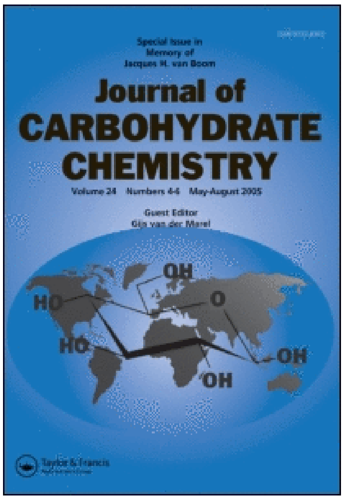

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POTENTIAL ENERGY SURFACES OF α-(1 → 3)-LINKED DISACCHARIDES CALCULATED WITH THE MM3 FORCE-FIELD

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ABSTRACT

The adiabatic conformational surfaces of sixteen 4′,6′,6-trideoxy-α-D-(1 → 3)-linked disaccharides were obtained using the MM3 force-field at two different dielectric constants. Calculations were carried out on disaccharides with different configurations at C2, C4 and C2′, which are neighbors to the glycosidic linkage, as well as that of the linked carbon (C3). The resulting maps were similar, indicating that the substituents do not play a major role in the conformational features of these disaccharides. However, the preferred minimum conformation and the flexibility were found to be slightly dependant on the configurations of the carbons. Although equatorial bonds and vicinal axial substituents tend to increase the overall flexibility, it was found that these factors can have a cross over effect; i.e., an axial hydroxyl group on C2 may decrease the flexibility if the glycosyl group on C3 is also axial. The relative stabilities of the minimal energy conformations of the 16 compounds also show deviations of the predicted increased stabilities of equatorially substituted compounds over axially substituted ones: these deviations occur mainly for the C2 substituent.

Key Words: Conformational analysis; Disaccharide maps; MM3; Axial; Equatorial

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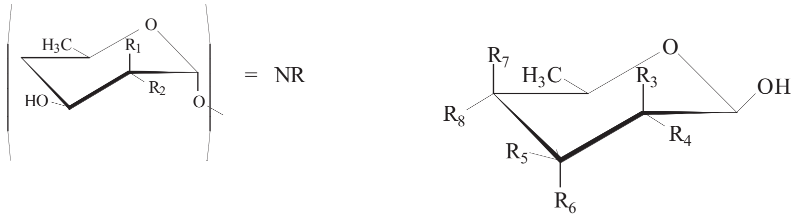
INTRODUCTION

Conformational analysis of disaccharides is usually accompanied by generation of a Ramachandran-like conformational map as a tool in understanding oligosaccharide conformational structures.[1,2] In these maps the energy is determined for all mutual orientations of the two monosaccharide residues, expressed by the glycosidic angles ϕ and ψ. Early studies were carried out by rigid residue analysis,[2] especially using the HSEA (Hard Sphere Exo-Anomeric Effect) of Lemieux and coworkers.[3] However, by allowing all variables to relax, Melberg and Rasmussen[4] initiated flexible residue analysis by 1979. Those studies were extended in the late 1980s,[5] giving rise to the first fully relaxed energy maps of disaccharides. Special features of the carbohydrates, such as the presence of hydrogen bonding, and anomeric and *exo*-anomeric effects complicated their study. However, the parameterization of the force-field MM3[6] considered these facts,[7a] and consequently has been applied to many different disaccharides as demonstrated by Dowd et al.,[8,9] and others. Another difficulty encountered with disaccharide modeling has its origins in the rotameric complexity of the exocyclic substituents (the ''multiple minimum problem''),[1,2] which has been circumvented using different approaches.[10] More recently, French and coworkers[11,12] applied ab initio or hybrid quantum mechanics-MM3 procedures to map disaccharides, in an attempt to achieve higher accuracy.

Rees[13] has brilliantly predicted that disaccharides with equatorial bonds will be more flexible than those with axial–equatorial bonds, and these in turn even more



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Conf.NR	Conf. R
1	H	OH	H	OH	NR	H	H	OH	D-*xylo*	D-*gluco*
2	H	OH	H	OH	NR	H	OH	H	D-*xylo*	D-*galacto*
3	H	OH	OH	H	NR	H	H	OH	D-*xylo*	D-*manno*
4	H	OH	OH	H	NR	H	OH	H	D-*xylo*	D-*talo*
5	OH	H	H	OH	NR	H	H	OH	D-*lyxo*	D-*gluco*
6	OH	H	H	OH	NR	H	OH	H	D-*lyxo*	D-*galacto*
7	OH	H	OH	H	NR	H	H	OH	D-*lyxo*	D-*manno*
8	OH	H	OH	H	NR	H	OH	H	D-*lyxo*	D-*talo*
9	H	OH	H	OH	H	NR	H	OH	D-*xylo*	D-*allo*
10	H	OH	H	OH	H	NR	OH	H	D-*xylo*	D-*gulo*
11	H	OH	OH	H	H	NR	H	OH	D-*xylo*	D-*altro*
12	H	OH	OH	H	H	NR	OH	H	D-*xylo*	D-*ido*
13	OH	H	H	OH	H	NR	H	OH	D-*lyxo*	D-*allo*
14	OH	H	H	OH	H	NR	OH	H	D-*lyxo*	D-*gulo*
15	OH	H	OH	H	H	NR	H	OH	D-*lyxo*	D-*altro*
16	OH	H	OH	H	H	NR	OH	H	D-*lyxo*	D-*ido*

Figure 1. The α-(1 → 3) linked disaccharides studied in this work: the non-reducing terminals are 4,6-dideoxy-α-D-hexosyl units, while the reducing terminals are 6-deoxy-β-D-hexose units.

flexible than those with axial–axial bonds. This fact was shown to be correct with disaccharide analogs in a recent paper by French and coworkers,[11] and in the comparison of the maps from an α-(1 → 3)-galactobiose when the non-reducing end was replaced by a 3,6-anhydro ring, leading to an equatorial–equatorial linkage.[14] When Rees[13] analyzed the factors leading to enhanced flexibility, he pointed out that bulky equatorial substituents vicinal to the glycosidically linked atoms reduce the flexibility of the linkage more than bulky axial substituents. Calculations carried out for sulfated disaccharides[14,15] agree with this prediction.

