Syracuse, New York. We thank S. Rumsey for aid with the molecular graphics and Y. C. Meinwald, G. Némethy, L. Piela, and S. Talluri for helpful discussions.

Registry No. 2,4-MePro-OBzl·TsOH, 103794-05-4; Ac-2,4-MePro-NHMe, 103794-02-1; Ac-2,4-MePro-OBzl, 103794-06-5; Ac-L-Tyr-2,4-MePro-NHMe, 103794-03-2; 2,4-MePro-NHMe, 103794-07-6; Boc-L-Tyr, 3978-80-1; Boc-L-Tyr-2,4-MePro-NHMe, 103794-08-7; L-Tyr-2,4-MePro-NHMe, 103794-09-8; 2,4-methanoproline, 73550-56-8; methylamine, 74-89-5.

Evidence for Conformational Equilibrium of the Sulfated L-Iduronate Residue in Heparin and in Synthetic Heparin Mono- and Oligosaccharides: NMR and Force-Field Studies

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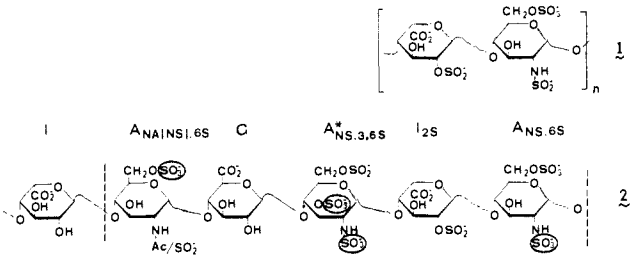
Abstract: The conformation of sulfated L-iduronic acid (I_{2S}) in different heparin sequences, including the specific pentasaccharide sequence representing the binding site to antithrombin III (AT-III), was investigated by ^1H NMR spectroscopy on suitable synthetic mono- and oligosaccharides. For the monomer methyl 2-*O*-sulfo-α-L-iduronate vicinal interproton coupling constants are all small (1.8–3.4 Hz), accounting for a predominant contribution of a $^1C_4$ chair. By contrast, some couplings (especially $J_{2,3}$) become larger (up to 6 Hz) when I_{2S} is inserted between two N,6-disulfated D-glucosamine residues as occurring in the regular sequences of heparin, and even larger (up to 7.5 Hz) when I_{2S} is glycosylated by the *N*,3,6-trisulfated D-glucosamine residue typical of the binding site to AT-III. The interproton coupling constants of the individual, nearly isoenergetic conformers of I_{2S} ($^1C_4$, $^2S_0$, and $^4C_1$) were evaluated for different heparin sequences by using molecular geometries obtained by a force field method. Other conformations were discarded on the basis of energy considerations. The conformer populations were obtained by least-squares fitting the average computed coupling constants of the conformer mixture to the observed values. When I_{2S} is part of regular heparin sequences, the skew-boat form $^2S_0$ becomes an important contributor (~40%) to the conformation of the sulfated iduronate residues. When the amino sugar residue glycosylating the I_{2S} is trisulfated (as in the binding site to AT-III), $^2S_0$ becomes predominant (>60%). Force field calculations suggest that such a drive toward the $^2S_0$ conformation is associated with electrostatic effects of the unique 3-sulfate group.

Heparin, a sulfated polysaccharide belonging to the class of glycosaminoglycans, widely distributed in animal tissues, is currently used in therapy as an anticoagulant and antithrombotic.[1] Heparin acts mainly by binding to antithrombin III (AT-III) and enhancing the inhibitory effect of this protein on a number of procoagulant proteases.[2,3] Other biological activities of heparin are associated with less specific but strong interactions with plasma proteins.[4]

The structure of heparin is largely accounted for by regular sequences of the trisulfated disaccharide **1** {(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranose)}. However, heparin also contains irregular sequences, including a unique one, the hexasaccharide **2** {*O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-2-deoxy [*or* 2-deoxy-2-sulfamido]-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-3,6-di-*O*-sulfo-α-D-glucopyranosyl-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfoamido-6-*O*-sulfo-α-D-glucopyranose)}.[5-8] Sequence **2** contains the structure responsible for binding to AT-III, i.e., the pentasaccharide sequence $A_{NA/NS,6S}$-G-$A^*_{NS,3,6S}$-I_{2S}-$A_{NS,6S}$ (between dashed lines in formula **2**).[9-14] Among the sulfate groups

Chart I



essential for binding to AT-III (encircled in formula **2**),[11] the unique 3-*O*-sulfo group of residue $A^*_{NS,3,6S}$ plays a critical role

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‖ Institut Choay.

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(9) Abbreviations used: I = α-L-iduronic acid; G = β-D-glucuronic acid; I_{2S} = 2-sulfate-α-L-iduronate; $A_{NA,6S}$ = *N*-acetyl-α-D-glucosamine 6-sulfate; $A_{NS,6S}$ = α-D-glucosamine *N*,6-disulfate; $A^*_{NS,3,6S}$ = α-D-glucosamine *N*,3,6-trisulfate; AT-III = antithrombin III.

