]. It is interesting that in many of the sulfated monosaccharides for which crystal structures have been determined, a significant difference between these bond lengths is actually not observed (Table 8). In the three sulfated glucosamines reported here, the bond length difference is obvious in the 6-sulfate (1.447(4) vs 1.431(4) Å), less so in the 3-sulfate (1.438(5) vs 1.424(5) Å), and insignificant in the 2(N)-sulfate (1.436(3) vs 1.430(3) Å). Although it appears that the bond length difference increases with increasing distance between the sulfate group and the ring oxygen atom, too few structures are available to show conclusively that this is a general trend.

As has been observed in other sulfated monosaccharides [9], the placement of the bulky sulfate group on a formerly hydroxylic oxygen atom in these glucosamine sulfates causes a lengthening of the carbon–oxygen bond. The C-6–O-6 bond length of 1.426(4) Å in the 2(N)-sulfate becomes 1.446(5) Å in the 6-sulfate, and the C-3–O-3 bond length of 1.423(3) Å in the 2(N)-sulfate and 1.424(4) Å in the 6-sulfate becomes 1.448(5) Å in the 3-sulfate. In methyl α-D-galactopyranoside 4-sulfate, the C-4–O-4 distance is even longer at 1.467(6) Å [7], perhaps as a result of additional steric strain due to the axial orientation of this bond. In contrast, placing the sulfate group on the nitrogen atom does not lengthen the C–N bond. In fact, the C–N bond in the 2(N)-sulfate (1.477(3) Å) is as short as the C–N bond in the 6-sulfate (1.483(5) Å) and is significantly shorter than the C–N bond in the 3-sulfate (1.496(4) Å).

In these structures the length of the S–O bond in the C–O–S linkage (approximately 1.600 Å in the two cases) is significantly different from those of the terminal S–O bonds. The three terminal S–O bond lengths of approximately 1.455 Å found in all three

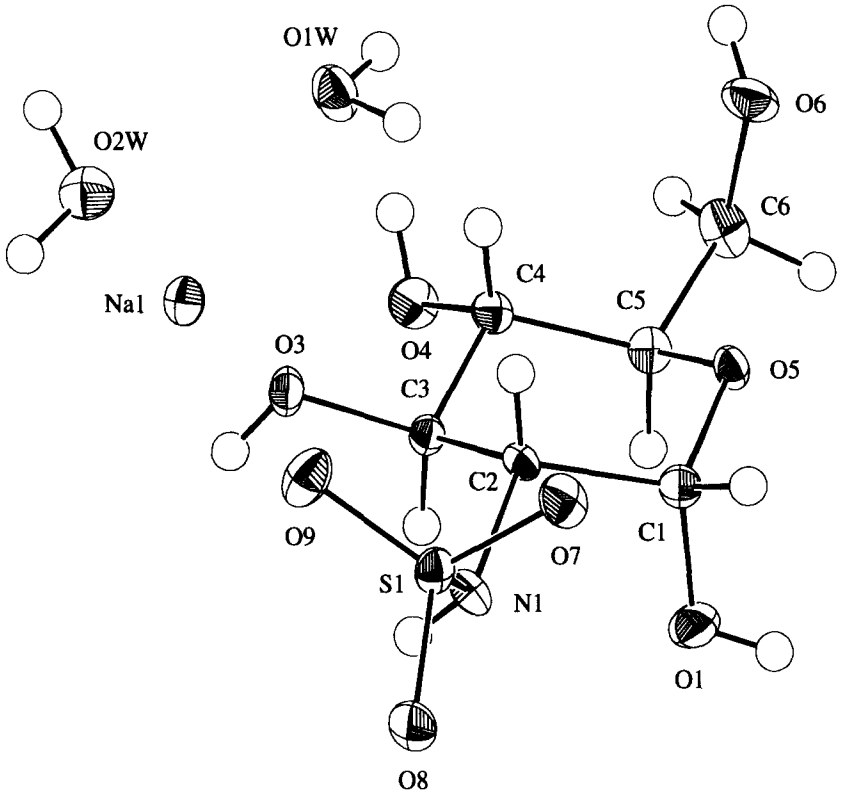

Fig. 1. *ORTEPII* drawing of the 2(N)-sulfate, showing atom numbering. For non-hydrogen atoms, 50% probability ellipsoids are shown.