Herein are presented the potential energy surfaces of sixteen disaccharides of the type 3-*O*-(4,6-dideoxy-α-D-hexosyl)-6-deoxy-β-D-hexose with different configurations at carbons 2, 3, 4 and 2′ (Figure 1), calculated using MM3 at either $\varepsilon = 3$ or $\varepsilon = 80$. Comparison with tetrahydropyran and acyclic derivatives provide help to learn about the factors governing the flexibility of these molecules.

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),[6] and compiled by the SparcCompiler 2.0.1. The MM3 routines were modified as suggested[16] by changing the maximum atomic movement from 0.25 Å to 0.10 Å. The dihedrals ϕ_H and ψ_H are defined by atoms H1′–C1′–O3–C3 and H3–C3–O3–C1′, respectively, with the usual sign conventions.[14] Minimization was carried out by the block diagonal Newton–Raphson procedure for grid points, using the full-matrix procedure for minima and transition states.

The minima were generated by an automated procedure: starting from a structure with a minima in the **B** region, the 243 conformers produced by rotating the exocyclic OH groups were generated, and those (3 to 12) with minimal energies were left. The procedure was repeated in the same manner to determine minima in the **A** and **C** regions. The main minimum in each region was used to locate minima in the **D** region. Starting from each of those **A**–**B**–**C**–**D** minima, using both the dihedral drivers 2 and 4, ϕ_H and ψ_H were fully varied using a 20° grid. 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 grid point was the lowest of any of the unique 15–40 different minima obtained previously. In this way, only the conformation of minimal energy for each ϕ, ψ combination was recorded and thus the conformational adiabatic maps, or potential energy surfaces as function of ϕ and ψ angles were produced. In one case, the map was made from the 243 different **B** conformers, using dihedral driver 4. The maps were plotted using Sigma Plot 4.01 (SPSS, USA). It has been suggested[12b] to drive torsion angles in terms of non-hydrogen atoms, given the different motions of the three atoms during driven rotation and the inaccuracy of hydrogen atom positions in diffraction studies. However, in order to keep up with our previous studies[10,14,15] we continued driving in terms of hydrogen atoms. For compounds in which flipping of the chair was assumed feasible (e.g., **4**, **8**, **12** and **16**), special care was taken to include only conformers in the map with the original chair conformations ($^4C_1$). Free energies were calculated from the vibrational analysis of the minima, with no special treatment for the low-frequency vibrations:[17] i.e., the effect of frequencies equal or lower than 20 cm^{-1} was added to the MM3 output values (which do not include those frequencies) of vibrational enthalpies and entropies.

The absolute flexibility was calculated as described by Koca et al.[7] First, the energies and geometries of the transition states between minimum energy regions were calculated: they were first estimated from the walk within the adiabatic maps, and then determined by a full-matrix analysis, confirming that only one negative eigenvalue appeared. When more than one transition state was present in the same region, that with the lower-energy was considered. The absolute flexibility[7] Φ was calculated as:

$$\Phi = \sum_{i=1}^{n}\left(\frac{e^{-Ei/RT}}{\sum_{k=1}^{n} e^{-Ek/RT}}\right) \times \left[\sum_{j=1}^{m}\left(e^{-(Ej-Egm)/RT}\right) \times \left(\frac{|\phi_i-\phi_j|+|\psi_i-\psi_j|}{720}\right)\right]$$

where E_{gm} is the energy of the global minimum, n is the number of minima (indexes i and k), m the number of transition states (index j) surrounding minimum i, the ϕ, ψ angles are given in degrees, R is the universal gas constant and T the temperature (set to $25°C = 298.16$ K).

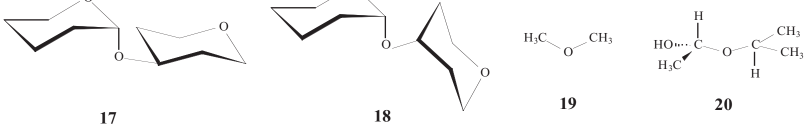
The partition functions were calculated as:[14]

$$q = \Delta\phi \times \Delta\psi \times \sum_{i=1}^{324} e^{-(Ei-Egm)/RT}$$

where $\Delta\phi$ and $\Delta\psi$ are the grid spacings (20° each in this case) and the summation is carried out over the entire ϕ, ψ surface (324 points). French et al.[11] used the name ‘‘probability volume’’ for this function. The percentage of allowed surfaces was calculated as a quotient of the number of points below certain energy and 324 (the total number of points in a $20° \times 20°$ grid).

RESULTS

The conformational maps of the sixteen 4′,6′,6-trideoxy-α-D-(1 → 3)-linked disaccharides shown in Figure 1 were calculated using MM3 at a dielectric constant of 3. For comparison purposes, the same analysis was carried out with **17**, the analog of **1–8** without exocyclic substituents (axially–equatorially linked 4-(tetrahydropyran-2-yloxy)tetrahydropyran), and **18**, the corresponding axially–axially linked analog of **9–16**. Other simpler analogs studied were the acyclic dimethyl ether **19**, and (*R*)-1-isopropoxyethanol **20** (the isopropyl hemiacetal of ethanal). Figure 2 shows the structure of those compounds.



17 **18** **19** **20**

Figure 2. Other compounds studied in this work.

