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# Crystal and molecular structure of a histo-blood group antigen involved in cell adhesion: the Lewis x trisaccharide

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**This work describes the first crystal structure ever reported of a histo-blood group carbohydrate antigen: $Le^x$. This study provides a detailed description of the conformation of two crystallographic independent molecules in a highly hydrated environment along with their hydrogen bonding properties and packing features. Some interactions observed between adjacent trisaccharides can provide the basis for involvement of $Le^x$–$Le^x$ interactions in cell–cell adhesion.**

*Key words:* histo-blood group/cell adhesion/carbohydrate-carbohydrate interactions/Lewis x/trisaccharide

## Introduction

Histo-blood group carbohydrate-dependent antigens are borne by glycolipids and glycoproteins which are expressed on human erythrocytes and also in the epithelia of glandular tissues, primary sensory neurons, and exocrine secretions in man and other mammals (Oriol, 1995; Henry and Oriol, 1995). Two antigens families, the ABH(O) and the Lewis determinants, constitute the major histo-blood group carbohydrate determinant (Clausen and Hakomori, 1989). They exhibit a pattern of appearance and disappearance on defined cells in particular tissues, representing developmental and specific functions.

The Lewis x ($Le^x$) determinant (Figure 1) is a trisaccharide [Galβ1–4[Fucα1–3]GlcNAcβ-1 (Hakomori *et al.*, 1981). In mammals, this oligosaccharide is a stage-specific embryonic antigen (SSEA) (Solter and Knowles, 1978), implicated in the compaction of the morula (Bird and Kimber, 1984; Fenderson *et al.*, 1984) and is also a tumor-associated marker (Feizi, 1985; Hakomori, 1989). This trisaccharide has been recently found on the cell surface of *Helicobacter pilori*, a human pathogenic bacteria associated with gastric ulcer and cancer (Aspinall *et al.*, 1994). It is also present at the surface of the infecting phase of *Schistosoma mansoni* (Vellupillai and Harn, 1994), having a signaling role in the interaction between this parasitic worm and its human host (Van Dam *et al.*, 1994). The sialyl $Le^x$ tetrasaccharide and its sulfated analog have also been recognized as important ligands in cellular adhesion, playing a role in the inflammatory response through their interaction with selectins (Lasky, 1992).

Conformational studies of histo-blood group carbohydrates first appeared in the beginning of the 1980s (Biswas and Rao, 1980; Lemieux *et al.*, 1980). Important achievement in the chemistry of synthetic oligosaccharides, along with the availability of conformational analysis methods coupled to high resolution NMR characterizations, set the foundation for such studies (see Pérez *et al.*, 1994, for review). They have provided a general description of the molecular conformations in solution, but no direct information concerning their hydrogen bonding properties and packing features. At the moment, no such biologically active oligosaccharides have yet been crystallized.

## Results

### *Description of the crystal*

Starting from chemically synthesized $Le^x$ trisaccharide methyl glycoside, single crystals could be grown. They were crystallized by slow evaporation of $Le^x$ dissolved in a water/ethanol mixture. Over a period of 2 years, more than 20 crystals having dimensions suitable for x-ray investigations have been obtained. One single crystal having dimensions of 0.5 × 0.25 × 0.05 mm was mounted on a glass pin with its long dimension along the pin axis. $Le^x$ crystallizes in the monoclinic space group $P2_1$ with unit cell parameters: $a$ = 12.147(6), $b$ = 27.552(9), $c$ = 8.662(6) A, β = 91.71°(5). In such a unit cell, the asymmetric unit contains two independent molecules, and an unusually high number of water molecules. All hydrogen atoms of the O-H groups and of the water molecules could be located in this crystal structure, allowing a straightforward assignment of hydrogen bonds. The coordinates of all atoms are listed in Table 1 and Table 2

### *The trisaccharide conformation*

The six monosaccharide units in the two $Le^x$ molecules adopt the typical $^4C_1$ chair conformation, with no significant deviation away from classical pyranose ring shape. The N-acetyl group adopts a *trans* conformation, whereas the primary hydroxyl groups of the Gal and GlcNAc are *trans-gauche* and *gauche-trans*, respectively. The two $Le^x$ molecules differ in their overall conformations (Figure 2). These differences are essentially located at the glycosidic torsion angles at the Galβ(1–4)GlcNAc linkage for which angle Φ1 differs by 10° (Table 3). Neither of the two trisaccharides exhibits any intramolecular hydrogen bonds. A strong interaction exists between the fucose and galactose ring, but only nonpolar van der Waals contacts are involved, each ring presenting its most hydrophobic face to the other one. Conformational studies using NMR and/or molecular modeling (Thogersen *et al.*, 1982; Ichikawa *et al.*, 1992; Miller *et al.*, 1992; Imberty *et al.*, 1995) generally

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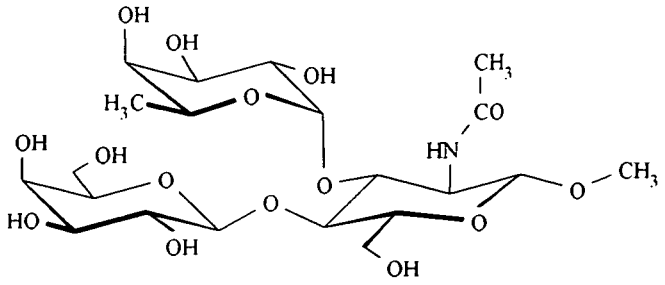

**Fig. 1.** Schematic representation of $Le^x$ methyl glycoside: methyl 2-acetamido-4-O-(β-D-galactopyranosyl)-3-O-(α-L-fucopyranosyl) -2)deoxy-β-D-glucopyranoside.

agree on a single conformation for Lewis x in solution, corresponding closely to the one reported here.

