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MM3 Potential energy surfaces of trisaccharide models of λ-, μ-, and ν-carrageenans☆

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Abstract—The adiabatic potential energy surfaces (PES) of six trisaccharides, sulfated derivatives of α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-α-D-Gal*p* and β-D-Gal*p*-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p* representing models of λ-, μ-, and ν-carrageenans were obtained using the MM3 force-field at $\varepsilon = 3$. Each PES was described by a single contour map for which the energy is plotted against the two ψ glycosidic angles, given the small variations of the ϕ glycosidic torsional angle in the low-energy regions of disaccharide maps. Most surfaces appear as expected from the maps of the disaccharidic repeating units of carrageenans, with less important factors altering the additive effect of both linkages. Only small interactions between the first and third monosaccharidic moieties of the trisaccharides are observed. The flexibility of the α-linkages appears nearly identical to that in their disaccharide counterparts, with only one exception, where it appears reduced by the presence of the third monosaccharide. On the other hand, the flexibility of the β-linkage appears to be equal or sometimes even higher than that observed for the corresponding disaccharide.

Keywords: Trisaccharides; Molecular mechanics; MM3; Potential energy surfaces; Carrageenans

1. Introduction

Red seaweeds are an important source of sulfated galactans. Among them, carrageenans are interesting because of their capacity to act as thickeners, gelling agents, and products with pharmacological activity. Carrageenan structures are based on linear chains of alternating 3-linked β-D-galactopyranosyl units, and 4-linked α-D-galactopyranosyl (or 3,6-anhydro-α-D-galactopyranosyl) units, usually sulfated in different positions.[2] The properties of these and other polysaccharides depend on their conformations and molecular flexibility, which is concentrated mostly on the glycosidic linkages. Some fiber diffraction studies of oriented carrageenan fibers were carried out,[3,4] as well as single crystal studies of low-molecular weight analogs.[5,6] Conformational studies were first complicated for the lack of parameters for the sulfate group. However, after the development of those parameters,[7–12] modeling studies of the disaccharide repeating units of carrageenans have been carried out using different force-fields.[13–25] These studies have been recently reviewed.[2] MM3[26,27] is considered an useful force-field for carbohydrates, given its considerations to hydrogen bond forces, anomeric, and *exo*-anomeric effects. It was systematically used to evaluate the potential energy surfaces of many disaccharides,[2,28] including the repeating units of carrageenans[15,16,19–21,24] using the parameters for sulfate described by Lamba et al.[11] for a dielectric constant of 3. First, attempts to extrapolate disaccharide modeling to higher oligosaccharides were made analyzing each glycosidic linkage taken independently.[29–33] However,[34] a clear correlation for the contour maps of disaccharides with the energy related to ϕ and ψ, and to x–y plots with the energy just related to ψ was found. This has opened the possibility of representing the potential energy surfaces of trisaccharides by contour maps in which the energy is plotted as a function of both ψ glycosidic angles. This method

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[☆] MM3 Potential energy surfaces of trisaccharides. Part 6. For Part 5, see Ref. 1.

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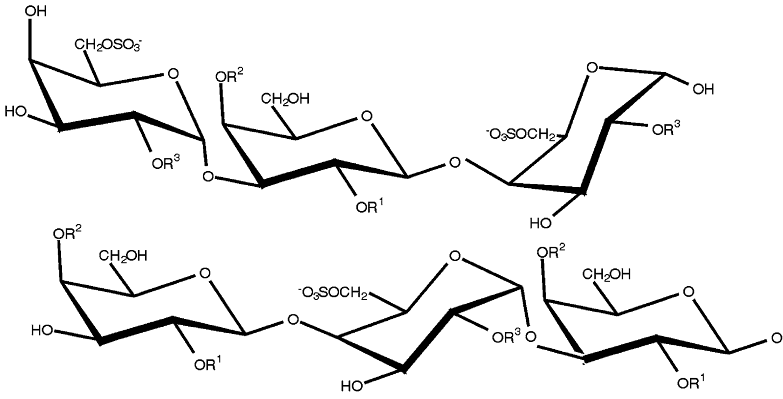


R^1	R^2	R^3	
SO_3^-	H	SO_3^-	**Lαβ**
H	SO_3^-	H	**Mαβ**
H	SO_3^-	SO_3^-	**Nαβ**
SO_3^-	H	SO_3^-	**Lβα**
H	SO_3^-	H	**Mβα**
H	SO_3^-	SO_3^-	**Nβα**

Figure 1. The six trisaccharides studied in this work.

was successfully applied to many trisaccharides,[1,35–38] including 3,6-anhydrogalactose-containing carrageenan models.[36] In most cases, each linkage behaves independently (i.e., they appear as expected from observing the disaccharide maps). However, in some cases interactions between the first and third monosaccharide moieties of the trisaccharides are produced,[36,37] thus changing sharply the expected shapes of the surfaces.

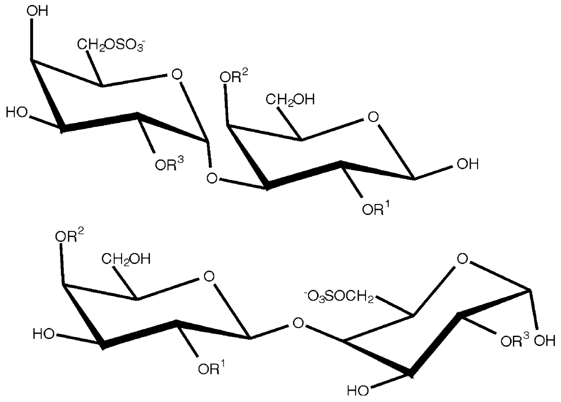
Herein, the potential energy surfaces of the two trisaccharides, which model the basic structure of non-gelling carrageenans, that is, 2,2′,2″,6,6″-pentasulfonato α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-α-D-Gal*p* (**Lαβ**), their 4′,6,6″-trisulfonato (**Mαβ**) and 2,2″,4′,6,6″-pentasulfonato (**Nαβ**) counterparts, and 2,2′,2″,6′-tetrasulfonato β-D-Gal*p*-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p* (**Lβα**), as well as its 4,4″,6′-trisulfonato (**Mβα**) and 2′,4,4″,6′-tetrasulfonato (**Nβα**) counterparts (Fig. 1) were determined using MM3 at $\varepsilon = 3$. For comparison, the minima of the disaccharides were also recalculated using this variant of the force-field. Shapes and flexibility measurements for the trisaccharides are compared with those of the disaccharide counterparts, as a step toward the knowledge of the shape of higher polymers.

