2nd Baltic Meeting on Microbial Carbohydrates

2-4 October, 2006
Rostock, Germany

Preface

In 1988, a meeting in Borstel (*Kdo-Meeting*) dedicated to one of the most important sugars in bacterial lipopolysaccharides, namely Kdo, was started and took place annually afterwards. After a few years the contents of the meeting extended to the whole field of carbohydrate chemistry and biochemistry. The Kdo-Meeting was proven to be one of the most important smaller meetings in that field in Europe. After 1995, the meeting was organized by Dr. Holst. In parallel to this meeting in Borstel, the German-East European Carbohydrate Workshop was founded at the University of Rostock. In particular, this workshop was dedicated to young researchers and to colleagues from East European countries in terms of a platform for the presentation of their work. After 1998, the workshop was organized by Dr. Vogel. In early 2001 Dr. Holst and Dr. Vogel agreed to merge both symposia to the new meeting *The Carbohydrate Workshop* which occurred annually alternating in Borstel and in Güstrow, a town close to Rostock. The meetings in Borstel (2002, 2004) were focused more on the topics that deal with bioorganic, natural product and medical chemistry of carbohydrates, whereas meetings held in Güstrow (2001, 2003) were focused on synthetic carbohydrate chemistry.

A second platform for the exchange of the latest knowledge on the chemistry and biochemistry of bacterial carbohydrates was the *Russian-Polish-German Meeting on Bacterial Carbohydrates*, held 2000 in Borstel, 2002 in Moscow, and 2004 in Wroclaw. During the *3rd German-Polish-Russian Meeting 2004* in Wroclaw (*1st Baltic Meeting on Bacterial Carbohydrates*) it was agreed by all organizers of the various above mentioned meetings to merge *The Carbohydrate Workshop* and the *Russian-Polish-German Meeting on Bacterial Carbohydrates* to the ***2nd Baltic Meeting on Microbial Carbohydrates*** which is taking place now at the University of Rostock.

We hope that our symposium will meet the interests of European scientists that deal with carbohydrate chemistry and biochemistry, and that The *Baltic Meeting on Microbial Carbohydrates* will work its way up the ladder to a highly successive and informative meeting. With this in mind, we cordially invite you to participate in this meeting at the University of Rostock in the new Institute of Chemistry.

Andrzej Gamian ***Otto Holst*** ***Yuriy Knirel*** ***Christian Vogel***

PROGRAM

Wednesday, October 4

14:00 – 21:00 **Registration**

19:30 **Informal Get-Together and snack**

Thursday, October 5

08:50 **Christian Vogel**, *Rostock, Germany*
OPENING

09:00 **Thomas Strothotte**, *President of Rostock University, Germany*
WELCOME ADDRESS

Chairperson: **Otto Holst**, *Borstel, Germany*

09:20 **Stefan Oscarson**, *Stockholm, Sweden*
APPROACHES TOWARDS ANTI-MICROBIAL GLYCO-CONJUGATE VACCINES BASED ON SYNTHETIC OLIGOSACCHARIDE STRUCTURES

09:50 **Rikard Slättegård**, *Stockholm, Sweden*
SYNTHESIS OF STABLE C-PHOSPHONATE ANALOGUES OF *NEISSERIA MENINGITIDIS* TYPE A CAPSULAR POLY-SACCHARIDE STRUCTURES

10:05 **Lina Rydner**, *Stockholm, Sweden*
APPROACHES TOWARDS A GLYCOCONJUGATE VACCINE AGAINST *CRYPTOCOCCUS NEOFORMANS* BASED ON SYNTHETIC OLIGOSACCHARIDE STRUCTURES.

10:20 **Johan Olsson**, *Stockholm, Sweden*
SYNTHESIS OF OLIGOSACCHARIDES CORRESPONDING TO INNER CORE LPS-STRUCTURES FROM *H. INFLUENZAE* AND *N. MENINGITIDIS*

10:35 **Coffee break**

Chairperson: **Andrzej Gamian**, *Wroclaw, Poland*

11:00 **Wieslaw Kaca**, *Kielce, Poland*

SEROTYPING OF CLINICAL *PROTEUS MIRABILIS* STRAINS BASED ON CHEMICALLY DEFINED OF O-SPECIFIC PART OF LIPOPOLYSACCHARIDES

11:30 **Iwona Konieczna**, *Kielce, Poland*
THE REACTION OF ANTIBODIES WITH PEPTIDES MIMICKING ANTIGENIC EPITOPE IN *HELICOBACTER PYLORI* UREASE

11:45 **Michal Arabski**, *Kielce, Poland*
THE HUMAN COMPLEMENT ACTIVATION BY SMOOTH AND ROUGH *PROTEUS MIRABILIS* STRAINS AND THEIRS LIPOPOLYSACCHRAIDES

12:00 **Cordula Lembke**, *Rostock, Germany*
CONTRIBUTION OF CARBOHYDRATES IN STREPTOCOCCAL BIOFILM FORMATION

12:15 **Photo date and lunch**

Chairperson: **Andrei V. Nikolaev**, *Dundee, Scotland, United Kingdom*

13:30 **Anna M. Shpirt**, *Moscow, Russian Federation*
TOTAL SYNTHESIS OF SIALIC ACID POLYPRENYL PHOSPHATE, A PROBABLE BIOSYNTHETIC INTERMEDIATE OF BACTERIAL POLYSIALIC ACID

13:45 **Anna V. Orlova**, *Moscow, Russian Federation*
A NOVEL GLYCOSYL DONOR — *N,N*-DIACETYLNEURAMINIC ACID GLYCOSYL CHLORIDE

14:00 **Lidia A. Nazarova**, *Moscow, Russian Federation*
SYNTHESIS OF O-BENZYLATED SIALIC ACID DERIVATIVES WITH VARIOUS SUBSTITUENTS AT N(5) AS POTENTIAL GLYCOSYL DONORS

14:15 **Nikolay N. Kondakov**, *Moscow, Russian Federation*
SYNTHESIS OF NOVEL LACTOSE—DODECABORATE CONJUGATES AS POTENTIAL AGENTS FOR BORON NEUTRON CAPTURE THERAPY

14:30 **Leonid O. Kononov**, *Moscow, Russian Federation*
INTERMOLECULAR HYDROGEN BONDING PATTERN IN SOLUTIONS OF SIALIC ACID DERIVATIVES DRAMATICALLY INFLUENCES THEIR PROPERTIES

14:50 **Nadezda Yu. Kulikova**, *Moscow, Russian Federation*
CAN HYDROGEN BONDED AGGREGATES OF N-ACETYL-GLUCOSAMINE DERIVATIVE BE SEPARATED?

15:05 **Vera Zimmermann**, *Rostock, Germany*
OPTIMISATION OF NEURAMINIC ACID SYNTHESIS BY APPLICATION OF IN SITU PRODUCT REMOVAL

15:20 **Coffee break**

Chairperson: **Buko Lindner**, *Borstel, Germany*

15:40 **Daniela Hameister**, *Rostock, Germany*
CARRIER FACILITATED EXTRACTION OF CARBOHYDRATES FROM AQUEOUS SOLUTION

15:55 **Ralf-Jörg Fischer**, *Rostock, Germany*
CARBOHYDRATES AND THE INITIATION OF SPORULATION
IN *CLOSTRIDIUM ACETOBUTYLICUM*

16:10 **Göran Hübner**, *Borstel, Germany*
STRUCTURAL ANALYSIS OF GLYCOLIPIDS BY HIGH RESOLUTION FOURIER TRANSFORMED MASS SPECTROMETRY (FT-MS)

16:25 **Anna Kondakova**, *Moscow, Russian Federation*
HIGH-RESOLUTION MASS SPECTROMETRY AS A TOOL FOR STRUCTURAL ELUCIDATION OF BACTERIAL LIPOPOLY- SACCHARIDES

16:40 **Elena Ciliberti**, *Borstel, Germany*
ANALYSIS OF DUST FROM COWSHEDS: PRESENCE OF ALLERGY PROTECTIVE COMPONENTS?

16:55 **Kay Vogel**, *Borstel, Germany*
ROLE OF CARBOHYDRATES FROM BACTERIAL AND FUNGAL SPORES IN ALLERGY PROTECTION

17:10 **Bernward Rittgerodt**, *Berlin, Germany*

SEPARATION OF BINARY MIXTURES WITH SIMULATED MOVING BED (SMB)

17:35 **Coffee break**

Chairperson: **Wieslaw Kaca**, *Kielce, Poland*

18:00 **Anna Hanuszkiewicz**, *Borstel, Germany*
STRUCTURAL INVESTIGATION OF THE LIPOPOLYSACCHARIDE FROM *ACINETOBACTER LWOFFII* F78

18:15 **Malte Hammer**, *Borstel, Germany*
INFLUENCE OF THE CORE OLIGOSACCHARIDE OF LIPOPOLY- SACCHARIDES ON THE ANTIBACTERIAL ACTION OF POLY- MYXIN B

18:30 **Yuliya P. Fedonenko**, *Saratov, Russian Federation*
IMMUNOCHEMICAL CHARACTERIZATION OF THE LIPO- POLYSACCHARIDES FROM A GROUP OF *AZOSPIRILLUM BRASILENSE* STRAINS

18:45 **Evelina L. Zdorovenko**, *Moscow, Russian Federation*
EXTRACELLULAR AND CELL-WALL-BOUND LIPOPOLY- SACCHARIDES: DIFFERENCES AND SIMILARITIES IN STRUCTURE AND BIOLOGICAL ACTIVITIES

19:00 **Inna N. Krasikova**, *Vladivostok, Russian Federation*
THE INFLUENCE OF LIPOPOLYSACCHARIDES AND LIPID As FROM SOME MARINE BACTERIA ON SPONTANEOUS AND INDUCED BY *ESCHERICHIA COLI* LPS TNFα RELEASE FROM PERIPHERAL HUMAN BLOOD CELLS

19:15 **Student's dinner, afterwards pub crawl in Warnemünde**

Friday, October 6

Chairperson: **Wieslaw Szeja**, *Gliwice, Poland*

09:00 **Elke Schweda**, *Huddinge, Sweden*
EXPLOITING GENOMICS IN PROFILING THE STRUCTURE OF SHORT-CHAIN NTHI LPS

09:25 **Katarzyna Anna Duda**, *Katowice, Poland*
INFLUENCE OF TEMPERATURE ON THE PRESENCE OF ECA IN LIPOPOLYSACCHARIDES FROM *YERSINIA ENTEROCOLITICA* O:3 R MUTANTS

09:40 **Mariola Paściak**, *Wrocław, Poland*
STRUCTURAL CHARACTERIZATION OF THE MAJOR GLYCOLIPIDS FROM *ARTHROBACTER GLOBIFORMIS* AND *ARTHROBACTER SCLEROMAE*

09:55 **Ewa Zuziak**, *Wrocław, Poland*
DETERMINATION OF ENDOTOXIN USING GLC-MS DETECTION OF CHEMICAL MARKER 2-KETO-3-DEOXYOCTULOSONIC ACID (KDO) AND BY LAL METHOD

10:10 **Arkadiusz Bartyś**, *Wrocław, Poland*
ANALYSIS OF ADVANCED GLYCATION END-PRODUCTS IN HUMAN SERUM SAMPLES USING THE ASSAY BASED ON MODEL COMPOUNDS SYNTHESIZED WITH DRY REACTION AT HIGH TEMPERATURE

10:25 **Bogumiła Szponar**, *Wrocław, Poland*
DISTRIBUTION OF ENDO- AND EXOGEOUS 3-HYDROXY FATTY ACIDS IN RAT TISSUES

10:40 **Coffee break**

Chairperson: **Chris Meier**, *Hamburg, Germany*

11:05 **Harun A. Shaikh**, *Kiel, Germany*
TOWARDS DETERMINATION OF FUNCTION OF GLYCOCALYX

11:20 **Michaela Wiegand**, *Kiel, Germany*
PHOTOACTIVE MANNOSE DERIVATIVES AND THEIR EVALUATION FOR LECTIN LABELING

11:35 **Katharina Elsner**, *Kiel, Germany*
SYNTHESIS OF GLYCOSYLATED LIPID MEMBRANE MODELS

11:50 **Jana Neumann**, *Hamburg, Germany*
SYNTHESIS OF NOVEL OLIGOSACCHARIDE MIMETICS AS INHIBITOR PROBES FOR NK CELL RECEPTORS

12:05 **Andreas Steinmann**, *Hamburg, Germany*
SYNTHESIS OF OLIGOSACCHARIDE LIBRARIES VIA AN UNUSUAL GLYCOSYLATION METHOD

12:20 **Lunch**

Chairperson: **Grigorij Kogan**, *Bratislava, Slovakia*

13:30 **Polina I. Abronina**, *Moscow, Russian Federation*
SYNTHESIS OF DI− AND TRISACCHARIDE PRECURSORS OF 2-CARBOXYETHYLTHIO GLYCOSIDE OF A LINEAR MANNO- PENTAOSIDE, A REPEATING UNIT OF O-ANTIGENIC POLY- SACCHARIDES FROM *ESCHERICHIA COLI* O9 AND *KLEBSIELLA PNEUMONIAE* O3

13:45 **Elena A. Khatuntseva**, *Moscow, Russian Federation*
SYNTHESIS OF PROTEIN BASED NEOGLYCOCONJUGATES BEARING GLYCOLIPID LIGANDS INVOLVED INTO MICROBIAL ADHESION

14:00 **Silke Wendicke**, *Hamburg, Germany*
A NOVEL ACCESS TO NUCLEOSIDE DIPHOSPHATE SUGARS

14:25 **Anna Kasprzycka**, *Gliwice, Poland*
STUDY ON THE SYNTHESIS OF α-L-FUCOPYRANOSIDES

14:45 **Sebastian Derfla**, *Gliwice, Poland*
THIOGLYCOSIDES IN THE SYNTHESIS OF GLYCALS

15:00 **Coffee break**

Chairperson: **Joachim Thiem**, *Hamburg, Germany*

15:25 **Thomas Jöge**, *Hanover, Germany*
NOVEL AMINOGLYCOSIDES AND CYCLITOLS

15:40 **Nico Paepke**, *Rostock, Germany*
SYNTHESIS AND APPLICATION OF METHYL-2,3-DIACETAMIDO-2,3-DIDEOXY-URONIC ACIDS

15:55 **Gnuni Karapetyan**, *Rostock, Germany*
SYNTHESIS OF SPECIAL *N*- AND *C*- GLYCOSIDES OF GALACTURONIC ACID

16:10 **Andrei V. Nikolaev**, *Dundee, Scotland, United Kingdom*
RECENT STUDIES IN SYNTHETIC CHEMISTRY OF GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHORS

16:40 **Suzannah J. Harnor**, *Dundee, Scotland, United Kingdom*

SYNTHESIS OF FRAGMENTS OF A GPI ANCHOR FROM *LEISHMANIA* LIPOPHOSPHOGLYCAN

16:55 **Olga V. Sizova**, *Dundee, Scotland, United Kingdom*
ELONGATING AND BRANCHING β-D-GALACTOSYL TRANSFERASES: NOVEL BIOSYNTHETIC ENZYMES IN *LEISHMANIA*

19:30 **Banquet dinner**

Saturday, October 7

Chairperson: **Janusz Madaj**, *Gdańsk, Poland*

09:00 **Irina M. Yermak**, *Vladivostok, Russian Federation*
THE MODIFICATION OF BIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF LIPOPOLYSACCHARIDE BY CARRAGEENAN.

09:25 **Viktoria N. Davydova**, *Vladivostok, Russian Federation*
THE DIFFERENT LIPOPOLYSACCHARIDES BINDING TO CHITOSAN

09:40 **Andrzej Nowacki**, *Gdańsk, Poland*
PRELIMINARY STUDY ON STRUCTURE OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS* EXOPOLY SACCHARIDES

09:55 **Peter Langer**, *Rostock, Germany*
SYNTHESIS OF THE FIRST INDIGO- AND INDIRUBIN *N*-GLYCOSIDES

10:15 **Coffee break**

Chairperson: **Leonid O. Kononov**, *Moscow, Russian Federation*

10:40 **Holger Feist**, *Rostock, Germany*
FROM BRANCHED-CHAIN OR CHAIN-ELONGATED MONOSACCHARIDES TO UNUSUAL *C*-NUCLEOSIDES

10:55 **Yvonne V. Schnitzler**, *Berlin, Germany*

SYNTHESIS OF KEY COMPOUNDS OF THE MAILLARD-REACTION

11:10 **Martin Pajtinka**, *Bratislava, Slovakia*
SORPTION CAPACITIES OF CELL WALL GLUCAN ISOLATED FROM *SACCHAROMYCES CEREVISIAE* TOWARDS PCP

11:25 **Grigorij Kogan**, *Bratislava, Slovakia*
COULD FUNGAL POLYSACCHARIDES FIGHT CANCER?

11:50 **Closing**

12:00 **Coffee break and snack**

13:00 **Saturday afternoon party**
Guided Sightseeing tour by bus - Rostock, Warnemünde, Heiligendamm; then a guided tour of the Minster Bad Doberan, a 14th century Cistercian abbey church closed by an organ recital; finally, a dinner in a really cosy fish restaurant directly located on the beach of the Baltic sea (small village: Nienhagen).

23:00 **Back in the hotel**

Sunday, October 8

Departure

ABSTRACTS

APPROACHES TOWARDS ANTI-MICROBIAL GLYCOCONJUGATE VACCINES BASED ON SYNTHETIC OLIGOSACCHARIDE STRUCTURES

Stefan Oscarson*, Johan Olsson, Lina Rydner, Rikard Slättegård

Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden
s.oscarson@organ.su.se

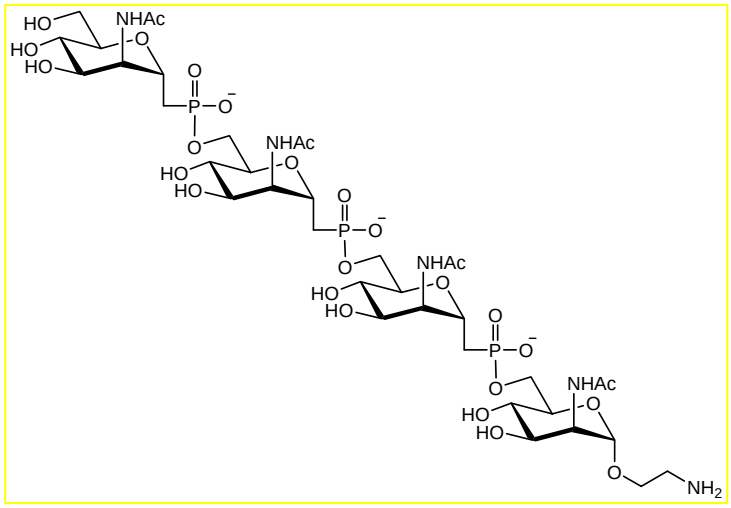
Carbohydrate structures, in the form of capsular polysaccharides (CPSs) or lipopolysaccharides (LPSs), are important surface antigens of bacteria and accordingly of interest for serotyping of and as vaccines against bacteria. The successful introduction of glycoconjugate vaccines, i.e. saccharide structures conjugated to a carrier protein, has dramatically increased the interest in this type of vaccines. There are now three efficacious commercial glycoconjugate vaccines, against *Haemophilus influenzae* type b (Hib), *Neisseria menigitidis* type C and *Streptococcus pneumoniae* (seven serogroups), all based on partly hydrolyzed native capsular polysaccharide structures. However, to use of native bacterial polysaccharides is sometimes combined with various problems, e.g., heterogeneity, instability, toxicity or molecular mimicry of these structures. An interesting alternative is then synthetic part structures or analogues. Owing to the fast progress in oligosaccharide synthesis during the last years the synthesis of these often most complex structures has become feasible. For the Hib vaccine there is now already a commercial vaccine based on chemically synthesized oligosaccharide structures. We will present our approach towards glycoconjugate vaccine candidates based on synthetic carbohydrate structures against infections caused by the fungi *Cryptococcus neoformans*, a major cause of death in AIDS patients, and the bacteria *Vibrio cholerae*, *Haemophilus influenzae* and *Neisseria meningitidis*.

SYNTHESIS OF STABLE C-PHOSPHONATE ANALOGUES OF *NEISSERIA MENINGITIDIS* TYPE A CAPSULAR POLY-SACCHARIDE STRUCTURES

Rikard Slättegård*, Peter Teodorovic and Stefan Oscarson

Arrhenius Laboratory, Stockholm Univesity, S-106 91 Stockholm, Sweden
rikard@organ.su.se

The construction of C-phosphonate analogues of the *Neisseria meningitidis* type A capsular polysaccharide structures is described. Using a modified Mitsunobu reaction (tris(4-chlorophenyl)phosphine, DIAD, excess of Et_3N) between an anomeric C-phosphonate monoester and a 6-OH ManNAc acceptor a very high yield (88%) of the dimer was obtained. After transformation of the dimer into a new 6-OH acceptor and further reaction with the elongating C-phosphonate monomer employing the same conditions, the trimer was obtained in 92% yield. Finally, the tetramer (Fig) was obtained in 85% yield using the same procedure.

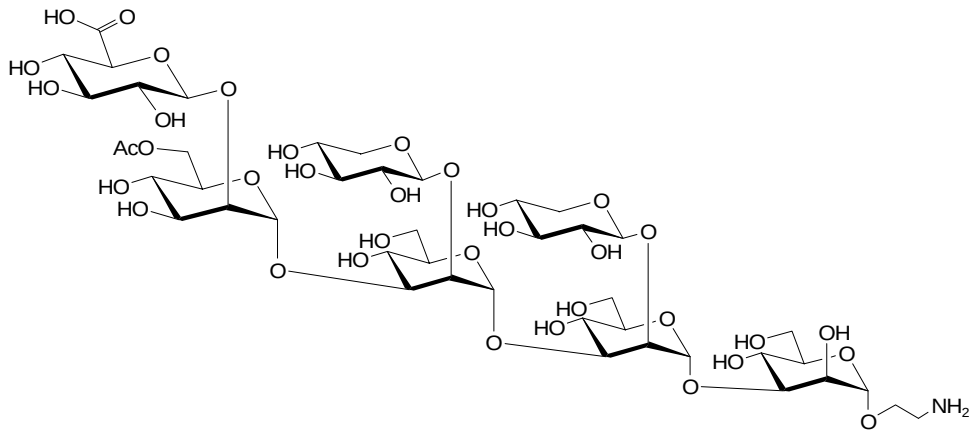


APPROACHES TOWARDS A GLYCOCONJUGATE VACCINE AGAINST *CRYPTOCOCCUS NEOFORMANS* BASED ON SYNTHETIC OLIGOSACCHARIDE STRUCTURES

Lina Rydner*, Jan Vesely and Stefan Oscarson

Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden
lina@organ.su.se

Conjugation of *C. neoformans* capsular polysaccharide to proteins results in highly immunogenic compounds that can elicit high titer antibody responses. However, there are difficulties associated in consistently eliciting protective antibody responses using GXM polysaccharide based vaccines. One alternative approach is to synthesize oligosaccharide-based vaccines to identify motifs that are immunogenic and potential vaccine candidates. We now report a synthetic pathway to almost any structural fragment of the GXM, including variant acetylation pattern. Because of the repetitiveness of the GXM polysaccharide structures a block synthesis approach is utilized. All the necessary di-and trisaccharide blocks have been synthesised as thioglycosides and their subsequent couplings carried out to give (so far) up to heptasaccharide structures (Fig). All the synthetic oligosaccharide target structures are equipped with a spacer to allow efficient conjugation to a carrier protein.



SYNTHESIS OF OLIGOSACCHARIDES CORRESPONDING TO INNER CORE LPS-STRUCTURES FROM *H. INFLUENZAE* AND *N. MENINGITIDIS*

Johan Olsson*, Karin Mannerstedt, Stefan Oscarson

Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden
johano@organ.su.se

Here we will present approaches towards the synthesis of inner core lipopolysaccharide (LPS) structures from the Gram-negative bacteria *Haemophilus influenzae* and *Neisseria meningitidis*. These two bacteria show large structural similarities in their LPS inner core region (Figure).

A synthetic route to a suitably protected derivative of the branched trisaccharide L-α-D-Hep*p*-(1→3)-[β-D-Glc*p*-(1→4)]-L-α-D-Hep*p*, which is used as a key intermediate for the synthesis of both *H. influenzae* and *N. meningitidis* structures, has been developed. The introduction, into this trisaccharide intermediate, of a third (1→2)-linked L-α-D-Hep*p* residue on the second heptose moiety and a (1→5)-linked α-Kdo residue at the reducing end, to give *H. influenzae* structures, will be described. Furthermore, the introduction of a (1→2)-linked α-D-Glc*p*NAc moiety, to give the core tetrasaccharide for *N. meningitidis*, will also be discussed.

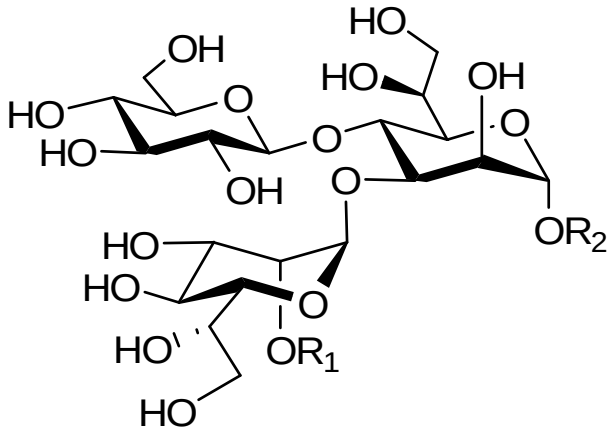


Fig. The inner core branched trisaccharide from *H. influenzae* and *N. meningitidis.*
R_1 = L-α-D-Hep*p* or D-Glc*p*NAc and R_2 = Kdo or Spacer.