*Hydration in the crystal*

The nine water molecules present in the asymmetric unit are arranged in a cluster-like fashion. They establish hydrogen bonds to other water molecules within the cluster and to the surrounding carbohydrate molecule. They are arranged to fill an empty space in the crystal packing. Six and seven water molecules, respectively, are involved in the hydration of the two trisaccharides (Figure 3). When comparing the two trisaccharides, the conserved hydration sites are the one accepting the amide hydrogen from the N-acetyl group and the one giving hydrogen to the oxygen O2fuc. In both trisaccharides, a hydrogen bond chain involving two water molecules links the oxygen atoms O6gln and O2gal but with different geometries.

*Hydrogen bonds network*

The two independent trisaccharides of the asymmetric unit are linked by four hydrogen bonds, two of them being part of a three-centered bond (O6gln→O7gln′, O3fuc′→O7gln, O2fuc′→O1gln, O2fuc′→O5gln). Almost the same interaction is produced by one unit translation along the *a* axis. In fact, a pseudo-symmetry element exists between the two independent molecules, therefore creating this apparent high level of symmetry in the *ab* plane (Figure 4a).

**Table I.** Fractional atomic coordinates (with estimated standard deviations in parentheses) for $Le^x$

| Atom | x | y | z | Atom | x | y | z |
|---|---|---|---|---|---|---|---|
| C1gln | −0.1944(7) | 0.0515(3) | 1.049(1) | C1gln′ | 0.3053(7) | 0.1167(3) | 0.7283(9) |
| C2gln | −0.3186(7) | 0.0553(3) | 1.0447(9) | C2gln′ | 0.1807(6) | 0.1121(3) | 0.720(1) |
| C3gln | −0.3698(6) | 0.0047(3) | 1.0468(9) | G3gln′ | 0.1269(6) | 0.1620(3) | 0.7167(9) |
| C4gln | −0.3292(6) | −0.0238(3) | 0.9068(9) | C4gln′ | 0.1704(7) | 0.1912(3) | 0.854(1) |
| C5gln | −0.2031(6) | −0.0232(3) | 0.909(1) | C5gln′ | 0.2952(6) | 0.1935(3) | 0.8498(9) |
| C6gln | −0..1575(7) | −0.0466(3) | 0.767(1) | C6gln′ | 0.3464(6) | 0.2205(3) | 0.985(1) |
| C7gln | −0.4069(7) | 0.1265(3) | 1.153(1) | C7gln′ | 0.0985(7) | 0.0408(3) | 0.602(1) |
| C8gln | −0.4552(8) | 0.1490(4) | 1.294(1) | C8gln′ | 0.0645(9) | 0.0150(4) | 0.455(1) |
| C9gln | −0.0360(7) | 0.1001(4) | 1.084(1) | C9gln′ | 0.4513(8) | 0.0614(4) | 0.682(1) |
| O1gln | −0.1496(5) | 0.0971(2) | 1.0383(7) | O1gln′ | 0.3491(5) | 0.0705(2) | 0.7521(8) |
| O5gln | −0.1624(4) | 0.0250(2) | 0.9153(6) | O5gln′ | 0.3396(4) | 0.1453(2) | 0.8572(7) |
| O6gln | −0.0411(4) | −0.0531(2) | 0.7813(6) | O6gln′ | 0.4622(4) | 0.2280(2) | 0.9706(7) |
| O7gln | −0.4211(5) | 0.1466(2) | 1.0236(7) | O7gln′ | 0.0785(5) | 0.0226(2) | 0.7317(7) |
| N2gln | −0.3572(5) | 0.0844(2) | 1.1729(8) | N2gln′ | 0.1471(5) | 0.0830(2) | 0.5874(7) |
| C1gal | −0.4103(6) | −0.0942(3) | 0.7807(9) | C1gal′ | 0.0835(7) | 0.2614(3) | 0.973(1) |
