Pozvané prednášky

RESEARCH ON CANCER FINDING AND CHEMOTHERAPY AT THE EARLY STAGE

SYNTHESES AND EVALUATIONS OF NOVEL SUGAR-BALL-DENDRIMER MRI CONTRAST AGENTS AND PHOSPHA SUGARS BY SUGAR MODIFICATION

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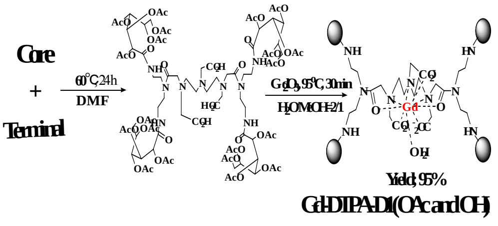
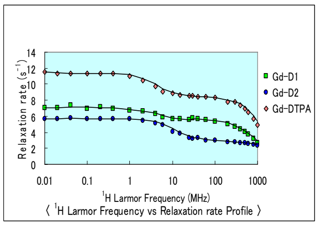
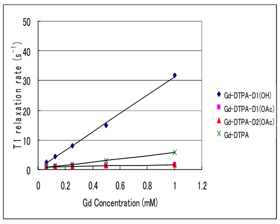
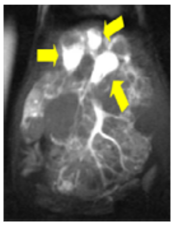
This paper deals with the preparation and evaluation of novel dendritic MRI contrast agents provided by sugar moieties on the outer shell of the sugar dendrimer with the core part of Gd-DTPA (Gadrinium-Di-ethylenetriaminepentaacetic acid) complexes, Gd-DTPA-D1-Glc-(OH) as well as phospha sugars prepared from phosphorus heterocycles. The *in vivo* evaluation of Gd-DTPA-D1-Glc(OH) by using rats revealed that the sugar modified dendritic Gd complex described clear MR images of blood vessels and tumors.

Gd-DTPA derivatives are widely used for MRI contrast agent, however, the agent has poor potentiality for imaging specific organs, blood vessels, and tumors. To improve these properties of Gd-DTPA, sugar-ball-dendrimer derivatives of Gd-DTPA (e.g., Gd-DTPA-D1-Glc(OAc), Gd-DTPA-D1-Glc(OH), Gd-DTPA-D2-Glc(OAc)), were prepared (Scheme 1) [1]. The T1 relaxation rates of Gd-DTPA-D1-Glc(OH) vs Larmor flequency (Fig. 1) and vs Gd concentration (Fig. 2) showed good results [2]. The *in vivo* evaluation of Gd-DTPA-D1-Glc(OH) showed an excellent MR imaging of tumors on the liver of rats (Fig. 3). The preparation and evaluation of phospha sugars for leukemia cells will also be presented so as to use the phospha sugars as the anti-cancer agents.









Scheme 1. Gd-DTPA-D1-Glc(OAc). Fig. 1. ^{1}H Larmor frequency. Fig. 2. T1 relaxation. Fig. 3. MRI of tumors.

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STUDY OF THE COMPETITIVE FIVE- VERSUS SIX-MEMBERED RING CLOSURE ADDITION REACTIONS USING A CASCADE REACTION SEQUENCE

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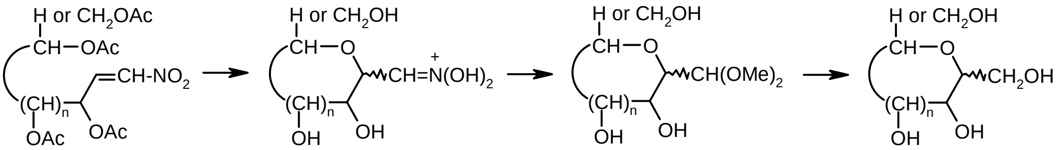
A strong preference of the five-membered ring closure over six-membered ring closure occurring in cyclization reactions of acyclic species during their irreversible or kinetically controlled reversible stages has been a rather anticipated than exactly studied phenomenon in carbohydrate chemistry. Formation of alkyl furanosides by mercury(II)-catalyzed alcoholysis of the corresponding aldose dialkyl dithioacetals [1] is a typical striking case known for a long time. Another example is recently developed method for regiospecific preparation of methyl glycofuranosides from acyclic 1-deoxy-1-nitroalditols using strong acid catalyzed methanolysis of their respective nitronates at –30 ºC [2]. This high preference for the five-membered ring closure over six-membered ring closure is in a good agreement with a two order difference in the relative rate constants for closing five- and six-membered lactone rings from the respective 4-bromobutanoate and 5-bromo-pentanoate [3]. Our recent finding of strong acid catalyzed methanolysis of 1,2-dideoxy-1-nitro-L-*arabino*-hex-1-enitol peracetate to L-arabinofuranosylmethanal dimethyl acetals [4] has offered a very convenient, one molecule model for a quantitative assessment of both the competitive ring closure addition reactions, also with respect to the stereochemical configuration. The methodology presented involves a cascade reaction sequence of the competitive nucleophilic additions of the in HCl/MeOH in situ generated C-5-OH or C-6-OH groups of **1** to its nitroalkene double bond and subsequent capture of thus generated nitronic acids by protonization to provide intermediates **2**, which spontaneously undergo the Nef methanolysis to ring-stable products **3**. These can be then, after simplification of their structures to compounds **4**, easily separated and quantified.