SEROTYPING OF CLINICAL *PROTEUS MIRABILIS* STRAINS BASED ON CHEMICALLY DEFINED OF O-SPECIFIC PART OF LIPOPOLYSACCHARIDES

Wiesław Kaca*[a] and Sebastian Grabowski [b]

[a] - Department of Microbiology, Swietokrzyska Academy, Swietokrzyska 15, 25-406 Kielce, Poland, [b] - Institute of Microbiology and Immunology, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Gram-negative rods *Proteus mirabilis* are common habitant lower part of human gastrointestinal tract. *P. mirabilis* strains are considered as opportunistic pathogens, responsible for urinary tract infections. *P. mirabilis* rods are also important in rheumatoid arthritis and nosocomial infections. The urease activities and outer–membrane lipopolysaccharides (LPS) are consider as the important virulence factors of *P. mirabilis* strains. The differences in the structure of O-antigens serves are the basis for the serological classification of *Proteus* bacteria. The serological classification scheme of Kaufman and Perch includes 49 different *P. mirabilis* and *P. vulgaris* O-serogroups.

The aim of presented studies was the serological classification of *P. mirabilis* strains, isolated from patients of urological hospital department.

For the serological classification the standard lipopolysaccharides isolated form representing serogroups *P. mirabilis* laboratory strains, (with O-polysaccharides complete structures known) were used. The schema of serotyping method is proposed. It is as follows: the preliminary ELISA results were then confirmed by immunoblotting method. The discrepancies between results of ELISA and immunoblotting tests were observed. Based on ELISA results, the antigenic mosaics of the thermostable, surface antigens of clinical *P. mirabilis* strains that were cross-reacted with rabbit-anti-O antibodies are presented. The migration patterns of laboratory lipopolysaccharides in SDS-PAGE and immunoblotting differ from patterns of LPSs isolated form clinical *P. mirabilis* strains. Only 36 % out of 99 strains, originated form Sweden urological clinic were classified. The majority of classified *P. mirabilis* clinical stains belong to O10, O23, O30 and O35 serogroups, representing by 9, 3, 6, and 3 strains, respectively. The low number of classified strains is probably due by the large heterogeneity O-antigens as well as strong cross-reaction of common core oligosaccharides of *P. mirabilis* lipopolysaccharides.

This work was supported by grant from State Committee of Sciences, Poland (KBN) 2 P04C 028 28.

THE REACTION OF ANTIBODIES WITH PEPTIDES MIMICKING ANTIGENIC EPITOPE IN *HELICOBACTER PYLORI* UREASE

Iwona Konieczna*[a], Sebastian Grabowski [b] , Magdalena Białek [c], Beata Kolesinska [c], Zbigniew Kamiński [c] , Wiesław Kaca [a]

[a] - Department of Microbiology, Swietokrzyska Academy, Swietokrzyska 15, 25-406 Kielce, Poland, [b] - Institute of Microbiology and Immunology, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland, [c] – Institute of Organic Chemistry, Lodz University, Zeromskiego 116, 90-924 Lodz, Poland

Helicobacter pylori is a major etiological agent of gastroduodenal ulcer diseases *Helicobacter*-induced gastritis is considered nowadays an epidemic, the prevalence of which is one of the highest world-wide (70%). Possible links between *H.pylori* infections and human arteriosclerosis have been suggested. The strong urease activity is one of the most important virulence factors of *H. pylori*. The human antibodies reacting with urease are considered as the valuable marker of *H.pylori* infection.

The aims of studies were to identify synthetic oligopeptides that mimicking the *H. pylori* urease epitopes and define the level and specificities of natural anti-urease antibodies in human sera.

The antibodies from arteriosclerosis effected patients sera were used. The determination of antibodies against *H. pylori* proteins UreA, VacA, CagA were done by Helico Blot 2.1 test. 77% of tested sera were H. pylori seropositive, and 70% were UreA positive. In next step, antibodies present in patients sera were tested with the synthetic oligopeptides, mimicking the UreB part of *H. pylori* urease epitope. The most effective in antibodies binding was oligopeptide: Gly-Gly-Leu-Phe-Lys-Thr. Stepping apart the peptides from the cellulose via glycine spacers increased their recognition by antibodies. However, tetra-glycine spacer significantly reduced the reactions with antibodies. The most selective and specific recognition was achieved by use of 18-mer peptide.

The levels of anti-urease antibodies in arteriosclerosis patient's and volunteer blood donor's sera were determined. As antigen in ELISA the Jack Bean urease was used. The level of those antibodies in arteriosclerosis patient's sera was significantly higher then in control group. The reaction of antibodies in arteriosclerosis patient's sera with Jack Bean urease do not always correlate with presence in the tested sera of anti-UreA *H. pylori* antibodies.

In addition, anti-*Proteus mirabilis* O10 lipopolysaccharides antibodies were identified in *H.pylori* infected, arteriosclerosis patient's sera. The presented results may suggest the role of anti- *H.pylori* and *P. mirabilis* antibodies on the inflammatory reactions ending in arteriosclerosis diseases.

This work were supported by grants from State Committee of Sciences, Poland (KBN) 2P04C 02828 and 4TO9A 18925.

THE HUMAN COMPLEMENT ACTIVATION BY SMOOTH AND ROUGH *PROTEUS MIRABILIS* STRAINS AND THEIRS LIPOPOLYSACCHRAIDES

Michal Arabski*[a], Mariusz Durlik [a], Wieslaw Kaca [a], Bożena Futoma-Kołoch [b], Gabriela Bugla-Płoskońska [b], Włodzimierz Doroszkiewicz [b]

[a] - Department of Microbiology, Swietokrzyska Academy, Swietokrzyska 15, 25-406 Kielce, Poland, [b] – Institute of Genetics and Microbiology, Wrocław University, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland

The lipopolysaccharides (LPSs) and outer membrane proteins (OMPs) located in the outer membrane of Gram-negative bacteria are an important targets for the bactericidal action of the complement system. The structure of the O - specific side chains and core oligosaccharides of LPS play important role in the resistance of bacterial cells to the lytic activity of complement. Also OMPs may be responsible for different level of sensitivity of Gram-negative Proteus mirabilis rods against the bactericidal activity of complement. *Proteus mirabilis* bacilli play an important role in human urinary tract infections, bacteremias, and in rheumatoid arthritis.

In the presented studies we try to correlate the C3 fragmentation with complement-mediated bacteriolysis and the role of core and O-polysaccharide part of *P. mirabilis* S1959 LPS on that reaction. The smooth S1959 LPS and two rough mutants of LPSs R110 and R45, Ra and Re types, respectively were used. We determined also the electrophoretic patterns of OMPs isolated from *P. mirabilis* S1959 and it's two R LPS mutants and we tried to find the correlation between the presence of some OMPs in outer membranes and the susceptibility of *P. mirabilis* strains to the bactericidal activity of human serum.

We were shown that Ra *P. mirabilis* and Re *P. mirabilis* mutants possess different composition of OMPs in relation to P. mirabilis S1959 strains. *P. mirabilis* S1959, R110 and R45 LPSs activated complement and fragmented C3 inducing the C3c neo-antigens exposure. The complement activation by *Proteus* lipopolysaccharides, in presence of rabbit anti-endotoxins antibodies, the hemolysis of human erythrocytes was observed. The amount of released hemoglobin from erythrocytes was determined by spectroscopy measurement in the range of wavelength 400-600 nm. The data demonstrated that R45 and R110 LPSs of rough mutants were more hemolyticaly effective than smooth S1959 LPS. It was found that the R110 LPS showed the strongest hemolytic activity measured at concentrations 10 and 50 μg/ml, 34.36%, 39.73%, respectively.

In conclusion: we have shown the role of the O-specific polysaccharide of LPS and the outer membrane proteins (OMPs) in protecting the parent *P.mirabilis* S1959 strain against the bactericidal action of serum. The complete S1959 LPS possesses reduced abilities to activated complement and induce human erythrocytes lysis, in contrary to R110 LPS (Ra type).

This work were supported by grants from State Committee of Sciences, Poland (KBN) 2P04C 02828

CONTRIBUTION OF CARBOHYDRATES IN STREPTOCOCCAL BIOFILM FORMATION

Cordula Lembke*, Bernd Kreikemeyer, Andreas Podbielski

University of Rostock, Medical Faculty
Department of Medical Microbiology, Virology and Hygiene
Schillingallee 70
D-18057 Rostock
Cordula.lembke@med.uni-rostock.de

Among the Gram-positive ß-hemolytic streptococci, *Streptococcus pyogenes* (group A *Streptococcus*, GAS) is an important pathogen which exclusively infects humans. Preferential GAS initial colonization sites on and entry ports into the human body are the mucous membranes (naso-/oro-pharynx) and the skin (face, lower legs). At these sites GAS encounter the residential bacterial biofilm flora that serves as a natural barrier to physically exclude and repel pathogens.

Single and multi-species biofilms are organized as bacterial cells embedded in a self-produced extracellular polymeric substance (EPS), a complex mixture of carbohydrates, proteins, DNA, cell membrane vesicles, and additionally, of absorbed substances. The EPS is responsible for mechanical biofilm stability and serves as a reservoir for self produced or absorbed essential nutrients.
It is well established for members of the local physiologic microflora such as *S. gordonii*, *S. mutans*, *S. oralis* that nutrient components in culture medium influence the biofilm formation process of this bacteria. We have recently shown that *S. pyogenes* is able to form biofilms, a potential virulence trait also influenced by nutrient components.

In general, carbohydrate metabolism regulates biofilm formation among many pathogenic bacteria. Several carbohydrate transport and metabolic turnover proteins were identified and functionally associated with the *S. mutans* and *S. gordonii* biofilm formation process, including extracellular glucosyltransferases (Gtf´s) and inducible fructose phosphotransferases.

In the current study, several substances of the extracellular polymeric components of biofilm-organized GAS were identified by lectin labeling analyses using fluorescence microscopy and confocal laser scanning methods (CLSM). Furthermore, carbohydrates potentially involved in the GAS biofilm formation process were characterized. Using a batch culture approach, growth media supplemented with glucose, sucrose, fructose, or mannose, and various detection methods, including scanning electron microscopy (SEM) and CLSM, we were able to demonstrate the influence of increased monosaccharide concentrations on the biofilm structure. GAS biofilms exposed to high glucose or fructose concentrations appeared altered and looser structure than biofilms formed in control media. By also using safranin staining and quantitative absorption measurements, increased sucrose levels as well as the absence of any carbohydrate supplement was shown to lead to decreased biofilm amounts.

Additionally, preliminary results revealed the majority of GAS bacteria enter a VBNC (viable but not culturable, i.e. spore-like) state after ≥48h biofilm growth. This effect appears to be pH-dependent.
The functional and chemical role of DNA in the biofilm-EPS could be essential for the early stages of biofilm development. Interestingly, we found that the presence of DNaseI decreased GAS biofilm growth and biofilm thickness.

In summary, our work documented that the presence of glucose, sucrose, fructose and mannose obviously influences the general GAS biofilm formation and structure. Further investigations on carbohydrate transport, integration, the impact of complex carbohydrates and the potential involvement of Gtf enzyme activity during GAS biofilm formation are currently in process.

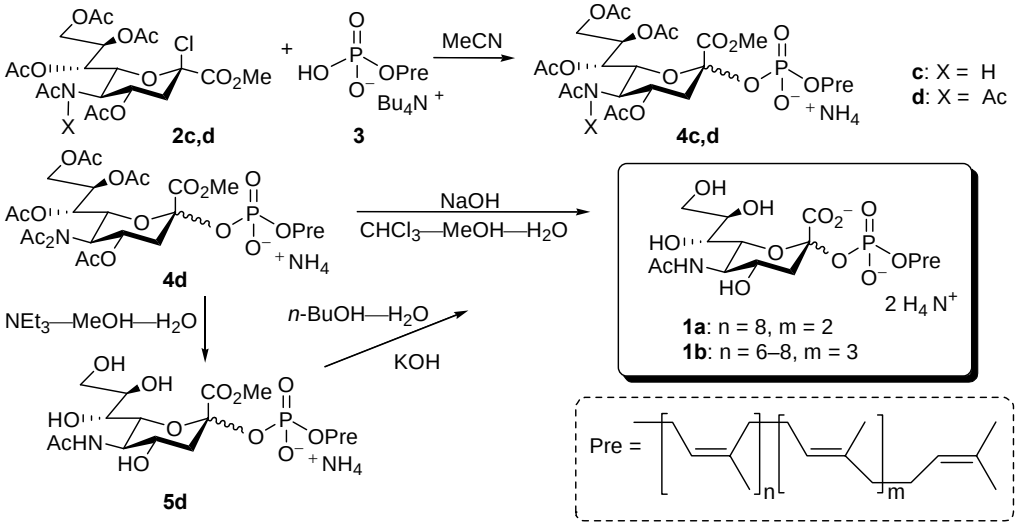
TOTAL SYNTHESIS OF SIALIC ACID POLYPRENYL PHOSPHATE, A PROBABLE BIOSYNTHETIC INTERMEDIATE OF BACTERIAL POLYSIALIC ACID

Anna M. Shpirt,* Leonid O. Kononov, Vladimir I. Torgov, Vladimir N. Shibaev

N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION
*E-mail: * asya@ioc.ac.ru; kononov@ioc.ac.ru*

Some details of biosynthesis of bacterial polysialic acid $(Nue5Ac)_n$ are still ambiguous. Neu5Ac undecaprenyl phosphate **1a** was postulated to be the key biosynthetic intermediate [1]. This compound has never been isolated and characterized. To clarify this point, we decided to synthesize its close analog Neu5Ac moraprenyl phosphate **1b**.

We found that Neu5Ac polyprenyl phosphate **4c** (α : β = 1:1.3) could be prepared directly from the Neu5Ac glycosyl chloride **2c** and tetrabutylammonium polyprenyl phosphate **3** in MeCN. Unfortunately, purification of **4c** was not straightforward.



The reaction between $Neu5Ac_2$ glycosyl chloride **2d** bearing two acetyl groups at the nitrogen atom [3] with tetrabytylammonium polyprenyl phosphate **3** proceeded stereoselectively to afford **4d** (α : β = 2.5:1, yield 70%) as the product, which was easy to purify. Deprotected methyl ester **5d** (yield 57%) was obtained by selective cleavage (NEt_3—MeOH—H_2O) of all *O*-acetyl groups and one of *N*-acetyl group. The target α- and β-isomers of Neu5Ac moraprenyl phosphate **1b** were prepared either from **5d** (KOH, *n*-BuOH—H_2O) or directly from **4d** (NaOH, $CHCl_3$—MeOH—H_2O) and purified by ion-exchange chromatography.

This work was supported by RFBR (projects No. 04-03-32854 and 05-03-32579).

REFERENCES

1. F. A. Troy II, *Glycobiology*, 1992, **2**, 5.
2. A. M. Shpirt, L. O. Kononov, V. I. Torgov, V. N. Shibaev, *Russ. Chem. Bull., Int. Ed.,* 2005, **54**, 481.
3. A. V. Orlova, N. Yu. Kulikova, A. M. Shpirt, L. O. Kononov, *Baltic Meeting on Microbial Carbohydrates 2006*, October 4—8, 2006, Rostock, Germany.

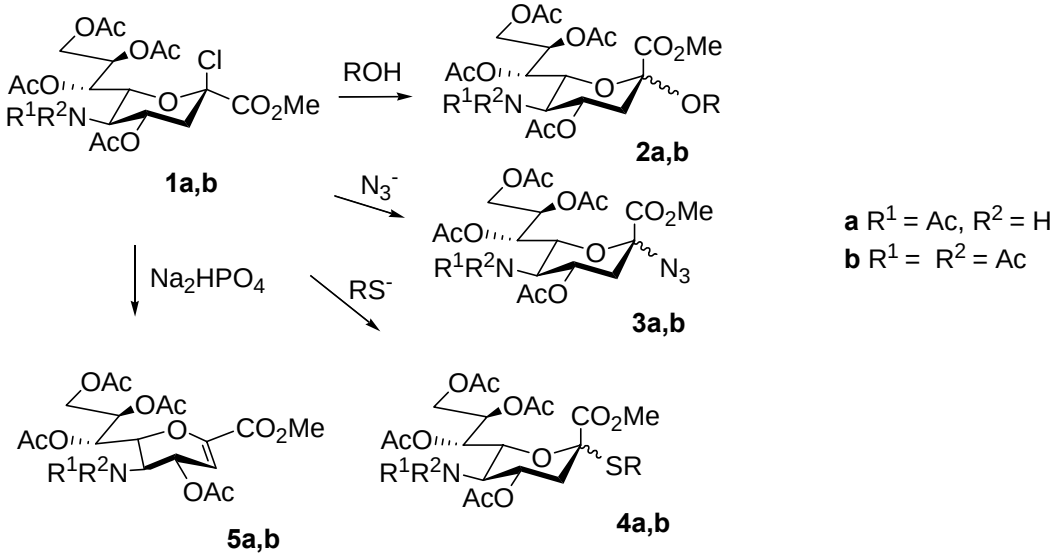
A NOVEL GLYCOSYL DONOR — *N,N*-DIACETYLNEURAMINIC ACID GLYCOSYL CHLORIDE

Anna V. Orlova[1]*, Nadezda Yu. Kulikova[1,2], Anna M. Shpirt[1], Leonid O. Kononov[1], Vladimir I. Torgov[1]

[1] N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION
[2] Higher Chemical College of the Russian Academy of Sciences, Miusskaya pl, 9, Moscow, RUSSIAN FEDERATION
*E-mail: * zoka@ioc.ac.ru, kononov@ioc.ac.ru*

One of important problems of modern carbohydrate chemistry is the search for novel efficient sialic acid glycosyl donors. Thioglycosides of *N,N*-diacetylneuraminic acid **4b** are known to be more efficient glycosyl donors than more traditional thioglycosides of *N*-acetylneuraminic acid **4a** [1]. We supposed that *N,N*-diacetylglycosyl chloride **1b** will also have advantages over *N*-acetylglycosyl chloride **1a** [1].

For this reason, we synthesized glycosyl chloride **1b** and studied the possibility of its interactions with O-, N- and S-nucleophiles in comparison with the known glycosyl chloride **1a**. We also studied the possibility of conversion of glycosyl chloride **1b** into the corresponding *N,N*-diacetylglycal **5b** under conditions that were recently described by us [2] for the preparation of *N*-acetylglycal **5a** from chloride **1a**.



This work was supported by RFBR (projects No. 04-03-32854 and 05-03-32579).

REFERENCES

[1] G.-J. Boons, A. V. Demchenko, *Chem. Rev.,* 2000, **100,** 4539.
[2] N. Yu. Kulikova, A. M. Shpirt, L. O. Kononov, *Synthesis*, 2006, *submitted*.

SYNTHESIS OF *O*-BENZYLATED SIALIC ACID DERIVATIVES WITH VARIOUS SUBSTITUENTS AT N(5) AS POTENTIAL GLYCOSYL DONORS

L. A. Nazarova,*[a,b] A. M. Shpirt,[b] N. N. Malysheva,[b] L. O. Kononov[b]

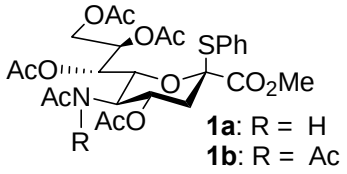
[a] *Higher Chemical College of the Russian Academy of Sciences, Miusskaya pl, 9, 125047, Moscow, RUSSIAN FEDERATION*

[b] *N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION*

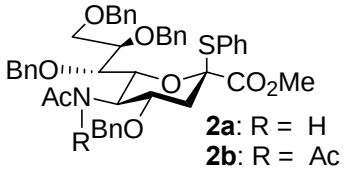
E-mail: kononov@ioc.ac.ru

One of important problems of modern carbohydrate chemistry is the search for novel efficient sialic acid glycosyl donors.[1] Thioglycosides of neuraminic acid (Neu5Ac) with modified substituents at N(5) are known to be more efficient glycosyl donors than the traditional 5-acetamido-counterparts.[2] It is important to note that the reasons for success of these modified sialyl donors still remain obscure.

Recently, we have demonstrated that the outcome of sialylation reaction can be modulated by changing ability of the molecules of sialic acid glycosyl donors to participate in *intermolecular* hydrogen bonding.[3,4] Oxygen atoms in ethers are known to be much weaker hydrogen bond acceptors than carbonyl oxygen atoms in esters. For this reason, one can expect that the behavior of *O*-benzylated thioglycosides in sialylation would dramatically differ from that of *O*-acyl derivatives.



1a: R = H
1b: R = Ac



2a: R = H
2b: R = Ac
2c: R = Bn

However, *O*-benzylated Nue5Ac derivatives are known to be difficult to prepare due to the known problem of "undesired" *N*-benzylation, and for this reason the derivatives with *O*-acyl protective groups (acetyl, chloroacetyl) were mostly used in sialylations. Using conditions developed for *O*-benzylation of Neu5Ac glycal,[5] we prepared novel *O*-benzylated thioglycosides **2a,b,c** with various substituents at N(5) and studied the possibility of their use as glycosyl donors in comparison with the known *O*-acetylated thioglycosides with one and two *N*-acetyl groups (**1a,b**).

This work was supported by RFBR (projects No. 04-03-32854 and 05-03-32579).

REFERENCES

1. G.-J. Boons, A. V. Demchenko, *Chem. Rev.,* 2000, **100**, 4539.
2. H. Tanaka, Y. Nishiura, T. Takahashi, *J. Am. Chem. Soc.,* 2006, **128**, 7124, and references cited therein.
3. L. O. Kononov, N. N. Malysheva, E. G. Kononova, O. G. Garkusha, *Russ. Chem. Bull.,* 2006, **55**, No.7, 1263.
4. L. O. Kononov, N. N. Malysheva, E. G. Kononova, *Baltic Meeting on Microbial Carbohydrates 2006*, October 4—8, 2006, Rostock, Germany.
5. Y. Ito, S. Nunomura, S. Shibayama, T. Ogawa, *J. Org. Chem.,* 1992, **57**, 1821.

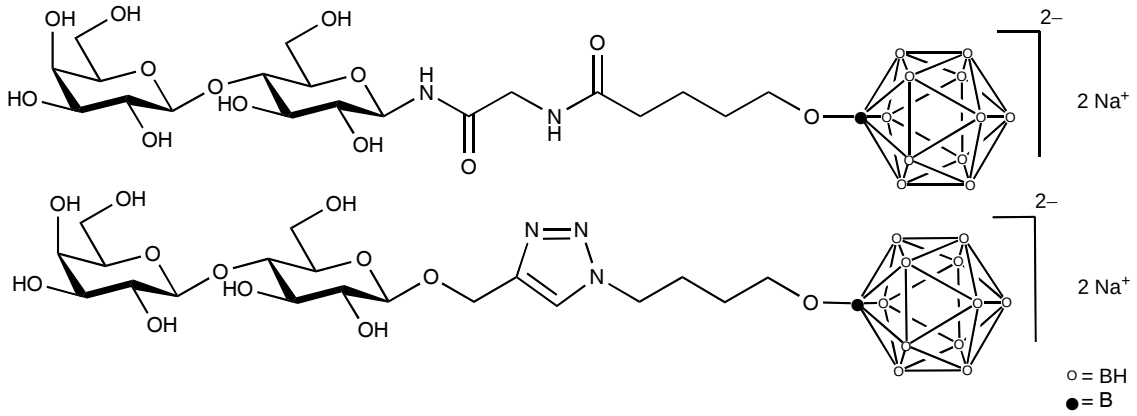
SYNTHESIS OF NOVEL LACTOSE—DODECABORATE CONJUGATES AS POTENTIAL AGENTS FOR BORON NEUTRON CAPTURE THERAPY

Nikolay N. Kondakov*[1], Anna V. Orlova[1], Olga G. Ovchinnikova[1], Leonid O. Kononov[1], Polina I. Abronina[1], Igor B. Sivaev[2], Vladimir I. Bregadze[2]

[1] N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION
[2] A.N.Nesmeyanov Institute of Organoelement Compounds Russian Academy of Sciences, Vavilova str., 28, 119991, Moscow, RUSSIAN FEDERATION
*E-mail: * nkondak@server.ioc.ac.ru, kononov@ioc.ac.ru*

Carbohydrate derivatives of the *closo*-dodecaborate anion, which can be used for glycotargeting, are promising agents for boron neutron capture therapy of cancer (BNCT).[1] However, only few carbohydrate derivatives of *closo*-dodecaborate anion have been described so far.[2]

In this communication we report syntheses of novel conjugates of *closo*-dodecaborate anion with carbohydrates. We have chosen lactose, a known ligand of lectins on the surface of melanoma cells, as a representative example. Several oligosaccharide derivatives of *closo*-dodecaborate anion were prepared (two examples are shown below).



Two types of reactions were used for conjugation of *closo*-dodecaborate anion with lactose. The first approach relies on a formation of an amide bond while the second one makes use of a [2+3] cycloaddition reaction between terminal alkynes and azides with copper ascorbate as a catalyst.

This research was financially supported by RFBR (project No. 06-03-33035-a).

REFERENCES

1. A. H. Soloway, W. Tjarks, B. A. Barnum, F.-G. Rong, R. F. Barth, I. M. Codogni, J. G. Wilson, *Chem. Rev.* (1998) **98,** 1515.
2. N. N. Kondakov, A. V. Orlova, A. I. Zinin, B. G. Kimel, L. O. Kononov, I. B. Sivaev, V. I. Bregadze, *Russ. Chem. Bull., Int. Ed.,* (2005) **54**, 1352, and references cited therein.