of these glucosamine sulfates are typical of those found in sulfated monosaccharides, although a very short S–O bond length of 1.414(4) Å in the structure of potassium β-D-glucopyranose 6-sulfate has been reported [3]. The length of the fourth bond to the sulfur atom varies with the type of atom to which the sulfur is bonded, the S–X distance increasing as X = O, then N, then C. This bond is an S–O bond in the 3-sulfated and 6-sulfated glucosamines, in which it has lengths of 1.600(3) Å and 1.602(3) Å, respectively. In the 2-sulfated glucosamine, in which S–X is an S–N bond, the length increases to 1.637(2) Å. In the crystal structure of 2-amino-2,6-dideoxy-α-D-glucopyranoside 6-sulfonic acid, this bond is an S–C bond having a length of 1.771(7) Å [4], and in the methyl glycoside of this sugar the S–C bond has a length of 1.785(3) Å [5].

By virtue of O-5–C-5–C-6–O-6 and C-4–C-5–C-6–O-6 torsional angles of −49.1(3)° and 71.5(3)° respectively, the glucosamine 2(N)-sulfate has a *gg* side chain conformation. The glucosamine 6-sulfate, with corresponding angles of 58.5(4)° and 177.0(3)°, has a *gt* side chain conformation. The major component of the disordered 3-sulfate (having torsional angles O-5–C-5–C-6–O-6 and C-4–C-5–C-6–O-6 of −54.5(6)° and 67.5(6)°, respectively) has a *gg* side chain conformation, and the minor

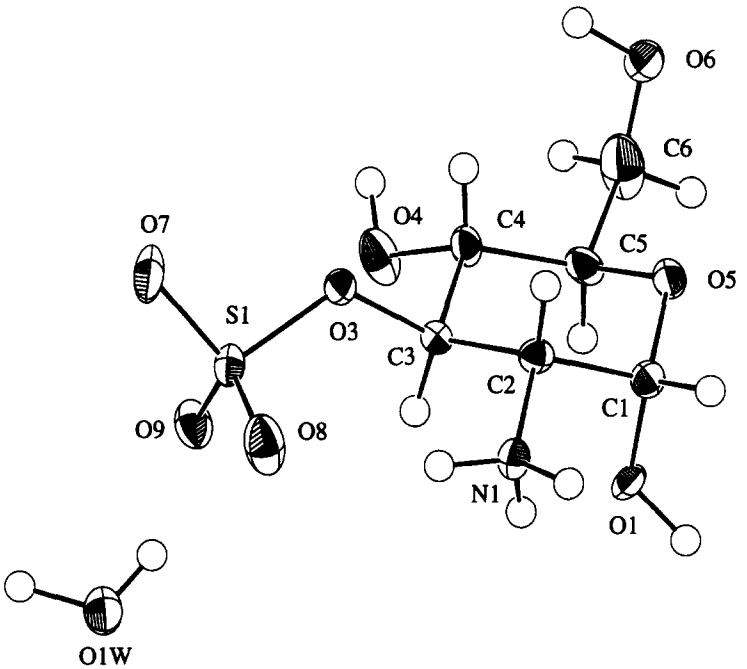

Fig. 2. *ORTEPII* drawing of the 3-sulfate, showing atom numbering. For non-hydrogen atoms, 50% probability ellipsoids are shown.

component (having torsional angles O-5–C-5–C-6—O-6A and C-4–C-5–C-6–O-6A of 37.8(9)° and 159.8(7)° respectively) has a *gt* side chain conformation [19].

In crystal structures of sulfated monosaccharides reported previously it is commonly found that the sulfate group is rotated slightly (typically 15°–20°) out of a perfectly staggered orientation with respect to the sulfate ester linkage. The three sulfated glucosamines described here follow this same trend, the sulfate group in each structure being rotated approximately 10° out of the perfectly staggered orientation. The sulfate conformations in these glucosamine sulfates vary little from structure to structure even though the crystalline environments about the respective sulfate groups are widely different. For example, the coordination of the 2(N)-sulfate group directly to a sodium ion does not make its torsional orientation much different from those assumed by the sulfate groups in the 3- and 6-sulfate structures, in which no similar interaction occurs. The fact that the immediate surroundings of the sulfate group have little influence on its conformation has been noted for other sulfated monosaccharides as well [9]. In related studies we have been pursuing the question of whether the sulfonate group, found on a variety of physiologically important molecules, exerts its biochemical influence by mimicking the sulfate group. With respect to these torsional preferences, we find that the two groups differ. The strong preference for the staggered orientation shown by the sulfate groups is not shown by sulfonate groups in other structures we have examined. For example, in a series of salts of the naphthalenedisulfonate dye Orange G, we have observed both staggered and eclipsed conformations of sulfonate groups with respect to

