Plagátové oznámenia

SYNTHESIS OF BRANCHED OLIGOSACCHARIDE-POLYETHER MODULES FOR ULTRA THIN BIOFUNCTIONALISED PEG-STAR-POLYMER LAYERS

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The carbohydrate moiety of glycoproteins and glycolipids within the extracellular matrix (ECM) plays an important role as information carrier system. One common characteristic of these glycans is the presentation of oligosaccharide ligands in a multivalent manner on antennae-like structures. Imitation of this natural situation implies the arrangement of antennary glycan structures on biofunctionalised surfaces.

Our synthetic strategy of a multivalent biohybrid system is based on the most important carbohydrate chain poly-*N*-acetyllactosamine (Gal□1-4GlcNAc□1-R, poly-LacNAc) and comprises the three steps: i) enzymatic poly-LacNAc synthesis, ii) the development of a synthetic multifunctional coupling system, iii) embedding of poly-LacNAc via orthogonal linkers as oligosaccharide-polyether modules in a PEG hydrogel. Multivalency should be achieved by the coupling of different poly-LacNAc chains to functionalised triol structures.

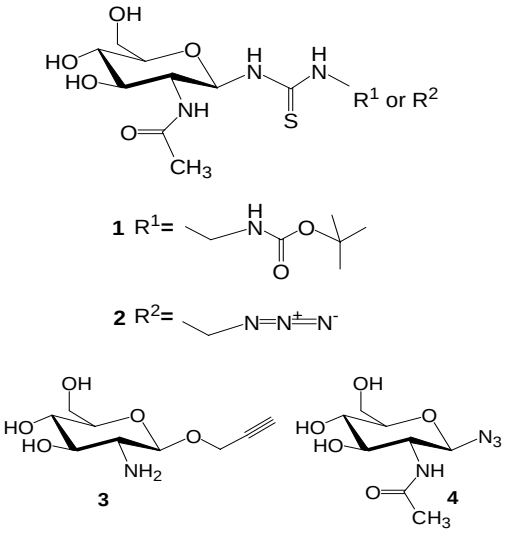

Figure 1: Acceptor structures for enzymatic synthesis of poly-LacNAc derivatives.

The starter molecules GlcNAcβ1-linker-amino-*t*Boc (**1**) and GlcNAc-linker-azide (**2**) were characterized as acceptor structures for the recombinant galactosyltransferase His_6-propeptide-β4GalT-1 [1] (**Figure 1**). Further we synthesized the modified starter molecules $GlcNH_2$-propargylether ($GlcNH_2$-Prop, **3**) and GlcNAc-azide (**4**) to enlarge our library of glycan structures as acceptor substrates of β4GalT-1. Towards building multivalent recognition structures, the complementary coupling molecules (**Figure 2**) were synthesized. Work is in progress to set up the synthesis of poly-LacNAc glycan chains and their characterisation by different galectins.

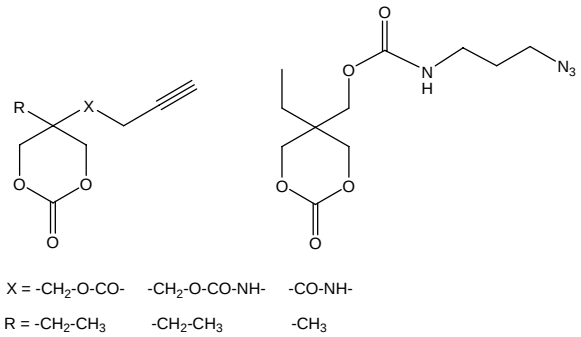

Figure 2: Multifunctional couplers with chemical complementary groups for the acceptor structures.

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Acknowledgements: Financial support by the DFG (project EL 135/8-1 and MO 682/8-1) is gratefully acknowledged.

THE DOSE-DEPENDENT COUGH SUPPRESSIVE EFFECT OF RHAMNOGALACTURONAN IN GUINEA PIGS

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Some polysaccharides of medicinal plants have been found to possess a variety of biological effects including antitussive activity. It has been shown that these polysaccharides are able to reduce cough comparably with commonly used antitussives without such severe side effects observed very often during the most active antitussives codeine type treatment.[1,2]

A. officinalis is known to contain relatively large amounts of mucilage which makes this medicinal plant excellent demulcent, emollient, expectorant, mucilaginous, etc. We have found that the main component responsible for antitussive activity of *Althaea* mucilage is rhamnogalacturonan[3], which can exert a soothing effect on the mucous membranes, including the upper and lower airways epithelium, confirmed by statistically significant ability of this polymer to reduce parameters of mechanically induced cough reflex in conscious cats[1].

The present studies were focused on effect of *Althaea* rhamnogalacturonan on the citric acid-induced cough reflex in dose-dependent manners and reactivity of airways smooth muscle *in vivo* as well as *in vitro* conditions. The best cough suppression was observed currently after administration of *Althea* rhamnogalacturonan. Since the cough reflex and bronchoconstriction are two very closely cooperated reflexes[4], we decided to determine the participation of bronchodilatory properties on antitussive activity of *Althea* rhamnogalacturonan *in vitro* as well as *in vivo* conditions.

This work was supported by the Slovak Scientific Grant Agency (VEGA), grants No. 1/3375/06 *and 2/0155/08*, *and the Science and Technology Assistance Agency (APVV), grant No*. *0030-07*.

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ANALYSIS OF WORT AND BEER CARBOHYDRATES USING A COMBINATION OF HPLC AND MASS SPECTROMETRY

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Saccharides play an important role in brewing process associated with taste, flavor, and nutritional quality of beer. Therefore, monitoring of saccharide composition sweet wort, wort and beer is of great significance. Recently, various efficient separation techniques (chromatography, electrophoresis) followed by mass spectrometry (MS) or nuclear magnetic resonance are used to analyze structure of saccharides [1, 2].

The main objective of this study was to develop the method for separation and characterization of dextrins in wort and beer samples. The dextrins were separated on HPLC column according to the number of glucose units. Particular factions were collected and subjected to off-line electrospray MS analysis. MS/MS spectra of both sodium adducts and deprotonated molecules derived from oligosaccharides revealed the evident differences compared to the corresponding standards of maltooligosaccharides. In the negative ion mode there were diagnostic B and ^{0,3}A fragment ions in the former case. The MS^n experiments provided more detailed information about structure. The on-line LC-MS analysis gave the similar results.

The results obtained for wort and green beer samples supported earlier observation, that the dextrins remains unchanged during the fermentation.

