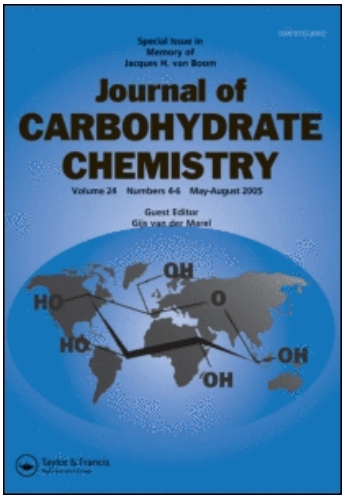

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CONFORMATIONAL ANALYSIS OF NEOCARRABIOSE AND ITS SULFATED AND/OR PYRUVYLATED DERIVATIVES USING THE MM3 FORCE-FIELD

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ABSTRACT

The adiabatic conformational surfaces of neocarrabiose (3,6-An-α-D-Gal*p*-(1→3)-β-D-Gal*p*) and of nine sulfated and/or pyruvylated derivatives were obtained using the MM3 force-field. These maps indicate greater flexibility of the glycosidic linkage than found for similar compounds that are based on α-D-galactose instead of 3,6-anhydrogalactose units. Sulfation of the β-D-galactose unit on position 2 shifts the global minimum to negative ψ_H ($\theta_{C1'\text{-}O3\text{-}C3\text{-}H3}$) angles, whereas sulfation at either position 4 of the same unit or at position 2 of the 3,6-anhydro-α-D-galactose unit has less effect. The results are consistent with the X-ray diffraction data on crystalline neocarrabiose and carrageenan fibers. Free energy calculations show that entropy is not uniformly distributed among confomers.

INTRODUCTION

Carrageenans are sulfated galactans which can be extracted from red seaweeds for use as thickeners and gelling agents. Their repeating unit [→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*(1→], usually sulfated (and seldom pyruvylated) in different positions, often has the

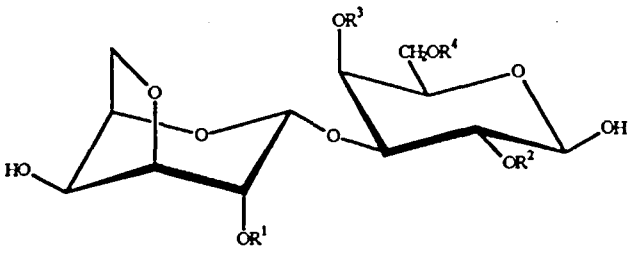
α-D-galactose unit replaced by 3,6-anhydrogalactose.[1] The useful physical properties of these polysaccharides depend on their conformations and molecular flexibility, which is concentrated mostly in the glycosidic linkages. Thus, conformational analysis of the various disaccharides which act as repeating structures should be useful in improving the understanding of the physical and biological properties of these macromolecules.

Structural studies of sulfated sugars were formerly hindered because they were difficult to crystallize for diffraction crystallography. Also, the necessary parameters for molecular mechanics modeling calculations were not available. In the past ten years, however, parameters for sulfated carbohydrates using different modeling force-fields[2-4] were developed based on *ab initio* calculations and on X-ray studies. A single crystal study of neocarrabiose (3,6-An-α-D-Gal*p*-(1→3)-β-D-Gal*p*) was carried out,[5] as were fiber diffraction studies of several oriented carrageenan fibers.[6-9] Modeling studies of neocarrabiose have been carried out with several methods that used either rigid or flexible monomeric residues,[5,10-12] and the sulfated disaccharidic units of κ- and ι-carrageenan have been studied with the Tripos force-field.[4] Other disaccharides that are found in carrageenans, such as carrabiose (β-D-Gal*p*-(1→4)-3,6-An-α-D-Gal*p*),[10,11,13] α-D-Gal*p*-(1→3)-β-D-Gal*p*,[14-16] and their sulfated derivatives[4,10,15,16] have now been modeled by molecular mechanics and also by molecular dynamics.[11,12]

Previously,[16] the conformational maps of α-D-Gal*p*-(1→3)-β-D-Gal*p* and eight sulfated derivatives were presented, based on the MM3 force-field with parameters for sulfate described by Lamba *et al.*[3] Herein, we show the conformational energy surfaces for ten derivatives of neocarrabiose (Fig. 1), calculated with MM3. By comparing these new maps with the previous ones, the influence of the 3,6-anhydro ring and the sulfate groups on the molecular conformation was also determined.

