**Institute of Chemical Technology, Prague**
**Czech Chemical Society, Czechoslovak Society for Microbiology**
**Institute of Microbiology, Prague**


**Current Chemistry and Biochemistry of Saccharides**

# Book of Abstracts

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**Program:**

8.00 - 9.00 Registration, poster installation
9.00 - 9.15 Opening, *Vladimír Křen*

***Invited Lectures, Oral Communications***

*Chairperson: Tomáš Trnka (Prague, Czech Republic)*

9.15 - 9.45 Oscarson S.: Approaches towards anti-microbial glycoconjugate vaccines based on synthetic oligosaccharide structures

9.45 - 10.00 Veselý J., Džoganová M., Trnka T., Ledvina M.: Preparation of 2-amino-2-deoxy-mannopyranosyl donors and the unexpected migration of ethylsufanyl group from the reducing end of sugar units

10.00 - 10.15 Duy-Phong Pham-Huu, ThachTruc Pham, Petruš L.: Improved synthesis of L-azasugars

***Coffee Break***

***Invited Lectures, Oral Communications***

*Chairperson: Stefan Oscarson (Stockholm, Sweden)*

11.00 - 11.30 Martínek V., Sklenář J., Šulc M., Bezouška K., Frei E., Stiborová M.: Protection against radicals formed from xenobiotics: a novel role for carbohydrate moieties in glycoproteins?

11.30 - 11.45 Sklenar J., Hofbauerova K., Pompach P., Bezouska K.: Inhibition of hexosaminidase secretion in *Aspergillus oryzae* causes changes in protein glycosylation

11.45 - 12.00 Ďurana R., Lacík I., Paulovičová E., Bystrický S.: Oxidation of mannans from pathogenic yeasts – preparation of precursors for conjugation reactions with respect to preservation of immunological properties.

12.00 - 12.15 Wimmer Z.: Alkyl glycosides and esters of glycuronic acids derived from cyclic alcohols: which are possibilities of their synthesis?

***Poster Session, Lunch***

***Invited Lectures, Oral Communications***

*Chairperson: Karel Bezouška (Prague, Czech Republic)*

14.00 - 14.30 Babjak M., Gracza T., Karlubíková O.: New Pd(II)-catalysed transformation of α-benzyloxy-alkenitols: an effective route to chiral tetrahydrofurans

14.30 - 14.45 Valentová K., Šimánek V.: β-Oligofructans from *Smallanthus sonchifolius* and human health

14.45 - 15.00 Synytsya A., Urbanová M., Setnička V., Tkadlecová M., Havlíček J., Raich I., Matějka P., Synytsya A., Čopíková J., Volka K.: Spectroscopic study of D-galacturonic acid complexation with metal cations

15.00 - 15.15 Trilčová A., Čopíková J., Coimbra M. A., Barros A. S., Synytsya A., Křístková H., Egert L.: Determination of cocoa powder quality by NIR and FTIR spectroscopy

***Coffee Break***

*Chairperson: Vladimír Farkaš (Bratislava, Slovak Republic)*

15.35 - 16.05 Křen V., Hušáková L., Bezouška K., Jiménez-Barbero J.: *N*-Acetylhexosamine triad in one molecule: glycosidase-catalysed synthesis of complex oligosaccharides

16.05 - 16.20 Ait-Mohand F., Stratilová E., Farkaš V.: Sensitive detection of transglycosylating activity of xyloglucan endotransglycosylase/hydrolase (XTH) after isoelectric focusing in polyacrylamide gels

16.20 - 16.35 Pišvejcová A., Rauvolfová J., Weignerová L., Křen V.: Library of fungal glycosidases as a powerful synthetic tool

16.35 - 16.50 Simerská P., Monti P., Riva S., Macková M., Křen V.: Purification, characterisation and synthetic application of unique α-D-galactosidase from *Talaromyces flavus*

16.50 - 17.00 Closing, *Tomáš Trnka*

***Poster Session:***

1. Synthesis and biological activity of lipid A mimics
   M. Baráth, J. Hirsch, M. Petrušová, A. Fišerová, V. Křen and L. Petruš

2. Synthesis of Allyl Type Alkylation Reagents and Their Use for Complexation Driven Regioselective Alkylation of Cyclodextrins
   Beran A., Jindřich J.

3. Characterisation of β-glucans isolated from *Pleurotus* sp.
   Blafková P., Míčková K., Synytsya A., Černá J., Čopíková J., Synytsya A., Jablonský I.

4. Preliminary Study of C2-Epimerization of 2-Azido-2-deoxyhexopyranoses
   Drašar L., Rohlenová A., Ledvina M.

5. Isomerisation of 1,6:2,3- and 1,6:3,4-dianhydro-D-hexopyranoses via 3-deoxy-3-iodo intermediates
   Džoganová M., Trnka T., Černý M.

6. Glycosyl Azides – New Efficient Substrates for Enzymatic Transglycosylation
   Fialová P., Carmona A.-T., Robina I., Ettrich R., Sedmera P., and Křen V.

7. RHF/DFT Calculation of structural and vibrational properties of w-azido-w-deoxy-D-xylitol
   Kaminský J., Buchalová K., Raich I.

8. Action of endoxylanases of glycoside hydrolase family 10 and 11 on glucuronoxylan and acidic xylooligosaccharides
   Kolenová K., Vršanská M., and Biely P.

9. Conformational analysis of the anomeric glycosyl radicals using DFT methods
   Kozmon S. and Tvaroška I.

10. Synthesis of fluoro amino derivatives of 1,6-anhydrohexoses
    Kroutil J., Jeništová K., and Karban J.

11. Isolation and structural characterization of a mannan from the yeast *Candida dubliniensis*
    Ližičárová I., Matulová M., Machová E., and Capek P.

12. Determination of polydextrose as a fat replacer in milk butter
    Míčková K., Čopíková J., Synytsya A.

13. Lymphocyte activation receptors: new structural paradigms in the group of *C*-type animal lectins
    Pavlíček J., Ettrich R., Kopecký V. Jr., Man P., Vrbacký M., Bezouška K.

14. *N*-Terminal propeptide of fungal β-N-acetylhexosaminidase plays role in enzyme's folding and dimerization
    Plíhal O., Kmoníčková,J., Man P., Pompach P., Křen V., and Bezouška K.

15. Molecular dynamics simulations and conformational analysis of Pullulan oligomers
    Raab M. and Tvaroška I.

16. Easy Preparation of Regioisomers of Mono-*O*-substituted Cyclodextrins
    Řezanka M., Jindřich J., Tišlerová I.

17. Study of pectin oligomers
    Sihelníková L., Luňáčková P., Synytsya A., and Čopíková J.

18. Performance of Empirical Force Fields in Modelling of Carbohydrate-Aromatic Interactions
    Spiwok V., Lipovová P., Skálová T., Dohnálek J., Dušková J., Hašek J., Králová B.

19. Synthesis of 6I-Alkenoylamino-6I-deoxy Derivatives of β-Cyclodextrin as Modifiers of Porous Silicon Sensoric Response
    Trojan T., Jindřich J.

20. Stereoselective synthesis of new $\alpha(1\rightarrow3)$-*C*-disaccharide
    Vích O., Štěpánek P., Kniežo L.

21. A Regiospecific Route to the C-D-Galactofuranosyl Compounds. Synthesis of 2-C-Glycosyl benzimidazols.
    Vojtech M., Petrušová M., and Petruš L.

22. 3-*O*-Sulfonylerythroses – new synthons for imino-*C*-glycosides
    Werner L., Kniežo L.

23. Porphyrine receptors containing glycosylated steroid and its synthesis
    Zelenka K., Trnka T., Drašar P.

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# Approaches Towards Anti-Microbial Glycoconjugate Vaccines Based on Synthetic Oligosaccharide Structures

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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, it is sometimes most difficult to use native bacterial polysaccharides due to, 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 against infections caused by the fungi *Cryptococcus neoformans*, a major cause of death in AIDS patients, and also the bacteria *Haemophilus influenzae* and *Neisseria meningitidis*. Chemical syntheses of CPS and LPS structures will be presented, as well as their conjugation to carrier proteins and use in immunization experiments.

# Preparation of 2-amino-2-deoxy-mannopyranosyl donors and the unexpected migration of ethylsufanyl group from the reducing end of sugar units

**Jan Veselý[a], Martina Džoganová[a],Tomáš Trnka[a], Miroslav Ledvina[b]**

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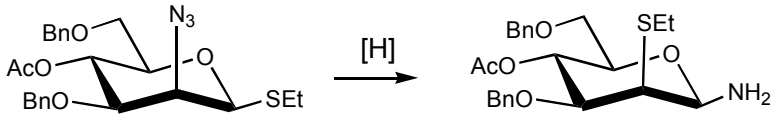
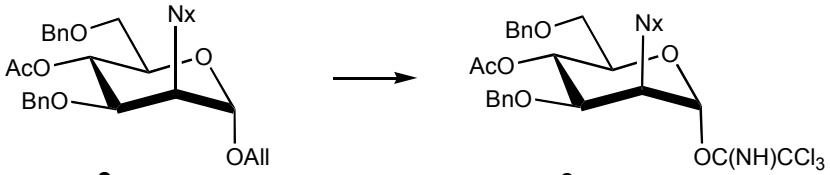
The synthesis of 2-amino-2-deoxy-hexopyranosyl donors for glycosylation reactions from D-glucosamine, D-galactosamine and D-mannosamine is described. We have focused our effect on the synthesis of mannopyranosyl units with $N_3$, NHTroc, and NPhth group at C-2 position; according to character of used protecting group these units can be used for construction of 1,2-*cis*, or 1,2-*trans*-glycosidic bond as glycosyl donors. Since direct functionalization of D-mannosamine proved to be difficult, we were interested in its preparation from *D-gluco* precursors.

In the first approach we prepared unit **1** from the corresponding *gluco* precursor *via* $S_N2$ substitution with $LiN_3$ or $NaN_3$. Reduction of the azido group was accompanied with unexpected migration of ethylsulfanyl group to C-2 position; compound **2** was formed.

Instead of usually used SEt group at C-1 position we used OAll group in the second approach and in this case no rearrangement during reduction step was observed. Prepared allylmannopyranosides **3**,**4**,**5** were transformed to corresponding trichloroacetimidates **6**,**7** and **8** for the use in glycosylation reactions.