2. Methods

The molecular mechanics program MM3 (92) (QCPE, Indiana University, USA), developed by Allinger and co-workers, was used,[26,27] modified as the MM3 (2000) version in O–C–C–O and O–C–O–H torsional parameters, O–H hydrogen bonding parameters and C–H electronegativity correction.[39] Besides, the maximum atomic movement was modified in the MM3 routines from 0.25 to 0.10 Å.[40] MM3 parameters for the sulfate group were taken from Lamba et al.,[11] 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, with no cations added. Minimizations were carried out by the block diagonal Newton–Raphson procedure for grid points (using default termination conditions) and using the full-matrix procedure for minima. The main minima were resubmitted to calculations with hundredfold more stringent termination conditions up until the energy remained constant. In this way, it was found that several geometries were reorganized. The constituting monosaccharides are numbered starting from the reducing end. The dihedrals $\phi_{2\to1}$ and $\psi_{2\to1}$ are defined by atoms O-5′–C-1′–O-1′–C-*n* and C-*n* + 1–C-*n*–O-1′–C-1′, respectively, while $\phi^{H}_{2\to1}$ and $\psi^{H}_{2\to1}$ are defined by atoms H-1′–C-1′–O-1′–C-*n* and H-*n*–C-*n*–O-1′–C-1′, respectively, where *n* is 3 for the α-(1→3)-linkage or 4 for the β-(1→4)-linkage. The equivalent dihedrals for the 3→2 linkage use the same conventions, but the non-primed-atoms should be primed, while those primed should be double-primed. The names ψ_β or ψ_α to denote the ψ dihedral angle corresponding to a β- or α-linkage, respectively, are also used. The orientation of the hydroxyl hydrogen atoms is indicated by χ_n, defined by the atoms H-*n*–C-*n*–O-*n*–H(O)-*n*, while χ_6 is defined by the atoms C-5–C-6–O-6–H(O)-6, and ω by the atoms O-5–C-5–C-6–O-6, single- or double-primed when necessary. Their values are described by a one-letter code:[41] **S** for angles between −30 and +30°, **g** for 30–90°, **e** for 90–150°, **T** for angles with absolute value larger than 150°, **E** for angles between −90° and −150°, and **G** for angles between −30° and −90°. When a sulfate group replaced a hydroxyl hydrogen, the same definition was used.

2.1. Generation of di- and trisaccharide minima used as starting points

The minima previously obtained[19,24] for the disaccharides (Fig. 2) were recalculated using the new variation of the force-field and the more stringent termination conditions (see above). New combinations of exocyclics were also added. A final set containing all the minima within 1 kcal/mol from the main minimum in each region was obtained. For the trisaccharides, the minima in each region were determined as explained elsewhere:[35] starting structures were generated with orientations of the hydroxyl and hydroxymethyl groups that yielded



R^1	R^2	R^3	
SO_3^-	H	SO_3^-	**L**α
H	SO_3^-	H	**M**α
H	SO_3^-	SO_3^-	**N**α
SO_3^-	H	SO_3^-	**L**β
H	SO_3^-	H	**M**β
H	SO_3^-	SO_3^-	**N**β

Figure 2. The disaccharides studied in this work.

low-energy minima in the disaccharide maps, taking into account those occurring at the reducing ends (in monosaccharides 1 and 2) and non-reducing ends (in monosaccharides 2 and 3). In an automated fashion, unrestrained MM3 full-matrix calculations were carried out for points starting at each of those orientations, each one at a different combination of the ϕ, ψ angles (in either of both linkages), which gives rise to a minimum in the disaccharide maps. For each minimum, only those orientations with an energy up to 1 kcal/mol above the lowest in that $\psi_{2\to1}$, $\psi_{3\to2}$ region were kept and served as starting points for the generation of the maps.

2.2. Generation of trisaccharide maps

The maps were generated following the general procedures described for di- and trisaccharides.[20,35,36] Both the dihedral drivers 2 (sequential) and 4 (from the initial structure) were used: the first one for all the starting points previously generated, and the second one for each unique orientation of exocyclic angles. The angles $\psi_{2\to1}$ and $\psi_{3\to2}$ were fully varied using a 24° grid. At each point, energies were calculated after minimization with restraints for these two angles but allowing the other variables (including both ϕ angles) to relax. The conformational adiabatic maps, or energy surfaces as function of both ψ angles were produced. In order to recalculate the surface in the region where the main minima appear (both ψ between 40–88° and 192–208°, depending on the compound), the starting points that yielded low-energy grid points in this region were submitted to calculations using the same procedure described above, but with an 8° grid. The maps were inspected carefully for the possible appearance of new minima in a different location. When such situation appeared, the minima were identified, and used as new starting points for the recalculation of the maps.

2.3. Flexibility measurements and generation of 2D maps

The partition function $q_{\psi,\psi}$ was calculated as:[35,36] $q_{\psi,\psi} = \Delta\psi^2 \times \sum_{i=1}^{\mathrm{ES}} \mathrm{e}^{-(Ei-Egm)/RT}$ where $\Delta\psi$ is the grid spacing (24° for full maps, 8° for amplifications) and the summation is carried out over the entire surface determined (e.g., ES = 225 for full maps). The largest value was actually used. The partial flexibility on each of the ψ torsional angles of the trisaccharides was evaluated by generating estimates of the adiabatic [energy vs $\psi_{3\to2}$] and [energy vs $\psi_{2\to1}$] relationships. These were created by recording the minimum energy in the amplified maps (8° grid) for each column and for each row of data, respectively, assuming that they were generated by a continuous path. From these data, partial $q_{\psi3\to2}$ and $q_{\psi2\to1}$ values were calculated using the above-mentioned equation. The 2D maps for each linkage of the trisaccharide were generated using the values of minimal energy within each path determined when calculating the partial flexibilities. For the disaccharides, the entire set of low-energy conformers in the **A**–**B** regions was used as starting conformers[1] to optimize with restrained ψ in 8° intervals, using both dihedral drivers and allowing the remaining variables to relax. The energy for each ψ point was considered to be the lowest of all the calculations, thus giving rise to a 2D conformational adiabatic plot as a function of the ψ angle. The flexibility was calculated using the same protocol.[1] The allowable surfaces AS_5 and AS_{10} were calculated as the quotient of the number of points below 5 and 10 kcal/mol (respectively) of relative energy and 225 (the total number of points in the 24° × 24° ψ, ψ grid) taken as percentage. For the disaccharides or for each separated linkage of the trisaccharide, the $AS_{5\text{-}\alpha}$ and $AS_{5\text{-}\beta}$ were calculated using the same principle, over the 2D energy versus ψ plot calculated at 8° intervals.