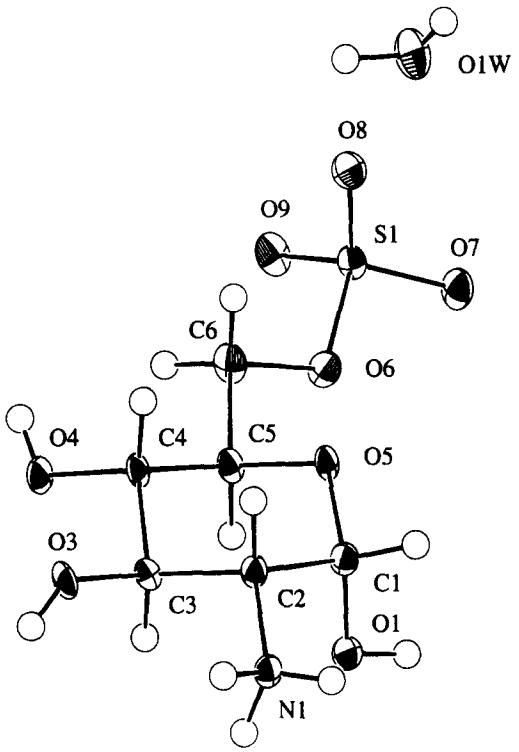

Fig. 3. *ORTEPII* drawing of the 6-sulfate, showing atom numbering. For non-hydrogen atoms, 50% probability ellipsoids are shown.

Table 5
Infrared frequencies (cm^{-1}) associated with the sulfate group (KBr pellet)

Compound	υ_{as} S=O	υ_{s} S=O	υ_{as} C–O–S
2 (N)–Sulfate	1202	1063,1032	
3 (O)–Sulfate	1242, 1207	1049,1026	830
6 (O)–Sulfate	1219	1067 (sh),1018 (st)	821

the naphthalene ring, suggesting that at least in this system the staggered orientation of the sulfonate is not greatly preferred to the eclipsed [21].

In the glucosamine 3-sulfate the location of the sulfate group on O-3 allows a close intramolecular contact with the protonated amino group on C-2. The O-8–N-1 distance is 2.923(4) Å, and the O-8–H-1N distance is 2.055(3) Å. The S-1–O-8 · · · H-1N angle is 112.1(2)°, and the N-1–H-1N · · · O-8 angle is 156.2(2)°.

All three glucosamine sulfates assume $^4C_1$ chair conformations in the crystal. Cremer–Pople puckering parameters [22,23] (calculated using the program *PLATON*-91 [24]) for the three structures are Q = 0.563(3) Å, $\theta = 3.9(3)°$, $\Phi = 296(4)°$ for the 2(N)-sulfate; Q = 0.574(4) Å, $\theta = 10.4(4)°$, $\Phi = 36(2)°$ for the 3-sulfate; and Q =

Table 6
Proton NMR peaks for the anomeric hydrogen (500 MHz, Me_2SO-d_6, 22°C)

Compound	H-1 signal
2 (N)–Sulfate	δ 5.02 (dd, J = 4.0 Hz, 1 H_{eq})
3 (O)–Sulfate	5.17 (dd, J = 4.0 Hz, H_{eq}) and 4.69 (dd, J = 9.0 Hz, H_{ax})
6 (O)–Sulfate	5.15 (dd, J = 4.0 Hz, 1 H_{eq})

Table 7
^{13}C NMR peak positions [a] (125 MHz, Me_2SO-d_6, 22°C)

Compound	C-1	C-2	C-3	C-4	C-5	C-6
2 (N)–Sulfate	91.8	58.3	71.9	71.1	72.8	61.4
3 (O)–Sulfate	99.9	53.3	76.5	72.6	68.7	60.8
6 (O)–Sulfate	89.1	54.5	69.8	70.5	70.7	65.8

[a] In ppm relative to TMS.

