

## EGC2

# MMC and LD simulations of $\alpha$ -D-Glcp-(1 $\rightarrow$ 2)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 3)- $\alpha$ -D-Glcp-OMe.

## A model for the terminal trisaccharide in glycoprotein precursors

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The conformational flexibility and the dynamics of  $\alpha$ -D-Glcp-(1  $\rightarrow$  2)- $\alpha$ -D-Glcp-(1  $\rightarrow$  3)- $\alpha$ -D-Glcp-OMe (**I**) has been investigated by Metropolis-Monte Carlo with the HSEA (Hard Sphere Exo-Anomeric) force field and Langevin dynamics simulations employing two different CHARMM (Chemistry at HARvard Molecular Mechanics) force fields, CHEAT95 and PARM22. The conformational space spanned by the molecule is similar for the two former force fields but differ significantly for the latter. Hydrogen bonding between O2'' and O4 of the title compound is analysed in comparison to NMR and preliminary results from X-ray powder diffraction studies.

**Keywords:** conformation; flexibility; Metropolis-Monte Carlo; Langevin dynamics; hydrogen bond

### Introduction

The spatial extension of oligosaccharides can be used for moderating interaction between molecules, resulting in different signalling and trafficking events. In the synthesis of glycoproteins the Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> oligosaccharide, transferred to an asparagine residue in the protein, contains a glucotriose unit which is removed after the transfer is complete [1]. The glycoprotein can subsequently be processed further into more complex structures.

The oligosaccharide  $\alpha$ -D-Glcp-(1  $\rightarrow$  2)- $\alpha$ -D-Glcp-(1  $\rightarrow$  3)- $\alpha$ -D-Glcp-(1  $\rightarrow$  3)- $\alpha$ -D-Manp-OPr (**II**), as a model for this terminal glucotriose unit, has been studied by proton NMR spectroscopy and HSEA calculations [2]. It was proposed that a hydrogen bond existed between O2'' of the terminal glucosyl and HO4 of the 3-substituted glucosyl residue. We have previously studied the terminal glucotriose as its methyl glycoside by NMR spectroscopy and energy minimizations employing the HSEA force field [3]. We report here further studies addressing the flexibility and hydrogen bonding of the title compound (**I**).

### Methods

The Metropolis-Monte Carlo simulation (MMC) was performed using the HSEA force field as implemented in the GEGOP 2.6 program [4]. The acceptance ratio for 500 000 macro steps was 62%. Langevin dynamics (LD) were performed using Quanta/CHARMM version 4.0 (Molecular Simulations Inc., San Diego, CA, USA) with PARM22 or CHEAT95<sup>5</sup> using collision frequencies of  $\gamma = 50 \text{ ps}^{-1}$  for carbon and oxygen atoms as previously described [6]. Atomic charges were taken from charge templates and the total charge adjusted to zero. One 10 ns run each was conducted with a time step of 1.0 fs, saving data every 0.1 ps for analysis. All simulations were started from structures obtained from the HSEA global energy minimum [3] after minimization with the appropriate force field. A temperature of 310 K and a constant dielectric of unity was used.

The HSEA force field uses rigid sugar residues, van der Waals interactions as well as a torsional potential for the  $\phi$  dihedral angle. The other two are full molecular mechanics (CHARMM) force fields, the difference between CHEAT95 and PARM22 being that the former uses extended atoms for the representation of hydroxyl groups which simulates the averaged effect of intra- and inter-molecular hydrogen bonding. Both MMC and LD can be used

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for conformational search but from the latter information about dynamics can also readily be obtained.

The glycosidic torsions are defined as follows:

$\phi_1$ : H1''-C1''-O2'-C2'

$\psi_1$ : C1''-O2'-C2'-H2'

$\phi_2$ : H1'-C1'-O3-C3

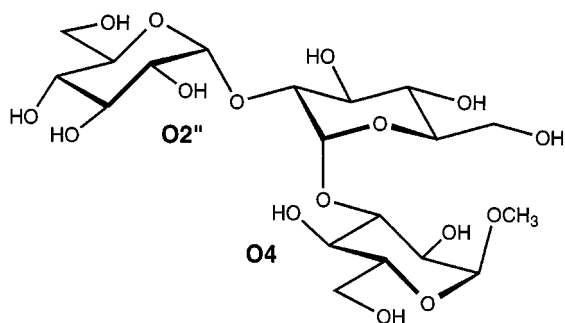
$\psi_2$ : C1'-O3-C3-H3

in which unprimed atoms belong to the 3-linked glucosyl residue, primed to the central 2-linked glucosyl residue and double primed to the terminal glucosyl residue.

