UNIVERSIDAD DE LA RIOJA

Posters

May 28-30, 2014

XI JORNADAS DE CARBOHIDRATOS

Posters

Logroño, La Rioja, Spain

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XI CARBOHYDRATE SYMPOSIUM

Glycan epitope presentation to lectins by NMR and molecular modeling

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Lectins are carbohydrate binding proteins that through the specific recognition of endogeneous glycans play important roles in a wide diversity of key biological events.[1] Lectins are usually classified according to their specificity toward mono or disaccharide epitopes.[2] However these glycan epitopes are differentially presented to their receptors as part of larger oligosaccharides.

Herein we have studied the recognition at a molecular level of biologically relevant glycan structures by different lectins. By using a combinaton of different NMR experiments, both from the ligand and from the protein points of view, and molecular modeling approaches we have been able to propose the corresponding binding modes, in which not only the presence of a specific epitope is important, but also its presentation is determinant.[3]

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XI JORNADAS DE CARBOHIDRATOS

Lanthanide-chelating carbohydrate conjugates as tools for structural studies of sugars and their recognition by receptors

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Paramagnetism offers a rich source of long-range structural restraints through the induction of residual dipolar couplings, paramagnetic relaxation enhancement (PRE) and pseudocontact shifts (PCS), which have been thoroughly employed for structural characterization of biological molecules.[1,2] Previously, we reported the synthesis of the first lanthanide-chelating linker attached to a sugar molecule,[3] which allowed us successfully measuring PCSs in the carbohydrate moiety. The use of paramagnetic restraints emerges as a very convenient approach for the structural elucidation of carbohydrate molecules, as there are often problems to obtain structural information on these systems by NOEs, due to signal overlapping, strong coupling, and/or the scarcity of key NOE information.

Importantly, we show that the so-produced paramagnetic sugars can also be used as tools for the study of structural aspects of their recognition by macromolecular receptors. In particular, we studied the recognition of paramagnetic mono- and disaccharides by a prototype lectin, i.e. the CRD of human galectin-3 (gal-3), demonstrating that paramagnetic effects can be transferred through the space to the protein from the non-covalently bound ligand, as shown in ^{1}H-^{15}N HSQC spectra.

Also, for those instances where recombinant production or isotopic labeling are not possible (e.g., proteins isolated from natural sources), we conceived a strategy suitable to detect binding events, involving the tagging of the protein by a fluorine-containing probe and the monitoring of paramagnetic perturbations, exerted by ligands, of the protein fluorine signals in 1D ^{19}F-NMR spectra.

Finally, we present additional studies on the molecular recognition of systems involving non-protein receptors, such as cyclodextrins. We show that paramagnetic tagging of hydrophobic guests of β-cyclodextrin increases the sensitivity of the characterization of their binding by NMR methods, and permits the extraction of topological information.

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XI CARBOHYDRATE SYMPOSIUM

Methyl orthoesters as glycosyl donors. Acid-washed molecular sieves (AW-MS) mediated glycosylations

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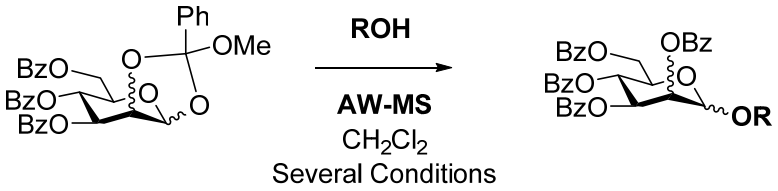
We have recently introduced the use of methyl orthoesters (MeOEs), e.g. **1**, as a donor in glycosylation protocols.[1] We have also found that this process is highly dependent on the choice of the acid promoter. Thus, when BF_3OEt_2 is used as the promoter the glycosylation takes place in moderate to good yields, whereas the use of different Lewis Acids ($Yb(OTf)_3$, TMSOTf, TsOH) led to poorer yields of the desired disaccharides.