Table 8
O-5–C-5 and O-5–C-1 bond lengths (Å) in some sulfated monosaccharides

Compound	O-5–C-5	O-5–C-1
α-D-glucosamine 2(N)-sulfate	1.436(3)	1.430(3)
D-glucosamine-3-sulfate	1.438(5)	1.424(5)
α-D-glucosamine-6-sulfate	1.447(4)	1.431(4)
potassium β-D-glucopyranose-6-sulfate [3]	1.431(7)	1.420(7)
2-amino-2,6-dideoxy-α-D-glucopyranose-6-sulfonic acid [4]	1.430 [a]	1.420 [a]
methyl 2-amino-2,6-dideoxy-α-D-glucopyranoside-6-sulfonic acid [5]	1.443(4)	1.415(4)
methyl α-D-galactopyranoside-3-(sodium sulfate)-monohydrate [6]	1.428(6)	1.421(5)
methyl α-D-galactopyranoside-4-(sodium sulfate)-dihydrate [7]	1.437(5)	1.431(5)
methyl α-D-galactopyranoside-2,6-bis(sodium sulfate)-dihydrate [8]	1.44(2)	1.43(1)

[a] Max. esd's are 0.007 Å.

0.603(4) Å, $\theta = 2.1(4)°$, $\Phi = 256(6)°$ for the 6-sulfate. Asymmetry parameters ΔC_S are 3.40(2) at C-2 for the 2(N)-sulfate, 6.40(4) at C-3 for the 3-sulfate, and 2.10(3) at C-2 for the 6-sulfate [25]. The 3-sulfate shows the greatest deviation from a perfect chair conformation and the 6-sulfate shows the least, consistent with the idea that the bulky sulfate group causes less distortion of the ring when it is located at the remote C-6 position. Another measure of the distortion effected upon the ring by the presence of the sulfate group is the chi-squared value associated with the C-2–C-3–C-5–O-5 reference plane. For the 2(N)-sulfate this value is 389.4, and for the 3-sulfate it is 227.6. For the 6-sulfate, in which an intervening carbon atom moves the sulfate away from the ring, chi-squared for the reference plane is only 38.8, indicating a significantly higher degree of planarity. Attachment of a sulfate directly to the ring evidently causes considerable deviation from the perfectly coplanar arrangement of C-2, C-3, C-5, and O-5 expected for an undistorted chair conformation.

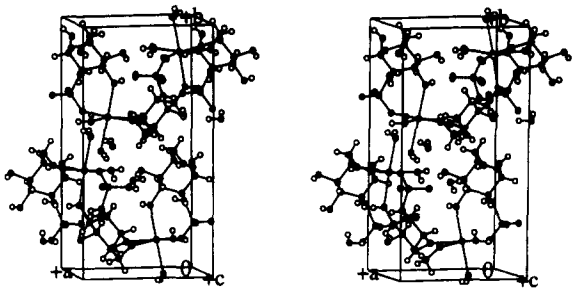

Fig. 4. *PLUTO* stereoview of the packing arrangement in the 2(N)-sulfate, showing coordination of the sodium ions.

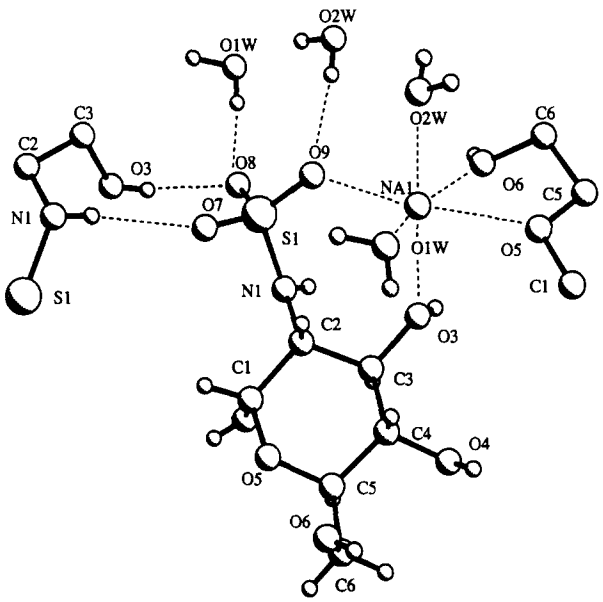

Fig. 5. *PLUTO* view of the crystalline environment of the sulfate group in the 2(N)-sulfate, showing close intermolecular contacts (see text and Table 9 for distances).