| C2gal | −0.4054(6) | −0.1481(3) | 0.8060(9) | C2gal′ | 0.0660(6) | 0.3139(3) | 0.9314(9) |
| C3gal | −0.4694(7) | −0.1757(3) | 0.684(1) | C3gal′ | −0.0025(7) | 0.3397(3) | 1.048(1) |
| C4gal | −0.5855(7) | −0.1555(3) | 0.668(1) | C4gal | −0.1046(7) | 0.3116(3) | 1.081(1) |
| C5gal | −0.5796(7) | −0.1015(3) | 0.638(1) | C5gal′ | −0.0774(7) | 0.2595(3) | 1.126(1) |
| C6gal | −0.6922(8) | −0.0761(3) | 0.630(1) | C6gal′ | −0.1779(8) | 0.2261(3) | 1.139(1) |
| O1gal | −0.3667(4) | −0.0724(2) | 0.9180(6) | O1gal′ | 0.1276(4) | 0.2398(2) | 0.8397(6) |
| O2gal | −0.2923(5) | −0.1632(2) | 0.8054(7) | O2gal′ | 0.1708(5) | 0.3378(2) | 0.9255(7) |
| O3gal | −0.4695(5) | −0.22622) | 0.7135(7) | O3gal′ | −0.0304(5) | 0.3866(2) | 0.9886(8) |
| O4gal | −0.6422(5) | −0.1630(2) | 0.8074(7) | O4gal′ | −0.1783(5) | 0.3105(2) | 0.9499(7) |
| O5gal | −0.5214(4) | −0.0769(2) | 0.7611(6) | O5gal′ | −0.0161(4) | 0.2365(2) | 1.0002(7) |
| O6gal | −0.7638(5) | −0.1000(2) | 0.5189(7) | O6gal′ | −0.2577(5) | 0.2488(2) | 1.2335(7) |
| C1fuc | −0.5411(7) | 0.0011(3) | 1.1795(9) | C1fuc′ | −0.0502(7) | 0.1606(3) | 0.581(1) |
| C2fuc | −0.6591(7) | 0.0175(3) | 1.152(1) | C2fuc′ | −0.1616(7) | 0.1367(3) | 0.600(1) |
| C3fuc | −0.7184(7) | −0.0152(3) | 1.036(1) | C3fuc′ | −0.2288(7) | 0.1638(3) | 0.715(1) |
| C4fuc | −0.7073(7) | −0.0676(3) | 1.085(1) | C4guc′ | −0.2361(7) | 0.2176(3) | 0.670(1) |
| C5fuc | −0.5909(7) | −0.0815(3) | 1.1225(9) | C5fuc′ | −0.1230(7) | 0.2386(3) | 0.645(1) |
| C6fuc | −0.5767(8) | −0.1321(3) | 1.191(1) | C6fuc′ | −0.124(1) | 0.2888(3) | 0.581(1) |
| O1fuc | −0.4872(4) | 0.0077(2) | 1.0384(6) | O1fuc′ | 0.0096(4) | 0.1557(2) | 0.7247(6) |
| O2fuc | −0.6631(5) | 0.0679(2) | 1.1116(7) | O2fuc′ | −0.1503(5) | 0.0861(2) | 0.6308(7) |
| O3fuc | −0.8321(5) | −0.0022(3) | 1.0247(8) | O3fuc′ | −0.3346(5) | 0.1429(3) | 0.7220(7) |
| O4fuc | −0.7759(5) | −0.0772(3) | 1.2144(7) | O4fuc′ | −0.3025(5) | 0.2216(2) | 0.5298(7) |
| O5fuc | −0.5383(5) | −0.0476(2) | 1.2302(6) | O5fuc′ | −0.0623(5) | 0.2090(2) | 0.5365(7) |
| Owat1 | −0.1013(6) | 0.3838(3) | 0.4516(8) | | | | |
| Owat2 | 0.2043(7) | 0.1265(3) | 1.3006(9) | | | | |
| Owat3 | −0.3239(7) | 0.0381(3) | 0.4668(8) | | | | |
| Owat4 | −0.3227 | −0.0632 | 1.4045 | | | | |
| Owat5 | −0.105(1) | −0.0773(3) | 1.283(1) | | | | |
| Owat6 | 0.154(1) | 0.3094(3) | 0.432(1) | | | | |
| Owat7 | 0.2978(7) | 0.3230(4) | 0.671(1) | | | | |
| Owat8 | 0.2810(9) | 0.2201(3) | 0.396(1) | | | | |
| Owat9 | 0.4967(7) | 0.2546(4) | 0.655(1) | | | | |



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**Table II.** Hydrogen atom fractional atomic coordinates for $Le^x$. Estimated standard deviations on x/a, y/b and z/c amount to 0.009, 0.004 and 0.009, respectively.