In this context, we have been interested in the development of an, operationally simple, experimental procedure for glycosylation that could allow the easy glycosyl coupling of natural products. On the other hand, commercially available 4A acid-washed molecular sieves (AW-MS) has been used to facilitate the rearrangement of orthoester by-products to their corresponding glycosides,[2] and more recently, Castillon and co-workers have reported on the use of AW-MS in the rearrangement of orthoester by-products leading to β-glycosphingolipids.[3]

We have evaluated the use of AW-MS as a heterogenous promotor in the glycosylation of different alcohols and polyols with methyl *manno*- and *gluco*-orthobenzoates. The glycosylation takes place to give good yields of saccharides, and good regioselectivity is observed when diols, or polyols are used as acceptors.



ROH : monosaccharides, natural products

Acknowledgements: The authors are grateful to the *Ministerio de Economía y Competitividad* and *Comunidad de Madrid* for grants CTQ2009-10343, CTQ2012-32114, and grant S2009/PPQ-1752, respectively.

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Synthesis of enaminone sugar/ferrocene derivatives

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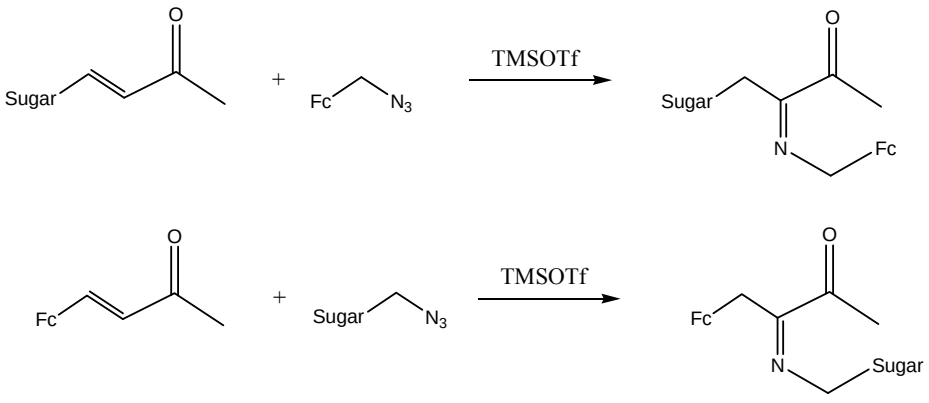
Water-soluble ferrocenes are particularly of interest as their reversible and tunable redox properties could have biological applications, for example, in the development of biosensors.[1]

The ferrocene attached carbohydrates are used as hematinic and anticancer agents in clinical therapy. This renders the ferrocene tethered C-glycosyl heterocycles more attractive in both chemistry and biological chemistry.[2] Enaminones, enamines of β-dicarbonyl compounds are compounds that demonstrated a potential as multipurpose synthetic intermediates in organic synthesis, in pharmaceutical development, and in heterocyclic synthesis.[3]

In this work we present the reaction of sugar derivatives with α,β-unsaturated ketone group, with ferrocenyl azides and the reaction of ferrocenyl derivatives with α,β-unsaturated ketone group with sugar azides (scheme 1).



Sugar = monosaccharide residue
Fc = ferrocenyl

Scheme 1

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XI CARBOHYDRATE SYMPOSIUM

Synthesis of fluorinated analogues of KRN7000

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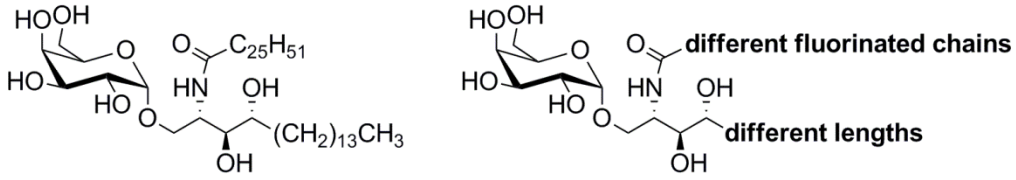
Glycosphingolipids are important biomolecules commonly found in eukaryotic cell membranes. They play a critical role in cell communication, growth, differentiation and programmed cell death and have also shown promising activities against diverse pathologies.[1] For example, the complex formed by the association of α-glycosphingolipid KRN7000 and CD1d proteins interacts with a component of the immune system, the Natural Killer T (NKT) cells, and upon its activation, NKT cells release cytokines, which are signaling molecules involved in cellular communication and immune response.[2] Several KRN7000 analogues have been synthesized featuring modifications in both the sugar and the lipid moieties, all of which with the aim of developing new structures for clarifying and exploring their biological role and therapeutic potential. Particularly relevant examples are those incorporating fluorine moieties in their structure, which are known to confer some interesting properties such as higher metabolic stability, binding and lipophilicity and membrane permeability.[3] Although several fluorinated KRN7000 analogues at the carbohydrate moiety have been synthesized as well as those with partial fluorination of the lipid portion, the preparation of derivatives with fully or partially perfluorinated acyl chains able to modulate the lipid-receptor interaction is unprecedented. Here we describe our progress on the development of a diversity-oriented synthesis approach to perfluorinated analogues of KRN7000 at the ceramide moiety to gain insight into the underlying mechanisms of glycolipid-protein interactions.