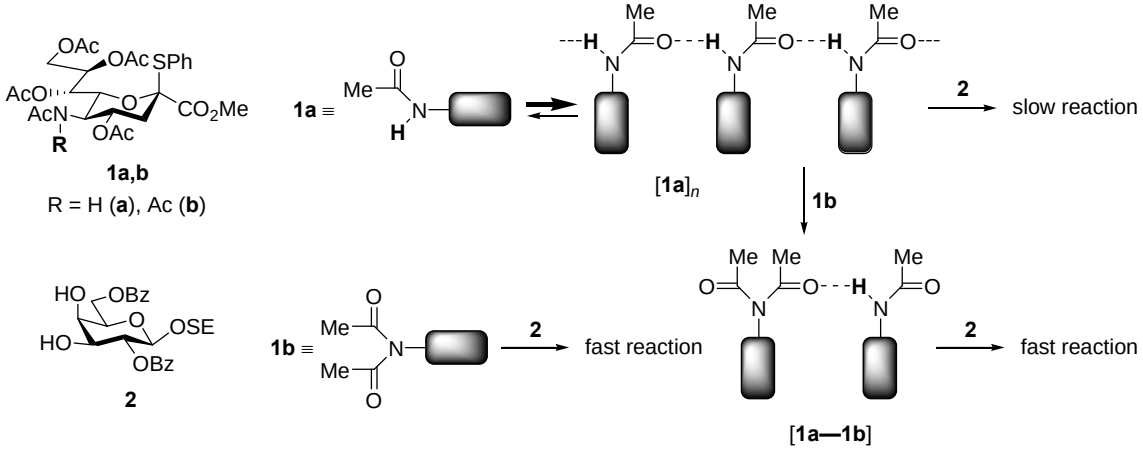
INTERMOLECULAR HYDROGEN BONDING PATTERN IN SOLUTIONS OF SIALIC ACID DERIVATIVES DRAMATICALLY INFLUENCES THEIR PROPERTIES

L. O. Kononov*, N. N. Malysheva, E. G. Kononova

N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION
E-mail: kononov@ioc.ac.ru

One of important problems of modern carbohydrate chemistry is the search for novel efficient sialic acid glycosyl donors.[1] Thioglycosides of neuraminic acid with modified substituents at N(5) are known to be more efficient glycosyl donors than the traditional 5-acetamido-counterparts.[2] However, the reasons for success of these modified sialyl donors still remain obscure.

Recently, synergistic activation of sialic acid *N*-acetyl-thioglycoside **1a** in the presence of *N,N*-diacetyl-thioglycoside **1b** in reaction with **2** has been discovered.[3] Glycosylation of **2** with the equimolecular mixture of **1a** and **1b** is complete within 5 min while it takes 3 h for **1a** alone to be consumed. Synergism is probably related to changes in hydrogen bonding network in solution of **1a** in MeCN upon addition of **1b**. The addition of **1b**, which is a hydrogen bond acceptor competing for hydrogen bond donors with **1a**, leads to "depolymerization" of hydrogen bonded "*oligomers*" $[\mathbf{1a}]_n$, whose reactivity is *lowered* in comparison to that of the unbound molecules of **1a**, and to *increased* apparent reactivity of **1a**. The postulated change in hydrogen bonding pattern in solutions of **1a** upon addition of **1b** is corroborated by IR-spectroscopy and by results of glycosylations in the presence of other amides. The proposed hypothesis makes possible novel interpretations of results of sialylations and opens new possibilities for optimization of reagents structure and reaction conditions.



This work was supported by RFBR (projects No. 04-03-32854 and 05-03-32579).

REFERENCES

1. G.-J. Boons, A. V. Demchenko, *Chem. Rev.,* 2000, **100**, 4539.
2. H. Tanaka, Y. Nishiura, T. Takahashi, *J. Am. Chem. Soc.,* 2006, **128**, 7124, and references cited therein.
3. L. O. Kononov, N. N. Malysheva, E. G. Kononova, O. G. Garkusha, *Russ. Chem. Bull.,* 2006, **55**, No.7, 1263.

CAN HYDROGEN BONDED AGGREGATES OF *N*-ACETYLGLUCOSAMINE DERIVATIVE BE SEPARATED?

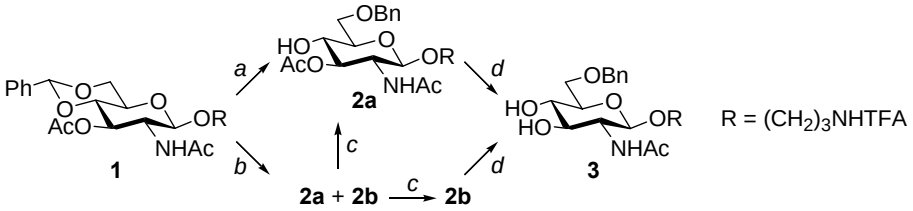
Nadezda Yu. Kulikova[1,2]*, Anna M. Shpirt[1], Alexander I. Zinin[1], Polina I. Abronina[1], Leonid O. Kononov[1]

[1] N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION
[2] Higher Chemical College of the Russian Academy of Sciences, Miusskaya pl, 9, 125047, Moscow, RUSSIAN FEDERATION
E-mail: kononov@ioc.ac.ru

Recently, in our laboratory it has been shown that methanesulfonic acid (MsOH) is an efficient and convenient substitute for ethereal HCl in reductive 4,6-*O*-benzylidene acetal ring-opening reaction with sodium cyanoborohydride in THF. Normal regioselectivity was observed, the 6-*O*-benzyl ethers with free 4-OH group being the major products of the reaction [1]. The best yields were achieved when the acidity of the reaction mixture was near pH 2.5—3.

We found that while the reduction of acetal **1** at pH 2.5—3 leads to the *single* product **2a**, the reduction at pH 2 results in formation of *two* glucosamine derivatives **2a** and **2b**. These compounds were separated by silica gel column chromatography (R_f 0.33 (**2a**), R_f 0.26 (**2b**), $CHCl_3$—MeOH, 8:0.7). Most differences in their ^1H and ^{13}C NMR spectra were concerned with C(2)NHAc fragment [**2a**: δ_H 6.42 (C(2)N<u>H</u>); δ_C 23.1 (<u>C</u>H_3CONH), 53.8 (C(2)), 171.1, 172.0 (C=O); **2b**: δ_H 6.99 (C(2)N<u>H</u>); δ_C 23.0 (<u>C</u>H_3CONH), 58.2 (C(2)), 170.6, 173.3 (C=O)]. The most abundant peak in the ESI-(+) mass spectrum of **2a** was that of quasi-molecular ion while the peak of dimer had the highest intensity in the mass spectrum of **2b** [(*m/z* (I_{rel}, %)), **2a**: 529.2 (100) [*M* + Na (*calc*.: 529.18)], 1034.8 (16) [M_2 + Na (*calc*.: 1035.36)]; **2b**: 529.2 (58) [*M* + Na (*calc*.: 529.18)], 1034.7 (100) [M_2 + Na (*calc*.: 1035.36)]]. Removal of *O*-acetyl groups by MeONa in MeOH from both compounds **2a** and **2b** led to the same product **3**.

These facts allowed us to suppose that **2a** and **2b** were different hydrogen bonded (via the AcNH group) aggregates of the same compound **2** that could be separated by chromatography.



Reagents and conditions: *a*: $NaBH_3CN$, MsOH, THF, MS 4Å, pH 2.5—3
b: $NaBH_3CN$, MsOH, THF, MS 4Å, pH 2
c: SiO_2 column chromatography
d: MeONa, MeOH

This work was supported by RFBR (projects No. 05-03-32579 and 06-03-33035).

REFERENCES

[1] A. I. Zinin, N. N. Malysheva, A. M. Shpirt, V. I. Torgov, L. O. Kononov, *Carbohydr. Res.,* 2006, *submitted*.

OPTIMISATION OF NEURAMINIC ACID SYNTHESIS BY APPLICATION OF *IN SITU* PRODUCT REMOVAL

V. Zimmermann*, U. Kragl

Institute of Chemistry, University of Rostock
vera.zimmermann@uni-rostock.de, udo.kragl@uni-rostock.de

Due to the increasing demand of pharmaceutical industry there is a constant interest in the improvement of N-acetylneuraminic acid synthesis[1]. Usually neuraminic acid is produced biotechnologically from N-acetyl-D-mannosamine and sodium pyruvate catalysed by N-acetylneuraminic acid aldolase (E.C. 4.1.3.3). N-acetyl-D-mannosamine, which is quite expensive, can be synthesised from its epimer N-Acetyl-D-glucosamine, which can be bought at good terms and can be gained by hydrolisation of shrimp shells. This epimerisation can be catalysed by an inorganic base like calcium or sodium hydroxide or by the enzyme N-acetyl-D-glucosamine 2-epimerase (E.C. 5.1.3.8). The enzymes catalysing those two reaction steps work at similar conditions, which offers the opportunity to perform both reactions in one pot[2]. However, both reactions suffer from low yields owing to thermodynamic equilibrium. One possibility to shift the equilibrium to the product is the selective removal of product from the reaction phase during reaction. This principle is called *In Situ* Product Removal (ISPR) and has found application mostly in biocatalytic processes using whole cells[3, 4]. Concerning reactions catalysed by isolated enzymes, the selective removal of one compound is much more difficult due to their chemical similarity. Therefore different parts of the process have to be optimised to reach sufficient selectivity. For that purpose a model describing the two-step synthesis has been developed. The model can be applied to simulate the reaction in different reactor types and in combination with ISPR methods. Scope of this work is the development of an ISPR method for the neuraminic acid system to improve yield and downstream processing of the process.

1. Maru, I.; Ohnishi, J.; Ohta, Y.; Tsukada, Y., Why is Sialic Acid Attracting Interest Now? Complete Enzymatic Synthesis of Sialic Acid with N-Acylglucosamine 2-Epimerase. *J. Biosci. Bioeng.* **2002,** 93, (3), 258-265.
2. Kragl, U.; Gygax, D.; Ghisalba, O.; Wandrey, C., Enzymatic Two-Step Synthesis of N-Acetyl-Neuraminic Acid in the Enzyme Membrane Reactor. *Angew. Chem. Int. Ed. Engl.* **1991,** 30, (7), 827-828.
3. Lye, G. J.; Woodley, J. M., Application of in situ product-removal techniques to biocatalytic processes. *Trends Biotechnol.* **1999,** 17, (10), 395-402.
4. Takors, R., Ganzzell-ISPR-Prozessentwicklung: Chancen und Risiken. *Chem. Ing. Tech.* **2004,** 76, (12), 1857-1864.

CARRIER FACILITATED EXTRACTION OF CARBOHYDRATES FROM AQUEOUS SOLUTION

Daniela Hameister*, Udo Kragl

University of Rostock, Institute of Chemistry, Department of Technical Chemistry, daniela.hameister@uni-rostock.de

The recovery of carbohydrates from aqueous media presents a problem due to their large, irregular and multivalent structure and the low solubility in organic solvents. A system to recover mono- and disaccharides selective from an aqueous solution was developed. Therefore it was necessary to investigate different solvents and carrier, which are suited for this extraction.

To determine the optimum conditions, parameters like temperature, time and amine/sugar-ratio had to be investigated. On the basis of an one-stage extraction a model solution of galactose was extracted to get the final product. The purity of the product was determined by HPLC, NMR and elementary analysis with the result of > 90 % of galactose in the product.

Finally, the extraction process was passed on a simulated cross flow extraction. After eight extraction stages a yield of 81 g (90%) could be obtained.

CARBOHYDRATES AND THE INITIATION OF SPORULATION IN *CLOSTRIDIUM ACETOBUTYLICUM*

Ralf-Jörg Fischer*, T. Rieger, H. Janssen, Hubert Bahl

Institute of Biological Sciences/ Division of Microbiology, University of Rostock
ralf-joerg.fischer@uni-rostock.de

The Gram-positive strictly anaerobic bacterium *Clostridium acetobutylicum* is well known because of its ability to produce industrial bulk solvents like acetone and butanol. Solvent production is induced at the end of the exponential growth phase. This metabolic shift from acid to solvent production is accompanied by substantial morphological changes like the appearance of so called clostridial stage cells preceding the initiation of the formation of endospores.

Tightly connected with the transition phase in the cell cycle of *C. acetobutylicum* is the transient production of specific intracellular and extracellular carbohydrates. One of them is granulose which is expected to be a glycogen-like polysaccharide. Granulose accumulates intracellularly in the form of large granules until the end of spore maturation. Although some biochemical aspects were analyzed in the case of *C. acetobutylicum* P262 (later reclassified as *C. saccharobutylicum* NCP262) nearly nothing is known about the composition of these complexes or the involved genes and their expression and regulation.

Thus, we have analysed the cell cycle of *C. acetobutylicum* under defined growth conditions in a synthetic medium. Based on this, we have started to isolate and to purify the granulose complexes and to analyse the transcription pattern of the putative granulose synthesis operon *glgCDA*.

Furthermore, we are interested in an extracellular polysaccharide which typically is found at the end of the exponential growth phase resulting in a dramatic increase of the viscosity of the culture liquid. The structure of this carbohydrate will be revealed in cooperation with Prof. O. Holst (FZ Borstel, Germany).

STRUCTURAL ANALYSIS OF GLYCOLIPIDS BY HIGH RESOLUTION FOURIER TRANSFORMED MASS SPECTROMETRY (FT-MS)

Göran Hübner[*], Catharina Crone, Buko Lindner[#]

Research Center Borstel, 23845 Borstel, Germany
 # Corresponding author: blindner@fz-borstel.de

Microbial glycolipids are not only amphiphilic molecules important for the organization and function of their membranes but play a major role in the activation of cells of the innate and adaptive immune system of a host. Furthermore, glycolipids in functional compartments of cytoplasma membranes are mediators of cell-cell recognition, cell adhesion and can be involved in transmembrane signal transduction. For an understanding of the underlying biological interaction mechanisms structural details of the glycolipids have to be known.

To perform such structural analysis in highly complex biological samples (nano)-electrospray ionization Fourier transform mass spectrometry (ESI FT-MS) was applied successfully. The high resolution (> 50 000 in broad-band modus), high mass accuracy(~ 2 ppm), sensitivity (pmol range) and online HPLC-coupling enhance the ability to identify single components in small amounts of isolated material without time consuming chemical analysis. Structural details like sugar sequence, degree of acylation, type and linkage position of fatty acid and functional groups can by obtained by the application tandem mass spectrometric methods. It is shown that sustained off resonance irradiation collision induced dissociation (SORI-CID MS/MS) and infrared multiphoton resonance dissociation (IRMPD-MS/MS) in general induce similar product ions of a selected parent ion. However, fragmentation is dependent on the ion mode and the type of pseudomolelar ion (e.g. protonated or cationized parent ion species). Experiments with various glycolipids (for example gangliosides, phosphatidyl inositol mannosides, lipid A) yielded complementary fragment ions demonstrating that the combination of MS/MS data generated in the positive and negative ion mode is an powerful approach for the structural analysis glycolipids.

This work was financially supported by Deutsche Forschungsgemeinschaft (SFB-TR22, Z01)

HIGH-RESOLUTION MASS SPECTROMETRY AS A TOOL FOR STRUCTURAL ELUCIDATION OF BACTERIAL LIPOPOLYSACCHARIDES

Anna N. Kondakova*[1,2], Buko Lindner[2]

[1] N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prospect, 47, 119991, Moscow, RUSSIAN FEDERATION
[2] Liebniz Center for Medicine and Biosciences, Research Center Borstel, D-23845, Parkallee 1-40, GERMANY
E-mail: annakond@gmail.com, blindner@fz-borstel.de

Lipopolysaccharides (LPS) of Gram-negative bacteria play an important role in the organization and functioning of the cell outer-membrane, and their fine structure defines the immune response of the host. Even minor changes in the LPS structure can have the major impact on their biological activity and properties of the bacterial cultures. However, a high degree of heterogeneity in the LPS structure often complicates structural studies by the conventional approach, which includes separation of LPS-derived mixtures of carbohydrates and lipids into individual compounds.

High-resolution mass spectrometry helps to overcome this difficulty as it allows identification of common structural fragments and recognition of the compounds with previously determined structures using small amounts of material.

We used various mass spectrometry techniques for structural studies of the isolated core oligosaccharides and R-type LPS of the bacteria of the genera *Proteus*, *Providencia*, *Citrobacter,* and *Yersinia pestis*. Conserved and strain-variable structural features of the core oligosaccharides were revealed, including the most common sugar substitution patterns and the major sources of heterogeneity. New structural variants of the core oligosaccharides were identified. Temperature-dependent variations were discovered in the *Y. pestis* LPS structure.

This research was financially supported by the President of Russian Federation Program for Support of the Young PhD (project No. MK-2204.2006.4) and RFBR (project No. 05-04-48439)

ANALYSIS OF DUST FROM COWSHEDS: PRESENCE OF ALLERGY PROTECTIVE COMPONENTS?

Elena Ciliberti*[1], Erika von Mutius[2] and Otto Holst[1]

[1]Division of Structural Biochemistry, Research Center Borstel, Leibniz Center for Medicine and Bioscience, 23845 Borstel, Germany.
[2]Dr. von Hauner´s Children Hospital, Ludwig-Maximillians-University of Munich, 80337 Munich, Germany.

At present, atopic diseases are the most common chronic illnesses in children living in the industrialized world. In general, asthma and athopy rates are higher in affluent, Western countries with a high degree of industrialization than in developing countries with a large rural population.[1] Recent epidemiological studies have shown that growing up on a traditional farm provides protection from the development of allergic disorders such as hay fever and allergic asthma.[2-7] Contact with livestock appears to reduce the risk to develop asthma in children, adolescent and young adults, and it has been speculated that respiratory exposure to endotoxin may play an important role[8] since keeping animals is associated with high exposure to bacterial endotoxin. Hence, the farm environment provides a valuable source for studying environmental determinants for the development of asthma and allergies. It has been suggested that the `farm effect` may result from elevated exposures to bacterial compounds in the microbial environment of farm stables.

In this frame we think it should be possible to isolate allergy-protective factors from cowshed and barns environment of traditional farms. Consequently, we are collecting cow-shed and barn dusts from such farms, and dust extracts are processed and biochemically characterized.[9] At the moment, our qualitative and quantitative analyses confirm the presence of proteins, glycoproteins, sugars and aminosugars. Our aim is to analyse whether such extracts (or fractions of these) could modify the allergic response in animal models of acute allergic asthma.

References:

1. M. Kabesch and R.P. Lauener *Journal of Leukocyte Biology,* **2004**; 75, 383-387.
2. Braun-Fahrländer C, Gassner M, Grize L, et al., *Clin Exp Allergy,* **1999**; 29, 28-34.
3. von Ehrenstein OS, von Mutius, Illi S, et al., *Clin Exp Allergy,* **2000;** 30, 187-193.
4. Riedler J, Eder W, Oberfeld G, et al., *Clin Exp Allergy* **2000**, 30, 194-200.
5. Downs SH, Marks GB, Mitakakis Z, et al., *Clin Exp Allergy,* **2001,** 31, 570-575.
6. Klintberg B, Berglund N, Lilja G, et al., *Eur J Respir Dis.,* **2001,** 17, 1151-1157.
7. Portengen L, Sigsgaard T, Omland, et al*., Clin Exp Allergy,* **2002,** 32, 247-253.
8. von Mutius E. *J Allergy Clin Immunol,* **2000**; 105, 9-19.
9. Peters M. et al., *Thorax*, **2006** 61; 134-139.

ROLE OF CARBOHYDRATES FROM BACTERIAL AND FUNGAL SPORES IN ALLERGY PROTECTION

Kay Vogel[1*], Torsten Goldmann[2], Johann Bauer[3], Holger Heine[4], Otto Holst[1]

[1]Division of Structural Biochemistry; [2]Division of Clinical and Experimental Pathology; [4]Division of Innate Immunity; Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Parkallee 4a, D-23845 Borstel, Germany.
[3]Chair of Animal Hygiene, Faculty of Agriculture and Horticulture, Technische Universität München, D-85354 Freising-Weihenstephan, Germany

Correspondig author: Otto Holst; e-mail: oholst@fz-borstel.de

According to the hygiene hypothesis, exposure to microbial components in early childhood plays an important role for the development of the immune system and the protection against allergies. Several epidemiological studies related the increasing number of asthma, hay fever and atopic sensitization in the last years to the lack of contact to microbial components. Allergic patients showed a dominant immune response by the so-called T_H2 pathway, leading for example to increased IgE levels. Inhalation of spore-containing air, after foddering, is one possible reason for the balanced, non-allergic T_H1/T_H2 immune response in children grown up on a farm. This contact to microbes stimulates the release of T_H1 cytokines. Interesting components of bacterial and fungal spores are for example peptidoglycan or partial structures thereof, or β-(1→3)-glucans, respectively.

The isolation from farm cowsheds, fermentation and purification of *Bacillus licheniformis* and *Mucoracae spp.* was the starting point for our studies. Identifying responsible spore components, stimulating the T_H1 pathway of the immune response and leading to an anti-allergic effect is the aim of our research. *Bacillus licheniformis* is a Gram-positive, motile, spore-forming, facultatively anaerobic rod that are able to start an asymmetric cell devision initiated by starvation, which is leading to a dormant spore surviving dryness and high temperature. Germination is possible as soon as specific nutrients are present again, enabling the spore to start a vegetative life cycle.
Mucoracae spores represent an example for fungal spores being a part of the mould's reproductive life cycle. They are dispersed in the air and could be found in high concentrations in farm cowsheds after foddering.

Immunological experiments showed a stimulation of innate immune receptors like TLR2 and the intracellular peptidoglycan receptor Nod2. These receptors recognize lipoproteins, lipoglycans and peptidoglycan, which are important components in Gram-positive spores. Gas chromatographic analysis and SDS-PAGE support the important role of these components, focusing our research on this class of spore components.

Acknowledgments: This work was financially supported by the Deutsche Forschungsgemeinschaft (SFB Transregio 22).

SEPARATION OF BINARY MIXTURES WITH SIMULATED MOVING BED (SMB)

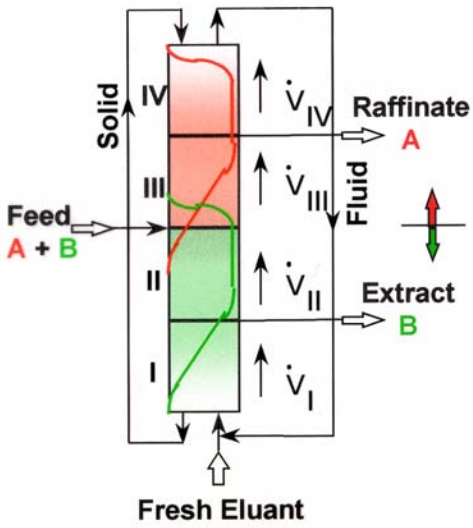
Bernward Rittgerodt

Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer GmbH, Hegauer Weg 38, D-14163 Berlin, Germany
rittgerodt@knauer.net

The advantage of the HPLC (Production of product with high purity) i soften combined with the disadvantage of the need of lots of eluent and the time consuming batch chromatography. The simulated counter current chromatography (simulated moving bed, SMB) does not have this disadvantages. SMB is working with continuous counter current between stationary and mobile phase.

Principle of separation:

Continuous Counter Current Chromatography (TMB): In the TMB-Process solid and mobile phase are moving counter current wise. In the middle of the separation region the sample (binary mixture) is applied. Than the compound with the better affinity to the solid phase will move together with the solid phase and the compound with the better affinity to the mobile phase will move with the mobile phase. Therefore there is he possibility of continuous injection and separation.



Simulated Moving Bed (SMB): The difference between SMB and TMB is the usage of a number of packed chromatography columns instead of a moving solid phase. The moving of the solid phase is simulated by a number of columns which moves in a circle counter current the mobile phase.

Normally the SMB allows a separation of binary mixtures as Glucose/Fructose with high purity and up to 1000kg per year.

Classic SMB Applications:

Fructose from sugar mixtures
Fructose from Molasses and Corn Syrup
42% Fructose in feed and to 94% Fructose in Extract
Glucose/Fructose
Xylose/Arabinose
Trehalulose/Fructose

STRUCTURAL INVESTIGATION OF THE LIPOPOLYSACCHARIDE FROM *ACINETOBACTER LWOFFII* F78

Anna Hanuszkiewicz[1*], Jennifer Debarry[1], Evgeny Vinogradov[2], Buko Lindner[1], Holger Heine[1], Otto Holst[1]

[1] Research Center Borstel, 23845 Borstel, Germany
[2] National Research Council Ottawa, Ontario K1A 0R6, Canada
Corresponding author Otto Holst; email: oholst@fz-borstel.de

It has been shown that growing up on a traditional farm protects against development of allergic diseases later in life. Daily contact with farm livestock as well as with its products like raw milk induces defensive mechanisms against asthma, hay fever and atopic sensitization in children. Farming microflora might have very strong influence on development of farm-related allergy protection. In order to understand the allergy-protective capabilities of farming environment, we focused on bacteria often present in cow-sheds of rural areas of south Germany. From many isolated species we have chosen two of the most abundant strains which undergo now intensive immunological as well as structural investigations. Here we present the isolation and structural examination of lipopolysaccharide (LPS) derived from *Acinetobacter lwoffii* F78 which showed strong allergy-protective effect in an *in vivo* mouse model. This LPS was examined utilizing chromatographic methods (GC, GC/MS, HPAEC) as well as high resolution Fourier transform mass spectrometry (FT-MS) and NMR spectroscopy. The carbohydrate backbone has the following general structure:

α-GalNAc-β-GalNAc-α-Glc-α-Kdo┐
β-Glc-α-Glc-α-Kdo-β-GlcN4*P*-α-GlcN1-*P*
α -Kdo┘

This work was financially supported by Deutsche Forschungsgemeinschaft (SFB-TR 22).