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INVESTIGATION OF THE ACTIVE SITE OF THE INTRACELLULAR *β*-D-XYLOSIDASE FROM THERMOBIFIDA FUSCA

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The gene encoded the *β*-D-xylosidase was isolated from *Thermobifida fusca* strain TM51 by the use of an earlier published expression library procedure (1). The gene encoded the *β*-D-xylosidase was inserted into pET28a vector, the resulting construct was transformed into *E. coli* BL21 (DE3) strain expressing *β*-D-xylosidase as a fusion protein with His_6-tag at the C-terminal.

The aminoacid sequence homology of the intracellularly produced *β*-D-xylosidase (EC 3.2.1.43) from *T. fusca* has placed the enzyme into the family GH43 having an inverting mechanism in the hydrolysis, which was proven by NMR studies, where we have followed the change of the anomeric configuration. NMR STD measurements have exhibited that both anomers of D-xylose bind to the active site indicating product inhibition.

β-D-xylosidase has shown very narrow substrate specificity, it reacts only with *β*-D-xylopyranoside derivatives. The classical kinetic characterisation was carried out with *p*-Nitrophenyl *β*-D-xylopyranoside as substrate at 50°C and at pH=6,0 as the optimal conditions (K_M=0.71±0.05mM, v_{max}=3,37±0,12 [nmol/min]/μg).

Active site topology of the wild type enzyme was investigated by using carboxylate specific chemical modifier 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) in the presence of glycine methyl ester as well as *N*-bromoacetyl-*β*-D-xylopyranosylamide (NBAXA) as a mechanism-based inactivator (2). The chemical modifications were carried out in presence of *β*-D-xylopyranosylazide as competitive inhibitor, which indicates that all the modified catalytic groups were involved in active site. Both chemical modifiers have completely inactivated the enzyme, which suggests that the inactivators covalently attached to the putative catalytic Glu203 or/and Asp31. Based on ESI/MS and MALDI-TOF studies, in case of NBAXA, the enzyme has bound four molecules of inactivator that could reflect the special arrangement of four carboxylate side chains (Asp31- Glu203, Asp144-Glu226) in the active centre.

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SACCHARIDE PRECURSORS OF THE POTENTIAL INHIBITORS OF GLYCOSYLTRANSFERASES

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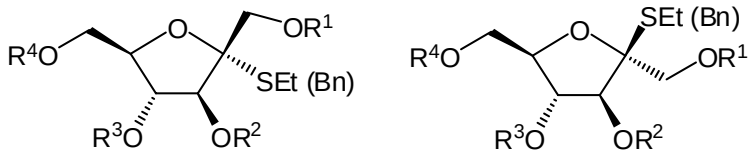
A new generation of inhibitors of glycosyltransferases based on carbohydrate mimetics has been recently emerged. The model compounds of this type are structurally altered analogs of carbohydrates designed to simulate their shape and functionalities of the natural substrates in the ground and/or the transition state (TS), respectively, with the aim to modulate their biological activity.

In this contribution, we report the synthesis of saccharide moiety of TS analog, which mimics donor UDP-GlcNAc – the precursors – suitable protected ethyl (benzyl) 2-thio-α-and-β-D-fructofuranosides with the free OH-group at C-1. The ethyl and/or benzyl group, respectively, mimics the acceptor part of the molecule and the '1-thio' linker provides the ~1.9 Å distance between the 'donor' and 'acceptor' part of the molecule and the charge distribution as it is in TS. The starting point in the synthesis of these model mimetics were ethyl (benzyl) 2-thio-α- and β-D-fructofuranosides (***I***) which are easily available from D-fructose in four reaction steps. Subsequently, the OH group at C-6 was regioselectively blocked with *tert*-butyldimethylsilyl chloride to give ***II***, followed by selective tritylation with dimethoxytrityl chloride in pyridine in order to protect OH group at C-1 affording compounds ***III***. Free OH groups at positions C-3 and C-4 were protected by classical acetylation to yield products ***IV***. Finally, the required nucleophiles ***V*** suitable for coupling reaction with dibenzyl (diethyl) chlorophosphates were obtained by detritylation with trifluoroacetic acid in the presence of triethylsilane in $CHCl_3$ (Fig. 1).



	R^1	R^2	R^3	R^4
I	H	H	H	H
II	H	H	H	TBDMS
III	DMTr	H	H	TBDMS
IV	DMTr	Ac	Ac	TBDMS
V	H	Ac	Ac	TBDMS

Fig. 1

TBDMS - *tert*-butyldimethylsilyl; DMTr - dimethoxy triphenylmethyl; Ac - acetyl

Acknowledgements: This work was supported by the grants APVV No. 51-004204 and VEGA No. 2/0128/08.

POLYSACCHARIDES EXTRACTED FROM THE LEAVES OF *FALLOPIA SACHALINENSIS*

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Giant knotweed (*Reynoutria or Fallopia sachalinensis*), a closely related species to Japanese knotweed (*Fallopia japonica*, previously classified as *Reynoutria japonica* or *Polygonum cuspidatum)* (1*),* is an invasive non-native species in Slovakia. It is regarded as a troublesome pest in many parts of the Earth because of its rapid invasion and domination of habitats. Giant knotweed is native to South Sachalin, the north of Japan, Korea, and the Kurile Islands and grows much taller (4-5 metres) than Japanese knotweed.

In view of the potential health benefits, there has been intense research on natural antioxidants derived from plants. Data from various studies (2,3) indicate that Chinese medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than common dietary plants. Polygonaceae has traditionally been used in Asia to treat skin burn, gallstone, hepatitis, inflammation, and osteomyelitis (4). From 30 Chinese medicinal plants (2), the extracts from stem and root of *Polygonum multiflorum* (boiling in water or 80% methanol) exhibited the highest antioxidant activities and, thus, are a rich sources of natural antioxidants. Except of the phenolic components of *Polygonum* species, there are no reports on other plant components, particularly, the polysaccharide ones.

The main objective of this study was the isolation and structural elucidation of polysaccharides extracted from the leaves of *Fallopia sachalinensis.* The polysaccharides recovered from the extracts were characterised by yield, chemical composition and FT-IR spectroscopy. The crude polysaccharide fractions were associated with different amounts of proteins and phenolics. Some polysaccharide fractions showed antioxidant activities in the DPPH test.