**1** **2**




**3** Nx = $N_3$
**4** Nx = NPhth
**5** Nx = NHTroc

**6** Nx = $N_3$
**7** Nx = NPhth
**8** Nx = NHTroc

References and notes:

1. This work was supported by grants No MSM 1131 00001 and GAUK 418/2004/B-CH/PrF.

# IMPROVED SYNTHESIS OF L-AZASUGARS

Duy-Phong Pham-Huu,[a] ThachTruc Pham,[b] Ladislav Petruš[c]

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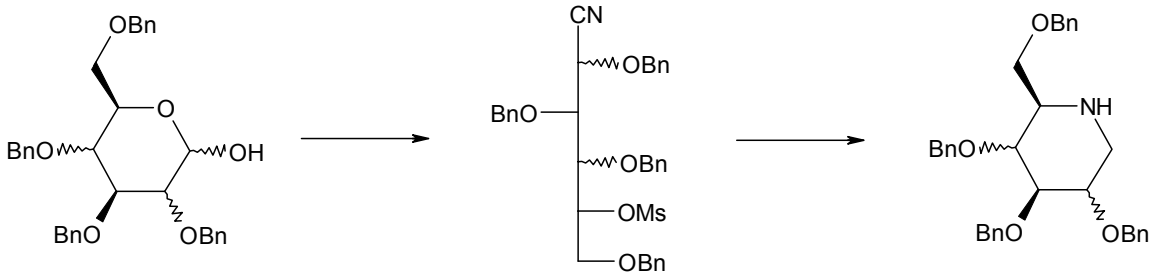
Polyhydroxylated piperidines (often referred to as azasugars) are known as specific and competitive inhibitors of glycosidases or glycosyl transferases.[1] These classes of compounds are useful tools for probing the details of enzyme catalytic mechanisms and have a potential for therapeutic applications including treatment of diseases such as diabetes, cancer, inflammation, viral and bacterial infections.[2] The stereochemical similarity between the inhibitor and the appropriate hexose sugar, or an enzyme-bound intermediate derived from it, is apparent. However, there is also a case where L-azasugars have been demonstrated to inhibit D-glycosidases. Recently, synthetic methods leading to L-azasugars have been developed. However, many of them either require expensive reagents and/or a high number of steps. As part of our effort for synthesis of selective glycosidase inhibitors,[3] we report herein a simple synthesis of L-azasugars from common D-glycopyranosides.




**I** **II** **III**

*O*-Benzylated D-glycopyranoses **I** were converted to nitriles **II** by treatment with hydroxylamine hydrochloride and sodium methoxide, followed by mesylation. Nitriles **II** were treated with borane to give L-azasugars **III** in very good yields.

*Acknowledgements: The work was in part supported by the APVT-51039802 and VEGA-2/3077/23 grants.*

References:

1. Winchester, B.; Fleet, G. W. J.: *Glycobiology* **1992**, *2*, 199.
2. Stütz, A. E.: *Iminosugars as Glycosidase Inhibitors*, Wiley-VCH: Weinheim, **1998**.
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# PROTECTION AGAINST RADICALS FORMED FROM XENOBIOTICS: A NOVEL ROLE FOR CARBOHYDRATE MOIETIES IN GLYCOPROTEINS?

Václav Martínek[1], Jan Sklenář[1,2], Miroslav Šulc[1,2], Karel Bezouška[1,2], Eva Frei[3], Marie Stiborová[1]
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Glycosylation is one of the most important posttranslational modification of protein [1]. It has been suggested previously that carbohydrate moieties may be involved in glycoprotein folding, protection against proteolysis, and participate in specific recognition functions [2]. We observed that radicals generated from two xenobiotics, 1-phenylazo-2-hydroxynaphthalene (Sudan 1) and ellipticine, by horseradish peroxidase readily attach to nonglycosylated proteins such as HSA, while glycosylated proteins such as the peroxidase itself were not modified. Sudan I is a liver and urinary bladder carcinogen in mammals known to be metabolically activated by both cytochromes P450 and peroxidases to reactive species that bind covalently to nucleic acids and proteins [3]. Ellipticine is an alkaloid with antineoplastic and anti-HIV activities that is oxidized by peroxidases generating radicals participating in its pharmacological efficiency [4]. We established a remarkable correlation between the resistance of glycoproteins (asialofetuin, fetuin, ovomucoid, Tamm-Horsfall glycoprotein and $\alpha_1$-acid glycoprotein) towards modification by the above radicals, and the degree of glycosylation. Comparison of these activities using RNase A (nonglycosylated) and RNase B (glycosylated) supported our findings on two proteins having an identical protein moiety and differing only in glycosylation. Our findings indicate a novel role for protein glycosylation, namely the protection of protein against the attack by radicals generated from a one-electron oxidation of the xenobiotics.

**References:** [1] Kornfeld S. (1998) J.Clin.Invest. 101, 1293. [2] Gagneux P., Varki A. (1999) Glycobiology 9, 747. [3] Stiborova M., Martinek V., Rydlova H., Hodek P., Schmeiser H.H., Frei E. (2000) Cancer Res. 62, 5678. [4] Frei E., Borek-Dohalska L., Wiessler M., Stiborova M. (2001) Proc.Am.Assoc.Cancer Res. 42, 252.

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# Inhibition of hexosaminidase secretion in Aspergillus oryzae causes changes in protein glycosylation

Jan Sklenar[2], Katerina Hofbauerova[2], Petr Pompach[2], and Karel Bezouska[1, 2]

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Aspergillus oryzae cultivated in GlcNAc based medium produces considerable level of β-hexosaminidase. While GlcNAc serves as a sole source of energy, and it is rapidly utilized, only small fraction of ManNAc is incorporated in medium containing a mixture of both saccharides. Under these conditions we measured hexosaminidase turnover by pulse-chase experiment. The speed of intracellular production was the same irrespective of the growth substrate used, but the speed of secretion was highest in GlcNAc based medium, and decreased proportionally with the rise of ManNAc concentration. Also, the total protein concentrations in mycelium reached the higher values in media with higher concentrations of ManNAc. These observations suggest that ManNAc can trigger an unknown mechanism causing slow down of hexosaminidase secretion.

To confirm the observation, we decided to analyze hexosaminidase glycosylation.

Assuming the slow down of protein secretion may lead to a change in the intracellular flow through the secretory compartments, we hypothesized this change might influence protein glycosylation. Analysis of N-linked glycans of hexosaminidase (by HPAEC-PAD and MALDI MS) secreted under different conditions with different strains of Aspergillus oryzae revealed small, yet significant, changes in ratios of distinct high-mannose oligosaccharides, which correlated with increasing ManNAc concentration. Thus glycosylation pattern sensitively indicates the intracellular fate of protein after translation.

Presented work provides the basis for delineation of the mechanism that modulates the protein secretion, and therefore might be of a great importance for heterologous expression in filamentous fungi.

*This work was supported by: Institutional Research Concept for the Academy of Sciences AVCR No. 50200510, and Grant Agency of The Czech Republic GACR 203/04/1045, and GACR 203/05/0172*

# Oxidation of mannans from pathogenic yeasts – preparation of precursors for conjugation reactions with respect to preservation of immunological properties.

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Three different reagents (sodium periodate, Dess-Martin periodinane (DMP), 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)/hypochlorite/bromide) were used to introduce aldehyde/carboxyl functions onto mannans from the four pathogenic yeasts of *Candida* genus (*Candida albicans*, *Candida tropicalis*, *Candida glabrata, Candida parapsilosis*) in order to prepare glycoconjugate vaccine precursors suitable for further conjugation reactions *via* amine function of protein. A combination of NMR, IR, potentiometric titration, Park-Johnson colorimetric assay and size exclusion chromatography (SEC) was applied for physicochemical characterization of the oxidized mannans. Correlation between molecular weight of periodate, DMP and TEMPO-mediated oxidized mannans and branching frequency as a characteristic structural feature of original cell wall mannans was found. It indicates that higher branching frequency of original mannan used in the reaction, lesser molecular weight decrease of oxidized mannan measured. This dependence determined by SEC upon oxidation agent can be expressed by following relationship: (TEMPO) > original > DMP >> periodate. Degrees of oxidation were as following: periodate – 11% (1:9.3, periodate/m.u.), DMP – 6% (2:1, periodate/m.u.), TEMPO – 25%. Immunological characteristics relevant to applications in vaccine technology (i.e. preservation of antigenic structural properties of polysaccharides) were analyzed by double immunodiffusion. It was revealed, that significant modification of immunological properties or damage of relevant epitopes due to structural changes *via* DMP and TEMPO-mediated oxidation can be excluded.

# Alkyl Glycosides and Esters of Glycuronic Acids Derived from Cyclic Alcohols: Which Are Possibilities of Their Synthesis?

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*Alkyl glycosides*: (i) Based on methods of activation of the monosaccharide molecule, four key approaches exist in the *chemical synthesis* of alkyl glycosides, in practice realized by a number of modifications of their parent synthetic methodologies [1-2]: (a) Koenigs-Knorr synthesis, (b) Fischer-Helferich synthesis, (c) anomeric *O*-alkylation method, and (d) a synthesis using trichloroacetimidate species. Our own investigation resulted in experience that only Koenigs-Knorr synthesis (which disadvantage is a need for multiple molar excess of heavy metal promoters) and the trichloroacetimidate method (which disadvantage is connected with the course of the reaction resulting in occurrence of both anomers of the target alkyl glycosides in the product mixture) may be used for the synthesis of alkyl glycosides of cyclic (secondary) alcohols. (ii) *Enzymic methods* (reverse hydrolysis and transesterification) represent considerable alternatives to the above synthetic approaches. Using cyclic alcohols, however, limited tolerance of the screened glycosidases towards these alcoholic substrates has been observed, resulting in the relatively low yields of the target alkyl glycosides, unless we take into account the principles of the "green" chemistry.
*Esters of glycuronic acids*: (i) Based on the general mechanisms of formation of the ester functionality, two basic mechanisms can occur: *O*-acyl fission and *O*-alkyl fission. Both mechanisms, but especially the latter one, disfavor esterification of enantiomerically pure alcohols. From the *synthetic chemistry*, six key approaches can be considered [3-5]: (a) catalyst-free esterification, (b) acid-catalyzed esterification, (c) esterification mediated by auxiliary reagents, (d) base-catalyzed transesterification, (e) alkylation of the carboxylate ion, and (f) alcohol acylation. Based on our investigation, none of those methods has been applicable in the esterification of glycuronic acids by cyclic alcohols. Lower primary alcohols and alcohols of the benzyl type react with protected and/or unprotected glycuronic acids, however, cyclic (secondary) alcohols favored side or no reactions, wherever possible. (ii) *Enzymic esterification*, using lipases, is a good method for esterification of fatty (primary) alcohols. Its limit consists in impossibility to use cyclic alcohol, bearing another ester functionality in its molecule, which makes our task more complicated. Future results can prove the power of biotechnology to substitute synthetic chemistry behind its present limits, when performing esterification of glycuronic acids.

References:
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[2] R. R. Schmidt, K.-H. Jung, in *Preparative Carbohydrate Chemistry* (S. Hanessian, Ed.), p. 283. Dekker (1997).
[3] J. Svoboda, in *Organická syntéza I*, p. 274. VŠCHT Praha (2000).
[4] H. B. Wood, Jr., in *Methods in Carbohydrate Chemistry II* (R. L. Whistler, M. L. Wolfrom, Eds.), p. 57. Academic Press, New York (1963).
[5] M. Poláková, D. Joniak, M. Ďuris, *Monatsh. Chem.* **131** (2000) 1197.

