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PCILO CALCULATIONS ON 2-ACETAMIDO-2-DEOXY-α-D-GALACTOSE AND *O*-(2-ACETAMIDO-2-DEOXY-α-D-GALACTOSYL)-(1→3)-2-ACETAMIDO-2-DEOXY-D-GALACTOSE*

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ABSTRACT

PCILO semi-empirical quantum-chemical calculations have been conducted on 2-acetamido-2-deoxy-α-D-galactose (GalNAc) and the α-D-(1→3)-linked di-GalNAc disaccharide component of the Forssman antigen, in order to determine the favored orientations of their side groups, and the mutual orientations of the two pyranoid rings in the latter. For the *N*-acetyl group, three stable positions have been located, in one of which the plane of the amide unit is normal to the mean plane of the pyranoid ring. The orientation of the hydroxyl group attached to the anomeric carbon atom is found to be in accord with the exo-anomeric predictions. Furthermore, all of the side-group orientations of GalNAc found in the present study are in good agreement with the results of X-ray investigations. A grid-search method was used to locate all low-energy conformational regions for the glycosidic dihedral angles of *O*-(2-acetamido-2-deoxy-α-D-galactosyl)-(1→3)-2-acetamido-2-deoxy-D-galactose. Two allowed regions were found, and energy minimization was then performed in each of them. For this disaccharide, three sets of favored orientations of the (reducing) pyranose ring relative to the nonreducing ring were found. One set of glycosidic torsion angles is very close to that found from our X-ray diffraction results, n.m.r.-spectral observations, and hard-sphere exo-anomeric calculations.

INTRODUCTION

The experimental difficulties in the determination of the structure of big molecules limit our understanding about their conformational states. Therefore, conformational-energy calculations are becoming very effective in elucidating how interatomic interactions dictate the stable conformations of biopolymers and of their intermolecular complexes[1–4].

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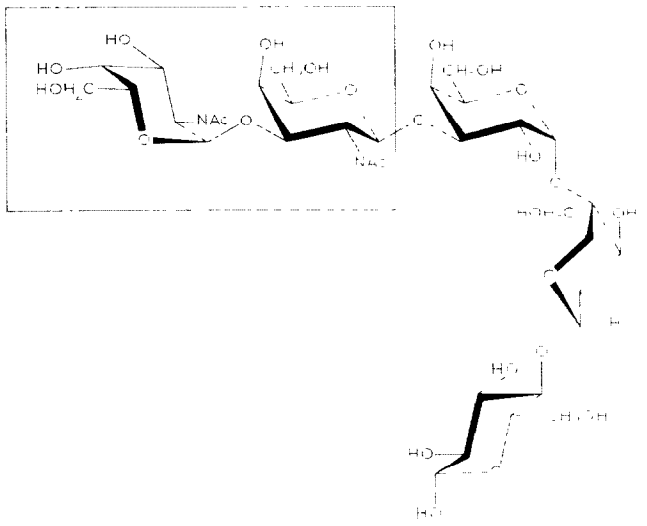


α-D-GalNAc-(1→3)-β-D-GalNAc-(1→3)-α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc

Fig. 1. Primary structure of the pentasaccharide of Forssman antigen

The total energy associated with the different atomic configurations of a system is directly evaluated in quantum-chemical computations. Therefore, their results are expected to be more reliable than those afforded by other theoretical methods. Nevertheless, such calculations on complete macromolecules are not possible at present. The information desired is, therefore, obtained on smaller units by mentally dividing the molecule into fragments. Stereochemical information on these subunits remains almost unchanged for the polymer chains, and helps in predicting their three-dimensional model. In the field of conformational analysis of oligosaccharides, extensive calculations have recently been conducted by empirical methods[5–11]; quantum-chemical studies have become available on the conformations of oligosaccharides[12], and some calculations on the conformations of carbohydrates, executed on the MNDO and PCILO levels have been made[13–16].

The main objective of the present series is to provide stereochemical information on the pentasaccharide component of the Forssman antigen, obtained by using quantum-chemical methods. The Forssman antigen[17] consists of a pentasaccharide chain (see Fig. 1) and a ceramide residue linked to cell membrane[18]. As a surface antigen, it regulates cell growth in mammals[19]. The pentasaccharide component, a total synthesis of which has been reported by Paulsen and Bunsch[20], is immunologically active, and its segments are also present in many glycosphingolipids.

Because, for the first quantum-chemical approach to the Forssman pentasaccharide, it seemed desirable to have comparable experimental data available, we first directed our attention to the terminal disaccharide (see Fig. 1), which also oc-

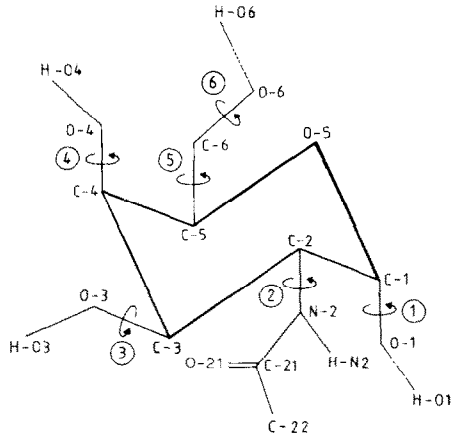


Fig 2. Numbering scheme for atoms and dihedral angles in 2-acetamido-2-deoxy-α-D-galactose (GalNAc). (Atoms are identified by sequence numbers. Dihedral angles are numbered on the curved arrows. They are defined in Table I.)