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,[17] and compiled by the SparcCompiler 2.0.1. The MM3 routines were modified as suggested[18] by changing the maximum atomic movement from 0.25 Å to 0.10 Å. The dihedrals ϕ_H and ψ_H are



	R^1	R^2	R^3	R^4	Repeating unit of carrageenan
1	H	H	H	H	β-
2	SO_3^-	H	H	H	α-
3	H	SO_3^-	H	H	
4	SO_3^-	SO_3^-	H	H	Alk.tr.λ-
5	H	H	SO_3^-	H	κ-
6	SO_3^-	H	SO_3^-	H	ι-
7	H	SO_3^-	SO_3^-	H	
8	SO_3^-	SO_3^-	SO_3^-	H	
9	H	H	H_3C C CO_2H		
10	SO_3^-	H			

Figure 1. The disaccharides studied in this work

defined by atoms H1'-C1'-O3-C3 and H3-C3-O3-C1', respectively. Other papers dealing with neocarrabiose conformations[5,7,9,11,12] used other conventions for the angles. 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. The orientation of the hydroxyl hydrogens is indicated by χ_n, defined by the atoms Hn-Cn-On-H(O)n, while χ_6 is defined by the atoms C5-C6-O6-H(O)6, and ω by the atoms O5-C5-C6-O6. Their values are described by one of these eight one-letter codes:[19] **S** for angles between − 30 and + 30°, **g** for 30-80°, **p** for 80-100°, **e** for 100-150°, **T** for 150-210°, **E** for 210-260°, **P** for 260-280°, and **G** for 280-330°.

MM3 parameters for the sulfate group were taken from Lamba *et al.*,[3] and a dielectric constant of 3.0 was used. In that model the charge on the sulfate groups is emulated by S-O bond dipoles. No cations were added. To generate each map, for each compound, *ca.* 25-30 conformers with varied exocyclic groups orientation were chosen as starting points. Those conformers were minima in different regions of ϕ,ψ space, found using an iterative method.[16,20] The pyruvic acid ketal was included with the usual *R*-configuration (axial carboxyl group).[21] Minimization was carried out by the block diagonal Newton-Raphson procedure for grid points and using the full-matrix procedure for minima. Using both the dihedral drivers 2 and 4, ϕ and ψ were fully varied using a 20° grid.[20] At each point, energies were calculated after minimization with restraints for these two angles but allowing the other variables to relax. The optimization was terminated

when the decrease in energy converged to a value lower than 2 cal/mol. The energy for each point was the lowest of any of the 25-30 different minima obtained previously. In this way, only the conformation of minimal energy for each ϕ,ψ combination was recorded. The conformational adiabatic maps, or energy surfaces as function of ϕ and ψ angles were produced. The partition functions were calculated as described previously:[15]

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

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 all calculations was set to 25 °C (298.16 K). Free energies were calculated from the vibrational analysis of the minima, with no special treatment for the low-frequency vibrations:[22] *i.e.*, the effect of frequencies equal or lower than 20 cm^{-1} was added to the MM3 output values of vibrational enthalpies and entropies.

RESULTS

The conformational map of the disaccharide neocarrabiose (3,6-An-α-D-Gal*p*-(1→3)-β-D-Gal*p* (**1**, Fig.1) calculated using MM3 was obtained. This force-field, specially parameterized for sulfated carbohydrates was also used for the study of some sulfated derivatives: In compound **3** the hydroxyl group at C2 of the β-D-galactose unit was sulfated, and in compound **5**, the hydroxyl group at C4 of the same unit was sulfated (repeating unit of κ-carrageenan). Calculations were also carried out with the same compounds, sulfated on position 2 of the 3,6-anhydrogalactose unit (disaccharides **2**, **4** and **6**, repeating units of α-, alkali-treated λ- and ι-carrageenans). The influence of sulfation on both position 2 and 4 of the β-galactose unit was analyzed in compounds **7** and **8**, while that of pyruvylation was checked on compounds **9** and **10** (Fig. 1). The resulting maps are shown in Figures 2 (**1**, **3**, **5** and **7**), 3 (**2**, **4**, **6** and **8**) and 4 (**9** and **10**), while the geometric and energy data (steric and free energy) on the minima are shown in Table I.

All the maps have very similar shapes (Figs. 2, 3 and 4), with four main minima each (Table 1). A fifth minimum in the **D** region (around ϕ,ψ=173,–65°) appears for the compounds not sulfated on C4 (**1-4**, **9** and **10**), with energies 0.1-2.0 kcal/mol higher than