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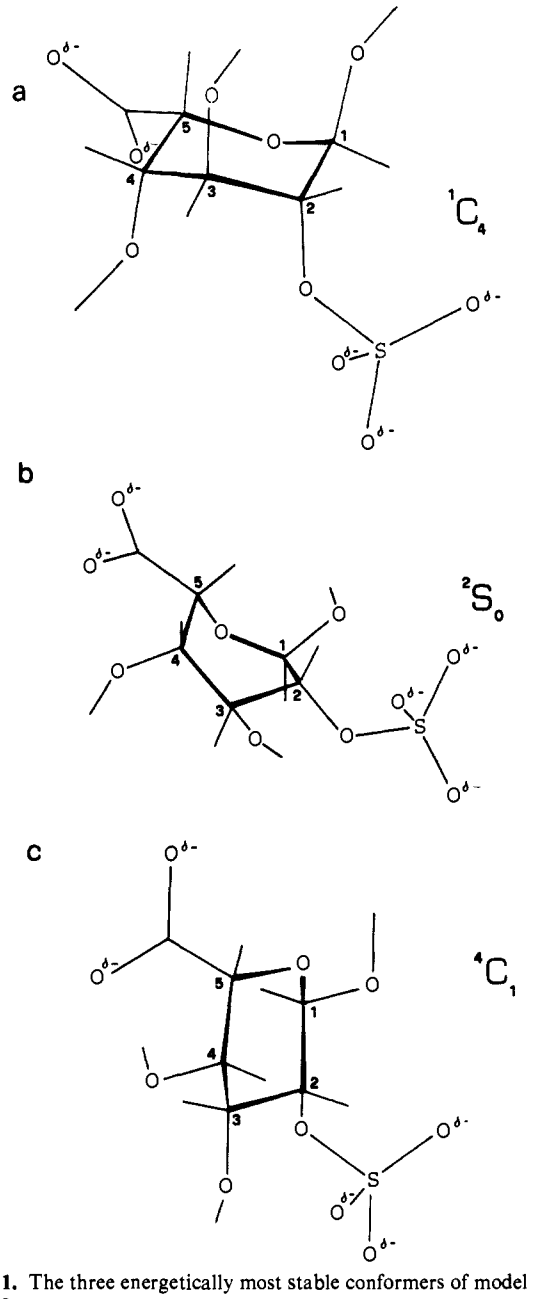

Figure 1. The three energetically most stable conformers of model compound **8**.

in determining the anticoagulant properties of heparin.[14]

^{1}H NMR studies on the foregoing pentasaccharide reproducing the structure of the minimal binding site to AT-III indicated that the conformation of the amino sugar residue $A^*_{NS,3,6S}$ is substantially similar to that of other amino sugar residues in the "active" pentasaccharide as well as in the regular regions of heparin.[15,16] The possibility remained that the 3-*O*-sulfo group affects the conformation of the pentasaccharide either by inducing substantial changes in the torsional angles (φ and ψ, as defined in ref 17) between adjacent residues, or by changing the conformation of the adjacent I_{2S} residue, or both.

The conformation of L-iduronic acid residues in glycosaminoglycans is a matter of controversy.[18,19] Whereas the most common monosaccharide residues in oligo- and polysaccharide chains invariably settle either in the chair form $^1C_4$ (Figure 1a) or in the alternative one ($^4C_1$, Figure 1c) predicted on the basis of steric interactions,[17] neither of these two forms can be rejected on the same ground for L-iduronic acid.[20] A broad discrimination between the two chair forms was possible through ^{1}H NMR studies, which ruled out a substantial contribution of the $^4C_1$ form because of the small vicinal interproton coupling constants observed for both the sulfated L-iduronic acid residues in the regular regions of heparin[21] and the nonsulfated residues in dermatan sulfate.[22] The coupling constants were also not compatible with a previously proposed skew-boat form ($^1S_3$).[23] However, some of the coupling constants (especially $J_{2,3}$) were somewhat larger than predicted for the $^1C_4$ form, thus leading to the proposal of a slightly distorted $^1C_4$ conformation as statistically depicting the shape of I_{2S} residues in the regular regions of heparin (sequences **1**).[21] Remarkably larger $J_{2,3}$ values (up to 7.5 Hz) were recently observed for the I_{2S} residue of the synthetic pentasaccharide **6** corresponding to the binding sequence of heparin to AT-III.[15] Coupling data combined with preliminary force field calculations suggested the skew-boat $^2S_0$ (Figure 1b) as a contributor to the conformation of I_{2S} residues in heparin sequences.[15,16] The contribution of skewed forms (including $^2S_0$) was subsequently proposed for a nonsulfated L-iduronate residue at the nonreducing end of a heparan sulfate tetrasaccharide.[24]

The availability, through chemical synthesis, of methyl 2-*O*-sulfo-α-L-iduronate (**3**)[25] and of the heparin oligosaccharides **4**–**7** containing an I_{2S} residue linked to either a disulfated ($A_{NS,6S}$) or a trisulfated ($A^*_{NS,3,6S}$) 2-amino-2-deoxy-α-D-glucose (D-glucosamine) residue[26,27] offered the unique opportunity of investigating the influence of sequence on the conformation of I_{2S} residues.

