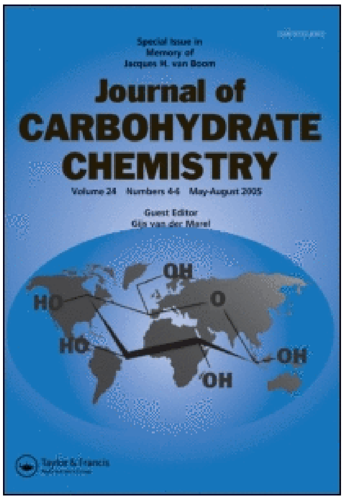

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CONFORMATIONAL ANALYSIS OF SULFATED α-(1→3)-LINKED D-GALACTOBIOSES USING THE MM3 FORCE-FIELD

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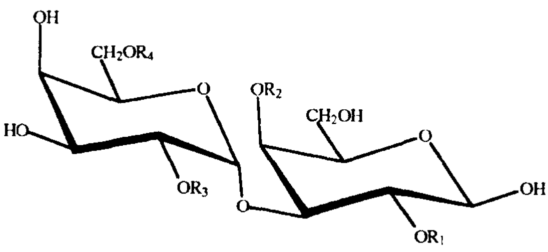
ABSTRACT

The conformational maps of eight derivatives of the disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* sulfated in different positions were obtained using the MM3 force-field specially parameterized for sulfate ester groups. As occurred with MM2, the conformational flexibility of the glycosidic linkage is only slightly hindered by sulfation. A substantial effect of sulfation of the β-D-galactose unit on position 4 shifts the global minimum to positive ψ_H (C1'-O3-C3-H3) angles, while sulfation at position 2 of the same unit deepens the well at negative ψ_H angles. On the other hand, sulfation on the α-D-galactose unit has a lesser effect, which in any case tends to stabilize the minimum at negative ψ_H angles.

INTRODUCTION

Sulfated polysaccharides enact important roles in plant and animal tissues. There are also natural mono- and oligomeric units with biological activities different from those of their parent, non-sulfated counterparts.[1] Conformational analysis of sulfated sugars has usually been difficult, due to complications in crystallization for X-ray studies, or because of the lack of parameters for molecular mechanics calculations. In 1986, the MM2[2] parameters for a sulfated iduronic acid were obtained and applied,[3] and in recent years X-ray crystallographic studies and *ab initio* calculations helped to develop new parameters



	R^1	R^2	R^3	R^4	Repeating unit of carrageenan
1	H	H	H	H	
2	SO_3^-	H	H	H	
3	H	SO_3^-	H	H	
4	SO_3^-	H	SO_3^-	H	ξ–
5	H	SO_3^-	SO_3^-	H	
6	SO_3^-	H	H	SO_3^-	
7	H	SO_3^-	H	SO_3^-	μ–
8	SO_3^-	H	SO_3^-	SO_3^-	λ–
9	H	SO_3^-	SO_3^-	SO_3^-	ν–

Figure 1. The disaccharides studied in this work.

for sulfated carbohydrates using MM3,[1] an MM2 variant,[4] Tripos,[5] AMBER[6] and CHARMM[6,7] force-fields.

The disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* and its sulfated derivatives are structural units of many carrageenan molecules. The conformational features of the disaccharides may explain physical or biological properties of the macromolecules. In a previous paper[8] the conformational maps of six sulfated derivatives were presented, using variants of the general purpose MM2 force-field. Herein, in order to emulate the behavior of the sulfate groups with appropriate parameters (as those described by Lamba *et al.*[1] for the MM3 force-field[9]) the maps corresponding to eight sulfated derivatives of this disaccharide (Fig. 1) were calculated with the MM3 force-field. These results are presented and the influence of sulfation on the main conformational features is discussed.

METHODS

Calculations were carried out on a Sun SparcStation 10 computer, running under the Solaris 2.4 operating environment, using the molecular mechanics program MM3 (92) (QCPE, Indiana University, USA), developed by Allinger and coworkers,[9] and compiled by the SparcCompiler 2.0.1. The MM3 routines were modified as suggested[10] by changing the maximum atomic movement from 0.25 Å to 0.10 Å. The dihedrals ϕ_H and ψ_H are defined by atoms H1'-C1'-O3-C3 and H3-C3-O3-C1', respectively. Their approximate relationships with ϕ (defined by the atoms O5'-C1'-O3-C3) and ψ (C2-C3-O3-C1') are $\phi = \phi_H + 120°$, $\psi = \psi_H - 120°$. For the dihedrals A-B-C-D the sign is considered positive, when looking at a Newman projection from B towards C, A is rotated clockwise with respect to D.