3. Results

The adiabatic conformational surfaces[35] of the trisaccharides **Lαβ**, **Lβα**, **Mαβ**, **Mβα**, **Nαβ**, and **N**βα (Fig. 1) were obtained using the MM3 force-field at $\varepsilon = 3$. The minima for the disaccharides **Lα**, **Lβ**, **Mα**, **Mβ**, **Nα**, and **Nβ** (Fig. 2) were also calculated using the same

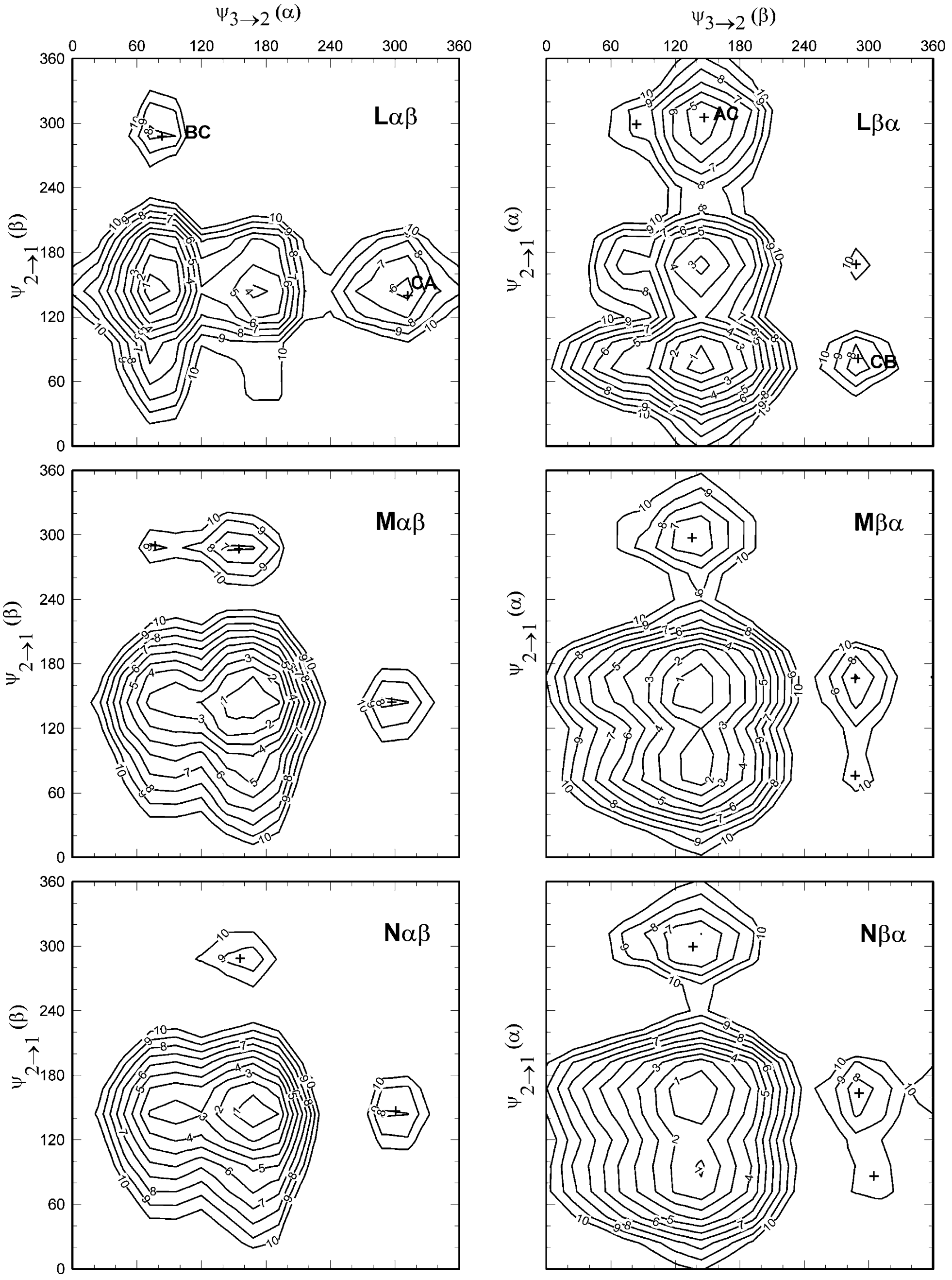


Figure 3. MM3 Adiabatic conformational maps (energy vs $\psi_{3\to2}$, $\psi_{2\to1}$) of the six trisaccharides under study at $\varepsilon = 3$. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum, up to 10 kcal/mol. The symbol (+) indicates some of the MM3 local minima.

force-field. Figure 3 depicts the conformational surfaces for the trisaccharides as contour maps. The three minima appearing for each disaccharidic unit produce nine minima for the trisaccharides, with different stabilities. A low-energy region appears at ψ values between 50° and 200°, with slightly different patterns in each case (Fig. 3). A recalculation of the low-energy regions of **Lαβ** and **Lβα** leads to the plots shown in Figure 4: four minima are observed, one of which is by far the global minimum. Figure 5 shows the plots corresponding to **Mαβ** and **Mβα** and Figure 6 shows those corresponding to **Nαβ** and **Nβα**, all of which show a two-minima pattern. Table 1 indicates the data (geometries and energies) on the minima obtained in each region for the disaccharides, whereas Table 2 shows the same data for the trisaccharides **Lαβ**, **Mαβ**, and **Nαβ**, and Table 3 shows the corresponding data for the remaining trisaccharides. The minima are named after a two-letter code according to the minimum energy region (**A**, **B**, or **C**)[19,24] of the 3→2 linkage (first letter) and the 2→1 linkage (second letter), as shown elsewhere[35,36] (see Figs. 3 and 4). Although the ϕ angles were allowed to relax in

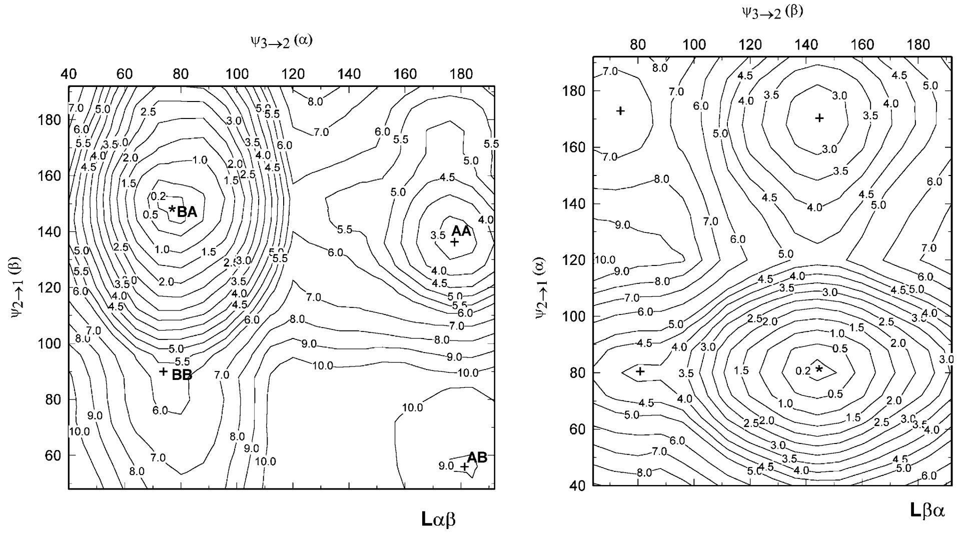


Figure 4. MM3 Adiabatic conformational maps of compounds **Lαβ** and **Lβα** recalculated in a low-energy region. Iso-energy contour lines are graduated in the indicated increments above the global minimum. The symbols indicate: (*) MM3 global minima; (+) MM3 local minima, some of the regions are labeled.