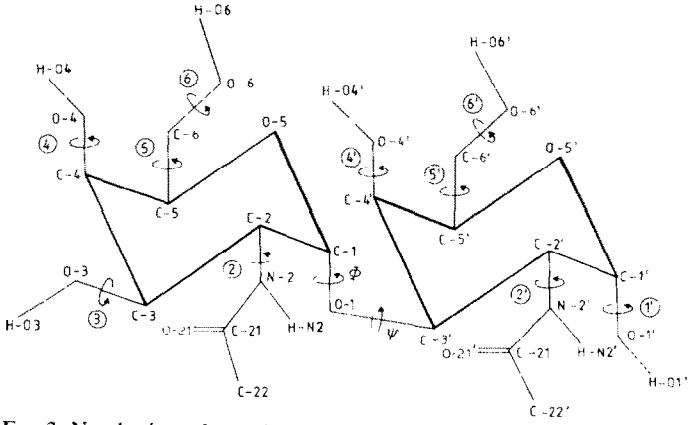


Fig. 3. Numbering scheme for atoms and dihedral angles in α-(1→3)-diGalNAc. (Dihedral angles are numbered on the curved arrows. They are defined in Table I.)

curs as a building unit of human blood-group determinants. Results of X-ray and n.m.r.-spectral investigations were already available[21] on the fully acetylated derivative, which was synthesized, and crystallized, by Paulsen and Bünsch[20].

In the present study, we concentrated on the application of PCILO (Perturbative Configurational Interaction of Localized Orbitals) to 2-acetamido-2-deoxy-α-D-galactose (GalNAc) and α-(1→3)-linked di-GalNAc in an effort to gain an understanding of the nature of the intramolecular interactions that stabilize their particular conformations, which are important as regards the three-dimensional structure of the pentasaccharide.

Nomenclature, conventions, geometry, and details of calculations. — The PCILO method[22,23] was selected for the present study because its successes in conformational-energy calculations have been very encouraging[2,15]. All of the calcula-

TABLE I

DEFINITIONS OF DIHEDRAL ANGLES OF GalNAc AND DI-GalNAc

Molecule	*Number for dihedral angle*	*Atoms defining dihedral angles*
GalNAc	1	H-O1-O-1-C-1-O-5
	2	C-2-N-2-C-2-C-1
	3	H-O3-O-3-C-3-C-4
	4	H-O4-O-4-C-4-C-5
	5	O-6-C-6-C-5-O-5
	6	H-O6-O-6-C-6-C-5
Di-GalNAc	2	C-2-N-2-C-2-C-1
	3	H-O3-O-3-C-3-C-4
	4	H-O4-O-4-C-4-C-5
	5	O-6-C-6-C-5-O-5
	6	H-O6-O-6-C-6-C-5
	1′	H-O1′-O-1′-C-1′-O-5′
	2	C-2′-N-2′-C-2′-C-1′
	4′	H-O4′-O-4′-C-4′-C-5′
	5′	O-6′-C-6′-C-5′-O-5′
	6′	H-O6′-O-6′-C-6′-C-5′
	ϕ	O-5′-C-1′-O-1′-C-3
	ψ	C-1′-O-1′-C-3′-C-3′-H

tions were conducted on a CDC CYBER 175 computer, using QCPE 272. In order to decrease the number of variables to be optimized, a dependency option[24] was introduced in the QCPE 272.

The polarity of all bonds was optimized during the overall calculations. The numbering scheme of atoms and dihedral angles is given in Figs. 2 and 3 for the mono- and di-saccharides, respectively. The definition of the dihedral angles is given in Table I. The atoms in residue 2 are numbered in the same way as for residue 1, except that primed numbers are used.

A positive rotation is defined as the rotation of a moving bond-vector in the clockwise sense when viewed from the fixed towards the moving bond. This definition is identical to the Klyne–Prelog[25] definition of signs of dihedral angles. Bond lengths, bond angles, and dihedral angles involving the atoms in the pyranoid ring, and the pendant oxygen atoms were taken from standard values reported by Arnott and Scott[26]. The geometry around the anomeric carbon atom was selected in accordance with the work of Jeffrey *et al.*[27]. A glycosidic bridge-angle of 116.5° was used. The pyranoid ring was held in the $^4C_1$(D) conformation reported by Arnott and Scott[26]. The bond lengths and bond angles for the amide group were those used by Momany *et al.*[28]. The O–H, N–H, and C–H distances were set equal to 0.95, 1.0, and 1.1 Å, respectively. The H–O–C and H–N–C valence-angles were made equal to 109.5° and 115°, respectively.

Strategy of selecting starting conformations. — During the whole calculation,

bond lengths and angles were kept invariant. The pyranoid rings and the amide groups were regarded as rigid bodies. With this proviso, the conformational space of GalNAc has six variables; these are the dihedral angles 1–6 listed in Table I. For the disaccharide, the number of conformational variables increases to 12 (see Table I; see also, Fig. 2), including the glycosidic dihedral angles Φ and ψ.

Supported by the PCILO program-operating mode, the overall strategy was to perform the calculation in two steps. First, a systematic *variation* of a number of selected variables was calculated, in order to obtain energy profiles, and second, using local minima of step 1 as input, simultaneous *optimization* of as many parameters as possible was considered.

A systematic and simultaneous variation of all of the variables requires extensive calculations; in practice, this is very difficult and expensive. Whenever it seemed justified from steric considerations, dihedral angles were varied separately, and, moreover, results of previous PCILO calculations[13] were utilized, if possible.

