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Additive effects in the modeling of oligosaccharides with MM3 at high dielectric constants: an approach to the 'multiple minimum problem'

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Abstract—The production of an adiabatic map for a di- or trisaccharide requires the generation of many relaxed maps, ideally 59,049 for a disaccharide or 4,782,969 for a trisaccharide composed by hexose residues, due to a combination of exocyclic angle torsions. As the production of this amount of maps is usually ruled out for time considerations, different approaches were exploited. When working at low dielectric constants, starting points originated in cooperative hydrogen bonds through the rings are usually sufficient to produce an adiabatic map, but at higher dielectric constants those circuits are meaningless, and many low-energy conformers appear in each energy well. Herein, different conformations of four disaccharides (β-4-linked mannobiose, and three galactobioses, linked α-(1→3), α-(1→4), and β-(1→4)) and one trisaccharide (β-4-linked mannotriose) were minimized using MM3 at $\varepsilon = 80$, and the difference in energy produced by changes in torsional angles was recorded. A remarkable additive effect was found to occur when the exocyclics were gathered in groupings of two or three neighboring angles. Thus, in most cases, each grouping can be studied separately, and the minimum energy conformers can be predicted without the need of resorting to thousands of calculations. In some cases where two protons of different groups show steric interactions in some specific conformations, small deviations of the additivity were encountered. Anyway, a complex system with many variables can be transformed in one with many fewer variables, thus simplifying further studies. An attempt to calculate the same effect at $\varepsilon = 3$ shows that hydrogen bonding and electrostatic interactions make impossible to find those additive effects, thus precluding its utilization at such low dielectric constants.

Keywords: Oligosaccharides; MM3; Multiple minimum; Exocyclic; Modeling

1. Introduction

Oligosaccharides act out an important task in biological structures. Thus, understanding their three-dimensional structures, either by experimental studies or by theoretically-based methods has acquired a rising interest.[1] Modeling of disaccharides is usually accompanied by the generation of a Ramachandran-like 3D contour map, where the energy is plotted for all mutual orientations of the two monosaccharide residues, expressed by the glycosidic angles ϕ and ψ.[2–5] Recently, it has been shown that trisaccharide conformations could also be represented by 3D contour maps where the energy is plotted against both ψ angles.[6–9] Many different potential functions were used to generate these maps, but most of the studies were done by molecular mechanics, using MM3[10,11] for the majority of the systematic studies, given its ability to handle directional hydrogen bonding and its parameterization of anomeric and exo-anomeric effects.[4,12] However, the generation of these maps is complicated by the rotameric complexity of the exocyclic substituents (the 'multiple minimum problem'[2,4,13]): when making these contour maps, it should be borne in mind that the actual conformational hypersurface of a disaccharide carries, for each ϕ, ψ combination, the

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influence of the orientations of secondary and primary hydroxyl groups, and hydroxymethyl groups, all of which may affect the calculated final energy by several kilocalories.[13,14] For a disaccharide formed by two hexopyranose residues, and considering the three distinct staggered positions for each exocyclic group, 59,049 (3^{10}) different starting points are possible for the construction of the map or finding the true minima. For an equivalent trisaccharide, the figure is 4,782,969 (3^{14}).

An adiabatic map is generated when plotting the lower energy values found at each grid point.[15] Thus, the true adiabatic map for a disaccharide can only be obtained after calculating 59,049 relaxed maps and determining for each ϕ, ψ conformation the one with lowest energy. Even in order to find the global minimum, thousands of minimizations (millions for a trisaccharide) could be required. This problem has been recognized since publication of the early flexible residue analyses.[5,15–19] However, considering that the secondary hydroxyl groups are likely to form a crown of cooperative hydrogen bonds oriented either clockwise or counterclockwise around the pyranose ring,[20] and by restricting the rotation of the hydroxymethyl groups to their preferential positions,[5] only a few (16–36) of those 59,049 starting conformations for a disaccharide may be used. This approach was applied by Dowd et al.[21–24] in a systematic study of different disaccharides by MM3 with 16 or 24 starting conformers, which afterwards became a standard procedure. Moreover, the same approach was adopted when working at high dielectric constants (to emulate aqueous solutions), where such circuits of hydrogen bonds should not form at all.[25,26] As expected, an attempt to produce a true adiabatic map at $\varepsilon = 80$ only from these starting points has failed, at least in one example[13] where an iterative, non-systematic procedure has found lower energy values throughout the entire map. No systematic procedure (different from a tedious full search) has been developed for finding the minima in these cases, though random sampling techniques,[14,19,27] molecular dynamics,[14,28–30] or Monte Carlo[31,32] simulations can be useful. Herein, the output of several MM3 calculations at $\varepsilon = 80$ of di- and trisaccharides (with axial and equatorial glycosidic linkages) are presented. The MM3 energy bears an independent effect of the orientation of groups of exocyclic dihedral angles, motivated by torsional and other steric interactions. This leads to constant additive contributions per rotameric effect, allowing one to calculate easily the lower energy conformers without the need of resorting to thousands or millions of calculations.