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UNUSUAL REARRANGEMENT IN THE GRIGNARD REACTION OF CARBOHYDRATE-ALDEHYDES WITH BENZYLMAGNESIUM HALIDE

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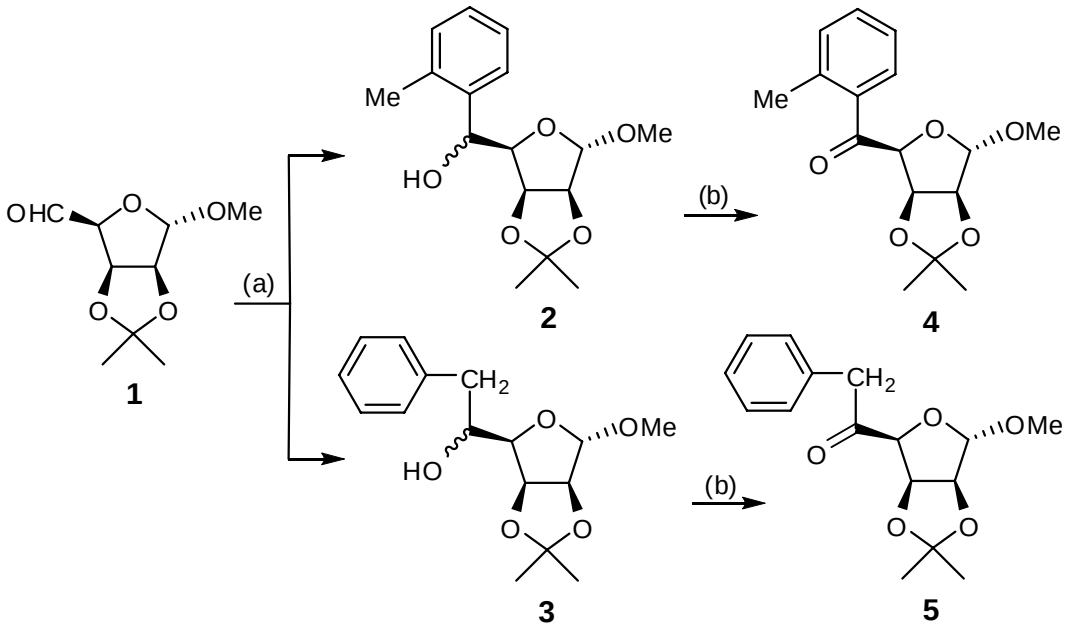
The Grignard reaction has been widely used in the chemistry of saccharides. It represents an important method for the synthesis of versatile branched carbohydrates because the obtained alcohols can be further modify by various manipulations (like oxidation, substitution, acylation, alkylation, etc).

In our study, the Grignard reaction of methyl 2,3-*O*-isopropylidene-α-D-*lyxo*-pentodialdo-1,4-furanoside (**1**) with benzylmagnesium chloride or bromide afforded preferentially the product of rearrangement – 5-(*o*-tolyl) carbinol **2** (a mixture of 5-*R* and 5-*S*-isomers) and the minority of expected „normal" product – 5-benzyl derivative **3** (a mixture of 5-*R* and 5-*S*-isomers). The ratio of **2** and **3** depends on the reaction conditions. A mixture of four unseparable isomeric alcohols was oxidized to give a mixture of corresponding crystalline ketones **4** and **5**. These were successfully separated and subjected to X-ray analysis.



Reaction conditions: a) BnMgCl or BnMgBr, Et_2O, cooling or r.t.; b) PDC, CH_2Cl_2, reflux.

Acknowledgements. This work was supported by the VEGA grant No. 2/6129/27 and by the APVV grant No. APVV-0366-07.

CONJUGATE VACCINES FROM SYNTHETIC CARBOHYDRATE ANTIGENS

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Our strategy for developing conjugate vaccines for bacterial diseases is based on the use of synthetic fragments of O-specific polysaccharides of bacterial pathogens as antigenic components. Accordingly, we synthesize oligosaccharides that mimic structure of the O-specific polysaccharides of bacterial pathogens and attach them chemically to protein carriers. Conjugation is essential because carbohydrates, classified as T-cell independent antigens, are poor immunogens. By conjugating carbohydrates to proteins we transform them into T-cell dependent antigens. As a result, multiple injections of such neoglycoconjugates can sharply boost antibody titers way beyond those observed following priming with TI antigens. This is, essentially, the rational behind the concept of conjugate vaccines. Currently, we work on developing conjugate vaccines for cholera and anthrax.

Cholera is an infectious, enteric, life threatening disease caused by some strains of *Vibrio cholerae.* There are over 200 strains of *Vibrio cholerae* but only strains O1 and O139 cause a disease in humans. We have made most significant progress in the development of a vaccine for cholera from the hexasaccharide mimic of the O-PS of *Vibrio cholerae* O:1, serotype Ogawa (Fig. 1, A).

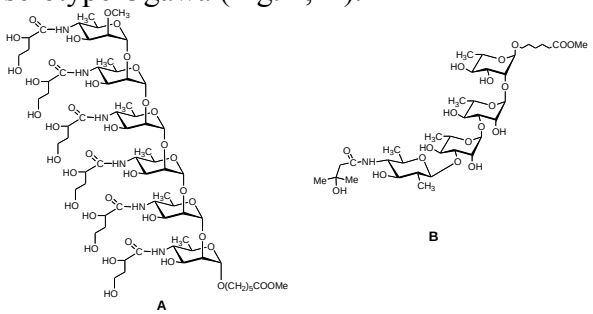

Fig. 1

Anthrax has been known as a serious disease for decades. It has not done much harm in the civilized world but new concerns regarding anthrax have recently emerged in connection with the use of some form of *Bacillus anthracis* as a biological weapon. Our work towards a conjugate vaccine for anthrax is based on the use of the synthetic tetrasaccharide (Fig.1, B) that a side chain of the major glycoprotein of the *Bacillus anthracis* exosporium as an antigenic component of the vaccine.

Of the many methods available for conjugation of synthetic carbohydrates to proteins we prefer the squaric acid chemistry. It gives constructs whose general structure is shown in Fig. 2.

Serological evaluation of mice immunized with our conjugates made from the hexasaccharide mimic of the O-PS of *Vibrio cholerae* O:1, serotype Ogawa showed their vibriocidal activity and protective capacity.

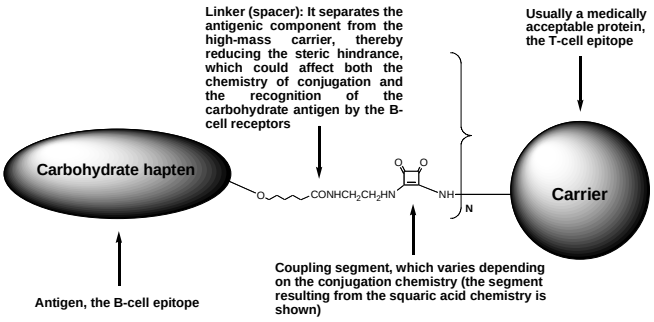

Fig. 2

Conjugates prepared from the tetrasaccharide side chain of the major glycoprotein of *Bacillus anthracis* exosporium were instrumental in development of a glycan array, which can be used to identify presence of B. anthracis spores, surveillance and diagnosis of anthrax infection, and can be helpful in development of novel vaccines for anthrax.

