3rd Baltic Meeting
on
Microbial Carbohydrates

Program
and
Abstracts

2-4 August, 2008
Sigtuna, Sweden




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Program

Friday, August 1

16.00-18.00 **Registration**

19.30 Dinner

Saturday, August 2

08.50-09.00 **Elke Schweda**
Opening

Chairperson: Stefan Oscarson

09.00 **Hans Kamerling**, Utrecht, The Netherlands
Studies towards potential synthetic carbohydrate-protein conjugate vaccines against *Streptococcus pneumoniae* serotypes

10.00 **Christian Vogel,** Rostock, Germany
Synthesis of diacetamido-dideoxy-uronic acids which represent rare modules of the bacterial envelope

10.30-11.00 Coffee break

Chairperson: Christian Vogel

11.00 **Anna V. Orlova,** Moscow, Russia
Synthesis of a suicide substrate-based tool to trap proteins with sialidase activity

11.20 **Polina Abronina,** Moscow, Russia
β-D-Arabinofuranosyl-containing Branched Hexasaccharides from *Mycobacterium tuberculosis*: A convergent Approach to the Synthesis

11.40 **Nikita Podvalnyy**, Moscow, Russia
Nucleophilic opening of 3-*O*-acyl-β-D-arabinofuranose 1,2,5-*O*-orthobenzoates: a new look at an old reaction

12.00 Photo date and lunch

Chairperson: Elke Schweda

13.45 **Jianjun Li**, Ottawa, Canada
Capillary electrophoresis and mass spectrometry strategies for bacterial glycomics

14.45 **Göran Hübner,** Borstel, Gemany
Application of capillary electrophoresis coupled to Fourier-transform ion cyclotron resonance mass spectrometry to the analysis of glycolipids

15.15-15.45 Coffee break

Chairperson: Yurij Knirel

15.45 .**Brigitte Twelkmeyer**, Stockholm, Sweden
Structural details of *Haemophilus influenzae* lipopolysaccharide as evidenced by mass spectrometry
16.05 **Michal Arabski**, Kielce, Poland
Laser interferometric method in measurement of colistin and ampicilin diffusion in presence of *Proteus sp* endotoxins
16.25 **Leonid O. Kononov,** Moscow, Russia
What can carbohydrate chemistry gain from the existence of supramers?

19.00 Dinner

Sunday, August 3

Chairperson: Jim Richards

09.00 **Derek Hood**, Oxford, United Kingdom
Haemophilus influenzae lipopolysaccharide: a highly adaptive molecule
10.00 **Philip Toukach**, Moscow, Russia
Diversity of the bacterial glycome

10.30-11.00 Coffee break

Chairperson: Otto Holst

11.00 **Yuriy A. Knirel,** Moscow, Russia
Relationships between O-antigens of enteric bacteria
11.30 **Julija Fedonenko,** Moscow, Russia
Structure-function studies of the surface polysaccharide of Azospirillum lipoferum type strain Sp59b
11.55 **Varvara Vitiazeva,** Stockholm, Sweden
The structural diversity of lipopolysaccharides expressed by non-typeable *Haemophilus influenzae* strains 1158 and 1159

12.15-13.30 Lunch

13.30-17.00 Excursion to Skokloster Castle

18.30-20.00 Poster session with refreshments

Justyna Samaszko, Gdansk, Poland
Synthesis of murein fragment of *S. aureus* cell wall
Mona Svensson, Stockholm, Sweden
Structural determination of the enteroaggregative Escherichia coli O175 O-antigen
Sabina Górska, Wrozlaw, Poland

Structural and immunochemical studies of exopolysaccharide of *Lactobacillus johnsonii* strain 142 isolated from mice with inflammatory bowel disease
Katarzyna Duda, Katowice, Poland
Resolving the structure of *Yersinia pseudotuberculosis* O:9 O-specific polysaccharide repeating unit to elucidate the receptor structure of phage ϕR1-37
Adam Choma, Lublin, Poland
Revised structure of the repeating unit of the O-specific polysaccharide from *Azospirillum lipoferum* strain SpBr17
Jadwiga Zawisza, Gliwice, Poland
Synthesis of glycoconjugates derivatives of flavonoids
Michał Jadwiński, Gliwice, Poland
Application of glycosyl thiocarbamates in orthogonal glycosidation
Iwona Komaniecka, Lublin, Poland
Periplasmic β-glucan from *Bradyrhizobium elkanii*
Janusz Madaj, Gdansk, Poland
Purification and preliminary structural investigation of exopolysaccharides from *Pseudomonas mutabilis* biofilms

20.00 Dinner

Monday, August 4

Chairperson: Leonid Kononov

09.00 **Lennart Kenne**, Uppsala, Sweden
Bacterial polysaccharides, their structures, properties and use
10.00 **Roberto Adamo**, Siena, Italy
SPR studies on the binding bewteen MAb 2G8 and laminarin

10.30-11.00 Coffee break

Chairperson: Andrzej Gamian

11.00 **Ndegwa Maina,** Helsinki, Finland
Structural and physico-chemical characterisation of dextran from *Weissella confusa* E392
11.20 **Johan Olsson,** Stockholm, Sweden
Synthesis of lipopolysaccharide structures from *Neisseria meningitidis*
11.40 **Wiesław Kaca**, Kielce, Poland
Serotyping of clinical isolates belonging to *Proteus mirabilis* serogroup O36 and structural elucidation of the O36-antigen polysaccharide
12.00 **Stefan Oscarson**
Concluding remarks

12.20 Lunch and goodbye

Studies towards potential synthetic carbohydrate-protein conjugate vaccines against *Streptococcus pneumoniae* serotypes

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Streptococcus pneumoniae is still a leading cause of life-threatening diseases such as otitis media, pneumonia, and meningitis. Vaccination with the available 23-valent capsular polysaccharide vaccines offers protection for healthy adults to invasive pneumococcal diseases. However, these vaccines are ineffective in the most important high-risk groups, such as small children and the elderly, because they do not respond adequately to the T-cell independent polysaccharides as antigens. Conjugation of the carbohydrate antigens to protein carriers results in T-cell dependent neoglycoconjugate antigens, which give efficient immune responses in the high-risk groups. Currently, neoglycoconjugate vaccines against *S. pneumoniae*, prepared by conjugation of isolated polysaccharides or of a mixture of polysaccharide-derived oligosaccharides to a protein carrier, have been introduced. We have shown via combined synthesis/immunology programs for different serotypes of *S. pneumoniae* the potential of well-defined synthetic oligosaccharides-protein conjugates as vaccine candidates. Aspects of the research program will be presented.

Synthesis of diacetamido-dideoxy-uronic acids which represent rare modules of the bacterial envelope

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Diacetamidodideoxyuronic acids are rare moieties of bacterial O-specific polysaccharides [1-4] or of bacterial secondary cell-wall polymers [5]. The synthesis of members of this class of seldom sugars has been described only in two papers [6,7]. In continuation of our efforts towards the synthesis of uronic acid derivatives, we investigated the preparation of protected 2,3-diacetamido-2,3-dideoxy-D-uronates with *gluco*-, *galacto*- and *manno*-configuration. The synthetic pathways and first examples of applying these uronates as glycosyl donors will be presented.

Literature

1. Knirel, Y. A.; Kocharova, N. A.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K. Carbohydrate Research 1981, 93, C12-C13.
2. Knirel, Y. A.; Vinogradov, E. V.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K. Carbohydrate Research 1982, 104, C4-C7.
3. Knirel, Y. A.; Vinogradov, E. V.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K. Carbohydrate Research 1983, 112, C4-C6.
4. Cummins, C. S.; White, R. H. Journal of Bacteriology 1983, 153, 1388-1393.
5. Schäffer, C.; Kählig, H.; R., C.; Schulz, G.; Zayni, S.; Messner, P. Microbiology 1999, 145, 1575-1583.
6. Dmitriev, B. A.; Kocharova, N. A.; Kochetkov, N. K. Bioorganicheskaya Khimiya 1982, 8, 1234-1241.
7. Szurmai, Z.; Rako, J.; Agoston, K.; Danan, A.; Charon, D. Organic Letters 2000, 2, 1839-1842.

Synthesis of a suicide substrate-based tool to trap proteins with sialidase activity

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Neuraminidase (sialidase, NA) is an exo-glycosidase that hydrolyzes the linkage of sialic acid residues, which are mostly found as terminal constituents of glycoconjugates. The search for proteins with sialidase activity could be an important area of glycobiology research in post-genomic era when sequences of all proteins are known while their functions in many cases still remain obscure. A valuable tool for this purpose could be a mechanism-based inhibitor of NA, the molecule of which consists of a sialic acid recognition head, an ortho-difluoromethylphenyl latent trapping device, a linker, and a biotin reporter group. An example of such compound was recently used for trapping influenza viruses [1].

