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Conformational Analysis of α-D-Gal-(1–4)-β-D-Gal-OR: An Oligosaccharide Substructure Recognized by P-Fimbriated *Escherichia coli*[1]

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The preferred ground-state conformations of the galabiose glycosides, α-D-Gal-(1–4)-β-Gal-OMe (1) and α-D-Gal-(1–4)-β-D-Gal-OEt (2), have been examined by molecular mechanics calculations and 2D NMR. The calculations predicted a rather rigid molecular shape with the deepest potential energy minimum in the range of −50° to −30° for ϕ and −20° to +20° for ψ. Experimental support for the global minimum energy conformation ($\phi = -50°$, $\psi = -20°$) was obtained by ^{1}H NMR measurements. Complete proton assignments were made by using two-dimensional homonuclear correlated spectra (COSY). A strong nuclear Overhauser effect (NOE) between the anomeric and aglycon protons at the disaccharide glycosidic linkage corresponding to an interatomic distance of 2.4 ± 0.2 Å was revealed by two-dimensional NOE spectroscopy (NOESY). This experimental result is consistent with the molecular mechanics calculations.

Introduction

The process of bacterial adherence to mucosal surfaces is generally regarded as the first step in the pathogenesis of urinary tract infection (UTI).[2] Pathogens (most often *Escherichia coli*) originating from the fecal flora can ascend the urinary tract via adherence and subsequent colonization of the vaginal and periurethral mucosa.[3] Two Swedish groups have extensively studied the mechanistic details of the adherence of P-fimbriated *E. coli* to urinary tract epithelial cells.[4] Hair-like organelles (fimbriae) extending from the bacterial surface specifically recognize and bind to glycolipid substructures on the uroepithelial cell surface.[5] The chemical structures recognized by these lectins all contain an α-D-Gal-(1–4)-β-D-Gal-OR disaccharide either at the nonreducing terminus or embedded in the oligosaccharide sequence.[6] Synthetic glycoside analogues of this disaccharide have also been shown to bind to these fimbriae.[7] Presently, the preferred conformation(s) of oligosaccharide cell-surface antigenic determinants is an active field of research.[8] Consequently, an understanding of bacterial adherence is rapidly evolving at the molecular level.

Table I. Conformational Energy Values of α-D-Gal-(1–4)-β-D-Gal-OMe Predicted by HSEA

confrmtn no.	ϕ, deg	ψ, deg	ΔE, kcal/mol	O3′–O5 distance, Å
1	−40	−10	0.000	3.264
2	−30	+10	0.081	2.736
3	−40	−20	0.178	3.531
4	−30	0	0.186	2.997
5	−30	+20	0.287	2.494
6	−30	−10	0.430	3.264
7	−40	0	0.512	2.997
8	−30	−20	0.866	3.530
9	−30	+30	1.075	2.284
10	−50	−20	1.207	3.530
11	−40	+10	1.283	2.736

Table II. HSEA Predicted Conformational Energy Values of α-D-Gal-(1–4)-β-D-Gal-OMe Refined by CHEM-X

confrmtn no.	ϕ, deg	ψ, deg	CHEM-X VDW repulsion E, kcal/mol	H1 to H4′ distance, Å
10	−50	−20	370.3	2.484
3	−40	−20	370.3	2.359
1	−40	−10	370.4	2.270
7	−40	0	371.4	2.203
11	−40	+10	373.1	2.162
8	−30	−20	376.1	2.246
6	−30	−10	376.4	2.163
4	−30	0	378.4	2.104
2	−30	+10	379.4	2.075
5	−30	+20	380.9	2.077
9	−30	+30	384.2	2.110

In connection with our studies of inhibitors of bacterial adherence in the urinary tract, the preferred three-dimensional shape of α-D-Gal-(1–4)-β-D-Gal-OR [methyl and ethyl galabioside (1 and 2)] was required to assist in the design of novel antiinfective agents. In this report the global minimum for 1 and 2 was derived by using the empirical molecular mechanics method, HSEA, and correlated to the preferred solution conformer(s) by using two-dimensional proton NMR spectroscopy.[7e,8]