α-GalCer (KRN 7000)

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XI JORNADAS DE CARBOHIDRATOS

Chemical interrogation of drug/RNA complexes: from chemical reactivity to drug design[1]

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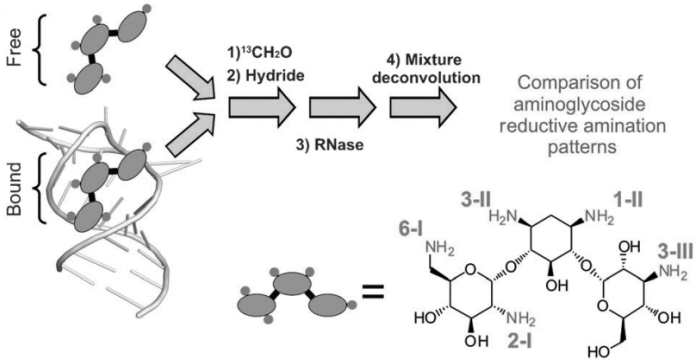
Medicinal chemistry efforts oriented to the optimization of bioactive compounds have traditionally relied on the synthesis and evaluation of a large number of structurally related chemical derivatives. This procedure, in most cases expensive and time consuming, represents a major challenge for complex molecular architectures containing numerous equivalent reactive positions. Unfortunately, such feature is non unusual among natural ligands as carbohydrates or polyamine RNA binders, as aminoglycosides (a family of antibiotic RNA-binding oligosaccharides).



Inspired by the principles of dynamic combinatorial chemistry,[2] we hypothesized that the chemical reactivity exhibited by the aminoglycosides in complexes with their target RNAs could provide valuable guidelines for accessing to a reduced number of binders. Thus, the synthesis and evaluation process of the potential drug derivatives would be highly facilitated. As a proof of principle, we have analyzed kanamycin-B methylation (a chemical modification of general importance for the molecular recognition of nucleic acids[3]) in the context of three different RNA fragments, whose structures in complex with this or closely related aminoglycosides have already been described. The proposed methodology is based on the detailed comparison of the drug *N*-methylation patterns obtained from reductive amination reactions performed with the free and RNA-bound drug. Our concept may be exploited and adapted to a variety of examples within the molecular recognition and drug design fields.

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XI CARBOHYDRATE SYMPOSIUM

Engineered glycated amino dendritic polymers as non-viral gene delivery vectors targeting the receptor for advanced glycation end products

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The receptor for advanced glycation end products (RAGE) is expressed in cells involved in pathological processes, as diabetes or angiogenesis in tumors.[1,2] Under pathological conditions, RAGE is overexpressed and stimulate signaling pathways that promote cell proliferation. The objective of the present work is to engineer the amino dendritic polymers PEI 25 kDa and alkylated derivatives of PAMAM-G2 by exploiting the non-enzymatic Maillard glycation reaction for the preparation of novel AGE-containing non-viral gene delivery vectors targeting the RAGE.[3] The glycated versions of those dendritic polymers were easily prepared and retained the capability to bind and protect DNA from endonucleases. Furthermore, while glycation decreased the transfection efficiency of the dendriplexes in the CHO-k1 cell line that does not express RAGE, glycated dendriplexes acted as efficient transfection reagents in CHO-k1 cells that stably express recombinant RAGE. In addition, pre-incubation with BSA-AGEs, a natural ligand of the RAGE or dansyl cadaverine, an inhibitor of the RAGE internalization, blocked transfection for the dendriplexes of the glycated vectors confirming their specificity for the RAGE. The transfection efficiency and specificity of the glycated dendriplexes was confirmed in NRK and RAW264.7 cell lines that naturally express the RAGE. The glycated compounds retain its transfection capability towards cells expressing RAGE in the presence of serum and are able to promote *in vivo* transfection in a mouse model. Together these findings suggest that the RAGE is a suitable molecular target from which the development of site-directed engineered glycated non-viral gene vectors is feasible.