We synthesized a close analog of the reported compound [1] and optimized several critical steps. In the reaction of sialylation of 2-formyl-4-nitrophenol with sialic acid glycosyl chloride under phase-transfer catalysis conditions we have replaced expensive cesium carbonate with sodium carbonate without considerable loss in yield of product. The stereochemical result of glycosylation depended on the nature of substituent at C-5 of glycosyl donor. While reproducing the known transformation of formyl group into difluoromethyl moiety by treatment with DAST we found a side process – O-methylation of 5-acetamido group of Neu5Ac. In our hands, the aldehyde reacted with DAST more slowly than it was described earlier (3 days instead of 1 day [1]) and gave acetimidate along with the target acetamide in approximately equal amounts. The O-methyl acetimidate was apparently formed during quenching the excess of DAST with MeOH. We found that the treatment of crude worked-up reaction mixture with 1% TFA in THF–H2O mixture resulted in selective cleavage of the imidate methyl group and regeneration of acetamido function giving the desired product in high yield after chromatography (94% instead of 47% reported in literature [1]).

This work was supported by the Russian Foundation for Basic Research (project No. 08-03-00839) and the Council on Grants at the President of the Russian Federation (project MK-3244.2008.3).

[1] C.-P. Lu, C.-T. Ren, Y.-N. Lai, S.-H. Wu, W.-M. Wang, J-Y. Chen, L.-C. Lo, *Angew. Chem. Int. Ed.*, **2005**, *44*, 6888.

β-D-Arabinofuranosyl-containing branched hexasaccharides from *Mycobacterium tuberculosis*: A convergent approach to the synthesis

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The development of efficient and stereoselective glycosylation methodologies has attracted a great deal of attention in recent years due to the biological significance of arabinofuranosyl-containing oligosaccharide fragments of mycobacterial arabinogalactan and a lipoarabinomannan [1].

We synthesised fully protected hexasaccharides of arabinofuranose with 2-chloroethyl aglycon as precursors of the potential novel therapeutic targets. 3-*O*-Acyl-b-D-arabinofuranose 1,2,5-*O*-orthobenzoate and 3,5-di-*O*-benzoyl-1,2-*O*-benzylidene-b- D-arabinofuranose were chosen as monosacharide building blocks. It should be noted, that the key step of the synthesis was stereoselective introduction of 1,2-*cis*-glycosidic linkages. Thioglycosides with 3,5-*O*-tetraisopropyldisiloxanylidene (TIPDS)[2], 3,5-*O*-di-tert-butylsilylene [3] protective groups and 2'-carboxybenzyl-tri-*O*-benzyl-D-arabinofuranoside [4] were applied as arabinofuranosyl donors. We studied the stereoselectivity of glycosylation under various conditions and found it to be greatly dependent on the nature of glycosyl donor.

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[1] M. Joe, Y. Bai, R. C. Nacario and T. L. Lowary, J. Am.Chem.Soc, 129, 9885 (2007).
[2] A. Ishiwata, H. Akao and Y. Ito, Org. Lett, 24, 5525 (2006).
[3] X. Zhu, S. Kawatkar, Y. Rao and G. J. Boons, J. Am.Chem.Soc, 128, 11948 (2006).
[4] Y. J. Lee, K. Lee, E. H. Jung, X. B. Jeon and K. S. Kim, Org. Lett, 15, 3263 (2005).

Nucleophilic opening of 3-*O*-acyl-β-D-arabinofuranose 1,2,5-*O*-orthobenzoates: a new look at an old reaction

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A growing interest in the construction of D-arabino-oligosaccharides related to cell wall components of Mycobacteria has been developed over recent years [1] due to the necessity of creating means for diagnostics and treatment of human tuberculosis, a worldwide health problem taking millions of lives annually.

During the work on the synthesis of oligosaccharide fragments of mycobacterial arabinans we needed a series of 2-*O*-benzoyl-β-D-arabinose building blocks with various protective groups at O-3 and O-5. Although various strategies have been developed for the preparation of selectively protected arabinofuranose building blocks, the seemingly most versatile approach is based on the use of 3-*O*-benzyl-β-D-arabinofuranose 1,2,5-*O*-orthoesters as the key intermediates [2]. Fascinated by the apparent elegancy of this approach we attempted a synthesis of 3-*O*-benzoyl-β-D-arabinofuranose 1,2,5-*O*-orthobenzoate (**1**) (L-enantiomer of (**1**) is known [3]) and subsequent opening reactions of it by O- and S-nucleophiles. We were surprised to find the situation much more complex than we expected initially. Now we present the results of our studies aimed at solving problems faced during the synthesis of the required building blocks.

3-*O*-Acyl-β-D-arabinofuranose 1,2,5-*O*-orthobenzoates with 3-*O*-benzoyl (**1**), -acetyl (**2**) and -chloroacetyl (**3**) groups were prepared from the known β-D-arabinofuranose 1,2,5-*O*-orthobenzoate (**4**) [2,3]. Nucleophilic ring-opening of orthoesters (**1**)-(**3**) with O- and S-nucleophiles was reinvestigated. Optimized conditions leading selectively to the formation of the respective monosaccharide adducts or 1-5-linked disaccharide thioglycosides were found in the case of reactions of (**1**)-(**3**) with 2-chloroethanol or EtSH. 2-Chloroethyl O-glycosides and ethyl thioglycosides were obtained with α-stereoselectively in 94-96% isolated yields while 1-5-linked disaccharide ethyl thioglycoside could be prepared in 94% yield. However, reactions of (**1**)-(**3**) with PhSH gave phenyl thioglycosides in 73-75% yields along with 20-25% of the respective disaccharide phenyl thioglycosides as by-products.

This work was supported by RFBR (project No. 07-03-00830).

[1] M. Joe, Y. Bai, R.C. Nacario, T.L. Lowary, *J. Am. Chem. Soc.* 2007, **129**, 9885-9901, and references cited therein.
[2] S. Sanchez, T. Bamhaoud, J. Prandi, *Eur. J. Org. Chem.* 2002, 3864-3873.
[3] N.K. Kochetkov, A.Ya. Khorlin, A.F. Bochkov, I.G. Yazlovetskii, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1966, **15**, 1966-1968.

Capillary electrophoresis and mass spectrometry strategies for bacterial glycomics

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Lipopolysaccharides (LPS) are glycolipids found on the outer membrane of Gram-negative bacteria and display significant structural diversity that has been linked to disease pathogensis. The structural analysis of these complex carbohydrates has proved challenging due to the natural heterogeneity and environmental glycoform selection. Applications of on-line CE tandem mass spectrometry open the door to elucidating structural detail of isomeric oligosaccharides in complex LPS mixtures. The microheterogeneity of the LPS molecules expressed by a broad range of gram-negative bacterias, such as *H. influenzae*, *N. meningitides*, and *C. jejuni* can be characterized by CE-MS. We have been successfully applied the technique to probe LPS glycoform populations of bacteria isolated from an in vivo source. For the characterization of bacterial polysaccharides we have developed in-source collision-induced dissociation technique. This approach was proven particularly useful for probing the subtle structural differences in monosaccharide composition and functionalities arising across bacterial species. N-glycosylation of proteins is one of the most common post-translational modification of proteins in eukaryotes. To study the specific functional roles of glycans and the relationship between their structures and functions, a common approach of exoglycosidase cleavage and permethylation has been widely used, i.e. releasing N-glycans using Peptide-N-Glycosidase F (PNGase F). However, this technique is applicable to prokaryotic cells because the carbohydrate-peptide linkages are different and the enzymes for releasing carbohydrate moieties are rarely available. In this presentation I will also describe a new glycomics technique combines non-specific proteolysis digestion and permethylation. The strategy was applied to the characterization of N-linked glycans in the total protein extracts from mucosal pathogen *Campylobacter jejuni*. The results demonstrated that the proposed method enables not only N-linked glycan detection, but also leading to the discovery of free glycans.

Application of capillary electrophoresis coupled to Fourier-transform ion cyclotron resonance mass spectrometry to the analysis of glycolipids

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Glycolipids such as gangliosides and lipopolysaccharides are important components of various biological membranes. These membranes provide the permeability barrier between cells and organelles and are also involved in various biological processes. Since most isolates of these amphiphilic molecules are complex and heterogeneous mixtures, their detailed structural analysis may be challenging and is subject of numerous investigations using a broad spectrum of analytical techniques and instruments, including mass spectrometry (MS).