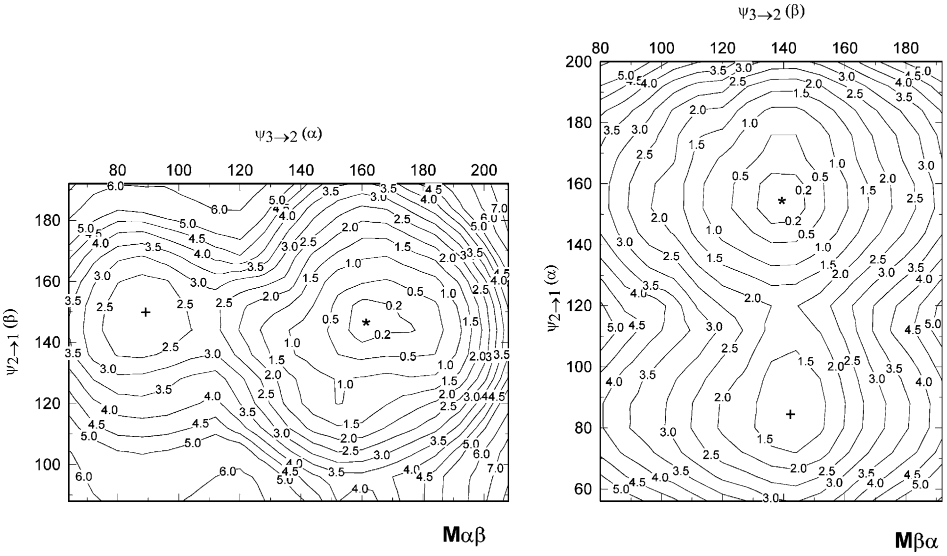


Figure 5. MM3 Adiabatic conformational maps of compounds **Mαβ** and **Mβα** recalculated in a low-energy region. Iso-energy contour lines are graduated in the indicated increments above the global minimum. The symbols indicate: (*) MM3 global minima; (+) MM3 local minima.

the calculation of the maps, their values remained within a small range (<35°) in most of the minima (with the exception of those labeled as **B′**, Tables 1–3). Figures 7 and 8 depict molecular drawings for the minimum-energy conformations of the six trisaccharides under study.

Table 4 shows the flexibility measurements carried out using the data obtained for the di- and trisaccharides under study. The hydrogen-bonding pattern was determined for the main minima in each region. Starting from these data, Table 5 was built up to show the main hydrogen bond arrangements established for the trisaccharides in each minimum, expected to appear in higher polymers.

4. Discussion

A recent review has illustrated the conformational characteristics of carrageenan models that were the subject of computational studies.[2] Using MM3, we have first studied the disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* and its sulfated derivatives,[15,16,19,20] and afterwards the most usual repeating units of gelling carrageenans 3,6-An-α-D-Gal*p*-(1→3)-β-D-Gal*p* and sulfated derivatives,[21] as well as their equivalents with reversed linkage.[24] We have also studied some trisaccharides containing 3,6-anhydrogalactose,[36] taking advantage of the possibility of depicting the potential energy surfaces of trisaccha-

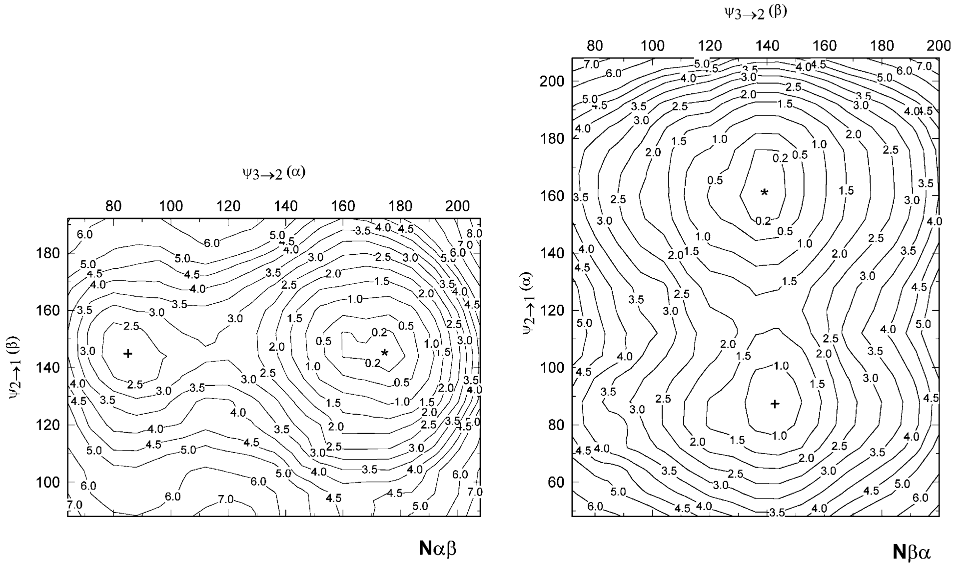


Figure 6. MM3 Adiabatic conformational maps of compounds **Nαβ** and **Nβα** recalculated in a low-energy region. Iso-energy contour lines are graduated in the indicated increments above the global minimum. The symbols indicate: (∗) MM3 global minima; (+) MM3 local minima.

Table 1. Torsion angles (°), relative steric energies (kcal/mol), and exocyclic angles for the minimum-energy conformations in the main regions obtained for the disaccharides under study using the MM3 force-field

	ϕ, ψ	(ϕ^{H},ψ^{H})	E_{rel}	Exocyclic torsion angles[a]		Literature data[19]	
				$\chi_{2'}\chi_{3'}\chi_{4'}\omega'\chi_{6'}$	$\chi_1\chi_2\chi_4\omega\chi_6$	(ϕ^{H},ψ^{H})	E_{rel}
Lα							
A	103, 169	−17, 53	2.74	Sgg gT	gGg gG	−20, 53	2.50
B	89, 80	−31, −40	0.00	Sgg gG	gSg gG	−33, 41	0.00
C	84, 306	−37, −171	3.85	Sgg gE	gSS gG	−37, −172	3.27
Mα							
A	89, 154	−32, 37	0.00	Tgg gT	ggS gG	−34, 44	0.00
B	69, 84	−52, −37	1.12	Tgg gE	gGS gG	−50, −34	1.61
C	103, 299	−18, −179	6.14	Tgg gT	gGS gG	−16, 180	7.33
Nα							
A	89, 162	−32, 45	0.00	Sgg ge	gSS gG	−30, 55	0.00
B	69, 85	−51, −36	0.81	Sgg gE	gGS gG	−52, −37	1.50
C	102, 301	−19, −176	5.57	Sgg gT	ggS gG	0.17, −179	6.15
				Exocyclic torsion angles[a]		Literature data[24]	
				$\chi_{2'}\chi_{3'}\chi_{4'}\omega'\chi_{6'}$	$\chi_1\chi_2\chi_3\omega\chi_6$	(ϕ^{H},ψ^{H})	E_{rel}
Lβ							
A	281, 146	43, 27	0.00	Sgg gG	GSg GT	36, 27	0.00
B	243, 90	4, −34	4.51	Sgg gG	GSg gG	9, −39	3.77
C	274, 287	36, 171	7.63	Sgg gG	GGT gT	35, 175	7.03
Mβ							
A	284, 143	46, 23	0.00	GgS gG	GTg GT	36, 25	0.00
C	274, 289	37, 173	7.73	GgS gG	GgT gE	38, 173	6.25
B′	208, 111	−34, −10	3.78	GgS gG	GTg Ge	−34, −4	2.95
Nβ							
A	283, 142	45,23	0.00	GgS gG	GSg GT	33,24	0.00
C	271, 292	34, 175	8.47	GgS gG	GST gE	34, 175	7.32
B′	207, 111	−35, −10	3.65	GgS gG	GSg Ge	−35, −4	2.82

Comparison with previous data is included.