HOMOLOGY MODELING OF THE *N*-ACETYLGLUCOSAMINYLTRANSFERASE V (GNT-V)

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N- and *O*-linked oligosaccharides of the glycoproteins are involved in many recognition processes like bacterial and viral and adhesion, inflammatory processes, and cancer invasion. Several glycosyltransferases that are involved in biosynthesis of these glycan strutures have been confirmed as primary targets in drug development of various human diseases. Experiments on mice with blocked β-1,6-*N*-acetylglucosaminyltransferase V (GnT-V) production (Mgat5 knockout) clearly show reduction in cancer metastasis [1]. Due to the clear evidence of the involvement GnT-V in the cancer metastasis, the understanding of the GnT-V structure and reaction mechanism is of great interest.

In the present study, we have attempted to develop the 3D model of the GnT-V structure. From available experimental data it is not clear to which GT fold family GnT-V belongs. Therefore, for homology modeling we investigated structures from both fold families, GT-A and GT-B, respectively. Then one structure from each family with the best primary sequence alignment with the GnT-V minimal catalytic domain [2] was used as structure templates. Thus, we have selected the structure of Core2 transferase (2GAM) [3] as the representative of the GT-A family and the structure of MurG transferase (1NLM) [4] as the representative of the GT-B family. We made several alignments employing different algorithms, for example Blosum62, 120PAM or 3D-PSSM. As a result, altogether 10 models of the GnT-V structure were generated. On the basis of the 3D-structure analysis we selected 5 models for the molecular dynamic equilibration in the water. All structure models have been equilibrated at 300 K during 4ns.

This work was supported by the grants from ESF number JPD 3BA 2005/1-031.

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QM STUDIES OF THE ACID CATALYSED HYDROLYSIS OF GLYCOSIDIC BOND – CONFORMATION, INTERMEDIATES AND DYNAMICS OF SELECTED HEXOPYRANOSES

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Methyl glycoside has been chosen as model compound for substrate undergoing glycosidic bond hydrolysis. Conformational preferences for both anomers has been calculated at the B3LYP/6-311++G(d,p) level of theory (1). Intermediates for the hydrolysis have been proposed and compared in terms of their energies and geometries. These intermediates are (i) glycosidic oxygen protonated methyl glycosides and (ii) oxocarbenium ions (2). Effect of conformation on the energy of intermediates and their stability are discussed.

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PHYSICO-CHEMICAL CHARACTERIZATION OF GLUCOSE MONOHYDRATE DEHYDRATION AND ANHYDROUS GLUCOSE REHYDRATION

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Dehydration of α-D-Glucose monohydrate is a common industrial practice for the elaboration of anhydrous α-D-Glucose (or Dextrose). Studying the kinetics of such a dehydration process revealed that removal of water from the hydrate may be considered as a chemical reaction :

Dextrose Monohydrate (solid) ⟶ dextrose anhydrous (solid) + water (vapor)

To understand the mechanism by which we move from a crystal form(monohydrate) to another (hydrate), establish at a structural basis the kinetics of polymorph transition and check the possibility of reversibility of dehydration-hydration reactions, the characterization of crystals was achieved.

Such characterization was made with X-Ray Diffraction control of the dehydration of the monohydrate as well as the hydration of anhydrous crystals submitted to varied ERH (Equilibrium Relative Humidity). Other techniques like DSC and Moisture content and Water Vapor Adsorption isotherms were used as well as the direct observation under camera microscope of the adsorption of water by a sample of anhydrous α–D-Glucose and its transformation into monohydrate.

Kinetics of the dehydration of the monohydrate crystals was controlled by following the change in standardized intensity of characteristic 2 θ angles. It was clearly shown that after a time-lag, the change in intensity varies linearly with time. Both decrease in anhydrous percentage and increase in monohydrate seem to follow a zero-order kinetics. The 2 lines representing the 2 kinetics intersect at 50%, which means that the disappearance of monohydrate crystals is simultaneously accompanied by the formation of anhydrous crystals.

Re-hydration of the anhydrous glucose was also controlled with XRD and DSC after equilibration with humid surrounding atmosphere. From the standardized intensities of characteristic 2θ angles, the same kinetic trend was found with much longer time needed for adsorption of water and crystallization of the monohydrate. The role of Crystal Size Distribution (CSD) was also evaluated. It does not seem to modify the kinetics, but time-lag is changed.

Characterization of the 2 polymorphs can help in understanding at a microscopic level the process of dehydration of a hydrate and eventually the behavior of the hydrate during storage at varied ERH. These information can be used to complete the modeling of the dehydration of Dextrose monohydrate in a fluidized bed dryer.

Keywords: Glucose monohydrate, Anhydrous glucose, crystal, dehydration, Re-hydration.

AN ARABINOGALACTAN ISOLATED FROM PURE *COFFEA ARABICA* FREEZE DRIED COFFEE

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Coffee is known for its wonderful aroma and stimulative effects on organism. It is one of the most consumed beverages prepared from roasted seeds of the coffee plant. The *Coffea* plant is native to subtropical Africa and southern Asia, and belongs to the family *Rubiaceae*. The most commonly grown species is *Coffea arabica*. About three-fourths of coffee cultivated worldwide is *C. arabica*. It is an evergreen shrub or small tree. The fruit berry is green when immature, but ripens to yellow and becoming black on drying. Berries ripen in seven to nine months. Annually is produced about 6-7 million metric tons of coffee.[1] Besides the caffeine the coffee is a rich source of many natural compounds. Between them, polysaccharides represent a significant group of polymeric compounds. Galactomannan and arabinogalactan were found to be the most known water-soluble polysaccharides in extracts of various roasted beans.[2,3] . Arabinogalactan from roasted coffee as well as from green coffee beans and instant coffee has been investigated, albeit not extensively, however the polymer primary structure has not yet been fully elucidated [4,5].

The present work reports on the isolation and structure determination of a low molecular-mass arabinogalactan from freeze dried coffee powder from dark roasted *Coffea arabica*. The polymer was composed of Gal (85%), Ara (8.2%), Man (2.7%), Glc (2.4%), Hep (2.7%) and traces of Xyl residues. The results of chemical and spectroscopic investigation revealed a 3-linked β-galactosyl backbone branched at C-6 by side oligomeric chains.

This project has been supported by the Illy Caffe grant, the VEGA agency, grant No. 2/0155/08 and the APVV agency, grant No. 0030-07.