## Results and Discussion

We have extended the previous studies to include MMC and LD simulations using three different force fields. The flexibility of glycosidic linkages can be visualized in scatter plots where highly populated conformational states show clustering. In the title molecule (**I**) both glycosidic linkages have the  $\alpha$  configuration and substitute equatorial positions of the aglycon sugar residues. The HSEA force field shows the lowest flexibility of the three force fields and the two linkages show a very similar conformational flexibility (Figure 1).

The dihedral angle averages during the MMC simulation (Table 1) are similar to the energy minimized structures in previous investigations [2,3]. The flexibility as measured by rms deviations is ca. 10–15°, with slightly larger flexibility for the  $\psi$  dihedral angles. The CHEAT95 force field shows a similar conformational space sampled, having rmsd values of the same magnitude. However, the dihedral angle averages differ by ca. 10–15°, a fact that will be of importance in the below discussion on hydrogen bonding possibilities. The last force field, PARM22, differs significantly in a number of aspects from the two above. The glycosidic dihedral angles show larger rmsd values. For the  $\phi$  dihedrals ca. 15° and for the  $\psi$  dihedrals ca. 30°. The  $\psi$  dihedral angles exhibit a significantly larger span ranging from ca. –80° to 80°. The most highly populated region for the (1 → 3)-linkage has been changed from a gauche(–) state to a gauche(+) state. A common feature for the two CHARMM force fields is the close resemblance of the most highly populated region for the (1 → 2)-linkage.