[a] For nomenclature of the angles, see Section 2.

rides as single contour plots (on the basis that there is no need to relate the glycosidic linkage features with angle ϕ).[34,35] The calculations were carried out at $\varepsilon = 3$ and with a set of dipole parameters for the sulfate group that

Table 2. Torsion angles (°), relative steric energies (kcal/mol), and exocyclic angles for the minimum-energy conformations in every region with relative energies lower than 10 kcal/mol obtained for trisaccharides **Lαβ**, **Mαβ**, and **Nαβ** using the MM3 force-field

	$\phi_{3\to2}$, $\psi_{3\to2}$	(ϕ^H,ψ^H)	$\phi_{2\to1}$, $\psi_{2\to1}$	(ϕ^H,ψ^H)	E_{rel}	Exocyclic torsion angles[a]		
Lαβ								
AA	93, 178	−27, 62	294, 137	55, 18	3.21	Sgg gT	Gg gG	GSg GT
AB	91, −179	−29, 65	267, 56	28, −69	8.83	Sgg ge	Gg gG	GST gT
BA	73, 77	−48, −45	280, 148	42, 29	0.00	Sgg ge	Sg gG	GSg GT
BB	75, 76	−45, −46	247, 91	9, −33	5.73	Sgg ge	Sg gG	GSg gE
BC	86, 83	−34, −37	272, 291	35, 175	6.82	Sgg gE	Sg gG	GGT gE
CA	83, 311	−38, −166	292, 139	54, 20	5.54	Sgg gE	SS gG	GSg GT
Mαβ								
AA	86, 161	−35, 44	286, 147	47, 27	0.00	Tgg ge	SS gG	GTg GT
AC	89, 154	−32, 37	272, 287	34, 171	6.54	Tgg gT	gS gG	GgT gT
BA	70, 89	−51, −32	286, 150	48, 31	2.00	Tgg ge	GS Gg	GTg GT
BC	66, 78	−54, −43	272, 289	34, 173	8.52	Tgg ge	gS gG	GgT gT
CA	104, 298	−16, −179	284, 144	45, 24	7.41	Tgg gT	GS gG	GTg GT
AB′[b]	91, 172	−30, 56	206, 113	−36, −9	5.48	Tgg ge	SS gG	GTg Ge
BB′[b]	67, 84	−53, −37	207, 110	−35, −12	5.89	Tgg ge	GS gG	GTg Ge
Nαβ								
AA	86, 175	−34, 58	285, 145	47, 26	0.00	Sgg gE	SS gG	GSg GT
AC	90, 159	−31, 42	271, 288	33, 172	8.60	Sgg gE	gS gT	GST gT
BA	69, 85	−51, −36	283, 145	45, 25	1.97	Sgg ge	GS gG	GSg GT
BC	66, 79	−54, −43	271, 290	33, 174	9.97	Sgg ge	gS gT	GST gT
CA	105, 300	−15, −178	281, 149	43, 29	7.35	Sgg gT	gS gG	GSg GT
AB′[b]	98, 164	−23, 47	203, 116	−40, −6	5.46	Sgg gT	SS gG	GSg Ge
BB′[b]	68, 85	−53, −37	207, 110	−35, −11	6.47	Sgg ge	GS gG	GSg Ge

[a] The angles are given in the order $\chi_{2''}\chi_{3''}\chi_{4''}\omega''\chi_{6''}\chi_{2'}\chi_{4'}\omega'\chi_{6'}\chi_1\chi_2\chi_3\omega\chi_6$. For nomenclature of the angles, see Section 2.

[b] They are true minima, but other conformations with the same ψ, ψ have lower energy. Thus, they do not appear within the adiabatic map.

allow to screen partially the strong electrostatic interactions occurring in vacuum calculations and thus to emulate better the molecules in solution or crystal state.[11]

It was already shown for the disaccharides that the glycosidic preferences of the α-linkage are strongly influenced by the sulfation pattern of the β-galactose unit.[19] Table 1 shows that the 2-sulfated compound (**Lα**) exhibits a minimum in the **B** region (negative ψ^H), whereas the 4-sulfated compounds (**Mα** and **Nα**) shift the minimum to the **A** region (positive ψ^H). This has consequences over several experimental values as NMR chemical shifts and optical rotations.[15,19] On the other hand, the conformation around the β-linkage is less influenced by the sulfation pattern.[24] Table 1 shows that a similar global minimum (**A**) occurs for the three compounds. For the 2-sulfated compound (**Lβ**), other minima (**B** and **C**) with different ψ value and higher energy appear. On the other hand, 4-sulfated disaccharides (**Mβ** and **Nβ**) give rise to the same **C** minimum, but none in the **B** region: another minimum appears (**B′**, probably stabilized by hydrogen bonding, see later) with the expected ψ value, but with a ϕ value outside the region stabilized by the exo-anomeric effect.

An examination of the adiabatic map of compounds **Lαβ** and **Lβα** (Figs. 3 and 4), and the energy data on the minima (Tables 2 and 3), evidences that it represents the crossing of the maps of each linkage behaving more or less independently. Thus, the three main minima appearing for each linkage are transformed into nine minima. However, as a difference with that occurring for the 3,6-anhydrogalactose counterparts,[35] the ϕ, ψ angles were not always the same as those encountered for the disaccharides (Table 1): the differences are from negligible to up to 15° in ϕ and/or ψ, but in one case, have shifted further away (minimum **B** for the β linkage, ϕ, $\psi = 243°, 90°$ for the disaccharide, 267°, 56° in one **B** minimum of the trisaccharide **Lαβ**). In any case, the global minimum for the trisaccharides appears as the combination of regions giving rise to the global minimum for each disaccharide, that is **AB** for **Lβα** and **BA** for **Lαβ**. As expected, **AA** appears as the next minimum, about 3 kcal/mol higher in energy (Tables 1–3). The adiabatic maps (Fig. 4) clearly reflect this behavior, by showing one main minimum and other less important minima.