Capillary electrophoresis (CE) is a high-resolution separation technique widely used for the analysis of complex mixtures of various kinds of molecules. As a continuous-flow analysis system, the coupling to mass spectrometers via electrospray ionization (ESI) ion sources is apparent. During the past years an increasing number of papers has been published which demonstrate the capabilities of on-line CE-MS, mainly for the analysis of peptides and proteins, but also for glycolipid analysis [1-5]. However, the on-line coupling of CE and mass spectrometry is limited i! n its possibilities due to the incompatibility of the ESI source to common CE buffer systems, which are usually made up of non-volatile liquids and contain high amounts of salts or detergents. Furthermore, CE-MS is generally based on aqueous buffer systems, which of course anticipate the analysis of highly hydrophobic molecules.

The capabilities and limitations of the application of CE coupled on-line to high resolution and high mass accuracy Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to the analysis of glycolipids are demonstrated in this work. Ganglioside standards were analyzed as well as lipid A and native deep rough mutant lipopolysaccharides isolated from different bacteria.

Acknowledgement:
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Literature:

[1] Chen, Y-R, Electrophoresis, 2005, 26(7-8), 1376-1382
[2] Li, J., Electrophoresis, 2004, 25(13), 2017-2025
[3] Li, J., Rapid Commun Mass Spectrom, 2005, 19(10), 1305-1314
[4] Wang, Z., Carbohydr Res, 2006, 341(1), 109-117
[5] Zamfir, A., Electrophoresis, 2004, 25(13), 2010-2016

Structural details of *Haemophilus influenzae* lipopolysaccharide as evidenced by mass spectrometry

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The Gram-negative bacterium *Haemophilus influenzae* is an exclusively human pathogen and exists in encapsulated and unencapsulated (non-typeable) forms. The non-typeable forms of *H. influenzae* (NTHi) cause otitis media in children as well as acute and chronic lower respiratory tract infections. Lipopolysaccharide (LPS) from *H. influenzae* is found to consist of a conserved inner core having oligosaccharide extensions mimicking host glycolipids. A range of non-carbohydrate substituents including phosphate, phosphoethanolamine, pyrophosphoethanolamine, phosphocholine, acetates and glycine are found [1].

Mass spectrometry is an indispensable tool in the structural analyses of NTHi LPS. O-deacylated LPS (LPS-OH) and core-oligosaccharide (OS) are analyzed by electrospray ionization mass spectrometry (ESI-MS). Positions of sialic acid in LPS-OH are determined using capillary electrophoresis ESI tandem MS. The positions of non-carbohydrate substituents in OS are determined using ESI multiple step tandem MS (MS^n). Chemically modified material, e.g. dephosphorylated and permethylated OS are analyzed using liquid chromatography ESI-MS^n yielding sequence and branching information.

References

1. E. K. H. Schweda, J. C. Richards, D. W. Hood, E. R. Moxon; 2007; Expression and structural diversity of the lipopolysaccharide of *Haemophilus influenzae*: implication in virulence; Int J Med Microbiol, 297(5), 297-306

Laser interferometric method in measurement of colistin and ampicilin diffusion in presence of *Proteus sp* endotoxins

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In our study we used firstly the interferometric system consisted of a Mach-Zehnder interferometer with a He-Ne laser, TV-CCD camera, computerised data acquisition system and a membrane system with two cuvettes separated by a horizontally placed cellulose membrane. Diffusion of the substance through a membrane separating solutions of different concentrations leads to the formation of concentration boundary layers (CBLs). Computer-assisted analysis of the antibiotic CBLs showed that the amount of ampicillin and colistin (polymyxin E) at concentrations 4 mg/ml transported through the membrane was not influenced by the presence of O25 *P. vulgaris* LPSs (100 μg/ml), in contrast to colistin diffusion, which depended on the O-deacylated form of O25 LPS. The diffusion coefficient of colistin was 1.7 times greater in the presence than in the absence of endotoxin. The ionic interaction between colistin and O25 LPSs may have facilitated the transport through the membrane. Secondly we proposed the modification of laser intererometric method that allowed testing the partially insoluble mixtures. Modification relies on the measurement of diffusion from 1% agarose. As model for our studies we used two *P. mirabilis* LPSs that differs on the polysaccharide content. Smooth (S) *P. mirabilis* S1959 strain synthesize complete LPS, whereas it's Re type mutant, strain R45. By laser interferometric and precipitation method we have shown that R45 LPS is more effective in colistin binding (2.74 times). Saponin rich plant extract partially enhanced interaction of colistin with S and Re types of *P. mirabilis* LPSs. Those results were confirmed with whole cells *Proteus* studies. In conclusion: the laser interferometric method is useful tools for studies of LPS: antibiotics interactions, even if tested substances are not fully dissolved in water.

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What can carbohydrate chemistry gain from the existence of supramers?

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Supramers are differently arranged supramolecular assemblies of the same molecular entities. The term has recently been coined to describe stereoisomerism at the supramolecular level (in the crystal) [1]. We have grounds to believe that similar supramers exist in solutions of carbohydrates as indirectly evidenced by NMR spectroscopy, optical rotation [2], IR spectroscopy, mass spectrometry and light scattering. In some cases supramers can be separated by chromatography.

One can expect different chemical reactivity for supramers which are supramolecular isomers [3]. This difference in reactivity can be easily understood if one assumes that in many cases the real reacting species are differently organized supramers rather than isolated molecular entities. Molecular structures of reactants and reaction conditions (solvent, temperature, concentration, presence of "non-reacting" compounds, etc.) would determine the aggregation type and the spatial arrangement ("structure") of supramers formed in each particular case. Accessibility of the reaction center in the supramers present would determine their reactivity and the outcome of a reaction.

This expected different reactivity of carbohydrate supramers can be extremely helpful for rationalizing a number of strange and puzzling facts. Among them are such different observations as a novel phenomenon of synergism in sialylation [4], significant influence of a remote functional group in aglycon on reactivity during chemical synthesis of HNK-1 pentasaccharide [5] or unprecedented dependence of hydrolytic stability of closo-carborane-lactose neoglycoconjugates on the nature of a spacer [6], to mention a few. All this means that formation of supramers in the reaction mixture should be considered as an important factor which is currently almost ignored during analysis of outcome of reactions in carbohydrate chemistry.

This work was supported by RFBR (projects No. 07-03-00830 and 08-03-00839).

[1] M. Czugler, N. Bathori, *Cryst. Eng. Comm.*, 2004, *6*, 494.
[2] L.O. Kononov, D.E. Tsvetkov, A.V. Orlova, *Russ. Chem. Bull.*, 2002, *51*, 1337; DOI: 10.1023/A:1020981320040. [3] http://old.iupac.org/goldbook/I03289.pdf.
[4] L.O. Kononov, N.N. Malysheva, E.G. Kononova, O.G. Garkusha, *Russ. Chem. Bull.*, 2006, *55*, 1311; DOI: 10.1007/s11172-006-0419-4.
[5] A.V. Kornilov, A.A. Sherman, L.O. Kononov, A.S. Shashkov, N.E. Nifant'ev, *Carbohydr. Res.*, 2000, *329*, 717.
[6] A.V. Orlova, L.O. Kononov, B.G. Kimel, I.B. Sivaev, V.I. Bregadze, *Appl. Organometal. Chem.*, 2006, *20*, 416.