MM3 parameters for the sulfate group were taken from Lamba *et al.*,[1] and a dielectric constant of 3.0 was used. In that model, the charge on the sulfate groups is emulated from the S-O bond dipoles. No cations were added. It is expected that for each compound, there will be up to 3^{10} conformers for each ϕ,ψ combination which yields a minimum, thus discouraging a full search. Starting geometries for calculation were then chosen: a) from minima obtained previously with a less sulfated disaccharide, b) from minima obtained by an MM2 calculation,[8] and c) from the lower-energy conformations of α- and β-galactose obtained in a full search of those monosaccharides.[11] In each case, the systems were allowed to minimize, and, for the minimal energy conformations, the effect of rotation of alternate pairs of hydroxymethyl and hydroxyl groups was checked. Minimization (by the block diagonal Newton-Raphson procedure) was terminated when the average movement converged to a value lower than 10^{-4} Å. Using the dihedral driver, ϕ and ψ were fully varied using a 20° grid size. In each point, energies were calculated after minimization with restraints for these two angles but allowing the other variables to relax. The energy for each point was calculated after starting from the different minima obtained previously. In this way, only the conformation of minimal energy for each ϕ,ψ combination was saved and recorded. The conformational adiabatic maps, or energy surfaces as function of ϕ and ψ angles were produced. The relative contributions of energy factors to the global energy was obtained from the output files of MM3.

Linkage rotation[12,13] for a particular conformer was calculated from the equation:

$$\Lambda = -105° - 120° (\sin \phi_H + \sin \psi_H)$$

The calculations of populations, atomic distances and partition functions were carried out using a home-made program. NOE theoretical calculations were computed from the matrix of H-H distances by an iterative procedure[14] and averaged with the statistical population of the minima as shown by Imberty *et al.*[15] Throughout this paper, NOEs are expressed as increases in ^{1}H NMR peak intensity when irradiating H1', relative to the

NOE on H2'. Average linkage rotations and distances were calculated by Boltzmann-weighed summation of their individual values over all the maps surfaces. The partition functions[16] were calculated as depicted previously: [8]

$$q = \Sigma \exp(-\Delta E/RT).\ \Delta\phi.\Delta\psi,$$

where the ΔE are the differences in energy between each grid point and the global minimum, $\Delta\phi$ and $\Delta\psi$ are the grid spacings (20° in this case) and the summation is carried out over all the ϕ, ψ surface. The temperature for calculating populations and partition functions was set to 25 °C. The theoretical optical rotation for the disaccharide can be calculated from tabulated optical rotations and the average linkage rotation:[12,13]

$$[\alpha]_{disac.} = ([\alpha]_1 M_1 + [\alpha]_2 M_2 + 100\ \langle\Lambda\rangle\ ) / M_{disac}$$

where M indicates molecular weights, and the subscripts 1 and 2 correspond to the methyl glycoside of the nonreducing sugar with the configuration (α or β) of the linkage in the disaccharide, and the free reducing sugar, respectively.

RESULTS

The conformational map of the disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* (**1**, Fig. 1) calculated using MM3 was obtained (Table 1). This force field, specially parameterized for sulfated carbohydrates was also used for the study of some sulfated derivatives.

In compound **2** the hydroxyl group at C2 of the β-galactose unit was sulfated, and in compound **3**, the hydroxyl group at C4 of the same unit was sulfated. Calculations were also carried out with disaccharide **4** (sulfated on both positions 2), and on compound **5**, sulfated on O2 of α-galactose and on O4 of β-galactose, and also on disaccharides **6** (sulfated on O6 of α-galactose and on O2 of β-galactose) and **7** (sulfated on O6 of α-galactose and on O4 of β-galactose, repeating unit of μ-carrageenan). Finally, the maps of compounds **8** and **9**, which carry an additional sulfate on O6 of the α-galactose unit, were also calculated. **8** and **9** are the repeating units of λ- and ν-carrageenans, respectively. The resulting maps are shown in Figures 2 (**1**), 3 (**2**, **4**, **6** and **8**) and 4 (**3**, **5**, **7** and **9**), while the data deduced are shown in Tables 1 and 2.