The α-linkage of the 4-sulfated disaccharides **Mα** and **Nα** appears with two more-leveled off minima, distant only around 1 kcal/mol from each other (Table 1). This behavior is also observed for the trisaccharides **Mβα** and **Nβα**, for which the global minimum (**AA**) is also ca. 1 kcal/mol more stable than the second one (**AB**, Table 3). For the reverse trisaccharides (**Mαβ** and **Nαβ**), the same qualitative effect appears, but the difference between the minimum **AA** and the second one (**BA**) rises to ca. 2 kcal/mol, suggesting that the presence of another linkage on the reducing end of the α-disaccharidic moiety may be partly destabilizing the **B** minimum. The geometries of the minima are more similar between the

Table 3. Torsion angles (°), relative steric energies (kcal/mol), and exocyclic angles for the minimum-energy conformations in every region with relative energies lower than 10 kcal/mol obtained for trisaccharides **Lβα**, **Mβα**, and **Nβα** using the MM3 force-field

	$\phi_{3\to2}$, $\psi_{3\to2}$	(ϕ^{H},ψ^{H})	$\phi_{2\to1}$, $\psi_{2\to1}$	(ϕ^{H},ψ^{H})	E_{rel}	Exocyclic torsion angles[a]		
Lβα								
AA	283, 144	44, 24	96, 171	−25, 55	2.57	Sgg gG	Sg GT	gGg gG
AB	280, 144	41, 25	87, 81	−33, −40	0.00	Sgg gG	Sg gT	gSg gG
AC	281, 144	43, 25	84, 306	−37, −171	3.96	Sgg gG	Sg gE	gSS gG
BA	251, 73	13, −52	97, 173	−23, 57	6.30	Sgg gG	ST gT	gGg gG
BB	246, 81	7, −44	86, 80	−33, −41	3.87	Sgg gG	ST gE	gSg gG
BC	243, 82	3, −43	86, 299	−35, 178	8.57	Sgg gG	ST gE	gSS gG
CA	273, 288	36, 172	103, 170	−17, 54	9.25	Sgg gG	GT gT	gGg gG
CB	273, 287	36, 171	75, 81	−44, −40	6.86	Sgg gG	GT gE	gSg gG
Mβα								
AA	281, 139	43, 20	88, 155	−33, 38	0.00	GgS gG	Tg gT	ggS gG
AB	284, 142	46, 23	70, 85	−50, −36	1.02	GgS gG	Tg GT	gGS gG
AC	285, 139	47, 20	102, 297	−19, 180	5.97	GgS gG	Tg GT	gGS gG
CA	274, 289	37, 173	92, 169	−29, 52	6.91	GgS gG	gT gE	gSS gG
CB	274, 289	37, 172	71, 77	−49, −44	8.87	GgS gG	gT gE	gGS gG
B′A[b]	208, 111	−34, −11	89, 171	−32, 54	3.93	GgS gG	Tg Ge	gSS gG
B′B[b]	202, 114	−40, −7	70, 86	−50, −35	5.15	GgS gG	Tg GT	gGS gG
B′C[b]	203, 113	−39, −9	103, 296	−17, 179	9.98	GgS gG	Tg GT	gGS gG
Nβα								
AA	280, 139	41, 19	89, 161	−32, 33	0.00	GgS gG	Sg gT	gSS gG
AB	282, 142	44, 22	71, 87	−50, −34	0.60	GgS gG	Sg GT	gGS gG
AC	285, 139	46, 19	100, 300	−20, −178	5.43	GgS gG	Sg GT	ggS gG
CA	274, 291	37, 174	92, 163	−28, 47	7.34	GgS gT	GT gE	gSS gG
CB	272, 305	36, −172	70, 82	−50, −39	8.70	GgS gG	gT gE	gGS gG
B′A[b]	208, 111	−34, −11	90, 171	−30, 55	3.39	GgS gG	Sg Ge	gSS gG
B′B[b]	208, 110	−34, −11	70, 87	−50, −34	3.96	GgS gG	Sg Ge	gGS gG
B′C[b]	203, 113	−39, −9	101, 299	−19, −178	9.28	GgS gG	Sg GT	ggS gG

[a] The angles are given in the order $\chi_{2''}\chi_{3''}\chi_{4''}\omega''\chi_{6''}\chi_{2'}\chi_{3'}\omega'\chi_{6'}\chi_{1}\chi_{2}\chi_{4}\omega\chi_{6}$. For nomenclature of the angles, see Section 2.

[b] They are true minima, but other conformations with the same ψ, ψ have lower energy. Thus, they do not appear within the adiabatic map.

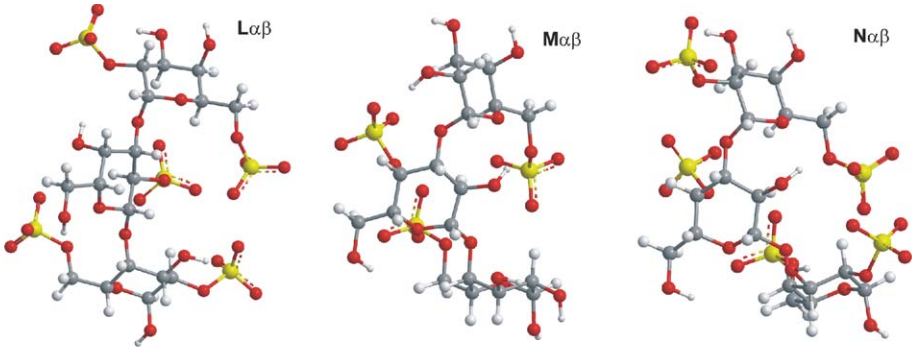


Figure 7. Drawings of the minimum-energy conformations of the trisaccharides **Lαβ**, **Mαβ**, and **Nαβ**.

di- and trisaccharides than those observed for the λ-carrageenan models. They are more or less alike for the minima of the β-linkage, and for the minimum **B** of the α-linkage (Tables 1–3). Only in three cases for the **A** minimum in the α-linkage, the ψ angle had a value 13–17° higher in the trisaccharide than in the disaccharide (Tables 1–3). The adiabatic maps in the low-energy region (Figs. 5 and 6) show the expected eight-shaped region comprising the two main minima. For the **Mβα** and **Nβα** trisaccharides, this region has an energy below 2 kcal. As expected, for the reverse trisaccharides (**αβ**) the eight-shaped region is lying down and has higher energy (3 kcal/mol). The minima comprising any **C** region are not important for the trisaccharides. The most important one is the **AC** region of the **Lβα** trisaccharide, around 4 kcal/mol above the global minimum (Table 3 and Fig. 3). The remaining ones are well above 5 kcal/mol, so that some **C** regions are barely seen in the adiabatic maps comprising points up to 10 kcal/mol (Fig. 3).

The angle taken by the exocyclic groups in the main minima of the trisaccharides follows some expected patterns: those of the non-reducing and reducing ends take conformations similar to those occurring for the disaccharides in an equivalent glycosidic conformation (cf.

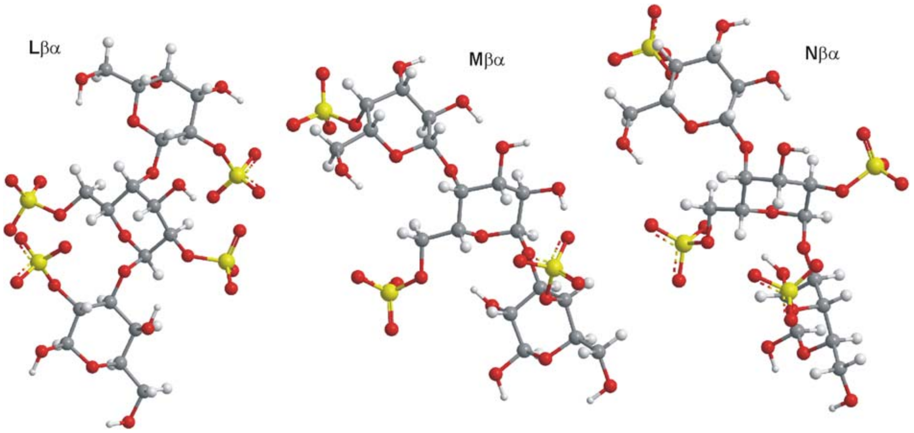


Figure 8. Drawings of the minimum-energy conformations of the trisaccharides **Lβα**, **Mβα**, and **Nβα**.