INFLUENCE OF THE CORE OLIGOSACCHARIDE OF LIPOPOLYSACCHARIDES ON THE ANTIBACTERIAL ACTION OF POLYMYXIN B

Malte U. Hammer*, Thomas Gutsmann,

Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Division of Biophysics, Parkallee 10, 23845 Borstel, Germany
mhammer@fz-borstel.de

The first target of polycationic antimicrobial peptides, in case of Gram-negative bacteria, is the outer leaflet of the outer membrane which is composed mainly of anionic lipopolysaccharides (LPS). We investigated the influence of the core oligosaccharide of LPS on the antibacterial action of polymyxin B (PMB). To this end, various strains of *Salmonella enterica* serovar Minnesota differing in the length and substitution with charged (e.g. phosphates) or uncharged groups of the core oligosaccharide of the LPS structure were used. The antimicrobial activity was determined by cell culture methods. As a measure for the surface potential, the ζ potential was determined for whole bacteria and for aggregates composed of isolated LPS from the respective bacterial strain in dependence on the PMB concentration. Furthermore, the incorporation of PMB into LPS-monolayers was determined by film balance measurements, and the lesion-forming capability was investigated using asymmetric planar bilayers. After binding to LPS, which is mainly electrostatically driven, PMB intercalates into the membrane and induces lesion formation, thus allowing further PMB molecules to permeate through the outer membrane and to reach their final locus of action, which is the cytoplasmic membrane or cytosolic targets. Our data allow to explain the different biological activities of PMB against different bacterial strains on the basis of their different core oligosaccharide structures.

IMMUNOCHEMICAL CHARACTERIZATION OF THE LIPOPOLYSACCHARIDES FROM A GROUP OF *AZOSPIRILLUM BRASILENSE* STRAINS

Yuliya P. Fedonenko[1]*, Alevtina S. Boiko[1], Olga N. Konnova[1], Evelina L. Zdorovenko[2], Svetlana A. Konnova[3], Vladimir V. Ignatov[1]

[1]*Institute of Biochemistry and Physiology of Plants and Microorganisms, RAS, Saratov,*
[2]*N.D. Zelinsky Institute of Organic Chemistry, RAS, Moscow, Russia*
[3]*Saratov State University, Saratov, Russia*
room308@ibppm.sgu.ru

In this work we report on the structures and serological properties of the O-antigens from four strains of the diazotrophic rhizobacterium *A. brasilense* - SR75, SR15, Cd, and S17 – isolated in various climatic zones. Lipopolysaccharides (LPSs) were extracted by the phenol-water method. Immunochemical analysis of the LPSs and O-antisera (against whole cells of *A. brasilense* Sp245, Sp7, and Cd and also against homologous LPSs) revealed common antigenic determinants. This finding allowed us to divide the bacteria into two groups: (1) *A. brasilense* SR75, SR15, and Sp245; (2) *A. brasilense* Cd and *A. lipoferum* Sp59b. No cross-reaction with the LPS of any other strain tested was found for the LPS of *A. brasilense* S17. Previously, we described the O-polysaccharides (OPS) structures for *A. brasilense* Sp245 and *A. lipoferum* Sp59b [1, 2]. The OPSs of *A. brasilense* - SR75, SR15, Cd, and S17 were isolated by mild acid degradation of LPSs, and their structures were determined by component and methylation analysis, and one- and two-dimensional ^{1}H- and ^{13}C-NMR spectroscopy. The data showed that the serological cross-reaction revealed in each bacterial group was due to the fact that the structures for the OPS repeating units (or, in the case of *A. brasilense* SR15, for the common fragment) were identical. For the LPS of *A. brasilense* S17, we showed the presence of a mixture of two structurally different neutral OPSs. However, one of these shared a common fragment with the OPS of *A. lipoferum* SpBr17 [3]. We believe that although the strains examined in this work were different origin, their development proceeded in a convergent fashion. Possibly, this process was largely influenced by the occurrence of the bacteria in similar ecological niches.

This work was funded in part RFBR (project 05-04-48123a) and the Foundation for Young Scientists of the Russian Federation (projects MK-1514.2005.4 and NSh.6177.2006.4).

1. Fedonenko, Yu.P.; Zatonsky, G.V.; Konnova, S.A.; Zdorovenko, E.L.; Ignatov V.V. *Carbohydr. Res.* **2002**, *337*, 869-872.
2. Fedonenko, Yu.P.; Konnova, O.N.; Zatonsky, G.V.; Konnova, S.A.; Kocharova, N.A.; Zdorovenko, E.L.; Ignatov V.V. *Carbohydr. Res.* **2005**, *340*, 1259-1263.
3. Choma, A.; Lorkiewicz, Z.; Russa, R. *Abstr. 9th Intern. Congress on Nitrogen Fixation*; Cancun, Mexico, 1992; p. 125.

EXTRACELLULAR AND CELL-WALL-BOUND LIPOPOLYSACCHARIDES: DIFFERENCES AND SIMILARITIES IN STRUCTURE AND BIOLOGICAL ACTIVITIES

Evelina L. Zdorovenko

N.D. Zelinsky Institute of Organic Chemistry, RAS, Moscow, Russia
evelina@ioc.ac.ru

Studies of biological activities (toxicity and antitumor activity) on mammalians of the lipopolysaccharides (LPSs) from phytopathogenic bacteria *Pseudomonas syringae* have revealed preparations with striking toxicity (>10 fold as compared to the classical endotoxin of *E. coli*) [1]. Although similar in structure [2,3], extracellular and cell-wall-bound LPS preparations of the same *P. syringae* strain have been found to differ in antitumor activity [1]. The aim of the present work was to find structural distinctions between extracellular and bound LPSs that define differences in their biological activities.

The structures of the three parts of the LPS molecule (lipid A, core oligosaccharide, O-specific polysaccharide) were studied by component and methylation analyses, ESI-FT mass-spectrometry, one- and two-dimensional ^{1}H- and ^{13}C-NMR spectroscopy. The structure of the O-specific polysaccharide was found to have no effect on the level of toxicity and antitumor activity. As in *E. coli*, lipid A with the maximal number (six) of fatty acids showed the highest toxicity. The degree of phosphorylation of the LPS was found to be critical for manifesting the antitumor activity.

This work was supported by the Council on Grants at the President of the Russian Federation for Support of Young Russian Scientists (project MK-3962.2005.4) and the Russian Science Support Foundation.

1. Zdorovenko G.M. et al. (1992) Proc. Intern. Conf. On Plant Pathogenic Bacteria, Versailes, France; Varbanets L.D. et al. (1995) Exp. Oncology, 17, No 3, 211-214.
2. Zdorovenko G.M. et al. (1991) In *Pseudomonas syringae* Pathovars, Proc. 4th Intern. Working Group on *Pseudomonas syringae* pathovars, Firenze, Italy, pp. 392-401.
3. Knirel Y.A. et al. (1997) In *Pseudomonas syringae* and related pathogens, Proc. 5th Intern. Working Group on *Pseudomonas syringae* pathovars and related pathogens, Berlin, Germany, pp. 475-480.

THE INFLUENCE OF LIPOPOLYSACCHARIDES AND LIPID AS FROM SOME MARINE BACTERIA ON SPONTANEOUS AND INDUCED BY *ESCHERICHIA COLI* LPS TNFα RELEASE FROM PERIPHERAL HUMAN BLOOD CELLS

Inna N. Krasikova*, Elena V. Vorobeva, Aleksandra S. Volk, Tamara F. Solov'eva

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, pr. 100 let Vladivostoku 159, 690022 Vladivostok, Russia

** Email: innakras@mail.ru*

Some endotoxic properties of lipopolysaccharides (LPSs) and lipid As (LAs) from marine Gram-negative bacteria *Chryseobacterium indoltheticum* CIP 103168^T, *Idiomarina zobellii* KMM 231^T, *Marinomonas communis* ATCC 27118^T, *Marinomonas mediterranea* ATCC 700492^T, *Marinomonas vaga* ATCC 27126^T, *Pseudoalteromonas haloplanktis* ATCC 14393^T, and *Shewanella alga* 48055 were studied. The preparations tested were shown to have high 50% lethal doses: from 1.46 (LA from *S. alga*) to 4.85 (LA from *P. haloplanktis*) and from 2.73 (LPS from *M. mediterranea*) to 12.65 (LPS from *M. communis*) μg per mouse. They were moderate (371 and 350 pg/ml at 10 μg/ml of *C. indoltheticum* and *S. alga* LPSs, and 320 pg/ml at 100 μg/ml of *P. haloplanktis* LPS), weak (148 pg/ml at 1 μg/ml of *M. mediterranea* LPS), and poor (LAs from *C. indoltheticum* and *M. communis*; LPSs from *I. zobellii*, *M. communis*, and *M. vaga* at all used concentrations, and LPS from *P. haloplanktis* at 1 - 10000 ng/ml) inducers of tumor necrosis factor α (TNFα) in peripheral human blood cells.

LPSs from *I. zobellii*, *M. communis*, and *M. vaga* were then tested as inhibitors of LPS-induced TNFα release from peripheral human blood cells. At concentration of 1 μg/ml, they were capable of inhibiting the response to LPS from *Escherichia coli* O55 : B5 (10 ng/ml) by 80 (LPS from *M. communis*), 91 (LPS from *M. vaga*), and 82 (LPS from *I. zobellii*) %. On the example of LPS from *M. communis*, it was shown that the inhibition could be overcome by increasing the concentration of *E. coli* LPS, and thus, these compounds appear to be competitive antagonists.

A competitive inhibitor of endotoxin should effectively block the biologic activities of any toxic LPS preparation. In addition to the LPS from smooth *E. coli* (LD_{50} = 0.012 μg/mouse), two other endotoxins from smooth (*Yersinia pseudotuberculosis*, LD_{50} = 0.06 μg/mouse) and rough (*Salmonella minnesota* R7, LD_{50} = 0.021 μg/mouse) bacterial strains were examined. With all endotoxins, LPS from *M. communis* blocked the production of TNFα in human whole blood cells. These results support the concept that LPS stimulates cells by interacting with a specific LA receptor.

Thus, LPSs from marine bacteria may be endotoxin antagonists with a universal type of action. It is interesting that their inhibitory concentrations were much lower than that of endotoxin antagonist E-5531, a well known synthetic analog of pentaacylated LA from *Rhodobacter capsulatus*. For E-5531, semi-maximal inhibition of TNFα release, induced by 10 ng/ml of LPS from *E. coli*, was observed at concentration of 3765 ng/ml, whereas LPSs from *I. zobellii*, *M. communis*, and *M. vaga* displayed similar results at concentration lower than 100 ng/ml. This makes further more complex investigation of LPSs from marine bacteria as potential endotoxin antagonists promising.

EXPLOITING GENOMICS IN PROFILING THE STRUCTURE OF SHORT-CHAIN NTHI LPS

Elke K. H. Schweda

Karolinska Institutet and University College of South Stockholm, S-141 86 Huddinge, Sweden
elke.schweda@kfc.ki.se

Non-typeable (acapsular) Haemophilus influenzae (NTHi) is a major cause of otitis media accounting for 25-30% of all cases of the disease. Lipopolysaccharide (LPS) is an essential and exposed component of the H. influenzae cell wall. A characteristic feature of H. influenzae LPS is the extensive inter- and intrastrain heterogeneity of glycoform structure which is key to the role of the molecule in both commensal and disease-causing behaviour of the bacterium. However, to characterize LPS structure unambiguously is still a major challenge due to the extreme heterogeneity of glycoforms that certain strains express. Over the last decade we have focussed on structural and biosynthetic studies of LPS from a representative set of NTHi clinical isolates [1]. H. influenzae LPS comprises a conserved glucose-substituted triheptosyl inner-core moiety L-α-D-HepIp-(1→2)-[PEtn→6]-L-α-D-HepIIp-(1→3)-[β-D-Glcp-(1→4)]-L-α-D-HepIIIp linked to lipid A via a Kdo 4-phosphate. This inner-core unit provides the template for attachment of oligosaccharide- and non carbohydrate substituents like PCho, PEtn, Ac and Gly. A high number of genes involved in expression of these structural features have been identified. Amongst those are genes that are required for oligosaccharide initiation from each of the three heptose residues in the inner core which are lgtF (HepI), lic2C (HepII), and lpsA (HepIII). Genes that are required for the biosynthesis of globotetraose extending from HepIII are lgtD (β-D-GlcNAcp), lgtC (α-D-Galp) and lic2A (β-D-Galp). PCho addition has been shown to be directed by the products of the lic1 locus.

Currently we are investigating NTHi strains that express novel features as well as particular epitopes, i.e. globotetraose (α-D-GalpNAc-(1→4)-α-D-Galp-(1→4)-β-D-Galp-(1→4)-β-D-Glcp) or truncated versions thereof, are part of chain extensions from more than one of the heptoses of the inner-core. In order to establish LPS structure unambiguously we have used material from genetically defined isogenic mutants that express targeted truncated LPS structures. Thus, strains containing a lpsA mutation lack elongations from HepIII and allow us to characterize epitopes from HepI and HepII more efficiently. Moreover, in addition to providing a powerful aid for structure determination, the combination of genetics and detailed structural analysis is key in determining the genetic blueprint for LPS biosynthesis. This combined approach will be presented here.

[1] H.H. Yildirim, J. Li, J.C. Richards, D.W. Hood, E. R. Moxon and E.K.H. Schweda Biochemistry, 44, 5207 (2005)

INFLUENCE OF TEMPERATURE ON THE PRESENCE OF ECA IN LIPOPOLYSACCHARIDES FROM *YERSINIA ENTEROCOLITICA* O:3 R MUTANTS

[1]Katarzyna Anna Duda[*], [2]Mikael Skurnik, [3]Otto Holst, [1]Katarzyna Teresa Duda, [1]Katarzyna Kasperkiewicz, [1]Joanna Radziejewska-Lebrecht

[1]Department of Microbiology, University of Silesia, Katowice, Poland, [2]The Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland, [3]Division of Structural Biochemistry, Research Center Borstel, Borstel, Germany.

Corresponding author: Joanna Radziejewska-Lebrecht, joaradle@us.edu.pl

Enterobacterial Common Antigen (ECA) is a cell surface lipoglycan that is present in all Gram–negative enteric bacteria. This polymer, built of trisaccharide repeating units [→3)-α-D-Fuc*p*4NAc-(1→4)-β-D-Man*p*NAcA-(1→4)-α-D-Glc*p*NAc-(1→]$_n$ is anchored in the outer membrane of the bacterial cell wall either *via* its own glycerophospholipid (ECA_{PG}) or *via* lipid A of lipopolysaccharide (LPS) (ECA_{LPS}). It can also be present in the periplasmic space in a cyclic form (ECA_{CYC}) with four to six repeating units. The biological role of ECA is not well understood.

Yersinia enterocolitica causes gastrointestinal diseases ranging from acute enteritis to mesenteric lymphadenitis. As all *Yersiniae*, it possesses a collection of genes that are temperature-regulated. Influence of temperature also on the ECA biosynthesis was observed in smooth forms of *Y. enterocolitica* O:3 (Muszyński *et al.,* submitted). However, the details of the temperature effect on ECA biosynthesis are not known yet.

For the present work five isogenic rough *Y. enterocolitica* O:3 mutant strains differing in their LPS structures were cultivated at 22°C and 37°C; one with a complete (YeO3-R1) and one (YeO3-c-trs22-R) with a truncated outer core region in LPS and three possessing only the inner core (YeO3-c-RfbR7, YeO3-c-trs8-R and YeO3-c-trs24-R). LPS preparations were obtained from dry bacterial masses by phenol/water extraction followed by phenol/chloroform/light petroleum extraction in order to isolate LPS/PCP that might contain ECA_{LPS} but not ECA_{PG}.

The LPS/PCP samples and standard preparation of ECA_{PG} were analysed by Western blotting, immunostained either with Mab898 (specific for ECA) or with monovalent antiserum against *E. coli* O14:K7 enriched in antibodies against ECA. Additionally, these preparations were analysed by an ELISA test using Mab898.

In both ELISA and Western blotting LPS/PCP preparations from bacteria grown at 37ºC showed stronger reaction with Mab898 than those from bacteria grown at 22ºC. An exception was strain YeO3-c-trs24-R that expressed trace levels of ECA at both temperatures. We therefore conclude that biosynthesis of ECA_{LPS} is temperature-dependent and that more ECA_{LPS} is expressed at 37ºC than at 22ºC.

STRUCTURAL CHARACTERIZATION OF THE MAJOR GLYCOLIPIDS FROM *ARTHROBACTER GLOBIFORMIS* AND *ARTHROBACTER SCLEROMAE*

Mariola Paściak*[a], Anna Duda[a], Ying Huang[b], Otto Holst[c], Andrzej Gamian[a]

[a]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland; [b]Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, PR China; [c]Borstel Research Center, Parkallee 22, Borstel, 23845 Germany

The genus *Arthrobacter* includes a heterogeneous group of aerobic, Gram-positive, non-fermentative actinobacteria. Members of this genus are widely distributed in the environment, especially in soil, and have recently been recognized as opportunistic pathogens. New *Arthrobacter* species were isolated from blood, skin infection, surgical wound, urinary tract infection and endophthalmitis.

The aim of the studies was isolation, purification and chemical studies of major glycolipids from *Arthrobacter* representatives. Two major glycolipids were isolated from *Arthrobacter globiformis* (type strain isolated from soil) and *Arthrobacter scleromae* (clinical strain isolated from swollen scleromata of a dermatosis patient). Glycolipids G1 and G2 were isolated using column adsorption chromatography and purified using GPC, TLC and HPLC methods. On the basis of sugar and methylation analysis, MALDI-TOF mass spectrometry and specific enzymatic degradations of carbohydrate moieties of major glycolipids G1 and G2, it was shown that they are glycerol linked dimannoside D-Man(1→3)-D-Man(1→1/3)–Gro and glycerol linked monomannoside D-Man(1→1/3)–Gro, respectively. The fatty acid composition was similar in both strains, as glycolipids contained *iso* and *anteiso* branched pentadecanoic acid, hexadecanoic acid and heptadecanoic acid.

These results indicate that major glycolipid G1 from two strains belongs to diglycosylglycerides with 1 glycerol, 2 mannose and 2 fatty acids residues. Glycolipid G2 belongs to monoglycosylglycerides with 1 residue of glycerol and mannose and 2 residues of fatty acids. The major glycolipids studied have potential value for taxonomic purposes. The glycolipid G1 of *Arthrobacter* spp. has the same TLC mobility as major glycolipid of *Rothia, Micrococcus* and *Saccharopolyspora* but glycolipid G2 could be used to differentiate *Arthrobacter* genus from other representatives of *Micrococaceae* family.

Chemical analysis of major glycolipids from *Arthrobacter globiformis* and *Arthrobacter scleromae* are fundamental to further structural studies of these compounds and could facilitate their identification in clinical specimens.

DETERMINATION OF ENDOTOXIN USING GLC-MS DETECTION OF CHEMICAL MARKER 2-KETO-3-DEOXYOCTULOSONIC ACID (KDO) AND BY LAL METHOD

Ewa Zuziak*[a], Jacek Rybka[a], Michał Zimecki[a], Danuta Witkowska[a], Leonard Kraśnik[c], Christian Pehrson[b], Lennart Larsson[b], Andrzej Gamian[a]

[a]Institute of Immunology and Experimental Therapy, Weigla 12, 53-114 Wroclaw, Poland; [b]Department of Medical Microbiology, Dermatology and Infection, University of Lund, Sölvegatan 23, 223 62 Lund, Sweden; [d]Department of Physiology, Medical Academy, Poznań, Święcickiego 6, Poland

Lipopolysaccharides (LPS, endotoxins) are major components of the outer membrane of Gram-negative bacterial cell envelope. LPS possess immunostimulatory and toxic properties to human organism even in trace amounts. Detection and quantitation of endotoxins in biological fluids, environment or biotechnological products is a matter of great importance for medicine as well as for the industry. Kdo (3-deoxy-D-*manno*-2-octulosonic acid) is one of the inherent constituents of bacterial endotoxin [1]. A gas-liquid chromatographic – mass spectrometric (GLC-MS) method was applied to the detection of Kdo as a possible marker of endotoxin [2]. In this Kdo method samples are methanolyzed, then dephosphorylated with alkaline phosphatase and acetylated prior to GLC-MS analysis. In the present work the Kdo method was applied for the estimation of the endotoxin content in various samples and results were compared with the endotoxin determination with LAL (Limulus Amebocyte Lysate) assay. In the first experiment the rat serum was analyzed for the Kdo and endotoxin content. Two groups of rats were treated with intraperitoneal injections of LPS from *E. coli* O127:B8, the first group received a single LPS dose of 20mg/kg of body weight, second group received LPS injections of 2mg/kg over ten days (total dose of 20mg/kg body weight), whereas a control group received saline only. The level of Kdo per ml of serum in the fist group was on average 10 times higher than in the second group and 60 times higher than in control group. Comparison of results obtained by both Kdo and LAL methods indicate, that GLC-MS provides information about the total LPS content, whereas LAL test detects only biologically active part of endotoxin present in the sample. For the determination of endotoxin in lactoferrin samples, the method of choice appeared the Kdo assay due to the interference of color in the LAL test. Both methods were suitable for the determination of endotoxin in samples of bacterial plasmids, outer membrane proteins preparations as well as industrial products like inorganic salts and water. The work was supported by grant 3 P04A 014 23 of Polish Ministry of Science and Higher Education.

[1] Holst O., Chemical structure of the core region of lipopolysaccharides. In: Endotoxins in Health and Disease, ed. by Brade H. et al., Marcel Dekker Inc., 1999, pp 115-154.

[2] Rybka J., Gamian A., J. Microbiol. Meth. 2006, 64, 171-184.

ANALYSIS OF ADVANCED GLYCATION END-PRODUCTS IN HUMAN SERUM SAMPLES USING THE ASSAY BASED ON MODEL COMPOUNDS SYNTHESIZED WITH DRY REACTION AT HIGH TEMPERATURE

Arkadiusz Bartyś*[a], Andrzej Gamian[a], Magdalena Staniszewska[a], Leszek Masłowski[b], Michał Sroka[b], Jerzy Leszek[c]

[a]Institute of Immunology and Experimental Therapy, Weigla 12, 53-114 Wroclaw, Poland; [b]Department of Angiology, Regional Specialistic Hospital, Kamieńskiego 73A, 51-124, Wrocław, Poland; [c]Department of Psychiatry, Medical University, Pasteura 10, 50-367 Wrocław, Poland

Advanced glycation end-products (AGE) accumulating in living tissues play an important role in diseases related to diabetes and aging processes [1]. The AGE formed during spontaneous reaction of aldehydes and proteins in the organism accumulate in tissues and modify several important proteins. Model compounds are synthesized in order to prepare the diagnostics and experimental tools for studying the mechanisms of pathogenesis. In our laboratory the high temperature glycation (HTG) method was applied to obtain a panel of model glycation products from several sugars [2]. Synthesis of freeze-dried mixture of substrates was performed at 115 °C for 30 minutes, then the products were fractionated on Sephadex G-200 column. The fractionated products were used to prepare anti AGE sera, because immunization with a mixture of glycation products produces antibodies in low titer against AGEs. The synthetic procedures allowed to elaborate specific immunochemical test for the measuring glycation in human organism, that involves the determination of protein AGE, anti-AGE antibody and immune complexes. That ELISA inhibition test for the determination of AGEs is based on an affinity purified rabbit antibody and synthetic AGE antigen, where analyzed serum sample treated with proteinase K is used as an inhibitor. The specificity of the elaborated tests concerns the epitopes, structure of which is under investigation. Further experiments revealed that the obtained model products are recognized by human serum, what indicates that the synthesized AGEs mimic the epitope present in human organism. Immunochemical experiments revealed the distinct lower level of circulating serum AGEs in patients with Alzheimer's disease, in relation to healthy controls [3]. Significant lower level of glycation products were also noted in sera of patients with diabetic nephropathy, whereas in type 1 and type 2 diabetic patients the level of Ages was increased comparing to healthy persons.

[1] Y. Al-Abed, A. Kapurniotu, R. Bucala, Meth. Enzymol. 1999, 309, 152-171.

[2] M. Staniszewska, S. Jarosz, M. Jon, A. Gamian, Arch. Immunol. Ther. Exp. 2005, 53, 71-78.

[3] M. Staniszewska, J. Leszek, K. Małyszczak, A. Gamian, Int. J. Geriatr. Psychiatry, 20, 2005, 896-897.

DISTRIBUTION OF ENDO- AND EXOGEOUS 3-HYDROXY FATTY ACIDS IN RAT TISSUES

Bogumiła Szponar*[1,3], Leonard Kraśnik[2], Lennart Larsson[3]

[1]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław; [2]Medical Academy of Poznań, Dept. of Physiology, Poland; [3]Lund University, Dept. of Laboratory Medicine, Sweden

corresponding author: szponar@iitd.pan.wroc.pl

3-Hydroxy fatty acids (3-OH FAs) of 10-18 carbon chain lengths have been identified in mammal serum and tissues by using GC-MSMS1,2. These acids may stem from mitochondrial β-oxidation, from lipid A fragment of bacterial lipopolysaccharides, or from other sources. Increased levels of the 3-OH FAs were found in tissues of healthy mammals following injection of endotoxin3. For being able to determine whether the 3-OH FAs found in such tissues originate from bacterial or non-bacterial sources, in the present study, we used a rat model where LPS of Pectinatus cerevisiiphilus was injected into the peritoneal cavity. The structure of this LPS is known4 and characterized by high content of 3-OH C13, a 3-OH FA that is absent in mammals. We used 3-OH FA analysis 1) to determine the distribution of such intraperitoneally delivered endotoxin in serum and liver and 2) to distinguish these molecules of bacterial and non-bacterial origin. State-of-the-art gas chromatography–triple-quadrupole tandem mass spectro-metry was used for optimal analytical specificity. Quantitative assessment of bacterial and non-bacterial 3-OH FAs was performed with help of internal standard (synthetic deuterized 3-OH C14). Additionally, analysis of chiral derivatives of the 3-OH FAs was applied to confirm whether these acids were of R and/or S-forms.