In this work, the interproton coupling constants were calculated from mono- and bidimensional 300- and/or 500-MHz spectra for the monosaccharide **3**, the trisaccharide **4**, the tetrasaccharide **5**, and the pentasaccharides **6** and **7**, the latter differing from **6** in lacking the unique 3-*O*-sulfo group in the central amino sugar residue and having practically no affinity for AT-III. In order to rationalize the experimental results, data were compared with sets of values predicted for molecular models with I_{2S} residue in the $^1C_4$, $^2S_0$, and $^4C_1$ conformation, as derived from force field calculations accounting for the contribution of polar interactions. The results clearly indicate that the I_{2S} residues are in conformational equilibrium between the $^1C_4$ and $^2S_0$ forms and that the ratio of these two forms is indeed a function of sequence.

Experimental Section

Materials. Compounds **3**–**7** were synthesized as reported elsewhere.[25-27]

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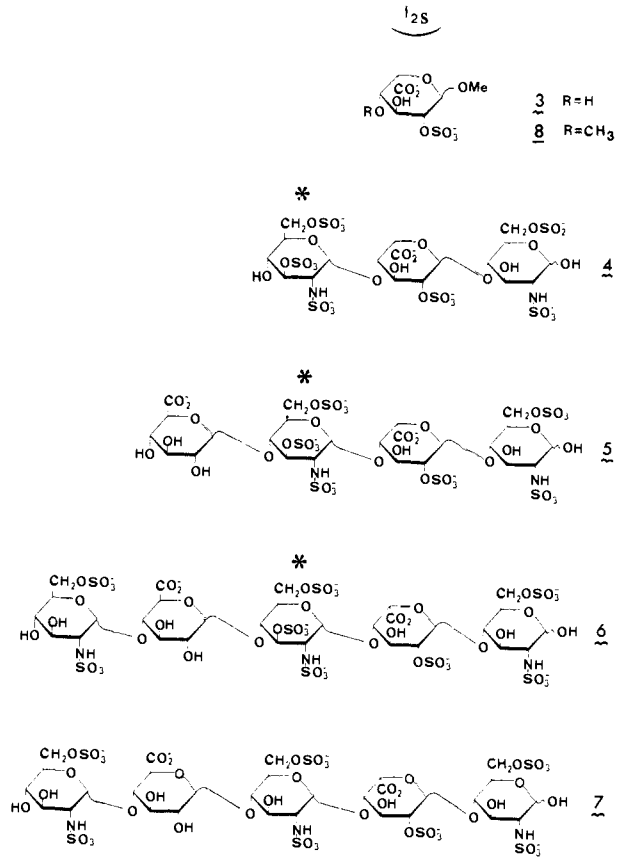
Chart II



NMR Spectroscopy. ^{1}H NMR spectra were obtained at 500 MHz for compound **6**, and at 300 MHz for compounds **3–5** and **7**, on Bruker spectrometers (WH-500 and CPX-300, respectively), on ~5% (w/v) solutions in D_2O at 23 °C. Chemical shifts were measured with reference to internal TSP. Coupling constants were calculated from spectra simulated with the PANIC.84 version of the LAOCOON computer program. Signal assignments were made by homonuclear spin-decoupling and confirmed by bidimensional (COSY) experiments.

Method of Calculation. The conformational structures of monosaccharide **3** and of the compounds used as models for **6** and **1** were computed by using the UNIVAC version of REFINE,[28] a computer program for energy minimization of macromolecules in Cartesian coordinates. The energy calculations were accomplished by means of a force field derived from Allinger's MM2 program,[29] which among other energy terms includes a contribution representing the so-called exo-anomeric effect. Details concerning the parameters of the force field are as reported in a recent study of the conformational characteristics of monosaccharide methyl 4-*O*-methyl-2-*O*-sulfo-α-L-idopyranosiduronate (**8**).[20] Here the electrostatic energy was computed by taking the value of 3 for the effective dielectric constant, and a net charge of 0.3 e was assumed for the polar groups COO^- and OSO_3^-.

The calculations for **3** were performed starting from previous results.[20] For each of the three forms $^1C_4$, $^2S_0$, and $^4C_1$, the molecular geometry was averaged over the values corresponding to the most stable structures differing for the local orientation of functional (mainly OH) groups.