Packing arrangements and intermolecular interactions.—In considering the packing arrangements assumed by these three sulfated glucosamines, we choose to focus on the intermolecular contacts made by the sulfate groups because of their probable resemblance to contacts made by the sulfate groups of large GAG molecules. In living systems the GAG sulfate groups interact with proteins, water molecules, and inorganic ions; a model for a direct GAG sulfate–inorganic ion interaction is found in the crystal structure of the glucosamine 2(N)-sulfate (Figs 4 and 5), where O-9 of the sulfate group is one of six oxygen atoms coordinated directly to the sodium ion. (The additional oxygen atoms are those from the two water molecules, hydroxyl oxygen O-3 from the same glucosamine molecule as sulfate oxygen O-9, and ring oxygen O-5 and hydroxyl oxygen

Table 9
Hydrogen bonds and other intermolecular contacts

	2(N)-Sulfate			
	To sulfate oxygen atoms			
Acceptor · · · H-Donor	A · · · D (Å)	H–D (Å)	A · · · H (Å)	A · · · H–D (°)
O-7 · · · N-1 [a]	3.026(3)	0.75(3)	2.28(3)	171(3)
O-8 · · · O-1W [b]	2.712(3)	0.80(3)	1.91(3)	173(3)
O-8 · · · O-3 [a]	2.779(3)	0.78(3)	2.00(3)	170(3)
O-9 · · · Na-1 [c]	2.358(2)			
O-9 · · · O-2W [d]	2.995(3)	0.84(3)	2.17(3)	170(3)
	To other atoms			
Acceptor · · · H-Donor	A · · · D (Å)	H–D (Å)	A · · · H (Å)	A · · · H–D (°)
O-1W · · · O-2W [e]	2.834(3)	0.86(3)	1.99(3)	167(3)
O-2W [f] · · · O-1	2.911(3)	0.77(3)	2.23(3)	148(3)
O-1 · · · O-6 [b]	2.927(3)	0.74(3)	2.29(3)	145(3)
O-1W [g] · · · O-4	2.755(3)	0.83(3)	3.15(3)	55(2)
	3-Sulfate [x]			
	To sulfate oxygen atoms			
Acceptor · · · H-Donor	A · · · D (Å)	H–D (Å)	A · · · H (Å)	A · · · H–D (°)
O-7 · · · O-1 [h]	2.925(5)	0.93	2.98	78
O-7 · · · O-1A [i]	2.706(9)	H not found (disordered oxygen)		
O-7 · · · O-6A [j]	2.99(1)	H not found (disordered oxygen)		
O-7 · · · N-1 [h]	2.881(5)	0.81	2.12	157
O-7 · · · N-1 [i]	2.919(4)	0.85	2.11	159
O-8 · · · O-1W [c]	2.768(4)	0.86	1.93	168
O-9 · · · N-1 [k]	2.854(5)	0.92	2.57	98
	To other atoms			
Acceptor · · · H-Donor	A · · · D (Å)	H–D (Å)	A · · · H (Å)	A · · · H–D (°)
O-6[1] · · · O-1	2.690(6)	0.93	1.77	171
O-3 [m] · · · O-1A	2.92(1)	H not found (disordered oxygen)		
O-1A · · · O-4 [m]	2.91(1)	0.99	2.89	81
O-1A · · · O-6A [n]	2.77(2)	H not found (disordered oxygen)		
O-1W[o] · · · O-4	2.617(4)	0.99	1.64	175
O-4 · · · O-6 [p]	2.813(5)	0.99	1.90	152
O-5 · · · O-1W [q]	2.855(4)	1.02	2.05	134
O-6A · · · O-1W [q]	2.90(1)	1.02	2.04	141
	6-Sulfate			
	To sulfate oxygen atoms			
Acceptor · · · H-Donor	A · · · D (Å)	H–D (Å)	A · · · H (Å)	A · · · H–D (°)
O-7 · · · O-3 [r]	2.699(4)	0.71(4)	2.00(4)	169(5)
O-7 · · · N-1 [s]	2.927(5)	0.84(4)	2.58(4)	106(4)
O-8 · · · N-1 [t]	2.846(5)	0.84(4)	2.02(4)	167(4)
O-9 · · · O-1W [c]	3.152(4)	0.88(5)	2.65(4)	117(4)
O-9 · · · O-4 [d]	2.741(4)	0.77(5)	2.02(5)	156(5)
	To other atoms			
Acceptor · · · H-Donor	A · · · D (Å)	H–D (Å)	A · · · H (Å)	A · · · H–D (°)
O-5 [s] · · · O-1	2.777(4)	0.87(4)	1.93(4)	164(4)
O-3 · · · O-1W [u]	2.728(5)	0.82(5)	1.91(5)	172(5)
O-3 · · · N-1 [v]	2.995(5)	0.95(4)	2.46(4)	116(3)
O-4 · · · O-1W [d]	2.786(4)	0.88(5)	2.09(5)	136(4)
O-1W [w] · · · N-1	2.700(5)	0.91(4)	1.81(5)	165(4)