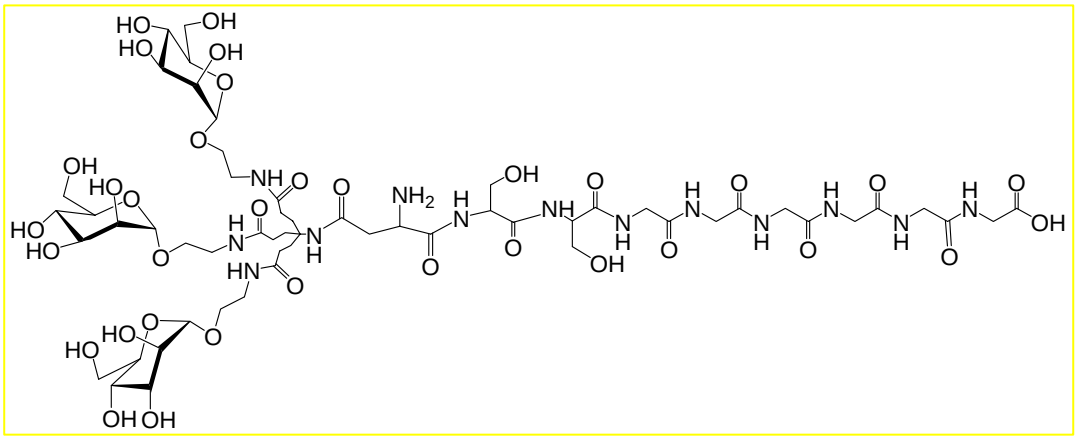
1. Szponar B, Norin E, Midtvedt T, Larsson L. Limitations in the use of 3-hydroxy fatty acid analysis to determine endotoxin in mammalian samples. J Microbiol Meth 2002, 50:283 -289.
2. Jones PM, Quinn R, Fennessey PV, Tjoa S, Goodman SI, Fiore S, Burlina AB, Rinaldo P, Boriack RL, Bennett MJ. Improved stable isotope dilution-gas chromatography-mass spectrometry method for serum or plasma free 3-hydroxy-fatty acids and its utility for the study of disorders of mitochondrial fatty acid beta-oxidation. Clin Chem. 2000, 46:149-155.
3. Szponar B, Krasnik L, Hryniewiecki T, Gamian A, Larsson L. Distribution of 3-hydroxy fatty acids in tissues after intraperitoneal injection of endotoxin. Clin Chem. 2003, 49:1149-1153.
4. Helander IM, Haikara A, Sadovskaya I, Vinogradov E, Salkinoja-Salonen MS. Lipopolysaccharides of anaerobic beer spoilage bacteria of the genus Pectinatus – lipopolysaccharides of a Gram-positive genus. FEMS Microbiol Rev 2004, 28:543-552.

TOWARDS DETERMINATION OF FUNCTION OF GLYCOCALYX

Harun A. Shaikh*, Thisbe K. Lindhorst

Christiana Albertina University of Kiel, Institute of Organic Chemistry, Otto-Hahn-Platz 3, 24098 Kiel, Germany

Curiosity about the biological function of the glycocalyx is one of the major incentives for our synthetic work. The glycocalyx is a fascinating macromolecular system covering every eukaryotic cell. Parts of the glycocalyx are anchored in the plasma membrane by conjugation to lipids and transmembrane proteins, respectively. The glycocalyx plays a crucial biological role in cellular communication processes. An important tool to investigate the biological function of the glycocalyx is synthetic glycoconjugates, which resemble portions of the structures found in the glycocalyx.



Glycocluster peptide

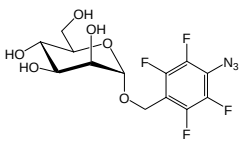
We have designed a new type of glycomimetic, a so-called '**glycocluster peptide**', which combines a cluster mannoside portion and a multifunctional peptide portion, which is synthesised on solid phase. In glycocluster peptides, the glycocluster moiety can be regarded as oligosaccharide mimic of a glycocalyx, while the peptide part resembles a portion of a transmembrane protein. With this molecule we would like to carry out several functional studies by attaching various labels to the peptide part of the molecule. These studies should allow assisting our understanding of the function of the glycocalyx in biological systems.

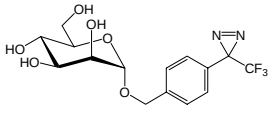
PHOTOACTIVE MANNOSE DERIVATIVES AND THEIR EVALUATION FOR LECTIN LABELING

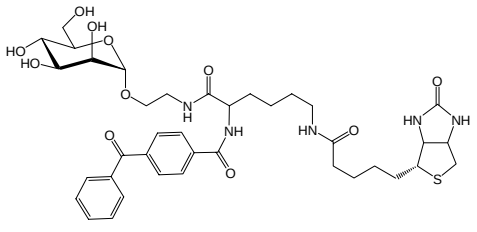
Michaela Wiegand*, Th. K. Lindhorst

Christiana Albertina University of Kiel, Institute of Organic Chemistry, Otto-Hahn-Platz 3, 24098 Kiel, Germany
mwiegand@oc.uni-kiel.de

Adhesion to the glycosylated surface of eukaryotic cells, mediated by carbohydrate- binding proteins called lectins, plays a significant role in cellular processes of living organisms such as cell-cell communication, cell division, cell maturing or inflammation, metastasis and apoptosis. To elucidate the mechanisms involved in adhesion to cell-surfaces and their biological consequences, the investigation of molecular interactions between carbohydrate recognition domains (CRDs) of lectins and their ligands is of important relevance. In this work, we have selected the photoaffinity labeling technique as powerful tool for exploring ligand binding to mannose-specific lectins. As target protein we chose the α-mannose-specific adhesin FimH, which is expressed at the tips of type 1 fimbriae of *Escherichia coli* bacteria.
To further investigate the carbohydrate binding properties of FimH, we have designed and synthezised a series of α-mannosides and glycopeptides derived thereof, which are equipped with a photoactive functional group (PAFG).[1] Introduced over 40 years ago[2] the general principle of photoaffinity labeling still holds: Upon irradiation with UV light, the photoactive functional group is converted into a highly reactive intermediate, which is able to form a covalent linkage with its receptor protein by insertion into N–H, O–H or C–H bonds, respectively.[3] The resulting crosslinked product can be visualized by different blotting methods (SDS–PAGE or immunoassays) and analyzed by mass spectrometry after enzymatic digestion of the labeled protein.
We will report about the synthesis of various α-mannoside derivatives with different photolabels, their photospectrometric features and their properties concerning irradiation in the presence of different target molecules. Moreover, we will report on the introduction of a biotin tag to facilitate purification of the crosslinked product using affinity chromatography and about mass-spectrometric analyses including various biochemical methods. A selection of the photoprobes prepared is depicted.







References:
[1] (a) M. Walter, Th. K. Lindhorst, *Synthesis* **2006**, 952-958. (b) M. Walter, M. Wiegand, Th. K. Lindhorst, *Eur. J. Org. Chem.* **2006**, 719-728. (c) M. Wiegand, Th. K. Lindhorst, *Eur. J. Org. Chem.* in press. [2] A. Singh, E. R. Thornton, F. H. Westheimer, *J. Biol. Chem.* **1962**, *237*, 3006-3008. [3] Y. Hatanaka, Y. Sadakane, *Curr. Top. Med. Chem.* **2002**, *2,* 271-288.

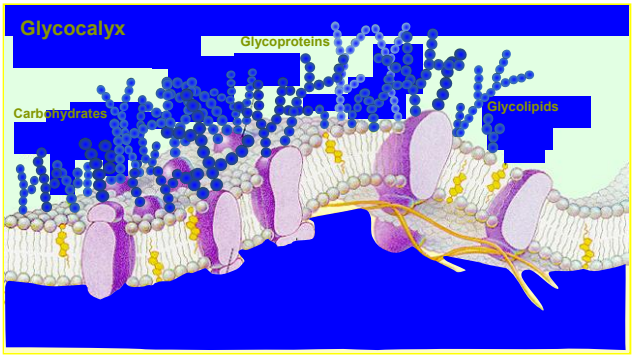
Synthesis of glycosylated lipid membrane models

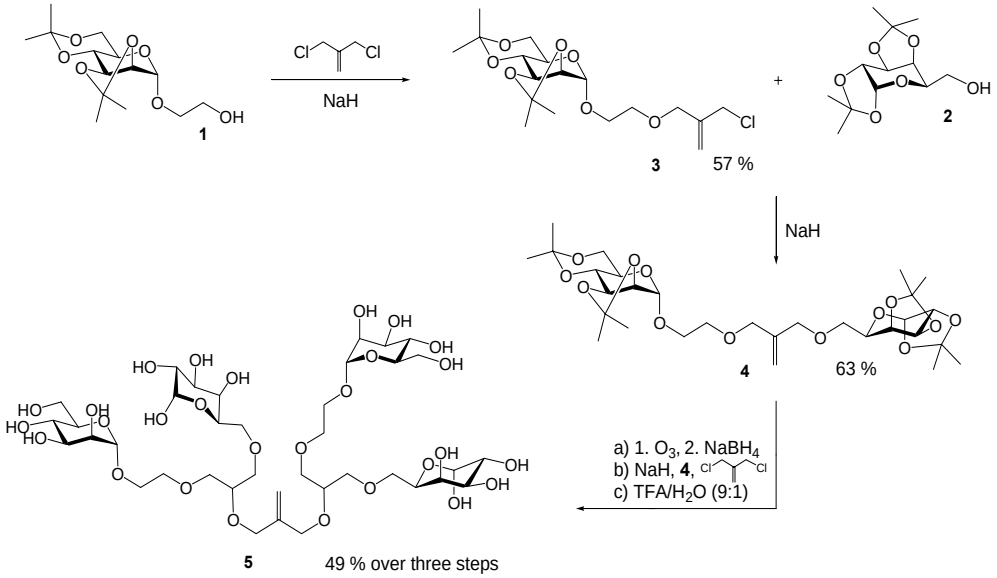
Katharina Elsner, Thisbe K. Lindhorst*

Otto Diels-Institut für Organische Chemie, Christian-Albrechts-Universität
Otto-Hahn-Platz 4, 24098 Kiel, Germany
kelsner@oc.uni-kiel.de

The plasma membrane of eukaryotic cells is a lipid bilayer made up of phospholipids and embedded proteins, which may be integral or associated membrane constituents. Both types of molecule cell membrane lipids as well as proteins are glycosylated with complex oligosaccharides and these are exposed to the extracellular site.

These carbohydrates are part of the so-called glycocalyx, which plays an essential role in cellular communication involving carbohydrate-protein and carbohydrate-carbohydrate interactions[1]. To elucidate the biological implications and underlying mechanisms of glycocalyx functions, glycomimetics have been seeked as molecular tools for glycobiology.





In this project, we have utilized the principles of dendrimer chemistry to mimic the hyperbranched character of the natural example structures and in addition, we are employing the focal point of oligosaccharide mimetics of a glycodendron type for functionalization, to approach a novel class of glycoprotein as well as glycolipid mimetics[2].

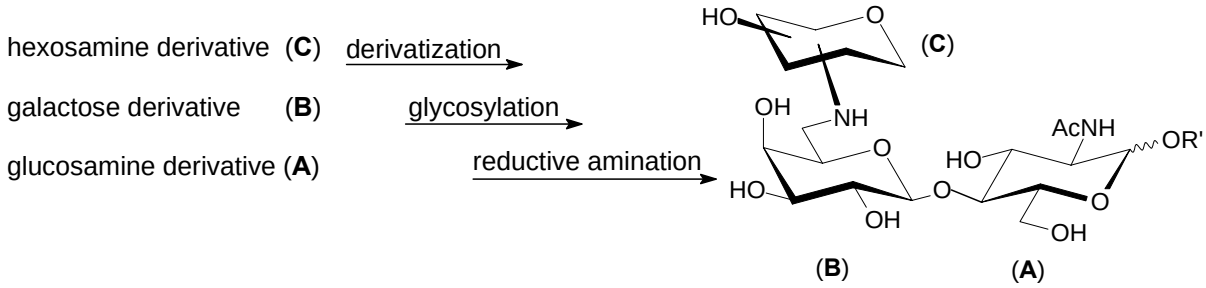
[1] S. V. Evans, C. R. MacKenzie, *J. Mol. Recog.* **1999**, *12*, 155-168.
[2] M. M. K. Boysen, K. Elsner, O. Sperling, Th. K. Lindhorst, *Eur. J. Org. Chem.* **2003**, *22*, 4376-4386.

SYNTHESIS OF NOVEL OLIGOSACCHARIDE MIMETICS AS INHIBITOR PROBES FOR NK CELL RECEPTORS

Janna Neumann*, Saskia Weingarten, Joachim Thiem

University of Hamburg, Faculty of Sciences, Department of Chemistry, Martin-Luther-King Platz 6, D-20146 Hamburg, Germamy

Oligosaccharides are of particular interest due to their involvement in biological recognition processes. It is attractive to explore the potential of simple methods to obtain oligosaccharides that can imitate natural carbohydrate structures. *N*-Acetyllactosamine represents a promising basis for the design of potential useful glycomimetics. To provide a certain resistance to enzymatic catabolism a third monosaccharide unit should be attached to the disaccharide *via* an unnatural sugar-sugar-bond, without participation of the anomeric center.
Therefore, efficient access to novel di- and trisaccharide mimetics was achieved by combining different methods, such as enzymatic glycosylation, selective derivatization and reductive amination.



Enzymatic glycosylation could be realized using β-galactosidase (*B. circulans*).[1] Novel disaccharides were obtained by modification of the monosaccharide building blocks prior to glycosylation.[2] The trisaccharides were obtained using reductive amination as a quite simple and selective method.
The generation of novel pseudo-disaccharides was achieved using Dess-Martin oxidation and a subsequent reductive amination. By oxidation of the primary alcohol group a C-6-carbaldehyde sugar was obtained which could be reacted with an amino sugar to give a nitrogen-linked disaccharide mimetic.

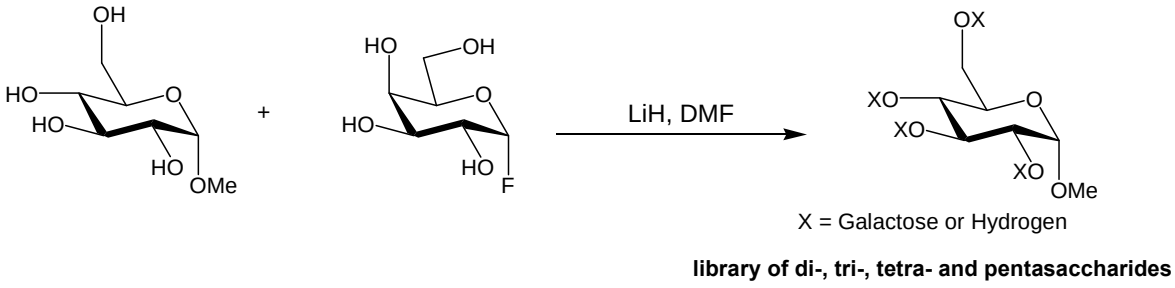
[1] T. Murata, M. Kosugi, T. Nakamura, T. Urashima, T. Usui, *Biosci. Biotechnol. Biochem.*, **2001**, *65*, 2456-2464.
[2] S. Weingarten, J. Thiem, *Org. Biomol. Chem.*, **2004**, *2*, 961-962.

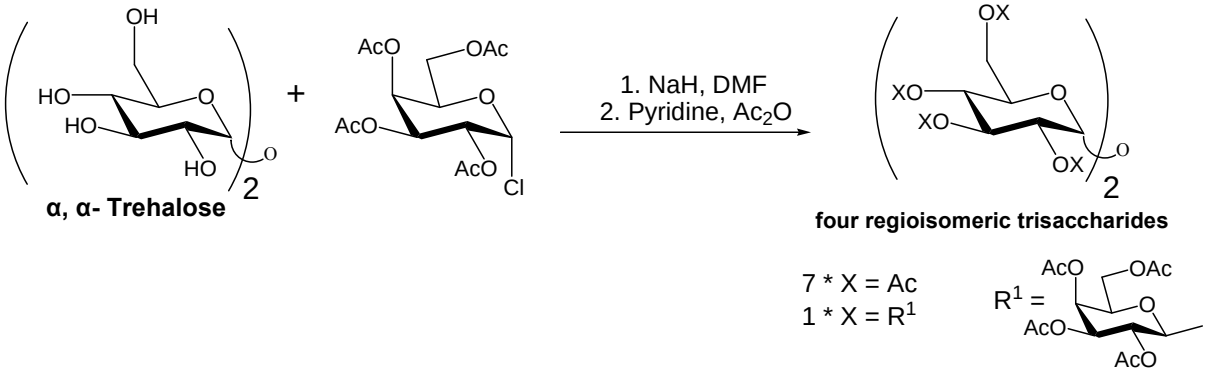
SYNTHESIS OF OLIGOSACCHARIDE LIBRARIES VIA AN UNUSUAL GLYCOSYLATION METHOD

Andreas Steinmann*, Julian Thimm and Joachim Thiem

University of Hamburg, Faculty of Sciences, Department of Chemistry
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Preparation of oligosaccharide libraries using classical protecting / deprotecting strategies is still a tedious exercise. There are only a few examples of random glycosylations of saccharides with several free hydroxyl groups [1, 2]**.** A reason may be that common glycosylation methods fail for unprotected sugars as we observed in attempts under varied conditions. This contribution will present the development of a glycosylation method for entirely unprotected mono- and disaccharides and also the analysis of the resulting product mixtures.





References

[1] Y. Ding, J. Labbe, O. Kanie and O. Hindsgaul, *Bioorg. Med. Chem*., **4** (1996) 683.
[2] B. Yu, B. Ling, G. Xing and Y. Hui, *J. Comb. Chem*., **3** (2001) 404 – 406.

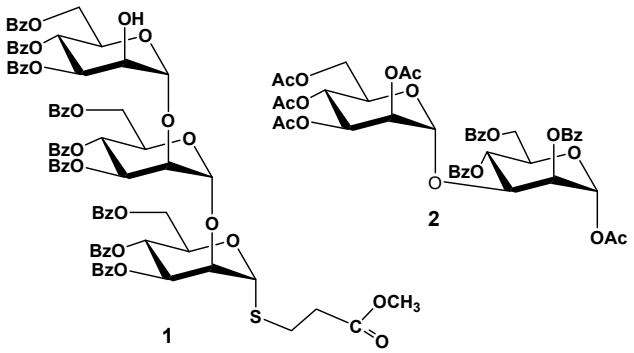
SYNTHESIS OF DI− AND TRISACCHARIDE PRECURSORS OF 2-CARBOXYETHYLTHIO GLYCOSIDE OF A LINEAR MANNO-PENTAOSIDE, A REPEATING UNIT OF O-ANTIGENIC POLY-SACCHARIDES FROM *ESCHERICHIA COLI* O9 AND *KLEBSIELLA PNEUMONIAE* O3

Polina I. Abronina*, Alexey A. Grachev, Leon V. Backinowsky

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect, 47, 119991,Moscow, Russia.
E-mail: polina-abronina@yandex.ru

O-Specific chains of lipopolysaccharides from *Escherichia coli* O9 and *Klebsiella pneumoniae* O3 with high adjuvant activities are built of pentasaccharide repeating units [1,2] [-3)-α-D-Man*p*-(1-3)-α-D-Man*p*-(1-2)-α-D-Man*p*-(1-2)-α-D-Man*p*-(1-2)-α-D-Man*p*-(1-].

We are planning to synthesise a pentasaccharide fragment of the polysaccharide as a 2-carboxyethylthio glycoside according to a [2+3] scheme. Here we present an access to the oligosaccharide precursors of the pentasaccharide, *viz*., a trisaccharide acceptor **1** and a disaccharide **2** as a glycosyl donor synthon, starting from 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranose and 1,3-di-*O*-acetyl-2,4,6-tri-*O*-benzoyl-α-D-mannopyranose, respectively, which carry 'permanent' *O*-benzoyl and 'temporary' *O*-acetyl protective groups. The latter is selectively removed upon mild acid-catalysed methanolysis leaving the former intact [3].



References

1. T. Yokochi, M. Fukada, M. Kawai, Y. Zhang, G. Jiang, and K. Takahashi, *Infect. Immun.* (1992), 60, No. 11, 4953-4956.
2. E. Vinogradov, E. Frirdich, L. L. MacLean, M. B. Perry, B. O. Petersen, J. Ø. Duus, and C. Whitfield, *J.Biol.Chem.* (2002), 277, No. 28, 25070-25081.
3. N. E. Byramova, M. V. Ovchinnikov, L. V. Backinowsky, and N. K. Kochetkov, *Carbohydr. Res.* (1983), 124, C8- C11.

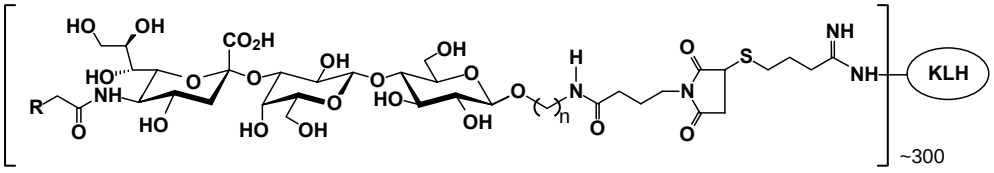
SYNTHESIS OF PROTEIN BASED NEOGLYCOCONJUGATES BEARING GLYCOLIPID LIGANDS INVOLVED INTO MICROBIAL ADHESION

Elena A. Khatuntseva*, Olga N. Udina, Yuri E. Tsvetkov, Alexey A. Grachev, Nikolay E. Nifantiev

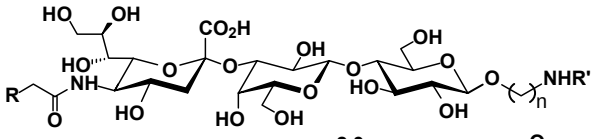
N.D.Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences. Leninsky 47,119991, Moscow, Russia. E-mail:nen@ioc.ac.ru

It is known that cell-surface glycolipids are involved into microbial adhesion. To carry out investigation of adhesion sites, labeled carbohydrate-specific antibodies are applied, which can be obtained upon immunization with neoglycoconjugates of oligosaccharide ligands with highly immunogenic proteins. Compounds of this type can also be trailed as potential carbohydrate onco-vaccines. In this context, neoglycoconjugates (**1**,**2**) of GM3 and N-glycolyl-GM3 trisaccharides with keyhole limpet hemocyanin were synthesized.

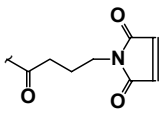
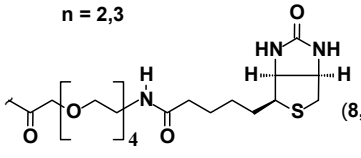
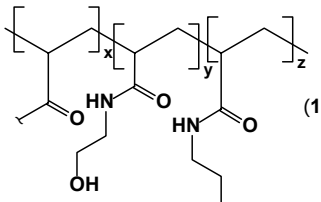
Starting from readily accessible biotechnological sialyl-3′-lactose (**3**), corresponding aminoalkyl β-glycosides of **3** (**4**) and its N-glycolyl analog (**5**) were synthesized and then converted to (4-maleimido-butanoylamino)alkyl derivatives (**6,7**). Interaction of these precursors with thiolated KLH resulted in neoglycoconjugates (**1,2**) containing about 300 trisaccharide ligands per KLH. Monovalent biotinylated conjugates with oligoethyleneglycol linker (**8,9**) and polyvalent conjugates with polyacrylamide (**10,11**) were also prepared as covering antigens for ELISA assay.



1 R = H (trisaccharide epitope of GM3 ganglioside), n = 3
2 R = OH (trisaccharide epitope of N-Gc-GM3 ganglioside), n = 2



n = 2,3

R' = H (**4,5**);  (**6,7**);  (**8,9**);  (**10,11**)

X = 0.2; Y = 0.4; Z = 0.4

We thank the Russian Foundation For Basic Research, grant # 05-03-08107.