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# New Pd(II)-Catalysed Transformation of α-Benzyloxy-Alkenitols: an Effective Route to Chiral Tetrahydrofurans

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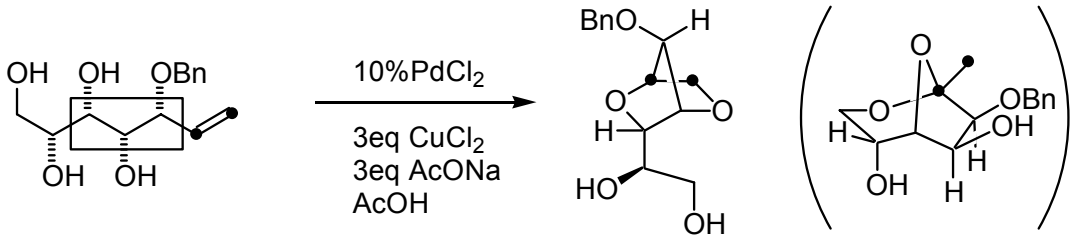
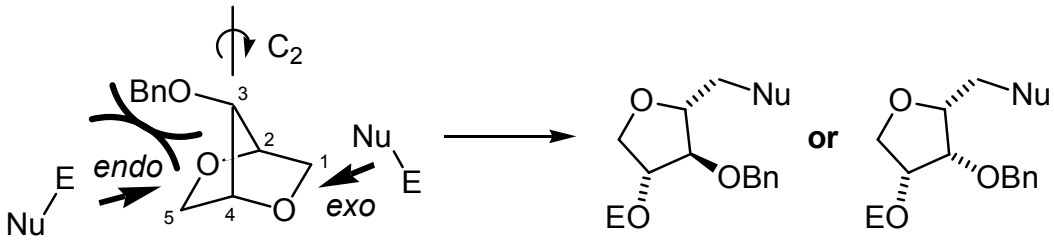
Entirely new transformation of α-benzyl protected alkenitols in "magic" $PdCl_2/CuCl_2$/AcOH catalytic system has been studied; the reaction shows exceptional demand for substrate configuration, when substrates bearing *xylo*- alignment at α,β,γ- carbons afford desired bicyclic dianhydroalditols, whereas non-*xylo* substrates undergo Wacker oxidation instead. Mechanistic considerations of the problem, based on semi-empirical calculations and 3D modeling, will be discussed in the first part of the lecture.




A simple stereoconvergent synthesis of C-2 symmetrical bicyclic product from D-glyceraldehyde, followed with directed diastereoselective ring opening [1], will be presented as a smart route to polysubstituted semi-protected tetrahydrofurans with rare D-*lyxo* configuration, which is complementary to D-*arabino*, routinely accessible from conventional iodocyclisation. [2]




[1]J. Kuszmann, *Carbohydr. Res.* **1985**, *142*, 71.

[2]F. Bravo, S. Castillón: *Eur. J. Org. Chem.* **2001**, 507.

# β-Oligofructans from *Smallanthus sonchifolius* and human health

VALENTOVÁ K., ŠIMÁNEK V.

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Tubers of *Smallanthus sonchifolius* (yacon, Asteraceae) contain mainly low glucose content fructans as storage compounds. The fructan structure is of the inulin type, i.e. β(2→1)fructofuranosylsucrose, the same as in other Asteraceae species, e.g. Jerusalem artichoke (*Helianthus tuberosus*). In comparison with the latter, yacon tubers contain fructans of a lower degree of polymerization. Low DP fructans have been used as sucrose substitutes; they are considered dietetic. They have a favourable influence on the human intestinal flora. Human enzymes do not hydrolyse the β(2→1)bond and thus β(2→1)fructans of the inulin type constitute dietary fibre or the indigestible residues of plant origin in the diet.

Oligofructans are classified as prebiotics, components of diet which are not digested in the human gastrointestinal tract but rather they are transported to the colon where they are fermented by selected species of gut micro-flora, in particular *Bifidobacterium* and *Lactobacillus*, both indicators of a balanced gut flora. Consumption of prebiotics modifies gut flora composition and metabolic activities. Probably through this action they also modulate lipid metabolism, calcium absorption, childhood immune systems and gut function. The prebiotic effect of yacon tuber extracts has been recently demonstrated as fermentation by several common gut bacteria *L. plantarum*, *L. acidophilus* and *B. bifidum*.

Yacon tubers are also rich in free fructose, glucose and sucrose. Saccharide content and related enzyme activities in tubers fluctuates during cultivation and storage; during cultivation, the degree of polymerization in the fructans increases while it declines during storage.

We investigated the proportion of individual fructooligosaccharides in tubers from various yacon clones cultivated in Czech Republic and studied some of their biological activities. The distribution of fructans was strongly dependent on the year of cultivation and on the genotype. An extract from the tubers modulated immune functions in mice (increased antibody and T-cell production). The combination of yacon with silymarin improved metabolism of triacylglycerols and glucose in patients with metabolic syndrome.

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# SPECTROSCOPIC STUDY OF D-GALACTURONIC ACID COMPLEXATION WITH METAL CATIONS

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Galacturonic acid (GalA) and its oligomers has been used as a simple model of pectin in the study of biologically important interactions with polyvalent metal cations. On the other hand, GalA itself represents an example of functionalised sugar demonstrating good complexation properties. Solid complexes of D-galacturonic acid (GalA) with cobalt(II), copper(II), nickel(II) and oxovanadium(IV) (**1**-**4**) were prepared and characterised. The metal-to-ligand molar ratio was 1:2 for complexes **1**-**3** and 1:1 for complex **4**. The α- and β-anomers of GalA were detected in all the complexes in solid state and in solutions. An addition of small amounts of the paramagnetic complexes to the $D_2O$ solution of pure ligand led to NMR line broadening of some $^{1}H$ and $^{13}C$ nuclei. This broadening was sensitive to the anomeric state of GalA in the case of complexes **1** and **4**. NMR and vibrational spectroscopic data confirm the formation of carboxylate complexes of all the cations, while non-carboxylic oxygens are also involved into the metal bonding in some cases. VCD spectra of complexes **1**-**4** in $D_2O$ and $Me_2SO$-$d_6$ solutions confirm that GalA carboxylic group may participate in the formation of optically active species around the metal cation. Possible ways of GalA coordination by metal cations of this study were proposed and discussed according to the proposed molecular mechanics models.

The work was supported by the research grants of Ministry of Education, Youth, and Sports of the Czech Republic (CB MSM 223400008) and Grant Agency of the Czech Republic (203/02/0328), and Grant Agency of Academy of Sciences (A4055104).

## DETERMINATION OF COCOA POWDER QUALITY BY NIR AND FTIR SPECTROSCOPY

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At present the use of reliable control methods to ensure the labeled quality of food is a task for organizations that have to limit or eliminate falsification. In food industry there are wide-spread products made from cocoa beans, e. g. chocolate, different chocolate confectionery, chocolate cookies, cocoa powder, instant chocolate drinks, etc. The Czech legislative deals only with moisture and the fat content of cocoa powder. NIR spectroscopy offers a number of important advantages over traditional chemical methods. It is a physical, non-destructive method, requiring minimal or no sample preparation and its precision can be high. It is a multi-analytical technique: several determinations can be made simultaneously. The method offers the possibility of measuring physical and chemical properties. Once calibrated, the NIR spectrometer is simple to operate. The chief disadvantages are the dependence on time-consuming and laborious calibration procedures and the complexity in the choice of data treatment. NIR is used for analyzing numerous foodstuffs, thus it is very useful to verify this method for quality monitoring of cocoa powder. The objective of this study was to analyze the major components of cocoa powder (moisture, fat content) using NIR. FTIR spectroscopy was used for the determination of carbohydrates. Carbohydrates show high absorbencies in region 1200-950 $cm^{-1}$ which is within the so-called “fingerprint” region, where the position and intensity of the bands are specific for every polysaccharide, allowing its possible identification. In this region it is not possible to assign absorbance at specific wavenumber to a specific bond or functional group due to overlaps. Traditional univariate calibration does not allow reliable predictions. Multivariate calibration can be used to overcome this problem. FTIR spectra and multivariate analysis, namely Principal component analysis (PCA) was used to evaluate the spectra of cocoa powder. This presentation describes the results of common analytical methods, NIR spectrometry and FTIR spectrometry applied to cocoa powder. Spectroscopy joined to statistical evaluation is a very powerful technique. The two spectrometric techniques are statistically processed allowing discrimination of authentic and fraudulent cocoa powder samples.

Acknowledgement. *We are thankful to the support by the grant* MSM 6046137305.

# *N*-ACETYLHEXOSAMINE TRIAD IN ONE MOLECULE: GLYCOSIDASE-CATALYSED SYNTHESIS OF COMPLEX OLIGOSACCHARIDES

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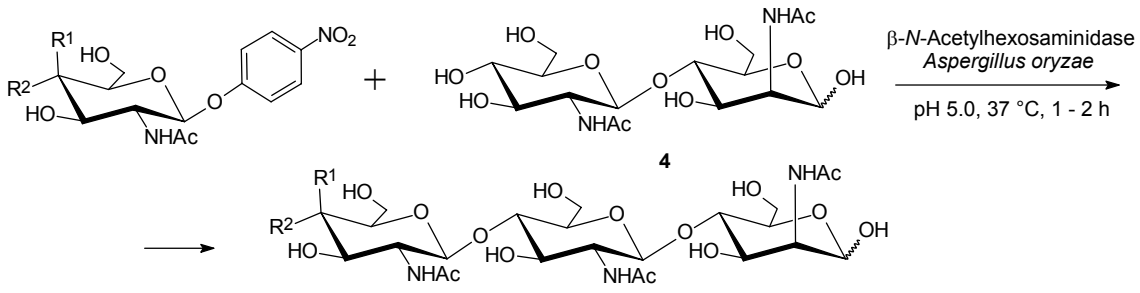
So far unknown trisaccharides containing all three common *N*-acetylhexosamines, e.g., GalNAcβ(1→4)GlcNAcβ(1→4)ManNAc (1) and GlcNAcβ(1→4)GlcNAcβ-(1→4)ManNAc (2) were prepared by enzymatic catalysis employing newly described discrimination activity of some hexosaminidases. β-*N*-Acetylhexosaminidase from *Aspergillus oryzae* selectively discriminates mixture of GlcNAcβ(1→4)GlcNAc (3) and GlcNAcβ(1→4)ManNAc (4). *N,N´*-Diacetylchitobiose (3) is selectively hydrolyzed by the β-*N*-acetylhexosaminidase, whereas its C-2 epimer (4) remains completely resistant to the enzymic hydrolysis [1]. Analogous discrimination was observed also with GalNAcβ(1→4)GlcNAc and GalNAcβ(1→4)ManNAc (4). Respective ManNAc containing disaccharides were prepared from their C-2 epimers by alkali-catalysed epimerisation affording thermodynamic mixture of two epimers followed by its enzymatic discrimination. Molecular modeling of β-*N*-acetylhexosaminidase from *A. oryzae* and docking experiments with both types of disaccharides revealed the mechanism of this unusual phenomenon.
Resistance of GlcNAcβ(1→4)ManNAc (4) to the hydrolytic attack of β-*N*-acetyl-hexosaminidase enabled us to use it as an acceptor for its further extension by enzymatic transglycosylation with β-GlcNAc or β-GalNAc unit in the yields of 30 – 40 % [2].
New trisaccharides 1 and 2 were used a molecular probes for study of the binding sites at the NK cell activation receptor NKR-P1 and both proved very high binding affinity confirming thus multiple binding sites for all three *N*-acetylhexosamines. By using NMR and molecular modeling as major tools we have demonstrated that trisaccharides containing GalNAc and ManNAc residues are also recognized by hevein domains [2].