Symmetry Code: [a] $= 1/2+x,\ 3/2-y,\ 2-z$; [b] $= x,\ y,\ 1+z$; [c] $= xyz$; [d] $= 1/2-x,\ 2-y,\ 1/2+z$; [e] $= 1/2-x,\ 2-y,\ -1/2+z$; [f] $= 1-x,\ -1/2+y,\ 3/2-z$; [g] $= -1/2+x,\ 3/2-y,\ 1-z$; [h] $= 1-x,\ -1/2+y,\ 2-z$; [i] $= 1+x,\ y,\ z$; [j] $= 1-x,\ -1/2+y,\ 1-z$; [k] $= 1-x,\ 1/2+y,\ 2-z$; [l] $= -x,\ 1/2+y,\ 1-z$; [m] $= -1+x,\ y,\ z$; [n] $= -x,\ -1/2+y,\ 1-z$; [o] $= x,\ y,\ -1+z$; [p] $= 1-x,\ 1/2+y,\ 1-z$; [q] $= -1+x,\ y,\ -1+z$; [r] $= 1-x,\ -1/2+y,\ 1/2-z$; [s] $= 3/2-x,\ 2-y,\ 1/2+z$; [t] $= 3/2-x,\ 2-y,\ -1/2+z$; [u] $= 1-x,\ 1/2+y,\ -1/2-z$; [v] $= -1/2+x,\ 5/2-y,\ -z$; [w] $= 1-x,\ 1/2+y,\ 1/2-z$.

[x] Hydrogen atoms were left in difference map positions and were not refined.

O-6 of a second glucosamine molecule related to the first by a crystallographic screw axis. Coordination distances and angles assume expected values.) Moreover, in this structure the sulfate group also interacts with the sodium ion by means of bridging water molecules; sulfate oxygens O-8 and O-9 are in contact with water oxygens O-1W and O-2W, respectively, which are both in direct contact with the sodium ion. Thus the sulfate group is in contact with the inorganic ion both *directly* through the O-9–Na-1 contact and *indirectly* through these water-mediated O-8 · · · O-1W · · · Na-1 and O-9 · · · O-2W · · · Na-1 contacts. Distances for these and additional intermolecular contacts for all three crystal structures are listed in Table 9.

Sulfate oxygen O-7 of the glucosamine 2(N)-sulfate does not participate in either direct or indirect coordination of the sodium ion. On the other hand, it does lie 3.026(3) Å from the sulfamide nitrogen of a neighboring screw-related molecule (located at $1/2 + x$, $3/2 - y$, $2 - z$). Finding that a sulfate oxygen approaches the sulfamide N-H suggests that the sulfate groups of glycosaminoglycans may be attracted similarly to the amide backbone N-H groups of peptides and proteins. This contact in the 2(N)-sulfate may be facilitated by an additional one between the sulfate group and the same neighboring molecule, an O-8 to O-3 approach of 2.779(3) Å. The sulfate group thus interacts with the neighboring molecule through a two-pronged contact, a sulfate "bridging" contact in which the H-1N–N-1–C-2–C-3–O-3–H-3O group of the neighboring molecule is spanned by the sulfate's O-7–S-1–O-8 group. Although this is the only example of a sulfate-bridging interaction we have found in these three sulfated glucosamines, we have found similar sulfonate bridging interactions in the crystal structures of the salts of the sulfonated azo dye Orange G noted previously [21] and in the structures of cocrystals of Orange G with the nucleic acid bases adenine and cytosine [26].