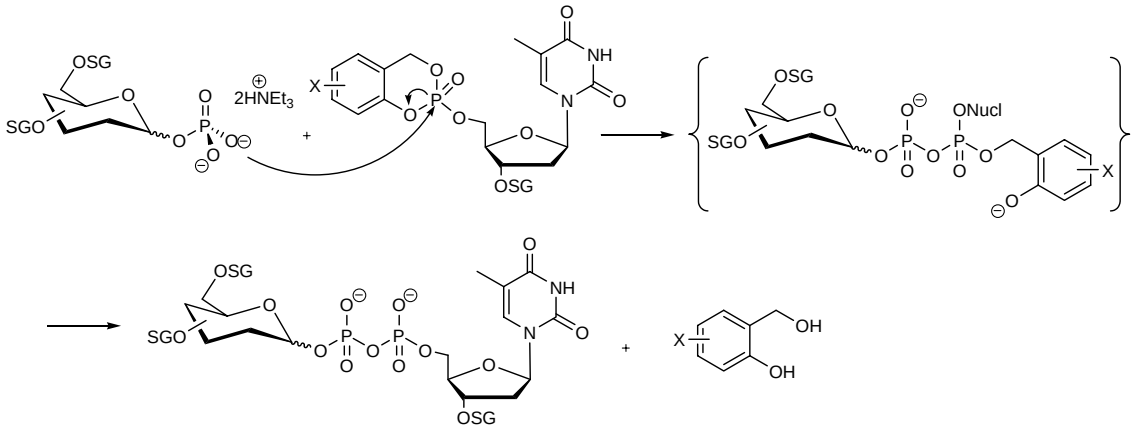
A NOVEL ACCESS TO NUCLEOSIDE DIPHOSPHATE SUGARS

Chris Meier, Silke Wendicke *

Department of Chemistry, Organic Chemistry, Faculty of Science, University Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg; Germany

chris.meier@chemie.uni-hamburg.de, wendicke@chemie.uni-hamburg.de

Nucleoside diphosphate sugars (NDP-sugars) are key metabolites in various biochemical processes, e.g. they serve as substrates of glycosyltransferases in the biosynthesis of all oligosaccharides and most of the polysaccharides.[1] Moreover, they play an indispensable role in the synthesis of deoxysugars, aminodeoxysugars, chain branched sugars, uronic acids and glycoconjugates. As a result, a number of methods have been developed for the preparation of naturally occurring NDP-sugars as well as for their analogues. Literature-known procedures for the synthesis of this important class of compounds proceed with very low yields. Here, we will present a new efficient chemical synthesis of NDP-sugars. Our new method uses *cyclo*Sal-nucleotides as active phosphate esters. Compounds of this type are originally used as prodrugs to deliver antiviral active nucleotides into cells.[2] However, the same type of compounds can also be used as active esters for synthetic application.



For the synthesis of NDP-sugars, the *cyclo*Sal-nucleotide is cleaved in a tandem reaction by an initial nucleophilic attack of the glycosylmonophosphate on the phosphorus center, yielding the product in 50-60 % after short reaction times. Moreover, the method allows the synthesis of NDP-sugars with a defined anomeric configuration. We optimized the reaction with regard to the solvent, the reaction temperature and the substituents at the *cyclo*saligenyl moiety. The method can be applied to the synthesis of a variety of nucleoside diphosphate sugars.

[1] L Leloir, *Science* **1971**, 1299.
[2] C. Meier, *Eur. J. Org. Chem*. **2006**, 1081.

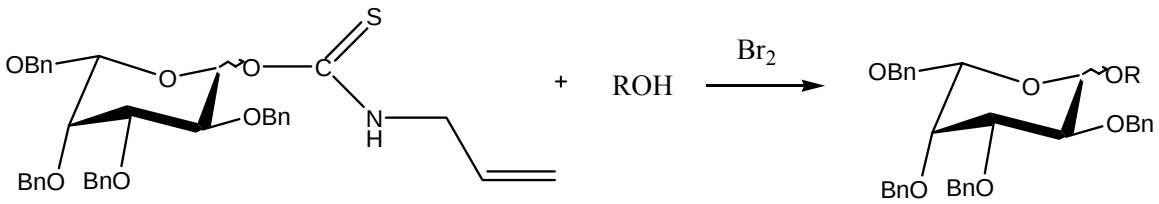
STUDY ON THE SYNTHESIS OF α-L-FUCOPYRANOSIDES

Anna Kasprzycka*, Wiesław Szeja, Christian Vogel

Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, 44-100 Gliwice, Krzywoustego 8, Poland
e-mail: akasprzycka@interia.pl, wieslaw.szeja@adres.pl.

The study of carbohydrates within biological systems has illustrated that they are involved in a number of fundamental biological functions such as cell-cell recognition and cell-external agent interactions[1].

The numbers of complex sugars biologically active constitute L-Fucose in the form of α-L-Fucopyranoside. Formation of this bond is not easy. Over recent years, N-allyl thiocarbamate of glycopyranoses have proved to be useful starting points in the stereoselective synthesis of O-glycosides[2]. We now present the application of this methodology in the synthesis of α-fucopyranosides. The method of preparation of glycosyl donor and synthesis of alkyl α-D-fucopyranosides di- and trisaccharides will be presented.



Literature:

[1]H. Lis and N. Sharon, *Chem. Rev.*, 98 **(1998)** 637 and references therein.

[2]A. Kasprzycka, G. Pastuch, A. Cyganek, W. Szeja, *Polish J. Chem.*, 77 **(2003)** 197.

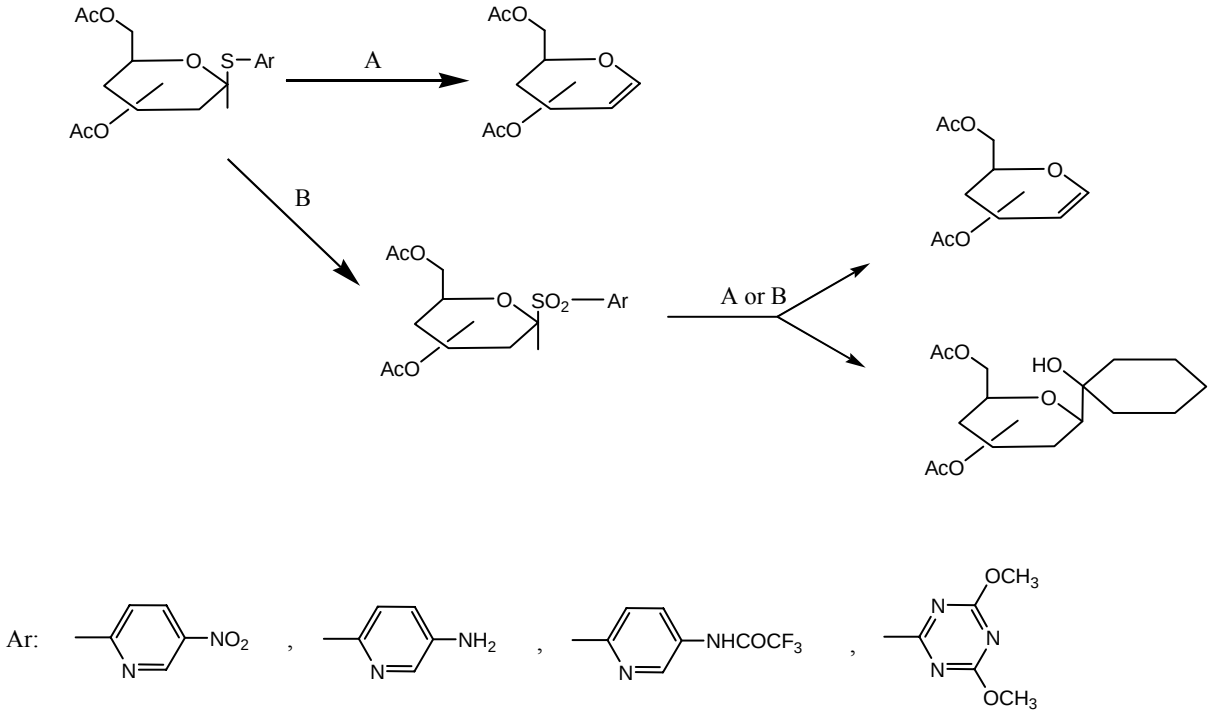
THIOGLYCOSIDES IN THE SYNTHESIS OF GLYCALS

Sebastian Derfla*, Gabriela Pastuch, Wiesław Szeja

Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, 44-100 Gliwice, Krzywoustego 8, Poland
Email: sderfla@wp.pl, gpastuch@wp.pl, wieslaw.szeja@adres.pl,

In recent years, 1,5-anhydro-2-deoxy-hex-1-enitals (glycals) have proved to be useful starting points/substrates in the synthesis of a wide range of carbohydrates [1].Particulary intresting is utilization of glycals in the preparation of oligosacharides containing 2-deoxysugar molecule [2].

The convenient method of the synthesis of glycals is via reductive elimination of an acetylated glucosyl halide. In our research on the synthesis of selectively protected glycosides by aryl groups, we have optimized the method for reductive elimination of anomeric sulfones[3].



A: SmI_2, HMPA, THF, r.t.
B: m-CPBA, CH_2Cl_2, r.t.
C: SmI_2, cycloheksanone, THF, r.t.

A number of sulfones have been examined in reductive elimination.The role of substituted hetaryl group in reductive elimination of glycosyl sulfones will be presented on the poster.

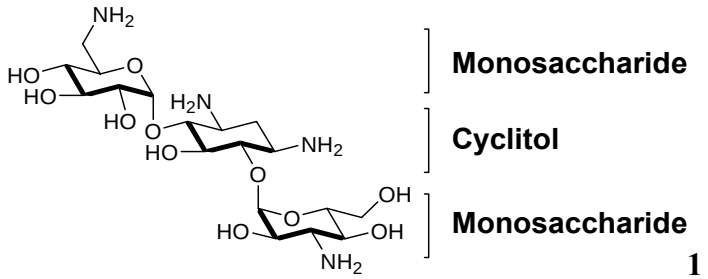
Literature:
[1] H.I. Osborne, *Carbohydrates*, Academic Press, **(2003)**.
[2] R. U. Lemieux and S. Levine, *Can. J. Chem.* **1964**, *42*, 1473.
[3] J. Thiem, H. Karl and J. Schwentner, *Synthesis* **1978**, 696.

NOVEL AMINOGLYCOSIDES AND CYCLITOLS

Thomas Jöge* **and Andreas Kirschning**

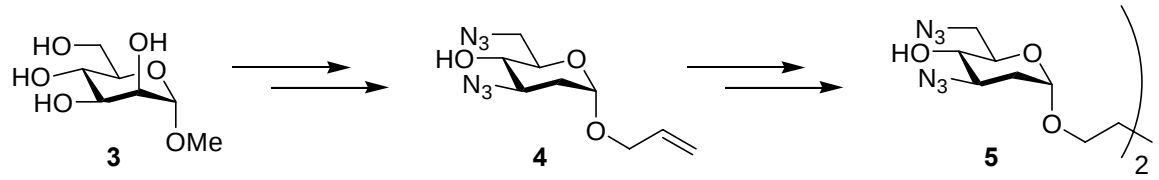
[*]Institute of Organic Chemistry, Schneiderberg 1b, 30167 Hannover, Germany
andreas.kirschning@oci.uni-hannover.de

Changing the conformation of nucleic acids at will would enable man to interfere with the life cycle of cells and viruses. RNA with its huge conformational diverse space (e.g. TAR RNA of HIV-1) is a very promising target for such an approach. Aminoglycosides like Kanamycin A **1** are prominent for their good binding properties to RNA.



1

This project focuses on the chemical and biological behavior of novel aminosugars. In this context, our efforts are governed by the goal to design novel "artificial" aminoglycosides or disaccarides like **4**. These novel structures consist of aminated sugar building blocks which are connected to each other by a flexible linker.



3 **4** **5**

Their oligomeric character containing several amino groups is essential for efficient binding and should lead to cooperative effects and hence tighter binding. Their synthesis is achieved by metathesis reactions starting from allyl linker building blocks like **3**. This synthetic strategy yields extended aminosugar structures like **4** in a few steps[1-3].

[1] A. Kirschning, G.-w. Chen, *Tetrahedron Lett*. **1999**, *40*, 4665-4668.
[2] A. Kirschning, G.-w. Chen, *Chem. Eur. J.* **2002**, *8*, 2717-2729.
[3] A. Kirschning, M. Lindner, *Tetrahedron* **2004**, *60*, 3505-3521.

SYNTHESIS AND APPLICATION OF METHYL-2,3-DIACETAMIDO-2,3-DIDEOXY-URONIC ACIDS

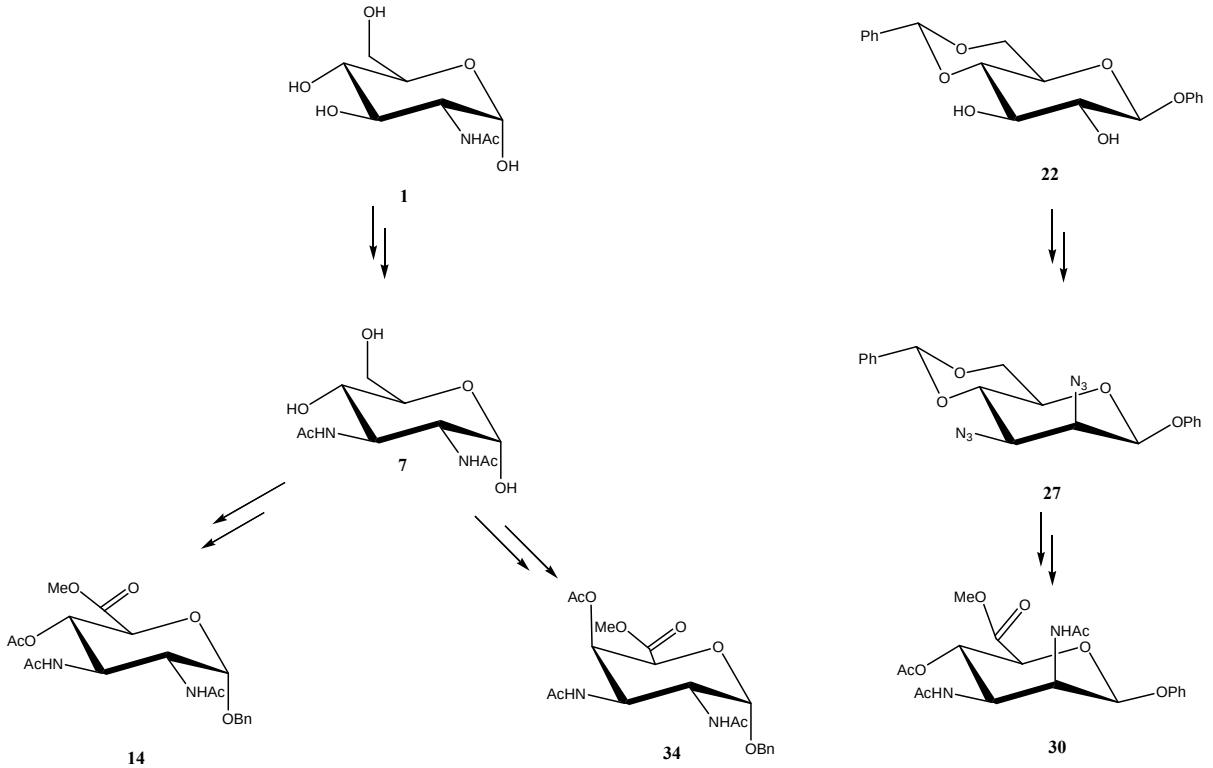
Nico Paepke*, Christian Vogel

Institute of Chemistry, University of Rostock

2,3-Diacetamido-2,3-dideoxy-uronic acids, as components of antigenic polysaccharides, were identified in several strains[1] of bacteria but the reports for the preparation is exclusively for D-gluco-configurated carbohydrates.

Based on the synthesis of 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid by Kochetkov et al.[2] we synthesized systematically 2,3-diacetamido-2,3-dideoxy-D-uronic acids with *gluco*[3]*-, galacto*[4]*- and manno*[5]-configuration.

For this we found different pathways to our target molecules. Starting from 2-Acetamido-2-deoxy-D-glucose (GlcNAc) **1** and Phenyl-4,6-benzylidene- D-glucopyranoside **22** the received 2,3-Diacetamido-uronic acids **14**, **30**, **34** were characterized and used for further tests.



For clinical studies the synthesized uronic acids were deprotected by acetolysis and basic deacetylation.

In further applications the synthetically obtained diacetamidouronic acids were transformed into derivatives with a protecting group pattern which makes these compounds suitable as either a donor or an acceptor in a glycosylation reaction.

[1] Knirel, Y.A., Kochetkov, N. K., (1987) *Microbiology Reviews* **46**: 381-385
[2] Dmitriev, B.A., Kocharova, N.A., Kochetkov, N.K. (1982) *Bioorganicheskaya Khimiya* **8**: 1234-1241 (Journal written in Russian)
[3] Reckendorf, W. Meyer zu, (1969) *Chemische Berichte* **102**: 4207-4208
[4] Reckendorf, W. Meyer zu, (1970) *Chemische Berichte* **103**: 995-996
[5] Guthrie, R.D., Murphy, D. (1962) *Journal of the Chemical Society*: 5288-5294

SYNTHESIS OF SPECIAL *N*- AND *C*-GLYCOSIDES OF GALACTURONIC ACID

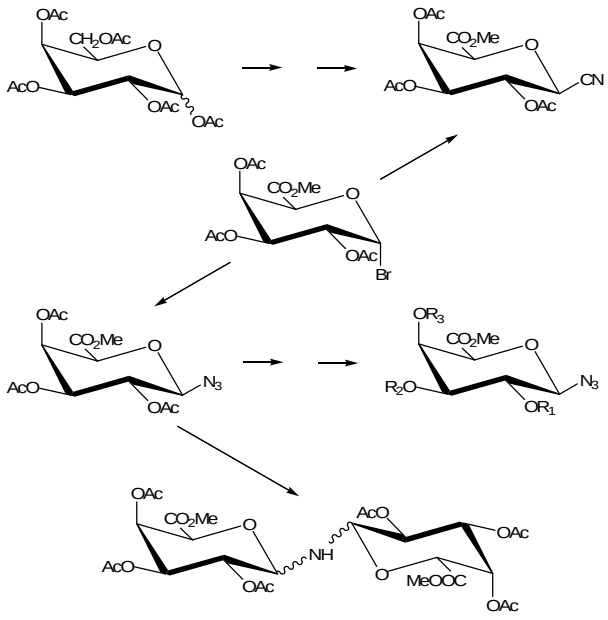
Gnuni Karapetyan*, Christian Vogel

Institute of Chemistry, University of Rostock, Albert-Einstein-Str. 3A, D-18059, Germany

N-glycosidic bonds are used to conjugate carbohydrates to other biomolecules, for example, polypeptides in glycoproteins and nucleic acids, RNA and DNA. The obvious importance of this linkage in biology has driven the search for efficient and stereoselective methodologies for the synthesis of N-glycosides.

Thus, methyl 2,3,4-tri-O-acetyl-1-azido-β-D-galactopyranuronate is available in a large scale from galacturonic acid by means of four–step sequence involving acetylation, esterification of uronic acid, activation as the glycosyl bromide, and stereospecific displacement with azide anion[1,2,3]. The azido group gives us further possibilities to introduce new functionalities, usually with retention of the β-stereochemistry. Using different protecting groups' manipulations we describe the synthesis of some new 1-azido-galacturonic acid derivatives as suitable building blocks and some novel "unusual" properties of them.

On the other hand, glycosyl cyanides are versatile intermediates for the synthesis of C-glycosyl derivatives, because the cyano group can be readily transformed into of variety of other functional groups. In fact glycosyl cyanides have been used as starting compounds for the synthesis of naturally occurring C-nucleoside antibiotics and many analogous[4]. Here we describe a new pathway for the synthesis of 2,3,4-tri-O-acetyl-1-cyano-β-D-galactopyranuronate.



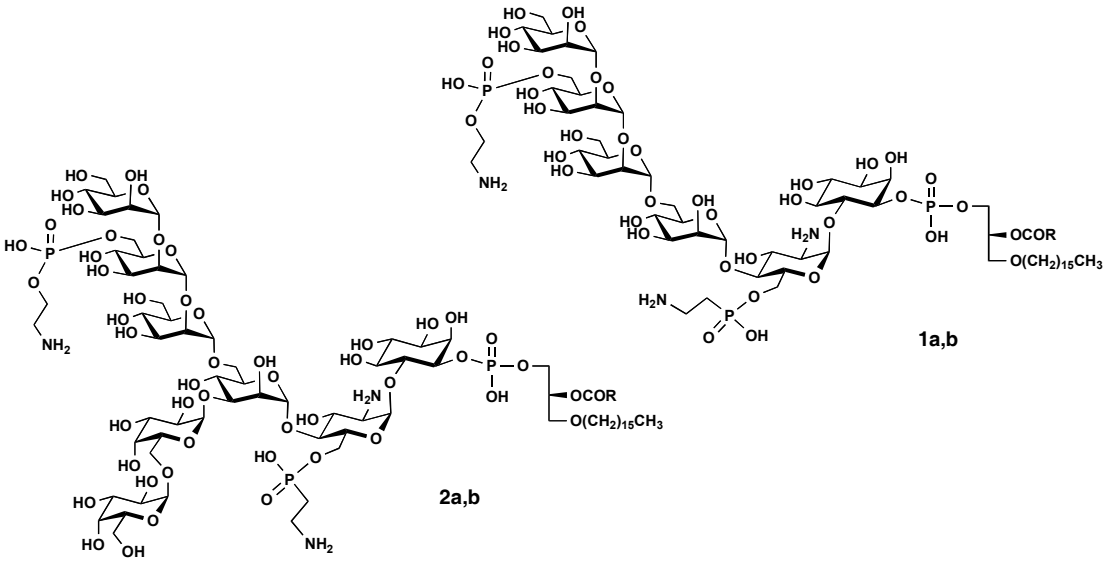
1. Gyoergydeak, Z.; Thiem, J. *Carbohydr. Res.* **1995**, *268*, 85-92
2. Li, C.; Ashok Arasappan; Fuchs, P. L. *Tetrahedron Lett.*, **1993**, *34,* 3535-3538
3. Ying, L.; Gervay-Hague, J. *Carbohydr. Res*. **2003**, *338*, 835-841
4. Levy DE, Tang C., *The Chemistry of C-glycosides*, 1995, Pergamon. Chapt. 2

RECENT STUDIES IN SYNTHETIC CHEMISTRY OF GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHORS

Dmitry V Yashunsky, Jennifer A Tee and Andrei V Nikolaev*

Faculty of Life Sciences, University of Dundee, Dundee, Scotland, UK
a.v.nikolaev@dundee.ac.uk

Glycosylphosphatidylinositols (GPIs) are a class of natural glycosylphospholipids that anchor proteins and glycoproteins (through their C-terminus) as well as phosphoglycans (through the reducing end of the chain) to the membrane of eukaryotic cell. Since the first full assignment of a GPI structure in 1988,[1, 2] a number of GPI anchors have been characterized. The discovered role of GPIs as mediators of regulatory processes (including insulin-mediated signal transduction, cellular proliferation and cell-cell recognition) makes the chemical preparation of the compounds and their analogues of great interest.[3-5] The lecture will comprise recent advances (including novel strategic approaches) in the preparation of GPI anchors from *Trypanosoma cruzi* trypomastigote mucins (compounds **1** and **2**). The synthesis of a GPI anchor from *Trypanosoma brucei* (**3**), which was prepared using MPEG (M = 5000) as a soluble polymer support, will be discussed as well.



1a, 2a R = $(CH_2)CH{=}CH(CH_2)_7CH_3$

1b, 2b R = $(CH_2)CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$

[1] M.A.J. Ferguson, et al., *Science* **1988**, *239*, 753; [2] S.W. Homans et al., *Nature* **1988**, *333*, 269; [3] T.G. Mayer, B. Kratzer, R.R. Schmidt, *Angew. Chem. Int. Ed*. **1994**, *33*, 2177; [4] A.S. Campbell, B. Fraser-Reid, *J. Am. Chem. Soc*. **1995**, *117*, 10387; [5] X. Liu, Y.-U. Kwon, P.H. Seeberger, *J. Am. Chem. Soc*. **2005**, *127*, 5004.

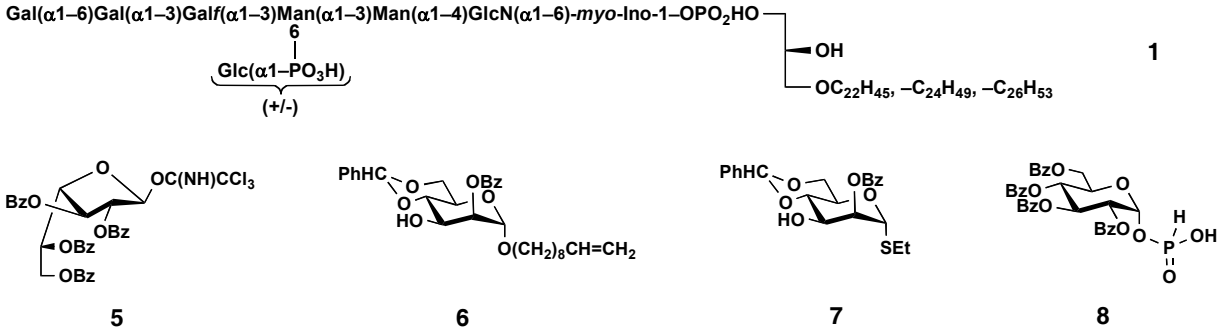
SYNTHESIS OF FRAGMENTS OF A GPI ANCHOR FROM *LEISHMANIA* LIPOPHOSPHOGLYCAN

Suzannah J Harnor* and Andrei V Nikolaev

Faculty of Life Sciences, University of Dundee, Dundee, Scotland, UK
a.v.nikolaev@dundee.ac.uk

Glycoconjugates on the cell surface of *Leishmania spp* play a role in determining parasite infectivity and survival. Many of these molecules are anchored to the plasma membrane via glycosylphosphatidylinositol (GPI) anchors. The *Leishmania* lipophosphoglycan (LPG) is the most abundant macromolecule on the surface of promastigote forms and its GPI anchor (**1**) contains a core hexasaccharide chain linked to a *lyso*-alkylphosphatidylinositol. The importance of the LPG for parasite infectivity and survival makes the enzymes responsible for its biosynthesis of great interest. Galactofuranose is an extensive component of cell wall polysaccharides in bacteria, protozoa and fungi, but is wholly absent in mammals, making galactofuranose-based glycoconjugates interesting target molecules for drug design.

A neutral disaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-O$(CH_2)_8CH{=}CH_2$ **2** and trisaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-O$(CH_2)_8CH{=}CH_2$ **3**, as well as a phosphorylated trisaccharide β-D-Gal*f*-(1→3)-[α-D-Glc*p*-(1-PO_3H-6)]-α-D-Man*p*-O$(CH_2)_8CH{=}CH_2$ **4**, all of which are fragments of the GPI anchor of *Leishmania* lipophosphoglycan, have been synthesised.