**1** $R^1$= OH, $R^2$= H, **2** $R^1$= H, $R^2$= OH

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# SENSITIVE DETECTION OF TRANSGLYCOSYLATING ACTIVITY OF XYLOGLUCAN ENDOTRANSGLYCOSYLASE/HYDROLASE (XTH) AFTER ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GELS

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We have developed a sensitive and rapid zymogram technique for detection of transglycosylating activity (XET) of xyloglucan endotransglycosylase/hydrolase (XTH; EC 2.4.1.207) in polyacrylamide isoelectric focusing gels. After the electrophoresis, the separating gel is overlaid and incubated with an agarose detection gel containing XET substrates: tamarind-seed xyloglucan as the glycosyl donor and sulphorhodamine-labelled xyloglucan-derived oligosaccharides (XGO-SRs) as the glycosyl acceptors. The transglycosylation catalyzed by XTH caused incorporation of the fluorescent label into the high-$M_r$ polysaccharide. Selective removal of unreacted XGO-SRs from the agarose replicas by washing with organic solvents revealed the zones corresponding to XET activity as bright pink fluorescent spots under UV-light. The method appears suitable for a number of purposes such as analysis of the isoenzyme composition of XTHs with XET activity in crude extracts from various plants and plant organs, monitoring the enzyme expression at various stages of plant development and/or for checking enzyme purity in the course of its isolation procedure.

# LIBRARY OF FUNGAL GLYCOSIDASES AS A POWERFUL SYNTHETIC TOOL

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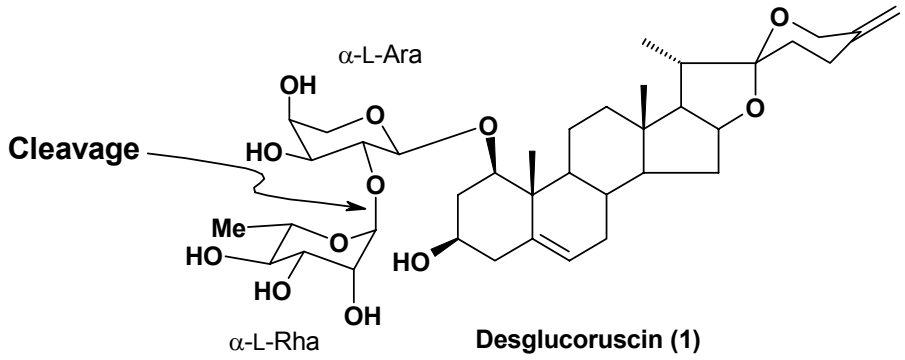
Laboratory of biotransformation keeps a large library of glycosidases produced by fungal strains from the Culture Collection of Fungi (CCF) or from the Culture Collection of the Institute of Microbiology (CCIM). These inducible extracellular enzymes are stored as $(NH_4)_2SO_4$ precipitates, which are sufficiently stable and pure for preparative and screening purposes.

An important set of glycosidases are β-*N*-acetylhexosaminidases (EC 3.2.1.52). As a result of an extensive screening, our library comprises more than 100 various types with different biochemical parameters. β-*N*-Acetylhexosaminidases have been used as catalysts of a range of transglycosylation and reverse glycosylations yielding a vast number of oligosaccharides, often possessing important biological properties. Moreover, these enzymes were found to be also suitable for the preparation of non-reducing disaccharides.

Another promising application of our enzymatic library is screening for new enzymes, such as α-L-rhamnosidases (EC 3.2.1.40). These enzymes may be used for modification of sugar moieties of biologically important substances, e.g., ruscosides, natural glycosides from rhizomes of *Ruscus aculeatus*, potent anti-inflammatory agents. An example is the cleavage of α-L-rhamnosidic moiety of desglucoruscin **(1).** We screened a series of fungal strains for the production of α-L-rhamnosidase in the presence of various inducers. The substrate specificity of selected enzymes and their stability in organic solvents were tested. The best of them were used for synthetic purposes in preparative scales.




**Desglucoruscin (1)**

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# PURIFICATION, CHARACTERISATION AND SYNTHETIC APPLICATION OF UNIQUE α-D-GALACTOSIDASE FROM *TALAROMYCES FLAVUS*

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New glycosidase activities can be obtained by exploring the natural diversity of fungal strains in different cultivation conditions. We have found rather unique enzyme, α-D-galactosidase from *Talaromyces flavus* CCF 2686, which is able to catalyze glycosylation of sterically hindered alcohols such as *tert*-butyl alcohol (Fig. 1). We have studied also glycosylation of thiols, mercaptoethanol and dithiothreitol.

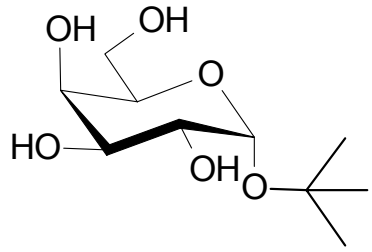

Figure 1. *tert*-Butyl α-D-galactoside.

This enzyme is produced exclusively in the presence of a specific inductor 6-deoxyglucose. Surprisingly, any of common α-D-galactosidase inductors or substrates (galactose, melibiose, raffinose, α-methyl galactoside, 6-deoxygalactose, 2-deoxyglucose) does not stimulate its production.

Purification of α-D-galactosidase from *T. flavus* was done by ion-exchange chromatography and three *iso*-enzymes (α-Gal1, α-Gal2, α-Gal3) with different substrate specificity were separated. Only *iso*-enzyme α-Gal1 (88 % of total activity) catalyses unique transglycosylation reaction with *tert*-butyl alcohol and does not catalyse cleavage of melibiose contrary to other two *iso*-enzymes α-Gal2 (10 % of total activity) and α-Gal3 (2 % of total activity) that cleave melibiose and do not glycosylate tertiary alcohols. The purified enzyme was characterised, its kinetic constants, pH and T optima and stabilities were found Inhibition by compounds relevant to potential application of the enzyme was investigated and it was observed that the enzyme is inhibited by galactose. *iso*-Enzyme α-Gal1 was immobilised on Eupergit C and α-D-galactosidase activity, stability and usage in transglycosylation reactions were studied.

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Support by the GACR grant 203/05/0172 and MSMT grant OC D25.002 (COST D25/01) is acknowledged.

# SYNTHESIS AND BIOLOGICAL ACTIVITY OF LIPID A MIMICS

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Lipopolysaccharides (LPS) as major amphiphilic components of the outer leaflet of the outer membrane of Gram-negative bacteria, exert in isolated form a variety of biological activities in mammals and are, thus called endotoxins[1]. One of these harmful responses is a fatal septic shock. LPS is also a highly potent stimulator of the immune system[2].

Chemically LPS consist of a hydrophilic heteropolysaccharide, which is covalently linked to a hydrophilic lipid portion, termed lipid A. Free lipid A has been shown to be responsible for the biological activity of LPS. The endotoxin is recognized by the co-operation of macrophage receptors (TLR-4, CD-14, MD-2), thus developing the septic shock[3].

During over a twenty-year endeavor to solve this serious, still lasting problem in human medicine, several approaches have been attempted, in which utilization of mimic structures of original substrates and supplanting them in biological processes seems to be effective procedures[4].

This contribution further develops a simple synthesis and biological facilities of a lipid A mimic described recently[5]. Here synthesized was a new lipid A mimic with a potential LPS antagonistic activities. Presented are also biological properties of lipid A mimic and their behavior as antagonist of lipid A.

*Acknowledgements: The work was supported by the APVT-51039802 and VEGA-2/3077/23 grants.*

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# Synthesis of Allyl Type Alkylation Reagents and Their Use for Complexation Driven Regioselective Alkylation of Cyclodextrins

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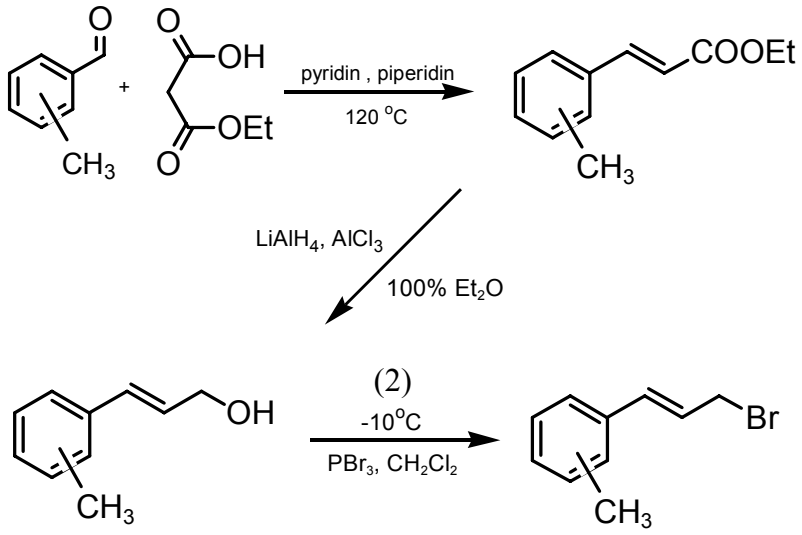
Cyclodextrins (CD) are cyclic oligosaccharides with truncated cone shape, where OH groups are situated outside, and C-H bonds inside of the CD ring. This property is the reason of a different polarity of the cavity and the ring surface and is the cause of their ability to make inclusion complexes with lipophilic compounds including organic reagents. Preparation of $3^{I}$-*O*-monosubstituted derivatives of β-CD in a good yield was up to recently very difficult. We found out that it can be done in a good yield (30 %) and regioselectivity (>90%) using cinnamyl bromide, most probably due to the complexation of the reagent[1].