All the maps have similar shapes (Figs. 2, 3 and 4), with four main minima each (Tables 1 and 2). A fifth minimum observed for all compounds, located around ϕ_H, ψ_H ~

Table 1. Torsion angles (°), relative energies (kcal/mol), calculated populations (%), linkage rotations (Λ, in °), relative NOE values and H1'-H3 distances (Å) for the minimum-energy conformations obtained for sulfated derivatives of the disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p*, using the MM3 force-field.

	Sulfate groups on				
	– **1**	O2 **2**	O2 & O2' **4**	O2 & O6' **6**	O2, O2' & O6' **8**
Minimum A					
ϕ_H,ψ_H	-32, 48	-18, 52	-23, 57	-20, 53	-20, 53
E_{rel}	0.41	1.03	1.40	2.11	2.50
Population	33.0	14.9	8.5	2.6	1.4
Λ	-131	-162	-159	-160	-160
NOE on H3[a]	0.56	0.53	0.47	0.52	0.51
NOE on H4[a]	-0.07	-0.07	-0.06	-0.06	-0.06
$d_{H1'-H3}$	2.28	2.31	2.37	2.33	2.33
Minimum B					
ϕ_H,ψ_H	-39, -40	-36, -38	-32, -39	-41, -39	-33, -41
E_{rel}	0.00	0.00	0.00	0.00	0.00
Population	66.2	84.7	91.4	93.6	98.2
Λ	+48	+39	+34	+49	+39
NOE on H3[a]	0.25	0.30	0.33	0.24	0.29
NOE on H4[a]	0.57	0.54	0.56	0.59	0.60
$d_{H1'-H3}$	2.58	2.50	2.46	2.57	2.51
Minimum C					
ϕ_H,ψ_H	-35,-178	-39, 180	-39, -178	-36, -175	-37, -172
E_{rel}	2.68	3.09	4.67	1.90	3.27
Population	0.7	0.5	0.0	3.8	0.4
Λ	-32	-29	-25	-24	-16
NOE on H3[a]	0.00	0.00	-0.01	0.00	-0.01
NOE on H4[a]	0.01	0.01	0.03	0.02	0.02
$d_{H1'-H3}$	3.69	3.71	3.72	3.70	3.72
Minimum D					
ϕ_H,ψ_H	58,66	60, 64	48, 54	56, 69	50, 60
E_{rel}	4.09	5.58	6.42	4.88	5.89
Population	0.07	0.01	0.00	0.02	0.01
Λ	-316	-317	-291	-317	-301
NOE on H3[a]	0.06	0.06	0.12	0.05	0.09
NOE on H4[a]	-0.01	-0.01	-0.01	-0.01	-0.01
$d_{H1'-H3}$	3.24	3.27	3.02	3.28	3.11
AVERAGE					
⟨Λ⟩	- 8	+1	+14	+39	+36
NOE on H3[a]	0.35	0.33	0.34	0.24	0.29
NOE on H4[a]	0.35	0.47	0.51	0.55	0.59
$\langle d_{H1'-H3}\rangle$	2.47	2.50	2.47	2.60	2.53

[a] By irradiating H1', relative to the NOE on H2'.