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Nanostructured weathering steel for matrix-free desorption ionisation mass spectrometry and imaging of metabolites, drugs and complex glycans

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Weathering steel has been employed for the first time to prepare sample plates for matrix-free laser desorption ionisation mass spectrometry (LDI-MS) of small molecules up to a mass range of around 1500 Da. The effective UV absorption, heat conductivity and porosity of the nanostructured inner rust layer formed during passivation determines the excellent performance in LDI-MS for a broad range of different analyte classes. The inexpensive material was evaluated in a series of relevant analytical applications ranging from the matrix-free detection of serum metabolites, lactose quantification, lipid analysis in milk to the glycoprofiling of antibodies and imaging mass spectrometry of brain tissue samples.

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XI CARBOHYDRATE SYMPOSIUM

Design of cationic amphiphilic carbohydrates as modulators of innate immunity

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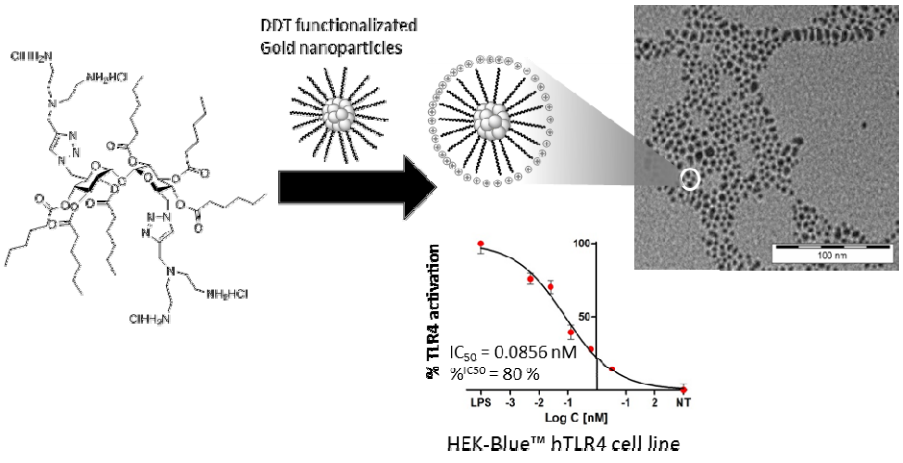
The identification of the bacterial endotoxin receptors for innate immunity, most notably TLR4 (Toll-like receptor 4),[1] has sparked great interest in the therapeutic manipulation of the innate immune system. Among microbial components, LPS and LOS (lipopoly- and lipoligosaccharides, respectively) and their bioactive portion, the lipodisaccharide lipid A, constitute the bacterial endotoxin and are potent stimulants of immune responses. An inherent charateristic of the endotoxin is its multi-tail amhiphilic character. Recently, several research groups have developed synthetic amphiphilic molecules capable of modulating the TLR4-mediated LPS signalling in animal and human cells.[2] Taking advantage of our previous experience in the design of carbohydrate-scaffolded polycationic facial amphiphiles,[3] we have synthesized a novel collection of derivatives based on methyl α-D-glucoside and α,α'-trehalose differing in the structure of the cationic heads and the number of lipophilic tails.[3] Self-assembly capabilities of these compounds have been evaluated by micellar critical concentration measurements and difusion light scattering techniques. Some of the compounds exhibited remarkable inhibitory activity of TLR4-mediated inmune response in the presence of lipid A when tested on HekBlue (human embrionic kidney) cells transfected with all the receptors involved in the TLR-4 route. Data for derivatives immobilized in gold nanoparticles will be also presented in this poster.