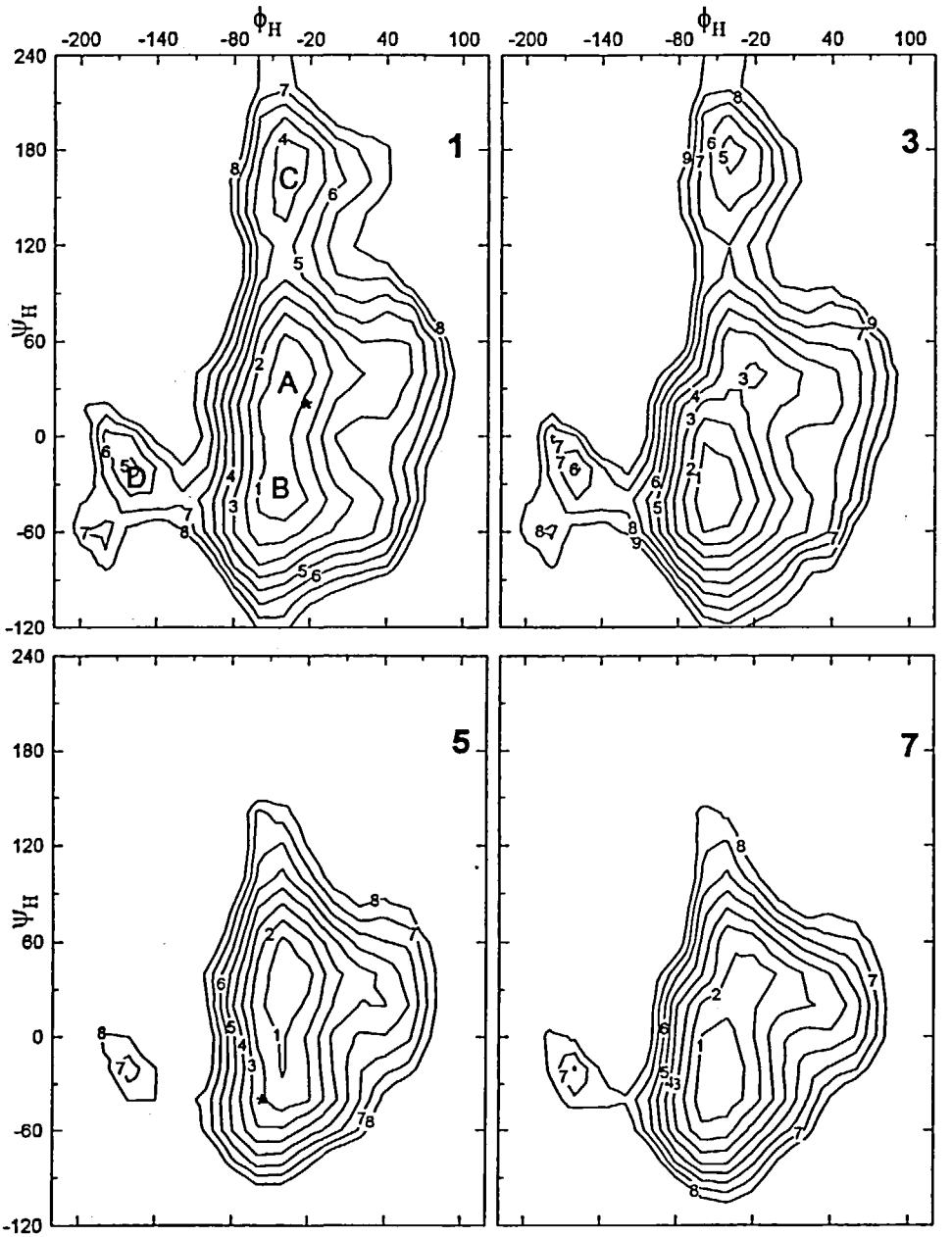


Figure 2. Conformational map of compounds **1**, **3**, **5** and **7**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum. The stars show the published crystal[5] and fiber[9] structures.

