Plenárne prednášky

SUGAR ANALOGS WITH NITROGEN IN THE RING: NEW SYNTHETIC APPROACHES, NOVEL BIOLOGICAL APPLICATIONS

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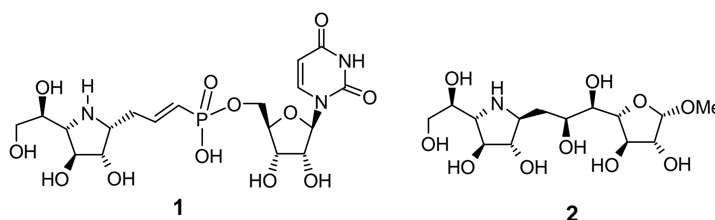
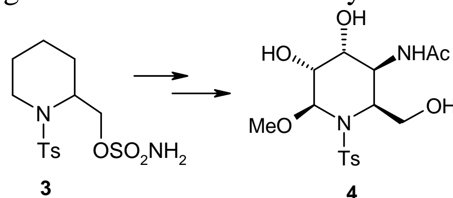
Sugar analogs with nitrogen in the ring, generally known as iminosugars (1), are gaining increasing importance as therapeutic agents for a diversity of diseases: two derivatives, namely *N*-butyl 1-deoxynojirimycin (NB-DNJ) and *N*-(2-hydroxyethyl)-DNJ have already been commercialized, for the treatment of Gaucher disease and of complications associated with type-2 diabetes, respectively, and several others are under investigations. The lecture will first provide an overview of these applications, showing for example that the strongest enzyme inhibitors designed to date belong to the class of iminosugars (2), and that iminosugars could act as chemical chaperones to rescue mutant proteins, thus providing an approach to the pharmacological treatment of certain genetic disorders (3).
In the second part of the lecture, recent synthetic methodologies to iminosugars and derivatives will be outlined. Traditional approaches starting from sugars will be illustrated by the synthesis of a series of novel pyrrolidine-based furanose sugar analogs such as UDP-Gal*f* mimics **1** (4), Gal*f*-disaccharide mimics such as **2** and related compounds, all from D-glucose. Such compounds are of interest as potential inhibitors of the enzymes involved in the biosynthesis of mycobacterial cell-wall galactans.



A novel and innovative strategy to piperidine-iminosugar derivatives will then be presented: by taking advantage of the intramolecular Rh(II)-catalyzed amination of saturated C-H bonds (the Breslow-Du Bois reaction (5)), the piperidine-2-methanol derivative **3** can serve as the precursor of a diversity of poly-functionalized piperidines derivatives including iminosugars. This process is remarkable in that it does not require preliminary activation of the different positions in the piperidine ring and therefore is extremely atom-efficient.



In this sequence, the sulfamoyloxymethyl group is directly or indirectly involved in the functionalization of every saturated methylene group of the piperidine ring at C-3, C-4, C-5 and C-6 and thus acts as a directing and activating arm. Application to the total synthesis of iminosugars such as **4** will serve to demonstrate the synthetic potential of this strategy.

Acknowledgement: I wish to acknowledge the outstanding work of two graduate students who accomplished most of the recent work from our research group described in the lecture, namely Sylvestre Toumieux and Virginie Liautard, in collaboration with Drs. P. Compain and V. Desvergnes, CNRS Research Fellows.

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STRUCTURES OF GLYCOSIDASES IN HUMAN HEALTH AND DISEASE

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Glycosidases, largely from microorganisms and plants, have been studied for many years. We have developed an interest in mammalian glycosidase that are associated with human physiological and disease processes. In this talk, our results from two such structure/function analyses will be presented.

1. **Golgi α-mannosidase II (GMII)**

The GH38 GMII is the final glycosidase in the maturation of N-glyans on glycoproteins. Inhibition of GMII by the natural product swainsonine in clinical trials led to evidence of tumour shrinkage and improved treatment response in advanced cancer patients. However, due, among other issues, to side-effects associated with cross-inhibition of other class II mannosidases and lack of mechanistic followup, this target was never developed to its full potential.

Several years ago, we exploited the technology for stable overexpression of complex, eukaryotic proteins in *Drosophila* S2 cells to produce the *Drosophila* GMII homologue in large amounts. Subsequent high resolution studies of the dGMII structure in complex with inhibitors and catalytic intermediates have culminated in a detailed model for the functioning of the enzyme.