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Exploring the structural basis for glycosidase inhibition by multivalent glycomimetics: the *inhibitory multivalent effect*

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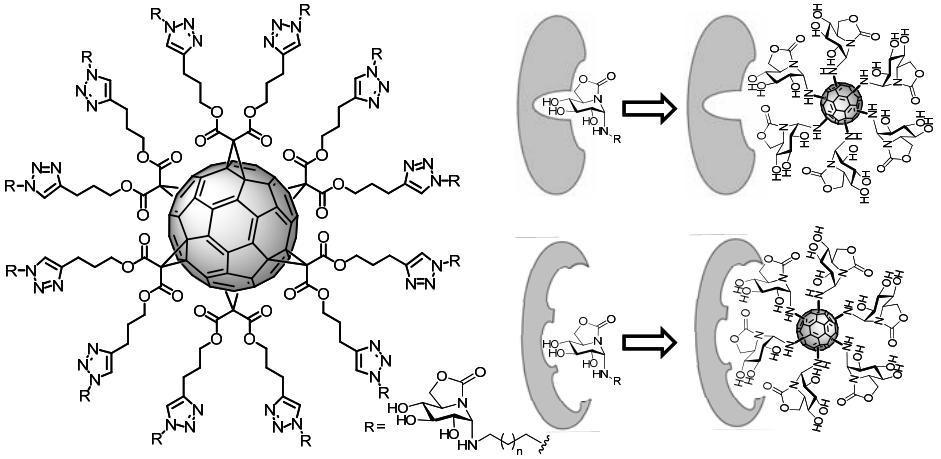
It was commonly assumed that the mechanisms by which lectins and glycosidases recognize their cognate sugar partners are intrinsically different: multivalency is a characteristic feature of carbohydrate-lectin interactions, whereas glycosidases bind to their substrates or substrate-analogue inhibitors in monovalent form. Recent observations on the glycosidase inhibitory potential of multivalent iminosugars displayed onto different platforms, including fullerene, cyclodextrin, porphyrines or self-assembled nanoparticles,[1,2] have questioned this paradigm and led to postulate an *inhibitory multivalent effect*. We have explored the structural basis for such effect by developing an sp^2-iminosugar glycomimetic acting as lectin ligand and glycosidase inhibitor and conducting lectin-glycosidase competitive binding experiments after displaying it onto a fullerene scaffold.[3] The ensemble of results point to a shift in the binding mode towards glycosidases on going from monovalent to multivalent systems: in the first case a typical "key-lock" model involving, essentially, the high-affinity active site can be assumed, whereas in the second a lectin-like behavior implying low-affinity non-glycone sites probably operates (see Figure). The differences in responsiveness to multivalency can then be rationalized in terms of the structure and accessibility of the corresponding carbohydrate binding regions.



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XI CARBOHYDRATE SYMPOSIUM

Relationship between foaming properties and polysaccharide composition of sparkling wines

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Sparkling wines elaborated following the champenoise method undergo a second fermentation in closed bottles of base wines, followed by aging of wines with lees for at least 9 months. The foam of sparkling wines is a key parameter of their quality but the compounds that are directly involved in foam quality are not yet completely established. Some authors have attempted to correlate the amount of mannoproteins in sparkling wines with the quality of their foam properties but there are few studies regarding other grape or yeast polysaccharides. Therefore, the aim of this work was to correlate the foaming properties with the polysaccharide composition in different white and rosé sparkling wines elaborated during three consecutive vintages. Foam instrumental parameters were analyzed by the Mosalux method.[1] Wine polysaccharides were recovered by precipitation after ethanolic dehydration and their carbohydrate composition was determined by GC-MS of their trimethylsilyl-ester O-methyl glycolsyl-residues.[2]

Table 1. Correlation coefficients (r) and significance levels (p) between parameters that determine foam instrumental properties (HM, HS, TS) and wine polysaccharides

