Proton NMR Studies of a Tetrasaccharide which is a Receptor for Uropathogenic *E. Coli* Bacteria *

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Glycosphingolipids occur in the plasma membrane of mammalian cells. The hydrophobic ceramide section is inserted into the external half of the lipid bilayer, whilst the hydrophilic oligosaccharide protrudes from the cell surface.[1,2]

The structures of the carbohydrate chains of glycosphingolipids are extremely variable, allowing them to function, for example, as blood group antigens and as receptors for bacterial toxins.[2] They may also serve as host receptors for adhesion of *E. coli* strains which cause urinary tract infections.[3,4] Pili, single helical coils of protein subunits (approx. M.W. 20 000), have been implicated as the bacterial ligands mediating this binding process.[5]

Preincubation of bacteria with a glycolipid fraction extracted from urinary sediment epithelial cells has been shown to inhibit bacterial attachment.[3] Globotetraosylceramide (*P* antigen, globoside) was the most efficient inhibitor. It has the structure βGalNAc-(1→3)-αGal-(1→4)-βGal-(1→4)-βGlc-(1→1)-Cer.*** The structurally related glycolipid, globotriasylceramide, was also active, but unrelated glycolipids were inactive.[4] The oligosaccharides of active compounds each contain αGal-(1→4)-Gal, binding activity may therefore reside in this disaccharide.[6] The tetrasaccharide (confirmed to be globotetraose in this study), cleaved from ceramide by ozonolysis [7] and alkaline degradation, also reduced the attachment of *E. coli* to human uroepithelial cells.[8]

The aims of this work were twofold: (1) to verify that it was indeed globotetraose that was isolated in the latter study;[8] (2) to assign the ^{1}H NMR spectrum of globotetraose in D_2O, as a prelude to studying its interaction with the protein receptor.

Experimental. A Bruker 270 MHz NMR instrument was used in the Fourier-transform mode. Spectra were typically taken up in 8 K memory with an acquisition time of 1.37 s and a spectral width of 3000 Hz. Chemical shift values are given in ppm relative to 2,2-dimethyl-2-silapentane-5-sulfonate. The time shared mode of homonuclear decoupling was used in some of the experiments. The globotetraose was dissolved in 99.998% deuterium oxide from Stohler Isotope Chemicals.

Results and discussion. In this study, the ^{1}H NMR spectrum of the tetrasaccharide was characterised by homonuclear decoupling experiments and comparison with spectra of glycosphingolipids possessing similar carbohydrate chains.[9,10] The spectrum (Fig. 1) shows five well-resolved resonances in the anomeric region (4.4 – 5.3 ppm). The α-anomeric protons of glucose and galactose have small coupling constants ($J_{1,2} \approx 4$ Hz) and appear at the low-field end of the spectrum, whilst the β-anomeric protons have larger coupling constants ($J_{1,2} \approx 9$ Hz) and appear at a somewhat higher field.

The reducing glucose residue of the tetrasaccharide is present both in α- and β-form, and gives rise to two doublets of lower intensity than the other anomeric resonances. Thus, the doublet at 5.23 ppm is easily assigned to the anomeric proton of α-glucose. The four peaks at 4.6 – 4.7 ppm were shown, by decoupling, to be due to two β-anomer doublets. The doublet to slightly higher field was of lower intensity and was therefore ascribed to the β-anomer of glucose. The percentages of the α- and β-anomers of glucose were found, by integration, to be 30 and 70%, respectively. The anomeric protons of 2-acetamido-2-deoxy-β-D-hexopyranosides appear between the α- and β-anomeric protons of the unsubstituted residues. Thus, the other doublet could be assigned to the 2-acetamido-2-deoxy-β-galactosyl group of the tetrasaccharide. The singlet at 2.05 ppm derives from the acetamido group of this sugar residue.

From their coupling constants and chemical shift values, the doublets at 4.95 ppm and 4.52 ppm were assigned to the anomeric protons of the α- and β-galactosyl residues, respectively.

The ring proton region (3.5 – 4.0 ppm) is very crowded and most resonances are overlapping. However, all H-2's could be assigned by selective irradiation of the appropriate anomer resonance. This was then checked by irradiating the H-2 resonances. The remaining ring proton assignments were made to narrow regions, and some of the H-3 and H-4 resonances could not be identified. A further complication is the small coupling constant between the H-3 and H-4 in galactosyl and 2-acetamido-2-deoxygalactosyl residues ($J_{3,4} \approx 1$ Hz),

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*** Abbreviations used: Gal, galactose; Glc, glucose; GalNAc, 2-acetamido-2-deoxygalactose; Cer, ceramide. All sugars are assumed to exist in the pyranose form, to be of the D series and to have a C1 chair conformation.