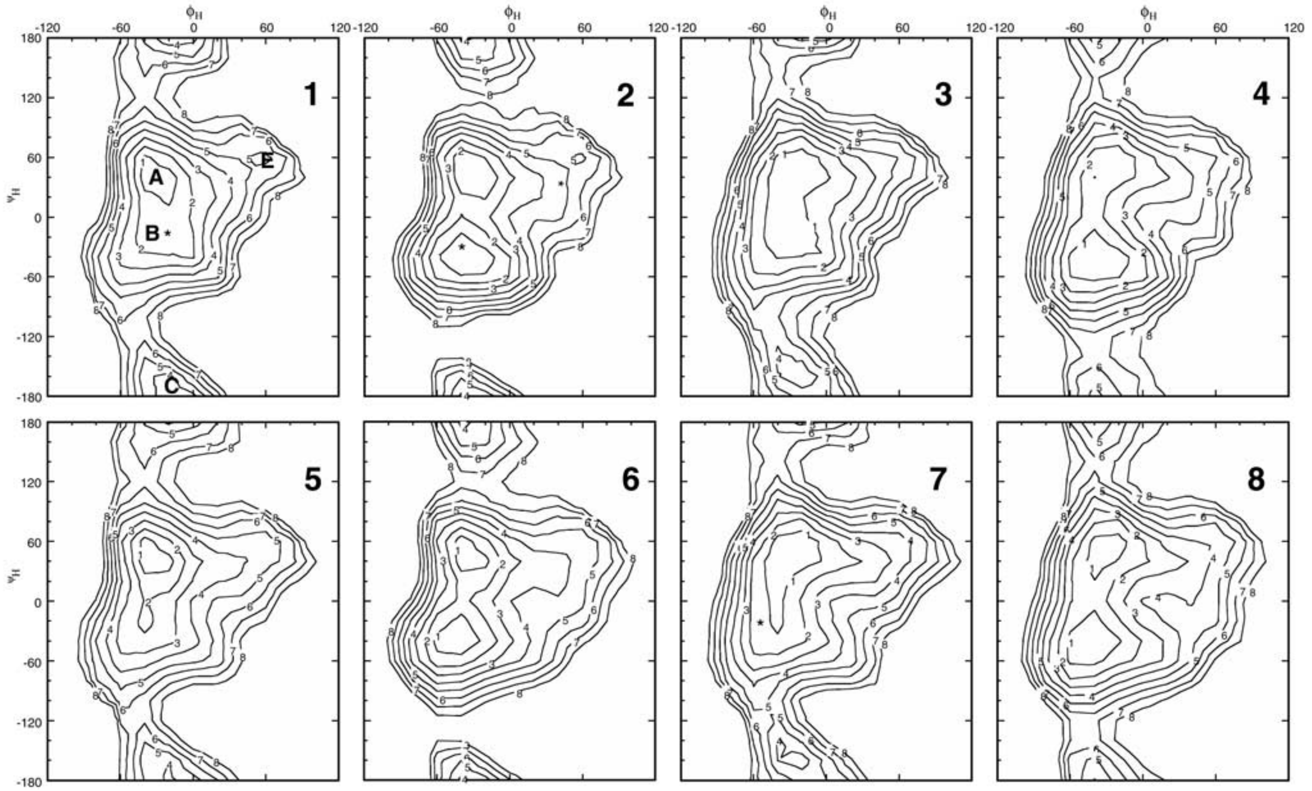


Figure 3. Conformational maps of compounds **1**–**8**, generated using MM3 at ε = 3. Iso-energy countour lines are graduated in 1 kcal/mol increments above the global minimum. The stars indicate the position of reported crystal structures. (From Ref. [18].)