The aim of this work was to find out if simple derivates of cinnamyl bromide (*ortho*-, *meta*- and *para*-methyl-cinnamyl bromide) can upon reaction with β-CD give higher yields or different regioselectivity of monosubstituted β-CD derivatives than cinnamyl bromide alone. The synthetized reagents gave lower yields of the β-CD monoderivatives and the same regioselectivity. For *orto*-methyl-cinnnamyl bromide 8.5%, for *meta*-methyl-cinnamyl bromide 4.9%. No reaction was observed between β-CD and *para*-methyl-cinnamyl bromide.




*This work was supported by grants GAUK 424/2004/B-CH/PřF, MŠM 113100001.*

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# Characterisation of β-glucans isolated from *Pleurotus* sp.

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*Pleurotus* sp. is interesting as medicinal mushroom due to the significant content of β-glucans. These polysaccharides are supposed to be responsible for some healthy properties of various mushrooms. There is evidence to study how β-glucan distribution in fruit body depends on genetic differences between strains of *Pleurotus* sp., cultivation conditions and harvest term. The aim of this study was characterisation of β-glucans and chitin-glucan complexes of four strains of *Pleurotus* sp. The contents of β-glucans were determined separately in pilei and stems by Megazyme (Ireland) enzymatic kit contained exo-1,3-β-glucanase and β-glucosidase. Soluble and insoluble glucans were isolated according to the modified procedure of Chenghua et al. (2000). Lyophilised samples obtained from dialysed extracts, as well as insoluble residues, were analyzed by spectroscopic and separation methods. Enzymatic analysis confirms significant differences in β-glucan contents among the strains. Harvest term did not influence significantly the content and distribution of these polysaccharides. In all cases β-glucan content was higher in stems than in pilei. The strain Italsp.77 showed maximal β-glucan content in stems among all strains studied. Spectroscopic analysis confirmed that solid fraction is mainly chitin-glucan complex without proteins, while lyophilised samples consist of protein and glucan components, so they could be protein-glucan complexes. The protein to sugar ratio is markedly higher for lyophilised samples obtained from pilei than those from stems. Therefore, stems of *Pleurotus* sp., which have low food quality in comparison with pilei, could be a valuable source of β-glucans for preparation of functional foods or food supplements.

*References*: D. Chenghua, Y. Xiangliang, G. Xiaoman, W. Yan, Z. Jingyan, X. Huibi (2000): *Carbohydr. Res.*, **328** (4), 629-633.

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# PRELIMINARY STUDY OF C2-EPIMERIZATION OF 2-AZIDO-2-DEOXYHEXOPYRANOSES

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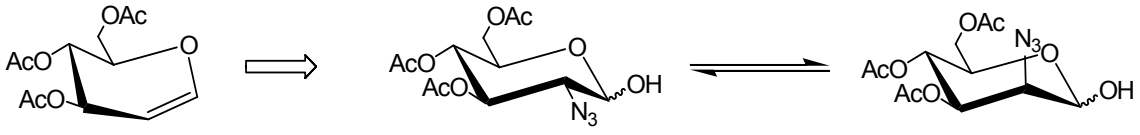
β-D-Mannopyranosyl and 2-acetamido-2-deoxy-β-D-mannopyranosyl residues occur frequently in natural polysaccharides and glycoproteins[1]. The simple ways to get mannosamine building blocks are investigated. Direct epimerization of GlcNAc to ManNAc at position C2 under the basic condition ($Ca(OH)_2$) showed among others serious problems with separation of the epimers[2]. Due to difficult accessibility of mannosamine the approaches using inversion after glycosylation step were elaborated to prepare oligosaccharides containing mannosamine units[3].

To prepare mannosamine blocs suitable for forming of β-glycosidic bond we used the alternative strategy based on azidonitration reaction[4] with glucal **1** and epimerization of azido-derivative **2a**. The non-participating azido group meets requirements for consequential *cis*-glycosidic bond forming.




**1** **2a** **2b**

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# Isomerisation of 1,6:2,3- and 1,6:3,4-dianhydro-β-D-hexopyranoses via 3-deoxy-3-iodo intermediates

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The behaviour of these compounds towards aqueous 5% potassium hydroxide has been studied.[1] It is known, that dianhydro derivatives having a hydroxyl group *trans*-oriented to the oxirane ring are rapidly equilibrated under alkaline conditions.[2] This reaction, called "epoxide migration", is an intramolecular $S_N2$ substitution of a vicinal oxide ion (originated from a hydroxyl group) on the oxirane ring. On the other hand, isomerisation of dianhydro derivatives having *cis*-oriented hydroxyl group and oxirane ring may be described as a pseudo-epoxide migration.

We have studied isomerisation of dianhydro derivatives effected by sodium iodide in acetone. The composition of the resulting equilibrium mixtures of isomeric epoxides is in great agreement with findings that have been published.[3]

Formation of 3-deoxy-3-iodo intermediates from the title dianhydro derivatives, in which the hydroxyl group and oxirane ring have a *cis* relationship, is acceptable for D-*allo* configuration (Fűrst-Plattner ring-opening condition). Therefore the finding, that slow stabilisation of equilibrium mixture of 1,6:2,3- and 1,6:3,4-dianhydro-β-D-*talo*pyranoses also occurs, is valuable. Equilibration of epoxide mixtures was accomplished by several different methods and their composition and the kinetics of of formation were estimated.

Some iodo derivatives were synthetised by cleavage of epoxide ring of dianhydrohexopyranoses with hydrogen iodide in dioxane.

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# GLYCOSYL AZIDES – NEW EFFICIENT SUBSTRATES FOR ENZYMATIC TRANSGLYCOSYLATIONS

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Enzymatic transglycosylation using glycosidases is a respected method in carbohydrate synthesis [1]. As the most used nitrophenyl glycosides have a number of disadvantages (low water solubility, complicated purification due to the released nitrophenol), alternative glycosyl donors are desirable [2,3]. The aim of this project is to introduce glycosyl azides as novel, highly soluble glycosyl donors, easy to synthesize.

2-Acetamido-2-deoxy-β-D-glucopyranosyl azide and 2-acetamido-2-deoxy-β-D-galactopyranosyl azide were prepared and subjected to a hydrolytic screening with 20 fungal β-*N*-acetylhexosaminidases. The enzymes willingly accepted the *gluco*-configuration, while the *galacto*-configuration was not accepted at all. This surprising result was discussed with regard to the molecular models of the substrates docked into the active site of the β-*N*-acetylhexosaminidase from *Aspergillus oryzae*. The hydrolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide by two selected enzymes was kinetically characterised ($K_M$, $k_{cat}$). An NMR study revealed that the β-*N*-acetylhexosaminidase from *A. oryzae* behaves towards these unconventional substrates (C-N bond) as a retaining glycosidase, as well as towards O-glycosides [1] .

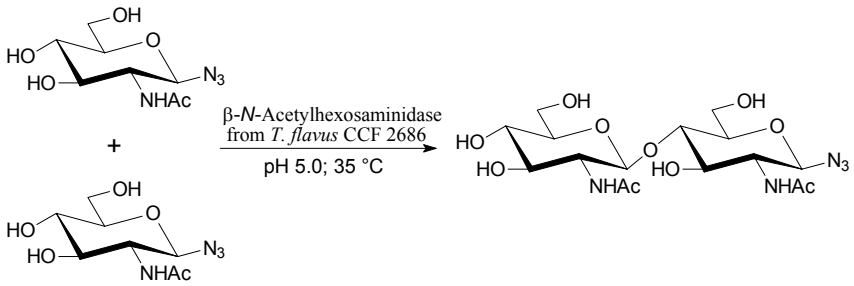


Figure 1. Synthesis of 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-aceta-mido-2-deoxy-β-D-glucopyranosyl azide.

Using 2-acetami-do-2-deoxy-β-D-glucopyranosyl a-zide as a glycosyl donor, we prepared *N*,*N*´-diacetylchitobiose in a yield comparable to the literature [4].

Furthermore, two novel disaccharides were prepared in transglycosylation reactions: 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyra-nosyl azide (Fig.1) and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-galactopyranosyl azide.

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# RHF/DFT CALCULATION OF STRUCTURAL AND VIBRATIONAL PROPERTIES OF ω-AZIDO-ω-DEOXY-D-XYLITOL

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Azido compounds with sugar moiety have been very popular not only since the AZT was discovered as an effective drug in the war against HIV. Azido polyols were prepared at our department in the last years and it was found that at least azido glycerol is a mutagenic agents but only for plants and microorganisms, not for the human cells.

We employed two different approaches of molecular modeling to understand the mechanism of forming of intramolecular versus intermolecular H-bonds in case of 5-azido-5-deoxy-D-xylitol, which should explain a bigger thermal stability of organic azides contrary to inorganic.

As the first way we tried to investigate a conformational space using *ab-initio* calculations. The 2592 starting geometries, that should cover the whole conformational space, were optimized at the PM3 level and the best conformers were re-optimized at several higher levels of theory (RHF, B3LYP, B3PW91, HCTH, PBEPBE, MP2 etc.). The calculations of representative NMR spin-spin couplings or ROA and VCD frequencies were used to confirm the correctness of minima on the PES found and were compared with our experimental results. The solvent influence was solved using the CPCM model. All calculations were performed using Gaussian[2] program suite.

The second approach for the solution of the conformational problem was a combination of Molecular Dynamic (MD) and Quantum Mechanic (QM) simulation. The 101ps MD simulations were taken as a base for the post-QM calculations of NMR properties. For MD calculation we used the Hyperchem[3] program with the MM+ force field.

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# Action of endoxylanases of glycoside hydrolase family 10 and 11 on glucuronoxylan and acidic xylooligosaccharides

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A comparison of action mode of endo-β-1,4-xylanases of glycoside hydrolase families 10 and 11 on glucuronoxylan and acidic xylooligosaccharides was done. Xylanases of family 10 are known to release from glucuronoxylan aldotetraouronic acid ($MeGlcA^3Xyl_3$) and xylanases of family 11 aldopentaouronic acid ($MeGlcA^3Xyl_4$) as the shortest acidic products. In this work five xylanases of family 10 (*Thermoascus aurantiacus* Xyn10A*, Streptomyces lividans* XylA, *Aspergillus oryzae* EX, *Cryptococcus albidus* EX and *Schizophyllum commune* XynB) were tested on their ability to cleave various acidic xylooligosaccharides. In respect of hydrolysis of glucuronoxylan the resistance of aldotetraouronic acid ($MeGlcA^3Xyl_3$) to the action of family 10 xylanases was not surprising. Interestingly, aldopentaouronic acid was also not attacked with exception of EX of *C. albidus*. Only aldohexaouronic acid ($MeGlcA^3Xyl_5$) served as a substrate for all family 10 xylanases and was hydrolysed to xylobiose and aldotetraouronic acid. These results indicated that binding of xylopyranosyl residue in the –2 subsite is prerequisite for cleavage of the substrate. Four tested xylanases of family 11 (*Schizophyllum commune* XynA, *Streptomyces lividans* XynC, *Sporotrichum thermophile* EX, *Thermomyces lanuginosus* EX) cleaved neither aldotetraouronic acid, nor aldopentaouronic acid, which is in agreement with their action on glucuronoxylan. Aldohexaouronic acid was cleaved to aldopentaouronic acid and xylobiose without any production of xylose suggesting, that a xylosyl transfer reaction is involved in the degradation of the substrate by xylanases of family 11.