1 **2** **3** **4** n = 1 or 2

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IS NATURAL PRODUCT MIMICRY AN EFFECTIVE STRATEGY FOR THE DESIGN OF INHIBITORS OF OLIGOSACCHARIDE-PROCESSING ENZYMES ?

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Natural products often provide the first clues as to which types of molecule might inhibit a given enzymatic activity. Unfortunately, the activities of such natural products are not always ideal and have proven difficult to improve with respect to features such as potency and selectivity simply by chemically introducing simple structural variations of the parent skeleton.

We have been interested in developing rapid syntheses' of function-orientated molecules with a view to perturb the activities of various oligosaccharide-processing enzymes.[1-5] Oligosaccharides are implicated in a plethora of biological processes and the inhibition of enzymes implicated in their biosynthesis has been identified as a therapeutic strategy for several diseases such as cancer, diabetes, etc). The function-orientated approach has allowed us to uncover a number of synthetic inhibitors that bear little obvious structural resemblance to any natural product counterparts. What is more, the totally synthetic inhibitors sometimes show improved selectivities and potencies for their target enzymes compared with those displayed by the natural compounds.

We present in this paper the design and synthesis of a number of synthetic compounds effective as glycosyl hydrolase inhibitors. The potency and selectivity of the compounds is rationalized in terms of the proposed mechanisms of action of the target enzymes and this is supported up by X-ray crystallographic, computational and thermodynamic studies.

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HYDROPHOBIC VS. HYDROPHILIC FORCES IN AN ANALOG OF CELLOTETRAOSE: CYCLOHEXYL 4'-*O*-CYCLOHEXYL β-D-CELLOBIOSIDE

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In carbohydrate structures, especially cellulose and its fragments, hydrogen bonding is thought to be important, but non-polar interactions are not so well understood. To better understand their roles, a molecule that has two glucosyl rings and two non-polar cyclohexyl rings was targeted for synthesis. Cyclohexyl 4'-*O*-cyclohexyl β-D-cellobioside **(1)** is otherwise similar to cellotetraose. Attempts to attach the 4'-*O*-cyclohexyl group failed until making the 4'-*O*, 6'-*O*-cyclohexylidene ketal. After peracetylation, the cyclohexylidene ketal ring was opened regioselectively, providing 65% of **1** after final deacetylation.

The crystal structure of **1** exhibited substantial effects from the non-polar groups. Three conformationally unique molecules (A, B and C) are in the triclinic unit cell, along with two solvent cyclohexanes. While cellotetraose has antiparallel (up-down) packing, A and B in **1** are oriented "down" in the unit cell while C is "up." "Down-down-up" packing is rare for carbohydrates, found only for three-fold helices (or double helices) that pack in trigonal unit cells. When viewed down the crystal's ***a***-axis, the array of C, A, and B looks like the letter N, with A inclined so that its cyclohexyl groups can stack with those of the reducing ends of the B and C molecules. The lower left and upper right points of the N are stacks of cyclohexyl rings on the non-reducing ends of B and C, interspersed with solvent cyclohexanes. Other unusual details include the finding of O6 in all three staggered orientations: one is *tg,* two are *gg* and three are *gt*. The *tg* O6 allows an intramolecular hydrogen bond to O2', as in native cellulose Iβ, and a similar but novel O6B···O2'B hydrogen bond is based on a slightly distorted *gg* orientation. The hydrogen bonds between parallel molecules are unique, with linkages between O2'A and O2'B, O3'A and O3'B, and O6A and O6B. C-H···O hydrogen bonds are extensive between A and C, as well as between B and C, along the [1-10] line of quarter-staggering. These unusual features expand the range of structural motifs to be considered for as-yet undetermined cellulose structures.