| Atom | x | y | z | Atom | x | y | z |
|---|---|---|---|---|---|---|---|
| H1gln | −0.160 | 0.037 | 1.145 | H1gln′ | 0.339 | 0.131 | 0.633 |
| H2gln | −0.344 | 0.078 | 0.950 | H2gln′ | 0.154 | 0.093 | 0.812 |
| H3gln | −0.353 | −0.013 | 1.144 | H3gln′ | 0.140 | 0.180 | 0.620 |
| H4gln | −0.359 | −0.010 | 0.806 | H4gln′ | 0.142 | 0.177 | 0.954 |
| H5gln | −0.175 | −0.039 | 1.006 | H5gln′ | 0.315 | 0.210 | 0.751 |
| H61gln | −0.175 | −0.026 | 0.673 | H61gln′ | 0.341 | 0.198 | 1.079 |
| H62gln | −0.192 | −0.079 | 0.750 | H62gln′ | 0.317 | 0.261 | 0.971 |
| H81gln | −0.491 | 0.181 | 1.269 | H81gln′ | 0.026 | −0.017 | 0.478 |
| H82gln | −0.512 | 0.127 | 1.339 | H82gln′ | 0.130 | 0.007 | 0.391 |
| H83gln | −0.396 | 0.155 | 1.377 | H83gln′ | 0.011 | 0.035 | 0.390 |
| H91gln | −0.011 | 0.135 | 1.073 | H91gln′ | 0.478 | 0.028 | 0.702 |
| H92gln | −0.025 | 0.090 | 1.194 | H92gln′ | 0.510 | 0.085 | 0.721 |
| H93gln | 0.009 | 0.079 | 1.017 | H93gln′ | 0.445 | 0.066 | 0.565 |
| HO6gln | 0.001 | −0.023 | 0.780 | HO6gln′ | 0.506 | 0.198 | 0.975 |
| *HN2gln* | −0.348 | 0.066 | 1.283 | *HN2gln′* | 0.167 | 0.095 | 0.488 |
| H1gal | −0.359 | −0.083 | 0.688 | H1gal′ | 0.131 | 0.256 | 1.069 |
| H2gal | −0.437 | −0.158 | 0.907 | H2gal′ | 0.029 | 0.316 | 0.826 |
| H3gal | −0.430 | −0.172 | 0.586 | H3gal′ | 0.043 | 0.343 | 1.145 |
| H4gal | −0.631 | −0.171 | 0.568 | H4gal′ | −0.147 | 0.330 | 1.162 |
| H5gal | −0.541 | −0.098 | 0.538 | H5gal′ | −0.033 | 0.261 | 1.226 |
| H61gal | −0.682 | −0.041 | 0.598 | H61gal′ | −0.156 | 0.193 | 1.179 |
| H62gal | −0.726 | −0.076 | 0.734 | H62gal′ | −0.213 | 0.221 | 1.030 |
| HO2gal | −0.250 | −0.156 | 0.901 | HO2gal′ | 0.164 | 0.369 | 0.871 |
| HO3gal | −0.489 | −0.236 | 0.816 | HO3gal′ | 0.028 | 0.410 | 1.011 |
| HO4gal | −0.668 | −0.196 | 0.814 | HO4gal′ | −0.197 | 0.341 | 0.912 |
| HO6gal | −0.753 | −0.091 | 0.410 | HO6gal′ | −0.268 | 0.232 | 1.329 |
| H1fuc | −0.498 | 0.018 | 1.265 | H1fuc′ | −0.004 | 0.147 | 0.496 |
| H2fuc | −0.703 | 0.018 | 1.251 | H2fuc′ | −0.211 | 0.136 | 0.486 |
| H3fuc | −0.688 | −0.009 | 0.930 | H3fuc′ | −0.194 | 0.159 | 0.822 |
| H4fuc | −0.735 | −0.088 | 0.995 | H4fuc′ | −0.274 | 0.243 | 0.752 |
| H5fuc | −0.554 | −0.080 | 1.020 | H5fuc′ | −0.089 | 0.239 | 0.751 |
| H61fuc | −0.612 | −0.157 | 1.121 | H61fuc′ | −0.166 | 0.312 | 0.649 |
| H62fuc | −0.614 | −0.134 | 1.294 | H62fuc′ | −0.047 | 0.301 | 0.571 |
| H63fuc | −0.497 | −0.140 | 1.207 | H63fuc′ | −0.162 | 0.289 | 0.474 |
| HO2fuc | −0.640 | 0.067 | 1.000 | HO2fuc′ | −0.132 | 0.076 | 0.737 |
| HO3fuc | −0.859 | −0.009 | 0.923 | HO3fuc′ | −0.359 | 0.145 | 0.825 |
| HO4fuc | −0.853 | −0.075 | 1.184 | HO4fuc′ | −0.364 | 0.244 | 0.536 |
| H1wat1 | −0.087 | 0.390 | 0.458 | H2wat1 | 0.004 | 0.397 | 0.351 |
| H1wat2 | 0.251 | 0.112 | 1.227 | H2wat2 | 0.233 | 0.158 | 1.315 |
| H1wat3 | −0.262 | 0.045 | 0.532 | H2wat3 | −0.346 | 0.006 | 0.501 |
| H1wat4 | −0.398 | −0.063 | 1.356 | H2wat4 | −0.316 | −0.098 | 1.408 |
| H1wat5 | −0.162 | −0.054 | 1.328 | H2wat5 | −0.089 | −0.095 | 1.380 |
| H1wat6 | 0.195 | 0.329 | 0.354 | H2wat6 | 0.083 | 0.317 | 0.392 |
| H1wat7 | 0.279 | 0.314 | 0.775 | H2wat7 | 0.226 | 0.319 | 0.619 |
| H1wat8 | 0.356 | 0.231 | 0.397 | H2wat8 | 0.246 | 0.248 | 0.349 |
| H1wat9 | 0.505 | 0.2563 | 0.767 | H2wat9 | 0.436 | 0.277 | 0.646 |