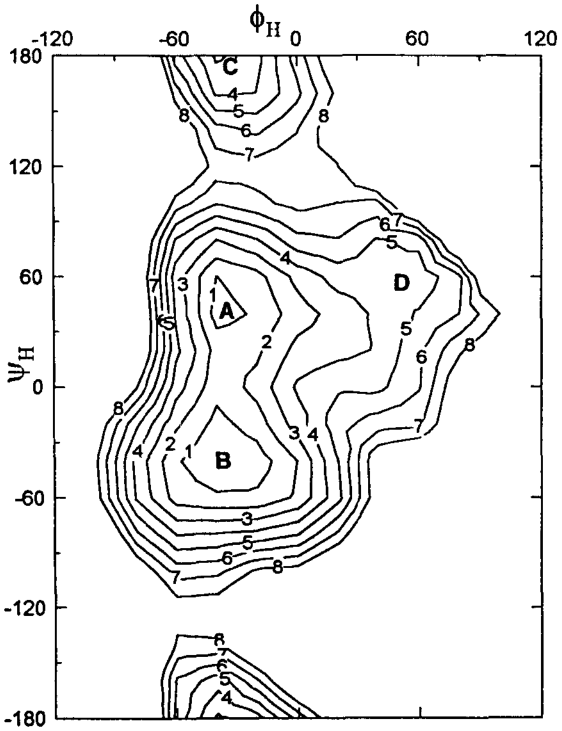


Figure 2. Conformational map of compound 1, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum.

-170°, -25°, is of very little influence on the map, as its energies are 9-12 kcal/mol higher than those of the global minima. The fourth minimum **D**, which corresponds to both glycosidic angles in the g^+ conformation, appears in a rather flat region, and was not observed in previous calculations.[8]

All of the minima appearing in Tables 1 and 2 showed that the exocyclic hydroxymethyl groups of both galactose units had a GT ($\theta_{O5\text{-}C5\text{-}C6\text{-}O6}$ ~ 60-70°) orientation. The corrected conformational partition function (see Methods) calculated for these maps[8] gives a value of 1400 for **1**, 690 for **4** and 800-820 for the other β-D-galactose 2-sulfated derivatives **2**, **6** and **8**. For the β-D-galactose 4-sulfated derivatives, **5** has a q with a magnitude of 980, but the rest are in the range 660-750.

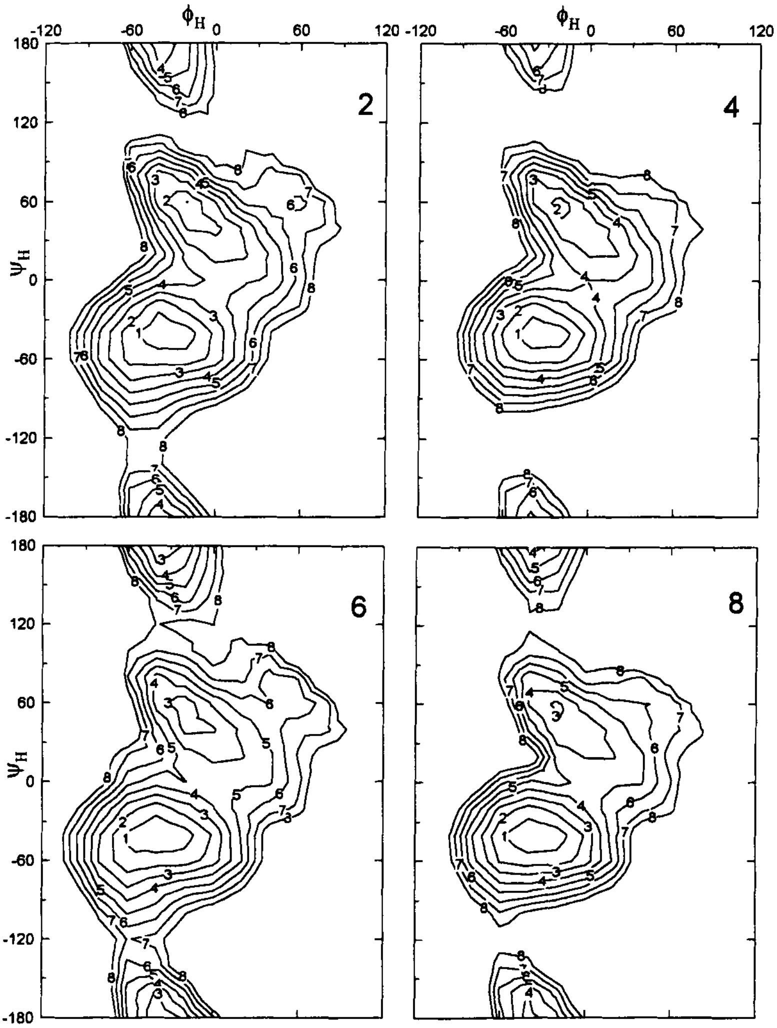


Figure 3. Conformational maps of compounds **2**, **4**, **6**, and **8**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum.