XYLOGLUCAN ENDO-TRANSGLYCOSYLASES FROM BARLEY: REACTION MECHANISM AND MOLECULAR STRUCTURE

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Molecular interactions between wall polysaccharides, which include cellulose, xyloglucans and (1,3;1,4)-β-d-glucans, are fundamental to cell wall properties in cereal plants (1). These interactions have been assumed to be non-covalent in nature. Experiments with near-homogenous barley xyloglucan endo-transglycosylase HvXET5 show formation of covalent linkages between tamarind xyloglucan and celluloses, and between xyloglucan and (1,3;1,4)-β-d-glucan, at least *in vitro*. The rate of covalent bond formation with hydroxyethylcellulose is comparable to that on xyloglucan, while that with (1,3;1,4)-β-d-glucan is slower (2). If the plant GH16 XET family catalyses these cross-linking reactions *in vivo,* then this will change quite dramatically, the way in which we view cell wall structure, remodelling and function in higher plants.

Relay experiments with a range of xyloglucans and xyloglucosides showed that HvXETs recognize xyloglucan polysaccharides as acceptors, and that distributions of molecular masses of xyloglucan molecules can be manipulated. Experiments in aqueous inorganic solvent mixtures indicated that HvXETs tolerate surprisingly well these milieux, compared to typical hydrolytic enzymes. A detailed kinetic analysis of HvXETs revealed that these enzymes adopt a ping-pong (Bi Bi) rather than sequential reaction mechanism (3). A potential biological significance of these findings is in the capacity of HvXETs to fine-graft and remodel xyloglucans *in situ*.

A combination of EST database searches and molecular cloning showed that the barley GH16 xyloglucan transferase gene family consists of about 28-30 members. Three-dimensional molecular models of several non-hydrolytic HvXETs in complex with donor and acceptor substrates, and comparisons with the crystal structure of *Tropaeolum* hydrolytic xyloglucan transferase (4) disclosed variable dispositions of loops in the vicinity of their active sites (3). A detailed understanding of hydrolytic *versus* transferolytic reaction mechanisms of these enzymes remains elusive.

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COMPARISON OF THREE *ASPERGILLUS NIDULANS* ENDO-BETA-MANNANASES

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Aspergillus nidulans endo-beta-1,4-mannananases AnMan5A (AN3358.2), AnMan5B (AN3297.2), AnMan5C (AN6427.2) of glycoside hydrolase family 5 were expressed in *Pichia pastoris*, affinity purified using His-tag and characterized. The protein sequence identities between these three enzymes are pairwise 32 – 39 %. Judged from the sequences the three *A. nidulans* mannanases appear to consist of sole catalytic modules and do not carry carbohydrate binding modules. The pH and temperature optima for AnMan5A were previously reported to be 5,5 and 52°C, respectively (1). We determined the corresponding values for the two other mannanases to be 6.0 and 60°C (AnMan5B) and 5.0 and 55°C (AnMan5C). Thus, in terms of temperature and pH preference, there are no dramatic differences between these three enzymes. However, the specific activity on locust bean gum varied. It was 2220, 370 and 5060 nkat/mg protein for AnMan5A, AnMan5B and AnMan5C, respectively. All three mannanases were also active on guar gum, konjac glucomannan, ivory nut mannanan and manno-oligosaccharides. The end products of ivory nut mannan and mannooligosaccharides (M_3-M_6) hydrolysis were mannobiose and mannotriose for AnMan5A, mannobiose for AnMan5B and mannose and mannobiose for AnMan5C. The end products reported for *Aspergillus niger* and *Trichoderma reesei* mannanases are mannobiose and mannotriose (2,3). It seems that AnMan5A is similar to these enzymes while AnMan5B and AnMan5C seem not to accumulate mannotriose but rather cleave it rapidly. The results indicate differences in substrate binding within the active sites of the three enzymes.