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DEOXYFLUOROGALACTOSYL PHOSPHONATES

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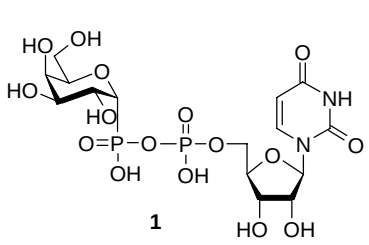
The phosphonates of natural occurring compounds represent a promising structural pool for the design of biologically active structures. Beside very efficient acyclic nucleoside phosphonates possessing remarkable antiviral activity, phosphonates of carbohydrates become more attractive target, as well. Moreover, phosphonates carrying a fluorine atom at organic part of the molecule have been identified as challenging structural motif quite recently[1].

As a part of our ongoing study on galactosyltransferase inhibitors based on a natural donor matrix we synthesized UDP-Gal analogues **1** and **2** using as a final step the modified



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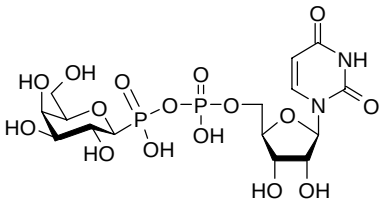

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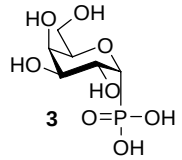

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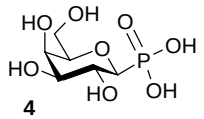

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Moffat-Khorana phophomorpholidate coupling of respective phosphonate with uridine monophosphate with 1*H*-tetrazole as catalyst[2]. Having in hands this well developed synthetic tool we turned our attention to the preparation of deoxyfluoro analogues of α(β),D-galactopyranosyl phosphonic acids[3] **3** and **4**. Formally, hydroxyl groups in both acids **3** and **4** were replaced step-by-step with fluorine but, in fact, the multistep syntheses had to be developed depending on the position of substitution.

The financial support of the grant MSM 6046137303 is gratefully acknowledged.

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STRUCTURE-FUNCTION STUDY OF CORE α1,3-FUCOSYLTRANSFERASE A FROM *ARABIDOPSIS THALIANA*

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Abnormal fucosylation in human leads to diseases associated with rheumatism, diabetes type II, inflammation, neural diseases, onco-diseases and allergy. Fucosylations are performed by enzymes called fucosyltransferases, which catalyze transfer of fucose from donor substrate GDP-fucose to acceptor substrate. According to their sequence similarities and mode of action, fucosyltransferases responsible for the formation of α1,3/4 linkage belongs to the same family (GT10). In plants as well as in some invertebrates a core α1,3-fucosylation has been described that does not occur in mammals and represents modification of innermost asparagine-linked GlcNAc with α1,3-linked fucose and thus forming immunogenic epitope (pollen allergy). Understanding of the catalytic mechanism and solving the 3D structure of a core α1,3-fucosyltransferase can be adapted to the other members of the GT10 family.

Sequence analysis of the GT10 family resulted in identification of 3 motives: 1st Cluster motif, 3FucT motif, and the CxxC motif present only in GT10 members of the higher eukaryotes. In order to elucidate the role of these amino acid residues which are associated with enzyme activity we prepared a series of mutants of core α1,3-fucosyltransferase A (FucTA) cloned from *Arabidopsis thaliana* and expressed in *Pichia pastoris.* As the 3FucT motif was already investigated early in human FucT VI, we studied the 1st Cluster motif by alanine screening of S218 and R226. Functional analysis of recombinant proteins showed that the replacement of any of these amino acid residues leads to a complete loss of activity. Furthermore, in order to delineate the minimal catalytic domain we examined the effect of N- and C-terminal truncation on FucTA activity. Deletion of 88 amino acid residues from the N-terminus did not affect significantly the activity while the deletion of 95 amino acids resulted in loss of activity. Deletion of the C-terminal domain (112 aa) C389-V501 which is unique to plant core α1,3-fucosyltransferases abolished the enzyme activity. Engineered recombinant FucTA is carrying three putative N-glycosylation sites (N337, N420, and N481) and two of them N420 and N481 are glycosylated, however none of this glycosylations is required for enzyme activity. AtFucTA is activated by Mn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, and Cd^{2+}, whereas it is inhibited by Cu^{2+}. Homology modelling of the conserved regions of AtFucTA was performed using template of α1,3-fucosyltranferase (FucT) from *Helicobacter pylori* with solved 3D structure.

UTILIZATION OF IMMOBILIZED GLUCOSE OXIDASE FOR REMOVAL OF GLUCOSE FROM SACCHARIDE MIXTURES

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Glucose oxidase (GOD) is a flavoenzyme catalysing the oxidation of β-D-glucose to δ-gluconolactone which is subsequently hydrolysing to gluconic acid. This enzyme has considerable importance in food industry for removal of glucose or oxygen from food and pharmaceutical products as well as for production of gluconic acid.
Present work was oriented on utilization of GOD immobilized on different supports (bead cellulose, Eupergit) for removal of glucose from mixture with low molecular dextran and mannose. GOD was immobilized on supports directly - chemically or biochemically through Concanavalin A. D-glucose oxidation in presence of low molecular weight dextran or mannose was performed in batch experiments using the preparations of immobilized GOD in phosphate buffer pH 6 under continual aeration with oxygen. Amount of 0.9 % (w/w) of glucose in 10 % (w/w) solution of low molecular weight dextran was decreased during 2.5 h to 3.8 – 15.6 % of its initial value depending on immobilization mode of GOD. The best glucose conversion was achieved (96.9 %) by an action of GOD immobilized on Eupergit CM (specific activity 24.6 $U.mg_{prot.}^{-1}$). Immobilized GOD on different supports was used also for removal of D-glucose from D-mannose solution obtained by epimerization of D-glucose. The removal of 0.04 % (w/w) of glucose from 0.96 % (w/w) of mannose was remarkably improved by the incorporation of an immobilized catalase (CAT) in biotransformation mixture in weight ratio 1:2 to immobilized GOD. At 30 °C and pH 6 and continuous aerating with oxygen the complete removal of glucose from solution was achieved during 2 h. Gluconic acid generated during the biotransformation was precipitated with $CaCl_2$ and filtrated away. GOD covalently immobilized on bead cellulose (GOD-TBC with specific activity 36 $U.mg_{prot}^{-1}$) showed the best operation stability of the three used types of immobilized GOD. This immobilized GOD was used also for determination of oxidation kinetics of other 11 saccharides: aldopentoses, aldohexoses and ketohexoses. The rate of oxidation of D-galactose, D-mannose, D-xylose and D-fructose at 0.01M saccharide concentration was ranging from 0.3 to 2.2 % as compared to glucose, but other saccharides tested were oxidized negligible.

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ATMOSPHERIC PRESSURE PHOTOIONIZATION MS OF PER-O-METHYLATED OLIGOSACCHARIDES RELATED TO D-XYLANS.