In our most recent results, we describe the atomic characteristics of three major subsites in the enzyme. The first, the catalytic site, is the site of the hydrolytic cleavage, and we have several high affinity compounds from previous work. This site is the most highly conserved in the related enzymes and, therefore, the compounds are not specific for GMII. A second, holding site interacts with a second scissile mannose residue. We have rationalized the sequence of cleavage reactions based on the nature of this site and its restrictive size. The third, anchor site binds tightly to an N-acetyl-glucosamine residue, the presence of which is essential to the function of GMII. We have presented ideas on how the occupancy of this site affects the activity in the catalytic site some distance away.

2. Intestinal Maltase-Glucoamylase (MGAM)

We have determined the structure of the first GH31 domain of MGAM, a major player in post-amylase starch digestion in the intestine. The results have provided the first glimpse into the mechanism of action, substrate specificity, and inhibition properties of this enzyme. These insights are relevant to understanding the complementary roles of the two GH31 subunits of MGAM and those of its partner enzyme, sucrase isomaltase (SI). Together these enzymes are responsible for the release of nutritional glucose from various starch structures under various physiological conditions. Modifiers of their activity are potential interventions for obesity and associated conditions, such as cardiovascular disease, Type II Diabetes and cancer.

HOW IS A WORM MORE COMPLEX THAN A FLY: COMPARATIVE N-GLYCOMICS AND ANTI-CARBOHYDRATE ANTIBODY CROSS-REACTIVITY

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In recent years major advances have been made as regards our genomic knowledge about a variety of species, whereas what is known about the glycomics and glycobiology of even model organisms is far from complete. However, screening of the N-glycans of the worm (Caenorhabditis elegans) and the fly (Drosophila melanogaster) in combination with molecular biological studies has yielded information as to the glycogenomic potential of these organisms and enabled some degree of a 'glycophylogeny' to be established. In contrast to the relative anatomical simplicity of the worm in comparison to the fly, the N-glycans of the former are more complex. This complexity is often even exacerbated in glycomutants, but demonstrates the flexibility of the worm as regards glycosylation and may offer some insights into the evolution of also parasitic species, with which the worm shares carbohydrate epitopes.

MECHANISMS AND APPLICATIONS OF XYLOGLUCAN-ACTIVE ENZYMES

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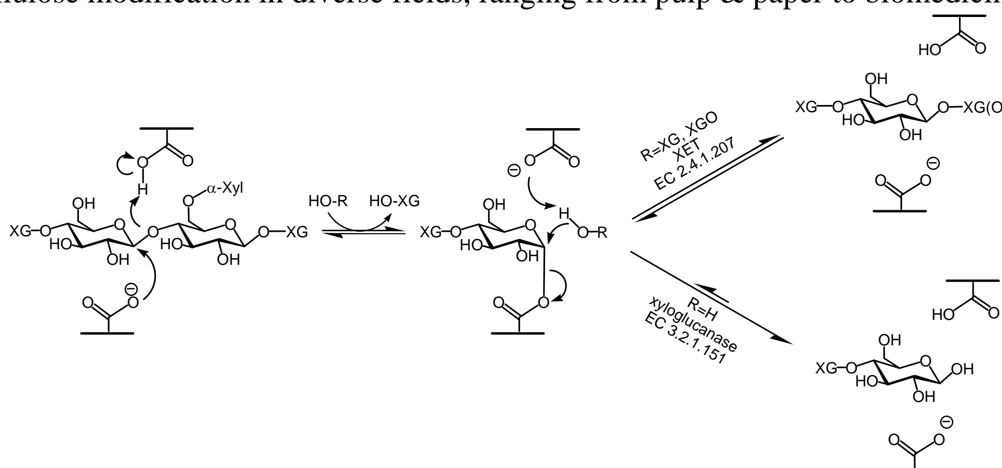
The xyloglucans (XG) comprise an important family of polysaccharides with diverse structural and energy-storage roles in many land plants. On one hand, (fucogalacto)xyloglucans are predominant cell wall matrix polysaccharides, which are believed to crosslink paracrystalline cellulose microfibrils by surface adsorption and chain intercalation, thereby modulating wall mechanical properties. In contrast, (galacto)xyloglucans are the principal seed polysaccharides in a number of plants, which are mobilised during germination. Plants have therefore evolved a key group of enzymes to restructure and degrade xyloglucans after biosynthesis and deposition: the xyloglucan *endo*-transglycosylases (XET, 2.4.1.207) and xyloglucan *endo*-hydrolases (XEH, EC 3.2.1.151).