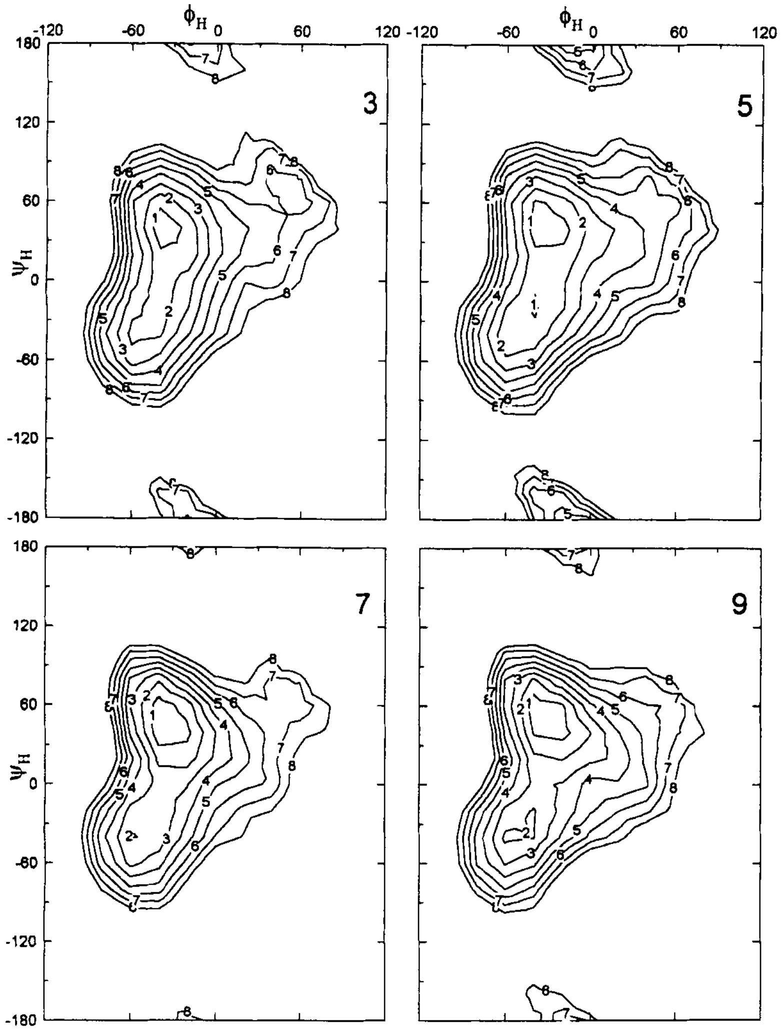


Figure 4. Conformational maps of compounds **3**, **5**, **7**, and **9**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum.

Table 2. Torsion angles (°), relative energies (kcal/mol), calculated populations (%), linkage rotations (Λ, in °), relative NOE values and H1'-H3 distances (Å) for the minimum-energy conformations obtained for disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* sulfated in different positions using the MM3 force-field.

	Sulfate groups on				
	–	O4	O4 & O2'	O4 & O6'	O4, O2' & O6'
	1	**3**	**5**	**7**	**9**
Minimum A					
ϕ,ψ	-32, 48	-35, 37	-33, 43	-34, 44	-30, 55
E_{rel}	0.41	0.00	0.00	0.00	0.00
Population	33.0	92.2	76.0	93.9	92.7
Λ	-131	-108	-121	-121	-143
NOE on H3^a	0.56	0.60	0.58	0.55	0.50
NOE on H4^a	-0.07	-0.07	-0.06	-0.07	-0.06
$d_{H1'-H3}$	2.28	2.22	2.24	2.27	2.34
Minimum B					
ϕ,ψ	-39, -40	-50, -39	-50, -34	-50, -34	-52, -37
E_{rel}	0.00	1.46	0.69	1.61	1.50
Population	66.2	7.8	23.9	6.1	7.3
Λ	+48	+62	+54	+54	+62
NOE on H3^a	0.25	0.17	0.22	0.21	0.18
NOE on H4^a	0.57	0.55	0.51	0.51	0.54
$d_{H1'-H3}$	2.58	2.70	2.61	2.61	2.67
Minimum C					
ϕ,ψ	-35,-178	-16, -179	-15, 178	-16, 180	-17,-179
E_{rel}	2.68	5.72	4.01	7.33	6.15
Population	0.7	0.0	0.1	0.0	0.0
Λ	-32	-70	-78	-72	-68
NOE on H3^a	0.00	0.00	0.00	0.00	0.00
NOE on H4^a	0.01	0.04	0.04	0.03	0.03
$d_{H1'-H3}$	3.69	3.66	3.66	3.66	3.66
Minimum D					
ϕ,ψ	58,66	53, 72	48, 66	56, 69	50, 61
E_{rel}	4.09	5.05	4.32	6.07	5.81
Population	0.07	0.02	0.05	0.00	0.01
Λ	-316	-315	-304	-317	-302
NOE on H3^a	0.06	0.05	0.08	0.05	0.09
NOE on H4^a	-0.01	-0.01	-0.01	-0.01	-0.01
$d_{H1'-H3}$	3.24	3.28	3.15	3.27	3.10
AVERAGE					
$\langle\Lambda\rangle$	- 8	-79	-73	-109	-119
NOE on H3^a	0.35	0.57	0.49	0.53	0.48
NOE on H4^a	0.35	-0.02	0.08	-0.03	-0.02
$\langle d_{H1'-H3}\rangle$	2.47	2.31	2.31	2.32	2.35