The crystal structure displays an extremely dense network of hydrogen bonds (Figure 5). Thirty-six hydrogen bonds are observed in the asymmetric unit, 30 of them implying water molecules. Each trisaccharide is involved in 21 hydrogen bonds, the ratio per glycosidic residue varying from 5 to 8. Such a high number of hydrogen bonds can be correlated to two facts: the high hydration level and the peculiar folding of the oligosaccharide, burying the hydrophobic faces of the residue and presenting the hydrophilic faces to the external part. The hydrogen bond network shown in Figure 5 has several features: one four-member ring of cooperative hydrogen bonds (→O2gal′→Owat5→Owat4→Owat7→) and three infinite chains. Whereas these features have been described previously in carbohydrate crystals (Jeffrey and Saenger, 1981), this is the fist example where they are shown to occur simultaneously.

*Description of the packing*

Analysis of the packing has been attempted by evaluating the energy of interaction between pairs of neighbors. The best

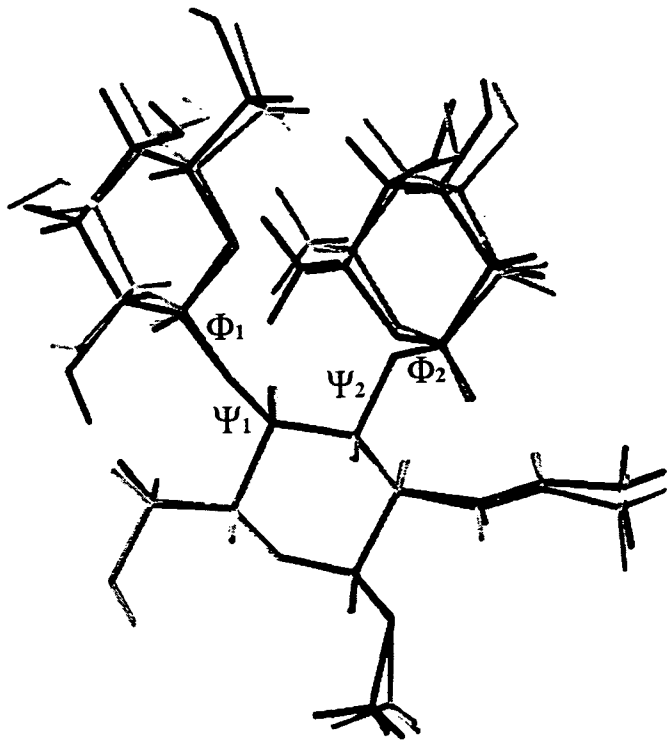


**Fig. 2.** Comparison of the crystalline conformations of the two independent $Le^x$ molecules.

ones, and almost equivalent ones, exist between the two independent molecules and between one molecule and the translation about *a* of the other. These two interactions create a strong molecular chain along the *a* axis. The two second best interactions result from the rotation about the $2_1$ axis. They also generate molecules in the *ab* plane. The *ab* plane displays therefore all the strongest interactions and can be seen as a sheet of Lewis x trisaccharide molecules with strong interconnections (Figure 4). A weaker but still energetically favorable interaction exists between each molecule and its related one along the *c* axis.

## Discussion

The interaction between adjacent trisaccharides observed in the crystal structure can be related to several biological effects, which were thought to involve $Le^x$–$Le^x$ interaction.

**Table III.** Comparison of the linkage torsion angles[a] in the crystal and in solution[b]

| Linkage | Φ/Ψ | | |
|---|---|---|---|
| | Crystal | | Solution[b] |
| Galβ(1-4)GlcNAc | −80.0/−104.6 | −70.5/−107.7 | −75/−104 |
| Fucα(1-3)GlcNAc | −72.5/139.2 | −76.7/139.0 | −81/151 |

[a]Torsion angles at the glycosidic linkages are defined as Ψ1 = (O5gal–C1gal–O1gal–C4gln), Ψ1 = (C1gal–O1gal–C4gln–C5gln), Φ2 = (O5fuc–C1fuc–O1fuc–C3gln), Ψ2 = (C1fuc–O1fuc–C3gln–C4gln).
[b]Lowest energy conformation (Imberty *et al.*, 1995) obtained with the MM3 force-field using a high dielectric constant in order to simulate the effect of water environment.