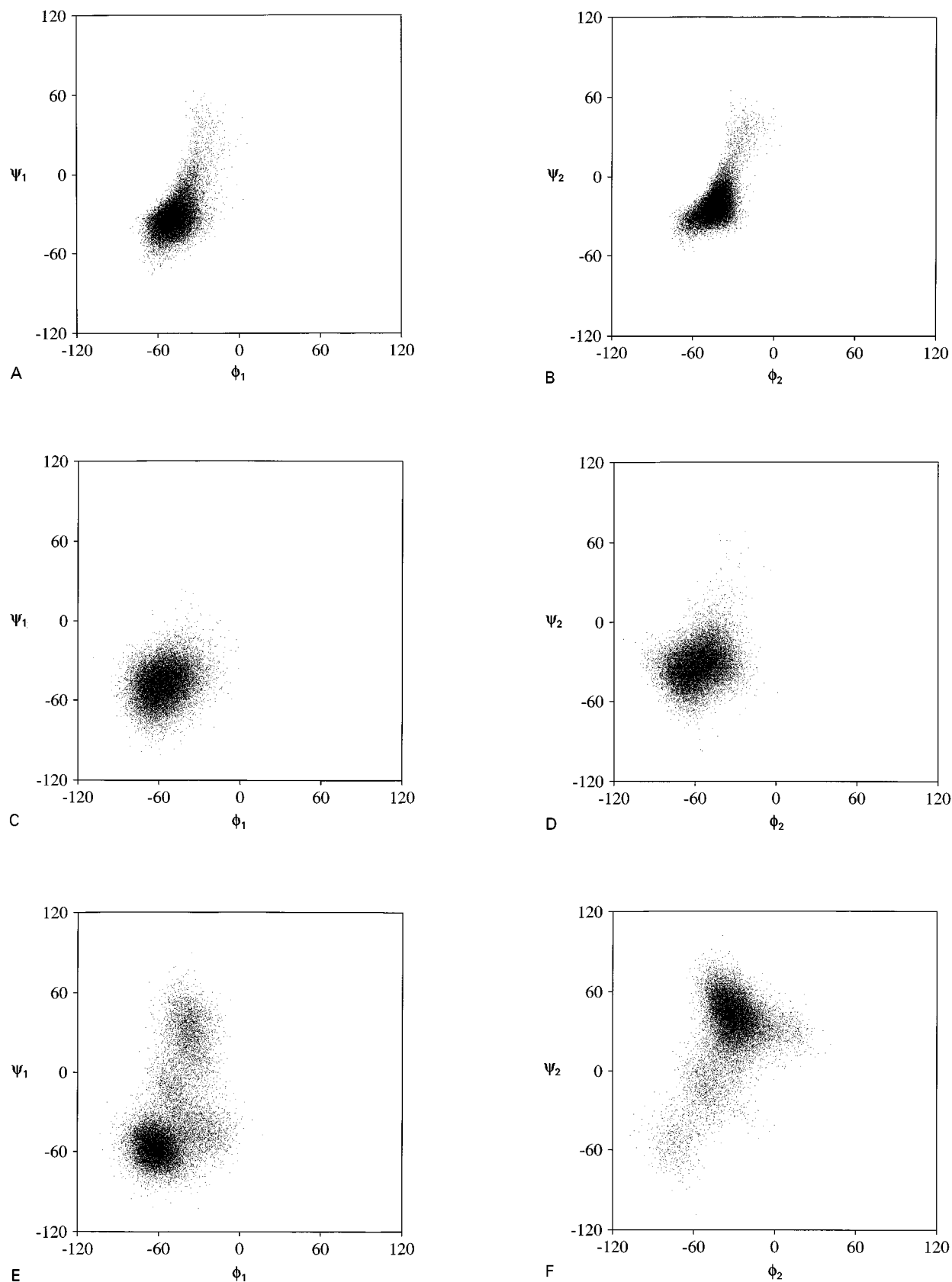


Schematic representation of Compound I

A recent structure determination based on X-ray powder diffraction of the title compound in the microcrystalline state identified two molecules in the unit cell (unpublished results). The geometries of the glycosidic dihedral angles are given in Table 1. These show overall similar values for the  $\phi$  dihedral angles in both the (1 → 2)- and (1 → 3)-linkages compared to the three force fields used in the present study. Large differences are observed for the  $\psi$  dihedral angle of the (1 → 2)-linkage whereas for the (1 → 3)-linkage the X-ray structures more resemble the geometry obtained by the PARM22 force field. In a NMR study of tetrasaccharide (**II**) in DMSO, Alvarado *et al.* [2] found a hydrogen bond between the hydroxyl group on O4 and O2'', based on the temperature dependence of the HO chemical shifts. In the present investigation the distance between O2'' and O4 at the global energy minimum employing the HSEA force field is 3.2 Å which is consistent with the possible presence of a hydrogen bond. On the other hand the global energy minimum in the CHEAT95 force field shows an O2''–O4 distance of 4.5 Å. Thus, the relatively small changes in dihedral angles going from HSEA to CHEAT95 lead to a conformation where a hydrogen bond is no longer possible (Figure 2).

Only occasionally is the O2''–O4 distance small enough during the dynamics simulation. In the simulation using PARM22 the average distance between O2'' and O4 is 3.1 Å (Table 2). As the force field includes explicit hydroxyl hydrogens (with partial charges) a geometrical analysis can readily be performed. From the distance and angle averages the geometry having the O4 atom as an acceptor instead of a donor seems more plausible. A closer inspection of the distribution of distances and angles for the O2''–HO2''–O4 and O2''–HO4–O4 geometries rules out the presence of the latter during the simulation. The distribution of HO2''–O4 distances shows a sharp peak at 1.9 Å whereas the reverse geometry, *ie* O2''–HO4, has a more diffuse maximum at 2.5 Å. Applying geometric criteria for hydrogen bonding, *ie* a maximum donor-acceptor distance of 3.4 Å and a minimum donor-hydrogen-acceptor angle of 135°, leads to a probability of 42% for the former geometry but only 6% for the latter arrangement. In the crystal structure one of the molecules shows the same geometrical preferences as above whereas the other one requires additional refinement before this question can be addressed. From the present PARM22 simulation and the preliminary crystal structure the preferred direction of hydrogen bonding is opposite to that previously proposed for (**II**) in solution. A similar difference in hydrogen bonding between crystal and solution structures has also been observed for maltose [7].