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SYNTHESIS OF DEOXYRIBOFURANOSYL-SPACER-*C*-NUCLEOSIDES

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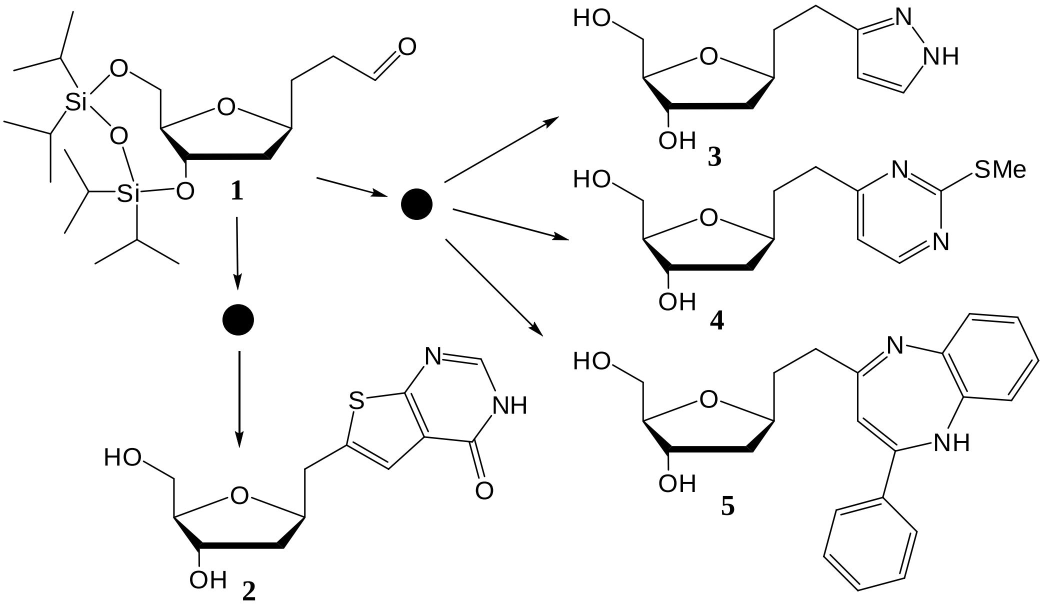
In order to synthesize new spacer-*C*-nucleoside analogues with potential biological activity two methods were used. First, the tetraisopropyldisiloxan-propanal **1** was treated with 2-cyanoacetamide in the presence of aluminium oxide to yield a pentene acid intermediate. Cyclization with sulphur and triethylamine was performed to provide a thiophene derivative which was then treated with triethyl orthoformate to furnish the thienopyrimidine nucleoside **2**.



The second pathway started from propanal **1**, again**.** The reaction with ethynylmagnesium bromide or lithium phenylacetylide in THF, followed by oxidation afforded pentinone intermediates. Treatment of these compounds with hydrazine, S-methylthiouronium sulfate and *o*-phenylendiamine furnished the corresponding pyrazole, pyrimidine or benzodiazepine derivatives in good to excellent yields. Deprotection was performed by using tetrabutylammonium fluoride to provide compounds **3**, **4** and **5** suitable for biological and pharmacological investigations.

CARBOHYDRATE METABOLISM IN BIFIDOBACTERIA; HUMAN SYMBIOTIC BACTERIA

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Bifidobacterium ssp. constitute up to 90% of microbial gut flora in the infant colon, but considerably less in adults. Carbohydrate metabolism in these bacteria is highly unusual. Data from four *Bifidobacterium* genomes indicates genes missing from glycolysis, gluconeogenesis, and the TCA cycle, including that for the key regulatory enzyme phosphofructokinase (PFK). A unique metabolic pathway, the "bifid shunt", is involved in a phospholytic cleavage of fructose-6-phosphate to erythrose-4-phosphate and acetyl phosphate, catalyzed by the enzyme fructose-6 phosphate phosphoketolase (F6PPK). Isotopic labeling experiments with ^{13}C-fructose and several other labeled sugars indicate that the bifid shunt is the predominant pathway for the degradation of carbohydrates by *Bifidobacterium* ssp., by-passing glycolysis. The fractional distribution of ^{13}C into carbohydrate and fatty acid metabolites was analyzed by GC-EI-MS. 2-Carbon units derived the bifid shunt are both secreted and incorporated into the *Bifidobacterium de novo* sugars and lipids. Moreover, several metabolic "chase" experiments have been undertaken using probiotic oligosaccharides, sialic acid, and human milk oligosaccharides, and differential pathways are reported for *B. longum* and *B. infantis* strains. These findings are consistent with the genome data for these strains, and suggest ways by which *B. infantis* has adapted to the environment of the infant human gut.