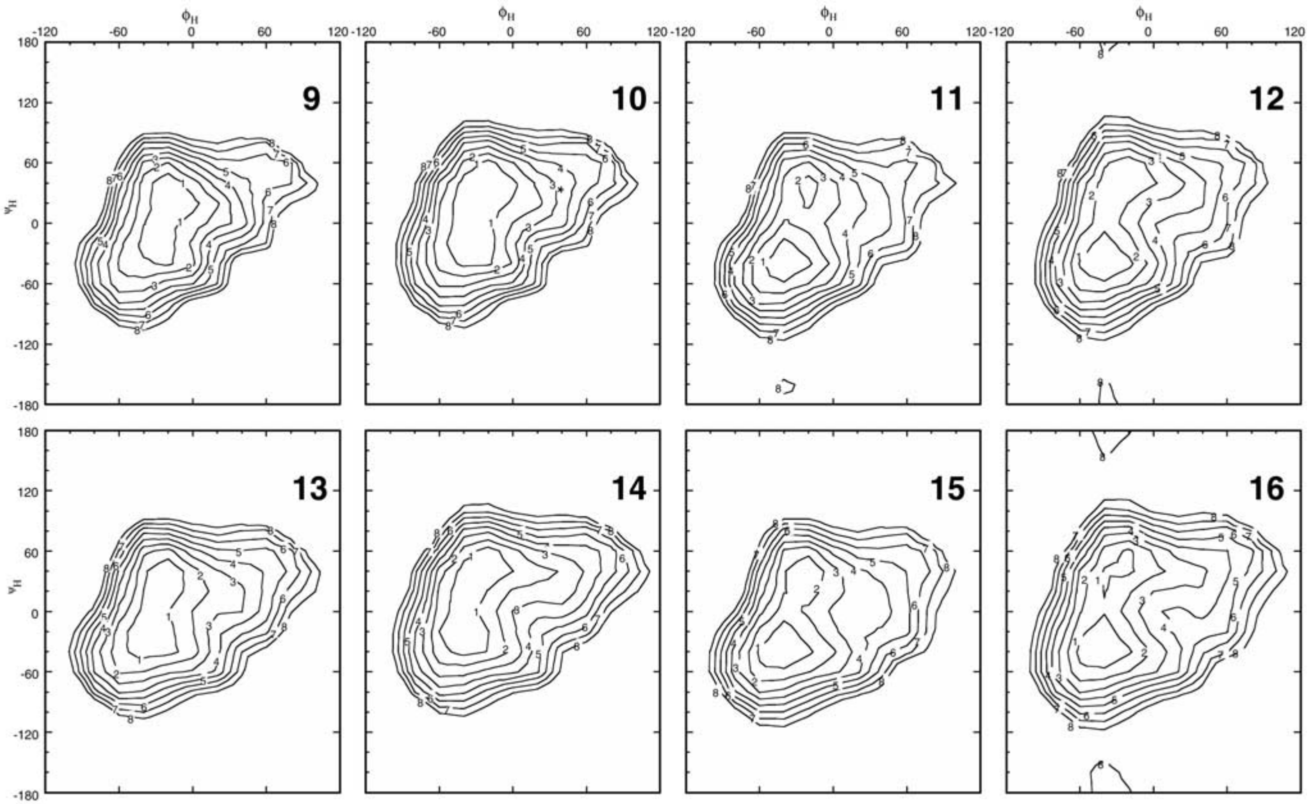


Figure 4. Conformational maps of compounds **9–16**, generated using MM3 at $\varepsilon = 3$. Iso-energy countour lines are graduated in 1 kcal/mol increments above the global minimum.

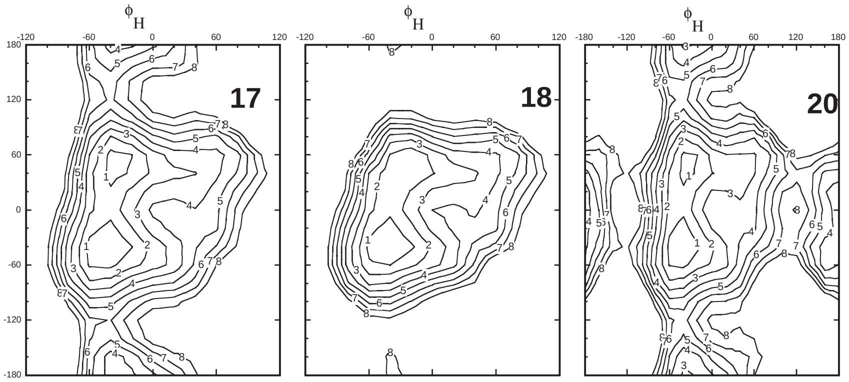


Figure 5. Conformational maps of compounds **17**, **18** and **20** generated using MM3 at ε = 3. Iso-energy contour lines are graduated in 1 kcal/mol increments above the global minimum. Observe the ϕ scale change for compound **20**.

The resulting maps are shown in Figures 3 (compounds **1**–**8**), 4 (compounds **9**–**16**) and 5 (compounds **17**–**20**), while the energy data (relative steric and free energies) on the minima are shown in Table 1. X-ray crystallographic studies[18] reported the solid-state structures for three compounds with the configuration of compounds **1**, **2** and **7**. Their torsional angles are also indicated in their corresponding maps (Figure 3).

Table 1. Relative Steric and Free Energies (kcal/mol) for the Minimum-Energy Conformations Obtained for the Compounds Under Study, Using the MM3 Force-Field at ε = 3

	O1′, O3	HO2′, HO2, HO4	Min. **A**		Min. **B**		Min. **C**		Min. **D**	
			ΔE	ΔG	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG
1	AE[a]	EEE	0.00	0.00	1.12	0.26	2.95	3.01	9.87	11.67
2	AE	EEA	0.58	1.29	0.00	0.00	3.62	5.01	9.74	11.82
3	AE	EAE	0.00	0.02	0.14	0.00	3.13	4.01	10.09	11.27
4	AE	EAA	0.86	1.12	0.00	0.00	4.56	5.77	11.93	12.53
5	AE	AEE	0.00	0.00	1.66	0.87	3.42	3.44	9.02	9.53
6	AE	AEA	0.00	0.63	0.10	0.00	3.60	4.58	8.92	9.62
7	AE	AAE	0.00	0.00	0.62	0.09	3.19	4.03	8.15	9.14
8	AE	AAA	0.32	0.60	0.00	0.00	3.91	5.64	9.52	10.22
9	AA	EEE	0.00	0.48	0.47	0.00	9.29	10.28	10.75	12.14
10	AA	EEA	0.00	0.39	0.18	0.00	9.42	10.46	11.50	12.51
11	AA	EAE	1.37	1.81	0.00	0.00	7.43	8.52	9.39	10.93
12	AA	EAA	0.96	1.20	0.00	0.00	7.48	8.19	10.90	11.65
13	AA	AEE	0.04	0.67	0.00	0.00	10.14	10.76	9.96	10.60
14	AA	AEA	0.08	0.12	0.00	0.00	8.88	9.66	9.01	9.66
15	AA	AAE	1.02	2.01	0.00	0.00	8.17	8.78	8.85	9.95
16	AA	AAA	0.53	0.90	0.00	0.00	6.95	7.62	8.57	9.21
17	AE	–	0.40	0.55	0.00	0.00	2.73	3.82	9.18	10.16
18	AA	–	0.85	1.09	0.00	0.00	7.48	8.28	9.31	9.86
20	–	–	0.60	0.99	0.00	0.00	2.63	3.51	3.08	3.90

[a] A = axial substituent, E = equatorial substituent.

Table 2. Major Hydrogen-Bond Arrangements ($E_{HB} > 0.6$ kcal/mol) in Minima **A** and **B** for the Sixteen Compounds Under Study, Using the MM3 Force-Field at $\varepsilon = 3$[a]

	O1′, O3	HO2′, HO2, HO4	Minimum **A**	Minimum **B**
1	AE[b]	EEE	H(O)2–O5′	
2	AE	EEA	H(O)2–O5′	
4	AE	EAA	H(O)4–O2	H(O)4–O2
5	AE	AEE	H(O)2–O5′	
6	AE	AEA	H(O)2–O5′	
8	AE	AAA	H(O)4–O2	H(O)4–O2
9	AA	EEE	H(O)4–O5′	H(O)2′–O2
11	AA	EAE	H(O)4–O5′	
12	AA	EAA	H(O)4–O2	H(O)4–O2
13	AA	AEE	H(O)4–O5′	
15	AA	AAE	H(O)4–O5′	
16	AA	AAA	H(O)4–O2	H(O)4–O2

[a] If a compound does not appear in the Table, no hydrogen bond with energies higher than 0.6 kcal/mol was found.