In the glucosamine 3-sulfate, the location and orientation of the sulfate group enable a close intramolecular contact to be made to the protonated amino group located on C-2. This type of contact may be a structural feature sufficiently distinctive to be important in the recognition of the analogous GAG fragment by a peptide or protein. The sulfate oxygens in this structure are also involved in intermolecular contacts to protonated amino groups (Figs 6 and 7). While O-8 interacts with the $-NH_3^+$ in the same molecule, O-9 interacts with a protonated amino group in a neighboring molecule (2.854(5) Å to

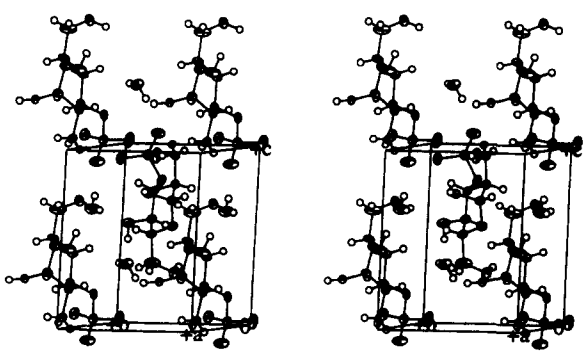

Fig. 6. *PLUTO* stereoview of the packing arrangement in the 3-sulfate.

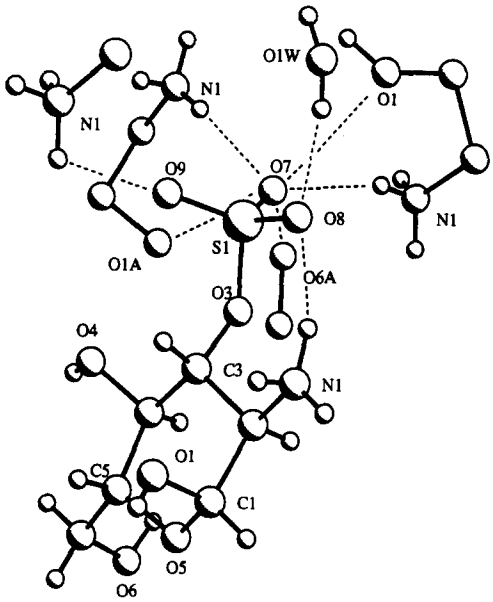

Fig. 7. *PLUTO* view of the crystalline environment of the sulfate group in the 3-sulfate, showing close intermolecular contacts (see text and Table 9 for distances).

N-1 of the molecule at $1-x$, $1/2+y$, $2-z$), and O-7 interacts with $-NH_3^+$ groups of two neighboring molecules (2.881(5) Å to N-1 of the molecule at $1-x$, $-1/2+y$, $2-z$; 2.919(4) Å to N-1 of the molecule at $1+x$, y, z). Of the sulfate oxygen atoms in the 3-sulfate structure, O-7 is unique in that it is the only one that comes into contact with the disordered atoms at the anomeric and primary hydroxyl positions. In fact, it is striking that none of the sulfate oxygen atoms in this sugar come into close, direct contact with an ordered hydroxyl group.

In contrast, O-7 in the glucosamine 6-sulfate structure closely approaches the O-3 hydroxyl group of a neighboring molecule (2.699(4) Å to O-3 of the molecule at $1-x$, $-1/2+y$, $1/2-z$). In fact, with the exception of the O-9 $\cdots$ Na-1 direct coordination approach in the 2-sulfate structure, this is the closest intermolecular approach to a sulfate oxygen observed in these three glucosamine sulfates. In the 6-sulfate structure, there is an additional sulfate oxygen contact to a hydroxyl and two contacts to protonated amino groups (Figs. 8 and 9). A feature that sets the 6-sulfate structure apart from the 2(N)- and 3-sulfate structures is the absence in the 6-sulfate of a close contact between a sulfate oxygen atom and a water molecule. In each of the other two structures there is at least one sulfate oxygen–water oxygen approach shorter than 2.8 Å, but the closest approach between a sulfate oxygen and a water molecule in the 6-sulfate structure is the 3.152(4) Å approach between O-9 and the water oxygen O-1W.