For compounds **6** and **1**, the trisaccharides $A^*_{NS,3,6S}$-I_{2S}-$A_{NS,6S}$ and $A_{NS,6S}$-I_{2S}-$A_{NS,6S}$ were utilized as model compounds, with both ends of the chain assumed to be methylated for simulating glycosylation (with saccharide residues). Extensive computations were first performed on dimers $A^*_{NS,3,6S}$-I_{2S} and I_{2S}-$A_{NS,6S}$: energy maps $E(\varphi,\psi)$, where each point represents the minimum energy with respect to all the other degrees of freedom for given values of the interglycosidic pair of dihedral angles, were computed for each form of the two disaccharides. The minima found in these maps were then utilized for building molecular models of $A^*_{NS,3,6S}$-I_{2S}-$A_{NS,6S}$, whose energy again was minimized. The energy calculations for obtaining the structures of $A_{NS,6S}$-I_{2S}-$A_{NS,6S}$ were performed by removing the 3-*O*-sulfo group from the corresponding models of **6** and again minimizing the molecular energy. The final structures adopted for the two trisaccharides correspond to the lowest energy minimum for each of the three conformers of I_{2S} ($^1C_4$, $^2S_0$, and $^4C_1$).

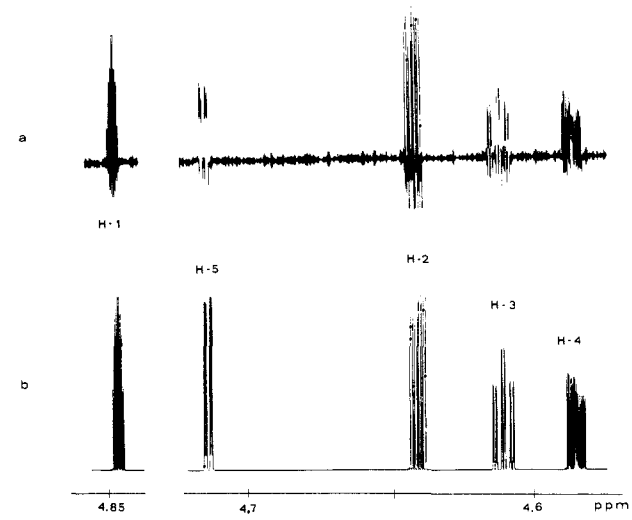


Figure 2. Experimental (a) and simulated (b) ^{1}H NMR spectra of monosaccharide **3** (300 MHz).

Table I. Chemical Shifts (δ) and Interproton Coupling Constants (^3J, Hz) for the I_{2S} Residue in Monosaccharide **3** and in Different Heparin Sequences

	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5
3[a]	4.85 (1.76)	4.64 (3.34)	4.62 (3.44)	4.59 (2.22)	4.72
4[b]	5.21 (3.5)	4.36 (8.3)	nd (4.2)	nd (3.0)	4.80
5[b]	5.19 (3.75)	4.32 (8.1)	4.21 (nd)	4.19 (3.0)	4.78
6[a]	5.19 (3.95)	4.32 (7.54)	4.17 (3.56)	4.15 (3.13)	4.77
7[b]	5.24 (2.8)	4.33 (6.1)	4.20 (3.3)	4.11 (3.3)	4.81
1[a,c]	5.22 (2.64)	4.35 (5.90)	4.20 (3.44)	4.11 (3.09)	4.82

[a] Refined values, from experimental data at 23 °C. [b] First-order values. [c] From ref 21.

Results and Discussion

NMR Spectra. Figure 2 shows the experimental and simulated ^{1}H NMR spectrum of the monomer **3**. The coupling constants between vicinal protons (^3J) as calculated from the simulated spectrum (given in Table I) are small (2.6 ± 0.9 Hz). **3** also shows two long-range (^4J) couplings larger than 1 Hz ($J_{1,3}$ = 1.21 Hz; $J_{2,4}$ = 1.02 Hz). Figure 3 compares the partial ^{1}H NMR experimental and simulated spectra of the pentasaccharide **6**. The figure also shows simulated spectra for individual residues. The ^3J values for the I_{2S} residue of **3** are compared in Table I with the corresponding values for sequences **4–7** and **1**. The sets of coupling constants for I_{2S} in **3**, **1**, and **6** remarkably differ from each other, especially for $J_{1,2}$ and $J_{2,3}$ (**3**: 1.76 and 3.34 Hz; **1**: 2.64 and 5.90 Hz; **6**: 3.95 and 7.54 Hz). The ^3J values for **3** strongly indicate the conformation $^1C_4$, also suggested by the long-range coupling between H-1 and H-3, as well as between H-2 and H-4 (^4J couplings reported for carbohydrates systems with a "W" arrangement of equatorial protons vary in magnitude form 1.2 to 1.6 Hz[30]). Here the $^4C_1$ conformation is practically ruled out by the ^3J and ^4J values. The ^3J values for **1** and (especially) for **6**, though still compatible with a $^1C_4$-like conformation and also ruling out a substantial contribution from $^4C_1$ conformers, are indicative either of some distorsion of the $^1C_4$ chair or of a contribution from another conformer.