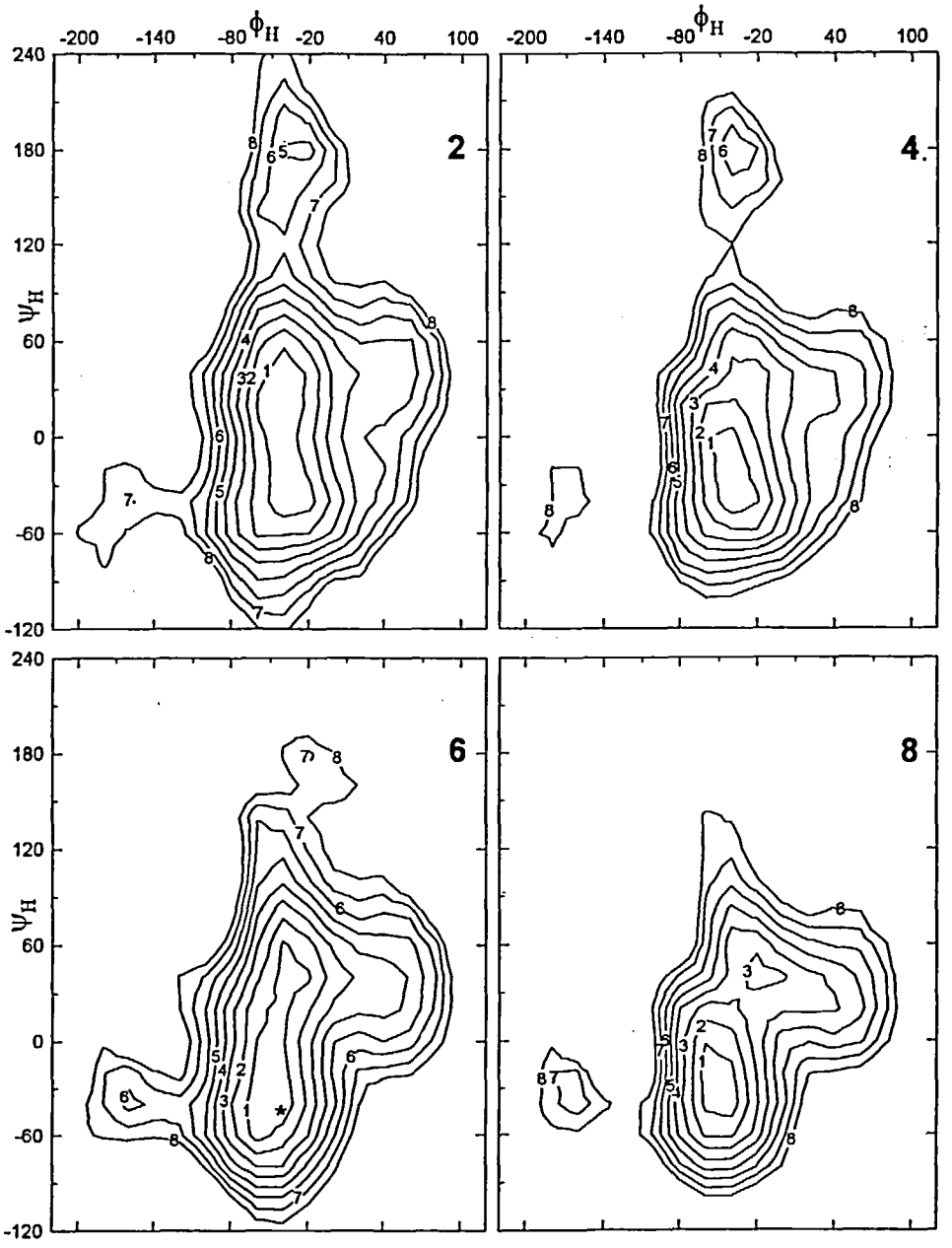


Figure 3. Conformational map of compounds **2**, **4**, **6** and **8**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum. The star shows the published fiber[7,9] structure.

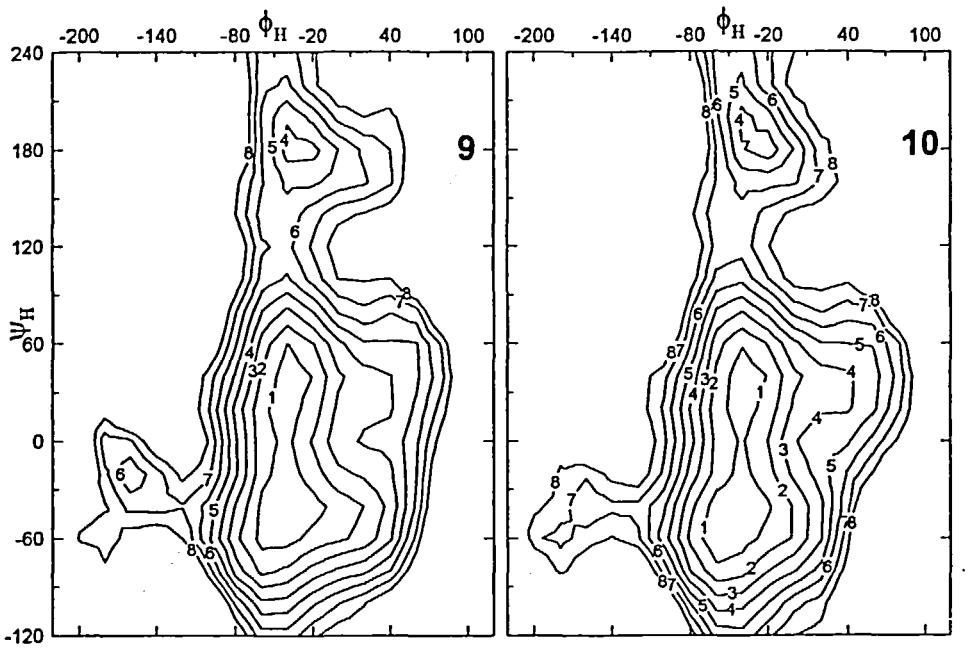


Figure 4. Conformational maps of compounds **9** and **10**, generated using MM3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum.

those reported for minimum **D** (Table 1). Almost all the lowest energy structures in each region had their only hydroxymethyl group in a GT ($\omega \approx 60\text{-}70°$) orientation, while its hydrogen keeps the χ_6 close to –60°. The conformational partition functions (see Methods) calculated for these maps are shown in Table 2. In some cases, minima in other regions were found, but they did not withstand a full matrix optimization (*i.e.*, they fell to other minima), or had one imaginary or zero vibrational frequency (*i.e.*, they were not actual minima). Table 3 shows the hydrogen bond energy and selected thermodynamic values, and Table 4 shows the inter-residue hydrogen bond arrangements determined for each of the four minima in compounds **1-10**.

DISCUSSION

Previous papers,[14-16] showed that the conformational maps around the glycosidic bonds of α-D-galactopyranosyl-(1→3)-β-D-galactopyranose and its sulfated derivatives

Table 1. Torsion angles (°), relative strain energies and free energies (kcal/mol) and exocyclic angles for the minimum-energy conformations obtained for sulfated derivatives of the disaccharide 3,6-An-α-D-Gal*p*-(1→3)-β-D-Gal*p*, using the MM3 force-field.