Table 4. Corrected partition functions q and allowed surfaces (AS, percentage of contour map surfaces below each energy range) calculated for the di- and trisaccharides under study

	$q_{\psi,\psi}$ (deg^2)	$q_{\psi\alpha}$ (°)	$q_{\psi\beta}$ (°)	AS$_5$ (%)	AS$_{10}$ (%)	AS$_{5\text{-}\alpha}$ (%)	AS$_{5\text{-}\beta}$ (%)
Lαβ	550	20.1	21.2	6	27	31	29
Lβα	580	19.4	26.1	12	30	60	31
Disaccharides **Lα** and **Lβ**		20.6	27.3			58	36
Mαβ	1080	36.7	28.4	10	25	44	38
Mβα	1250	38.3	31.0	15	30	47	40
Disaccharides **Mα** and **Mβ**		39.8	26.5			47	36
Nαβ	850	31.9	25.6	9	22	42	33
Nβα	1550	46.4	32.4	16	30	49	42
Disaccharides **Nα** and **Nβ**		46.5	25.7			49	38

Tables 1–3). On the other hand, the middle monosaccharide takes preferentially an exocyclic conformation equivalent to that occurring at the reducing end of the equivalent disaccharide, that is, it suffers a higher influence of the conformation of the previous glycosidic linkage (linked to its C3 or C4). Several differences were found, especially regarding the conformation of χ_6. It has been found that the **B** conformer of the α-linkage shows very small differences in energy for χ_6 in the α-galactose unit being in e (ca. 105°) or E (ca. −115°) conformations. A similar effect occurs in other compounds with the T, G, or g conformations of this angle.

The hydrogen bonding pattern of the trisaccharides is similar to that expected from the observation of the disaccharides.[19,24] When comparing 2- and 4-sulfated oligosaccharides (Table 5), the hydrogen bonding pattern is helpful to understand the presence of a different preferential minimum for the α-linkage: 4-sulfated polymers (μ- or ν-carrageenans) carry hydrogen bonds between H(O)-2 of the β-galactose unit and O-5, O-6 and/or O(S)-6 of the α-galactose unit, only possible for the **A** minimum. On the other hand, this hydrogen bond is precluded for 2-sulfated products like λ-carrageenan. In those polymers, a hydrogen bond between H(O)-4, free in this case, and the O-2 of the α-galactose unit is produced for the **B** minimum, thus making it more stable. The minimum **B′** appearing for the β-linkage of μ- and ν-carrageenans is stabilized by two hydrogen bonds, namely those between the H(O)-2 of the β-galactose unit and the O-3 of the α-galactose moiety, and that between H(O)-6 of the β-galactose unit and O-6 of the α-galactose unit (Table 5). Although this minimum is real, the minima for the trisaccharide involving this conformation do not appear in the adiabatic maps, as there are other conformations with the same ψ, ψ that carry lower energies. Some minima also show a hydrogen bond between the first and the third residues of the trisaccharides (Table 5). However, this occurs only for high-energy minima, and thus no special stabilization is found to occur as observed previously.[36]

Flexibility of the glycosidic linkage can be measured by different ways.[42] The partition function q is highly dependent on the size of the regions of the map with very low energy and, thus, is highly influenced by the entropy of the global minimum. Another parameter, used earlier as a semiquantitative measurement in rigid residue analysis,[43] can be defined in terms of allowed surfaces (AS), offering the notion of the percentage of the surface with an energy below a given value. It is also related with the entropy of the minima, but in a more linear fashion than q, and thus less subject to calculation errors. It has been shown previously that flexibility

Table 5. Hydrogen bond arrangements expected to occur in the polysaccharides with the substitution patterns of λ-, μ-, and ν-carrageenans[a]

Hydrogen bond	Carrageenan	Minima
Unit α-D-Gal-(1→3)-β-D-Gal		
H(O)2–O2′	**μ-, ν-**	**C** (0.61-1.05)
H(O)2–O5′	**μ-, ν-**	**A** (<0.67)
H(O)2–O(S)6′ or H(O)2-O6′	**μ-, ν-**	**A** (<0.93)
H(O)4–O2′	λ-	**B** (<0.61)
H(O)4–O5′	λ-	**C** (<0.61)
H(O)4–O(S)6′	λ-	**C** (<0.77)
H(O)2′–O2	**μ-**	**C** (0.89–0.92)
H(O)2′-O(S)4	**μ-**	**A** and **B** (0.67–0.94)
Unit β-D-Gal-(1→4)-α-D-Gal		
H(O)3–O(S)2′	λ-	**B** (0.73–0.84)
H(O)3–O5′	λ-, **μ-, ν-**	**C** (0.76–0.96)
H(O)2′–O3	**μ-, ν-**	**B′** (0.70–1.08)
H(O)6′–O(S)6	λ-, **μ-, ν-**	**B** (<0.75) in λ-, **B′** (0.62–0.89)
Between the first and third unit of trisaccharide		
H(O)2–O(S)6″ or H(O)3–O(S)6″	**Mαβ**	**AB′** (<0.88)
H(O)3–O(S)6″	**Nαβ**	**AB′** (0.83–0.86)
H(O)6″–O(S)2	**Lβα**	**BA** (0.80–0.82)

[a] The actual hydrogen bonding pattern also depends on the exocyclic arrangements. Those hydrogen bonds occurring mostly are mentioned. The data are obtained from the hydrogen bonds with $E_{HB} > 0.6$ kcal/mol observed for the six trisaccharides. The energy involved in the hydrogen bond is given in parentheses (in kcal/mol). The first two headings correspond to the bonds expected to occur in each disaccharidic repeating unit.

measurements for trisaccharides followed the trend shown for the corresponding disaccharides.[35–38] A similar effect is observed here: the 4-sulfated trisaccharides have larger flexibilities than the 2-sulfated ones (Table 4), either considering $q_{\psi,\psi}$ or AS$_5$ values. The AS$_{10}$ surfaces appear much more leveled (actually that of **Lαβ** is slightly larger than that of its **M** and **N** counterparts, Table 4 and Fig. 3). A similar effect (but smaller in magnitude) was observed for 3,6-anhydrogalactose-containing trisaccharides.[36] By the same token, the flexibility values for the **βα** compounds are higher than those for the **αβ** compounds, by either measurement (Table 4). This effect is small for the **L** trisaccharides, moderate for the **M** ones, and very high for the **N** trisaccharides, and has already been observed, with no exception for the 3,6-anhydrogalactose-containing trisaccharides.[36]