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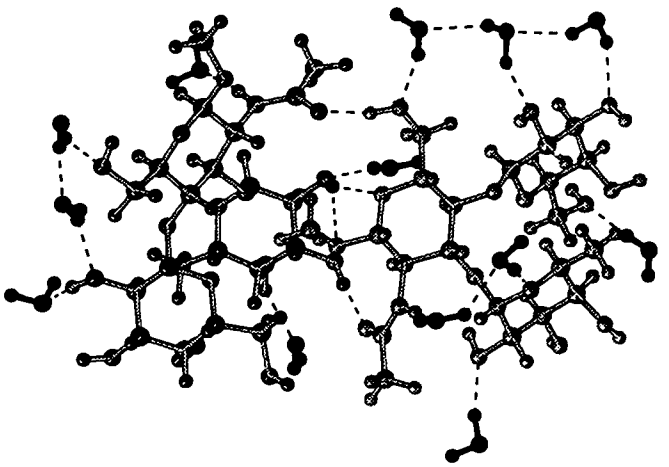

**Fig. 3.** Hydration shell of the dimer of $Le^x$ trisaccharide in the crystalline form. The two independent molecules have been given a different gray shading, water molecules are shown in black, and dashed lines depict hydrogen bonds.

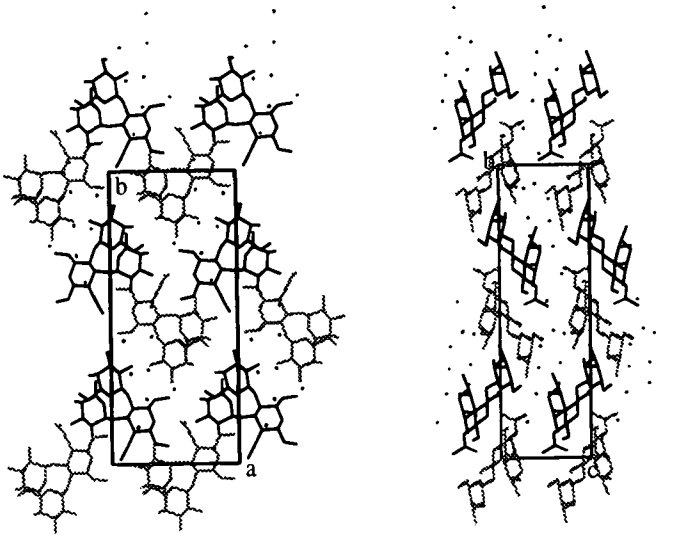


**Fig. 4.** Packing of $Le^x$ trisaccharide in the *ab* and *bc* planes. The two independent trisaccharide molecules have been given different gray shading. Hydrogen atoms are not displayed.

In several cases, the histo-blood group oligosaccharides have been shown to be more active when associated in clusters (Dean *et al.*, 1993; Varki, 1994). Such clusters are likely to exist at the surface of the cell, either due to high density of O-glycosylation sites on mucin-like proteins, or to the association of the glycosphingolipids in patches in the membrane. From the present crystal structure, the arrangement of trisaccharides along the *c* axis is compatible with this hypothesis (Figure 6). This translation maintains the orientation of the trisaccharide, therefore creating rows of identical molecules compatible with insertion of their carrier in a bilayer membrane. Calculations are in progress to model two-dimensional arrays of glycolipids, using this pairing as starting point.

The specific interaction of $Le^x$ with $Le^x$ has been proposed to be the basis of cell adhesion in morula compaction and autoaggregation of F9 teratocarcinoma (Eggens *et al.*, 1989). This hypothesis has been reinforced (Kojima *et al.*, 1994) based on autoaggregation studies of plastic beads coated with glycoconjugates bearing this trisaccharide determinant in the presence of $Ca^{2+}$. Indeed, this type of interaction, involving fucose and galactose residues, is observed in the crystal, between rows of trisaccharides (Figure 6). The two rows display a head-to-head arrangement which would be compatible with the arrangement required for a cell–cell recognition event. This interaction is one of the most favored in terms of energy since three hydrogen bonds are established (O4fuc→O3gal′, O4gal→O6gal′, O4gal′→O3fuc).