Results and Discussion

HSEA Calculations. Oligosaccharides are fairly rigid structures due to the large energy difference between boat and chair forms in the individual carbohydrate residues. Consequently, their conformational mobility is limited to the glycosidic hinge, where it is reduced even further by the exo anomeric effect.[8a] Therefore, the conformation of a simple disaccharide such as 1 or 2 is adequately defined by the spatial relationship between the two pyranose rings. By convention,[8] this is expressed with the torsion angles

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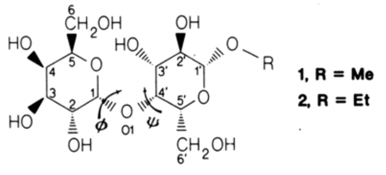

1, R = Me
2, R = Et

Figure 1. Structural formula and atomic numbering of α-D-Gal-(1–4)-β-D-Gal-OR.

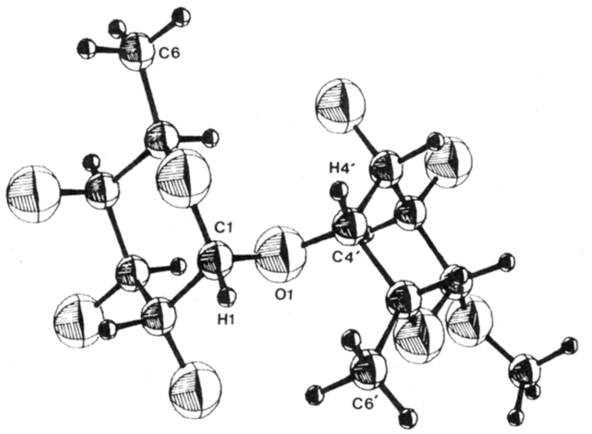

Figure 2. ORTEP plot of the global minimum energy conformation of symmetrically substituted (see Experimental Section) α-D-Gal-(1–4)-β-D-Gal-OMe. φ(H) = −50° and ψ(H) = −20°.

φ(H) = H1–C1–O1–C4′ and ψ(H) = C1–O1–C4′–H4′ (Figures 1 and 2).

In order to understand the conformational preferences of 1, we undertook a hard-sphere exo anomeric (HSEA) search around φ(H) and ψ(H) at a torsion angle resolution of 10°. The results of these calculations (Table I) revealed a distinct steric energy well containing the HSEA global minimum at φ(H) = −40° and ψ(H) = −10°. Consistent with the exo anomeric effect the molecule is quite rigid at φ(H) but relatively more flexible at ψ(H) as indicated by this narrow trough in the potential energy surface. This global energy basin is described by values in the range of φ(H) = −50° to −30° and ψ(H) = −20° to +30° (Table I). Since the HSEA method includes only nonbonded steric and glycosidic torsional (exo anomeric) potentials, the net van der Waals (VDW) repulsion energy was also calculated by using CHEM-X.[9] The latter method includes an electrostatic term,[10] which takes into account dipole–dipole interactions. Only the φ, ψ values defining the energy well as predicted by HSEA were included in these calculations. These additional results (Table II) predict that for φ(H) values of both −40° and −30°, the VDW repulsion energy steadily increases as ψ(H) values become more positive. On the basis of these data exclusively, the bottom of the energy well appears to be best defined by φ(H) values of −50° to −40° and ψ(H) values of −20° to −10°. The calculated global minimum energy conformer, φ(H) = −50° and ψ(H) = −20°, is shown as an ORTEP plot in Figure 2.

These calculated results differ from the solid-state conformation of 1, R = H,[11] in which φ(H) = −18° and ψ(H) = 35°. The stabilization of this relatively higher energy conformer in the solid state is accomplished by an intramolecular hydrogen bond between O3′-H and O5. The interatomic distance between these two oxygen atoms is 2.787 Å.[11] Only four of the points defining the low energy well as predicted by HSEA place these two oxygen atoms within hydrogen-bonding distance (2.6–3.0 Å) (Table I). Since neither the HSEA nor the CHEM-X calculations were parameterized for intramolecular hydrogen bonding, these four conformations could conceivably be stabilized by an additional 3–6 kcal/mol[12a] and on this basis would deepen the energy well. However, in an aqueous medium intramolecular hydrogen bonds should be minimized by the competing solvent.[12b] Consequently, this factor was discounted from our correlation between solution and computational results.