2. Methods

Molecular mechanics calculations were carried out using the program MM3(92) (QCPE, Indiana University, USA), modified as the MM3(2000) version in the O–C–C–O and O–C–O–H torsional parameters, O–H hydrogen bonding parameters, and C–H electronegativity correction.[12] Besides, a suggested modification of the MM3 routines[33] was made by changing the maximum atomic movement from 0.25 to 0.10 Å. The dielectric constant indicated in Section 3 was used (usually held at 80). The default minimization method and termination conditions were used: each minimum was submitted to a full matrix calculation in order to check that it really corresponds to a minimum, and then submitted to a block diagonal calculation in which MM3 energy was recorded. 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*, whereas 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:[34] S for angles between −20 and +20°, g for 20–80°, T for angles with absolute value larger than 150°, and G for angles between −20° and −80°. All starting points had their chairs in the most stable, $^4C_1$ conformation. The nomenclature of the minima (**A**, **B**, **AA**, etc.) follows the conventions already established in the original study of the di- and trisaccharide maps presented here. The adiabatic maps of the (1→4)-linked galactose disaccharides at $\varepsilon = 80$ were generated as explained elsewhere,[9,13,35] starting from the conformers in the **A** and **B** regions with less than 0.5 kcal/mol above each local minimum (97 for the α-linked disaccharide, 73 for the β-linked disaccharide), and using a 20° grid for both the ϕ and ψ angles.

In order to proceed with the present determination, for each di- or trisaccharide, the exocyclic angles were arbitrarily gathered in groupings, two for each monosaccharide (Fig. 1): one corresponded to the free hydroxyl groups linked to the ring (e.g., $\chi_{2'}\chi_{3'}\chi_{4'}$ for the non-reducing end, Fig. 1), and the other to the combination of hydroxymethyl group and primary hydroxyl moiety (i.e., $\omega\chi_6$). In a preliminary study, several groupings were selected by testing the effects of changing in turn the bunch of exocyclic angles in each group. Only those groupings leading to lower energies (usually with an effect smaller than 0.5 kcal/mol) were recorded. In an automated fashion, structures carrying the combination of all those recorded groupings were generated, and submitted to MM3 minimization, leading to an output containing many conformers with their final exocyclic angles and energies. The tables were cleaned out for repetitions and were then allowed to calculate the energy shifts due to change in exocyclics. For example, for β-mannobiose, five different arrangements of $\chi_{2'}\chi_{3'}\chi_{4'}$ (grouping 1) were recorded, as well as three arrangements for $\omega'\chi_{6'}$ (grouping 2), five for grouping 3, and four for grouping 4. The 300 ($5 \times 3 \times 4 \times 5$) combinations were minimized with the ϕ, ψ angles in both the **A** and the **B** regions. Table 1 shows the results obtained,

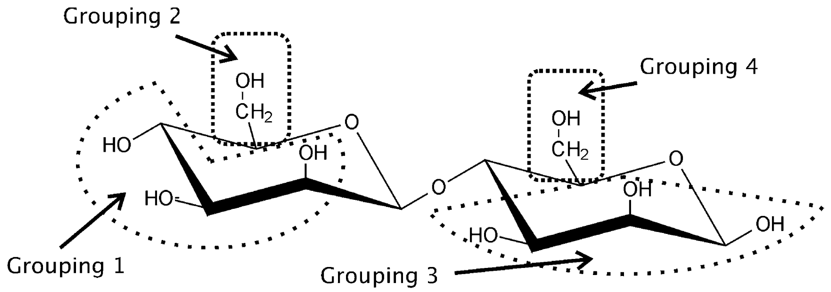

Figure 1. The disaccharide β-D-Man*p*-(1→4)-β-D-Man*p* with the division in groups of exocyclic angles.

Table 1. Average energy shift and standard deviations (kcal/mol) after changes in exocyclic angle arrangements for the main minima in β-mannobiose

Exocyclic angles	Min **A** Δ*E*	σ	*n*	Min **B** Δ*E*	σ	*n*
$\chi_{2'}\chi_{3'}\chi_{4'}$						
ggG→GgG	0.064	0.005	60	0.082	0.003	33
→gGG	0.113	0.002	60	0.115	0.001	33
→GGG	0.144	0.006	60	0.165	0.004	32
→ggg/ggS	0.460	0.004	45	0.468	0.003	13
$\omega'\chi_{6'}$						
gT→gG	0.105	0.011	95	0.315	0.012	36
→gg	0.430	0.002	95	0.425	0.004	48
$\chi_1\chi_2\chi_3$						
gGG→ggG	0.027	0.005	57			
→ggg	0.337	0.013	57			
→gGg	0.339	0.008	57			
→TGG	0.391	0.006	57			
gGg→ggg				−0.003	0.010	53
→TGg				0.417	0.008	34
$\omega\chi_6$						
gT→gG	0.188	0.005	75	0.194	0.005	34
→GT	0.381	0.007	60	0.463	0.010	33
→gg	0.444	0.003	75	0.435	0.004	36

including the average energy shifts between conformers carrying only one different grouping (and the other three identical), the number of conformer pairs for which this difference was calculated (*n*), and the standard deviation (σ) for this average. As expected, each change for the first grouping can only be made for up to 60 (3 × 4 × 5) replications, 100 (5 × 4 × 5) for the second grouping, and so on, depending on the number of conformations recorded for each of the remaining three groupings. The actual number *n* may be less than this maximum (Table 1), as many starting structures actually lead, after minimization, to the same final conformation. Attempts to reduce the size of the groupings to just one exocyclic failed: the reported combination of angles is necessary to produce the additive effect shown herein. For the trisaccharide, 5760 different combinations (5 × 3 × 4 × 4 × 6 × 4) were tested in each of the four minimum-energy regions. A similar calculation was carried out for β-mannobiose at $\varepsilon = 3$, taking into account the lower energy conformers determined in a previous work.[35]