## Conformational analysis of the anomeric glycosyl radicals using DFT methods.

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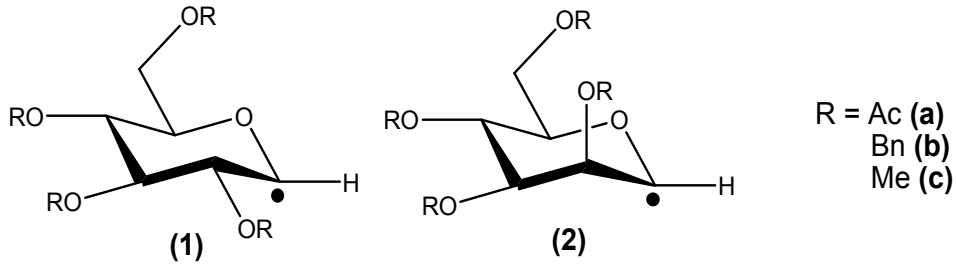
Anomeric carbohydrate radicals play an important role in the stereoselective synthesis of carbohydrate derivatives. For example, free radical reactions have been successfully applied in synthesis of C-glycosides [1] and preparation of 2-deoxy-□-glycosides [2]. Conformation and stability of glycosyl radicals are very important for the stereochemical outcome of these reactions and several studies [3,4] showed that these radicals prefer conformation in which the semi occupied orbital is in the axial position and the anomeric carbon has the pyramidal conformation.

To shed more light on the conformational preference of glycosyl radicals we have carried out DFT computations on various conformers of carbohydrate radicals with ADF program [5]. In this study we present results on the stability of selected boat and chair conformers for the 2,3,4,6-tetra-*O*-acetyl-D-glucopyranos-1-yl (1a), 2,3,4,6-tetra-*O*-acetyl-D-mannopyranos-1-yl (2a), 2,3,4,6-tetra-*O*-benzyl-D-glucopyranos-1-yl (1b), 2,3,4,6-tetra-*O*-benzyl-D-mannopyranos-1-yl (2b), 2,3,4,6-tetra-*O*-methyl-D-glucopyranos-1-yl (1c), 2,3,4,6-tetra-*O*-methyl-D-mannopyranos-1-yl (2c) radicals. All these radicals have been fully optimized using unrestricted DFT method with Becke-Perdew functional and TZP basis set.

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## Synthesis of fluoro amino derivatives of 1,6-anhydrohexoses

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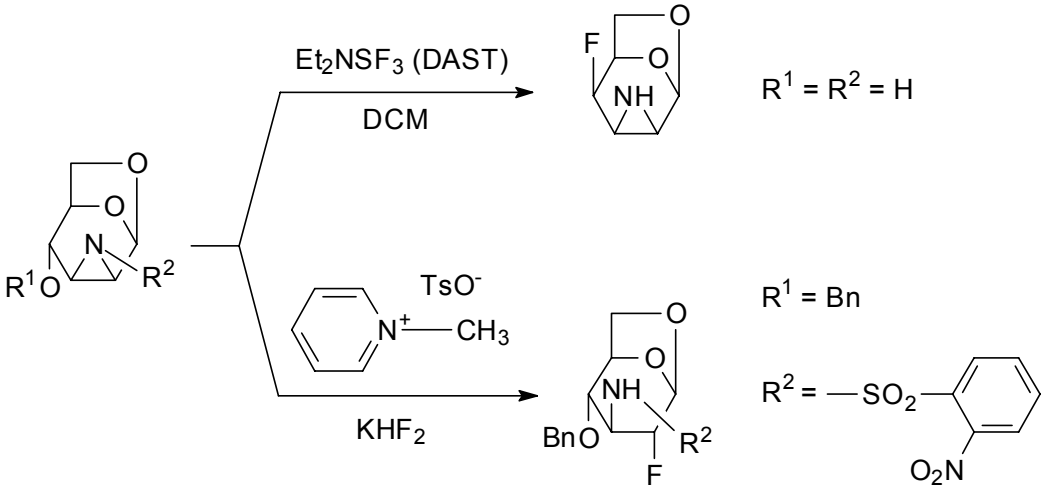
Sugar aziridines of five 1,6-anhydrohexoses with the D-*manno*, D-*allo*, D-*galacto*, and D-*talo* configurations (such as 2,3-D-*manno*-epimine depicted bellow) have been utilized for the preparation of fluoro amino hexopyranoses. Free epimines were treated with diethyl amino sulphur trifluoride in dichloromethane to obtain vicinal fluoro aziridines via direct displacement of DAST-activated hydroxyl group by fluoride. In no case the aziridine ring was affected by DAST.

Aziridine ring-opening reactions with $HF_2^-$ anion were performed to introduce fluoride atom onto 6,8-dioxabicyclo[3.2.1]octane skeleton in the same series of configurational isomers of sugar aziridines. The substitution of the aziridine ring with strongly activating *o*-nitrobenzenesulphonyl group was necessary to reach the ring fission since free or *N*-tosylated epimines are poor reactive towards fluoride or hydrogenfluoride anions. Ionic-liquid solvent (*N*-methyl pyridinium tosylate) at elevated temperature has been also required to obtain reasonable reaction yields for opened products. As predicted by the Fürst-Plattner rule, *trans*-diaxial isomers of the opened products were exclusively formed.




*This work was supported by Grant Agency of the Czech Republic (project No. GAČR 203/02/D047).*

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**Isolation and structural characterization of a mannan from the yeast *Candida dubliniensis***

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*Candida dubliniensis* is a recently described opportunistic pathogen that is closely related to *C. albicans* but differs from it with respect to epidemiology, certain virulence characteristics, and the developed fluconazole resistance *in vitro* (1-2). This strain was originally identified by Sullivan et al. (3) in oral specimens from Irish HIV-infected and AIDS patients with recurrent oral candidiasis.

Mannan, the outer-most layer of cell wall, is a major virulence, protective factor and a immunodominant antigen of the human opportunistic pathogen. The mannan conjugate can be considered as actual vaccine candidate for clinical evaluation.

A cellular *C. dubliniensis* mannan was isolated by autoclaving lyophilized biomass, precipitation of supernantant to ethanol, potassium hydroxide treating of ethanol precipitate (mannan-protein) and followed precipitation with Fehling's solution gave a homogeneous polysasccharide. It was composed of mannose (96%) and glucose (4%) residues only. Sugar linkage analysis, acetolysis as well as $^{1}H$ and $^{13}C$ NMR Spectroscopy were employed in structural elucidation of the *C. dubliniensis* mannan. The results obtained pointed to a highly branched comb-like structure of the polymer. The corea consisted of 1,6-linked mannose units, about 86% of which were substituted in position C-2 by side mannosyl chains of DP 2-5.

This research was supported by the Slovak Scientific Grant Agency (No. ) and APVT (No. 20-017304).

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## Determination of polydextrose as a fat replacer in milk butter

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Polydextrose® (PD) is used in several countries as a low caloric sugar and fat substitute (bulking agent). It is prepared by condensation of glucose, glucitol and citric acid (89: 10: 1). The resulting condensation product has no chemically defined structure, but represents a mixture of polymerisation products covering a molecular weight range from 150 to 20 000 [1]. Determination of PD in milk butter is complicated owing to large excess of fats and to the presence of other hydrocolloids, mainly proteins. FT-IR spectroscopy seems to be a sufficient method for detection of PD in samples derived from food products. The content of PD was determined after removing fats by extraction with petrolether and deproteinization with Sevag reagent [2] or trichloracetic acid. Dried extracts of milk butter without and with known amount of PD were prepared and analysed by FT-IR spectroscopy. IR marker bands of PD centred at 1150, 1076 and 1040 $cm^{-1}$ were found only for the sample contained the standard addition. Therefore, FT-IR analysis did not confirm the presence of PD in the studied sample of butter.

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# LYMPHOCYTE ACTIVATION RECEPTORS: NEW STRUCTURAL PARADIGMS IN THE GROUP V OF C-TYPE ANIMAL LECTINS

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From the point of view of structural biology, our understanding of the structure – function relationships in the C-type lectin family remains fragmentary. By far the best characterized molecule of the whole family is the soluble mannose binding protein studied protein crystallography. This protein binds to calcium ions in the ligand-binding domain, and the binding of calcium is intimately linked to the recognition of carbohydrates. Some of the receptors of natural killer lymphocytes have been also structurally characterized. Since this group is evolutionarily most divergent, interesting structural paradigms have been observed with regard to binding of calcium, carbohydrates, and other ligands. In NKR-P1, calcxium may not be easily removed by chelating agents because of its unique chemical nature. In CD69, binding of calcium causes a structural shift in amino acids important for binding of carbohydrates. Structural studies have also allowed us to understand an interesting preference of these receptors for either linear (NKR-P1) or branched (CD69) carbohydrate sequences.sugars. Remodeling of the binding surface in CD94 or Ly-49 opens the way for specific recognition of protein and peptide ligands.

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## *N*-Terminal propeptide of fungal β-*N*-acetylhexosaminidase plays role in enzyme's folding and dimerization

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β-*N*-Acetylhexosaminidase from *Aspergillus oryzae* is an extracellular enzyme known to be involved in chitin degradation. Sequencing of the enzyme, and cloning of the corresponding *hexA* gene revealed that the enzyme was produced as a preproprotein, which was processed early in the enzyme's biosynthesis. We found that the propeptide forms a non-covalent complex with the mature protein and it is vital for correct protein architecture and enzymatic function. Particularly, detailed investigation of the kinetics of enzyme production and secretion using pulse-chase labeling and protease inhibitors revealed that propeptide processing was essential for activation, dimerization, and secretion of the enzyme. We found that the most frequent form of the extracellular enzyme complex was composed of two molecules of *O*-glycosylated, processed propeptide associated with the mature protein homodimer (composed of two zincin-like domains and two *N*-glycosylated catalytic domains of glycosylhydrolase 20 family). Monomeric catalytic units devoid of propeptide could be detected only as minor intracellular species and they were enzymatically inactive. Moreover, reconstitution experiments with variable amount of propeptide combined with soluble but enzymatically inactive β-*N*-acetylhexosaminidase catalytic subunit allowed us to re-establish the catalytic activity of the enzyme complex. These data strongly suggest that the propeptide plays a crucial role as an intramolecular chaperone, facilitating the correct folding and activation of β-*N*-acetylhexosaminidase.