The lipopolysaccharide of *Haemophilus influenzae: A* highly adaptive molecule

Derek Hood

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Haemophilus influenzae (*Hi*) is a common human commensal bacterium that is also able to cause disease. Lipopolysaccharide (LPS) is the major glycolipid on the bacterial cell surface and is a critical determinant of colonisation, evasion of host clearance and tissue injury in the pathogenesis of *Hi* diseases, including otitis media (OM). Most episodes of OM are caused by unencapsulated, nontypeable (NT) strains. Investigation into the genetics of LPS biosynthesis and the structure of the molecule has been of crucial importance to understanding its biological roles. LPS biosynthetic gene discovery, together with the construction of defined mutants, has enabled detailed elucidation of the structure of the highly variable glycoforms that are a hallmark of *Hi* LPS. We have focussed our research on an epidemiologically diverse collection of both disease and carriage NT*Hi* strains obtained for the Finnish Otitis Media Study Group. The extraordinary diversity in LPS structure seen across strains is due to the heterogeneity of sugars and other substituents comprising the oligosaccharide outer core extensions. These extend from a conserved tri-heptose containing inner core structure that is common between strains. This diversity of LPS structure is determined by differences in the repertoire of biosynthesis genes between strains, DNA sequence (allelic) polymorphisms and importantly by phase variation. The phase variable expression of multiple LPS epitopes in any given strain is mediated through slippage of simple sequence (tetranucleotide) repeats within a subset of LPS biosynthetic loci. The structural complexity of *Hi* LPS glycoforms makes detailed structural determination absolutely key to any study. An important challenge is to determine how this abundant structural diversity determines biological variation in the adaptive biology of *Hi.*

As an exemplar of how structure-function correlates of LPS inform on pathogenesis, we have studied the role of sialylated glycoforms in the pathogenesis of NT*Hi* OM. Abrogation of LPS sialylation results in severe attenuation of *Hi* in an *in vivo* model of infection and sialylation of LPS impedes complement-dependent host clearance of the bacterium. Sialylation of *Hi* LPS is dependent upon scavenging the required N-acetyl neuraminic acid (Neu5Ac) precursor from the host and the action of up to 4 independent sialyltransferases present in any individual strain. Neu5Ac is also a potentially important carbon and energy source for *Hi* and has been hypothesised as being important for biofilm formation. *Hi* has evolved a system for controlling the balance between incorporation of Neu5Ac into LPS to generate sialylated glycoforms and utilizing the sugar as a catabolite, through genetic regulatory mechanisms.

Hi LPS sialylation and other structural diversity have implications for both the commensal and pathogenic behaviours of the bacterium.

Diversity of the bacterial glycome

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Bacteria synthesize glycans which show considerable differences compared to mammalian organisms. Particularly, in contrast to most eukaryotic carbohydrates, bacterial glycans are often composed of repeating units. Their diverse functions range from structural reinforcement to adhesion, colonization and camouflage. As their glycans are typically displayed at the cell surface, they are important interaction partners with the environment, and thus of great biomedical importance.

With this study we present for the first time a systematic analysis of the bacterial glycome derived from Bacterial Carbohydrate Structure Database (BCSDB) content, which covers more than 80% of published bacterial glycan structures. It summarizes the current knowledge of bacterial glycan architecture and reveals putative targets for the rational design and development of therapeutical intervention strategies by the comparison of the structural elements of bacteria and mammals. The study examines the principal glycan heterogeneity of the higher bacterial taxonomic classes, based on the knowledge deposited in BCSDB.

The findings in bacteria are compared to mammalian data taken from GlycoSCIENCES database, showing both similarities and unique features. Distributions of common and unique monosaccharides, typical modifications and deduced structural patterns are analyzed and discussed. Bacterial glycans have approximately tenfold greater variety on both the monosaccharide and disaccharide pattern level, as compared to mammalian glycome. Certain bacterial subclasses contain distinctive features on the monosaccharide, modification and linkage level.

On the monosaccharide level, in concordance with literature data, the main monosaccharides for the different branches of the tree of life could be extracted from the databases. Additionally, we could identify several monosaccharides unique to certain subclasses of bacteria, which could prove useful as molecular markers for these classes. Similarly, certain structural modifications of monosaccharides seem to be totally absent or overrepresented in certain taxonomic classes.

The linkage analysis of bacterial glycans revealed the predominant and non-existing monosaccharide connectivities in the taxonomic sets, which lead to a comparison of the linkage space of bacteria and mammals. This comparison provides an overview of the common and differing transferase activities in bacteria and mammals, thus linking to differential cross-species expression analysis and guiding towards a deeper understanding of immunogenic patterns present in pathogenic bacteria.

Relationships between O-antigens of enteric bacteria

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Bacteria of the genera *Salmonella, Citrobacter, Escherichia,* and *Shigella* are closely related members of the family Enterobacteriaceae. Many of them are well-known human pathogens that are an often reason of diarrhea; some of them cause more severe diseases, e.g., typhoid fever (*Salmonella* Typhi and *Salmonella* Paratyphi), bacilliary dysentery or shigellosis (*Shigella*), hemorrhagic colitis and hemolytic uremic syndrome (*E. coli* O157). Based on O-antigens (O-polysaccharide chains of the lipopolysaccharides), the enteric bacteria are classified into a number of O-serogroups. Most of the O-antigens are heteropolysaccharides built up of linear or branched tri- to hexa-saccharide repeats (O-units). Recent structural studies showed that whereas some O-antigen structures are specific to strains of one genus, some others are shared by two or more genera. The most closely related are the O-antigens of *E. coli* and *Shigella,* more than half of the *Shigella* O-antigens being shared by *E. coli* strains.

Functions of the genes involved with biosynthesis of the O-antigens were tentatively assigned based on DNA sequence data and for some genes confirmed by mutation tests. For most strains, a good correlation was observed with the corresponding O-antigen structures. Particularly, in strains having similar or identical O-antigens, the O-antigen gene clusters were found to be correspondingly related too. The elucidation of the structural and genetic relationships between the O-antigens is important for learning the evolutionary history of the O-antigen diversification in Enterobacteriaceae and for understanding the role of the O-antigens in bacterial virulence. These data may also have impact on improvement of the serological classification of the enteric bacteria.

Structure-function studies of the surface polysaccharide of *Azospirillum lipoferum* type strain Sp59b

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Bacteria of the genus Azospirillum are gram-negative nitrogen-fixing plant-growth-promoting rhizobacteria. It is known that bacterial attachment to plant roots guarantees the effectiveness of a plant-microbial association. This process occurs in two stages, namely adsorption and anchoring, in which an important role is played by surface polysaccharides, present in the capsule and in the outer membrane of the bacterial cell wall. The capsule of *A. lipoferum* type strain Sp59b was shown to contain a lipopolysaccharide-protein complex (LPPC) and a polysaccharide-lipid complex (PSLC). These high-molecular-weight bioglycans were found to contain 2-keto-3-deoxyoctulosonic acid (KDO), 3-hydroxy fatty acids, and carbohydrates. This finding permitted us to suppose that each of these complexes is an extracellular form of lipopolysaccharide (LPS). However, none of the capsular complexes showed a serological cross-reaction with the membrane LPS, as demonstrated by using anti-LPS antibodies. Mild acid degradation of LPPC and PSLC cleaved the lipid portions to give long-chain O-polysaccharides, which were isolated by gel chromatography. Chemical studies of intact and O-deacetylated polysaccharides, including methylation analyses with sodium methylsulphinylmethanide along with 1D and 2D NMR-spectroscopic studies, resulted in determination of the structure of the branched tetrasaccharide repeating unit with three residues of L-Rha (in 60 % of the repeating units; one of them is O-acetylated in position 2) and D-Glc in the side chain. Thereby, the polysaccharides from both LPPC and PSLC of *A. lipoferum* Sp59b have identical structures, which differ from the structure of the O-polysaccharide from the Sp59b membrane LPS composed of a branched hexasaccharide repeating unit with D-Gal, D-Man, and D-Rha at a 2:1:3 ratio.

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The structural diversity of lipopolysaccharides expressed by non-typeable *Haemophilus influenzae* strains 1158 and 1159

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Haemophilus influenzae is a Gram-negative bacterium, which resides on mucosal surfaces particularly in the nasopharynx of humans. *H. influenzae* strains can be subdivided into encapsulated (types a-f) and non-encapsulated (NT*Hi*) forms. NT*Hi* are common cause of otitis media, sinusitis, conjunctivitis and respiratory tract infections. The lipopolysaccharide (LPS) is a major surface component of *H. influenzae* and plays an important role in infection caused by NT*Hi*.

NT*Hi* strains 1158 and1159 were obtained from the same patient with otitis media on the same day from the left and right middle ear. The structure of the LPS of NT*Hi* strains 1158/1159 has been elucidated using NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS) on *O*-deacylated LPS (LPS-OH) and core oligosaccharide (OS) material, as well as ESI-MS^n on dephosphorylated and permethylated OS. The results show that LPS from both strains comprised the common structural element L-α-D-HepIII*p*-(1→2)-[*P*Etn→6]-L-α-D-HepII*p*-(1→3)-[β-D-Glc*p*-(1→4)]-L-α-D-HepI*p*-(1→5)-[*PP*Etn→4]-α-Kdo*p*-(2→6)-Lipid A. ESI-MS^n data demonstrated a very heterogeneous mixture of glycoforms in both strains. The most abundant glycoform contained a D-α-D-Hep*p*-(1→6)-β-D-Glc*p* disaccharide unit linked to HepI and β-D-Glc*p* attached to the HepIII residue. This structure was further substituted by non-carbohydrate residues such as: phosphocholine, phosphoethanolamine, acetate and glycine. The ESI-MS^n experiments also indicated glycoforms that have chain elongation from HepII. The elongation from HepII was only found in glycoforms, which lack the additional heptose in the outer core region. The structures of these glycoforms were confirmed by structural analyses of a 1158*losB2* mutant strain. LosB2 is a candidate to add LD-Hep to the outer core of LPS in strain R2846[1] but instead in strain 1158 it seems to be responsible for addition of DD-Hep.