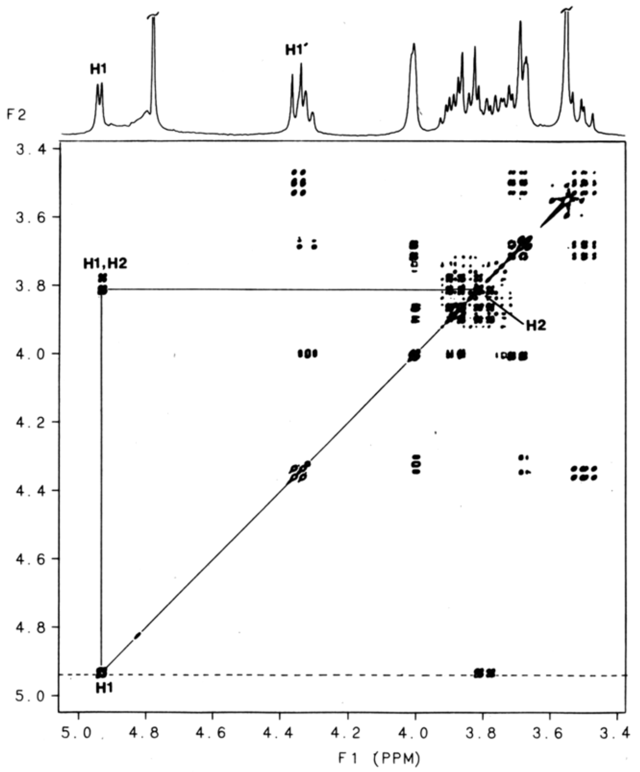

Figure 3. Contour plot of the two-dimensional homonuclear correlated (COSY) spectrum of 1. Cross-peaks for pairs of J-coupled protons are identified by joining their resonances by horizontal and vertical lines on either side of the diagonal, as illustrated for H1–H2. A cross-sectional spectrum through the dashed line is shown in Figure 4.

NMR Assignments and Conformation in Solution. Experimental support for the global minimum energy conformer was obtained by ^1H NMR measurements. The assignment of the one-dimensional ^1H NMR spectrum of 1 is complicated because of severe resonance overlap (as shown on top of Figure 3). Therefore, two-dimensional NMR pulse sequences were required to make complete proton and carbon assignments. We began by identifying the anomeric protons H1 and H1′ by their diagnostic downfield chemical shifts (4.2–5.0 ppm range). The β-anomer (H1′) was readily distinguished from the α-anomer (H1) by its coupling constant (trans diaxial scalar coupling, J = 7.9 Hz). With the H1′ and H1 assignments firmly established, the remaining protons were assigned from the two-dimensional homonuclear correlated spectrum (COSY), which establishes proton–proton connectivity through scalar or J coupling.[13,14] In the COSY contour plot (Figure

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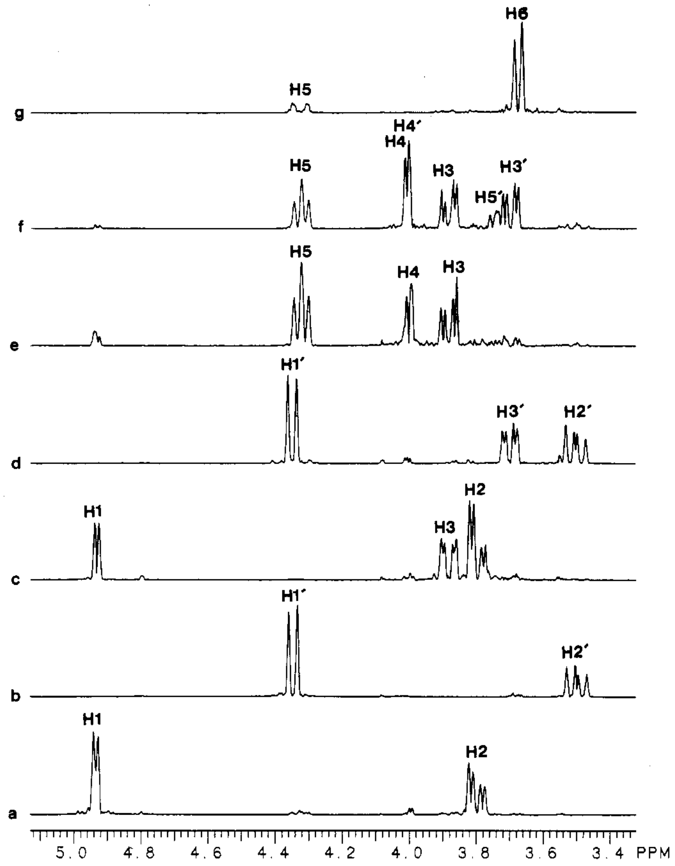