The geometry for GalNAc is very similar to that of 2-acetamido-2-deoxy-β-D-glucose (GlcNAc), except for the fact that, in the latter, the hydroxyl groups on C-1 and C-4 are attached equatorially. Therefore, some of the local conformational minima of the C-2, C-3, and C-5 side-groups of GlcNAc were considered to predict the low-energy conformers of GalNAc. These were (*a*) three local minima

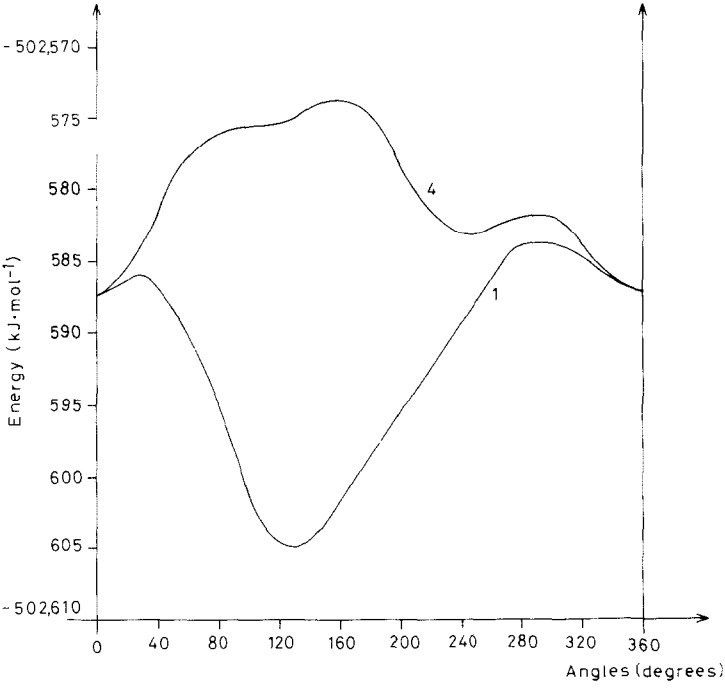


Fig. 4. The variation of potential energy as a function of dihedral angles 1 and 4, taken one at a time, for GalNAc. (In preparing the profiles, the dihedral angles 2, 3, 5, and 6 were held fixed in their positions obtained for the lowest-energy conformation[13] of β-D-GlcNAc.) The zero position in the scale of angles corresponds to $-60°$ and $-170°$ for dihedral angles 1 and 4, respectively.

TABLE II

SOME OF THE LOW-ENERGY MINIMA OF α-D-GalNAc

Conformer	*Dihedral angles (degrees)*						*Relative conformational energy (kJ mol^{-1})*
	1	*2*	*3*	*4*	*5*	*6*	
1	67	161	145	−173	60	40	0.0
2	66	161	145	−173	61	−177	5.9
3	67	161	145	−175	−177	179	6.3
4	68	161	−144	−177	69	177	6.7
5	68	161	144	−174	−69	177	7.1
6	67	161	−145	173	54	56	7.5
7	68	161	145	−175	−64	49	8.4
8	67	161	145	−173	178	73	10.0
9	68	161	−143	71	−163	−177	10.9
10	68	161	−145	175	−179	74	11.3
11	67	161	143	73	64	177	12.6
12	72	56	72	173	60	−40	13.8
13	67	161	−143	76	57	−57	14.2
14	71	−59	173	−175	−60	−40	15.0
15	72	56	70	175	−176	180	18.4
16	71	−56	71	−173	62	176	19.7
17	72	−56	70	−174	71	175	19
18	71	−59	172	−177	−176	180	20.3
19	63	−62	75	−174	−60	−40	21.3
20	72	59	173	−177	71	176	21.8
21	64	−63	171	−176	−60	40	21.8
22	63	61	74	−176	70	176	23.0
X-Ray[29]	73	82	165	20	−60	84	

for the amide group on C-2, (*b*) three for the OH group on C-3, and (*c*) nine for the CH_2OH group.

The interaction between the axial substituents on C-1 and C-4 was considered minimal. Therefore, the complete conformational-energy profiles (in 10° increments) for dihedral angles 1 and 4 were calculated independently, keeping the other side groups in their favored orientations, as in GlcNAc. The results are shown in Fig. 4.

In order to ascertain the mutual effects of the neighboring groups on each other's orientation, the simultaneous optimization of all side groups was considered. The variables 1–6 contributed with 1, 3, 3, 1, 3, and 3 local minima as starting values for the six dihedral angles of GalNAc, resulting in a total of 81 conformations to be optimized.

The most important aim of a study of oligosaccharide conformations is to determine the most favorable relative orientation of successive sugar rings in a chain. To locate the favored regions of glycosidic dihedral angles for α-(1→3)-diGalNAc, Φ–ψ maps using the low-energy conformers of GalNAc (see Table II) found in this study had to be considered. On inspecting a model of α-(1→3)-diGalNAc, and tak-

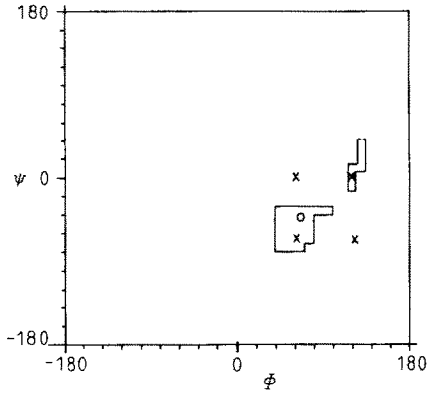


Fig. 5. Steric map for the glycosidic dihedral angles Φ and ψ of α-(1→3)-diGalNAc, using the PCILO method. [The side groups were kept fixed in the positions found in the lowest-energy conformation of GalNAc (conformer 1, Table II). The enclosed contour is within 20.9 kJ.mol^{-1} (5 kcal/mol) above the global minimum marked "○" in the Figure. The symbol x marks the Φ–ψ sets chosen for minimization.]

ing into account the steric hindrances, it was found that the side groups that come into close contact with each other are the *N*-acetyl groups of both residues and also the OH-4 group of the reducing residue. As may be seen from Table II, there are three favored sets of values for the *N*-acetyl group. Thus, nine possible orientations of these two bulky groups for α-(1→3)-diGalNAc should be considered in preparing the steric maps for Φ and ψ.