[b] A = axial substituent, E = equatorial substituent.

Table 3. Relative Steric and Free Energies (kcal/mol) for the Minimum-Energy Conformations Obtained for the Compounds Under Study, Using the MM3 Force-Field at $\varepsilon = 80$

	O1′, O3	HO2′, HO2, HO4	Min. **A**		Min. **B**		Min. **C**		Min. **D**	
			ΔE	ΔG	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG
1	AE[a]	EEE	0.00	0.15	0.03	0.00	2.50	3.33	10.89	12.49
2	AE	EEA	1.12	1.12	0.00	0.00	4.79	5.02	10.88	12.03
3	AE	EAE	0.00	0.00	0.68	0.26	4.40	4.73	10.48	11.45
4	AE	EAA	0.34	0.64	0.00	0.00	5.44	5.71	10.68	11.52
5	AE	AEE	0.00	0.10	0.09	0.00	2.54	3.40	8.55	9.96
6	AE	AEA	1.08	1.14	0.00	0.00	4.81	5.08	9.08	10.02
7	AE	AAE	0.00	0.00	0.74	0.30	4.44	4.80	8.31	9.13
8	AE	AAA	0.25	0.56	0.00	0.00	5.44	5.72	8.90	9.50
9	AA	EEE	1.12	0.86	0.00	0.00	9.54	10.26	11.22	12.24
10	AA	EEA	0.01	0.26	0.00	0.00	8.32	9.19	10.24	11.24
11	AA	EAE	2.21	2.12	0.00	0.00	8.39	9.15	10.85	12.04
12	AA	EAA	0.73	0.98	0.00	0.00	6.65	7.32	10.42	11.40
13	AA	AEE	1.05	0.80	0.00	0.00	9.46	10.13	9.00	10.88
14	AA	AEA	0.00	0.02	0.19	0.00	8.36	8.93	8.22	8.72
15	AA	AAE	2.09	2.06	0.00	0.00	8.29	8.99	9.04	9.99
16	AA	AAA	0.56	0.79	0.00	0.00	6.51	7.21	8.81	9.15
17	AE	–	0.35	0.50	0.00	0.00	3.15	3.80	8.74	9.57
18	AA	–	0.84	1.28	0.00	0.00	7.34	8.14	8.98	9.59
20	–	–	0.57	1.15	0.00	0.00	2.64	3.70	3.25	4.52

[a] A = axial substituent, E = equatorial substituent.

All the maps show similar shapes, with three minima having the ϕ_H angle around that predicted by the *exo*-anomeric effect (ca. $-60°$). The minima in the regions **A** and **B** (ψ_H g^+ and g^-, respectively) have similar energies (Table 1), while minimum **C** (ψ_H t) is only important for compounds **1**–**8** and **17** in which the glycosidic linkage to O3 is equatorial. Minimum **D** (ϕ_H ca. 180°) is very unfavorable (except for the acyclic compound **20**), while in some compounds (**1**, **2**, **9**, **11** and **12**), a minimum with ϕ, ψ around 60°, 60° (in a flat region) has been found. Table 2 shows the major hydrogen-bond arrangements for the compounds under study. In order to check a possible lack of adiabaticity[10] due to the restricted number of starting structures, the map of **1** was also calculated using the 243 possible conformers with all the exocyclic staggered positions as starting points: only four grid points appeared with lower energies, arising from exocyclic angles combinations which do not give low-energy minima, but yield lower energy values in map regions distant from the minima. As these differences are small (ca. 0.5 kcal/mol), and in high-energy regions (>5 kcal/mol), the conclusions drawn below on map shapes, flexibilities and relative energies are not affected at all.

The same studies were carried out at a dielectric constant of 80. Table 3 shows the energy data on these compounds. No major differences in the map shape

Table 4. Corrected Partition Functions (‘‘Probability Volumes’’) q, Absolute Flexibilities Φ and Percentages of the Map Surfaces Below Each Energy Range (‘‘Allowed Conformations’’) for the 20 Compounds Under Study Using the MM3 Force-Field

	HO2′, HO2, HO4	$\varepsilon = 3$ q (deg^2)	Φ ($\times 10^4$)	% Allowed (kcal)			$\varepsilon = 80$ q (deg^2)	Φ ($\times 10^4$)	% Allowed (kcal)		
				2	5	10			2	5	10
1	EEE[a]	960	82	3.7	14	36	2180	210	5.6	17	37
2	EEA	1230	13	4.3	15	35	1120	33	4.3	13	35
3	EAE	2670	243	7.1	19	38	1460	186	4.6	14	36
4	EAA	1530	57	5.2	16	39	1570	73	4.9	14	36
5	AEE	730	16	2.5	14	40	2250	211	5.9	20	45
6	AEA	1430	18	4.6	17	41	1180	38	4.6	16	40
7	AAE	1830	81	6.2	19	43	1470	184	5.6	17	43
8	AAA	1890	86	5.6	20	43	1740	88	4.9	18	43
9	EEE	1650	105	5.2	11	24	1220	116	4.0	11	24
10	EEA	2610	404	5.9	13	26	2290	361	5.6	14	29
11	EAE	850	19	3.4	12	30	930	25	2.5	11	26
12	EAA	1230	64	4.3	13	32	1320	78	4.6	13	33
13	AEE	2010	113	5.6	15	26	1340	134	4.6	15	29
14	AEA	2340	364	5.9	16	33	2200	345	6.2	17	35
15	AAE	1160	28	4.0	14	31	1030	23	3.4	14	29
16	AAA	1560	104	4.9	16	39	1560	103	5.6	17	40
17		1650	72	4.9	21	48	1740	72	4.9	23	49
18		1390	78	4.9	16	38	1430	78	5.2	17	39
19		11950	54	36	97	100	11950	54	36	97	100
20		1770	94	6.2	30	40	1970	119	6.5	30	41

[a] A = axial substituent, E = equatorial substituent.