3. Results and discussion

The struggle for the greatest possible accuracy on the actual adiabatic character of the contour maps generated for di- and trisaccharides has gained success when working at low dielectric constants.[13,21–24] In those cases cooperative hydrogen bonding between the exocyclic hydroxyl groups leads to preferential conformations. When these conformers are used as starting points, the whole ϕ, ψ surface is suitably covered. However, attempts to extrapolate this procedure to simulations of water solutions through raising the dielectric constant to around 80, failed to yield an adiabatic map.[13] One example showed that such a map has energies averaging 0.6–0.9 kcal/mol above the adiabatic map, depending on the type of dihedral driver used.[13] Actually, working at these high dielectric constants neglects almost completely hydrogen bonding and electrostatic interactions, thus leading to results not expected when the force field was parameterized. The value of 80 is much higher than that expected for water in the first solvation shell (at least in ionic solutions), where it can reach values as low as six.[13] However, many authors have made calculations at dielectric constants close to 80,[25,26,36,37] and in some cases they found a good coincidence of the minima calculated and the experimental data in solution,[13,36,37] in spite of the argued theoretical drawbacks. Thus, it is important to develop a way of determining the more stable starting structures at this dielectric constant without resorting to full search procedures. For 4-linked β-mannobiose at $\varepsilon = 80$,[35] the most stable combinations of exocyclic angles were tested in groupings (Fig. 1), which corresponded to either a combination of the dihedral angles of the secondary hydroxyl groups of each monosaccharide, or to a combination of the dihedral angle of the hydroxymethyl group (ω) and that of its primary hydroxyl group. The structures produced by a permutation of changes in these groupings were minimized by MM3, and their energies and geometries were recorded. After cleaning out for repetitions, the energy shift produced by changing in turn each of the four groups (while leaving the remaining three constant) was evaluated (Table 1). The results show that the effect of the conformation of each grouping is almost independent from that of the other groupings, as indicated by the very low standard deviations observed for each change. The largest standard deviation is just about a hundredth of a kilocalorie, and this occurs for both the **A** and **B** regions. Furthermore, a deeper study of the largest deviations indicates that they are not obtained from random variations, but from small steric interactions. For example, the change in energy for the **B** region when passing from $\omega = \mathrm{g}$ to $\omega = \mathrm{G}$ (0.463 ± 0.010) can be further divided into two sets: 0.448 ± 0.002, and 0.468 ± 0.002, depending on the conformation of χ_1. The groupings 1 ($\chi_{2'}\chi_{3'}\chi_{4'}$) and 4 ($\omega\chi_6$) are less influenced by the ϕ/ψ combination: the shift is similar in the **A** and **B** regions. On the other hand, grouping 2 ($\omega'\chi_{6'}$) shows a quite different shift for the gT→gG rotation in both regions, whereas for grouping 3 ($\chi_1\chi_2\chi_3$) the combination of angles of minimal energy for each of the two regions is different (Table 1). The energy shifts produced for the corresponding trisaccharide[35] are shown in Table 2, and they replicate the independent behavior of each group. As expected, the shifts for the groupings $\chi_{2''}\chi_{3''}\chi_{4''}$ and $\omega''\chi_{6''}$ in regions **AA** and **AB** (Table 2) give identical results to those of the groupings $\chi_{2'}\chi_{3'}\chi_{4'}$ and $\omega'\chi_{6'}$ in the region **A** of the disaccharide (Table 1). The same effect is obviously observed for the same groupings in regions **BA** and **BB** of the trisaccharide and in region **B** of the disaccharide. A similar effect is observed for the reducing end: the groupings $\chi_1\chi_2\chi_3$ and $\omega\chi_6$ in both the di- and trisaccharide show the same shifts in similar regions (region **A** of the disaccharide and regions **AA** and **BA** of the trisaccharide, region **B** of the disaccharide and regions **AB** and **BB** of the trisaccharide). The middle unit of the trisaccharide shows shifts with characteristics of their own, but related to those of the disaccharide: the group $\chi_{2'}\chi_{3'}$ shows shifts resembling those found at the reducing end of the disaccharide, as expected considering that they show a larger influence of the glycosidic conformation of the Man″→Man′ linkage (i.e., in the **AA** and **AB** regions, they behave like those in the **A** region of the disaccharide). On the other hand, the other middle group ($\omega'\chi_{6'}$) of the trisaccharide shows energy shifts similar to those observed at the non-reducing end of the disaccharide. Thus, the glycosidic conformation of the Man′→Man linkage exhibits larger influences on its magnitude, giving in the **AA** and **BA** regions energy shifts similar to those observed in the **A** region of the disaccharide (cf., Tables 1 and 2). A careful analysis of the standard deviations in mannotriose also indicates that the larger values do not originate from a natural

Table 2. Average energy shift and standard deviations (kcal/mol) after changes in exocyclic angle arrangements for the main minima in β-mannotriose