For quantum-chemical calculations of this type, the consideration of all of these conformations is a very expensive and difficult task. Therefore, the classical, potential functions were first applied, to ascertain the nine Φ–ψ steric maps for the nine orientations of the *N*-acetyl groups. The main result of these empirical calculations was that, in all cases, the lowest-energy regions were almost identical, and very close to those for the conformation obtained from the X-ray investigation. From this, it follows that the different orientations of the *N*-acetyl groups have only a minor influence on the glycosidic orientation. We then decided to take only the lowest-energy conformation (conformer 1; Table II) of GalNAc to calculate the PCILO Φ–ψ map. As shown in Fig. 5, two allowed regions within 20.9 kJ.mol^{-1} (5 kcal/mol) have been found.

For the PCILO optimization of the disaccharide structure, two sets of Φ–ψ values, namely (60, −60) and (120, 0), which fall in the allowed regions were considered as starting values. Additionally, we chose two more (Φ, ψ) pairs, namely (120, −60) and (60, 0), in the partially allowed regions. These two were chosen because each has one coordinate in the allowed range and the other outside it (see Fig. 5).

As starting conformations contributing from the monosaccharides, the three low-energy minima for the *N*-acetyl group (*i.e.*, three values for variables 2 and 2′) and the three minima of the OH-3 group were considered, whereas it was decided

that the CH_2OH groups be kept randomly in their orientations obtained at the monomer level. Therefore, 4 (Φ–ψ) and 27 combinations of side groups provided 108 conformations for simultaneous optimization of α-(1→3)-diGalNAc. The starting positions of the axial groups were taken as found in the lowest-energy conformation of GalNAc (see Table II, conformer 1).

RESULTS AND DISCUSSION

(A) 2-Acetamido-2-deoxy-α-D-galactose (GalNAc)

Of the 81 starting conformers of α-D-GalNAc (see Strategy section) only the 22 conformers having their energies within a range of 23 kJ.mol^{-1} (5.5 kcal/mol) were considered. Their torsion angles are summarized in Table II and the stereo plot of the lowest-energy conformation (conformer 1, Table II) is depicted in Fig. 6. From this stereo plot, it is clear that conformer 1 is stabilized by three hydrogen bonds of the types OH-3 · · · O-21, OH-4 · · · O-3, and OH-6 · · · O-4. The torsion angles of the different side-groups are next discussed.

(i) Orientation of the N-*acetyl group.* — As in the PCILO calculations[13] on GlcNAc, only one torsion angle, involving atoms C-1, C-2, N-2, and C-21 (see Fig. 2) is considered for positioning the *N*-acetyl group, because the amide bond was held fixed in the planar *trans* position, and no rotation around the C-21–N-2 bond was allowed. The hydrogen atoms of the CH_3 group were placed in staggered positions with respect to the C-21–N-2 bond. Three sets of values, namely, 160°, −60°, and 60° are found to stabilize the *N*-acetyl group. The conformation of lowest energy was found to occur at 160°. This conformation is stabilized by a hydrogen bond between the 3-hydroxyl group and the carbonyl group of the $NHCOCH_3$ group attached to C-2. The hydrogen-bond distance O-3 · · · O-21 is found to be 2.568Å, which is shorter in this molecule than in[13] GlcNAc. In one of the other two low-energy conformers (torsion angle 2, ~ −60°), the amide unit is oriented approximately normal to the mean plane of the pyranoid ring. In both conformations, the *N*-acetyl group has minimal contacts with its neighboring atoms in the ring, or with atoms directly attached to the ring.

A value of 60° for torsion angle 2 was not favored in[13] GlcNAc; instead, 120° was found to be favored, and this may be due to the fact that OH-1 is axial in GalNAc. Furthermore, in the crystal structure of GalNAc (Neumann *et al.*[20]), a value of 82° for this torsion angle was reported, and, in an X-ray investigation[24] on fully

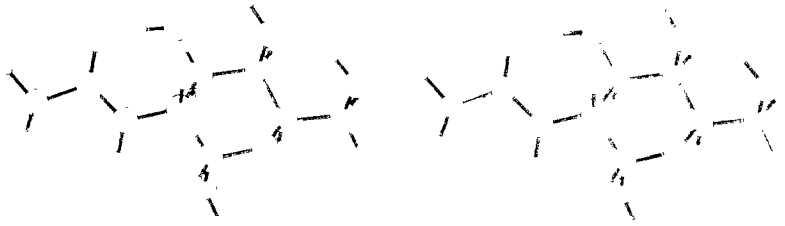


Fig. 6. The stereoplot of the lowest-energy conformation (conformer 1, Table II) of GalNAc

TABLE III

DIFFERENT POSSIBLE VALUES OF DIHEDRAL ANGLES FOR EACH GROUP IMPORTANT IN INTERACTIONS BETWEEN THE TWO RESIDUES OF DI-GalNAc[a]

Dihedral angle	*Value (degrees)*
2	−60, 60, 160
2′	−60, 60, 160
3′	145, 170, 70

[a]All 27 combinations of these dihedral angles were, together with the 4 sets of (Φ, ψ) values, taken as starting conformations.

acetylated α-(1→3)-diGalNAc, a similar value was found. This is surprising, because the coformers (see Table II) favoring the 60° value for dihedral angle 2 are 21 kJ.mol^{-1} (~5.0 kcal/mol) higher than the lowest conformer 1, wherein torsion angle 2 is stabilized around a value of 160°. However, it may be noted that in none of the crystal structures is the aforementioned OH-3 · · · O-21 hydrogen bond observed. The three values for the torsion angle of the *N*-acetyl do not change significantly after optimization at the di-GalNAc level (see Table IV).