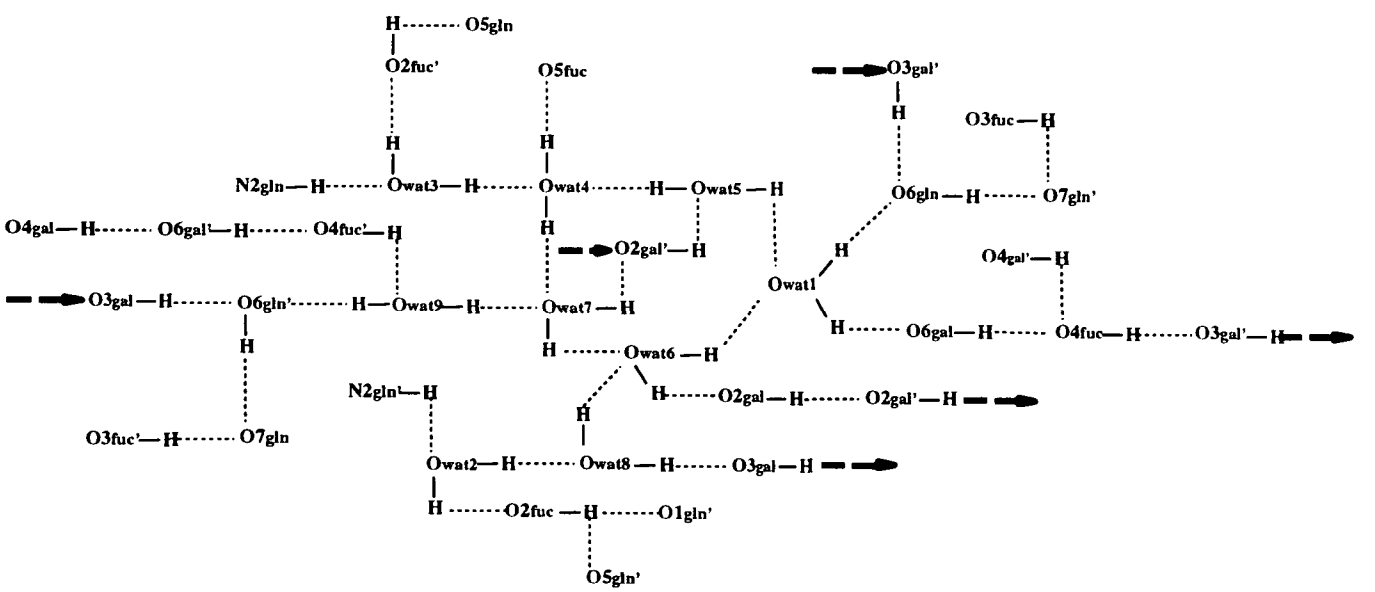


**Fig. 5.** Schematic representation of the hydrogen bond network in the crystal of $Le^x$ trisaccharide. The three infinite chains are indicated by bold arrows. The atom names are followed by the letters gal, gln, and fuc according to whether they belong to the galactose, N-acetyl galactosamine, and fucose residue, respectively. The two independent molecules are referred to as unprimed and primed. Water molecules are labeled as wat, from 1 to 9.



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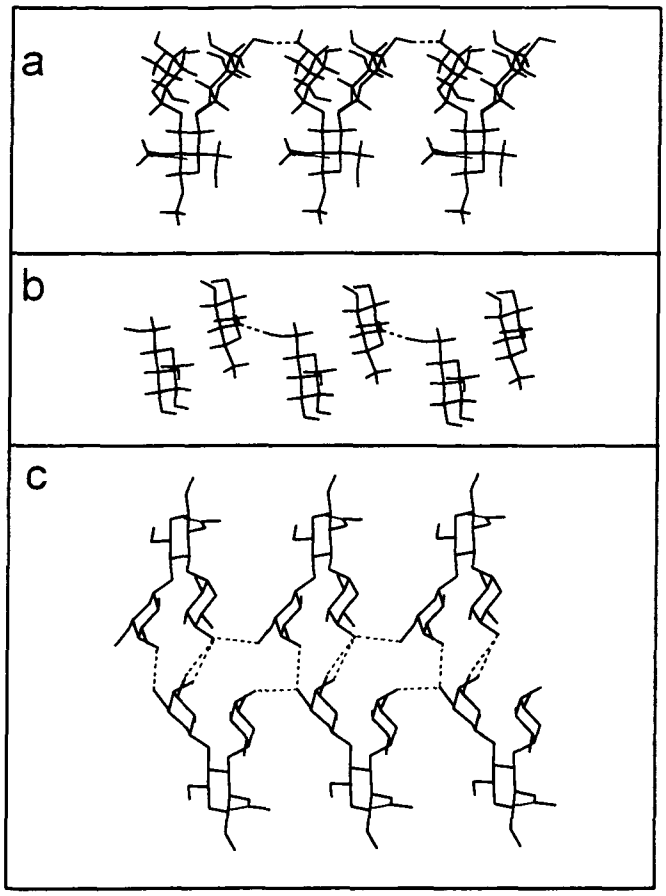


**Fig. 6.** Packing of $Le^x$ trisaccharide in the crystalline state which may mimic $Le^x$–$Le^x$ contact in biological conditions. (**a**) Row along *c* axis, (**b**) orthogonal representation of (**a**) without displaying the GlcNAc residues; (**c**) head-to-head contacts between two rows of parallel molecules (hydrogen atoms are not displayed).

## Material and methods

*Synthesis of Lewis x trisaccharide methyl glycoside*

Synthesis of Lewis x trisaccharide methyl glycoside (**1**) was accomplished by stepwise elongation of the oligosaccharide chain starting from the formation of lactosamine fragment (see Figure 7). Methyl 6-O-benzoyl-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**4**) was used as first glycosyl acceptor. It was prepared by acid hydrolysis of methyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**) (Alais and David, 1990) and subsequent regio-selective 6-O-benzoylation of diol (**3**) (Schwartz *et al.*, 1985). Presence of Bz-group in **4** at O-6 was confirmed by low-field location of the signals of H-6a and H-6b protons and up-field location of the signal of H-4 in $^1$H-NMR spectrum.