Figure 4. Subspectra a–g are cross sections of the COSY spectra of 1.

3), the diagonal peaks are equivalent to the one-dimensional proton spectrum. The off-diagonal or cross-peaks appear for pairs of protons (e.g., H1–H2), provided they have mutual scalar or through-bond coupling (e.g., J_{H1-H2}). This cross-peak is illustrated in Figure 3. The proton/proton scalar connectivity may not be readily apparent from the COSY contour plot; however, much more detail is available from cross sectional spectra (Figure 4). In Figure 4, spectrum a is a cross section through the dashed line shown in Figure 3. These cross sections (Figure 4, a–g) are a set of subspectra, which include coupled protons exclusively. Figure 4, spectrum a readily identifies H2 and its coupling constants. Similarly spectrum c identifies H3, while e and g reveal H4, H5, and H6. In this manner the assignments for the protons in the α-anomer were completed. The β-galactopyranoside protons (H2′, H3′, H4′, and H5′) are similarly assigned from the subspectra b, d, and f (Figure 4). The H6a′ and H6b′ protons had to be assigned from a heteronuclear proton/carbon chemical shift correlation experiment.[15] Complete tabulation of proton assignments and $J_{H,H}$ coupling constants for α-D-Gal-(1–4)-β-D-Gal-OMe is shown in Table III. The coupling constants were confirmed from both two-dimensional homonuclear J-resolved spectroscopy (HOM2DJ) together with the COSY subspectra shown in Figure 4. The ^{13}C assignments given in Table III were obtained from heteronuclear ^{1}H and ^{13}C chemical shift correlation (HETCOR) spectroscopy.[16]

Table III. ^{1}H and ^{13}C NMR and $J_{H,H}$ Assignments for α-D-Gal-(1–4)-β-D-Gal-OMe

^{1}H (ppm)	^{13}C (ppm)	assignmnt	$J_{H,H}$, (Hz)[b]
3.51	70.90	H2′	8.0, 10.5
3.54	57.12	CH_3	singlet
3.67	60.46	H6a, H6b	1.4, 6.0
3.69	72.37	H3′	3.3, 10.3
3.80	74.90	H2	3.6, 10.5
3.77	68.59	H5′	4.7, 11.2
3.82 3.86 [a]	60.05	H6′a, H6′b	7.5, 11.5
3.87	69.03	H3	3.3, 10.3
4.02	68.86	H4	3.5
4.00	77.27	H4′	unresolved
4.33	70.74	H5	6.1
4.36	103.81	H1′	7.9
4.95	100.22	H1	3.9

[a] Based on $^{1}H/^{13}C$ HETCOR. [b] Based on J-resolved spectra + COSY cross sections.