Previous work has also been carried out determining the partial flexibility on each glycosidic angle of trisaccharides. For those with single repeating structures (cellotriose, maltotriose, fucotriose, kojitriose, sophorotriose, and mannotriose), a decrease in flexibility of the linkage when passing from a disaccharide to a trisaccharide was usually found, as well as the flexibility of the 2→1 linkage (closer to the reducing end) diminished with respect to that of the 3→2 linkage.[1,35,37,38] On the other hand, for the trisaccharides models of 3,6-anhydrogalactose-containing carrageenans, it was found that the α-linkage appears to be less flexible in the trisaccharide than in the disaccharide notwithstanding its position, whereas the β-linkage keeps approximately the same flexibility in the disaccharides and the trisaccharides[36] in agreement with the observed fact that α-linked compounds might have larger restrictions in flexibility when passing from di- to trisaccharides.[35] The present study suggests a slightly different picture: the α-linkage in the trisaccharides models of λ- and μ-carrageenans (**Lαβ**, **Lβα**, **Mαβ**, and **Mβα**) has a flexibility similar to that of their disaccharidic counterparts (**Lα** and **Mα**), or just slightly lower. On the other hand, the α-linkage in **Nαβ** appears sharply diminished with respect to those of the disaccharide **Nα** or the trisaccharide **Nβα** (Table 4). The AS$_{5\text{-}\alpha}$ appears much larger for **Lβα** and **Lα** due mainly to the presence of points below 5 kcal/mol in the **C** region. Regarding the β-linkage, 2-sulfated and 4-sulfated compounds behave differently. For the λ-series the trisaccharides have their β-linkage less flexible than the disaccharide. For the μ- and ν-models, the **βα** trisaccharides have more flexibility in their β-linkage than the disaccharide, whereas the **αβ** have a similar one (Table 4), suggesting that the presence of a third unit toward the reducing end increases the low-energy regions. Figures 9 and 10 show the low-energy regions for each linkage and compound represented as 2D plots.[42] Figure 9 shows how for the α-linkage the behavior of the disaccharides is very similar to that of the **βα** trisaccharides, whereas those of the **αβ** trisaccharides are different. For **Lαβ** the distinction appears in the higher energy **A** region; thus, no major influence on the partition function q is observed. For **Mαβ** and **Nαβ**, the energy of the **B** region is higher and the shape of the **A** region is changed. The change is just a shift for **Mαβ**, but it appears less shallow in **Nαβ** thus reducing largely q in the latter case (Table 4). For the β-linkage (Fig. 10) some details of the curves differ, helping to explain the flexibility values: the more pronounced shallowness of the curve for the **Mβα** and **Nβα** trisaccharides (especially in the 80–130° region) helps to understand their higher partition function.

Besides those made in our laboratory with MM3, no other modeling studies have been carried out on models of μ- and ν-carrageenans. On the other hand, Le Questel et al. have studied both linkages of λ-carrageenan using the Tripos force-field.[12] Their results clearly agree with ours, with a minimum in the **A** region ($\phi,\psi = 260°, 140°$) for the β-linkage, and one in the **B** region ($\phi,\psi = 60°, 90°$) for the α-linkage. These data led to five possible, stable right-handed single helices, all of them having the β-linkage in the **A** region, and the α-linkage in several different conformations within the **A**–**B** region. The diffraction pattern of fibers of λ-carrageenan was considered to be too poor[44,45] for a definite structural determination. However, it was deduced that it might be well represented by a threefold helix with a

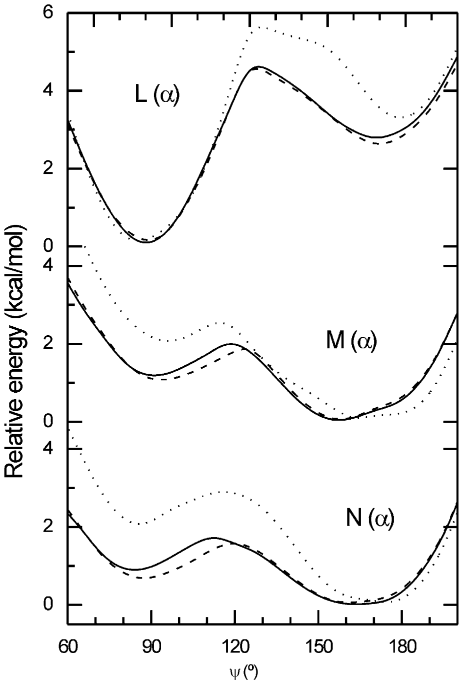


Figure 9. MM3 rotational profiles (energy vs ψ angle) around the low-energy regions of the α-glycosidic linkages of **L**-, **M**-, and **N**-models. Key: disaccharide (solid line), **αβ** trisaccharide (dotted line), **βα** trisaccharide (dashed line).

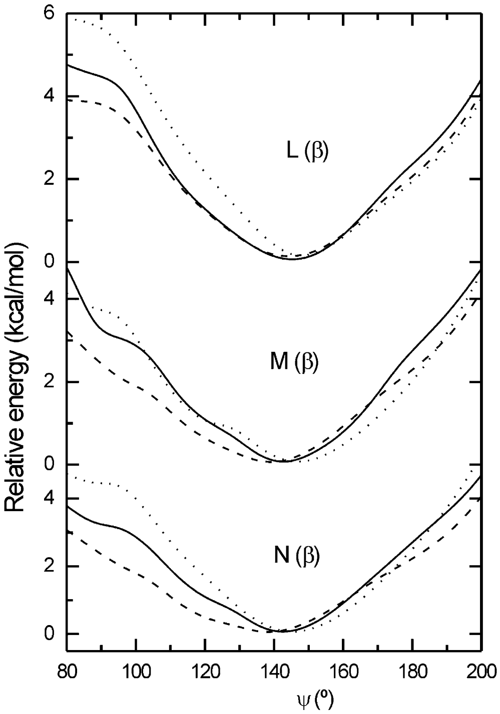


Figure 10. MM3 rotational profiles (energy vs ψ angle) around the low-energy regions of the β-glycosidic linkages of **L**-, **M**-, and **N**-models. Key: disaccharide (solid line), **αβ** trisaccharide (dotted line), **βα** trisaccharide (dashed line).

pitch of 25.2 Å (roughly compatible with the present minima, according to Le Questel et al.[12]). Early modeling studies indicated the possibility of left- and right-handed helices,[45] though later work only suggested possible right-handed helices.[12] It was stated that actually λ-carrageenan may be better described in terms of a twisted ribbon and not an open helix, double helices being prohibited by sterical reasons.[45]

The present work extends the previous information on the mapping of many disaccharidic carrageenan models[19–21,24] and a few trisaccharidic ones.[36] As shown earlier, most of the general features encountered for the disaccharides can be linearly extrapolated to the trisaccharides. However, although no new minimum-energy conformation has appeared as occurred in a previous paper,[36] minor effects on the geometries, energies, and flexibility have been found to occur due to the presence of a third unit. This work emphasizes the necessity of determining the maps of trisaccharides (even when those of the disaccharides are known), in order to proceed further toward the actual modeling of polysaccharidic chains.

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