	ϕ_H,ψ_H[a]	E_{rel} (G_{rel})	Exocyclic torsion angles[a,b] χ_2,χ_4 $\chi_1\chi_2\chi_4$ $\omega\chi_6$
1 Region A	-36,42	0.00 (0.38)	Gg Ggg gG
Region B	-43,-35	0.12 (0.00)	Gg Ggg gG
Region C	-36,175	3.18 (4.23)	Gg gGg gG
Region D	-162,-17	4.45 (6.00)	Gg gGg gG
2 Region A	-43,21	0.02 (0.28)	Sg Ggg gG
Region B	-36,-32	0.00 (0.00)	Sg Ggg gG
Region C	-30,-178 (-35,173)[a]	4.33 (5.06)	Sg Ggg gG (Sg gGg gG)[a]
Region D	-159,-34 (-163,-30)	6.81 (7.81)	Sg Ggg gG (Sg gGg gG)
3 Region A	-24,45 (-27,49)	2.64 (3.04)	Gg GSg gG (Gg gGg gG)
Region B	-50,-38 (-49,-37)	0.00 (0.00)	Pg GSg gG (Pg gSg gG)
Region C	-37,179 (-36,177)	4.34 (5.24)	Gg GSg gG (Gg gSg gG)
Region D	-163,-20 (-162,-22)	5.72 (6.42)	Gg GSg gG (Gg gSg gG)
4 Region A	-25,43 (-28,47)	2.43 (3.37)	Sg GSg gG (Sg gGg gG)
Region B	-39,-30 (-39,-28)	0.00 (0.00)	Sg GSg gG (SG gSg gG)
Region C	-35,180 (-35,178)	4.97 (5.74)	Sg GSg gG (Sg gSg gG)
Region D	-165,-30 (-165,-30)	7.19 (8.18)	gg GSg gG (gg gSg gG)
5 Region A	-35,41	0.00 (0.00)	Gg GgS gG
Region B	-38,-24	0.87 (0.45)	Gg GgS gG
Region C	8,163	8.37 (8.40)	Gg gGS gG
Region D	-160,-19	6.32 (6.44)	Gg gGS gG
6 Region A	-32,47	0.42 (0.38)	Sg GgS gG
Region B	-48,-33	0.00 (0.00)	Sg GgS gG
Region C	-17,173 (-7,164)	6.63 (7.59)	Sg GgS gG (Sg gGS gG)
Region D	-158,-35 (-158,-35)	5.60 (6.36)	Sg GgS gG (SG GgS gG)
7 Region A	-24,44 (-27,48)	1.29 (2.15)	Gg GSS gG (Gg gGS gG)
Region B	-49,-34	0.00 (0.00)	Gg GSS gG
Region C	-23,173 (-20,172)	8.31 (9.50)	Gg GSS gG (Gg gSS gT)
Region D	-161,-22 (-161,-24)	5.82 (6.35)	Gg GSS gG (Gg gSS gG)
8 Region A	-18,45 (-23,49)	2.55 (2.07)	Sg GSS gG (Sg gGS gG)
Region B	-49,-31	0.00 (0.00)	Sg GSS gG
Region C	-21,171 (-17,168)	7.88 (8.78)	Sg GSS gG (Sg gSS gG)
Region D	-164,-31	6.17 (6.63)	gg GSS gG
9 Region A	-36,40	0.30 (0.82)	Gg Gg[c]
Region B	-42,-40	0.00 (0.00)	Gg Gg
Region C	-32,-178	3.32 (4.50)	Gg gG
Region D	-160,-19	5.13 (6.79)	Gg gG
10 Region A	-37,37	0.26 (0.66)	Sg Gg[c]
Region B	-56,-56 (-39,-40)	0.00 (0.00)	Gg Gg (Sg Gg)
Region C	-29,-174	3.17 (5.04)	Sg Gg
Region D	-158,-36	6.33 (7.82)	Sg Gg

[a]When the strain energy minimum and the free energy minimum structures do not match, data in parentheses are for the free energy minimum. [b]For nomenclature, see Methods. [c]Only angles χ_2,χ_4, $\chi_1\chi_2$ are tabulated; the angles in the ketal ring C(Me)-C-C=O and C-C-O-H were T (~180°).

Table 2. Conformational partition functions (deg^2) and potential barriers between minima **A** and **B** (kcal/mol) for **1-10**, using the MM3 force-field.

Compound	q	ΔE_{barr}	
		From A	From B
1	2140	0.6	0.5
2	2160	0.3	0.3
3	1040	0.1	2.7
4	1090	0.2	2.5
5	1320	0.9	<0.1
6	1780	0.3	0.9
7	1500	<0.1	1.4
8	780	0.1	2.6
9	2200	0.5	0.8
10	2220	0.6	0.9

Table 3. Hydrogen bond energies ($-E_{HB}$), relative zero-point energies (ΔE_Z), vibrational enthalpies[a] (ΔH_v) and entropic terms[b] ($-T\Delta S$), all expressed in kcal/mol, for the ten compounds under study at the local minima.[c]