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APPI is a relatively recent ionization technique, which has considerable attention in the past few years. Initially restricted to small hydrophobic molecules, its use was then extended to larger, polar compounds. To our knowledge, this technique has not been extended to the study of oligosaccharides.

Three, D-xylan type per-O-methylated trisaccharides, with various types of linkages between the D-xylopyranose units were examined by APPI MS in the positive ion mode. The most interesting feature of a thermospray mass spectrum using the APPI source with UV lamp switched off, is the exclusive production of $[M + Na]^+$ adduct ions. Those are the most abundant species in the case of APPI mass spectrometry. The second ionization process has no analogy in the case of substances studied using APPI to date. This aspect involves the addition of a water molecule to the molecular ion of a per-O-methylated saccharide, giving rise to $[M + H_2O]^{+\cdot}$ adduct ions. The $[M + H_2O]^{\cdot+}$ species are readily detected at *m/z* 544, and are clearly visible for all three isomers studied. The MS/MS spectrum of $[M + Na]^+$ ions contains a base peak at *m/z* 375, produced by a Y-type cleavage of the trisaccharide, along with a hydrogen rearrangement on the terminal interglycosidically linkage glycosidic oxygen atom. The $[M + H_2O]^{+\cdot}$ species fragment largely give rise to ions at *m/z* 175, 143 and, as a result, the *m/z* 111 ion is unique to nonreducing terminal units.

This is the first report of both linear and branched oligosaccharides examined under UV radiation at a dense medium and under atmospheric pressure. The predominant pathway involved the production of glycosidic fragments without cross ring fragments. Tandem CID fragmentation of $[M + Na]^+$ ions result in fragment ions. The advantage of this technique is, that the spectra show no internal residue loss. These results differ from spectra obtained by ESI and MALDI, in which cross ring cleavages are the dominant feature. This finding demonstrates the potential value of the APPI technique in the detection of per-O-methylated saccharides, even in trace amount.

The investigation was supported by APVV project 51-017905 and VEGA 2/6132/27.

CARBON CHAIN DEGRADATION OF 2-KETOSES WITH TOLLENS' REAGENT

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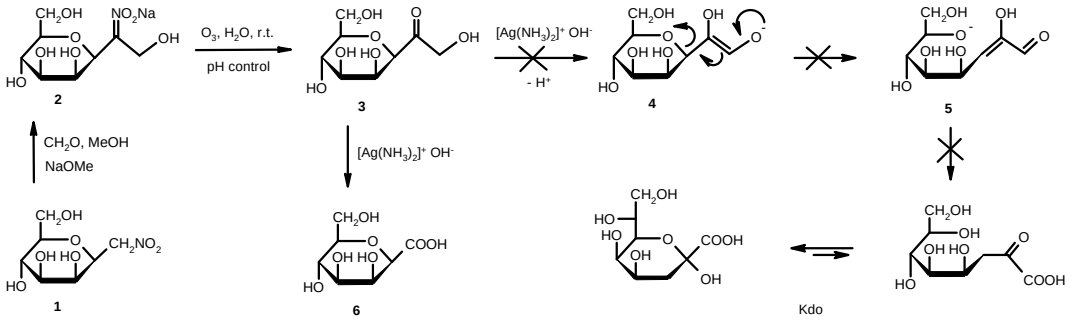
Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid), which is found in bacterial polysaccharides [1], have been synthesized in several ways [2]. 3,7-Anhydro-D-*glycero*-D-*galacto*-oct-2-ulose (**3**, Scheme 1), which is easily available from β-D-mannopyranosylnitromethane (**1**) by the procedure described earlier [3], could offer another simple approach for synthesis of Kdo, provided that ketose **3** could undergo the isomerization to enediolate **4** under the alkalinity of Tollens' reagent. Since the internal 3-*O*-substitution in enediolate **4** should introduce opening of its pyranoid ring via internal β-elimination of its 3-*O*-substituent, the enolate form of the aldehyde precursor of Kdo **5** was expected to be the next intermediate, which should be oxidizable with Tollens' reagent directly to Kdo.



Although the silver(I) ions are known to oxidize acrolein to acrylic acid [4], the treatment of 3,7-anhydro-octulose **3** with Tollens' reagent made of silver trifluoroacetate and ammonia, which does provide the alkaline conditions required both for its isomerization and intramolecular β-elimination to **5**, did not lead to the expected Kdo but resulted in the formation of acid **6**. Isolation of almost a quantitative yield of acid **6** after a 2 h treatment of 3,7-anhydro-octulose **3** was easily possible due to using Tollens' reagent made of silver trifluoroacetate instead of the classic silver nitrate made reagent, what enabled a simple removal of the anion coming from the reagent. Similarly D-fructose, on treatment with Tollens' reagent, gave D-*arabino*-pentono-1,4-lactone instead of the expected lactones of D-gluconic or D-mannonic acids. The behaviour of other common reducing sugars submitted to the treatment with $[Ag(NH_3)_2]OH$ is presented as well.

Acknowledgments: The work was supported by the APVV-51-046505 and VEGA-2/6129/26 grants.

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SYNTHESIS OF *N*-GLYCOSYLMETHYLATED IMINOALDITOL BETAINES

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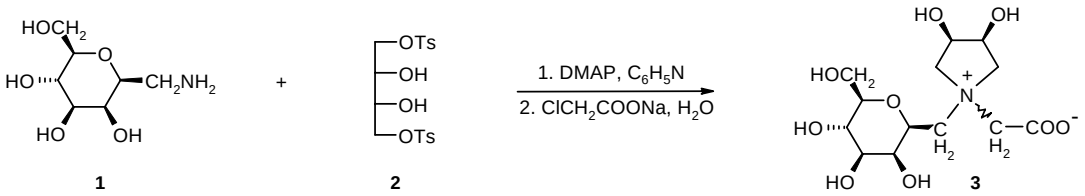
Many iminoalditols are efficient inhibitors of glycoside hydrolases. Both *N*- and *O*-substitutions of 1,5-dideoxy-1,5-iminoalditols and 1,4-dideoxy-1,4-iminoalditols greatly influence their inhibitory activities against sugars processing enzymes. The significance of iminosugars has been continuously increased as many other biological activities of the compounds have been found in recent years. They inhibit also glycosyl transferases, glycogen phosphorylase, nucleoside-processing enzymes, metallo-proteinases, etc. [1]. In addition, derivatives of iminosugars, especially *N*-alkylated piperidinols, were found to exhibit antiviral activities and to have a protective effect in patients with Gaucher disease, an inherited lysosomal disorder. Recently, we have reported synthesis of *N*-β-D-xylopyranosylmethyl-1,5-dideoxy-1,5-iminoxylitol from *C*-β-D-xylopyranosylmethyl-amine and 2,3,4-tri-*O*-benzyl-D-xylopyranose [2]. In this work, we describe synthesis of *N*-β-D-mannopyranosylmethyl-1,4-dideoxy-1,4-iminoerythritol and its quarternization to another group of disaccharide mimics with remarkable structural properties.