a. By irradiating H1', relative to the NOE on H2'.

DISCUSSION

In previous papers,[8,13] it has been shown that the conformational map around the glycosidic bond of α-D-galactopyranosyl-(1→3)-β-D-galactopyranose (**1**) has three energy minima, suggesting substantial conformational flexibility for the glycosidic linkage. Different parameterization led to different energy values, although in most of them the minimum called **B** (with ϕ_H and ψ_H in near g^- conformation) is the global minimum. The calculations carried out here show the same trend. Both the optical rotation obtained from the linkage rotation (+149°, see Methods) and the NOE value on H3 (0.35) calculated using MM3 agree with the experimental values (+155°, and 0.32, respectively),[13,17] as occurred with the MM2 calculations.[8]

In the map, a trough centered at a more or less fixed ϕ_H angle (between -10 and -60°) is observed and contains the three main minima, each of which exhibits a clearly different ψ_H angle. The ϕ_H value matches that expected as an expression of the *exo*-anomeric effect, although the preferred ϕ_H angle may also come from steric factors, as shown by MM2 calculations[14] in which this effect has a weaker parameterization.

As occurred with MM2 studies,[8] sulfation does not change the gross features of the maps. The flexibility is lowered slightly, as shown by the partition functions. It was already noted[8] that the position of sulfation of the β-D-Gal unit has major influences over the relative energies of the main conformers, while sulfation on the α-D-Gal unit has lesser influence. However, these results show clearly that the position of sulfation of the α-D-Gal unit has a constant (grossly additive) effect on the relative stability of the minima. Furthermore, the sulfation of the α-D-galactose unit has a larger effect on the relative stability of the **C** minimum conformers. Table 3 shows the effects of sulfation on the energy differences between minima.

For the products sulfated on the position 2 of the β-D-Gal unit (**2**, **4**, **6** and **8**), the region **B** is deepened relative to **A** in 0.6 kcal/mol. Additions of sulfate on position 2, and particularly on position 6 of the α-D-Gal unit significantly increases the instability of minimum **A** with respect to **B** (Table 3). The energy of minimum **C** stays approximately constant by β-D-Gal sulfation on C2, but suffers a substantial change by sulfation of the

Table 3. Effects of sulfation on the energy differences (kcal/mol) between minima.

Effect	E_B-E_A	E_C-E_A	E_D-E_A	E_C-E_B
On 2 of β-Gal	−0.6	−0.2	+0.9	+0.4
On 4 of β-Gal	+1.9	+3.5	+1.4	+1.6
β-2-sulfated				
On 2 of α-Gal	−0.4	+1.2	+0.5	+1.6
On 6 of α-Gal	−1.1	−2.3	−1.8	−1.2
β-4-sulfated				
On 2 of α-Gal	−0.7[a]	−1.7[a]	−0.7[a]	−1.0
On 6 of α-Gal	+0.2[a]	+1.6[a]	+1.0	+1.4

a. An additional of 0.5 should be added if both sulfates are present.