	Compound									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Region A										
$-E_{HB}$	2.2	3.3	2.2 (0.8)[c]	3.2 (1.8)	3.3	1.3	3.3 (1.9)	1.4 (-0.1)	1.7	1.2
ΔE_Z	0.1	0.2	0.3 (0.0)	0.1 (0.0)	0.2	0.0	0.3 (0.0)	0.3 (0.0)	0.1	0.1
ΔH_v	0.1	0.0	0.2 (0.3)	0.0 (0.2)	0.0	0.0	0.0 (0.2)	0.1 (0.3)	0.1	0.1
$-T\Delta S$	0.5	0.6	0.9 (0.5)	1.9 (1.5)	0.5	0.4	1.3 (0.9)	0.8 (0.0)	0.6	0.6
Region B										
$-E_{HB}$	1.2	2.3	3.3 (2.2)	3.3 (1.8)	2.1	0.6	3.0	1.5	0.7	1.7 (0.3)
ΔE_Z	0.0	0.0	0.2 (0.1)	0.4 (0.1)	0.0	0.2	0.1	0.5	0.0	0.5 (0.0)
ΔH_v	0.3	0.1	0.2 (0.3)	0.1 (0.3)	0.2	0.2	0.2	0.1	0.2	0.0 (0.2)
$-T\Delta S$	0.0	0.4	0.4 (0.0)	1.1 (0.0)	0.0	0.1	0.7	0.6	0.0	1.3 (0.0)
Region C										
$-E_{HB}$	1.7	2.8 (1.3)	3.0 (1.5)	2.5 (1.0)	0.6	1.9 (0.1)	2.0 (0.3)	1.4 (-0.1)	0.4	1.7
ΔE_Z	0.6	0.6 (0.4)	0.9 (0.7)	0.6 (0.4)	0.3	0.6 (0.2)	0.8 (0.4)	0.9 (0.8)	0.4	0.6
ΔH_v	0.1	0.0 (0.1)	0.0 (0.2)	0.0 (0.2)	0.3	0.0 (0.2)	0.1 (0.3)	0.0 (0.2)	0.2	0.0
$-T\Delta S$	0.8	1.2 (0.0)	1.4 (0.1)	1.5 (0.5)	0.1	1.4 (0.0)	1.6 (0.5)	1.4 (0.5)	0.8	1.7
Region D										
$-E_{HB}$	1.6	1.6 (0.4)	3.7 (2.4)	1.9 (0.3)	1.3	1.3 (1.3)	3.4 (2.1)	1.6	1.0	1.1
ΔE_Z	0.8	0.6 (0.5)	0.5 (0.6)	0.7 (0.5)	0.5	0.5 (0.3)	0.7 (0.5)	1.0	0.7	0.4
ΔH_v	0.0	0.1 (0.2)	0.1 (0.2)	0.2 (0.3)	0.1	0.1 (0.1)	0.1 (0.2)	0.1	0.0	0.0
$-T\Delta S$	1.2	0.9 (0.5)	1.1 (0.2)	1.2 (0.8)	0.1	0.8 (0.7)	0.7 (0.0)	0.5	1.2	1.4

[a]Relative change in vibrational enthalpies between 0 and 298.16 K (*i.e.*, excluding the zero-point energy). [b]At 298.16 K. [c]When the strain energy minimum and the free energy minimum structures do not match, data in parentheses are for the free energy minima.

Table 4. Inter-residue hydrogen bond arrangements established in each minimum energy region for the ten compounds under study.

	Minimum			
	A	**B**	**C**	**D**
1	H(O)2-O5'	-	H(O)4-O5'	H(O)2'-O2
2	H(O)2-O5' & H(O)4-O(S)2'	H(O)4-O(S)2'	H(O)4-O5' & H(O)2-O(S)2'	H(O)2-O(S)2'
3	-	H(O)2'-O(S)2	H(O)4-O5'	H(O)2'-O2 & H(O)2'-O(S)2
4	H(O)4-O(S)2'	H(O)4-O(S)2'	H(O)4-O5'	-
5	H(O)2-O5' & H(O)2'-O(S)4	H(O)2'-O(S)4	-	H(O)2'-O2
6	H(O)2-O5'	-	H(O)2-O(S)2'	H(O)2-O(S)2'
7	H(O)2'-O(S)4	H(O)2'-O(S)2	-	H(O)2'-O2 & H(O)2'-O(S)2
8	-	-	-	-
9	H(O)2-O5'	-	-	H(O)2'-O2
10	H(O)2-O5'	H(O)2-O(S)2'	H(O)2-O(S)2'	H(O)2-O(S)2'

have three main energy minima, suggesting substantial conformational flexibility for the glycosidic linkage. Usually the minimum called **B** (with ϕ_H and ψ_H in near g^- conformation) was the global minimum, with small energy differences with the so called minimum **A** (in which ψ_H has positive values). Sulfation on the β-D-galactose unit at position 2 led to a deepening of the well at the **B** region, whereas sulfation on position 4 shifts the global minimum to the **A** region. This effect agrees with the expectations from the ^{13}C NMR chemical shifts of the polysaccharides containing those repeating units.[16]

The present work, in which the α-D-galactose unit has been replaced by its 3,6-anhydro derivative shows the same trend. 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 the expression of the *exo*-anomeric effect. However, a fourth minimum (**D**), very high in energy for the non-cyclized disaccharide[16] (*i.e.*, without the 3,6-anhydro ring) has a considerably lower energy. In previous papers with other force-fields this was actually the global minimum,[12] or it had only a small energy difference with the global minimum.[4,9]