The disaccharide **2** was formed via the glycosylation of the mannose acceptor **6** with the β-D-galactofuranosyl trichloroacetimidate glycosyl donor **5**. Linear trisaccharide **3** was produced from the building blocks **5**, **7** and **6** using step-wise chain elongation. The phosphotrisaccharide **4** was synthesised by coupling of **5** and **6** followed by acid hydrolysis and condensation of the formed disaccharide 4,6-diol with the H-phosphonate derivative **8**.

The saccharides **2-4** have been designed to work and will be later tested (in a biochemical assay) as acceptor substrates for a novel enzyme in *Leishmania*: the GPI pathway β-D-galactopyranosyl transferase I (GT-I). The results of their testing will be used for identification and characterisation the GT-I enzyme.

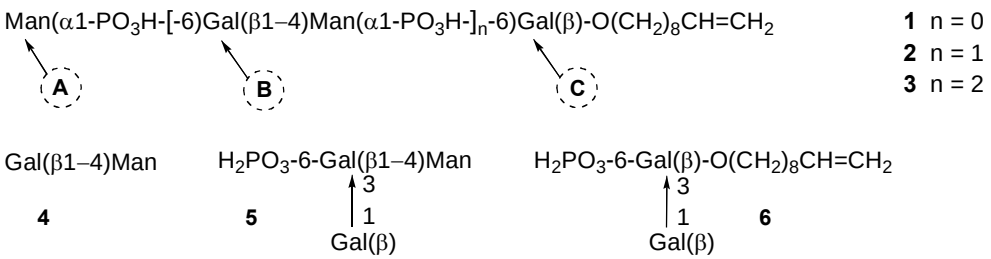
ELONGATING AND BRANCHING β-D-GALACTOSYL TRANSFERASES: NOVEL BIOSYNTHETIC ENZYMES IN *LEISHMANIA*

Olga V Sizova*, Andrew J Ross, Michael A J Ferguson and Andrei V Nikolaev

Faculty of Life Sciences, University of Dundee, Dundee, Scotland, UK
a.v.nikolaev@dundee.ac.uk

Lipophosphoglycan (LPG) molecules, which are ubiquitous at the cell surface of *Leishmania* protozoan parasites, are of vital importance for the parasite infectivity and survival both in the insect vector and in the mammalian host. The LPG contains a polymeric section consisting of [-6)-(R→3)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-]$_n$ repeating units, where the nature of the R group varies with species of *Leishmania*: in *L. donovani* R = H, whereas in *L. major* R is predominantly β-D-Gal*p*.[1] The biosynthesis of the LPG backbone was shown to be performed by sequential action of the *Leishmania* elongating α-D-mannosylphosphate transferase and the elongating β-D-galactosyl transferase.[2] There is also expected to be a branching β-D-galactosyl transferase, which introduces side-chain galactose residues in *L. major*,[3] but not in *L. donovani*.

A biochemical assay was developed for a galactose transfer in crude membrane preparation from either *L. donovani* or *L. major*, using UDP-[^{3}H]Gal (as a β-D-Gal*p* donor substrate) and synthetic LPG fragments **1**, **2** or **3**[4] (as exogenous acceptor substrates). Only phosphoglycans **2** and **3** produced [^{3}H]-labelled products of galactosylation. In order to analyse the structures and to identify the galactosylation sites (which are expected to be **A**, **B** or **C**), the products were hydrolysed with mild acid (that cleaves the glycosyl phosphate bonds only) and analysed by TLC. Only disaccharide **4** (corresponding to **A**-galactosylation) was detected in the hydrolysate of the product formed with *L. donovani* membranes, but both **4** and phosphotrisaccharide **5** (corresponding to the galactosylation sites **A** and **B**, respectively) were found after hydrolysis of products produced in the *L. major* assay. This indicates the presence of the elongating enzyme in both species and the presence of the branching galactosyl transferase in *L. major* only. No phosphodisaccharide **6** was detected in the *L. major* products hydrolysate, thus showing the galactosylation does not happen at the site **C**. Further studies of substrate specificity of the enzymes will be discussed.



[1] M.J.McConville, M.A.J.Ferguson, *Biochem. J.* **1993**, *294*, 305; [2] M.A.Carver, S.J.Turco, *J. Biol. Chem*. **1992**, *295*, 309; [3] D.E.Dobson et al., *J. Biol. Chem*. **2003**, *278*, 15523; [4] A.J.Ross et al., *Tetrahedron Lett*. **2000**, *41*, 2449.

THE MODIFICATION OF BIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF LIPOPOLYSACCHARIDE BY CARRAGEENAN

Irina M. Yermak[1] , Anna O. Barabanova[1] , Viktoria N. Davydova[1], Artur Ulmer[2], Ute Buwitt-Beckmann[2], Tamara F. Solov`eva[1], Mariusz Durlik[3], Wieslaw Kaca[3].

[1]Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia
[2]Forschungscentrum Borstel, Borstel, Germany
[3] Institute of Biology, Swietokrzyska Academy , Kielce .:Poland
iren@piboc.dvo.ru

LPS of gram-negative bacteria released into the body causes a series of acute physiological reaction: fever, dysmetabolism, disseminated intravascular coagulation and in high doses - tissue necrosis, severe intoxication, and death. We previously showed that chitosan, a natural polysaccharide, forms stable complexes with LPS, thus appreciably reducing their toxicity and modulating biological properties of LPS. Here we studied the possibility of using other polysaccharide- carrageenan for this purpose. Carrageenans – kappa-, kappa-beta and lambda-type were isolated from red algae *Chondrus armatus and Tichocarpus crinitus*, collected from the Russian Pacific coast .

The protective effect of carrageenan on the damaging effect of LPS was studied *in vivo* and *in vitro*. Carrageenan was shown to increase mouse resistance to the toxic effect of LPS from *Y. pseudotuberculosisi* and *E.coli*. The degree of protection depends on type – carrageenan, polysaccharides concentration and administration time and route.

In the murine monocyte cell line RAW 264.7 LPS from *E.coli* and kappa-carrageenan induced in dose-dependent manner TNF- α. In the human mononuclear cells kappa- and kappa/beta carrageenans were able to activate IL-6 and to increase ability LPS to induce cells synthesis IL-6. For lambda–carrageenan this property is expressed insignificantly. Using HEK 293 cells transfected with TLR4 in combination with MD2, we showed that kappa-carrageenan was able to induce the formation of the Il-8 in these cells. After the pretreatment with carrageenan the cells expressing TLR-4 is grown very essentially to activation by LPS. Our data appear to indicate that activation of cells by carrageenan occurs through specific for LPS TLR4 receptors and the ability of carrageen to induce cytokine production plays an important role in the modification of the toxicity of LPS. In comparison of mixtures of LPSs and carrageenan and LPSs alone the deposition of human C3c complement were statistically larger on the *E. coli , P. mirabilis* S 1959 LPS, it lipid A part. In contrary, the presence of *P. mirabilis R110* LPS did not influence the C3c deposition on the carrageenan.

In experiments *in vivo* carrageenan increase resistance of organism under LPS-induced endotoxemia. The capability of carrageenan to correct parameters of the hemostatic system, and also its influence on the homeostatic and immune system in the course of treatment of patients with alimentary toxicoinfection of *Salmonnela* etiology was investigated. Carrageenan restored system of a hemostatic and parameters of immune system is more active, in comparison with control group.

The presented data indicate that carrageenan modulates the biological properties of LPSs. The effects depend on both carrageens and LPSs types.

Carrageenan by interacting with LPSs change its physico-chemical properties, ultrastructure and molecular -mass characteristics.

THE DIFFERENT LIPOPOLYSACCHARIDES BINDING TO CHITOSAN

Viktoria N. Davydova*[1], Wieslaw Kaca[2], Irina M. Yermak[1], Vladimir I. Gorbach[1], Tamara F. Solov`eva[1]

[1]Pacific Institute of Bioorganic Chemistry, Far East Branch of Russian Academy of Sciences, Vladivostok, Russia
[2] Institute of Biology, Swietokrzyska Academ , Kielce, Poland
viktoria@piboc.dvo.ru

The lipopolysaccharides (LPS, endotoxin) are widely known as one of major contributing factors of pathogenesis of gram-negative infections. The problem of detoxification of LPS preventing bacterial sepsis is still actual.

Due to a negative charge, the LPS macromolecules represented an important target for polycationic compounds which are discussed as new potential antiseptic drugs. One of such molecule is a widespread natural polycation chitosan (Ch) β-1,4-D-glucosaminoglucan, which widely used in various biochemical specimens due to its availability and resistance to bacterial degradation. In particular, Ch was used for removing endotoxins from biological liquids.

The interaction of Ch with lipid A region of LPS - a toxophoric center of endotoxin, was demonstrated using LPS isolated from *Yersinia pseudotuberculosis* B598. An alteration of the LPS toxicity in the process of complex formation was suggested. We have shown that LPS-Ch complex (1:5 w/w) possess much lower endotoxicity to mice, in comparison with the LPS *Y. pseudotuberculosis* alone.

The binding of chitosan with LPS isolated from *Proteus vulgaris* O25 and *Escherichia coli* O55:B5 was shown using methods velocity sedimentation and absorbing spectroscopy. The process of interaction was depending on temperature. The binding LPS from *P. vulgaris* O25 and *Y. pseudotuberulosis* B598 occurred only at raised temperature 37˚ C. Binding constants of chitosan with LPS from *Y. pseudotubeculosis*, *E. coli* and *P. vulgaris* were determinated by method with using anionic dye – tropeolin. The LPS from *P. vulgaris* possess higher affinity to chitosan in comparison with two others samples.

The binding LPS to chitosan altered the ability of investigated LPSs to induce TNF-α, IL-8, IL-6 biosynthesis by stimulated human mononuclear cells. For LPS from *E. coli* was established that this effect depends on initial concentration of LPS and component ratios in complexes.

The ability of constitute of LPS *P. vulgaris* parts - lipid A, polysaccharide in complex with chitosan to induce cytokine synthesis was study. Decreasing of TNF and IL-6 synthesis by these complexes was noted only for complete lipopolysaccharide structure.

PRELIMINARY STUDY ON STRUCTURE OF STAPHYLOCOCCUS *AUREUS* AND *STAPHYLOCOCCUS* EPIDERMIDIS EXOPOLYSACCHARIDES

Andrzej Nowacki*[1], Monika Kuźma[1], Jannie M. Dilber[2], Martina. Maier[2], Poonam Gulati[2], Justyna Samaszko[1], Agnieszka Łakomiec[1], Janusz Madaj[1]

[1]*University of Gdańsk, Faculty of Chemistry, Sugar Chemistry Group*
J. Sobieskiego 18, 80-952 Gdańsk, Poland
[2]*University of Houston-Downtown, Department of Natural Science, Houston, Texas, USA*
januszm@chem.univ.gda.pl

Staphylococcus aureus and *Staphylococcus epidermidis* are gram positive bacteria. They are found primarily on the skin and in the mucous membranes of humans and warm-blooded animals. *S. aureus* is carried by ~30% of healthy humans, most commonly in the anterior nares [1]. These two bacteria are recognized as a major cause of nosocomial infections. *Staphylococcus aureus* is the leading cause of bacteremia, metastatic abscesses, primary septic arthritis, endocarditis, osteomyelitis, and wound infections [2]. *S. epidermidis*, is presently known to be an opportunistic pathogen, causing infections in immunocompromised hosts or patients with implanted medical devices, such as intravascular and peritoneal dialysis catheters, prosthetic heart valves, or orthopedic prostheses [3]. *Staphylococcus aureus* and *Staphylococcus epidermidis* (and many others) are able to attach to the surface (implanted medical device for instance) forming biofilms i.e. a microbially derived sessile community characterized by microbial cells embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription [4]. This ability to form biofilms make the microorganisms more resistant on environmental conditions, such as extreme temperature changes, dryness, water flow, and various pH conditions. It is believed now that biofilms make the microorganisms more resistant to antibiotics and to host defense mechanisms and are responsible for so called "chronic polymer-associated infections" [3].
Exopolysaccharides material was extracted from biofilm of two strains using glutaraldehyde. Each sample was subjected to size exclusion chromatography on Bio-Gel P-2 and then important fractions on Bio-Gel P-100. Preliminary GC, GC-MS an MALDI-ToF-MS analyses were done.

References:

[1]	García-Lara, J.; Masalha, M. and Foster, S.J., *DDT*, 10, (2005) 643-651.
[2]	Pfaller, M.A.; Jones, R.N.; Doern, G.V. and Kugler, K., *Antimicrob. Agents Chemother*. 42, (1998) 1762-1770.
[3]	Sadovskaya, I.; Vinogradov, E.; Flahaut, S.; Kogan, G. and Jabbouri, S., *Infect. Immun.*, 73, (2005) 3007-3017.
[4]	Donlan, R.M. and Costerton, J.W., *Clin. Microbiol. Rev.*, 15, (2002) 167-193.

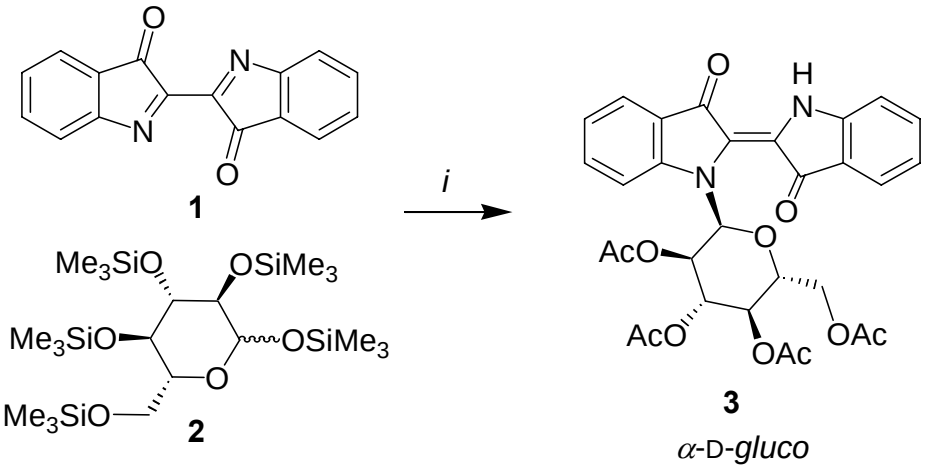
Acknowlegdements:
This work was partially financed by grant BW/8000-5-0300-6 and DS/8361-4-0134-6

SYNTHESIS OF THE FIRST INDIGO- AND INDIRUBIN *N*-GLYCOSIDES

Martin Hein, Stefanie Libnow, and Nguyen Thi Bich Phuong, Peter Langer*

Institut für Chemie, Universität Rostock, Albert-Einstein-Straße 3a, 18059 Rostock, Germany, e-mail: peter.langer@ uni-rostock.de

A few years ago, the first *N*-glycosides of indigo – the akashines A, B and C – were isolated.[1] In contrast to pharmacologically inactive parent indigo, the akashines show a considerable activity against various human tumor cell lines. We developed two independent syntheses of indigo *N*-glycosides. The first approach is based on the glycosylation of *N*-benzylindigo and subsequent rearrangement.[2] The second approach relies on the reaction of dehydroindigo (**1**) with in situ generated glycosyl iodides (*Scheme 1*).[3] In addition, the first indirubin *N*-glycosides were prepared by reaction of isatine *N*-glycosides with indoxyl acetate under basic conditions.[4]



Scheme 1. Synthesis of the acetylated *N*-(α-D-glucosyl)indigo **3**, *i*: a) **2**, CH_2Cl_2; b) Me_3SiI 20 °C, 30 min; c) **1**, 0 °C, 30 min; d) *n*PrSH, 0→20 °C, 1 h; e) Ac_2O/ pyridine = 3:1, KHF_2, 70 °C, 3 h

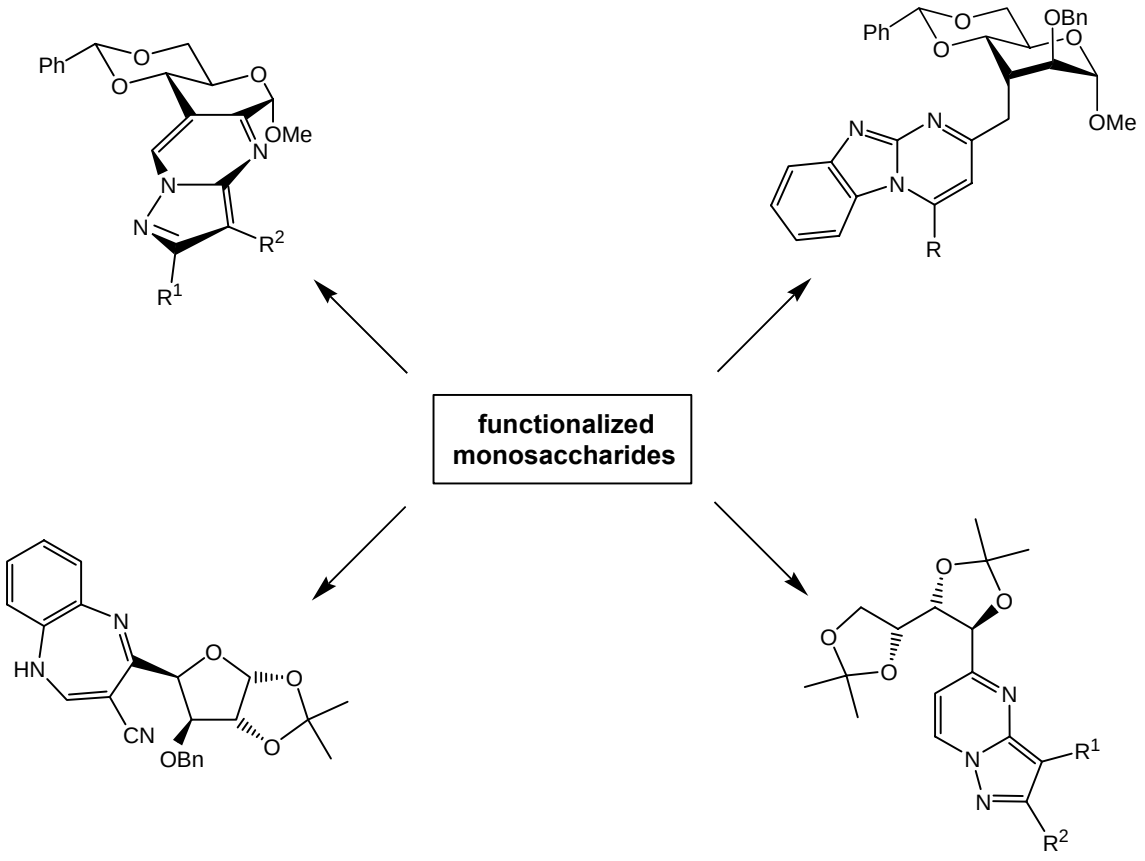
1. R. P. Maskey, I. Grün-Wollny, H. H. Fiebig, H. Laatsch, *Angew. Chem.* **2002**, *114*, 623; Angew. Chem. Int. Ed. **2002**, *41*, 597.
2. M. Hein, D. Michalik, P. Langer, *Synthesis* **2005**, *20*, 3531.
3. M. Hein, T. B. P. Nguyen, D. Michalik, H. Görls, M. Lalk, P. Langer, *Tetrahedron Lett.* **2006**, *47*, 5741.
4. S. Libnow, M. Hein, D. Michalik, P. Langer, *Tetrahedron Lett.* **2006**, in print.

FROM BRANCHED-CHAIN OR CHAIN-ELONGATED MONOSACCHARIDES TO UNUSUAL *C*-NUCLEOSIDES

Holger Feist*, Imran Ali Hashmi, Marcus Kordian, Iran Otero Martinez, Klaus Peseke

Institut für Chemie, Universität Rostock, Albert-Einstein-Str. 3a, D-18051 Rostock
holger.feist@uni-rostock.de

Due to the carbon-carbon bond between the anomeric atom of the sugar moiety and the heterocyclic unit *C*-nucleosides presenting a considerably increased enzymatic and hydrolytic stability, compared with the corresponding nucleosides. Often caused by this characteristic feature numerous natural occurring and synthetic *C*-nucleosides are showing interesting biological properties like antiviral, antibiotic, anticancer or glycosidase inhibitory activities.
To enlarge this class of carbohydrates synthetic pathways will be present for the synthesis of heterocyclic anellated pyranoses, iso-*C*-, 'reversed'- and acyclo-*C*-nucleoside analogues. All of them are relatively seldom mentioned in the literature. As starting point for these unusual *C*-nucleosides branched-chain or chain-elongated functionalized monosaccharides were used.



SYNTHESIS OF KEY COMPOUNDS OF THE MAILLARD-REACTION

Schnitzler,Y. V.*, Kroh, L. W.

Technical University of Berlin, Institute of Food Technology and Food Chemistry, Secretary TIB 4/3-1, Gustav-Meyer-Allee 25, 13355 Berlin
yschnitzler@gmx.de

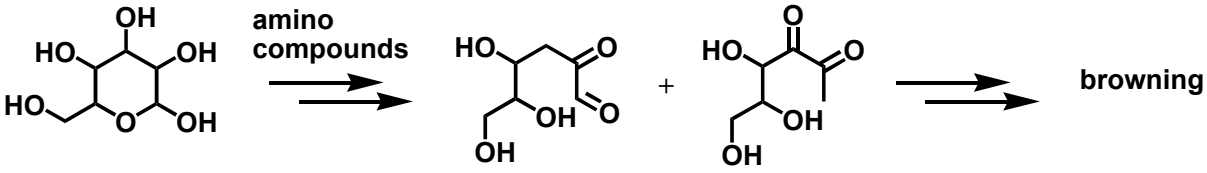
The non-enzymatic browning includes as important reactions the caramelisation and the MAILLARD-REACTION proceeding via a multitude of different reaction-mechanisms. α-Dicarbonyl-compounds, which are generated from carbohydrates and amino acids, are significant for the formation of colour and flavouring as well as antioxidant activity.[1]

These α-dicarbonyl-compounds are high reactive and key intermediates of the MAILLARD-REACTION. They play an important role as precursors for the formation of melanoidins, volatile compounds, reductones and many more.[2]

To qualify and quantify the α-dicarbonyl-compounds were trapped with *o*-diaminobenzene to yield the chinoxaline derivates and then detected by HPLC/DAD and GC/MS^n.

Despite the many structures published α-dicarbonyl-compounds available as authentic references for detailed studies are very limited.

As a consequence it is very difficult to follow the MAILLARD-REACTION in order to elucidate the reaction conditions for the formation of specific compounds.



A promising concept could be to follow the MAILLARD-REACTION starting only from one α-dicarbonyl-compound prepared by means of organic synthesis techniques.

Preliminary results towards the synthesis of 3-desoxyosones and 1-desoxyosones are presented.

Literature:

[1] F. Ledl, E. Schleicher, *Angew. Chem.* **1992**, *102*, 597.
[2] A. Hollnagel, L.W. Kroh, *J. Agric. Food Chem.* **2000**, *48*, 6219.

SORPTION CAPACITIES OF CELL WALL GLUCAN ISOLATED FROM SACCHAROMYCES CEREVISIAE TOWARDS PCP

Martin Pajtinka[1*], Zuzana Sejáková[2], Grigorij Kogan[1], Katarína Dercová[2]

[1]Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia
[2]Department of Biochemical Technology, Institute of Biotechnology and Food Sciences, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia
**corresponding author's e-mail chemmapa@savba.sk*

Contamination with pentachorophenol (PCP) represents serious environmental risk due to their widespread occurrence, toxicity, and recalcitrance. PCP has been used as herbicide, pesticide, fungicide, as the main component of wood preservative, and also is a microbial breakdown product of many pesticides commonly used in agriculture, e.g. lindane. PCP sorption-desorption investigation was performed using insoluble yeast glucan isolated from the cell walls of *Saccharomyces cerevisiae* (β-D-glucan). For a comparison, another fungal polysaccharide from the mycelium of filamentous fungus *Aspergillus niger* (chitin-glucan complex, CG) and its carboxymethylated derivative (CM-CG) were used as well. Microcrystalline cellulose (MC) was used as another glucan matrix for comparison with the yeast β-D-glucan.

β-D-Glucan revealed significantly better sorption ability towards PCP than CG and CM-CG. The sorption capacity of the prepared yeast β-D-glucan matrix (2 g/100 ml H_2O) reached 99 % PCP extracted from the 100 mg/l water solution. Even reduced amounts of glucan demonstrated very high sorption capacity: 0.5 g/100 ml suspension bound almost 90 % PCP. Due to the very high sorption capacity of β-D-glucan, more sorption experiments were performed with lower mass of glucan sorbent. The sparing amounts of polysaccharides were 0,25 g and 0,1 g per 100 ml H_2O.

In the sorption-desorption experiments, when the sorbent with the bound PCP was eluted with water (pH 4.0), the lowest desorption (i.e. the highest retention) parameters were shown by CG. Desorption revealed by CG was in a range 0.25-2.1 % of the sorbed PCP, whereas β-D-glucan and CM-CG had desorption values between 0.23-2.29 % and 1.19-4.89 %, respectively. The sorption data obtained with MC were up to 28 % lower than those observed for yeast glucan at the same conditions. This significant difference can be explained by the fact that while cellulose is a linear molecule, yeast β-D-glucan adopts triple helical conformation, which might more tightly enclose the PCP molecule. Additional studies to investigate the interactions between various polysaccharides and PCP and the effect of pH and temperature on the sorption-desorption processes are needed to fully understand their sorptive characteristics. The data obtained indicate that yeast cell wall material could be used at the decontamination processing.

