spectrometry to establish basic features of the LOS populations, such as the number of components present, their molecular weights, and their structural homology. Mild acid hydrolysis of the Hib A2 LOS mixture, which contained eleven components, produced free oligosaccharides which were analyzed by chemical methods, liquid secondary ion and tandem mass spectrometry, and 2D NMR spectroscopy. These studies established that the oligosaccharides were triantennary structures containing a heptose trisaccharide core with primarily glucose residues as branch sugars. Structures with nonreducing terminal Gal1→4GlcNAc were minor components in the oligosaccharide fraction which were found to be quantitatively sialylated in the O-deacylated LOS mixture. These data support the view that presenting a complex profile of LOS surface structures offers Hib added flexibility in evading host defense mechanisms. This work was supported by grants from NIAID (AI21620 and AI24616) and NIH (RRO1614).

S19.4
Mass Spectrometry of Glycoconjugates after Specific Chemical Modifications

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Mass spectrometry of glycoconjugates is the most sensitive method for characterization of glycoconjugates in terms of monosaccharide sequence and molecular weight. Further structural information can in general not be obtained by mass spectrometry. Therefore, in order to extend the structural information to include determination of glycosidic linkage positions specific chemical modifications have to be introduced. Chemical reactions like trifluoroacetolysis and periodate oxidation have been employed to modify the monosaccharide residues in glycoprotein oligosaccharides and glycosphingolipids (1, 2, 3).

Several choices are available to analyse the products e.g. peacetylation permethylation, GLC-MS and FAB-MS. Examples from glycoproteins and glycosphingolipids will be presented.

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S19.5
Computer-Assisted Method of the Structural Analysis of the Regular Branched Polysaccharides

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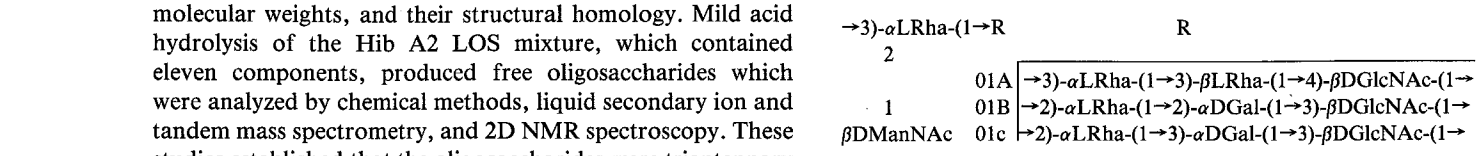
A computer-assisted approach to the structural analysis of regular branched polysaccharides has been described[1]. In this communication we report on the futures of the method on the example of the structural analysis of *Escherichia coli* 01A, 01B, and 01C specific polysaccharides[2], containing branched pentasaccharidic repeating units:



[1] G. M. Lipkind, A. S. Shashkov, N. E. Nifant'ev, N. K. Kochetkov, *Carbohydr. Res.*, **237** (1992) 11 – 22.
[2] N. E. Nifant'ev, A. S. Shashkov, G. M. Lipkind, N. K. Kochetkov, B. Jann, K. Jann, *Carbohydr. Res.* (1993), submitted.

S19.6
FAB-MS Sequencing of Mycobacterial Glycolipid Antigens

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In collaboration with Brennan's group (G. S. Besra, M. R. McNeil, D. Chatterjee and P. J. Brennan, Department of Microbiology, Colorado State University, Fort Collins, Colorado), our laboratory has been involved in developing concerted derivatisation/Fast Atom Bombardment-Mass Spectrometry (FAB-MS strategies to sequence a variety of glycolipids from *Mycobacteria* including the glycopeptidolipids (GPL), lipooligosaccharides (LOS) and the lipoarabinomannan (LAM) and lipomannan (LM).

The strategies typically involve FAB-MS analyses of underivatised samples in both positive and negative ion modes to define the molecular weight, followed by analyses of permethyl and/or perdeuteroacetyl derivatives to define the sequence. Both acid (trifluoroacetic anhydride/acetic acid) and base (pyridine or 1-methylimidazole/acetic anhydride) catalysed peracetylation procedures employed were found to fully retain the acetyl and/or fatty acyl substituents, affording abundant sodiated molecular ions using the *m*-nitrobenzyl alcohol matrix. The mass shifts compared to native samples define the number of free hydroxyl groups on the saccharide moieties as well as potential derivatisable functions on the peptide core (for the GPLs). Characteristic A-type oxonium ions together with glycosidic and/or ring cleavages of various modes afford unambiguous sequencing of the saccharide determinants and localisation of various *O*-methylation, *O*-acylation, pyruvylation *etc* commonly found on the saccharide moieties of these mycobacterial antigens. The delineation of fatty acyl substituents (number, chain length and heterogeneity) was conveniently effected by mild methanolysis/FAB-MS analysis of the native samples, further corroborated by the abundant fragment ions afforded by the perdeuteroacetyl derivatives. Alternatively, Prehm methylation yields fully methylated glycolipids retaining the acyl functions whilst sodium hydroxide methylation effected selective partial de-*O*-acylation of the GPLs and LOSs, resulting in a composite 'map' of the fatty acyl substitution pattern. NaOH permethylation also effected *N*-methylation of the amides of the peptide of GPLs which provided favoured mass spectrometric cleavage sites, yielding abundant *N*-terminal sequence ions, during subsequent FAB-MS analysis. These ions, with or without the attached saccharide appendages, allowed unambiguous sequencing of the tetrapeptide core of the GPLs. Selected recent data obtained on sequencing the pyruvylated LOSs from *M. smegmatis* mutants; the trimethylammonium containing positively charged LOS from *M. gordonae;* the GPLs from *M. butyricum* and *M. senegalense;* and the further elucidation of the phosphatidyl inositol