Force Field Calculations. In order to rationalize the observed vicinal coupling constants the equilibrium between different conformers of the iduronate moiety was assumed, and the empirical generalization of the Karplus equation proposed by

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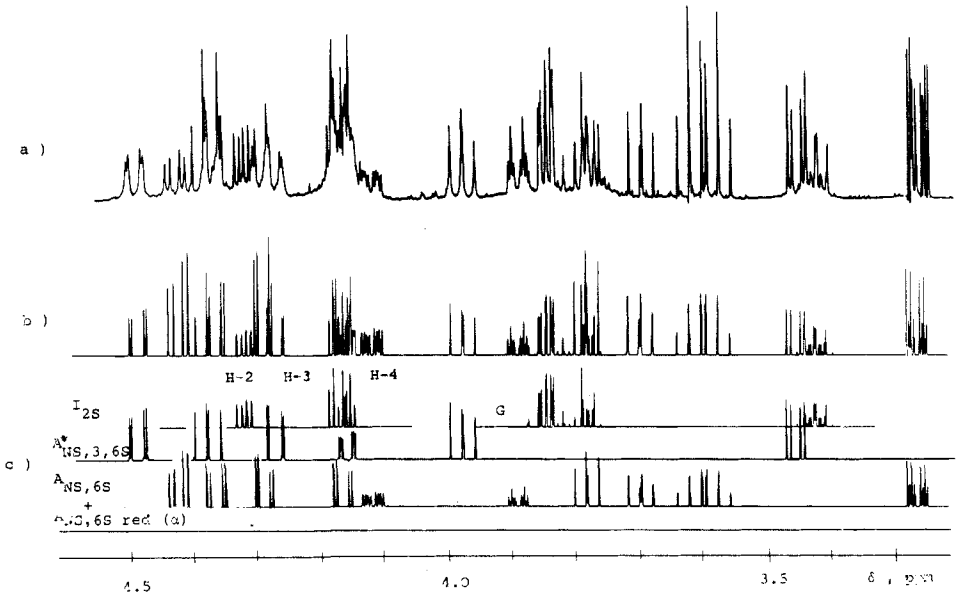

Figure 3. Experimental (a) and simulated (b) ^1H NMR spectra (500 MHz) of pentasaccharide **6**. Simulated spectra are also shown (c) for individual residues. Signals of anomeric hydrogens are omitted for simplicity.