Additionally, sorption capacities of the crude material - lyophilized and dried cells of *Saccharomyces cerevisiae* - were determined. Yeast cell walls could present a potential more economical alternative of PCBs sorbent for decontamination of the polluted areas.

Financial support from the Grant Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences, grants VEGA 2/4143/26 and VEGA 1/1309/04, as well as of Center of Excellence of Slovak Academy of Sciences CEDEBIPO is gratefully acknowledged.

COULD FUNGAL POLYSACCHARIDES FIGHT CANCER?

Grigorij Kogan[1*], Darina Slameňová[2], Peter Rauko[2], Andrej Staško[3], Melánia Babincová[4], Tatjana A. Korolenko[5]

[1]Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia
[2]Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovakia
[3]Chair of Physical Chemistry, Department of Chemical and Food Technology, Slovak University of Technology, 812 37 Bratislava, Slovakia
[4]Department of Biophysics and Chemical Physics, Comenius University, 842 15 Bratislava, Slovakia
[5]Institute of Physiology, Siberian Branch of Russian Academy of Medical Sciences, 630117 Novosibirsk, Russia
**corresponding author's e-mail chemkogi@savba.sk*

In the recent decades, an increasing attention is being paid to the natural substances of plant or microbial origin as potential substitutes for synthetic cytostatic drugs and other anticancer agents. β-D-Glucans are major fungal cell wall polysaccharides that determine stability of fungal cell walls and cell morphology. Many laboratory and clinical tests have demonstrated that fungal (1→3,1→6)-β-D-glucans exert pronounced immunomodulating properties, due to which they have found application in anti-infective and antitumor therapy. We have prepared water-soluble derivatives of β-D-glucan isolated from the cell walls of baker's yeast *Saccharomyces cerevisiae*, which revealed significant anti-oxidant effect comparable to those of the well-known anti-oxidants. The ability to scavenge free radicals was proven directly by using EPR spectroscopy. The antioxidant capacity to inhibit lipid peroxidation was also corroborated by their DNA-protective activity against oxidative damage established by means of comet assay. Antimutagenic and antigenotoxic activity of the β-D-glucan derivatives was demonstrated using yeast, bacterial, and algal models. In cell revitalization assay using the mouse leukemia cells, the studied yeast polysaccharides enhanced cytotoxic/cytostatic effect of the anticancer drug Vumon. In the in vitro tests, glucan derivatives exerted potent enhancement of TNF-α release from murine macrophages.Since free radicals are implicated in development and progression of many neoplastic diseases, we have investigated activity of the glucan derivatives in the murine models of Lewis lung carcinoma and lymphosarcoma. Use of β-D-glucans especially in combination with cyclophosphamide cytostatic resulted in a marked inhibition of tumor growth and incidence of metastases. At the same time, administration of the water-soluble β-D-glucan derivatives led to increased levels of the inhibitors of cysteine proteases stefin A and cystatin C in tumor tissue, as well as to the elevated concentration of the cathepsins B, L, and D. The presented results indicate significant protective antioxidant, antimutagenic, and antigenotoxic activities of the yeast polysaccharides and imply their potential application in anticancer prevention/therapy.

The work was supported by the Scientific Grant Agency of Slovak Academy of Sciences and Ministry of Education of Slovak Republic, VEGA grants numbers 2/4143/26, 2/3093/23, 2/4056/24, Slovak Academic Information Agency SAIA, grant 39s4, Center of Excellence of Slovak Academy of Sciences CEDEBIPO, and INTAS grant 2001-0592.

Participants

Dr. Polina I. Abronina
N.K. Kochetkov Laboratory of Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148
Email: polina@server.ioc.ac.ru

Dr. Michal Arabski
Department of Microbiology
Swietokrzyska Academy
Ul. Swietkrzyska 15
PL-25-406 Kielce
POLAND
Phone : +48 41 349 63 07
Fax : +48 41 349 62 92
Email: arabski@pu.kielce.pl

Adelia Luciana Barros Torres
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6432
Fax: +49-(0) 381-498-6412
Email: eq01033@fe.up.pt

Prof. Dr. Hubert Bahl
University of Rostock
Institute of Biological Sciences
Division of Microbiology
Albert-Einstein-Str. 3
D-18051 Rostock
GERMANY
Phone: +49-(0) 381-498-6150
Fax: +49-(0) 381-498-6152
Email: hubert.bahl@uni-rostock.de

Artur Bartyś
Laboratory of Medical Microbiology
Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Weigla 12
PL-53-114 Wroclaw
POLAND
Phone: +48-71-337-1172 ext. 389
Fax: +48-71-337-1382
Email: bartys@iitd.pan.wroc.pl

Michel Boretti
Institute of Organic Chemistry
University of Hamburg
Martin-Luther-King Platz 6
D-20146 Hamburg
GERMANY
Phone: +49-(0) 40-42838-4334
Fax : +49-(0) 40-42838-2495
Email: michelboretti@o2online.de

Sabine Borowski
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6435
Fax: +49-(0) 381-498-6412
Email: sabine.borowski@uni-rostock.de

Yudith Zamora Castell
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6435
Fax: +49-(0) 381-498-6412
Email: yudithz_apr@yahoo.com

Elena Ciliberti
Research Center Borstel
Leibniz-Center for Medicine
and Biosciences
Division of Structural Biochemistry
Parkallee 4a
D-23845 Borstel
GERMANY
Phone: +49-(0) 4537-188-246
Fax: +49-(0) 4537-188-745
Email: eciliberti@fz-borstel.de

Dr. Viktoria N. Davydova
Pacific Institute of Bioorganic Chemistry
Far-Eastern Branch of the
Russian Academy of Sciences
Pr. 100-Let Vladivostoku 159
690022 Vladivostok
RUSSIAN FEDERATION
Phone: +7-4232-310719
Fax: +7-4232-314050
Email: viktoria@piboc.dvo.ru

Sebastian Derfla
Laboratory of Bioorganic Chemistry and
Biotechnology
Department of Chemistry
Silesian Technical University
Ul. B. Krzywoustego
PL-44-101 Gliwice
POLAND
Email: sderfla@wp.pl

Katarzyna Anna Duda
Institute of Microbiology
Silesian University
Jagiellonska 28
PL-40-032 Katowice
POLAND
Phone: +48-(0) 322009357
Fax: +48-(0) 322009361
Email: kduda@uranos.cto.us.edu.pl

Katharina Elsner
Otto Diels-Institute of Organic Chemistry
Christian-Albrechts University
Otto-Hahn-Platz 4
24098 Kiel
GERMANY
Phone : +49-(0) 431-880-1937
Fax : +49-(0) 0431-880-7410
Email: kelsner@oc.uni-kiel.de

Dr. Yuliya P. Fedonenko
Institute of Biochemistry and Physiology
of Plants and Microorganisms
Prospekt Entuziastov 13
410049 Saratov
RUSSIAN FEDERATION
Phone: +7 8452 97 04 44
Fax: +7 8452 97 03 83
Email: konnova@ibppm.sgu.ru

Dr. Holger Feist
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6418
Fax: +49-(0) 381-498-6412
Email: holger.feist@uni-rostock.de

Dr. Ralf-Jörg Fischer
University of Rostock
Institute of Biological Sciences
Division of Microbiology
Albert-Einstein-Str. 3
D-18051 Rostock
GERMANY
Phone: +49-(0) 381-498-6153
Fax: +49-(0) 381-498-6152
Email: ralf-joerg.fischer@uni-rostock.de

Prof. Dr. Andrzej Gamian
Laboratory of Medical Microbiology
Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Weigla 12
PL-53-114 Wroclaw
POLAND
Phone: +48-71-370-9982
Fax: +48-71-337-1382
Email: gamian@iitd.pan.wroc.pl

Dr. Thomas Gutsmann
Research Center Borstel
Leibniz-Center for Medicine and Biosciences
Division of Biophysics
Parkallee 10
23845 Borstel
Germany
Phone: +49-(0)4537-188-291
Fax: +49-(0)4537-188-632
e-mail: tguts@fz-borstel.de

Daniela Hameister
University of Rostock
Institute of Chemistry
Dept. Technical Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6457
Fax: +49-(0) 381-498-6452
Email: daniela.hameister@uni-rostock.de

Malte U. Hammer
Research Center Borstel
Leibniz-Center for Medicine and Biosciences
Division of Biophysics
Parkallee 10
23845 Borstel
GERMANY
Phone: +49-(0)4537-188-631
Fax: +49-(0)4537-188-632
Email: mhammer@fz-borstel.de

Anna Hanuszkiewicz
Research Center Borstel
Leibniz-Center for Medicine and Biosciences
Division of Structural Biochemistry
Parkallee 4a
D-23845 Borstel
GERMANY
Phone: +49-(0) 4537-188-320
Fax: +49-(0) 4537-188-745
Email: ahanuszk@fz-borstel.de

Suzannah J. Harnor
Faculty of Life Sciences
Division of Biological Chemistry and Molecular Microbiology
University of Dundee
Dundee DD1 5EH
UK
Phone: +44-1382-3862-33 or -34.
Fax: +44-1382-386373
Email: s.j.harnor@dundee.ac.uk

Prof. Dr. Otto Holst
Research Center Borstel
Leibniz-Center for Medicine and Biosciences
Division of Structural Biochemistry
Parkallee 4a
D-23845 Borstel
GERMANY
Phone: +49-(0) 4537-188-472
Fax: +49-(0) 4537-188-745
Email: oholst@fz-borstel.de

Göran Hübner
Research Center Borstel
Leibniz-Center for Medicine and Biosciences
Division of Immunochemistry
Parkallee 10
D-23845 Borstel
GERMANY
Phone: +49-(0) 4537-633
Fax: +49-(0) 4537-632
Email: ghuebner@fz-borstel.de

Thomas Jöge
Institute of Organic Chemistry
University of Hannover
Schneiderberg 1B
30167 Hannover
GERMANY
Phone: + 49-(0) 511-762-4623
Fax. : + 49-(0) 511-762-3011
Email: thomas.joege@oci.uni-hannover.de

Prof. Dr. Wieslaw Kaca
Department of Microbiology
Swietokrzyska Academy
Ul. Swietkrzyska 15
PL-25-406 Kielce
POLAND
Phone : +48-41-349-6308
Fax : +48-41-349-62-92
Email: wkaca@pu.kielce.pl

Dr. Sabine Kapelle
Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38
D-14163 Berlin GERMANY
Phone: +49-(0) 30-9990-0825
Fax: +49-(0) 30-801-5010
Email: sabine.kapelle@t-online.de

Gnuni Karapetyan
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6432
Fax: +49-(0) 381-498-6412
Email: gnuni.karapetyan@uni-rostock.de

Lutz Krasny
VACUUBRAND GMBH + CO KG
Vertriebsbüro Berlin/Brandenburg
Schneeglöckchenring 4
D-14772 Brandenburg
GERMANY
Phone: +49-(0) 3381-707068
Fax: +49-(0) 3381-707075
Email: krasny@vacuubrand.de

Dr. Ania Kasprzycka
Laboratory of Bioorganic Chemistry and Biotechnology
Department of Chemistry
Silesian Technical University
Ul. B. Krzywoustego
PL-44-101 Gliwice
POLAND
Email: akasprzycka@interia.pl

Dr. Elena A. Khatuntseva
Laboratory of Glycoconjugate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-1356433
Fax: +7-495-1358784
Email: nen@ioc.ac.ru

Prof. Dr. med. Johannes K.-M. Knobloch
University Medical Center Schleswig-Holstein
Campus Luebeck
Department of Medical Microbiology and Hygiene
Ratzeburger Allee 160
D-23538 Luebeck
GERMANY
Phone: +49-(0) 451-5002822
Fax.: +49-(0) 451-5002749
Email : Johannes.Knobloch@uk-sh.de

Dr.* sc. *Grigorij Kogan
Institute of Chemistry
Slovak Academy of Sciences
Dubravska cesta 9
845 38 Bratislava
SLOVAKIA
Phone: +421-2-5941 0305
Fax: +421-2-5941 0222
Email: chemkogi@savba.sk

Dr. Anna Kondakova
N.K. Kochetkov Laboratory
of Carbohydrate Chemistry
N.D. Zelinsky Institute
of Organic Chemistry
Leninsky Prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-6148
Fax: +7-495-137-6148
Email: annakond@gmail.com

Nikolay N. Kondakov
N.K. Kochetkov Laboratory of Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148
Email: nkondak@server.ioc.ac.ru

Dr. Leonid O. Kononov
N.K. Kochetkov Laboratory of Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148, +7-495-135-5328
Email: kononov@ioc.ac.ru
leonid.kononov@gmail.com

Iwona Konieczna
Department of Microbiology
Swietokrzyska Academy
Ul. Swietkrzyska 15
PL-25-406 Kielce
POLAND
Phone : +48 41 349 63 07
Fax : +48 41 349 62 92
Email: iwona.konieczna@pu.kielce.pl

Dr. Inna N. Krasikova
Pacific Institute of Bioorganic Chemistry
Far-Eastern Branch of the
Russian Academy of Sciences
Pr. 100-Let Vladivostoku 159
690022 Vladivostok
RUSSIAN FEDERATION
Phone: +7-4232-310719
Fax: +7-4232-314050
Email: innakras@mail.ru

Prof. Dr. Udo Kragl
University of Rostock
Institute of Chemistry
Dept. Technical Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6450/6451/6351
Fax: +49-(0) 381-498-6452
Email : udo.kragl@uni-rostock.de

Prof. Dr. Lothar Kroh
Technical University of Berlin
Instiute of Food Technology
and Food Chemistry
TIB 3/4-1
Gustav-Meyer-Allee 25
13355 Berlin
GERMANY
Phone:+49-(0)30-31472-584
Fax: +49-(0)30-31472-585
Email: Lothar.Kroh@TU-Berlin.de

Nadezhda Yu. Kulikova
N.K. Kochetkov Laboratory of Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148
Email: neu5ac@mail.ru

Bianca Kunz
Otto Diels-Institute of Organic Chemistry
Christian-Albrechts University
Otto-Hahn-Platz 4
24098 Kiel
GERMANY
Phone : +49-(0) 431-880-1936
Fax : +49-(0) 431-880-7410
Email: bkunz@oc.uni-kiel.de

Monika Kuźma
Division of Sugar Chemistry
Faculty of Chemistry
University of Gdańsk
Sobieskiego 18
PL-80-952 Gdańsk
POLAND
Phone: +48 58 3450461
Fax: +48 58 3450472
Email: anowacki@chem.univ.gda.pl

Prof. Dr. Peter Langer
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6410
Fax: +49-(0) 381-498-6412
Email: peter.langer@uni-rostock.de

Cordula Lembke
Dept. of Med. Microbiology,
Virology & Hygiene
University Hospital Rostock
Schillingallee 70
D – 18057 Rostock
GERMANY
Phone: +49-(0) 381-494-5939
Fax: +49-(0) 381-494-5902
Email: lembkeco@med.uni-rostock.de

Eran Lerer
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6432
Fax: +49-(0) 381-498-6412
Email: lererer@hotmail.com

Dr. habil. Janusz Madaj
Division of Sugar Chemistry
Faculty of Chemistry
University of Gdańsk
Sobieskiego 18
PL-80-952 Gdańsk
POLAND
Phone: +48 58 3450364
Fax: +48 58 3450472
Email: januszm@chem.univ.gda.pl

PD Dr. Buko Lindner
Research Center Borstel
Leibniz-Center for Medicine and Biosciences
Division of Immunochemistry
Parkallee 10
D-23845 Borstel
GERMANY
Phone: +49-(0) 4537-188-233
Fax: +49-(0) 4537-188- 632
Email: blindner@fz-borstel.de

Prof. Dr. Chris Meier
Institute of Organic Chemistry
University of Hamburg
Martin-Luther-King Platz 6
D-20146 Hamburg
GERMANY
Phone: +49-(0) 40-42838-4324
Fax: +49-(0) 40-42838-2495
Email: chris.meier@chemie.uni-hamburg.de

Galina Morales Torres
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6435
Fax: +49-(0) 381-498-6412
Email: galina_morales@yahoo.com

Lidia A. Nazarova
N.K. Kochetkov Laboratory of Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148
Email: alizzee@mail.ru

Janna Neumann
Institute of Organic Chemistry
University of Hamburg
Martin-Luther-King Platz 6
D-20146 Hamburg
GERMANY
Phone: + 49-(0) 40-42838-2810
Fax: +49-(0) 40-42838-4325
Email: jannaneumann@web.de

Dr. Andrei V. Nikolaev
Faculty of Life Sciences
Division of Biological Chemistry
and Molecular Microbiology
University of Dundee
Dundee DD1 5EH
UK
Phone: +44-1382-384325
Fax: +44-1382-386373
Email: a.v.nikolaev@dundee.ac.uk

Dr. Andrzej Nowacki
Division of Sugar Chemistry
Faculty of Chemistry
University of Gdańsk
Sobieskiego 18
PL-80-952 Gdańsk
POLAND
Phone: +48 58 3450461
Fax: +48 58 3450472
Email: mkuzma@chem.univ.gda.pl

Johan Olsson
Department of Organic Chemistry
Arrhenius Laboratory, Stockholm University
S-106 91 Stockholm
SWEDEN
Phone: +46 8 674 72 63
Fax: +46 8 15 49 08
Email: johano@organ.su.se

Dr. Anna V. Orlova
N.K. Kochetkov Laboratory of Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148
Email: zoka@ioc.ac.ru

Professor Dr. Stefan Oscarson
Department of Organic Chemistry
Arrhenius Laboratory, Stockholm University
S-106 91 Stockholm
SWEDEN
Phone: +46 8 16 24 80
FAX: +46 8 15 49 08
Email: s.oscarson@organ.su.se

Nico Paepke
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6432
Fax: +49-(0) 381-498-6412
Email: nico.paepke@uni-rostock.de

Martin Pajtinka
Institute of Chemistry
Slovak Academy of Sciences
Dubravska cesta 9
845 38 Bratislava
SLOVAKIA
Phone: +421-2-59410274
Fax : +421-2-5941 0222
Email: chemmapa@savba.sk

Dr. Mariola Paściak
Laboratory of Medical Microbiology
Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Weigla 12
PL-53-114 Wroclaw
POLAND
Phone: +48-71-390-9922
Fax: +48-71-337-1382
Email: pasciak@iitd.pan.wroc.pl

Hmayak Poghosyan
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6432
Fax: +49-(0) 381-498-6412
Email: hmayakpoghosyan@yahoo.com

Prof. Dr. Dr. Andreas Podbielski
Dept. of Med. Microbiology,
Virology & Hygiene
University Hospital Rostock
Schillingallee 70
D – 18057 Rostock
GERMANY
Phone: +49-(0) 381-494-59-00/01
Fax: +49-(0) 381-494-5902
Email: andreas.podbielski@med.uni-rostock.de

Bernward Rittgerodt
Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38
D-14163 Berlin GERMANY
Phone: +49-(0) 30-809-72738
Fax: +49-(0) 30-801-5010
Email: rittgerodt@knauer.net

Lina Rydner
Department of Organic Chemistry
Arrhenius Laboratory, Stockholm University
S-106 91 Stockholm
SWEDEN
Phone: +46 8 16 24 82
Fax: +46 8 15 49 08
Email: lina@organ.su.se

Alexander Schierholt
Otto Diels-Institute of Organic Chemistry
Christian-Albrechts University
Otto-Hahn-Platz 4
24098 Kiel
GERMANY
Phone : +49-(0) 431-880-1932
Fax : +49-(0) 431-880-7410
Email: aschierholt@oc.uni-kiel.de

Yvonne V. Schnitzler
Technical University of Berlin
Instiute of Food Technology
and Food Chemistry
TIB 3/4-1
Gustav-Meyer-Allee 25
13355 Berlin
GERMANY
Phone: +49-(0) 30-31472297
Fax: +49-(0) 30-31472823
e-mail: yschnitzler@gmx.de

Prof. Dr. Elke Schweda
University College of South Stockholm &
Karolinska Institute
Clinical Research Centre
Huddinge Hospital, NOVUM
S-141 86 Huddinge
SWEDEN
Phone: +46 8 585 838 23
Fax: +46 8 585 838 20
Email: elke.schweda@kfc.ki.se

Kirsten Schwekendiek
Otto Diels-Institute of Organic Chemistry
Christian-Albrechts University
Otto-Hahn-Platz 4
24098 Kiel
GERMANY
Phone : +49-(0) 431-880-1936
Fax : +49-(0) 431-880-7410
Email: kschwekendiek@oc.uni-kiel.de

Harun A. Shaikh
Otto Diels-Institute of Organic Chemistry
Christian-Albrechts University
Otto-Hahn-Platz 4
24098 Kiel
GERMANY
Phone:+49-(0) 431-880-1932
Fax: +49-(0) 431-880-7410
Email: hshaikh@oc.uni-kiel.de

Anna M. Shpirt
N.K. Kochetkov Laboratory of
Carbohydrate Chemistry
N.D. Zelinsky Institute of Organic
Chemistry
Russian Academy of Sciences
Leninsky prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7-495-137-7570
Fax: +7-495-137-6148
Email: asya@ioc.ac.ru

Dr Olga V. Sizova
Faculty of Life Sciences
Division of Biological Chemistry
and Molecular Microbiology
University of Dundee
Dundee DD1 5EH
UK
Phone: +44-1382-384325
Fax: +44-1382-386373
Email: olga_sizova@yahoo.co.uk

Rikard Slättegård
Department of Organic Chemistry
Arrhenius Laboratory, Stockholm
University
S-106 91 Stockholm
SWEDEN
Phone: +46 8 674 72 63
Fax: +46 8 15 49 08
Email: rikard@organ.su.se

Andreas Steinmann
Institute of Organic Chemistry
University of Hamburg
Martin-Luther-King Platz 6
D-20146 Hamburg
GERMANY
Phone: + 49-(0) 40-42838-2820
Fax: +49-(0) 40-42838-4325
Email: asteinmann@web.de

Arno Strähmel
Technical University of Berlin
Instiute of Food Technology
and Food Chemistry
TIB 3/4-1
Gustav-Meyer-Allee 25
13355 Berlin
GERMANY
Phone:+49-(0) 30-31472297
Fax: +49-(0) 30-31472823
e-mail: arno_straehmel@gmx.de

Prof. Dr. Wieslaw Szeja
Laboratory of Bioorganic Chemistry and Biotechnology
Department of Chemistry
Silesian Technical University
Ul. B. Krzywoustego
PL-44-101 Gliwice
POLAND
Phone: +48-32-237-1654
Fax: +48-32-237-1614
Email: wieslaw.szeja@adres.pl

Dr. Bogumila Szponar
Laboratory of Medical Microbiology
Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Weigla 12
PL-53-114 Wroclaw
POLAND
Phone: +48-71-390-9922
Fax: +48-71-337-1382
Email: szponar@iitd.pan.wroc.pl

Prof. Dr. Joachim Thiem
Institute of Organic Chemistry
University of Hamburg
Martin-Luther-King Platz 6
D-20146 Hamburg
GERMANY
Phone: + 49-(0) 40-42838-4241
Fax: +49-(0) 40-42838-4325
Email: thiem@chemie.uni-hamburg.de

Prof. Dr. Christian Vogel
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6430
Fax: +49-(0) 381-498-6412
Email: christian.vogel@uni-rostock.de

Kay Vogel
Research Center Borstel
Leibniz-Center for Medicine
and Biosciences
Division of Structural Biochemistry
Parkallee 4a
D-23845 Borstel
GERMANY
Phone: +49-(0) 4537-188-320
Fax: +49-(0) 4537-188-745
Email: kvogel@fz-borstel.de

Alice Voß
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6431
Fax: +49-(0) 381-498-6412
Email: alice.voss@uni-rostock.de

Heike Wächtler
University of Rostock
Institute of Chemistry
Dept. Organic Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6432
Fax: +49-(0) 381-498-6412
Email: heike.waechtler@uni-rostock.de

Silke Wendicke
Institute of Organic Chemistry
University of Hamburg
Martin-Luther-King Platz 6
D-20146 Hamburg
GERMANY
Phone: +49-(0) 40-42838-4334
Fax: +49-(0) 40-42838-2495
Email: wendicke@chemie.uni-hamburg.de

Michaela Wiegand
Otto Diels-Institute of Organic Chemistry
Christian-Albrechts University
Otto-Hahn-Platz 4
24098 Kiel
GERMANY
Phone : +49-(0) 431-880-1937
Fax : +49-(0) 431-880-7410
Email: mwiegand@oc.uni-kiel.de

Dr. Irina M. Yermak
Pacific Institute of Bioorganic Chemistry
Far-Eastern Branch of the
Russian Academy of Sciences
Pr. 100-Let Vladivostoku 159
690022 Vladivostok
RUSSIAN FEDERATION
Phone: +7-4232-310719
Fax: +7-4232-314050
Email: iren@piboc.dvo.ru

Dr. Evelina L. Zdorovenko
N.D. Zelinsky Institute of Organic Chemistry
Leninsky Prospect 47
119991 Moscow B-334
RUSSIAN FEDERATION
Phone: +7 495 137 61 48
Fax: +7 495 135 53 28
Email: evelina@ioc.ac.ru

Vera Zimmermann
University of Rostock
Institute of Chemistry
Dept. Technical Chemistry
Albert-Einstein-Str. 3a
D-18059 Rostock
GERMANY
Phone: +49-(0) 381-498-6457
Fax: +49-(0) 381-498-6452
Email: vera.zimmermann@uni-rostock.de

Ewa Zuziak
Laboratory of Medical Microbiology
Institute of Immunology and Experimental Therapy
Polish Academy of Sciences
Weigla 12
PL-53-114 Wroclaw
POLAND
Phone: +48-71-337-1172 ext.337
Fax: +48-71-337-1382
Email: zuziak@iitd.pan.wroc.pl