Table 5. Relative Steric and Free Energies for the Minimum Energy Conformer of Each of the Disaccharides Under Study, Using the MM3 Force-Field at ε = 3 and 80

	O1′, O3	HO2′, HO2, HO4	ε = 3 ΔE	ε = 3 ΔG	ε = 80 ΔE	ε = 80 ΔG
1	AE[a]	EEE	0.00	0.00	0.63	0.20
2	AE	EEA	0.78	0.32	0.00	0.00
3	AE	EAE	1.85	1.47	1.03	1.24
4	AE	EAA	1.70	1.98	1.53	1.76
5	AE	AEE	0.64	0.74	1.20	0.86
6	AE	AEA	1.75	1.52	0.60	0.68
7	AE	AAE	2.42	2.15	1.55	1.85
8	AE	AAA	2.65	2.75	2.15	2.50
9	AA	EEE	2.88	2.89	2.50	2.85
10	AA	EEA	3.59	2.77	2.75	2.77
11	AA	EAE	3.00	2.62	2.34	2.56
12	AA	EAA	2.24	2.35	2.94	3.07
13	AA	AEE	4.00	3.80	3.07	3.42
14	AA	AEA	4.46	4.01	3.23	3.58
15	AA	AAE	3.62	3.30	2.97	3.27
16	AA	AAA	3.02	3.20	3.62	3.82

[a] A = axial substituent, E = equatorial substituent.

were found, although the minima in the region B become the global ones for most of the compounds.

Flexibility calculations for these compounds are shown on Table 4. This Table indicates the conformational partition functions (or probability volumes[11]), the allowed surfaces, and the absolute flexibilities.[7] Table 5 shows the relative energies for the minimal conformations of each of the sixteen disaccharides under study.

DISCUSSION

The force-field MM3 has been widely used to analyze the potential energy surfaces of many disaccharides.[8–10] In the present work, an attempt to correlate the potential surfaces with the configurations of the carbons bearing hydroxyl groups vicinal to the glycosidic linkage, as well as that of the glycosidic linkage itself has been carried out for α-(1 → 3)-linked disaccharides composed of two D-hexose residues. In order to facilitate the calculations, and to achieve a more reliable adiabaticity of the map,[10] hydroxyl groups located far from the glycosidic linkage have been eliminated. Thus, the non-reducing termini were 4,6-dideoxy-α-D-hexopyranosyl residues, while the reducing ones were 6-deoxy-β-D-hexoses. The general conclusions should apply also to the fully hydroxylated disaccharides as, for example, the maps for compounds **1** and **2** are very similar to those previously determined for β-nigerose[9] and α-D-galactopyranosyl-(1 → 3)-β-D-galactopyranose.[15]

Shape of the Potential Surfaces

For all the maps, a trough centered at a more or less fixed ϕ_H angle (between $-10°$ and $-60°$, matching the expression of the *exo*-anomeric effect) is observed and contains the three main minima, each of which exhibits a clearly different ψ_H angle. The same shape of the map was observed for the simple acyclic hemiacetal **20** (Figure 5, Table 1). A fourth minimum (**D**), with ϕ_H ca. 180° appears, but has very high energies (Table 1), as expected considering that they carry at least one axial linkage.[11] Only in the acyclic compound **20** is its energy lower (3.08 kcal/mol above the global minimum). A fifth minimum (**E**) in a plateau region appears for some compounds. Its appearance was previously reported to be non-systematic.[9] The relative energies of the minima **A** and **B** are very close. Compounds **1** and **5** are unique by exhibiting **A** as the global minimum by a considerable difference. They both have the reducing moiety with a D-*gluco* configuration. Other compounds with a predominance of minimum **A** are **3**, **7** and **9**. They all have in common an equatorial HO4. On the other hand, compounds **11** and **15** (reducing sugar with a D-*altro* configuration) have their global minima definitely in region **B**, while a similar effect, but smaller in magnitude has been observed for the compounds **2**, **4**, **8**, **12** and **16**, with axial HO4. Yet, **10**, **14** (reducing sugar with a D-*gulo* configuration) and **6** also have an axial HO4, but not the same effect. The effects of the substituents on the relative energies of **B** with respect to **A** (E_B-E_A) are not additive, but interrelated. However, there are clear stabilizing effects for the **A** minimum when O2 and/or O4 are equatorial. With O4 this effect is observed only when the linkage on C3 is also equatorial. In this case, also observed is a destabilization of the minimum **A** due to an equatorial HO2′. Given these facts, as expected, the compound with the highest E_B-E_A is **5**, with equatorial substituents on C2, C3 and C4, and an axial substituent on C2′. These effects cannot be explained exclusively on grounds of hydrogen-bonding, which would account for a stabilization of minimum **A** in equatorially linked compounds carrying an equatorial O2, or in axially linked compounds carrying an equatorial O4 (Table 2), as the hydrogen atom bound to these equatorial oxygens binds to O5′ in minimum **A** but not in **B**. The crystal structures reported[18] for compounds with the configuration of **1**, **2** and **7** have their glycosidic torsion angles in the **B** region, even though the calculations for **1** and **7** (as well as that of nigerose in a previous paper[9]) give their global minima in the **A** region.

Major differences for the **C** region have been found. Those with equatorial linkage (**1**–**8**) have an accessible **C** minimum (<5 kcal/mol), while this minimum appears almost prohibited (>7 kcal/mol) for compounds with an axial linkage at C3 (**9**–**16**), as predicted.[11] The same effect has been observed for the unsubstituted analogs **17** and **18**, and gives a different overall silhouette to the maps (cf Figures 3 and 4). Within the first set of compounds, an increase in the **C** energy due to an axial HO4 is observed. In the second set, the substituent producing the larger effect is HO2. When it is axial, minimum **C** appears stabilized. Minimum **D** always carries high energies (8–12 kcal/mol above the global minimum). The main factor that has an influence on its magnitude is the configuration of HO2′ (when it is axial, minimum **D** is stabilized).