(ii) Orientation of the CH_2OH group. — Nine distinct local-energy minima have been found for torsion angles 5 and 6 of the CH_2OH group, of which only the three values for torsion angle 5 need detailed discussion. The positions found, namely, −60°, +60°, and +180°, are customarily denoted *gauche-gauche* (*gg*), *gauche-trans* (*gt*), and *trans-gauche* (*tg*).

Close inspection of Table II suggested that all three orientations are almost equally probable. The lowest-energy conformer (No. 1 in Table II) is found to be *gauche-gauche,* allowing the hydrogen atom of OH-6 to form an intramolecular hydrogen-bond to the oxygen atom of the axial OH-4 group (see, also, Fig. 5). Interestingly, the *gg* orientation was also found in the X-ray structure[29] of GalNAc, whereas, in our crystal structure of acetylated di-GalNAc, this orientation was found in the first residue, but, in the second residue, the *trans-gauche* orientation was observed. In the X-ray structures of α- and β-D-galactose[30,31], the *tg* and *gt* orientations are present.

Originally, the *gg* orientation of the primary alcohol group was considered less likely for *galacto* derivatives. In Jeffrey's 1978 review article[32] on carbohydrate structures, it was concluded, from the experimental results so far obtained, that *gt* and *tg* orientations predominate. In the *galacto* case, the only *gg* examples cited were GalNAc and the D-galactopyranosyl group in planteose dihydrate[33]. In both, this unusual orientation is stabilized by an intramolecular hydrogen-bond between O-6 and O-4.

Our present PCILO results agree totally with the experimental findings. For the *gg* orientations, a hydrogen bond between O-4 and O-6 is also calculated and, as the results in Table II suggest, the *gt* and *tg* orientations are elsewhere equally probable. It should, however, be noted that, even in the absence of hydrogen

TABLE IV

REPRESENTATIVE MINIMA FOR α-D-(1→3)-DIGalNAc[a]

Conformer	Dihedral angles (degrees)												Relative conformational energy (kJ mol^{-1})
	2	3	4	5	6	2′	4′	5′	6′	1′	Φ	ϕ	
1	164	−147	−174	−58	−41	168	−173	−61	−40	67	63	−37	0.0
	(161	−145	−173	−60	−41	161	−173	−60	−41	69	60	0)	
2	47	168	177	57	41	172	157	60	39	67	79	50	2.1
	(60	−140	−173	−60	−41	160	−173	−60	−41	69	60	0)	
3	50	172	−177	−57	−41	59	156	59	−38	70	81	52	4.6
	(60	−145	−173	−60	−41	60	−173	−60	−41	69	60	0)	
4	164	−147	−175	62	−175	167	−173	60	177	66	63	−37	9.2
	(161	172	−173	60	−177	161	−173	60	−177	69	120	60)	
5	−70	169	−178	−57	−42	57	−166	−61	−38	71	84	−37	10.0
	(−60	−145	−173	−60	−41	60	−173	−60	−41	69	60	0)	
6	46	72	−175	63	−176	170	155	60	177	66	80	−48	10.5
	(161	70	−173	60	−177	161	−173	60	−177	69	120	−60)	
7	164	−146	−175	−57	42	−62	−178	−61	−40	64	55	−42	10.5
	(161	−145	−173	−60	−41	−80	−173	−60	−41	69	120	−60)	
8	164	−147	−175	62	−175	167	−173	60	−177	66	63	37	11.3
	(161	−145	−173	60	−177	161	−173	60	−177	69	120	−60)	
9	47	168	−177	63	−175	171	155	60	−178	66	80	−49	11.3
	(60	−145	−173	60	−177	161	−173	60	−177	69	90	60)	
10	48	168	−177	62	175	171	155	60	−177	65	80	−50	11.7
	(60	−145	−172	60	−177	161	−173	60	−177	69	60	0)	
11	49	70	−176	63	175	60	154	61	−177	69	81	51	12.6
	(60	70	−173	60	−177	60	−173	60	−177	69	120	−60)	
12	50	171	−178	63	−175	60	154	61	−177	69	81	−51	13.8
	(60	172	−173	60	−177	60	−173	60	−177	69	120	−60)	
13	50	171	−178	63	−175	60	154	61	−177	69	82	−51	13.8
	(60	−145	−173	60	−177	60	−173	60	177	69	60	0)	
14	164	−147	−174	−55	−43	74	55	−61	78	70	97	43	14.2
	(161	−145	−173	−60	−41	−80	70	−60	−41	69	60	0)	
15	164	−147	−173	−56	40	−76	−177	−61	−40	72	98	44	15.1
	(161	−145	70	−60	41	80	−173	−60	41	69	60	0)	
16	164	−147	−175	−57	−42	−66	−177	−61	−40	66	98	46	15.1
	(161	−145	−173	−60	−41	−60	−173	−60	41	69	60	0)	
17	164	−147	−176	63	−175	63	174	61	−176	70	83	−17	15.9
	(161	−145	−173	60	177	60	−173	60	−173	69	90	−60)	
18	−70	166	−179	−57	−42	134	169	−60	−37	68	111	3	17.2
	(−60	−145	−173	−60	−41	161	−173	−60	−41	69	60	0)	
19	−71	169	−179	59	−174	59	−171	61	−176	70	85	12	18.0
	(−60	−145	−173	60	177	60	−173	60	−177	69	90	−60)	
20	164	−147	−174	179	71	167	−171	180	73	67	63	−36	18.0
	(161	−145	−173	−170	60	161	−173	−170	60	69	60	0)	
21	−71	169	−179	59	−174	60	−171	61	−176	70	85	−12	18.4
	(−80	−145	−173	60	177	60	−173	60	−177	69	60	0)	
22	−75	168	−178	−57	−41	−59	174	−61	38	62	102	3	19.3
	(−60	−145	−173	−60	−41	−60	−173	−60	−41	69	60	0)	
23	−75	168	−178	−57	−41	−59	174	−61	−38	62	102	3	19.3
	(−60	−145	−173	−60	−41	−60	−173	−60	−41	69	60	0)	
24	−72	168	−179	62	−171	145	−149	60	−176	67	85	−26	21.8
	(−80	−145	−173	60	−177	161	−173	60	−177	69	60	0)	
25	−72	167	−179	62	−171	145	−169	61	−176	67	86	−27	21.8
	(−60	−145	−173	60	−177	161	−173	60	−177	69	90	−60)	