	HM		HS		**TS**	
	r	*p*	*r*	*p*	*r*	*p*
Total polysaccharides	0.071	0.679	0.189	0.270	**0.641**	**0.000**
Polysaccharides from yeast	-0.081	0.637	0.054	0.753	**0.533**	**0.001**
Polysaccharides from grapes	0.184	0.283	0.280	0.098	**0.684**	**0.000**
Mannoproteins	0.150	0.383	0.157	0.360	**0.465**	**0.004**
Glucans	-0.225	0.187	-0.041	0.811	**0.396**	**0.017**
Polysaccharides rich in arabinose and galactose	0.042	0.806	0.285	0.092	**0.723**	**0.000**
Homogalacturonans	0.290	0.087	0.209	0.221	**0.577**	**0.000**
Rhamnogalacturonans type II	0.602	0.589	0.240	0.846	0.204	0.869

None of the wine polysaccharides was correlated with the foam maximum height (HM) or the foam stability height (HS), indicating that they would not affect the foamability of sparkling wines. On the contrary, positive correlations were found between foam stability time (TS) and all wine polysaccharides with the exception of rhamnogalacturonans type II. Polysaccharides rich in arabinose and galactose showed the highest correlations.

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The authors thank the INIA for financing this study through the project RTA2009-029-C02.

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XI JORNADAS DE CARBOHIDRATOS

Design and synthesis of a modular drug delivery system based on monovinyl sulfone β-cyclodextrin

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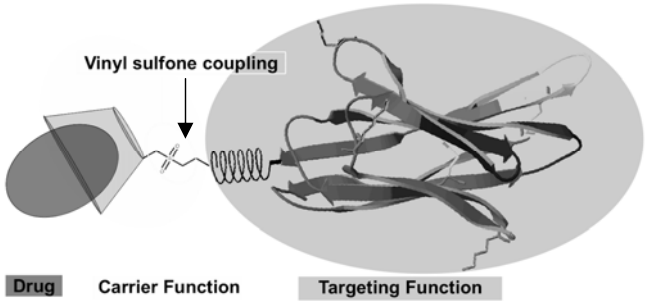
Among the different strategies to deliver drugs to specific physiological sites where pharmacological action is required, active targeting is an appealing approach that relies on the use of specific interactions with the target to direct and concentrate the drug at the site of action. The coupling of the therapeutic agent to the targeting agent and the stability of the linkage are important issues that have been approached by rigid designs that link a particular drug to a specific carrier, constraining the generalization of this approach.[1,2]

We have hypothesized that a modular design that decouples the carrier function from the targeting function leads to a flexible system that allows the targeting of different organs with different drugs. As a proof of concept we have synthesized monovinyl sulfone β-cyclodextrin (VSCD) as a key element that combines the good reactivity of the vinyl sulfone group toward biomolecules (targeting function) with the ability of β-cyclodextrin to form inclusion complexes with a wide range of drugs.



Our hypothesis was put to test by targeting different drugs to Trypanosoma sp [3]. VSC was directed linked to the targeting element (a nanobody raised against Trypanosoma sp) or via protein A (i.e. a protein with high affinity for IgG) and loaded with nitrofurazone, 5-chloro-2-mercaptobenzimidazol or camptothecin. The effect on cultures of parasites was evaluated.

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UNIVERSIDAD DE LA RIOJA

XI CARBOHYDRATE SYMPOSIUM

Unraveling the chiroptical response of sugar-protected gold nanoparticles through their Gold(I) sugar-thiolate precursors