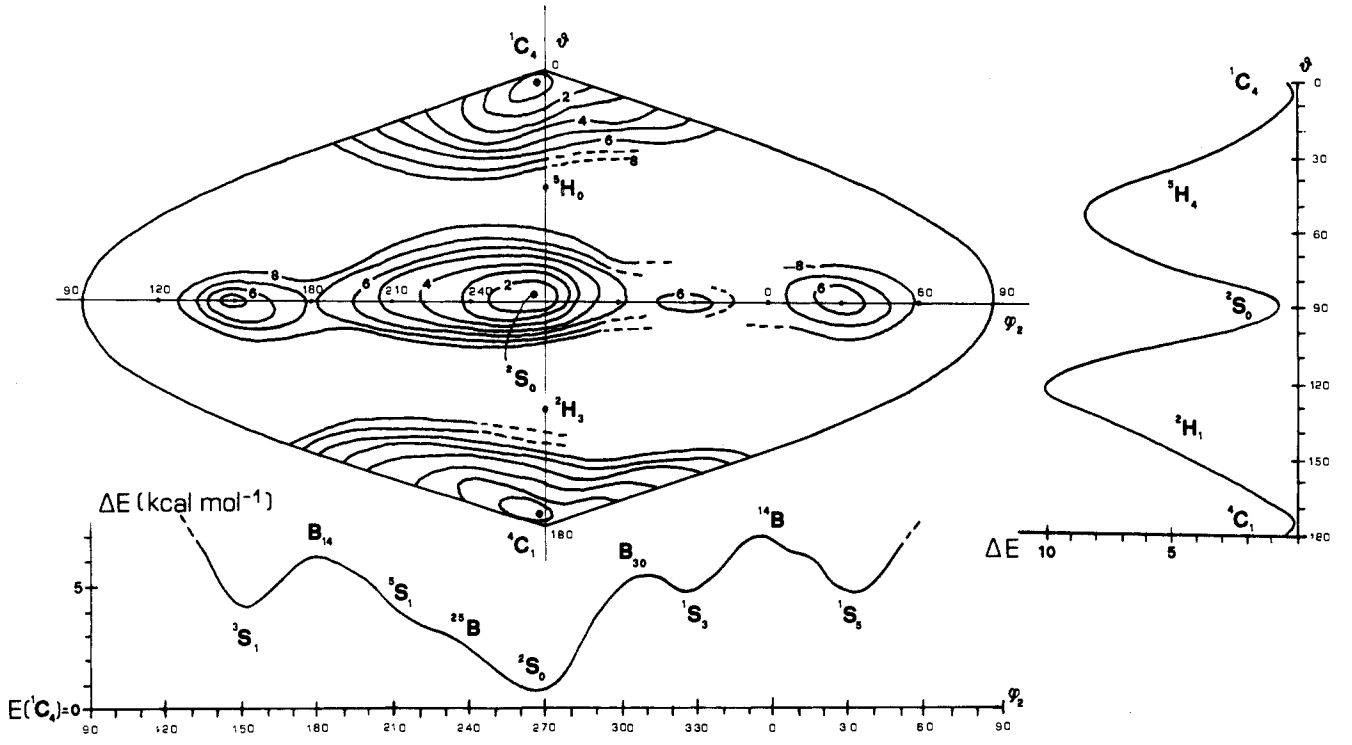

Figure 4. Results of energy computations on model compound **8**: equivalent projection of the energy map (the spherical surface represents the ring conformations as defined in ref 32) and energy profiles along the equatorial (pseudorotational) path (drive on φ_2) and along the minimal energy path connecting the chair forms (drive on ϑ). Approximate positions for selected ring conformations are shown. Details are found in ref 20, from which this figure has been reworked.

Haasnoot et al.[31] was utilized for computing the coupling constants for each conformer. Haasnoot et al.'s equation relates the vicinal coupling constants to the torsional angle between the coupling protons and accounts for the effects of electronegativity and relative position of substituents attached to the H–C–C–H fragment. Application of such a relation requires that the H–C–C–H torsional angles be computed for geometrical models of each conformer, usually provided by molecular-mechanical calculations. In our case this was accomplished by a method which involves the minimization of the molecular energy with respect to all degrees of freedom, a necessary condition for analyzing the flexibility of the pyranose ring.

Previous force field calculations[20] indicated that for the model compund methyl 4-*O*-methyl-2-*O*-sulfo-α-L-idopyranosiduronate (**8**) the skew-boat conformation $^2S_0$ is nearly equienergetic to the two chair forms $^1C_4$ and $^4C_1$. This is clearly illustrated in Figure 4 (reworked from ref 20), showing the isoenergetic contours represented on a spherical surface, as well as the energy profiles along two different conformational paths. The figure also shows relative minima (less stable by over 4 kcal mol^{-1}) near the skew-boat structures $^1S_3$, $^1S_5$, and $^3S_1$. Structures in the half-chair regions have energies about 10 kcal mol^{-1} higher than the stable

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Table II. Calculated Proton Coupling Constants (^3J, Hz) for the Monosaccharide **3**, the Disaccharide Sequences **1**, and the Pentasaccharide **6**

	3[a]				**1**[b]				**6**[a]			
	calculated			best fit	calculated			best fit	calculated			best fit
	$^1C_4$	$^2S_0$	$^4C_1$	to exptl	$^1C_4$	$^2S_0$	$^4C_1$	to exptl	$^1C_4$	$^2S_0$	$^4C_1$	to exptl
$J_{1,2}$	1.98	6.21	8.00	2.39	1.82	6.32	7.95	3.66	1.80	6.35	7.96	4.69
$J_{2,3}$	2.61	10.15	10.45	3.34	2.57	9.85	9.52	5.53	2.54	9.87	9.50	7.21
$J_{3,4}$	3.01	5.21	8.91	3.23	2.58	4.43	9.29	3.34	2.59	4.43	9.24	3.76
$J_{4,5}$	1.06	3.47	5.52	1.30	1.34	2.79	5.15	1.92	1.31	2.80	5.20	2.26

[a,b] From experimental data (Table I) at 23 °C (*a*) and at 37 °C (*b*).

Table III. Relative Contributions of $^1C_4$ and $^2S_0$ Conformations (Calculated from Experimental Coupling Constants) for the I_{2S} Different Sequences

		% $^1C_4$	% $^2S_0$
3	I_{2S}	90	10
1	$--A_{NS,6S}[I_{2S}-A_{NS,6S}]_n--$	59[a]	41[a]
7	$A_{NS,6S}-G-A_{NS,6S}-I_{2S}-A_{NS,6S}$	60[b]	40[b]
6[c]	$A_{NS,6S}-G-A^*_{NS,3,6S}-I_{2S}-A_{NS,6S}$	36	64

[a] At 90 °C, the population of $^2S_0$ rises at 47%. [b] Approximate values, from partial analysis of the spectrum. [c] Similar values are predicted from first-order analysis of the spectra for **4** and **5**.

forms. These results showed that $^2S_0$ is a well-defined conformer, so that changes in the coupling constants should correspond to changes in the conformational equilibrium. The need of considering the $^2S_0$ conformer for interpreting unusual coupling constants for idopyranoside derivatives was recently suggested by Augé and David.[33] However, they ascribed the variability of the observed ^3J values to the flexibility of the ring around the skew-boat form, rather than to the coexistence of various definite conformers.