α-unit: the sulfate on 2 destabilizes this minimum, while the sulfate on 6 stabilizes it markedly, to the point that in compound **6**, minimum **C** has lower energy than minimum **A**. As expected, the deepening of region **B** increases the net linkage rotation to positive values (Table 1).

On the other hand, for the products sulfated on position 4 of the β-D-Gal unit, the region **A** becomes the global minimum, with a stabilization of about 2 kcal with respect to minimum **B** (Table 3). The presence of sulfate at position 2 of the α-D-Gal unit decreases the magnitude of this stabilizing effect, but sulfation on C6 of that unit has little effect on the relative stability of the two minima (Table 3). For minimum **C**, the effect of sulfation of the α-D-Gal unit reverses the previous trend: sulfation on C2 stabilizes this minimum, while sulfation on C6 is destabilizing. Sulfation of the β-unit in either position destabilizes minimum **D**. Table 3 also shows that sulfation on C6 of the α-D-Gal unit produces a different effect according to the sulfation pattern on the β-D-Gal unit: it stabilizes minimum **A** when it is 4-sulfated, and destabilizes it when it is 2-sulfated. In 4-sulfated disaccharides minimum **A** is destabilized by sulfation of the α-unit in position 2. The 4-sulfated derivatives exhibit negative linkage rotations (Table 2).

In β-D-Gal 4-sulfated compounds, region **A** is the main one, with *ca.* 90% of the conformers, while 2-sulfated compounds clearly favor region **B**. Sulfation on C6 of the α-

D-Gal unit increases this trend. The change in minimum-energy regions produces a shift in the expected optical rotations of 16-18° when passing from the 4-sulfated to the 2-sulfated disaccharide (when C6 is not sulfated), going to 24-27° for the C6 sulfated ones (from Tables 1 and 2). Also, the NOE on H4 changes from 47-59% of the magnitude on H2' on compounds **2**, **4**, **6** and **8** to small (or even negative) values on **3**, **5**, **7** and **9**. A shortening of the average H1'-H3 distances is also observed (Tables 1 and 2). The values of the angles ϕ_H and ψ_H of the minima also shift with the position of sulfation of the β-D-Gal unit (Tables 1 and 2). These results show a sharper difference due to sulfate than those produced by variants of MM2 with a general parameterization.[8] The map for **8** calculated using the Tripos force field gives qualitatively similar results,[5] although their energies and geometries are different from those obtained in the current paper.

Figure 5 shows molecular drawings for the minimum energy conformations of compounds **6** and **7** in each of the three regions. The differences in relative stability of the minima upon sulfation on C2 and C6 of the α-D-Gal units arise mainly from hydrogen bonding and electrostatic forces. However, the latter are partly screened by the use of a dielectric constant of 3. In the **A** minima, the oxygen atoms facing each other (and thus, capable of producing inter-unit hydrogen bonds) are O2 and O6', in **B** the same couple, plus O4 and O2', while in **C** the pairs are O4–O6' and O2–O2' (Fig. 5). Therefore, the stabilization of minimum **C** in β-D-Gal 2-sulfated disaccharides upon 6'-sulfation can be rationalized as a function of a new hydrogen bond between the HO4 of the β-D-Gal unit and the charged oxygens, an interaction not occuring for minima **A** and **B**. Another concurrent factor is the disappearance of hydrogen bonding between HO6' and the O(S)2 in **A** and **B** after C6' sulfation (Fig. 5). Upon sulfation on C2', the effect on **C** is destabilizing (Table 4), as hydrogen bonding between HO2' and O(S)2 is precluded, and electrostatic repulsion appears. The stabilization of **B** relative to **A** upon C6' sulfation has many foundations (including a counter-acting change in bending forces), but the main ones are due to the relative orientations of the C5'-C6'-O6'-S (or H) dihedral angle. For the non-sulfated derivative, minimum **A** keeps this angle in a *g*- relationship, while minimum **B**, probably to allow for better hydrogen-bonding, gives a minimum in a g^+ relationship. A hydrogen bond from HO6' to O5' stabilizes conformer **A**. On the other hand, in the 6'-