Together, these enzymes form a distinct subfamily of glycoside hydrolase family 16 (GH16), which employ the canonical "retaining" mechanism to affect glycosyl transfer to either water (XEHs) or xyloglucan saccharide (XET) acceptors (Figure). In continuing studies of the diversity of plant xyloglucan-active enzymes from GH16, we have combined detailed kinetic analysis, molecular phylogeny and enzyme tertiary structure determination to highlight key features that dictate the fate of the glycosyl-enzyme intermediate (1). A firm understanding of the reaction mechanism is, in turn, guiding the effective application of these enzymes for *ex vivo* cellulose modification in diverse fields, ranging from pulp & paper to biomedicine (2,3).



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CARBOHYDRATE ESTERASES – EMERGENCE OF NEW BIOCATALYSTS

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Several plant cell wall polysaccharides are partially acetylated or esterified with cinnamic (phenolic) acids. As shown on examples of chemically synthesized model glycosides, the acyl groups protect sugar residues against hydrolysis by the corresponding glycoside hydrolases. The acetylation of polysaccharides also hinders their digestion in animals and also their hydrolysis by glycanases as the first step of their bioconversion. Enzymatic hydrolysis of such polysaccharides becomes therefore much easier after alkaline pretreatment of biomass, which loosens the plant tissue structure also by destroying majority of ester linkages. In 1985 we first reported on existence of microbial enzymes, which deacetylate hardwood *O*-acetyl-D-glucuronoxylan, called acetylxylan esterases (AcXEs) [1]. Since then other types of carbohydrate esterases (CEs) have been discovered, including feruloyl esterases (FeEs) [2]. At present, these two types of CEs represent the most diverse microbial enzymes involved in plant cell wall degradation. For instance, AcXEs, classified on the basis of primary and and tertiary structure similarity, can be found in 7 of 15 families of CEs (CAZY, [3]). Most of the families represent serine-type esterases, and there is also a family of metallo-enzymes [4]. However, the structure-function relationship among these esterases has not been properly established, therefore, the reason for their great variety still remains unclear. FeEs remain still unclassified despite reported differences in their substrate specificities.

We started to differentiate AcXEs on the basis of the deacetylation mode of acetylated derivatives of methyl β-D-xylopyranoside (Me-Xyl*p*). The enzymes were found capable of deacetylating both positions 2 and 3 [5]. Activity of some enzymes, especially those from CE family 4, was dependent on the presence of free vicinal OH group at position 3 and 2. It became clear that the deacetylation of Me-Xyl*p* at both positions 2 and 3 were due to the formation of two types of productive complexes of enzymes with acetylated Me-Xyl*p*s (2,4-di-*O*-acetyl and 3,4-di-*O*-acetyl Me-Xyl*p* bound in a reversed orientation) [5]. In contrast to these observations, all purified AcXEs tested on monoacetyls of 4-nitrophenyl β-D-xylopyranoside [6] in a β-xylosidase-coupled assay [7] showed high specificity for deacetylation of position 2. This positional specificity is complementary to that of non-hemicellulolytic esterases such as wheat germ lipase, orange peel acetylesterase and *Candida* lipases, which exhibited a preference for deacetylation of position 4. A number of crude cellulolytic and hemicellulolytic systems also exhibited considerable activity towards 3- and 4-monoacetates of NPh-Xyl. These results were due to the presence of another type of hemicellulolytic esterases, which operate on acetylated oligosaccharides but not on polymeric substrate [8]. These acetyl esterases form a new CE family (unpublished data). They show a unique ability of regioselective acetylation of saccharides in aqueous medium saturated with vinyl acetate as the acetyl group donor [9,10]. AcXEs of family 1 catalyzed reverse reaction in a microemulsion system in hexane [11], while FeEs in organic solvents [12] and detergent-less microemulsions [13]. Despite this achievement, the synthetic potential of majority of CEs remains still unknown and requires further investigations. However, the most important application of CEs should be envisaged in enzymatic hydrolysis of plant residues as the basis for an environmentally friendly process of lignocellulose bioconversion. Our goal should be the use of CEs to replace the alkaline pre-treatment of plant biomass.