The main purpose of the present calculations was to see whether the three stable conformers found for **8** maintain their identity also when the iduronic residue is inserted in the polysaccharide chain. This was found to be the case: the results for monosaccharide **3**, for the disaccharides $A^*_{NS,3,6S}$-I_{2S} and I_{2S}-$A_{NS,6S}$, and for the trisaccharides $A^*_{NS,3,6S}$-I_{2S}-$A_{NS,6S}$ and $A_{NS,6S}$-I_{2S}-$A_{NS,6S}$ indicate that the skew-boat form $^2S_0$ of the I_{2S} residue has an energy close to that of the chair forms, hence it is a possible contributor to the conformational equilibrium in these compounds and presumably also in longer sequences. The $^2S_0$ form essentially maintains its shape in all the compounds examined, although nonnegligeable changes in the geometry of the ring were found, which are reflected in the small differences between the computed ^3J values for each form of **3**, **1**, and **6** (Table II). In agreement with NMR data, the present calculations also indicate that minor conformational perturbation is induced in the amino sugar $A^*_{NS,3,6S}$ by the presence of the 3-*O*-sulfo group.

It must be pointed out here that the force field calculations just described were utilized in this work only for obtaining the conformer geometries, and the coupling constants via the Haasnoot et al.'s equation,[31] *not* for estimating the conformer populations. The force field presently adopted does not appear to be sufficiently reliable to deal with the energy differences which determine the conformational thermal equilibrium.

Conformer Populations. The conformer populations were obtained by least-squares fitting the coupling constants computed for a mixture of the $^1C_4$, $^2S_0$, and $^4C_1$ forms to the experimental values. The fitting procedure showed that for the compounds examined it was sufficient to consider the equilibrium between the $^1C_4$ and $^2S_0$ conformers, as the addition of the $^4C_1$ form did not improve the agreement at all. Only for compound **3** the presence of a small amount of the form $^4C_1$ cannot be excluded on the basis of the present data, whereas it is definitely ruled out for **1** and **6**.

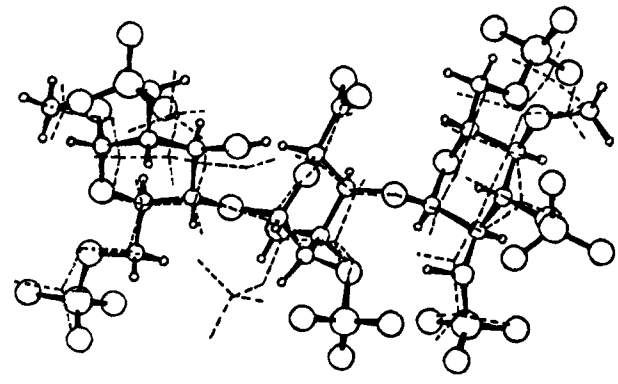

Figure 5. Comparison between two minimum-energy structures of model trisaccharide **4** with the iduronate ring in the $^1C_4$ (ball and sticks) and $^2S_0$ (dash) form, showing the striking similarity in the overall shape (in particular in the end-to-end) in spite of the conformational transition of the I_{2S} ring.

Table I reports the experimental values of chemical shifts and interproton coupling constants (^3J) for the I_{2S} residue of the monosaccharide **3**, and in different heparin sequences. The ^3J values calculated for each conformer are listed in Table II together with the best-fitting computed values. The agreement with experiment is not always satisfactory, systematic deviations being observed for J_{12} and J_{45}. This may partly arise from systematic errors in calculating hydrogen positions with the present force field,[31] but also effects due to the electronegativity of the ionic substituents in positions 2 and 5 should not be disregarded.

Table III reports the relative contributions of $^1C_4$ and $^2S_0$ conformers (calculated from experimental coupling constants at 23 °C) for the I_{2S} residue in different sequences. These results show that the monosaccharide **3** is predominantly (more than 90%) in the $^1C_4$ conformation. In the regular heparin sequences, as in **1** and **7** (i.e., when I_{2S} is between two disulfated amino sugars), the skew-boat form $^2S_0$ becomes an important contributor (~40%) to the conformational equilibrium of the sulfated iduronate residue. In the heparin sequences containing the active site for AT-III, i.e., when the iduronate residue is glycosylated in position 4 by a trisulfated amino sugar as in **4**, **5**, and **6**, $^2S_0$ becomes predominant (>60%).