[a]The starting values are enclosed in parentheses

bonds, the *gg* orientation may also occur, as is shown by the X-ray results for the fully acetylated α-(1→3)-diGalNAc.

(iii) Orientation of the OH groups. — The orientation of the hydroxyl groups is found to be strongly influenced by the neighboring CH_2OH and $NHCOCH_3$ groups. As already discussed, in the lowest-energy conformation, the OH-3 group forms a hydrogen bond with the carbonyl group of the acetyl group on N-2. This hydrogen bond provides an extra amount of energy of stabilization, and is probably one of the principal reasons for the conformation to have the lowest energy. In addition to a value of −145°, two more values, namely, 170° and 70°, have been found for torsion angle 3. These values are in agreement with earlier observations[13] on GlcNAc. It is again interesting that, in the X-ray investigation by Neumann *et al.*[29], a value of 165° for torsion angle 3 was reported, which is very close to the value of 170° found in the present study.

As stated earlier (in the Strategy section), separate energy-profiles were prepared for locating the low-energy regions for torsion angles 1 and 4. These profiles are shown in Fig. 4, from which it is clear that the minimum lies at about −170° for dihedral angle 4, responsible for the orientation of the axial hydroxyl group attached to C-4. Another minimum that is ~4.2 kJ.mol^{-1} (~1.0 kcal/mol) less stable than the lowest one is at ~70°, which is 240° away from the first minimum. It may be mentioned that, in the crystal structure of GalNAc given by Neumann *et al.*[29], a value of ~20° was obtained. However, for the orientation of −170° the OH-4 group forms a hydrogen bond with O-3, as is clearly seen in Fig. 6.

A most interesting observation made in the present study concerns the orien-

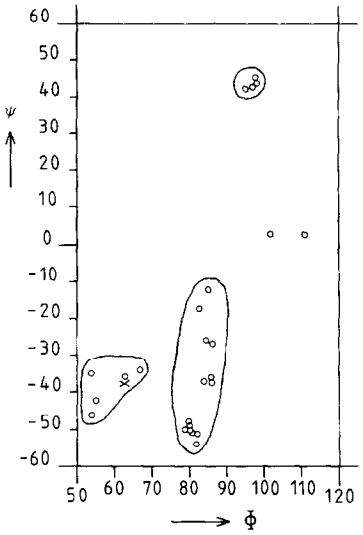


Fig 7. A distribution of low-energy conformers of α-(1→3)-diGalNAc obtained in the present study. [The symbol x stands for the lowest-energy conformation (conformer 1, Table IV), whereas ○ stands for the other low-energy conformers within 21.0 kJ.mol^{-1} above the global minimum obtained in this study.]

tation of the anomeric OH group. As is clear from Table II, a value of ~70° is always found for dihedral angle 1. According to the exo-anomeric effect[34] for α anomers, torsion angle 1 should have values in the range of 60 to 70°. As is clear from Fig. 4, the energy profile of torsion angle 1 has only one (deep) minimum, at ~70°. Also, all of the optimized conformers in Table II suggest only one value, at ~70°, for torsion angle 1. This finding in the present investigation is in excellent agreement with the value of 73° obtained in the X-ray study by Neumann *et al.*[29]. Also, in earlier studies[14] on GlcNAc, very good agreement was observed between quantum-chemical calculations and exo-anomeric predictions.

(B) O-(2-Acetamido-2-deoxy-α-D-galactosyl)-(1→3)-2-acetamido-2-deoxy-D-galactose [α-(1→3)-diGalNAc]

The optimization of 108 conformations (as discussed in the Strategy section) led to many low-energy conformers lying very close to each other, and it is difficult to include all of them in a Table. Therefore, only some of the representatives of these conformers, within ~21 kJ.mol^{-1} (~5 kcal/mol) above the global minimum found in this study, are listed in Table IV. Also, in order to provide a graphical view, a Φ *versus* ψ distribution plot for the low-lying-energy conformers is shown in Fig. 7. Table IV and Fig. 7 show that the different values for (Φ, ψ) obtained after optimization fall into three regions having their centers at approximately (60°, −40°) (80°, −30°), and (100°, 45°). The set (Φ = 80°, ψ = −30°) is in excellent agreement with the findings of a n.m.r.-spectral and X-ray investigation[23]. However, in the lowest-energy conformation (conformer 1, Table IV), the (Φ, ψ) values are found to be (63°, −37°). A comparison of Figs. 5 and 7 suggests that simultaneous optimization of all of the side-group dihedral-angles does not affect the favored values of Φ and ψ. Except for some minor differences, Figs. 5 and 7 provide the same orientational features for the glycosidic linkage.