Furthermore, several sialylated and disialylated glycoforms were identified by capillary electrophoresis coupled to ESI-MS^n (CE-ESI-MS^n) in NT*Hi* strains 1158/1159.

1. S.L. Lundström, J. Li, M.E. Deadman, D.W. Hood, E.R. Moxon, E.K.H. Schweda *Biochemistry,* 47 (2008) 6025-38

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Bacterial polysaccharides, their structures, properties and use

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Bacterial polysaccharides are known for their diversity in structure, monosaccharide components and non-sugar substituents. Both homo- and heteropolysaccharides are produced and there are a large number of different monomers building up repeating units of varying sizes. Bacteria are often used in the production of polysaccharides of commercial value and the polysaccharides have become important in different applications within biotechnology and medicine but also as functional food in which they have for example probiotic properties. Aspects on the polysaccharide structures and their physical properties will be presented as well as on their use in different applications.

SPR studies on the binding bewteen MAb 2G8 and laminarin

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Deep-seated mycoses represent a serious medical problem because the partial inability of antifungal drugs to eradicate the infections in immunocompromised hosts, which leads still to a high mortality rate, particularly in severely immunocompromised patients.
Recently it has been shown that when laminarin (Lam), a poorly immunogenic glucan extracted from the brown alga *Laminaria digitata,* was conjugated to the diphteriae toxoid CRM_{197}, the resulting Lam-CRM conjugate proved to be both immunogenic and protective against infections induced by *Candida albicans.*[1]

An anti-b-glucan monoclonal antibody (MAb 2G8) exhibited to inhibit the growth of b-glucan-exposing *Candida albicans* cells as well to enhance the anti-Candida protection conferred by the Lam-CRM conjugate.[1,2] Laminarin is commonly considered a prototypal β-(1, 3) glucan, yet it presents very short β-(1, 6)-branches which produce a degree of cross reactivity with β-(1, 6) glucans, such as pustulan.

Within the framework of our research project on a glycoconjugate vaccine against *Candida albicans*, we chose SPR as a reliable method to study the specificity of antibodies directed against the Lam-CRM conjugate. After immobilization of MAb 2G83 on a BiaCore CM5 chip by EDC-NHS coupling chemistry, the measured K_D for binding with laminarin was 100-fold smaller than with pustulan, showing a higher specificity for β-(1, 3) linkages. Binding studies between MAb 2G8 and the linear β-(1, 3) glucan curdlan or Lam-CRM conjugates are currently ongoing.

Kinetic constants obtained from these experiments will clarify the role of β-(1, 6) branching in promoting the protective immunity against *Candida albicans*.
BiaCore CM5 chips have been also prepared with a Lam-HSA conjugate and two fully synthetic linker equipped oligosaccharides obtained from Ancora Pharmaceuticals Inc., one presenting a 15 units linear structure and another having a 17 units chain with 5 β-(1, 6) branches.

The Lam-HSA chip showed a higher sensibility compared to the chips covered with synthetic oligosaccharides in detecting anti-β-glucan present in sera obtained from mice immunized with Lam-CRM conjugates, and it is under evaluation as a platform for fast screening of sera coming from immunization studies.

1. A. Torosantucci, C. Bromuro, P. Chiani, F. De Bernardis, F. Berti, C. Galli, F. Norelli, C. Bellucci, L. Polonelli, P. Costantino, R. Rappuoli, A. Cassone J. Exp. Med. 202 (5), 2005, 597–606.
2. A. Rachini, D. Pietrella, P. Lupo, A. Torosantucci, P. Chiani, C. Bromuro, C. Proietti, F. Bistoni, A. Cassone, A. Vecchierelli Infect. Immun. 75 (11), 2007, 5085–5094.
3. MAb 2G8 was kindly provided by A. Cassone Department of Infectious Diseases, ISS, Rome.

Structural and physico-chemical characterisation of dextran from *Weissella confusa* E392

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Dextrans are homopolysaccharides with a linear backbone made of α-(1→6) linked D-glucopyranosyl units and α-(1→2), α-(1→3) or α-(1→4)-linked branches.[1-3] Unlike most exopolysaccharides, dextrans are synthesized extracellularly from sucrose by dextransucrases. Commercially produced dextran is used for various purposes in its native or chemically modified form. Native dextrans of different molecular weights serve as standards for size exclusion chromatography and as ingredients in cosmetics and food products. Chemcial modification is used to produce bioactive dextran derivatives and in the production of Sephadex columns.[4] The structure of a specific dextran depends on the producing microbial strain. *Leuconostoc, Streptococcus*, *Lactobacillus* and *Weissella* are the main species that secrete dextrans. The aim of this study was to investigate that structure and physico-chemical properties of dextran produced by *Weissella confusa* E392. For comparison, dextran from *L. mesenteroides* B512F was utilized. Results from the analysis of dextran from *W. confusa* E392 and *L. mesenteroides* B512F by NMR spectroscopy will be presented. Additionally, the intrinsic viscosity, molecular weight and Mark-Houwink co-efficient of the dextrans determined by high performance size exclusion chromatography with a multiple detection system (HPSEC-MD) and rheology measurements will be shown.

1. Seymour, F. R.; Knapp, R. D. *Carbohydrate Research* 1980 (51)*, 1,* 67-103.
2. Cheetham, N. W. H.; Fiala-Beer, E.; Walker, G. J. *Carbohydrate Polymers* 1990*, 2,* 149-158.
3. Naessens, M.; Cerdobbel, A.; Soetaert, W.; Vandamme, E. J. *Journal of Chemical Technology & Biotechnology* 2005*, 8,* 845-860.
4. Heinze, T.; Liebert, T.; Heublein, B.; Hornig, S. Functional Polymers Based on Dextran. In *Polysaccharides II;* Klemm, D., Ed.; Springer Berlin: Heidelberg, 2006; Vol. 205, pp 199-291.

Synthesis of Lipopolysaccharide structures from *Nesseria meningitidis*

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The lipopolysaccharide of *Nesseria meningitidis* are of the rough type, i.e., it lacks the polysaccharidic O-antigen component and contains only the core and the Lipid A part.[1] The core part of these bacteria show a lot of heterogeneity but a conservative inner core structures have been identified (Fig 1).

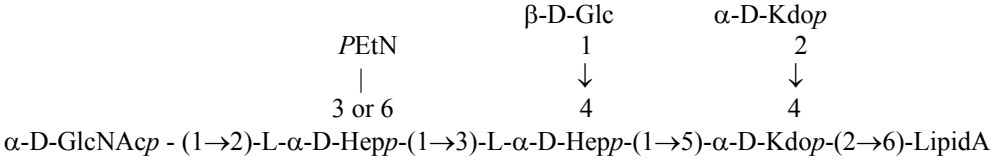

Figure 1. Structure of the conserved inner core of *N. meningitidis* LPS.

As part of a programme aiming at developing glycoconjugate vaccines based on the LPS structures, we are synthesising oligosaccharides corresponding to these motifs. Both outer and inner core motifs as well as lipid A parts are included in the target molecules. The syntheses of compounds corresponding to the conserved structure of *N. meningitidis* LPS will be presented. The syntheses go through a common trisaccharide intermediate[2] and its conversion[3] to target structures will be discussed (Fig. 2).

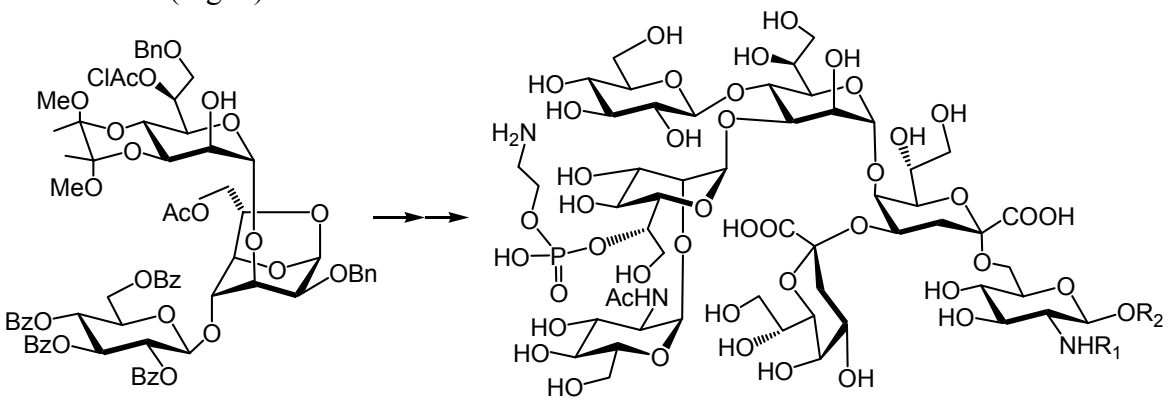

Figure 2. Structure common trisaccharide intermediate and core of *N. meningitidis* LPS.