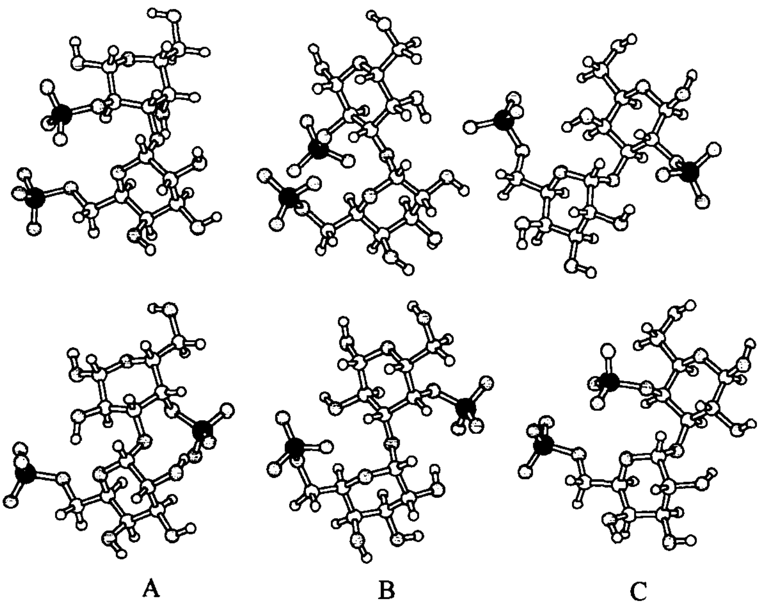


Figure 5. Molecular drawings of the minimum-energy conformers of compounds **6** (above) and **7** (below) in the **A**, **B** and **C** regions. Compounds **6** and **7** were chosen as examples of β-D-Gal 2- and 4-sulfated compounds, respectively.

sulfated derivative, minimum **A** keeps this angle in a *trans* relationship, because of steric reasons, while in the **B** minimum, this angle can take values of -90 to -115°. In this arrangement, the electrostatic forces (specially regarding O5') are highly stabilizing, producing a final 1.1 kcal/mol difference. As expected, C2' sulfation has little effect on the relative energies of conformers **A** and **B** (Table 4). The same geometric factors may help to explain the influence of C6'-sulfation on β-D-Gal-4 sulfated disaccharides: minimum **C** gets largely destabilized as a new hydrogen bond occurs between HO2 and the O(S)6' in minimum **A** (not in **B**, as HO2 has to take another conformation), while in **C** sulfation disables bonding between HO6' and O4. As stated above, C6' sulfation stabilizes **A** against **B**, by the new hydrogen bond between HO2 and O(S)6' in **A**, and the disappearance of the bond between HO6' and O5' in **B**. This fact is compensated by bending factors when comparing **3** to **7**, but not when contrasting **5** to **9**. In the latter case, *i.e.*, when both sulfates are present in the α-D-Gal unit, minimum **A** gains an extra stability

(0.5 kcal/mol, Table 4). Again, C2' sulfation has little effect on the relative energies of minima **A** and **B**, although this is the effect of counterbalancing factors. On the other hand, **C** appears stabilized by this kind of sulfation, due to the hydrogen bonding originating between HO2 and the O(S)2' in **C**, and the disappearance of the HO2' with O1' and O(S)4 in **A** and **B**.

The ^{13}C NMR signal corresponding to C1 of the α-galactose unit of a ν-carrageenan is almost 7 ppm downfield from that of a λ-carrageenan.[18] These carrageenans differ only in the position of sulfation of the β-galactose unit (4- in the first case, 2- in the latter), and it is also known that those chemical shift differences are related to the distance H1'-H3.[19] As this distance depends on the optimum conformation (shorter in region **A**) the resulting maps agree with this experimental fact, although the large magnitude (7 ppm) cannot be expected from these considerations alone.[19a] Another experimental fact which agrees with these results is that ν-carrageenans usually show lower optical rotations than λ-carrageenans,[20] a fact certainly related to the differences in linkage rotations, although two other factors are influential in this case: the effect of sulfation on the β-(1→4) linkage rotation, and the unavoidable presence of 3,6-anhydrogalactose in the natural preparations of ν-carrageenans. However, the disaccharidic conformational features can only be extended to those of the parent polysaccharides with care, considering the presence of the other, β-(1→4) linkage, and the polyelectrolyte nature of the polymer.

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