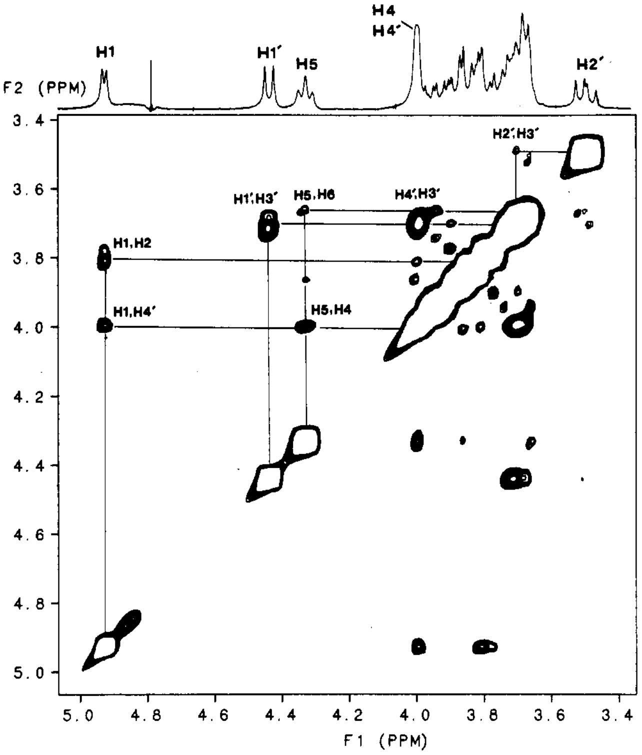

Figure 5. Contour plot of the two-dimensional NOESY spectrum of 2 in absorption mode. Mixing time of 600 ms was used. Cross-peaks for pairs of interacting protons are identified by joining their resonances by vertical and horizontal lines as illustrated. Resonances relevant to discussion are labeled.

After identification of all of the coupled protons for 1 and 2, a two-dimensional NOESY experiment was carried out that revealed a correlation between the solution and calculated low energy conformation(s) of 1 and 2. A contour plot of the NOESY spectra of α-D-Gal-(1–4)-β-D-Gal-OEt (2) is shown in Figure 5 with a mixing time of 600 ms. In this plot, the diagonal peaks represent spin magnetization that does not cross-relax during the mixing time. Their projection to either axis is equivalent to a one-dimensional spectrum as shown at the top of Figure 5. The cross-peaks that arise from cross-relaxation correlate protons that are coupled by a dipolar interaction and are in close spatial proximity (i.e. <3.5 Å apart).[17,18] The cross-peak intensities at 300 ms are reduced by a factor of ca. 2, as compared with those at 600 ms, implying that cross-peak intensities are linear in this range of mixing

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(15) Assignments for H6a′ and H6b′ were based on a routine $^{1}H/^{13}C$ heteronuclear chemical shift correlation spectrum.

(16) Routine homonuclear J-resolved and HETCOR spectra have not been included in this manuscript.

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times. Several cross-peaks arising from *intraresidue* proton–proton interactions labeled in Figure 5 have short internuclear distances (ca. 2.3–2.6 Å) as derived from the X-ray data.[19] A strong *interresidue* cross-peak observed between H1 of the α-anomer and H4′ of the β-anomer clearly indicates that these two protons must be in close spatial proximity in solution. The through-space distance between H1 and H4′ was quantitatively estimated from the NOESY experiment.[20] This distance was derived by relating its NOE to the corresponding NOE between H5 and H4,[21] which have a fixed (X-ray data) internuclear distance of 2.46 Å. The calculated H1–H4′ distance is 2.40 Å. The approximations involved in the equation[20] and uncertainties in the distance of the internal reference may introduce an error of <0.2 Å in the estimated distance.[22]

Correlation between NMR and HSEA Calculations. This NOE value for the H1 to H4′ distance is in best agreement with the computationally derived conformations 10 and 3 (Table II). These conformations ($\phi = -50°$ to $-40°$, $\psi = -20°$) were also predicted to be at the bottom of the potential energy well (Table II) on the basis of the molecular mechanics method HSEA as further refined by VDW (CHEM-X). Thus, the energy minimized model for **1** and **2** (Figure 2) is supported by proton NMR spectroscopy.