Angles	**AA** $\Delta E \pm \sigma$ (n)	**AB** $\Delta E \pm \sigma$ (n)	**BA** $\Delta E \pm \sigma$ (n)	**BB** $\Delta E \pm \sigma$ (n)
$\chi_{2''}\chi_{3''}\chi_{4''}$				
ggG→GgG	0.065 ± 0.005 (1152)	0.067 ± 0.005 (576)	0.083 ± 0.004 (574)	0.084 ± 0.004 (288)
→gGG	0.114 ± 0.002 (1152)	0.113 ± 0.002 (576)	0.115 ± 0.001 (574)	0.115 ± 0.001 (288)
→GGG	0.146 ± 0.006 (1152)	0.147 ± 0.006 (576)	0.167 ± 0.004 (573)	0.166 ± 0.004 (288)
→ggS	0.460 ± 0.004 (1152)	0.459 ± 0.004 (576)	0.467 ± 0.003 (290)	0.466 ± 0.002 (113)
$\omega''\chi_{6''}$				
gT→gG	0.103 ± 0.010 (1920)	0.102 ± 0.010 (960)	0.317 ± 0.012 (858)	0.316 ± 0.012 (396)
→gg	0.429 ± 0.003 (1920)	0.430 ± 0.003 (960)	0.425 ± 0.004 (769)	0.425 ± 0.004 (385)
$\chi_{2'}\chi_{3'}$				
gG→GG	0.005 ± 0.007 (1440)	0.023 ± 0.006 (720)		
→gg	0.309 ± 0.007 (1440)	0.309 ± 0.008 (720)		
→Gg	0.344 ± 0.005 (1440)	0.360 ± 0.005 (720)		
gg→Gg			0.035 ± 0.011 (1263)	0.052 ± 0.010 (625)
$\omega'\chi_{6'}$				
gT→gG	0.093 ± 0.010 (1440)	0.319 ± 0.012 (720)	0.101 ± 0.010 (628)	0.324 ± 0.012 (312)
→gg	0.433 ± 0.003 (1440)	0.432 ± 0.005 (720)	0.425 ± 0.004 (632)	0.422 ± 0.005 (314)
→GT	0.438 ± 0.006 (1440)	0.433 ± 0.006 (720)	0.525 ± 0.006 (633)	0.522 ± 0.006 (313)
$\chi_1\chi_2\chi_3$				
gGG→ggG	0.026 ± 0.005 (960)		0.026 ± 0.005 (421)	
→ggg	0.340 ± 0.012 (960)		0.339 ± 0.012 (421)	
→gGg	0.344 ± 0.009 (960)		0.343 ± 0.009 (418)	
→TGG	0.392 ± 0.006 (960)		0.391 ± 0.006 (421)	
ggg→gGg		0.004 ± 0.009 (960)		0.002 ± 0.009 (417)
→TGg		0.422 ± 0.013 (960)		0.420 ± 0.013 (416)
$\omega\chi_6$				
gT→gG	0.188 ± 0.005 (1440)	0.195 ± 0.005 (720)	0.187 ± 0.005 (636)	0.194 ± 0.005 (312)
→GT	0.374 ± 0.009 (1440)	0.460 ± 0.010 (720)	0.374 ± 0.009 (634)	0.460 ± 0.010 (312)
→gg	0.442 ± 0.003 (1440)	0.435 ± 0.004 (720)	0.443 ± 0.003 (634)	0.435 ± 0.004 (312)

dispersion due to errors in calculations, but to systematic differences that originate in different conformations of neighboring groups. In any case, these shifts are really small.

The disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* was studied with different force fields and dielectric constants.[13,38–40] It also shows two main minima with very small differences in energy. The change in energy produced by different exocyclic arrangements (Table 3) also follows a constant additive effect, with very small standard deviations (0.001–0.009 kcal/mol). However, a special effect is produced in region **A** when $\chi_{6'}$ is G and χ_2 is g (Fig. 2). This combination leads to an unfavorable non-bonded steric interaction between both hydrogens, which increases the energy of the conformer in additional 0.29 kcal/mol (Table 3), and distorts some angles: $\chi_{6'}$ from the usual −69° to −76°, and χ_2 from −25° to −29°. Anyway, χ_2 takes those small values in minimum **A** to avoid interactions not observed in minimum **B**, where this angle takes values about 20° larger.

Table 3. Average energy shift and standard deviations (kcal/mol) after changes in exocyclic angle arrangements for the main minima in α-D-Gal*p*-(1→3)-β-D-Gal*p*

Exocyclic angles	Min **A** ΔE	σ	n	Min **B** ΔE	σ	n
$\chi_{2'}\chi_{3'}\chi_{4'}$						
Ggg→gGg	0.038	0.003	27	0.024	0.004	27
→ggg	0.124	0.002	27	0.113	0.004	27
→GGg	0.229	0.001	27	0.224	0.001	27
→Tgg	0.365	0.002	27	0.496	0.009	27
$\omega'\chi_{6'}$						
gT→gG	0.244[a]	0.005	44	0.156	0.004	63
→gg	0.423	0.005	67	0.406	0.006	63
$\chi_1\chi_2\chi_4$						
gGg→ggg	0.265[b]	0.004	45	0.120	0.008	63
→TGg	0.872	0.005	66	0.903	0.003	63
$\omega\chi_6$						
gT→gG	0.199	0.003	67	0.200	0.003	63
→gg	0.459	0.003	66	0.459	0.003	63

[a] This value is valid when χ_2 is G; the shift is 0.529 ± 0.003 ($n = 21$) when χ_2 is g.

[b] This value is valid when $\chi_{6'}$ is T or g; the shift is 0.551 ± 0.002 ($n = 21$) when $\chi_{6'}$ is G.