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**Molecular dynamics simulations and conformational analysis of Pullulan oligomers**

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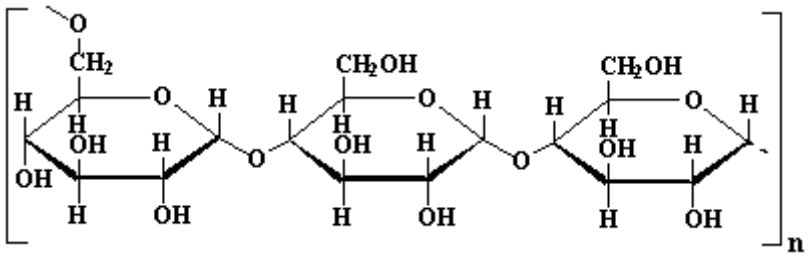
Pullulan (α-1,4'- ;α-1,6'-Glucan) is a linear homopolysaccharid of glucose (*Scheme 1*), which is produced by the fungus *Aureobasidium pullulans.* Having a different cellular location,there seems to be a correlation between glycogen and pullulan production, yet the reaction pathway is still uncertain.



**Scheme 1**

Molecular dynamics (MD) simulations of pullulan oligomer (n = 4) were carried out in order to investigate the three-dimensional structure of pullulan in gas phase and water solution. The GROMACS force-field and the SPC water models were employed. The analysis of MD trajectories at 300K brought interesting information about the conformational behavior of the studied oligomer. The examination of glycosidic dihedral angles revealed several structures appearing in the MD trajectory. These structures were then selected for further investigation and were fully optimized using MM approach. As a result, two stable conformations were observed in the gas phase, differing in just one CH-O-CH2-CH dihedral angle with small impact on the structure.

# Easy Preparation of Regioisomers of Mono-*O*-substituted Cyclodextrins

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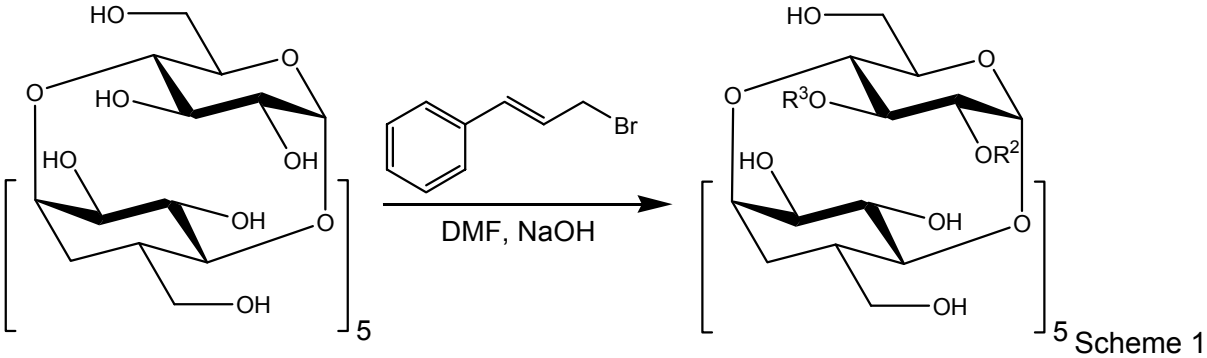
Cyclodextrins (CDs) and their derivatives have good complexation abilities due to the rigid, cone-shaped cavity formed by α-1,4-linked D-glucopyranose units. Our research is focused on the preparation of a set of exactly defined dimers of α-, β-, γ-CD linked with different spacers for using in analytical methods.

The first and the main step is the preparation of a monosubstituted derivative of CD, which has easily transformable functional group – e.g. cinnamyl group. Although many syntheses of regioisomers were described, there was no general and easy way (low yields, necessity to use HPLC) to prepare pure (especially $3^{I}$-*O*) regioisomers in larger amounts.

We prepared a mixture of $2^{I}$- and $3^{I}$-*O*-cinnamyl-α-cyclodextrin by the reaction of α-CD with cinnamyl bromide (Scheme 1), which could be easily separated by column chromatography. Position of the cinnamyl group was determined after peracetylation by 2D NMR techniques. In a similar way we prepared and isolated two monosubstituted derivatives of γ-CD (confirmed by MS). Determination of the structure of these isomers is now in progress.




Scheme 1

| $R^2$ | $R^3$ | Yield |
|---|---|---|
| cinnamyl | H | 9,4 % |
| H | cinnamyl | 11 % |

*This work was supported by grants GAUK 424/2004/B-CH/PřF, MŠM 113100001.*

# Study of pectin oligomers

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Pectins are complex polysaccharides found in the cell walls of higher plants. They are commonly used in the food industry as gelling agents, thickeners, texturizers, emulsifiers and stabilizers, in the pharmaceutical industry because pectins reduce blood cholesterol, prolong gastric emptying half-time and promote sustained release of medicine, and in other industrial applications. Although pectin is utilized in so many areas, its applicability can be even widen by fragmentation and derivatisation. Hydrolysis of pectin and pectin derivatives (some are totally insoluble) enhance solubility in water. Pectin is a soluble fibre and it is not absorbable in small gut. However, pectin hydrolysis yields smaller molecular weight molecules, which are more absorbable. Pectic oligosaccharides can consequently compete with natural ligands of galectins and inhibit the metastatic process.

This project deals with the preparation, characterisation and modification of pectin fragments. Oligosaccharide fractions were obtained by acid and alkaline degradation of citrus pectin and characterised by high performance size exclusion chromatography (HPSEC) and mass spectroscopy with the electrospray ionisation (ESI MS). The optimal conditions were found for the acid hydrolysis whereas the alkaline degradation caused large looses of the products. ESI-MS spectra of the hydrolysates proved presence of the fragments having degree of polymerisation (DP) 1 to10. Oligomeric fractions of DP from 6 to 18 were detected by HPSEC. The modified fragments, esterified and amidated derivatives, were studied by diffusion reflectance FT-IR spectroscopy. New characteristic IR bands in the DRIFT spectra confirmed that the oligosaccharide fractions obtained are suitable for esterification and following amidation.

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# Performance of Empirical Force Fields in Modelling of Carbohydrate-Aromatic Interactions

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Carbohydrate-protein interactions are often assisted by aromatic rings of aromatic amino acid residues with an involvement of CH/$\pi$ interactions. Carbohydrate-aromatic interactions are also likely to play an important role in supramolecular organic chemistry and in related fields. Therefore, an ability to model these interactions accurate is critical for application of molecular modelling techniques (e.g. force field methods) to areas of glycochemistry and glycobiology. *Ab initio* interaction energies for number of model carbohydrate-aromatic complexes were calculated in our previous studies [Spiwok *et al. Carbohydr. Res.* 2004, **339**, 2275-2280, Spiwok *et al. submited*]. Herein, comparison of these *ab initio* energies with values calculated using universal (OPLS, GROMOS) or carbohydrate (OPLS, CSFF, CHEAT, Glycam) force fields is presented. We observed unexpectedly high correlations between *ab initio* and force field values for some tested force fields. Carbohydrate-tuned version of OPLS-AA force field as well as CSFF and Glycam04 force fields turned out to be the most accurate in modelling of carbohydrate-aromatic interactions. Also the energy profiles as a functions of intermolecular distance are in agreement with *ab initio* values.

*Authors would like to gratefully acknowledge the support of the Grant Agency of the ASCR (GAAV KJB500500512) and the Ministry of Education of the Czech Republic (MSM 6046137305).*

# Synthesis of 6$^{I}$-Alkenoylamino-6$^{I}$-deoxy Derivatives of β-Cyclodextrin as Modifiers of Porous Silicon Sensoric Response

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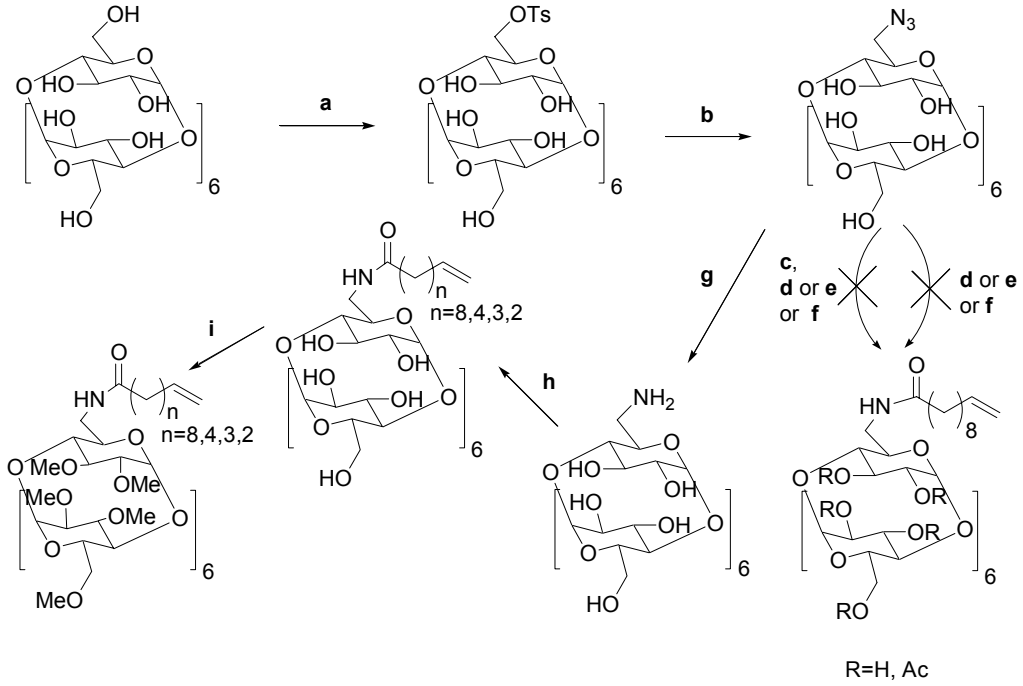
Cyclodextrins (CDs) are cyclic oligosaccharides. Very important characteristic of CDs and their derivatives is the ability to form inclusion complexes with organic molecules. Their selective complexation properties can be used as a recognition element of chemosensors. It can be expected, that CD attached to the surface of porous silicon will influence the luminescence, which porous silicon (PS) exhibits when exposed to UV radiation. Si-H bonds, which are present on the PS surface, can react by hydrosilylation reaction with CD derivatives containing terminal double bond. Synthesis of these derivatives is shown in the scheme.




a) Ts-Imz, NaOH, $NH_4Cl$, $H_2O$; b) $NaN_3$, DMF; c) $Ac_2O$, pyridine; d) $Ph_3P$ or $Bu_3P$, $RCO_2H$, Δ, DMF or toluene; e) $Bu_3P$, $RCO_2H$, 0°C, $CH_2Cl_2$; f) $Ph_3P$ or $Bu_3P$, $RCO_2H$, hν, DMF or toluene; g) $Ph_3P$, DMF, $NH_3$ aq.; h) DIC, HOBt, $RCO_2H$, DMF; i) MeI, NaH, DMSO