Free Energy Calculations

It was previously shown that the conformational entropy is not uniformly distributed.[14,17] It is expected that strongly hydrogen-bonded conformers will find an unfavorable entropic contribution, and thus relatively higher free energies.[12b,14] Table 1 shows that considering free energies, the energies of the **B** minima are diminished relative to the **A** minima. This agrees with the calculated hydrogen-bond arrangements (Table 2), usually more likely to occur in minima **A** than in **B** minima. Free energy calculations give **B** as the global minimum for all the compounds, with the exception of **1**, **5** and **7**. The same trend is observed when the calculations are carried out at a dielectric constant of 80 (Table 3). This is not surprising, as these calculations also diminish the strength of hydrogen bonds. However, they operate in a different manner. For compounds in which the reducing sugar has the D-*manno* configuration (**3** and **7**), the calculation at $\varepsilon = 80$ is actually destabilizing minimum **B**. In these compounds, no major hydrogen bond is observed in either **A** or **B** minima (Table 2). Free-energy calculations also give higher values of relative energies for minima **C** and **D**. However, this effect is negligible for the minima **C** in compounds with D-*gluco* configuration.

Flexibilities

The flexibility of the glycosidic linkage has been measured in terms of a partition function,[14,15] also called probability volume.[11] This parameter is highly dependant on the energy differences between the minima **A** and **B**, its magnitude being very sensitive to the size of the regions of the map with very low energy and thus, highly influenced by the entropy of the global minimum. Another parameter, which has been applied to carbohydrates,[7] is the ‘‘absolute flexibility,’’ which gives an indication of the conformational interconversions of the lower-energy minima, and is very sensitive to the height of the lower potential barriers. A third parameter to envision fully the flexibility can be defined in terms of ‘‘allowable surfaces.’’ Rees and Scott[19] used this value as a semiquantitative measurement in their rigid residue analysis. It can offer the notion of the percentage of the surface with an energy below a certain value, and is also related with the entropy of the global minimum, but in a more linear fashion than the partition function. Table 4 shows the results of calculating these parameters for the twenty compounds under study. Amazingly, the allowed surfaces to 2 kcal/mol for **1**, **2** and **7** (reducing end with D-*gluco*, D-*galacto* and D-*manno* configuration, respectively) are very close to those encountered by Rees and Scott[19] in 1971 for α-1,3-glucan, galactan and mannan, respectively.

It was previously predicted that equatorially linked disaccharides are more flexible than those that carry at least an axial bond.[13] This was shown to be true in several cases.[11,14] The inspection of allowable surfaces and absolute flexibilities indicate that the axially–equatorially linked compounds are more flexible than the axial–axial ones, especially at high dielectric constants, where steric and torsional effects play a major role (Table 4, cf **17** and **18** and **1**–**8** with **9**–**16**). The use of absolute flexibilities give the same trend at $\varepsilon = 80$ for compounds with equatorial HO4, and at $\varepsilon = 3$ for compounds with the D-*manno* configuration, but the opposite for the remaining structures. Compounds **10** and **14** (reducing end with the D-*gulo* configuration), with a diaxial linkage appear as the most flexible by any method, suggesting that a second factor (also predicted by Rees[13]), the configuration of the bulky groups vicinal to the linkage, is also influencing the flexibility. At low dielectric constants, the flexibility of compounds is very low when the reducing sugar carries a D-*gluco* or D-*galacto* configuration, with equatorial linkage and substituent on C2. Flexibility is also low in compounds **11** and **15** (D-*altro* configuration). Using partition functions, an increase of the dielectric constant leads to a small decrease in the flexibilities, with the exception of compounds with a D-*gluco* configuration, due to the leveling of the minima **A** and **B**. On the other hand, the absolute flexibilities of most compounds increase with dielectric constant, possibly due to a decrease of the potential barriers.

The effect of the configuration of the hydroxyl groups on the flexibilities can be summarized in the following manner: 1) the replacement of an equatorial HO2′ by an axial one usually leads to small increases in the partition functions (especially at $\varepsilon = 80$), while absolute flexibilities are almost unaffected, especially at $\varepsilon = 80$; 2) the replacement of an equatorial HO4 by an axial one leads to an increase in the flexibility when the linkage on C3 is axial. When the linkage is equatorial, the behavior is opposite when using absolute flexibilities, and non-systematic when using partition functions; 3) the replacement of an equatorial HO2 by an axial one increases the flexibility if the linkage on C3 is equatorial, but decreases it when the linkage is axial. Thus, it is not always true that bulky equatorial substituents vicinal to the glycosidic linkage decrease the flexibilities.

The allowed surfaces up to 10 kcal/mol are largely enhanced by an equatorial bond (Table 4). This effect is due mainly to the fact that the **C** region carries lower energies. Compound **16**, with a **C** minimum with relatively low energy, is an exception. In this compound, the linkage is axial, the three vicinal substituents at C2, C4 and C2′ are axial, and the flexibility is increased. In general, a good correlation is observed between the partition function q and the percentage of allowed surfaces below 2 kcal/mol, as expected considering their grounds. Each method to calculate the flexibility has a different physical basis (see METHODS), and with complex surfaces like those studied in this work, possibly each method by itself does not give the whole picture.