Larger coupling constants observed for the nonsulfated monomer methyl α-D-iduronate[34] indicate that lack of 2-sulfation increases the contribution of other forms. As nonsulfated iduronate residues of dermatan sulfate essentially show the same coupling constants as the sulfated residues in the regular regions of heparin,[22] insertion of the iduronate residue in a sequence, in both heparin and dermatan sulfate, is the major factor determining the destabilization of the $^1C_4$ and the presence of a substantial population of the $^2S_0$ form.

Since the further drive toward the $^2S_0$ conformation of I_{2S} in sequences **4**, **5**, and **6** appears to be associated with the presence of the 3-*O*-sulfo group on the preceding amino sugar, the possible role of this group in such a conformational shift was checked. The present calculations indeed indicate that, in the model trisaccharide

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4, the additional 3-O-sulfo group causes—already at the level of the trisaccharide—a stabilization of the skew conformation by about 0.5 kcal mol^{-1}. This stabilization arises from electrostatic interactions, the other energy contributions being unaffected. Caution, however, calls for more refined computations, capable of providing a more comprehensive understanding of the conformational equilibrium.

It is noteworthy that in heparin sequences conversion of one I_{2S} residue from conformation $^1C_4$ to $^2S_0$ can occur through an energy barrier of height similar to that computed for the monosaccharide,[20] without substantially affecting the position of the distant residues. Figure 5 shows molecular models of 4, respectively, for the lowest $^1C_4$ minimum and for the corresponding (i.e., with the closest φ and ψ values) $^2S_0$ minimum. It is apparent that major differences only occur at the iduronate moiety, where the orientation of some substituents drastically changes, while the other parts of the trisaccharide models are almost superimposable: the end-to-end distance (between O(4) of $A^*_{NS,3,6S}$ and O(1) of $A_{NS,6S}$) is 11.85 and 11.76 Å for $^1C_4$ and $^2S_0$, respectively, the mean difference between the atomic coordinates for the end groups being only 0.2 Å. This implies that even the complete conversion from $^1C_4$ to the $^2S_0$ form does not necessarily involve a significant change in the overall shape of the heparin chain.

In conclusion, the present work shows that the conformation of sulfated iduronate residues in heparin can be described in terms of an equilibrium between the $^1C_4$ and $^2S_0$ forms. The relative contribution of these conformers of I_{2S}, and therefore the orientation of its sulfate, carboxylate, and hydroxyl groups, is different in different sequences. This raises the question whether the biological activities associated with binding at the level of these groups are modulated by the unusual conformational flexibility of the iduronate residues. It is interesting in this respect that 3-sulfation, which is crucial for AT-III binding and thus for anticoagulant activity, indeed induces a noticeable change in the conformer equilibrium.

Registry No. 3, 104155-85-3; 4, 104172-26-1; 5, 88096-18-8; 6, 88096-19-9; 7, 92745-16-9; I_{2S}, 69098-37-9; heparin, 9005-49-6.

Structural Analysis of a Glycolipid Head Group with One- and Two-State NMR Pseudoenergy Approaches

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Abstract: The solution conformation of the oligosaccharide head group of the glycolipid globoside has been determined with the use of ^1H two-dimensional NMR methods. The intensities of cross peaks in cross relaxation correlated experiments have been analyzed in terms of interproton distances, and the distances have been incorporated as a pseudoenergy in a conformational energy calculation. Minimum energy conformations have been determined by using both one- and two-state models. Conformations are consistent with a previously proposed "L" shape for the molecule. Comparison of the results from the two models suggests the possibility of conformational flexibility to exist for the terminal residue of the head group.

It has long been recognized that NMR cross-relaxation data can provide information on the solution conformation of molecules through the inverse sixth power dependence of intramolecular cross-relaxation rates on internuclear distances. Data in the past have most frequently been obtained through selective irradiation or inversion of a single resonance in a one-dimensional spectrum, followed by the observation of steady state or dynamic Nuclear Overhauser Enhancements (NOEs) on other resonances. More recently, the introduction of two-dimensional analogues of these experiments[1] has improved data acquisition efficiency and resolution to the point where the determination of the solution conformation of biological macromolecules can be attempted. Noteworthy illustrations include applications by Wüthrich and co-workers[2-4] and Kaptein and coworkers[5] to a number of small proteins and polypeptides.

Despite the success of these applications, it is widely recognized that analysis is limited by two assumptions, first that sufficient distance constraints will be observed to permit the unequivocal determination of solution conformation, and second that the observed cross-relaxation rate can be interpreted in terms of a single static conformer rather than a weighted average over all conformers present in solution. Violation of either of these assumptions gives rise to the possibility that the conformer determined to best fit the observed data does not accurately represent the dominant conformer, or in fact any conformer, present in solution.

In this paper we shall explore methodologies which relax these assumptions. We plan to improve structural definition in cases where only limited cross-relaxation data are available through the integration of potential energy calculations with distance constraint

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