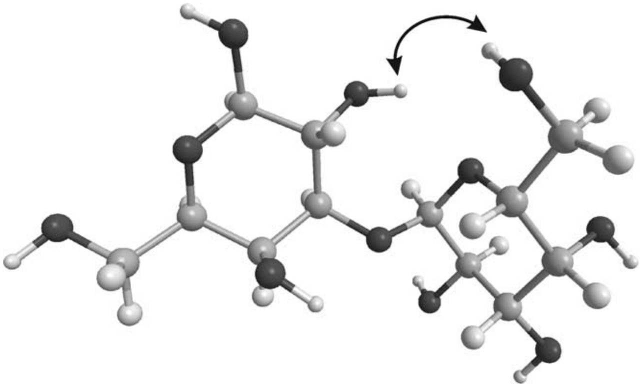

Figure 2. Local minimum determined by MM3 in the **A** region of disaccharide α-D-Gal*p*-(1→3)-β-D-Gal*p* carrying $\chi_2 = \text{g}$ and $\chi_{6'} = \text{G}$. The steric interaction between H(O)-2 and H(O)-6′ is indicated.

For a disaccharide with both glycosidic linkages axial like α-D-Gal*p*-(1→4)-α-D-Gal*p*, the same additive was found to occur (Table 4). However, the larger standard deviations indicate stronger interactions between groups of exocyclic angles. In the **A** region this effect is actually small (largest standard deviation is 0.017 kcal/mol), and it occurs because of a complex interaction of the external atoms involved in $\chi_{2'}$, $\chi_{3'}$, $\chi_{6'}$, χ_2, and χ_3. Within the **B** region, a stronger effect is observed: it occurs only when χ_3 is G, as this proton may have unfavorable non-bonded interactions with H(O)-6′ when it is not *anti* to C-5′ (Fig. 3). This interaction accounts for an extra 0.07 kcal/mol when $\chi_{6'}$ is G, and 0.12 kcal/mol when $\chi_{6'}$ is g. As expected, this leads to shifts, which when taken globally show standard deviations as large as half the maximum shift (0.06 kcal/mol, Table 4). When the shifts are divided in sets depending on the relative conformations of χ_3 and $\chi_{6'}$, the standard deviations fall down to lower values (see footnote in Table 4).

Table 4. Average energy shift and standard deviations (kcal/mol) after changes in exocyclic angle arrangements for the main minima in α-D-Gal*p*-(1→4)-α-D-Gal*p*

Exocyclic angles	Min **A** ΔE	σ	n	Min **B** ΔE	σ	n
$\chi_{2'}\chi_{3'}\chi_{4'}$						
Ggg→gGg	0.058	0.006	45	−0.105	0.006	45
→ggg	0.143	0.004	45	−0.017	0.005	45
→GGg	0.233	0.002	45	0.230	0.002	45
→Tgg	0.699	0.015	45	0.232	0.006	45
→TGg	0.911	0.015	45	0.446	0.007	45
$\omega'\chi_{6'}$						
gT→gG	0.113	0.017	90	0.157[a]	0.032	90
→gg	0.454	0.015	90	0.427[b]	0.057	90
$\chi_1\chi_2\chi_3$						
GgG→GGg	0.032	0.011	54	0.073[c]	0.051	54
→Ggg	0.116	0.010	55	0.165[d]	0.047	54
→GGG	0.263	0.014	54	0.236	0.004	54
→GTg	0.429	0.010	55	0.449[e]	0.051	54
$\omega\chi_6$						
gT→gG	0.209	0.004	90	0.214	0.005	90
→gg	0.462	0.003	90	0.477	0.005	90

[a] Divisible in two sets: 0.132 ± 0.002 ($n = 54$) when χ_3 is g and 0.196 ± 0.002 ($n = 36$) when χ_3 is G.

[b] Divisible in two sets: 0.381 ± 0.005 ($n = 54$) when χ_3 is g and 0.496 ± 0.007 ($n = 36$) when χ_3 is G.

[c] Divisible in three sets: 0.013 ± 0.008 ($n = 18$) when $\chi_{6'}$ is g, 0.069 ± 0.005 ($n = 18$) when $\chi_{6'}$ is G, and 0.136 ± 0.003 ($n = 18$) when $\chi_{6'}$ is T.

[d] Divisible in three sets: 0.112 ± 0.006 ($n = 18$) when $\chi_{6'}$ is g, 0.159 ± 0.004 ($n = 18$) when $\chi_{6'}$ is G, and 0.224 ± 0.002 ($n = 18$) when $\chi_{6'}$ is T.

[e] Divisible in three sets: 0.388 ± 0.007 ($n = 18$) when $\chi_{6'}$ is g, 0.447 ± 0.003 ($n = 18$) when $\chi_{6'}$ is G, and 0.511 ± 0.003 ($n = 18$) when $\chi_{6'}$ is T.

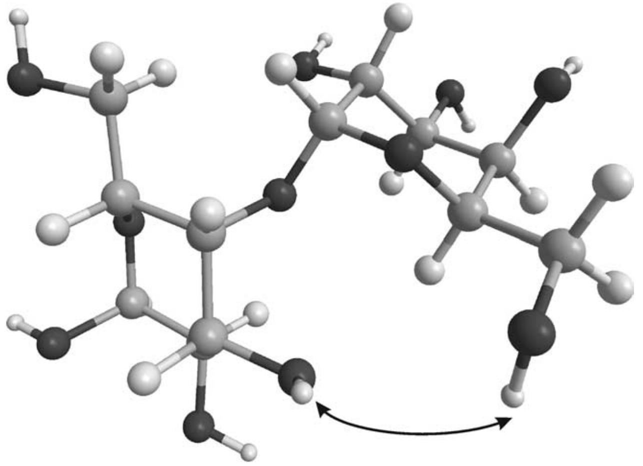

Figure 3. Local minimum determined by MM3 in the **A** region of disaccharide α-D-Gal*p*-(1→4)-α-D-Gal*p* carrying $\chi_3 = \text{G}$ and $\chi_{6'} = \text{g}$. The steric interaction between H(O)-3 and H(O)-6′ is indicated.