[1] A. D. Cox, J.C. Wright, M. A. J. Gidney, S. Lacelle, J. S. Plested, A. Martin, E. R. Moxon and J. C. Richards. *Eur. J. Biochem*. (2003) *270*, 1759.
[2] E. Segerstedt, K. Mannerstedt, M. Johansson and S. Oscarson, *J. Carbohydr. Chem*. (2004) *23*, 443.
[3] K. Mannerstedt, E. Segerstedt, J. Olsson and S. Oscarson, Org. Biomol. Chem. (2008) *6*, 1087.

Serotyping of clinical isolates belonging to *Proteus mirabilis* serogroup O36 and structural elucidation of the O36-antigen polysaccharide

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The O-specific polysaccharide (OPS) isolated from the lipopolysaccharide of *Proteus mirabilis* O36 was found to have a pentasaccharide repeating unit of the following structure:
→2)-β-D-Rib*f*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*NAc6Ac-(1→4)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→.
The structure is unique among *Proteus* OPS, which is in agreement with the classification of this strain into a separate *Proteus* O-serogroup. Remarkably, the *P. mirabilis* O36-polysaccharide has the same structure as the OPS of *Escherichia coli* O153 except that the latter is devoid of *O*-acetyl groups. In the present study, two steps of serotyping *Proteus* strains are proposed: screening of dry mass with ELISA and immunoblot with the crude LPSs. This method allowed serotyping 99 *P. mirabilis* strains infecting human urinary tract. Three strains were classified to serogroup O36. The migration pattern of these LPSs fraction with long O-specific PSs was similar to the standard laboratory *P. mirabilis* O36 (Prk 62/57) LPS. The relatively low number of clinical strains belonging to serogroup O36 did not correspond to the presence of anti-*P. mirabilis* O36 antibodies in the blood donors' sera. Twenty-five percent of tested sera contained statistically significant elevated level of antibodies reacting with thermostable surface antigens of *P. mirabilis* O36. The presence and amount of antibodies was correlate with Thr399Ile TLR4 polymorphism types (p=0,044).

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POSTERS

Synthesis of murein fragment of *S. aureus* cell wall

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Staphylococcus aureus also has known as "Golden Cluster Seed" or golden staph, is responible of many kind of staph infections. It is a Gram-positive bacteria, frequently living on the skin or in the nose of a person. Approximately 20–30% of the general population are "staph carriers", but staph infections, including MRSA and VRSA, occur most often among persons in hospitals and healthcare facilities (such as nursing homes and dialysis centers) who have weakened immune systems.[1)]

The most important component of bacterial cell wall is murein also known as peptidoglycan. It is compoused by polysacchride chains linked to short peptide components which occupy about 80-90 % the whole structure. Polycarbohydrets fragments are construct by disacchride repaeting units (N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-muramic acid (MurNAc), linked together by β(1→4) O-glycoside bond), where the carboxyl group of MurNAc is also the point of linkage to the peptide fragments. Peptide fragment contains both D- and L-amino acids, usually is a pentapeptide fragment: L-Ala-D-iGlu-L-Lys-D-Ala-D-Ala. Peptide subunits are cross-linked to other peptides which come by a neighboring glycan strand. Glycan strand can conteins from 5 to 30 subunit, but this depending on the bacterial species. In *S. aureus* the glycan strands are connected by pentaglycine bridge.[2,3)]

Even those that the Gram-positive bacteria structure is quite simple, the good method of treating against this bacteria no exist and nowadays there are many new strains expressing increasing levels of resistance to the glycopeptide antibiotics. The most popular antibiotic used against *S. aureus* is vancomycin. It has been used clinically since 1956. Mechanism of action this antybiotic is lining on selectivly bind with prekursor of peptidoglycan fragments UDP-MurNAc- L-Ala-D-iGlu-L-Lys-D-Ala-D-Ala, exactly the terminal fragment D-Ala-D-Ala by five hydrogen bonds, creating a stable complex. This complex regulate the biosynthesis by stop growing and accumulating murein fragments, but also prevent the degradation of vancomycin on complex CDP-I and CDP-II. Need only replacement the last residue D-Ala on D-Lac or D-ser ones to lose the activity about 1000-times/hold.[4,5,6)]

It is very important recognized the whole interactions between vancomycin and bacterial cell wall. This is a reason why we present the chemical synthesis of native peptidoglycan fragment build by two important fragments (the sugar fragments and peptide fragments: MurNAc- L-Ala-D-iGlu-L-Lys-D-Ala-D-Ala) which will be used to compoused a new method of study interactions between bacteria cell wall fragments and vancomycin. The sugar fragments (MurNAc derivative) was prepared on two ways: using the *N*-acetyl-D-glucosamine or D-glucal like a substrate, the peptide fragments was prepared using standard procedure used in peptide chemistry.

1. Heyman, D. *Control of Communicable Diseases Manual* 18th Edition. Washington DC: American Public Health Assocation, 2004.
2. Navarre W.W.; Schneewind O. *Microbiology and Molecular Biology Reviews*, 1999, **63(1)**, 174-229.
3. Williams D.H., Bardsley B. *Angew. Chem. Int. Ed.*, 1999, **38**, 1172-1193.
4. van Heijenoort, J. *Glycobiology*, 2001, **11(3)**, 25R-36R.
5. Adamczyk, A.; Grote, J.; Moore, J.A.; Rege, S.D.; Yu, Z. Bioorganic&Medical Chemistry Letters, 2000, **10**, 1613-1615.
6. McAtee, J. J. ; Castle, S.L.; Jin, Q.; Boger**,** D.L. *Bioorganic & Medicinal Chemistry Letters***,** 2002, **12(9)**, 1319-1322

Structural determination of the enteroaggregative *Escherichia coli* O175 O-antigen

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Lipopolysaccharides (LPS) are anchored to the outer cell wall in Gram-negative bacteria and can cause immunological response in a host organism. The LPS is divided into three regions: the lipid A, the core oligosaccharide and the O-antigen, which is specific for each serotype. The O-antigen polysaccharide consists of repeating units, ranging in size from 2 to 7 monosaccharides. Determination of these repeating units gives the means to increase the knowledge about the function of the LPS.

Escherichia coli O175:H18 was first isolated from a case of human diarrhea. Analyses of virulence factors indicate that this strain belongs to the group of enteroaggregative *E. coli*. The O-antigen of O175 has shown cross-reactions to *Escherichia coli* O22 and O83, meaning that they share antigenic determinants. NMR-analysis of the E. coli O175 O-antigen shows that it contains five sugar residues in the repeating unit, one of which is an N-acetylated amino-sugar, anticipated to determine the biological repeating unit. The primary structure of the repeating unit was determined by 1D and 2D NMR experiments.

Structural and immunochemical studies of exopolysaccharide of *Lactobacillus johnsonii* strain 142 isolated from mice with inflammatory bowel disease

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Some strains of *Lactobacillus* genus, the lactic acid bacteria, which are also natural inhabitants of human gastrointestinal tract, show probiotic activity on the host, i.e. the beneficial influence on its health [1]. Lactic acid bacteria (LAB) are known to synthesize polysaccharides as cell wall components and storage polymers, and in many species, as extracellular macromolecules – exopolysaccharides (EPS). The physical properties of these biopolymers provide a highly viscous local environment for the bacteria, trapping water and nutrients, helping bacteria to survive in otherwise hostile environments [2,3,4]. This cell surface antigens are also thought to play an essential role in the adhesion phenomenon. It has been shown that EPSs from LAB can modify the adhesion of pathogenic bacteria to intestinal mucus [5,6]. During the investigation concerning the immunological activity of various exopolysaccharides from probiotic bacterial strains, the information about the molecular structure of the polysaccharide is essential. Whilst structures of a number of LAB EPSs are known [7]. Our studies are focused on *Lactobacillus* strains isolated from healthy mice and from mice with experimentally induced inflammatory bowel disease (IBD). IBD is actually a group of gastrointestinal disorders, usually chronic in nature, which are characterized by an increase in the number of inflammatory cells found in the lining of the stomach or intestinal tract. The causes of IBD are variable, and often no single cause can be identified in a given patient. Comparative studies of the EPS from *Lactobacillus* strains isolated from healthy and IBD mice may shed the light on the mechanisms of the pathogenesis of this disease. Here we report the structural and immunochemical data on the extracellular carbohydrate material from Lactobacillus johnsonii strain 142, which was isolated from the mouse with experimentally induced IBD.