The flexibility of the glycosidic linkage has been noticeably enhanced. Minima **A** and **B** show almost the same energy (Fig. 1, Table 1), with potential barriers between them below RT (Table 2). All the compounds which are not sulfated on position 2 of the β-D-galactose unit exhibit a low energy "hallway" between these minima, and the other two minima only a few kilocalories above. The presence of large low energy regions cause that flexibility, shown by the partition function being markedly higher than with the non-cyclized derivative. This fact can be rationalized on the basis that for the non-cyclic disaccharide (α-D-galactose with the $^4C_1$ conformation), the glycosidic oxygen is connected by an axial and an equatorial bond, while in neocarrabiose (the 3,6-anhydrogalactose having the $^1C_4$ conformation) both bonds become equatorial. It has already been predicted[23] that axial bonds restrict the flexibility. The inspection of low energy regions and partition functions for maltose/cellobiose,[24,25] and other disaccharides based on glucose[25] and mannose,[26] also led to this conclusion. A concomitant factor leading to increased flexibility (see below) is the change of the C2' substituent from the equatorial to the axial position.

Sulfation does not change the gross features of the maps. Furthermore, sulfation on position 2 (axial) of the 3,6-anhydrogalactose unit has only a slight effect on energies and flexibilities (Tables 1 and 2, cf. **1** and **2**, **3** and **4**, **9** and **10**). This has already been predicted from calculations and X-ray diffraction analysis.[4] When position 4 of the neighboring unit is sulfated, sulfation of C2 stabilizes minimum **B** with respect to **A** by about 1.3 kcal/mol (cf. **5** and **6**, **7** and **8**), but when free energies are considered (Table 1), stabilization is reduced to 0.1-0.8 kcal/mol. Sulfation on position 2 of the β-D-galactose unit has the largest effect by making **B** the global minimum, as occurred with the non-cyclized disaccharide. In the present case, the effect is much larger (>2 kcal/mol). It has already been predicted that large equatorial groups adjacent to the linkage restrict the flexibility.[23] Position 2 of the β-D-galactose is equatorial; sulfation on this position lowers markedly the values of the partition functions (Table 2). However, those values are still higher than those on the non-cyclized disaccharide.[16] Sulfation on position 4 (when C2 of the 3,6-anhydrogalactose is not sulfated) shows the opposite trend, by slightly deepening minimum **A**, but markedly increases the energy of the **C** region, and shifts the positions of those minima. On the other hand, pyruvylation only slightly affects the energies and

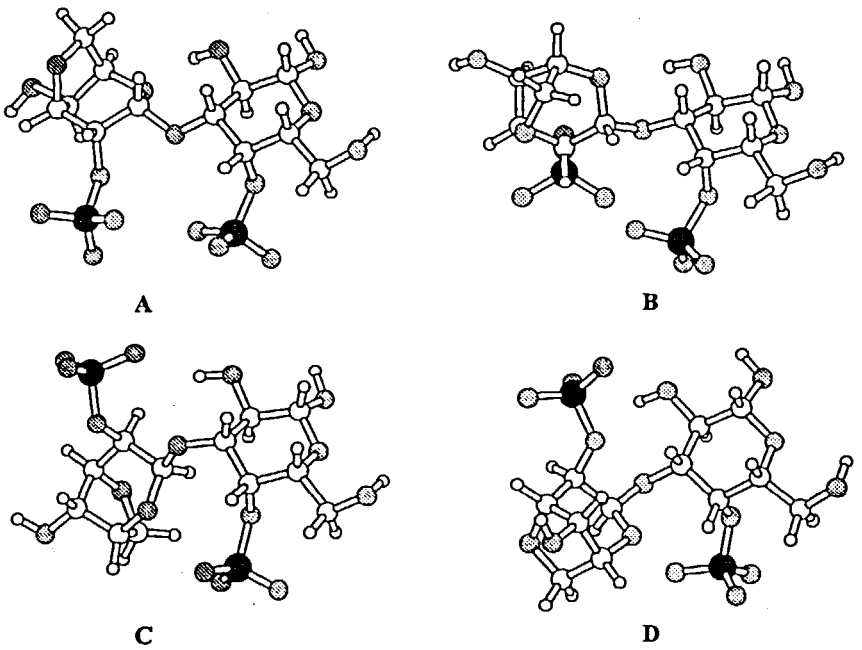


Figure 5. Drawings of the minimum-energy conformers of compound **6** in the four minimum-energy regions

geometries of the structures at the minima, and the flexibility is not reduced, as shown by the partition functions. Figure 5 shows the minimum energy conformations of compound **6** (as an example) in each of the four regions.

The arrangements of the inter-residue hydrogen bonds (Table 4) may help to explain the relative stabilities of some minima. For instance, minimum **B** appears stabilized with respect to **A** in β-D-galactose 2-sulfated compounds (cf. **1** and **3**). This may be due to two factors: a) in **B** a hydrogen bond joins H(O)2' and the negatively charged sulfate oxygen, and b) in **A**, the hydrogen bond between H(O)2 and the ring oxygen O5' is precluded by sulfation (cf. Fig. 5). By the same token, the high energy of minimum **C** in 4-sulfated compounds may be rationalized by the preclusion of hydrogen bonding between H(O)4 and O5' effected by 4-sulfation (Tables 3 and 4).