The disaccharide (considering those studied in the current work) presenting the largest effects on shifts produced by interactions between groupings of exocyclics is β-D-Gal*p*-(1→4)-α-D-Gal*p*, one of the repeating unit of carrageenans.[40,41] In the **A** region (Table 5) a small effect (0.08 kcal/mol) occurs only when two angles present characteristic values: $\chi_{2'}$ G and χ_3 T. On the other hand, in the **B** region, larger shifts are observed when H(O)-3 and H(O)-2′ interact. This occurs only when $\chi_{2'}$ is G and χ_3 is not g. If χ_3 is G, the extra shift amounts to 0.18–0.22 kcal/mol, but if χ_3 is T, it winds up to a 0.39–0.42 kcal/mol extra shift. Curiously, the place within the range depends on a third angle, namely that of $\chi_{6'}$ (Table 5). When this angle has a G orientation, the shift for $\chi_{2'}\chi_3 = \text{GG}$ goes to its maximum value within the range (0.22 kcal/mol), and that for a GT orientation to its minimum value within the range (0.39 kcal/mol). The other extreme values are observed for $\chi_{6'}$ in a g or T orientation. This small shift actually arises from an indirect effect, since no interaction is observed between H(O)-6′ and either H(O)-3 or H(O)-2′. It occurs because when the $\chi_{6'}$ angle has a G orientation, it forces the ϕ glycosidic angle to lower values, thus originating a different interaction between H(O)-3 and H(O)-2′.

The adiabatic maps at $\varepsilon = 80$ for the mannobiose and α-(1→3)-linked galactose disaccharide were published elsewhere.[13,35] Their shapes are similar (but not identical) to those occurring at $\varepsilon = 3$. Figure 4 shows the adiabatic maps at $\varepsilon = 80$ for both 4-linked galactose disaccharides, whereas Table 6 shows the geometry and energy of the corresponding minima. The low-energy troughs extend in a rather diagonal fashion through the ϕ, ψ space, giving rise to two main minima with similar differences in the angles ϕ and ψ (Table 6). This fact has already been shown to occur[41] for the β-(1→4)-linked disaccharide at $\varepsilon = 3$, being quite different from the maps of most of the disaccharides, where ϕ keeps more or less constant through the low-energy well.[40]

In order to determine if the additive behavior also occurs at lower dielectric constants, the energies of different conformers of the same mannobiose disaccharide[35]

Table 5. Average energy shift and standard deviations (kcal/mol) after changes in exocyclic angle arrangements for the main minima in β-D-Gal*p*-(1→4)-α-D-Gal*p*

Exocyclic angles	Min **A** $\Delta E \pm \sigma$ (n)	Min **B** $\Delta E \pm \sigma$ (n)	$\Delta E \pm \sigma$ (n)	$\Delta E \pm \sigma$ (n)
$\chi_{2'}\chi_{3'}\chi_{4'}$				
Ggg→GGg	0.231 ± 0.003 (63)	0.231 ± 0.003 (63)		
		With $\chi_3 = \text{T}$	With $\chi_3 = \text{G}$	With $\chi_3 = \text{g}$
→gGg	0.262 ± 0.030[a] (63)	−0.144 ± 0.008 (9)	0.079 ± 0.039[b] (27)	0.275 ± 0.018[b] (27)
→ggg	0.325 ± 0.027[a] (63)	−0.070 ± 0.007 (9)	0.144 ± 0.037[b] (27)	0.331 ± 0.017[b] (27)
$\omega'\chi_{6'}$				
gT→gG	0.143 ± 0.009 (87)	0.196 ± 0.028[c] (87)		
→gg	0.461 ± 0.003 (89)	0.442 ± 0.004 (92)		
$\chi_1\chi_2\chi_3$				
GGg→Ggg	0.099 ± 0.002 (40)	0.099 ± 0.005 (41)		
→GTg	0.381 ± 0.004 (39)	0.379 ± 0.003 (39)		
		With $\chi_{2'} = \text{g}$	With $\chi_{2'} = \text{G}$	
GGg→GgG	0.004 ± 0.004 (38)	−0.091 ± 0.007 (18)	0.104 ± 0.017[b] (22)	
→GGG	0.235 ± 0.004 (40)	0.135 ± 0.005 (18)	0.323 ± 0.003 (21)	
→GTG	0.600 ± 0.007 (39)	0.498 ± 0.006 (18)	0.687 ± 0.017[b] (21)	
→GgT	0.916 ± 0.039[d] (38)	0.368 ± 0.012 (18)	0.777 ± 0.007 (18)	
$\omega\chi_6$				
gT→gG	0.214 ± 0.004 (89)	0.224 ± 0.008 (89)		
→gg	0.468 ± 0.003 (88)	0.493 ± 0.006 (91)		

[a] It can be broken up depending on the value of χ_3.

[b] It can be further split depending on the value of $\chi_{6'}$.

[c] It can be split depending on the value of χ_3 and $\chi_{2'}$.

[d] It can be split depending on the value of $\chi_{2'}$.