In most cases, the side groups of each conformation of α-(1→3)-diGalNAc are in almost the same positions as found for the local minima of GalNAc. There are, however, certain final conformations in which some of the side groups do depart significantly from their initial positions. These are those that affect the orientation of the *N*-acetyl groups, and, thereby, the relative orientations of the pyranoid rings. Such changes are notable in conformers 3 and 4 in Table IV. The value of torsion angle 3 is, after optimization, ~170° from the initial value of −145° in conformer 3, whereas, in conformer 4, its initial value of 172° is changed to a final −145°. It may also be seen from Table IV that, in agreement with the finding for GalNAc, torsion angle 3 favors only three values, namely, −145°, 170°, and 70°, also at the diGalNAc level.

In general, when the *N*-acetyl group is kept at a value of either −60° or +60°, the OH-3 group is found to be oriented at ~170°, irrespective of whether the starting positions were at −145° or 170°. However, the initial value of 70° for this torsion angle remains almost the same after optimization, and this feature is found to be the same for all three orientations of the *N*-acetyl group. The conformers having torsion angles 2 and 2′ of ~ −60° and torsion angle 3 at 70° were found to be

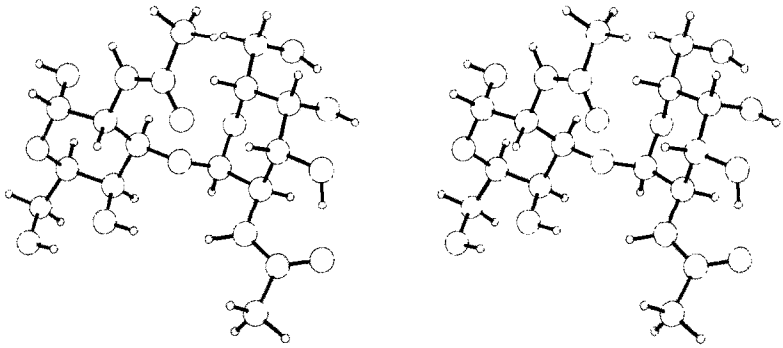
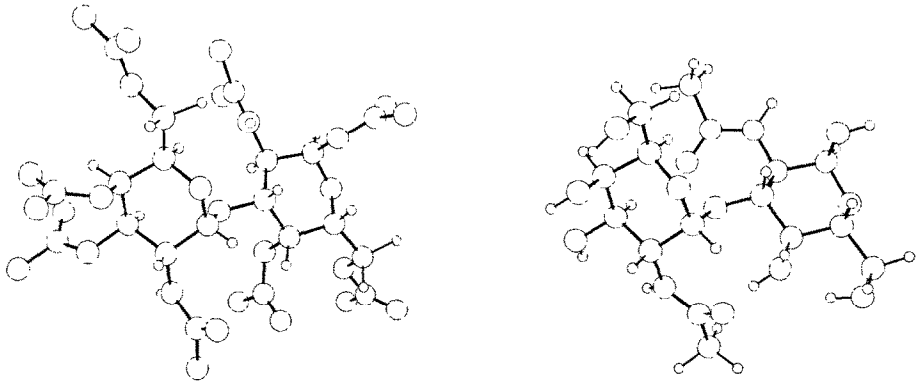


Fig. 8. The stereoplot of the lowest-energy conformation (conformer 1, Table IV) of α-(1→3)-diGalNAc.



A B

Fig. 9. A comparison of the crystal structure (A) and one of the low-energy conformations (B) (conformer 2, Table IV) of α-(1→3)-diGalNAc. [The crystal structure was determined[23] for acetylated α-(1→3)-diGalNAc.]

~33.5 kJ.mol^{-1} (~8 kcal/mol) less stable than the lowest conformer 1, and they are not listed in Table IV.

Similarly, the dihedral angle 4′ also favors a value of ~150° (as against its initial value of −173° in some of the final conformations). Interestingly, such a change for dihedral angle 4 is observed for all three positions of the *N*-acetyl group in residue 2. However, no such change has been noted for torsion angle 4, except in conformer 15 (see Table IV), wherein a value of −173°, as against its initial value of 70°, is favored.

The stereo plot of conformer 1 (see Table IV) is depicted in Fig. 8. As is clear from the stereo plot, there exists a hydrogen bond between the carbonyl group of $NHCOCH_3$ and the OH-3 group in residue 1. A similar hydrogen bond was also found in the conformation of GalNAc of lowest energy. Interestingly, this hydrogen bond is still present in the lowest-energy conformer of α-(1→3)-diGalNAc. Because the OH-3′ group is involved in the glycosidic linkage, no such hydrogen-bond

TABLE V

COMPARISON OF THE GLYCOSIDIC ANGLES OBTAINED BY DIFFERENT METHODS

Method	Φ (degrees)	ψ (degrees)
X-Ray	82	31
N.m.r. spectroscopy	80	32
PCILO set 1	60	−40
set 2	80	30
set 3	100	45
HSEA	73	−35

formation is possible in the second residue. Nevertheless, the *N*-acetyl group in the second residue is still retained at ~160°. On the other hand, in conformers 2 and 3, the *N*-acetyl group is stabilized at ~60° in the first residue, and therefore the aforementioned hydrogen bond does not exist, but the oxygen atom of the $NHCOCH_3$ group of the first residue is involved in hydrogen-bond formation with the OH-4 group of the second residue. It seems that, because of this inter-residue hydrogen-bond formation, conformers 2 and 3 are very close to conformer 1. Conformer 2, together with the molecule obtained from the crystal-structure determination, is depicted in Fig. 9. As is evident from this Figure, a close resemblance exists between the X-ray and PCILO results.

In our earlier quantum-chemical studies[15] on β-(1→4)-diGlcNAc, a conformer having a hydrogen bond between the carbonyl oxygen atom of $NHCOCH_3$ and the 3-hydroxyl group was also found to have the lowest energy. Furthermore, according to Scheraga and co-workers[35], in their extensive empirical conformational-energy calculations on enzyme–substrate complexes of lysozyme, such a type of hydrogen bonding is quite feasible, and would be even more likely to occur in the absence of neighboring hydrogen-bonding molecules.