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# STEREOSELECTIVE SYNTHESIS OF NEW α-(1→3)-*C*-DISACCHARIDE

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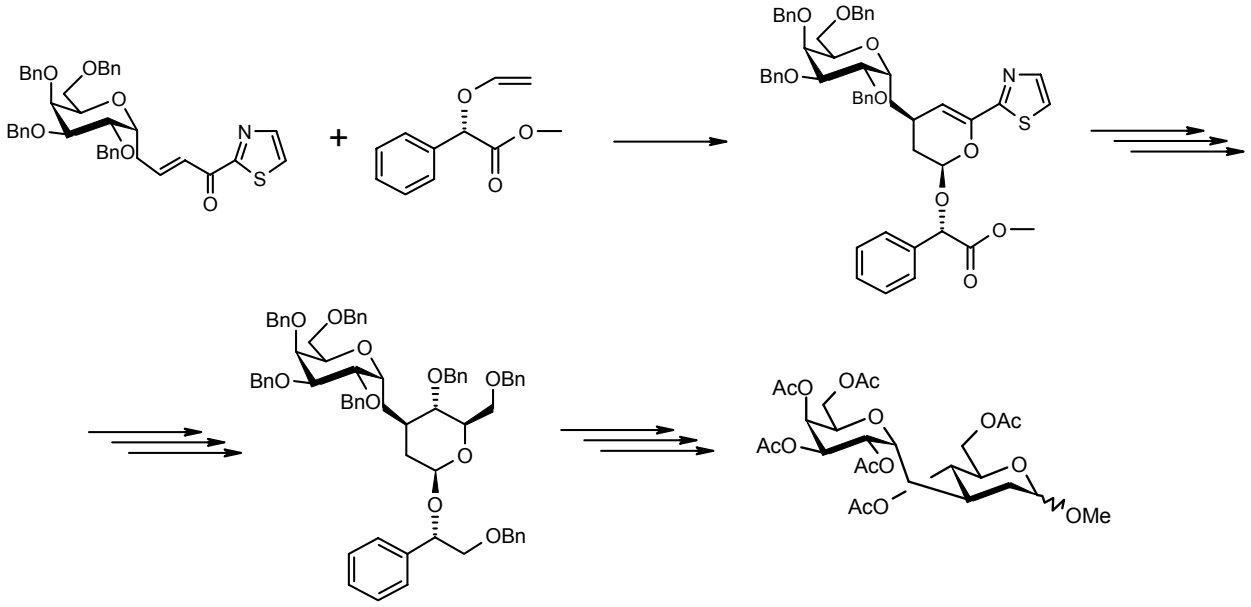
In our recent publication[1] we described efficient way leading to two α-(1→3)-*C*-disaccharides containing 2-deoxyhexopyranose (of D- and L- configuration). Afterwards we decided to inovate the procedure and explore a potential of stereocontrolled access to just one cycloadduct instead of mixture of diastereoisomers. The method of choice[2] were chiral vinyl ethers derived from mandelic acid. In this way, we converted starting oxadiene to individual cycloadduct in dependence on configuration of used mandelic acid derivate. Expected high facial (96:4) and *endo*/*exo* (>99:1) selectivity was observed in both cases. Then we used sligtly modified procedure then in previous case to prepare final α-(1→3)-*C*-disaccharide. Our results confirmed that this reaction sequence can be used for preparation of several new analogues of *C*-disaccharides (for mannose and galactose as starting compounds).

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# A Regiospecific Route to the *C*-D-Galactofuranosyl Compounds. Synthesis of 2-*C*-Glycosylbenzimidazols.

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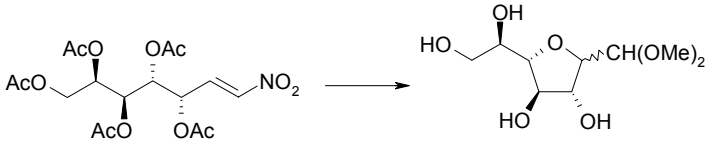
D-Galactofuranose is one of the sugar units that are essential for the production of the polysaccharides[1] found in the cell walls of many pathogenic bacteria causing such diseases as tuberculosis and leprosy[2,3] or parasitic protozoa causing e.g. leishmaniasis[4]. Thus, the compounds that mimic D-galactofuranose unit and interfere with the biosynthetic enzymes (especially UDP-Gal*p* mutase, E.C. 5.4.99.9) that produce and utilise this sugar in its furanoid form may represent a novel therapeutic strategy for the treatment of these infections[5].

An array of *C*-D-galactofuranosyl compounds that originate in 2,5-anhydro-3,4,6,7-tetra-*O*-benzoyl-D-*glycero*-L-*manno*-heptononitrile (β-D-galactofuranosylcyanide) and target at the aforementioned applications has been described recently[6]. This contribution suggests an alternative approach and is based on a recently described, regiospecific 2,5-anhydro ring-closure of the *in situ* generated 1,2-dideoxy-1-nitroald-1-enitols that are consecutively transformed by a Nef related reaction to stable *C*-glycofuranosyl compounds[7].

Thus, 1,2-dideoxy-1-nitro-D-*galacto*-hept-1-enitol peracetate (***1***), on treatment with methanolic hydrogen chloride gives an "anomeric" mixture of D-galactofuranosylmethanal dimethyl acetals (***2***).




***1*** ***2***

Starting from the corresponding glycosylmethanal dimetyl acetals, the contribution describes also an original method of the synthesis of 2-*C*-glycosylbenzimidazoles with α-D-galactofuranosyl, β-D-galactofuranosyl, β-D-glucopyranosyl, β-D-galactopyranosyl, β-D-mannopyranosyl, α-D-arabinopyranosyl, and β-L-rhamnopyranosyl configurations.

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# 3-*O*-SULFONYLERYTHROSES NEW SYNTHONS FOR IMINO-*C*-GLYCOSIDES

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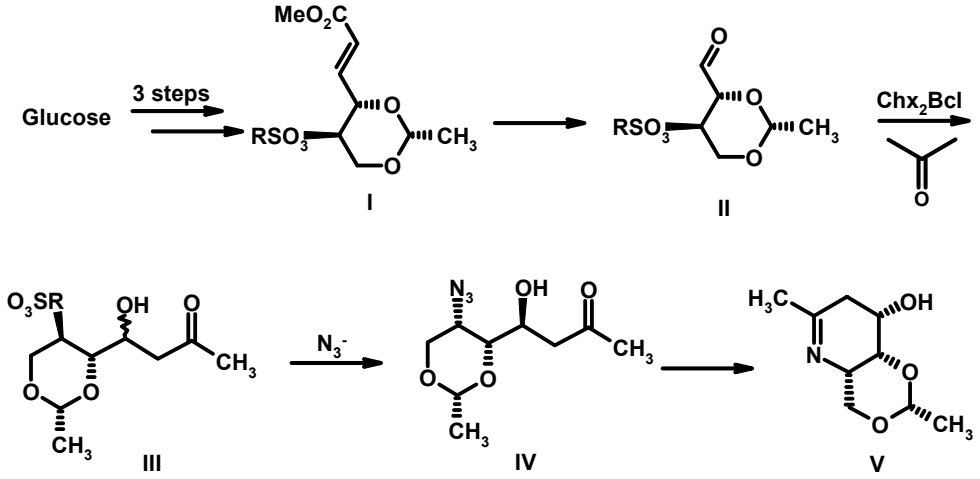
As result of their biological activity iminosaccharides are growing into one of the most significant class of carbohydrate mimetics. These compounds act especially as glycosidase or glycosyltransferase inhibitors[1,2]. Since these enzymes play a crucial role in biosynthesis of cell-surface oligosaccharides that are important for intracellular communication, their use as biochemical tools or therapeutic agents can be expected. The lability of the *O/N*-acetal function under hydrolytic conditions constitutes serious limitation in utilization described above. One possible means of generating interesting analogs of iminosugars consist in replacing the exocyclic oxygen atom of the *O*-*N*-acetal by a methylene group, thus forming "imino-*C*-glycosyl" compounds that are resisting acidic as well as enzymatic hydrolysis.

We proposed new synthetic route to such compounds based on cross-aldol reaction of 3-*O*-sulfonylerythroses with and any methylketone. Synthesis of 3-*O*-sulfonylerythroses is based on simple protocol involving protection of C4 and C6 position of glucose, periodate cleavage, "protection" of a carbonyl group as carboxymethylmethylene and conversion of C3 hydroxy group to appropriate sulfonate **Ia**,**b**. Ozonolysis of double bond lead to desired derivatives of erythrose **II**. $Chx_2BCl$ promoted cross-aldol reaction of **IIa**,**b** with acetone gave in both cases mixture of two diastereoisomes **IIIa**,**b** in ratio 5:1. At present we investigate substitution of sulfonates groups to azide **IV** and its subsequent reduction to cyclic imine **V**.




**a) R=$CH_3$**
**b) R= 4-tolyl**

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# PORPHYRINE RECEPTOR CONTAINING GLYCOSYLATED STEROID AND ITS SYNTHESIS

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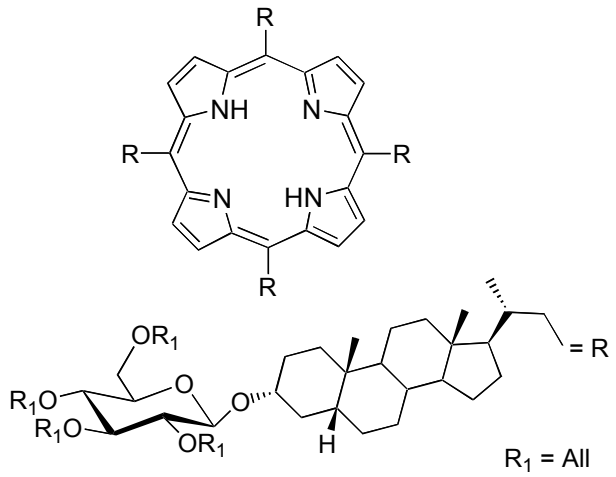
The suitable synthetic pathway for the new type of meso-substituted porphyrin (***X***) enables the study of this new kind of supramolecules and supramolecular synthons. These are being utilized in electrochemistry[1], membrane super assembly and biological studies, etc. This work shows the preparation of a new complex type of conjugate and opens rather vast territory of their possible exploitations. The authors aimed to demonstrate the possibility of combining the known receptor molecules with carbohydrates that can serve as anchors, chiral selectors, or polarity modifiers, depending on their level and kind of protection. The receptor was based on glycosylated steroid-porphyrines[2] that can have many interesting properties. In recent works, porphyrine structures substituted in meso positions by steroid units with some interesting properties were prepared[3,4].




**X**

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