Glycosylation of acceptor **4** by 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (**5**) under promotion with silver triflate gave selectively substituted methyl lactosaminide derivative (**6**) in 83% yield. β-Configuration of the inter-residual linkage was confirmed by the value of coupling constant $J_{1',2'}$ of 7.8 Hz. Glycosylation of **4** by 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose catalyzed by trimetylsilyl triflate (Ogawa *et al.*, 1981; Nifant'ev *et al.*, 1996) and by ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside under promotion with nitrosyl tetrafluoroborate (Pozsgay and Jennings, 1987) were less effective (yield of **6** was 40%) and accompanied by O-6→O-4 migration of benzoate in **4** and formation of (1→6)-linked allo-lactosaminide product (Nivant'ev *et al.*, 1988).

Hydrazinolysis of disaccharide **6**, followed by complete acetylation and debenzylation gave lactosaminide derivative **8** with free OH-group at C-3. Glycosylation of **8** by fucosyl bromide **9** under halide-ion catalysis and subsequent removal of protecting groups gave target trisaccharide **1**.

*Crystallography*

Lattice constants were determined by a least-squares fit to the setting angles at high 2θ values measured with a Philips PW1100 diffractometer on an x-ray generator, Ni-filtered Cukα radiation, wavelength λ = 1.541 Å. The intensities of 4144 independent reflections were measured inside the sphere limited by $2\theta < 125°$ of which 3139 such as $I > 3\sigma(I)$ were considered as observed. Lorenz and polarization corrections were applied, but no correction was made for absorption. The atomic scattering factors used were taken from the International Tables for X-Ray Crystallography (1974). The structure was solved by direct methods, allowing the location of all, C, O, and N atoms. The last refinement cycles were performed using an anisotropic thermal temperature factor for the nonhydrogen atoms. All the hydrogen atoms of the two trisaccharide molecules were located by successive difference Fourier maps and isotropic refinement. Half of the hydrogen atoms of the water molecules were

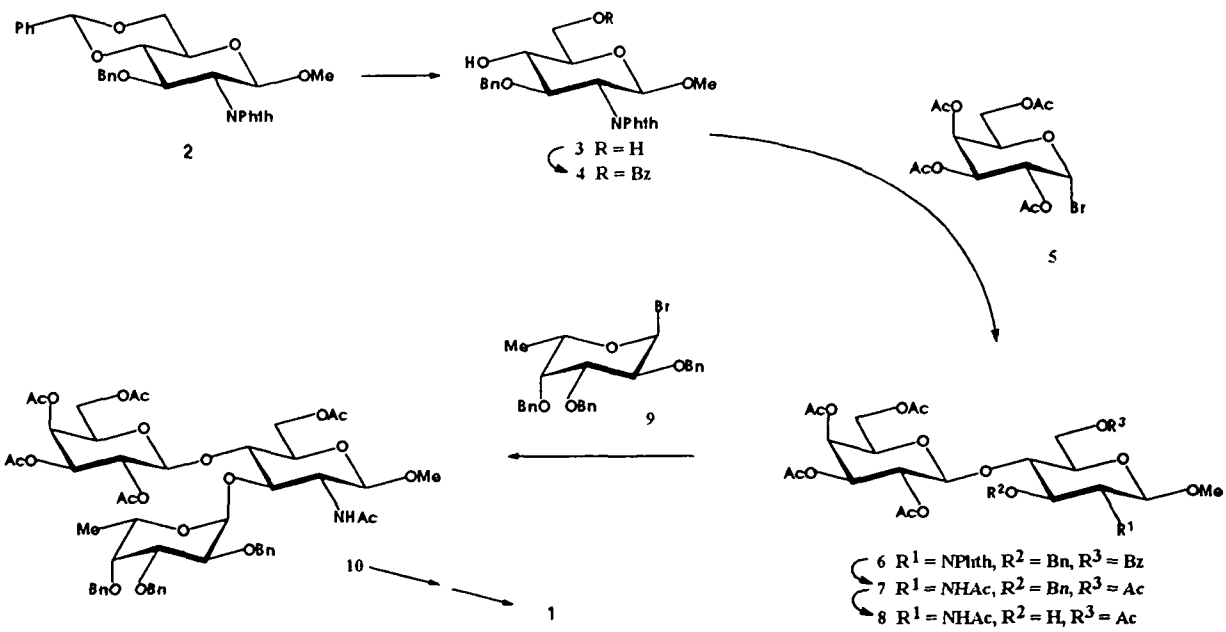


**Fig. 7.** Synthetic route for $Le^x$ trisaccharide methyl glycoside.



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located using the same procedure: consideration of these atomic positions in relation to possible hydrogen bonding schemes were sufficient to define the locations of the remaining hydrogen atoms without ambiguity. Introduction of these geometrically defined atoms at the final stage of the refinement did not increase the magnitude of the reliability index. The final $R$ value was 0.051 and $R_w$ = 0.054. A final electron density map showed no significant residual density. Averaged standard deviations are 0.01 Å for bond lengths, 0.7° for bond angles, and 1.5° for torsion angles.

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## Abbreviations

Le$^x$, Lewis x trisaccharide; Gal, galactose; GlcNAc, N-acetylglucosamine; Fuc, fucose.

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## Note added in proof

While our manuscript was processed, a paper describing an indipendent X-ray determination of the crystal structure of the Lewis x trisaccharide was published (Yvelin *et al.*, 1996).



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