Interestingly, the oxygen atom of the CH_2OH group is again *gauche-gauche* to both the C-5–O-5 and the C-5–C-4 bond in both residues in the lowest-energy conformation also at the disaccharide level; however, the *gauche-trans* orientation (conformer 4, Table IV) is also equally probable, but, in our X-ray investigation, the *gauche-gauche* position for the oxygen atom of CH_2OH is favored for the first residue, a *trans-gauche* for the second[23]. Again in connection with our earlier work, the PCILO studies[15] on the allowed conformations for β-(1→4)-diGlcNAc, a *trans-gauche* orientation was favored for both residues, and *gauche-trans* and *gauche-gauche* were relatively less favored.

It should be noted that the three stable positions of the *N*-acetyl group, namely, at 160°, −60°, and 60°, found in the monomer, remain almost unchanged after minimization at the diGalNAc level. However, in some of the conformers, a change of 10 to 20° is observed, particularly in the second residues. In the lowest-energy conformation (conformer 1, Table IV) both $NHCOCH_3$ groups favor values of ~160°. However, in conformer 2, which is only 2.1 kJ.mol^{-1} (0.5 kcal/

mol) less stable than the first, torsion angle 2 is ~50°, whereas torsion angle 2′ is again stabilized at ~160°. Dihedral angle 3, which is responsible for the orientation of the OH-3 group, is stabilized at about −145° in the lowest-energy conformer 1, as well as in most of the other conformers. In this orientation, it makes a hydrogen bond with the carbonyl group of $NHCOCH_3$ when the latter is held at 160°. Moreover, when dihedral angle 2 is initially set at about −60° or +60°, torsion angle 3 favors a value of ~170°, as against its initial value of −145°.

Furthermore, almost no change is noted in the initial value of 70° for dihedral angle 3 after optimization. On the other hand, when torsion angle 3 is 70°, torsion angle 2 is stabilized at ~50°, as against its initial value of 161° in conformer 6 (see Table IV).

As is clear from Table IV, irrespective of the starting values of 120 or 60° for Φ, the optimized conformations mostly favor values between 60 and 90°. Only in some exceptional cases do the values also reach to 100°. In order to ascertain whether or not the PCILO method really provides values for dihedral angles that are in agreement with the exo-anomeric effect, we tested a further set of conformations by assigning to Φ negative values, from −60 to −120°, as starting values for optimization, although, as is clear from the Φ and ψ map (see Fig. 5), negative values of Φ are not possible. Where the optimization of starting positions with negative Φ values was attempted, the resulting conformers were ~4–8 $MJ.mol^{-1}$ (~1000 to 2000 kcal/mol) higher than the lowest-energy conformer 1 (see Table IV) in this study.

A close inspection of Table IV suggests that, on each residue of α-(1→3)-di-GalNAC, the groups that are not potentially available for direct inter-residue interactions can be placed in any of the appropriate local GalNAc minima for either set of Φ and ψ values. Only their interactions within the monomer determine their positions.

Concluding remarks. — Such theoretical studies as the present one are actually valid for free molecules, because the interactions of the latter with their surroundings are not taken into account. Therefore, the reliability of the present PCILO results is open to discussion. There is little doubt that crystal-field effects or the effects of solvents have an influence on the overall conformational properties of crystalline materials, as compared to the compounds in aqueous solution or in the gas phase. However, in theoretical studies, it is generally presumed that the effect of solvent or crystal fields (or both) and other external factors may be expected to alter the relative importance of the conformational features, rather than to lead to entirely new minima. Comparison of the theoretical results with available experimental data makes possible an estimate of the extent to which the conformations observed corresponds to the intrinsically favored ones, and of the extent to which they are influenced by environmental forces. Moreover, theoretical conformational computations provide an amount of information that, from many points of view, is larger than that available experimentally, in the sense that they yield energy distributions instead of conformations favored in a given environment. It

must, however, be mentioned that computations of the present type are valid only for isolated molecules at rest; in real biology, these molecules are neither isolated nor at rest. However, it is known that a very disfavored conformation of a subunit of a large molecule cannot be stabilized only by solvent or other environmental effects. In fact, studies on isolated molecules provide a selection of possible conformers to be considered for construction of a larger molecular arrangement.

In the present PCILO study the crystalline as well as the solution conformation of α-(1→3)-diGalNAc, and the solid-state conformation of GalNAc have been reproduced (see Table V). Additionally, the present study provides more conformational possibilities (see Table IV) than are available experimentally.

One major aspect of the present PCILO computations is that the orientations around the anomeric carbon atom are found to be in excellent agreement with the considerations of the exo-anomeric effect. In that way, the results are also in agreement with the hard-sphere exo-anomeric (HSEA) calculations performed on α-(1→3)-di-GalNAc (see Table V). However, it should be noted that the hard-sphere approach needs an additional term for description of the exo-anomeric effect, whereas on the other hand in the quantum-chemical approach, such as the present one, no such exo-anomeric considerations are imposed externally. In distinction to the empirical approaches, the quantum-chemical computations aim at a direct evaluation of the total molecular energy associated with the different atomic configurations of the system, and thus, at a direct prediction of the favored molecular conformations. However, among such currently used semi-empirical methods as CNDO, INDO, MINDO/3, and MNDO, the PCILO method offers a rational, reliable, and less expensive approach for conformational studies.

From our experience on the application of the PCILO method to conformational studies, it therefore seems that the method is very good for orientational studies of the glycosidic linkage, at least for medium-sized oligosaccharides. Nevertheless, these studies are necessary, because the information acquired provides a possibility for selecting conformers for construction of a larger molecular arrangement.

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