C-β-D-Mannopyranosylmethylamine (**1**) [3] and 1,4-di-*O*-(toluene-4-sulfonyl)erythritol (**2**) [4] were the starting compounds in the expeditious synthetic procedure. Alkylation of the starting amine **1** began with the 1,4-di-*O*-tosylated bifunctional reagent **2** and the intermediate 1,4-iminoalditol was further treated with sodium chloroacetate to provide the required betaine epimers **3**. Analogous procedures with other di-*O*-sulfonylated alditols and appropriate *C*-glycosylmethylamines for preparation of a library of *N*-glycosylmethylated iminoalditols and their betaines as potential inhibitors of sugar-processing enzymes are being developed in our laboratory.

Acknwledgments: The work was supported by the APVV-51-046505 and VEGA-2/6129/26 grants.

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THE BINDING PROPERTIES OF THE SIALIC ACID AND ITS ANALOGS IN COMPLEX WITH THE INFLUENZA VIRUS NEURAMINIDASE

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Neuraminidases (NA) encompass a group of exo-glycosyl hydrolases that cleave the terminal sialic acid linked to the host cell's oligosaccharide chain. The function of neuraminidases is crucial for the virus reproduction cycle; therefore they are common targets for therapeutic intervention. With the occurring resistance to the available drugs, the design of novel inhibitors is of great importance. As the catalytic mechanism of the sialic acid hydrolysis remains unclear, the understanding of the mechanism of the resistance development for the common transition-state analogs is not straightforward.

We have performed QM/MM calculations and both MM and QM docking studies of the common neuraminidase inhibitors and natural substrates in several neuraminidases, including the reported resistant mutants. We have analysed and compared the potential surface of the active sites, in aim to understand the different binding preference of the ligands to the neuraminidase mutants.

This work was supported by the Slovak Research and Development Agency – APVT-51-004204.

AMIDATED PECTIN DERIVATIVES: PREPARATION AND CHARACTERISATION

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Four alkylamidated pectin derivatives **1**–**4** were prepared to be used as possible components of composites with new properties. Initial highly methylated (HM) pectin (*DM*=70%) was amidated with *n*-propylamine (**1**), 3-amino-1-propanol (**2**), 1, 3-diaminopropane (**3**), or 1, 7-diaminoheptane (**4**) under anhydrous conditions. Products **1**–**3** were prepared without a solvent; dimethylsulfoxide was used as solvent for preparation of **4**. Obtained derivatives were characterized by FTIR, FT Raman, 2D NMR (soluble in D_2O), TG/DTA and organic elemental analysis. Vibration spectroscopy confirmed amidation of initial HM pectin and purity of the products. The *DM* and *DA* values were determined from FTIR by the multiple Voigt peak decomposition of the carbonyl vibration bands. Obtained values were in agreement with organic elemental analysis. While *N*-propylpectinamide (**1**, *DA*=70.8%) and *N*-(3-oxy)propylpectinamide (**2**, *DA*=70.8%) were well soluble in water (95%), the *N*-(3-amino)propylpectinamide (**3**, *DA*=71.9%) had 46% solubility and *N*-(7-amino)heptylpectinamide (**4**, *DA*=67.3%) only 3%. The TG/DTA data in nitrogen and air environment demonstrated increased residue formation and decreased resistance of the products towards degradation and oxidation in comparison to the initial HM pectin.

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XYLOGLUCAN ENDOTRANSGLYCOSYLASES (XETS) FROM NASTURTIUM SEEDS DO NOT POSSESS HYDROLYTIC ACTIVITY

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Five forms of xyloglucan endotransglycosylase (XET) differing in isoelectric points (6.9, 6.3, 5.5, 4.7 and 4.4) were observed in the protein mixture obtained from nasturtium (*Tropaeolum majus*) seeds. The XETs were purified to apparent SDS-PAGE homogeneity and characterized. All forms were glycoproteins, deglycosylation led to decrease in molecular mass from approximately 29 to 26.5 kDa. A broad pH optimum was observed for all XETs with different level of activity. The enzymes behaved as typical transglycosylases since no xyloglucan- or cellulose- degrading activity was observed in the absence of xyloglucan oligosaccharides in the viscometric assay. Partial sequence determination of major XET form (pI ≈ 6.3) followed by comparison of appropriate sequences with other known XETs showed more similarity with XET enzymes from other plant sources (*Cucumis sativus*, *Hordeum vulgare*, *Oryza sativa* or *Zea mays*) than with NXG1 and XET1 enzymes previously found in nasturtium[1-3].

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POLYMERIC SURFACTANTS PREPARED BY TRANSESTERIFICATION OF TRIACYL GLYCERIDES WITH CARBOXYMETHYLCELLULOSE

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Partially hydrophobized carboxymethylcellulose (CMC) derivatives represent important biodegradable polymeric surfactants [1, 2]. An interesting alternative of the conventional hydrophobization methods, based on the esterification of carbohydrates with acid halides, anhydrides or by transesterification, represents the transesterification reaction of CMC with triacyl glycerides. This contribution deals with partial hydrophobization of CMC (DS = 1) with triacyl glycerides (rape seed oil) in system H_2O/DMF with LaCOOK as catalyst under various reactions conditions. The reaction was carried out analogical as described Aburto et al. [3], but with using microwave irradiation as heating source. The obtained CMC derivatives were characterized by FT-IR spectroscopy. The surface-active (surface tension, critical micelle concentration, emulsifying efficiency), functional properties (washing power and antiredepositive efficiency) and associate properties (capillary viscosimetry measurements, spectroscopy) were estimated. Prepared derivates were water-soluble, showed high emulsifying activity for oil/water type emulsions comparable to that of the commercial surfactant Tween 20 and had excellent washing power and antiredepositive efficiency. The results from associative measurement indicated the existence of macromolecular interactions ascribed to intramolecular associations of the hydrophobic substituents. These CMC derivatives are potential polymeric surfactants, which might be exploited in various technical applications. Application of microwave energy might contribute to a more economic process due to substantial shortening of reaction time.