In conclusion, the empirical molecular mechanics method HSEA was used to predict the global low-energy conformer of methyl and ethyl galabioside (**1** and **2**). These results were further refined by calculating the net VDW repulsion energy, which includes an electrostatic potential energy term using VDW in CHEM-X. Two-dimensional proton NMR spectroscopy was used to make complete proton and carbon assignments for **1** and **2** in solution. Two-dimensional NOESY NMR spectroscopy revealed an internuclear distance of 2.4 ± 0.2 Å between H1 and H4′. The calculated and experimental distances between H1 and H4′ are in excellent agreement. On this basis our data suggest that the conformer shown in Figure 2 is the preferred low-energy three-dimensional shape of the methyl and ethyl galabiosides (**1** and **2**).

Experimental Section

Methyl galabioside (**1**) was prepared as described by Garegg and Hultberg.[7a] Ethyl galabioside (**2**) was purchased from Carbohydrates International AB (Sweden).

The HSEA molecular mechanics program (version 1) was obtained from the Canadian Depository of Unpublished Data[8c] and compiled on a VAX 11/780. We began with the X-ray coordinates for methyl α- and β-D-galactopyranoside as input geometries.[19] The crystallographic data was obtained from the Cambridge Structural Database[23] and conveniently converted to structure files by using the molecular modeling software CHEM-X.[9] Following a standard approximation,[8a,e,24] the hydroxyl groups at C6 and C6′ as well as the hydroxyl hydrogens were removed to provide only symmetrical ring substituents. Using the WRITE FOREIGN command in CHEM-X, the structure files were converted to HSEA readable format. Additionally, the Kitaygorodsy function was used and the glycosidic bond angle was set to 117°.[11,25] A conformational search was performed by scanning around ϕ and ψ from −180° to +180°. A single potential energy well was revealed in the range of $\phi = -60°$ to $+30°$ and $\psi = -30°$ to $+50°$. The bottom of the well is defined by the values in Table I. Using the READ FOREIGN command in CHEM-X, the HSEA coordinates for the global minimum energy conformer were read into CHEM-X, where all of the H1–H4′ and O3′–O5 distances as well as the VDW repulsion energies were calculated (Tables I and II).

All NMR experiments were carried out at room temperature on a Varian XL-300 spectrometer. Proton two-dimensional homonuclear chemical shift correlated (COSY) spectra of **1** were obtained in 99.98% D_2O by using the standard pulse sequence[13,14] $(90°-t_1-90°-t_2)_n$, where t_1 is the evolution time and t_2 is the detection time. The spectra were recorded with 1024 time-domain data points for a sweep width of 700 Hz and 256 t_1 increments. A 16-step phase cycling was used for a quadrature detection in the second dimension. For data processing, the FIDs were zero-filled to 2K × 2K and were apodized in both dimensions using a pseudo-echo weighting function. Both symmetrized and unsymmetrized data sets were examined. Spectra presented are in absolute value mode and are symmetrized.

Proton two-dimensional NOESY spectra were obtained for both **1** and **2** in 99.98% D_2O and in nonspinning samples. Spectra were recorded in the phase-sensitive mode[26,27] by using a $[90°-t_1-90°-\tau_{\text{mix}}-90°-t_2]_n$ pulse sequence.[17] NOESY data were collected with mixing times of 300 and 600 ms by using 1024 complex points with a sweep width of 700 Hz for **1** and 1400 Hz for **2**. The experiment used 128 t_1 values with 64 transients per t_1 increment with a recycle time of 2.5 s. Quadrature detection was used in both dimensions.[26] The FIDs were zero-filled to 1K × 1K and were apodized by a Gaussian function, in conjunction with a line broadening of 0.1 Hz in both dimensions. The absorption mode spectra were symmetrized although the unsymmetrized data were also examined. The strong peak from HDO was suppressed by using presaturation.

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$$r_{kl} \approx r_{ij}\left[\frac{N_{ij(t)}}{N_{kl(t)}}\right]^{1/6} \approx r_{ij}\left[\frac{a_{ij(t)}}{a_{kl(t)}}\right]^{1/6}$$

where r_{ij} is the known fixed distance, r_{kl} is the distance to be measured, N_{ij} and N_{kl} are the observed NOEs between protons i and j and between protons k and l, respectively. The NOEs are directly related to the corresponding cross-peak intensities (volume integrals) a_{ij} and a_{kl} obtained with a mixing time t in the NOESY spectra.

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