In conclusion, the conformational flexibility and dynamics of the title compound have been investigated by MMC and LD simulations employing three different force fields. The overall shape of the HSEA and CHEAT95 force fields are similar whereas the PARM22 force field showed a larger flexibility in the  $\psi$  dihedral angles and a change in the global



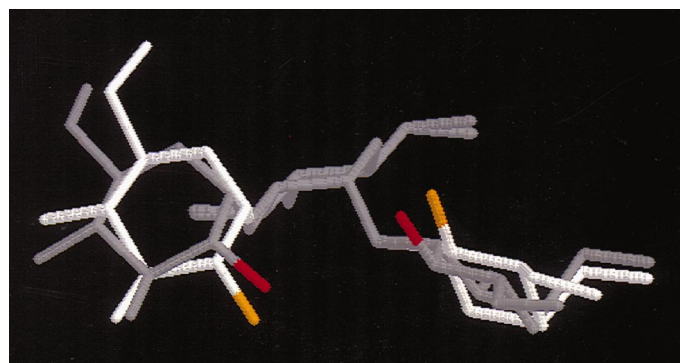
**Figure 1.** Scatter plots from MMC and LD simulation of title compound (**1**). Force fields and dihedral angles are shown as follows: (A) HSEA  $\phi_1$  vs  $\psi_1$ ; (B) HSEA  $\phi_2$  vs  $\psi_2$ ; (C) CHEAT95  $\phi_1$  vs  $\psi_1$ ; (D) CHEAT95  $\phi_2$  vs  $\psi_2$ ; (E) PARM22  $\phi_1$  vs  $\psi_1$ ; (F) PARM22  $\phi_2$  vs  $\psi_2$ .

**Table 1.** Glycosidic torsions.

Method	$\phi_1$ (H1''-C1''-O2'-C2')	$\psi_1$ (C1''-O2'-C2'-H2')	$\phi_2$ (H1'-C1'-O3-C3)	$\psi_2$ (C1'-O3-C3-H3)
HSEA	-47° (10°) <sup>a</sup>	-31° (14°)	-43° (9°)	-20° (13°)
CHEAT95	-56° (12°)	-47° (13°)	-57° (13°)	-32° (14°)
PARM22	-53° (16°)	-39° (34°)	-31° (16°)	34° (26°)
X-ray <sup>b</sup>	-71°	0°	-41°	20°
	-47°	-11°	-32°	6°

<sup>a</sup>RMS deviations in parenthesis.<sup>b</sup>Hydrogens in the crystal structure were placed using geometric criteria.**Table 2.** Hydrogen bonding.

Method	O2''-O4	O2''-HO2''-O4	HO2''-O4	O2''-HO4-O4	O2''-HO4
CHEAT95	4.6 Å	—	—	—	—
PARM22	3.1 Å	130°	2.5 Å	100°	2.9 Å
X-ray <sup>a</sup>	3.1 Å	180°	2.2 Å	140°	2.3 Å
	2.3 Å	130°	1.6 Å	100°	2.0 Å

<sup>a</sup>Calculations of H-bond geometry in the crystalline state are based on a H-O distance of 0.96 Å, a C-O-H bond angle of 108.9° and the C-O-O angles and O-O distances from the crystal structure.**Figure 2.** Overlay of global energy minimum HSEA and CHEAT95 structures. The atoms O2'' (to the left) and O4 (right) are colored red and orange in the HSEA and CHEAT95 structures, respectively.

energy minimum for the  $\psi$  dihedral angle of the (1 → 3)-linkage. Unfortunately, experimental evidence from, for example, NMR spectroscopy would not differentiate these three force fields easily. The molecular mechanics simulations were also compared with structures generated from X-ray powder diffraction data where differences were largest for the  $\psi$  dihedral angles. Finally, hydrogen bond geometries were investigated in which the direction of an inter-

residue hydrogen bond is more probable in the opposite direction to that previously reported. We hope to give a more definite answer to this problem through further work on the refinement of the powder X-ray structure.

### Acknowledgement

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