Another task in front of us is to find more about biotechnological and synthetic potential of recently discovered microbial carbohydrate esterase, called glucuronoyl esterase

[14]. The function of this enzyme might be hydrolysis of ester linkages between 4-O-methyl-D-glucuronic acid residues of glucuronoxylans and lignin alcohols [15]. Phylogenetic tree constructed on the basis of amino acid sequences of various CEs confirmed that glucuronoyl esterases is also a distinct type of plant cell wall degrading enzyme.

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TARGETING THE MOLECULAR DIVERSITY OF HEPARIN AND HEPARAN SULFATE BY CHEMICAL SYNTHESIS

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Heparin and heparan sulfate are sulfated polysaccharides belonging to the glycosaminoglycans. Both heparin and heparan sulfate show a great deal of structural heterogeneity due to structural variations in their carbohydrate backbone, and mainly, as a consequence of their highly varied sulfation pattern. Heparin, well-known as a blood anticoagulant, expresses a series of additional biological activities, such as antitumor, antiviral, anti-inflammatory, and antiasthmatic effects. The diverse biological activities of heparin are the results of its interactions with various proteins; heparin-protein interactions modulate a series of important physiological processes. More than a hundred heparin-binding proteins have been identified so far (1). Understanding heparin-protein interactions at the molecular level is of critical importance both from the scientific aspect and also for the potential pharmaceutical implications.

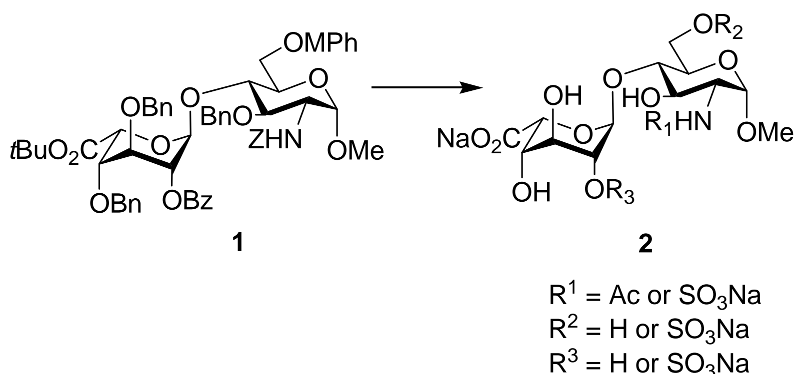
In most cases, relatively small oligosaccharide units of heparin, and not the whole polysaccharide, are accountable for heparin-protein interactions. It is commonly assumed that individual proteins recognize different oligosaccharide epitopes within the heparin chain in specific interactions.

The identification of oligosaccharide ligands of heparin-binding proteins could, in principle, be easily accomplished by testing the proteins against a heparin oligosaccharide library. Well-defined, homogeneous heparin oligosaccharides are, however, available only by extreme difficulties by the chemical degradation of the natural polysaccharide. Pure heparin oligosaccharides can be accessed by chemical syntheses, but current syntheses are lengthy, laborious, multistep processes which provide only one oligosaccharide from each synthesis and therefore are not suitable for oligosaccharide library generation.

We have developed a new synthesis strategy by which a multitude of heparin oligosaccharides can be generated from a small number of common intermediates (2).

Our strategy is based on orthogonal protection: oligosaccharides are synthesized in such protected forms, in which positions of possible sulfation are protected by orthogonal protecting groups. Selective removal of any of these protecting groups – or a combination thereof – followed by *O*-sulfation, total deprotection and final *N*-substitution affords all the possible sulfoforms of the oligosaccharide backbone.

In this way, for example, eight different target compounds (**2**) were easily generated from a single orthogonally protected disaccharide (**1**).



The strategy was extended for the synthesis of higher oligosaccharides. Thus, all possible tetrasaccharide units (576 different tetrasaccharides) of heparin can be simply accessed from only four protected intermediates.

The synthesis of azasugar analogs of heparin oligosaccharides, designed to inhibit the enzyme heparanase, will also be discussed.

In relation to the above syntheses, new methodologies – including the introduction of new protecting groups, new protecting group transformations, and a new glycosylation method – have also been developed and will be highlighted.

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