Infrared and NMR spectroscopy.—The use of spectroscopic techniques for identifying the sulfation pattern and the axial-equatorial orientation of the sulfate groups of sulfated sugars has been a topic of interest for many years [27,28]. Spectroscopy is of particular usefulness and importance in characterizing the substances obtained in the

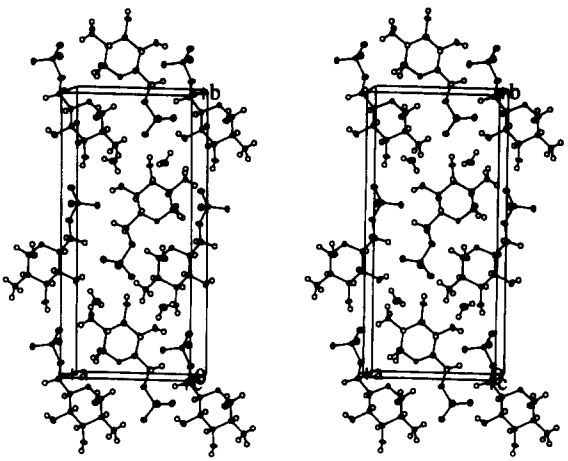

Fig. 8. *PLUTO* stereoview of the packing arrangement in the 6-sulfate.

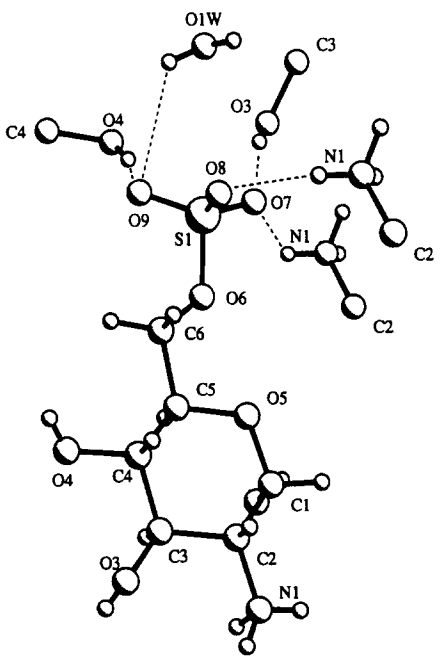

Fig. 9. *PLUTO* view of the crystalline environment of the sulfate group in the 6-sulfate, showing close intermolecular contacts (see text and Table 9 for distances).

synthesis of artificial glycosaminoglycans, where the resulting polysulfated polysaccharides are not usually crystalline or even homogeneous. Diagnostic IR band positions for sulfated mono- and polysaccharides have been summarized by Turvey [27]. According to these guidelines, sulfated sugars and polysaccharides give a band at about 1250 cm^{-1}

corresponding to an S–O stretching vibration, while the sulfate group's C–O stretching vibration appears in the 1030–1050 cm^{-1} region. Additional bands, attributable to the C–O–S vibration, appear in the 815–860 cm^{-1} region. In the spectra of hexoses a band at 810–820 cm^{-1} is characteristic of a 6-sulfate (with the sugar in the C1(D) conformation). A band found in the 850–860 cm^{-1} region indicates an axial secondary sulfate, and a band at about 830 cm^{-1} indicates an equatorial secondary sulfate. The band positions we find in the IR spectra of our three glucosamine sulfates are in good agreement with these guidelines (Table 5). On the other hand, we have found the NMR spectra of these three sulfated glucosamines to be rather complex. It has been reported that in spite of the complexity of carbohydrate NMR spectra, the peak position for the anomeric proton is often readily identified and can provide useful diagnostic information [29]. In addition, it has been observed that sulfation of the primary hydroxyl group of an aminated amylose causes a downfield shift of approximately 6 ppm in the position of the ^{13}C NMR peak for C-6 [30], suggesting that a downfield shift of roughly this magnitude may be diagnostic for the position of sulfation. We list in Table 6 the NMR data for the anomeric protons of our three glucosamine sulfates. In Table 7 are listed the ^{13}C NMR peak positions for the carbon atoms of these three compounds. The peak position for C-2 of the 2(N)-sulfate is located roughly 5 ppm downfield from its position in the 3- and 6-sulfates. This downfield shift in peak position due to sulfation at the corresponding carbon atom is also observed in the 3-sulfate and 6-sulfate. This information may prove useful to researchers interested in spectroscopic identification of the products they obtain in the preparation of GAG analogues.

Acknowledgements

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