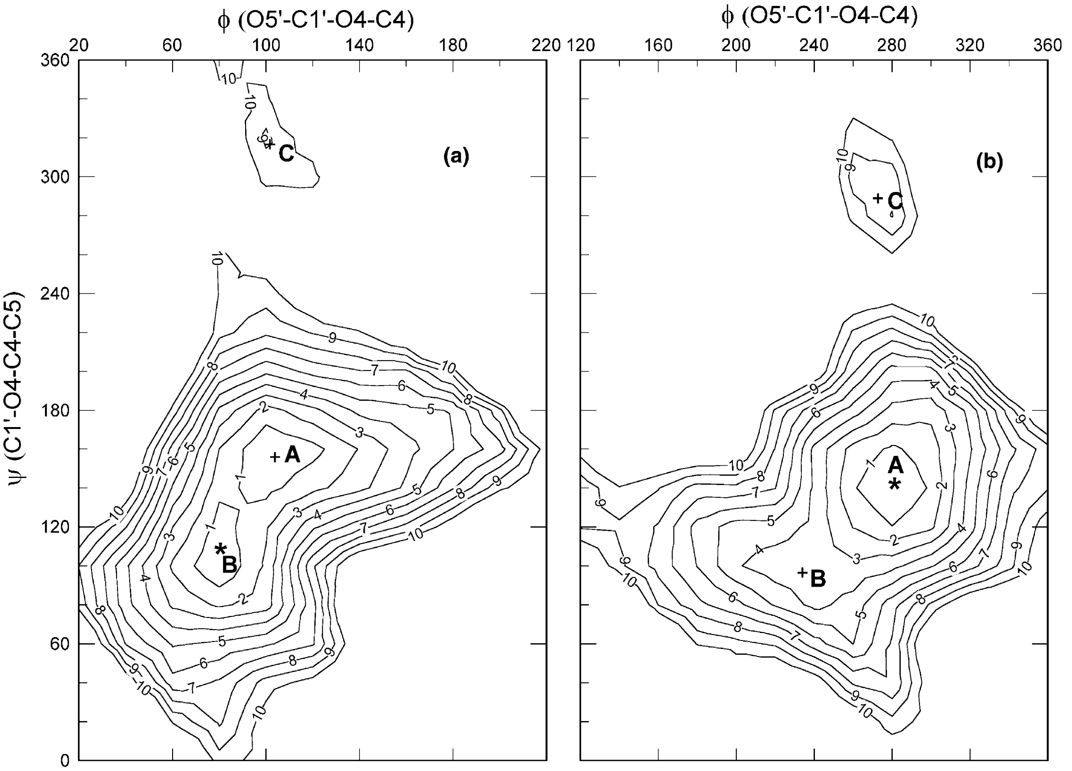

Figure 4. MM3 adiabatic conformational maps of the disaccharides α-D-Gal*p*-(1→4)-α-D-Gal*p* (a) and β-D-Gal*p*-(1→4)-α-D-Gal*p* (b) at $\varepsilon = 80$. Isoenergy contour lines are graduated in 1 kcal/mol increments above each global minimum. The symbols indicate: (∗) MM3 global minima, (+) MM3 local minima.

Table 6. Torsion angles (°), relative strain energies (kcal/mol) and exocyclic angles for the minimum-energy conformations obtained with MM3 at $\varepsilon = 80$ for two 4-linked galactobioses

	ϕ, ψ	ϕ_H, ψ_H	E_{rel}	Exocyclic torsion angles[a]			
				$\chi_{2'}\chi_{3'}\chi_{4'}$	$\omega'\chi_{6'}$	$\chi_1\chi_2\chi_3$	$\omega\chi_6$
α-D-Gal*p*-(1→4)-α-D-Gal*p*							
Region **A**	103, 158	−17, 40	0.04	Ggg	gT	GgG	gT
Region **B**	80, 103	−40, −19	0.00	gGg	gT	GgG	gT
Region **C**	105, 314	−14, −164	8.66	gGg	gT	GgG	gT
β-D-Gal*p*-(1→4)-α-D-Gal*p*							
Region **A**	281, 141	42, 21	0.00	Ggg	gT	GgG	gT
Region **B**	234, 97	−6, −26	3.18	Ggg	gT	GGg	gT
Region **C**	273, 290	36, 174	7.61	gGg	gT	GgG	gT

[a] For nomenclature, see Section 2.

studied in Table 1 were also determined at $\varepsilon = 3$. Results are shown in Table 7. Many differences were found when comparing with the results obtained working at $\varepsilon = 80$, namely: (a) the energy shifts between conformations appear much larger, i.e., there are highly preferential conformations, mainly established by hydrogen bonding capabilities; (b) the main conformations are different from those obtained at $\varepsilon = 80$ (cf. Tables 1 and 7); and (c) the standard deviations are very high (0.05–0.69 kcal/mol). This last issue is fundamental to disqualify this methodology at low dielectric constants: every attempt to subdivide the energy shifts into sets taking into account secondary factors results in a splitting that still contains energy shifts with quite large standard deviations (see footnote in Table 7). Thus, many of the effects actually depend on the whole conformational pattern, rather than on individual interactions, making predictive determinations with the present approach almost impossible.