1. Ruas-Madiedo P., J. Hugenholtz, and P. Zoon. 2002. Int. Dairy J. 12:163–171.
2. Granato D., F. Perotti, and I. Masserey.1999. Appl. Environ. Microbiol. 65:1071–1077.
3. Del Re B., B. Sgorbati, M. Miglioli, and D. Palenzona. 2000. Lett. Appl. Microbiol. 31:438–442.
4. Mukai T. and S. Kaneko. 2004. Int. J. Food Microbiol. 90:357–362.
5. Fooks L. J., Fuller, R. and G.R. Gibson. 1999. Int. Dairy J. 9:53–61.
6. Vesterlund S., M. Karp, S. Salminen and A.C. Ouwehand. 2006, Microbiol.-Sgm 152:1819–1826.
7. Górska S., P. Grycko, J. Rybka and A. Gamian. 2007. Postêy Hig. Med. Dosw. 61:805-818.

Resolving the structure of *Yersinia Pseudotuberculosis* O:9 o-specific polysaccharide repeating unit to elucidate the receptor structure of phage ϕR1-37

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Bacteriophage ϕR1-37 uses the LPS outer core hexasaccharide of *Yersinia enterocolitica* O:3 (Ye O:3) as a receptor. Its three proximal residues, Gal, GalNAc and the recently elicited 4-keto sugar Sug*p*, comprise the minimal requirement for the phage receptor. Surprisingly, a smooth strain of *Y. pseudotuberculosis* O:9 (Ypstb O:9) is also ϕR1-37 sensitive, whereas its R mutants are resistant to the phage. Therefore, at least *part* of the phage receptor must be located in the O-specific polysaccharide (OPS) of Ypstb O:9. We also expected that the phage receptor would be a common structural motif present in both the Ye O:3 and Ypstb O:9 LPSs. While the structure of Ye O:3 LPS is fully elucidated that of Ypstb O:9 is completely unknown.

Aim: To determine structure of the OPS repeating unit of Ypstb O:9 LPS.

Materials and Methods: Ypstb O:9 OPS was isolated from LPS by mild acid hydrolysis and separation using Sephadex G-50. The chemical and structural studies were carried out using colorimetry, gas chromatography, mass spectrometry and nuclear magnetic resonance (NMR) techniques.

Results: The sugar component analyses of LPS demonstrated the presence of Glc, Gal, GlcN in great abundance and smaller amounts of 6-deoxy-hexosamine and hexosaminuronic acid. The NMR studies revealed the acyl group substitutions and allowed the final determination of the Ypstb O:9 OPS repeating unit structure as a branched tetrasaccharide composed of 3-*O*-acetyl-*N*-acetylglucosaminuronic acid, *N*-acetimidoylfucosamine, *N*-acetylglucosamine and galactose.

Conclusions: As galactose is the only identical sugar residue present in both the Ypstb O:9 OPS repeating unit and the Ye O:3 outer core hexasaccharide, the structural motif that serves as ϕR1-37 receptor could not be resolved simply by comparison of the sugar residues and glycosidic linkages. Therefore it is likely that the phage receptor of Ypstb O:9 is formed by spatially correctly oriented side groups in the sugars and not by identical sugar residues. These possibilities will be discussed.

Revised structure of the repeating unit of the O-specific polysaccharide from *Azospirillum lipoferum* strain SpBr17

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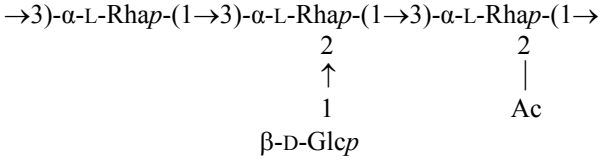
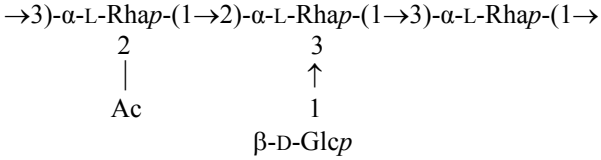
Azospirilla are Gram-negative diazotrophs living in close association with roots of grasses, cereals and other monocotyledonous plants. They are known as plant-growth-promoting-bacteria as they secrete into the rhizosphere many active substances which have a positive influence on plant development and growth. Up to now, seven species belonging to the *Azospirillum* genus have been described: *Azospirillum lipoferum*, *A. brasilense, A. amazonense*, *A. halopraeferens*, *A. irakense*, *A. largimobile* and *A. doebereinerae*. Surface polymers such as EPS, CPS and LPS play an important role in the interaction between a plant and bacteria. Studies concerning the chemical characteristics of azospirillum lipopolysaccharides, the main constituent of the outer leaflet of the outer membrane of those bacteria, have been performed only for four strains: *A. lipoferum* SpBr17 and Sp59b, *A. brasilense* Sp245 and S17, as well as *A. irakense* KBC1. The structure of the O-specific polysaccharide from *A. lipoferum* SpBr17 has been presented in poster form at the International Congress on Nitrogen Fixation in Cancun (Mexico, 1992). The polysaccharide chain was found to be a rhamnan containing exclusively α(1→3) glycosidic bonds. This structure was determined using methylation analysis and ^{13}C NMR data and is depicted below:



In the present study, the polysaccharide was reinvestigated using ^{1}H and ^{13}C NMR spectroscopy, including HSQC, HMBC and NOESY experiments for linkage and sequence estimation. The revised structure of the *A. lipoferum* SpBr17 O-specific polysaccharide repeating unit was established as follows:



Synthesis of glycoconjugates derivatives of flavonoids

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Soy isoflavonoids such as daidzein and genistein have recently attracted icreased attention for their estrogenic activity, and their potention for use in menopausal hormone replacement therapy [1] and the treatment of cancer has been suggested [2].
Our aim is to synthesize glycoconjugates derivatives of genistein. Biological experiments have proven their biological activity as antitumour agents [3].

Here we report a brief synthesis of glycoconjugates that can be conducted on large scale to produce over 5g of the find product.

The principal aim of our studies was to synthesis of glycoconjugates, which were the sugar part joined with isoflavon through the carbonic chain. The reaction conditions were optimized and we obtained products with high α-stereoselectivity.

[1] Adlercreutz H., Mousave Y., et al., *J. Steroid Biochem. Med. Biol*., **(1992)**, 41, 331
[2] Lamartiniere, C.A., *Am. J. Clin. Nutr*., **(2000)**, 71, 1705; Polkowski, K., et al., *Cancer Letters*, **(2004)**, 203, 59-69; R.A. Dixon, Isoflavonoids: Biochemistry, Molecular Biology and Biological Functions, in *Comprehensive Natural Product Chemistry* (D.H.R. Barton, Ed.,) Elsevier,vol.1 **(1999)** 773
[3] Polish Patent Appl.