The crystal structure for neocarrabiose[5] shows ϕ_H,ψ_H angles of –23°, 20°, *i.e.*, in the **A** region (Fig. 2), and the hydroxymethyl group is in the GT orientation. The present

work shows that MM3 has detected precisely this region and hydroxymethyl orientation as the minimum-energy one, although with very low energy difference with the **B** region (actually the free energy-minimum region). The rigid-residue analysis of Lamba *et al.*[5] also encountered region **A** as the lowest-energy one, but it switched to region **B** when a relaxed MM2CARB study was carried out. The work by Ueda and Brady[12] using a CHARMM-type force-field[27] indicated **D** as the global minimum, and TG as the hydroxymethyl orientation, whereas it failed to identify the region **A-B** as low-energy. Another approach with a variation of the same force-field,[11] found **B** as the global minimum, whereas **A** has an energy 1.1 kcal/mol higher. The rigid-residue analysis of Urbani *et al.*[10] indicated little flexibility around the **A-B** region. The fiber diffraction analysis[7] suggests that ι-carrageenan has an ordered conformation with ϕ_H,ψ_H angles around –43°, –40° and the hydroxymethyl group in the GT orientation.[7,9] These data match with the MM3 calculations, where the minimum falls in the **B** region (Table 1, Fig. 3). Similar results were found in the rigid-residue analysis of Le Questel et al.,[4] although their maps showed reduced flexibility and a different shape of the minimum energy region, which extends "horizontally" (*i.e.*, with low energies for **D** conformers and higher energies for the **A** rotamers). For κ-carrageenan, fiber diffraction analysis also gave a conformation in the **B** region.[4,9] This matches with the calculations carried out by Urbani *et al.*,[10] and Le Questel *et al.*[4] However, the minimum with the present calculations is in the **A** region, but with an energy only slightly lower than that of the **B** minimum, a difference that is reduced even further when free energy is calculated. The **D** conformer was predicted to have low-energy in previous calculations with other force-fields,[4,5,12] but it has never been detected experimentally. MM3 predicts an energy for those conformers that is probably closer to reality; this may be ascribed to a better parameterization of the exo-anomeric effect, leading to conformers with lower-energies in the **A-B** region. Some of the other force-fields[4,5,12] found minima in regions where the present work does not. Actually, it should be considered that some of these minima were also found in the present calculations using block-diagonal Newton-Raphson optimizations. However, further full-matrix minimization and vibrational analysis discarded their presence (see Results).

Engelsen and Rasmussen[22] have pointed out the need of calculating free energies as they showed that, for a lactose conformational map, the conformational entropy is

neither negligible nor uniformly distributed. This is reinforced in the present work (Tables 1 and 3). MM3(94) free energy calculations for carbohydrates were validated by comparison with *ab initio* molecular orbital calculations.[28] In order to obtain the free energy from the strain energy, some constant and three variable terms should be added. These are the zero-point energy, the change in vibrational enthalpy from 0 to 298 K, and the entropic term. Although hydrogen bonding causes a decrease in the strain energies, an opposite trend is shown for the other free energy terms. It has been suggested that this is caused by the expected loss of entropy upon formation of the hydrogen bond,[29] and by an increase in its enthalpy by the zero-point energy correction.[30] This work shows the expected relationship between hydrogen bonding energies and entropy when comparing two conformers on the same region (Table 3). However, no such relationship can be established when two conformers in different regions are compared. Thus, factors different from hydrogen bonding are altering the entropy of minima located in different regions.[29] Differences in zero-point energy values follow the same trend as entropic factors, but have usually lower magnitudes (Table 3), while the remaining vibrational enthalpies show a reverse trend, with small differences (< 0.3 kcal/mol). The effect of calculating free energies has allowed determination of some trends: a) for most compounds (but for **6** and **8**, sulfated together on positions 4 of the β-galactose unit, and 2 of the 3,6-anhydrogalactose unit) minimum **A** is destabilized with respect to **B**, and b) minima **C** and **D** are less favored when free energies are calculated: this may be explained either by their lower entropy content (Table 3) or by the higher steric energy values carried by their conformers with comparable or higher entropy. In many cases, the minimal energy conformer in each region deduced from steric energies is changed when free energies are calculated (Table 1). In most of the cases, the differences between both groups of conformers arise from the torsional angles around the C1-O1 and C2-O2 bonds. Enthalpies tend to favor negative χ_1 and positive χ_2 values, (Ggg, Table 1) carrying stronger hydrogen bonds (Table 3), while reversed orientations are predicted from free energy calculations (gGg, Table 1).

The ^{13}C NMR signal corresponding to C1 of the 3,6-anhydro-α-D-galactose unit of a ι-carrageenan is almost 4 ppm upfield from that of an alkali treated λ-carrageenan.[31] These carrageenans differ only in the position of sulfation of the β-D-galactose unit (4- in

the first case, 2- in the latter). It was postulated that those chemical shift differences are related to the H1'-H3 distance.[32] The opposite trend has been found for ν- and λ-carrageenan,[31,33] which was explained on grounds of their main minima being at the **B** and **A** regions, respectively.[15,16] For the 3,6-anhydro derivatives, the same tendency is shown, but the chemical shifts show opposite field effects.

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