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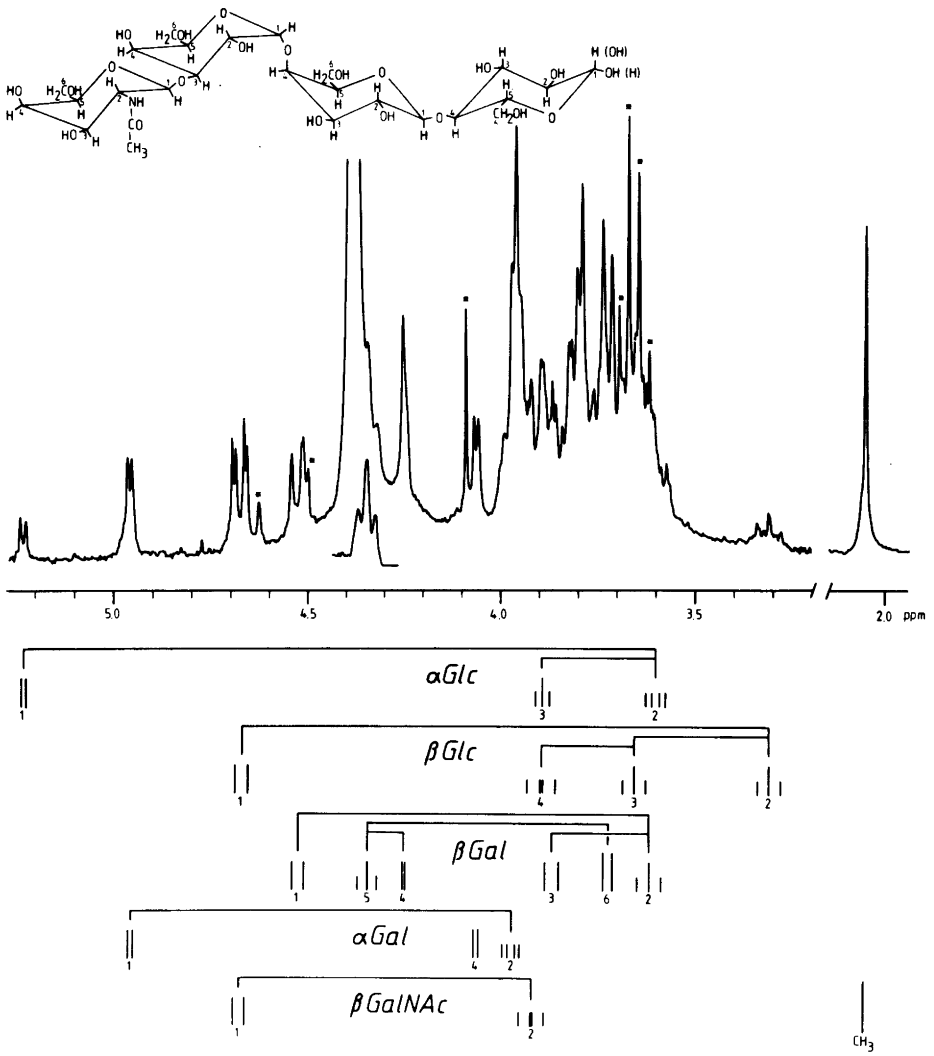

Fig. 1. Assignment of the globotetraose (~2 mg/ml) ^{1}H NMR spectrum at 270 MHz in deuterium oxide at 333 K. This spectrum was recorded in 16 K memory and 1000 transients were collected. Inserted is the triplet-structure at 4.33 ppm recorded at 295 K. The methyl resonance at 2.05 ppm is reduced to half intensity. Resonances marked (*) are due to impurities or spinning sidebands. The structure of globotetraose is indicated above the spectrum, the α-glucose configuration being shown in parenthesis. The C-4, 5 and 6 protons have been tentatively assigned (see text).

which makes it very difficult to detect H-4 signals by irradiation of H-3 signals.

There are three non-anomeric resonances shifted down-field, outside the bulk of the overlapping ring proton resonances. The triplet structure at 4.33 ppm (partly hidden by the HDO resonance at 333 K) is coupled to a resonance in the ring proton region at 3.76 ppm. These resonances arise from H-5 and H-6, respectively, of a galactosyl residue. The triplet also appears to be coupled to the signal at 4.24 ppm, which is a doublet structure, hidden beneath the HDO spinning sideband in Fig. 1. The latter signal and the third of the low-field ring proton resonances, at 4.05 ppm, can be assigned to the H-4's of the two galactosyl residues, since substituted galactosyl H-4's have very low-field shifts compared

to other ring protons.[11]

Dabrowsky *et al.*[11] have suggested that resonances in the spectrum of globoside in dimethyl sulphoxide having corresponding positions to the low-field triplet and doublet structures (4.33 and 4.24 ppm), belong to the α-galactosyl residue. We have not been able to correlate these assignments with the spectrum of globotetraose in D_2O. Neither decoupling experiments nor comparison with other spectra allow unambiguous assignment of these H-4, 5 and 6 signals to the α-galactosyl and/or β-galactosyl residues. One difficulty is that α- and β-galactosyl residues invariably occur together in the glycolipids so far available to us. However, tentative assignments are given in Fig. 1. Further studies are in progress to clarify the origin of these signals. Comparison with the spectrum of the corresponding globoside incorporated into SDS micelles[11] showed the two spectra to be very similar. The differences in the globoside spectrum were due to the reducing glucose residue being bound to ceramide, and to resonances arising from the ceramide itself. The study is also in good agreement with previous studies made in different organic solvents.[9,11] Thus, our study confirms that the isolated tetrasaccharide was globotetraose.

The spectrum of globotetraose has been sufficiently well assigned to allow interaction studies in a physiologically relevant environment. It should now be possible to observe the interaction between purified pili (the bacterial ligand) and the globotetraose (the specific receptor) in D_2O. For these studies, it may be possible to use the isolated sugar in solution, or the intact glycolipid inserted into micelles or vesicles.

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