Application of glycosyl thiocarbamates in orthogonal glycosidation

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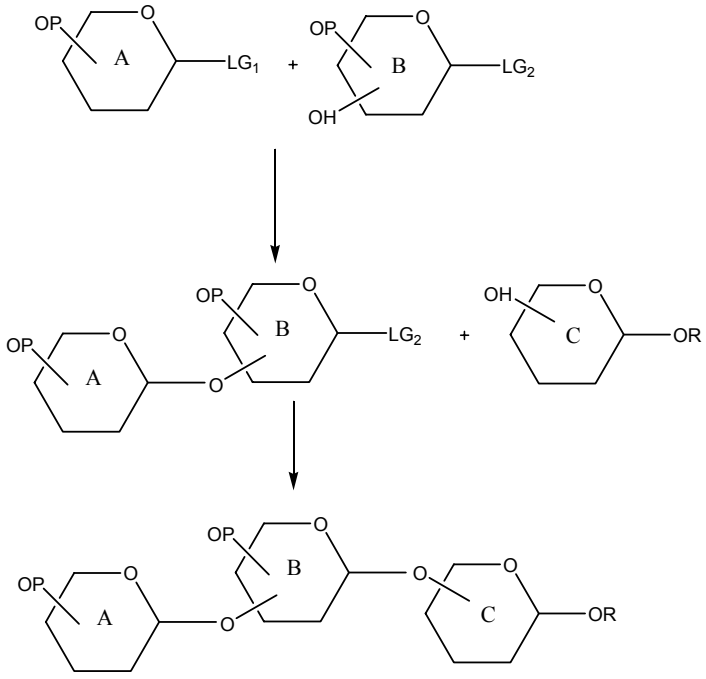
In orthogonal glycosidation a range of glycosyl donors that bear different leaving groups (LG^1/ LG^2) and that can be selectively activated in the presence of each other, are utilized. A highly reactive donor is required for the first glycosidation in the orthogonal sequence [1].
The O-glycosyl-N-allyl thiocarbamates (A) readily obtained from anomerically-unprotected sugars [2] are very reactive glycosyl donors. They can be readily activated with bromine. On the other hand the O-glycosyl-N-metyl thiocarbamates (B) can be used as glycosyl acceptors. They can be activated with thiophilic reagents. The application this method to the synthesis of trisaccharides will be presented.



LG_1: OC(S)NHAllil
LG_2: $OC(S)NHCH_2$

[1] J. Pietruszka in Carbohydrates (H.M.L. Osborn Ed.) Academic Press, 2003, 219-239
[2] Barili P.L., Catelani G., D'Andrea F., De Rensis F., Falcini P. *Carbohydrate Res.* **1997**, *298*, 75-84; Ott A.J., Brackhagen M., Davtyan A., Nolting B., Boye H., Schoknecht A. and Vogel Ch. J. Carbohydrate Chemistry, **2001**, *20*, 611-636; Abbas S.A., Barlow J.J. and Matta K.L. *Carbohydrate Res.* **1981**, *88*, 51-60, Kasprzycka A., Ślusarczyk A. and Szeja W. *Polish J. Chem.* **2001**, *75*, 1303-1308; Kasprzycka A. and Szeja W. *Polish J. Chem.* **2005**, *79*, 329-333

Periplasmic β-glucan from *Bradyrhizobium elkanii*

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Bradyrhizobium elkanii, a slow-growing rizobia, create nitrogen-fixing nodules on soybean plant (*Glycine max*). Extracellular polysaccharides (EPS), lipopolysaccharides (LPS) as well as β-glucans are critical for an effective symbiosis in *Rhizobium* species. For example, *Bradyrhizobium japonicum* which was not capable to synthesis β-(1-3),(1-6) glucans was also defective in motility, sensitive for hypoosmotic medium, and formed ineffective nodules. Other strain, a mutant producing truncated β-glucan was not able to create effective symbiosis. These observations lead to conclusion that not only the type but appropriate structure is important for establishing an effective symbiotic relationship.
Periplasmic, cyclic β-glucans isolated from *B. elkanii* USDA 76 strain cells are composed of 10 - 13 glucose residues. The main fraction contain oligosaccharides built up of 12 hexose units (M_w= 1945.363 Da). Glucose residues are linked by β-(1-3) or β-(1-6) type of glycosidic bound. The ratio of β-(1-3) to β-(1-6) linked glucose is 1:2. Methylation analysis proved presence of terminal, non-reducing as well as branched (3 and 6 substituted) glucoses. Thus, the basic structure of investigated compounds are similar to *Bradyrhizobium japonicum* and *Azorhizobium caulinodans* periplasmic β-glucan. Cyclic oligosaccharides from *B. elkanii* are substituted presumably by two phosphocholine moieties per ring. Investigated β-glucans are rich decorated with acetyl and succinyl residues. Those substituents are arranged diversly in the β-glucan molecules population.

Glucan concentration in *B. elkanii* periplasmic space is osmotically regulated and increase in response to a decrease of medium osmolarity.

Purification and preliminary structural investigation of exopolysaccharides from *Pseudomonas mutabilis* biofilms

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During the fermentation of a mutant strain of *P. mutabilis* (IR2#2), genetically constructed for the overproduction of a biotin, variable colony and cell morphologies were repeatedly observed during batch and continuous cultures. Transparent colony variants appeared on Luria Bertani (LB) agar plates with antibiotic selection after overnight incubation at 37°C from a sixth day fermentation sample. Microscopic observations of cell morphology revealed twitching motility and cell aggregation. It was initially determined from these observations and research of published accounts that the change in colony morphology of IR2#2 was possibly indicative of biofilm formation. In this study, the variant colony, we refer to as "time 6 ghost" (T6), was isolated and characterized in comparison to wild- type *P. mutabilis* (ATCC 31014), the mutant *P. mutabilis* #1F9 (acquired through chemical mutagenesis of wild-type *P. mutabilis*), and IR2#2 (transformed with plasmid containing biotin operon). Biofilm formation of T6 was indirectly evaluated through crystal violet (CV) staining and compared to all other aforementioned strains, as well as wild-type *P. aeruginosa.* The results confirmed that T6 was stable for biofilm production for at least five repetitive agar platings. The CV staining indirectly confirmed the formation of biofilm with an increased level of CV per cell density in comparison to other strains studied.

Exopolysaccharides (EPSs) were isolated from *P. mutabilis* biofilms using glutaraldehyde extraction. DNAse and Proteinase K was added to remove contaminating DNA and protein. Size exclusion chromatography was used to exopolysaccharides purification. The sugar components of EPS were identified by GLC, GLC-MS and HPLC method. Preliminary elucidation of the structure of *P. mutabilis* exopolysaccharides were also carried out by methylation analysis.

Approaches to glycoconjugate vaccines based on synthetic *Cryptococcus neoformans* CPS Structures

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The fungi *Cryptococcus neoformans* is an opportunistic species causing severe diseases, i.e. meningitis, and death, especially in immunodeficient patients. *C. neoformans* is divided into different serotypes, A-D, depending on the structure of the GXM surface capsular polysaccharide (CPS), which is an important virulence factor. Conjugation of *C. neoformans* capsular polysaccharide to proteins results in highly immunogenic compounds that can elicit high titer antibody responses. However, because of the heterogeneity in the GXM structure there are difficulties associated in consistently eliciting protective antibody responses using GXM polysaccharide based vaccines.

One alternative approach is to synthesize well-defined oligosaccharide-based vaccines to find immunogenic and protective motifs. Because of the repetitiveness of the GXM polysaccharide structures a block synthesis approach is utilized. Earlier all the necessary di, tri -saccharide blocks have been synthesised as thioglycosides and their subsequent couplings carried out carried out to give up to heptasaccharide structures (Fig 1) [1]. The synthetic oligosaccharide target structures are equipped with a spacer to allow efficient conjugation to a carrier protein, which has been performed to give vaccine candidates [2].

We now report improved synthetic pathways [3] to the different building blocks and the synthesis of a tetrasaccharide block.

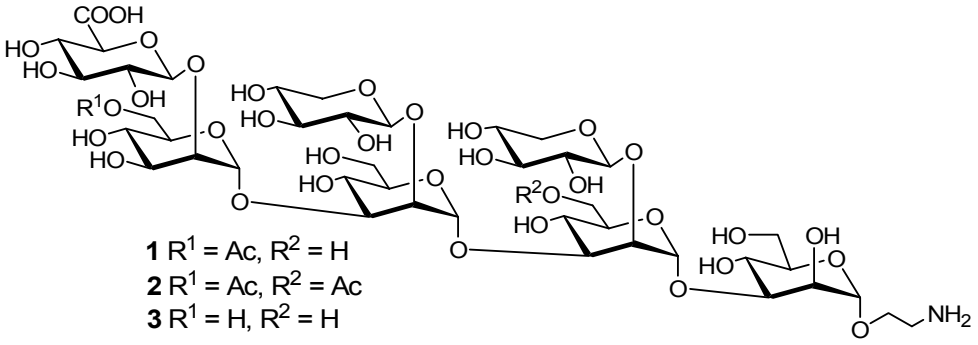

Fig 1. Synthesised heptasaccharide structure with variant acetylation pattern.

[1] M. Alpe, P. Svahnberg, S. Oscarson. *J. Carbohydr. Chem*, 2003, *22*, 565; 2004, *23*, 403.
[2] S. Oscarson, M. Alpe, P. Svahnberg, A. Nakouzi, A. Casadevall, *Vaccine*, 2004, *23*, 3961
[3] J Vesely, L Rydner, S Oscarson, *Carbohyd. Res*., 2008, *12*, 2200

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