Comparison of the Energy Surfaces

It is worthy of note that the presence or absence of bulky substituents does not have a major effect on the general shape of the maps and/or the flexibilities. The adiabatic maps and flexibilities of compounds **17** and **18** are very similar to those of their hydroxylated/ methylated counterparts (Tables 1 and 4), and even an acyclic model like **20** gives a map and a flexibility with similar characteristics. This fact emphasizes the prime role of the torsional energies in determining energy surfaces. For the dimethyl ether (**19**), an evident increase in the flexibilities calculated using partition functions or allowed surfaces is observed, given the symmetry and the low energy values all around the map. However, the absolute flexibility is low, due to the height of the barrier, higher than those usually found for the transition between minima **A** and **B**. In any circumstance, it should be kept in mind that the variations produced by the presence and configuration of the substituents are indeed small, as shown by the similarity of map shapes and the impossibility of reaching higher flexibilities (their theoretical maxima are, for q, 129,600 deg^2, and for Φ, 1, i.e., $10{,}000 \times 10^{-4}$).

Relative Stabilities of Compounds 1–16

Regarding the relative steric energies of the global minimum of each compound (Table 5), as expected, compound **1**, with equatorial substituents is the most stable. However, when calculating at $\varepsilon = 80$, compound **2** becomes more stable. For cyclohexanol conformers, the energy difference of 0.7–0.9 kcal/mol favors the equatorially substituted ring. An average value of 0.8 kcal is observed (Table 4) as the difference between compounds with axial and equatorial HO4 if HO2 is also equatorial. If HO2 is axial, an axial HO4 leads to a favorable energy difference of ca. 0.3 kcal. This is certainly due to a strong intramolecular hydrogen-bond arrangement between the two axial groups in a 1,3-diaxial array (Table 2). A 0.8 kcal effect of an axial HO2′ is found to be additive. On the other hand, the effect of the configuration of HO2 is dependant on other factors: it is largely favorable to the equatorial position (1.8 kcal) when O3 and HO4 are also equatorial, moderately favorable (0.9 kcal) when O3 is equatorial and HO4 axial, negligible when O3 is axial and HO4 equatorial, and clearly favorable to the axial conformer (1.3 kcal) when O3 and HO4 are axial (hydrogen-bonding, Table 2). The axial linkage on C3 represents a large penalty (ca. 3 kcal) when HO2 is equatorial, but a lesser one when it is axial: it amounts to 1.2 kcal if HO4 is equatorial, and to only 0.4 kcal if HO4 is axial. The previously explained effects are valid for calculations carried out at low dielectric constant, and using steric energies. If free energies are used, the relative energies of the compounds having HO2 and HO4 in different configurations (one axial and the other equatorial) are diminished, while those carrying both axial (because of diminished hydrogen-bonding) or both equatorial increase their energies. As explained previously, at $\varepsilon = 80$, **2** is more stable than **1**, while **6** is more stable than **5**. An axial HO4 becomes stabilized at high dielectric constants when both HO2 and O3 are equatorial, probably given that the compounds carrying those substituents do not yield strong hydrogen bonds (Table 2). The use of a high dielectric constant lessens the energy differences among compounds. However, the decrease in energy is larger for compounds that have HO2 and HO4 in different configurations, i.e., the same effect as using free energies. Thus, at this dielectric constant, the compound with all axial substituents (**16**) becomes the one with higher energy (because of its diminished 1,3-diaxial hydrogen-bonding) while at low dielectric constant, **14** has a considerably higher energy, given the effect of the equatorial HO2.

Stereochemical Consequences

The spatial interaction of protons in different monosaccharide moieties has been considered the basis of the ^{13}C NMR glycosylation effects in disaccharides.[20] Thus, according to several stereochemical factors (linkage configuration, monosaccharide series, configuration of neighboring carbons, chair conformation, etc.), two different groups can be distinguished: one with a large shift due to glycosylation on both linked carbons, but a negligible shift on the neighboring carbons (EII), and a second group (EI) with a smaller shift due to glycosylation on the linked carbons, but a substantial β-effect (≈ 3 ppm) on one of the carbons of the reducing terminus vicinal to the linked carbon.[20b] In a more empirical approach, Bock and coworkers[21] related the glycosylation shift with the expected proton–proton distances, determining out a continuous effect feasible for a linear regression relationship. The Boltzmann-averaged inter-proton distances calculated for the 16 disaccharides under study led to the determination that the distance H1′–H3 can have two different ranges of values: 1) short (2.26–2.32 Å) for those compounds with equatorial O3 and equatorial HO4 (**1**, **3**, **5** and **7**), or those with an axial O3 and an equatorial HO2 (**9**, **10**, **13** and **14**). According to the stereochemical rules given by Shashkov et al.,[20b] those compounds should be gathered within group EII, with large glycosylation effects on C1′ and C3; and 2) medium (2.47–2.52 Å) for the rest of the compounds (**2**, **4**, **6**, **8**, **11**, **12**, **15** and **16**), included in group EI, giving rise to smaller glycosylation effects on C1′ and C3. As expected from the relationship empirically found,[21] short inter-proton distances give rise to larger glycosylation effects. These effects have been proven to exist as stated experimentally in many compounds.[20] In addition, the analysis of the distances of H1′ with the protons on the neighboring carbons (H2 and H4) led to the conclusion that these distances should be short (< 3 Å) in the following cases: H1′–H4 is short only for compounds with an equatorial O3 and an axial HO4 (**2**, **4**, **6** and **8**), while H1′–H2 is short when O3 is axial and HO2 equatorial (compounds **9**, **10**, **13** and **14**). Therefore, the glycosylation effects on C1′ and C3 according to the groups EI and EII are probably correct in the interpretation of Shashkov et al.[20b] However, when O3 is axial, according to the calculated distances, it should be possible to find some differences for their β-effects: 1) in those compounds within group EI (**11**, **12**, **15** and **16**) a negligible glycosylation effect on either C2 or C4 should be found; 2) for compounds within group EII, a considerable β-effect on C2 is expected to appear. As has been stated,[20b] the configuration of HO2′ is irrelevant as to the glycosylation effect or distance, due to the strong preference for the compounds to have the ϕ angle predicted by the *exo*-anomeric effect, which shifts the ''aglycone'' apart from C2′.

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