The comparison of the shifts produced in the four disaccharides under study has also showed some constant effects using MM3: the most stable rotamer for the hydroxymethyl-primary hydroxyl grouping is always gT, followed by gG. For the reducing end, the latter is

Table 7. Average energy shift and standard deviations (kcal/mol) after changes in exocyclic angle arrangements for the main minima in β-mannobiose at $\varepsilon = 3$

Exocyclic angles	Min **A** ΔE	σ	n	Min **B** ΔE	σ	n
$\chi_{2'}\chi_{3'}\chi_{4'}$						
gTG→gGg	1.177	0.205	23	1.429	0.253	9
→GGg	1.178[a]	0.547	79	1.559	0.322	40
→ggG	1.361	0.076	83	1.328	0.051	41
→GgG	1.502	0.358	79	1.655	0.113	35
→GGG	1.525	0.085	21	1.744	0.108	30
$\omega'\chi_{6'}$						
gT→gG	−1.432[b]	0.661	92	0.784	0.133	12
→Gg	−0.334[c]	0.693	78	1.213	0.160	3
→gg	0.620	0.062	102	0.556	0.104	82
$\chi_1\chi_2\chi_3$						
TGG→ggG	1.594	0.345	100	1.655	0.255	55
→gGG	1.677	0.157	104	1.636	0.158	57
$\omega\chi_6$						
gG→Gg	0.057	0.083	81	0.222	0.086	43
→gT	0.911	0.211	82	0.838	0.222	46
→GT	1.326	0.115	81	1.388	0.127	44
→gg	1.611	0.168	81	1.488	0.209	44

[a] Divisible in at least four sets: 0.302 ± 0.077 ($n = 15$) when $\omega'\chi_{6'}$ is Gg and χ_3 is G, 0.932 ± 0.075 ($n = 20$) when $\omega'\chi_{6'}$ is gG and χ_3 is G or when $\omega'\chi_{6'}$ is Gg and χ_3 is g, 1.464 ± 0.085 ($n = 35$) when $\omega'\chi_{6'}$ is gG and χ_3 is g or when $\omega'\chi_{6'}$ is gg/gT and χ_3 is G, and 2.075 ± 0.078 ($n = 9$) when $\omega'\chi_{6'}$ is gg/gT and χ_3 is g.

[b] Divisible in at least five sets: -2.865 ± 0.140 ($n = 8$) when $\chi_{2'}$ is G and χ_3 is g, -2.309 ± 0.059 ($n = 12$) when $\chi_{2'}$ and χ_3 are g, -1.683 ± 0.132 ($n = 10$) when χ_3 and $\chi_{2'}$ are G and χ_2 is g, -1.296 ± 0.088 ($n = 24$) when $\chi_{2'}$ and χ_2 are g and χ_3 is G, or when χ_2, $\chi_{2'}$ and χ_3 are G, and finally -0.880 ± 0.138 ($n = 39$) when χ_2 and χ_3 are G, and $\chi_{2'}$ is g.

[c] Divisible in at least six sets: -1.989 ± 0.068 ($n = 4$) when $\chi_{4'}$ and χ_3 are g, -1.171 ± 0.076 ($n = 9$) when $\chi_{4'}$ and χ_2 are g and χ_3 is G, or when $\chi_{4'}$ and $\chi_{2'}$ are G and χ_3 is g, -0.790 ± 0.093 ($n = 19$) when χ_3 and χ_2 are G and $\chi_{4'}$ is g, or when χ_3 and $\chi_{2'}$ are g and $\chi_{4'}$ is G, -0.332 ± 0.072 ($n = 5$) when χ_3, $\chi_{4'}$, and $\chi_{2'}$ are G and χ_2 is g, 0.060 ± 0.078 ($n = 20$) when χ_2, χ_3, $\chi_{2'}$, and $\chi_{4'}$ are G, or when χ_2 and $\chi_{2'}$ are g and χ_3 and $\chi_{4'}$ are G, and finally 0.438 ± 0.085 ($n = 20$) when χ_3, $\chi_{4'}$, and χ_2 are G and $\chi_{2'}$ is g.

around 0.2 kcal/mol less stable, whereas for the non-reducing or middle units, the difference is more variable (0.1–0.3 kcal/mol). Other rotamers (gg, GT) carry even greater energies. Experimental results in solution indicate that carbohydrates with gluco or manno configurations have their hydroxymethyl groups (as indicated by the ω angle) in g and G conformations, in about equal proportions.[42] On the other hand, for those with galacto configuration, the g rotamer is the most stable.[42] The appearance of a high predominance of rotamers with $\omega = \mathrm{g}$ using elaborate force fields has already been shown to occur.[43] A similar effect (but lower in magnitude) appears even working at high QM levels.[44] Molecular dynamics determinations showed a large variability of the ω angles.[30] In the present work, for the galactose disaccharides two preferential arrangements occur for the secondary hydroxyl groups of the non-reducing end: Ggg or gGg (Tables 3–5).

The approach to the multiple minimum problem presented here should probably apply at dielectric constants lower than 80: it was shown that hydrogen bonding and electrostatic forces are actually almost neglected at dielectric constants much lower than 80 ($\varepsilon = 10$–20),[38] as expected, considering that these forces depend on the reciprocal of the dielectric constant (the default ε in MM3 is 1.5). The additivity determined in the present paper eases up considerably the efforts necessary in order to establish the lower energy conformers of a di- or trisaccharide at high dielectric constants. Considering three staggered conformations for each exocyclic angle, a full search for each grouping needs 27 (3^3) or 9 (3^2) calculations. Thus, only 72 MM3 calculations ($3^3 + 3^2 + 3^3 + 3^2$) are needed for each energy well of a disaccharide: the energy differences between groupings can be calculated, and a further simple algebraic calculation is sufficient to predict the energy of the 59,049 (3^{10}) possible rotamers, assuming that no interaction between different groupings occurs. In this way, the starting structures up to a given energy can be generated and used for the construction of an adiabatic map in a systematic way that can be automated easily.

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