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NEW HOMOLOGUES OF LYSOSOMAL α-MANNOSIDASES CLONED FROM FRUIT FLY *DROSOPHILA MELANOGASTER*

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The α-mannosidase of lysosomal type belongs to the sequence-based glycoside hydrolase family 38 (GH38). It is involved in the ordered degradation of the asparagine-linked carbohydrates of glycoproteins. Deficiency of this enzyme in human leads to the lysosomal storage disorder – α-mannosidosis. In our study, we applied the method of functional expression of the putative homologues of lysosomal mannosidases from *D. melanogaster* (dmLMs), previously identified by searching the FlayBase by using the human homologue of lysosomal mannosidase and by application of *PFAM* motif of glycosylhydrolase family GH38. These mannosidases, according to their sequence homology, belong to the family of those mannosidases, cloned earlier, with solved 3D structure.
Using RT-PCR amplification, with different gene-specific oligo-primers and mRNA isolated from adult flies *D. melanogaster,* we cloned corresponding DNA fragments into *Pichia pastoris* expression vector pPICZα under the precise control of AOXI induction promoter. After expression in *Pichia pastoris*, homologues from three cloned putative dmLMs showed significant activity using the synthetic pNP- α -mannopyranosid as a substrate. The recombinant dmLMs show activity over the range of pH from 4.0 to 6.0, while the reaction maximum being in the region of pH from 5.0 to 5.2. The reaction temperature dependency presents their optimum in the scale of 25 ˚C to 40 ˚C, with the Km values for pNP-α-mannopyranoside from 2 mM to 4 mM. dmLMs are reversibly inhibited by Swainsonine and Mannostatin A in nanomolar and micromolar range, respectively.

COMBINED CHEMOENZYMATIC SYNTHESIS OF MODIFIED NEO-GLYCOAMINOGLYCANS – WAYS TO NEW GLYCO-DRUGS AND -MATERIALS

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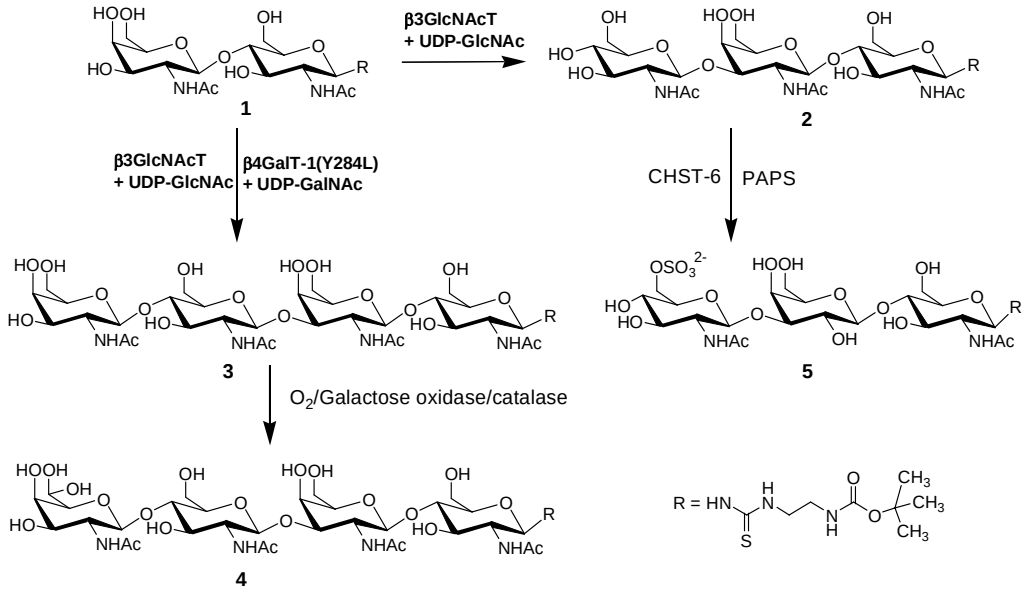
Immunoefficient glycostructures activating natural killer cells (NK cells) by binding to cell surface receptors are preferably composed of *N*-acetyl-D-hexosamines (GalNAc, GlcNAc, ManNAc) and carry additional modifications (COOH, SO_3H) of the terminal GalNAc moieties. Our Preliminary work proposes that the combination of glycosyltransferases with galactose oxidase and sulfotransferases should be an ideal multi-enzyme system to obtain modified neo-glycosaminoglycans. The basis for novel modified neo-glycosaminoglycans will be the LacdiNAc, GalNAc(β1-4)GlcNAc(β1-R (**1**), disaccharide structure synthesized by a UDP-GalNAc specific mutant of human β1,4galactosyltransferase-1 (His_6-propeptide-β4GalT-1 (Y284L) which is fused to the propeptide of the lipase from *S. hyicus* for soluble expression in *E. coli*. The trisaccharide, GlcNAc(β1-3)GalNAc(β1-4)GlcNAc(β1-R (**2**) and di- (**3**) or even multi-linear poly-LacdiNAc structures may result from the combination of His_6-propeptide-β4GalT-1 (Y284L) with β3GlcNAcT from *H. pylori*. Modification reactions are possible by galactose oxidase resulting in (**4**) or by sulfation of terminal GalNAc moieties (**5**) with sulfotransferases, respectively. The oxidized/sulfated neo-glycosamino-glycan structures shall be tested for the activation of NK cells and for the biofunctionalization of nanoparticle and biomaterial surfaces.



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HOW IS CRYPTOCOCCUS CAPSULA REARRAGED DURING BUDDING?

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Cryptococcus laurentii virulence is mediated mainly by polysaccharide capsule which surrounds its cell wall. Glucuronoxylomannan (GXM) makes about 90% of the capsule mass. GXM consist of an unbranched mannose backbone with varying amounts of xylose, glucuronic acid and O-acetyl substitution in various cryptoccocal strains. The two other minor constituents are galactoxylomannan and glucomannanprotein (1, 2).
During budding, the capsule often lost its well-rounded shape in the area where budding occurred, and the transit of a new cell through the capsule of the mother cell was usually accompanied by an invagination of the capsule surface at the site of budding (3). It suggested that budding and separation of the bud from mother cell required capsular degradation and/or reorganization. In 1960 Gadebusch (4) reported the discovery of an bacterial enzyme, that degraded capsular polysaccharide of Cryptococcus neoformans. Given the number of glycosidic bounds in GXM, it is most likely that the enzyme preparation was crude mixture of enzymes. Last studies show, it could be a complex of at least four enzymes work together: GXM-O-acetylhydrolase, β-xylosidase, α-manosidase and β-glucuronidase (http://www.freepatentsonline.com/6146868.html).Although enzymes from soil microbes can degrade capsules, no cryptococcal or human enzymes with this activity have been found. In our laboratory we identified in the yeast Cryptococcus laurentii, O-acetylhydrolase and β-xylosidase activity with kinetically very similar reactions. It is possible, that these enzymes are involved in capsule rearrangement during budding. A fuller understanding of this process will require the parallel application of genetic, biochemical and physico-chemical techniques.

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