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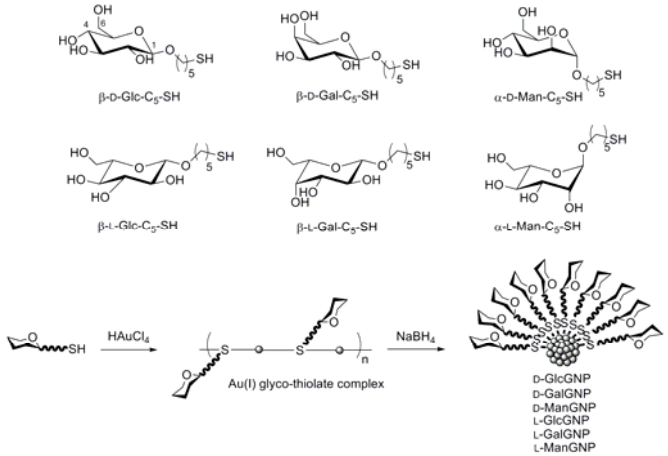
The concept of chirality has intrigued the scientific community since the 1870's but in the last years the optical activity of nanostructures has attracted a great deal of attention and discussion[1]. As a consequence of their particular properties and potential applications, thiolate-protected gold nanoparticles and nanoclusters have become a focus of increased interest[2]. Particularly, understanding the origin of the chirality in these nanostructures has demanded detailed studies on the structure-property relationship. Nevertheless, the dependence of the structure and chirality on the cluster-size remains not being completely understood[3]. Herein, the evidence of a strong chiroptical response by Circular Dichroism (CD) in the byproduct isolated from the preparation of 1.6 nm sugar-thiolated gold glyconanoparticles (GNPs) is presented. This byproduct was also characterized by UV-Vis, ^{1}H-NMR, Size-Exclusion Chromatography (SEC), X-ray Photoelectron Spectroscopy (XPS) and X-ray Diffraction (XRD) proving that its structure and properties are similar to those of a Au(I)-thiolate polymer obtained by the direct reaction of Au(III) salts with glycoconjugate-thiol species in water. Both byproduct and polymer were studied by mass spectrometry showing a major peak corresponding to a cyclic tetrameric unit $[Au(I)SC_5R]_4$. Thus, it is concluded that Au(I)-thiolate species are the origin of the ellipticity observed for the first time in gold glyconanoparticles. The results were also reproducible throughout the series of gold nanoparticles protected by enantiomeric pure thiol-conjugates of the natural monosaccharides D-glucose (Glc), D-galactose (Gal) and D-mannose (Man) and their corresponding non-natural L-enantiomers.



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A mannan polysaccharide enhances the allergenic vaccines effect stimulating the immunity response

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Immunotherapy anti-allergic treatments are based on the administration of therapeutic vaccines which contained allergen-antigens. These allergens are proteins from pollens, dust mites, epithelia, etc. It is widely accepted that the clinical efficiency of these vaccines are associated to the dose of allergen administered, for which the OPS and the consensus guides of the numerous companies scientific recommend that the vaccines should be prepared by a sufficient concentration of allergens. It is required to reduce the risk of adverse effects in the allergic patients. In order to achieve this goal, there is an increase use of vaccines based on modified allergens (allergoids) with minor capacity of reaction with the antibodies IgE (decrease inflammatory response).[1,2]

The dendritic cells are specialized to stimulate T-lymphocytes by means of endocytosis, using receptors. There are a great variety of receptors implicated in endocytosis, such as mannose receptors (CD206, CD209), stimulating immunity response.

Herein, we have developed an inmunogenic complex (allergoid) that contains a mixture of the antigen and the mannan polisaccharide that forms a polimeric matrix. The polisaccharide was obtained from *S. cerevisae* yeast. The mannan core is formed by manα(1-6)-man repeating units with manα(1-2)branches. This polysaccharide is attached to the proteins through an O-glycosidic bond. The antigen-mannan complex presented in this work will stimulate the T-lymphocites inmune response and decrease inflamatory response. Therefore, this inmunogenic complex will be used to generate allergenic vaccines, against: plants (gramineas: phelum pratense, lolium perenne..), dust mites (Dermatophagoides pteronyssinus, Dermotophagoides farinae..), etc.

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Synthesis of 1-phospha-sugars by ARF-Arbuzov-cyclization reactions

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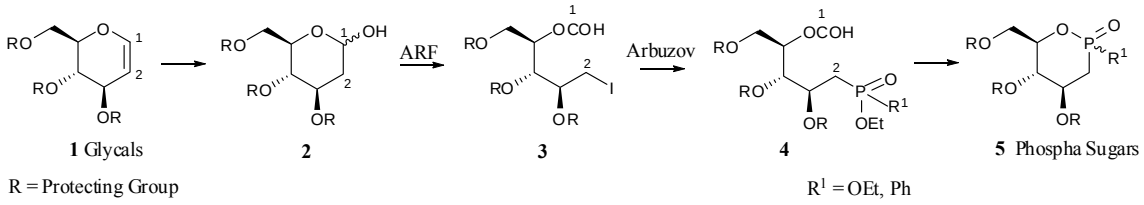
1-Phospha-sugars are analogs in which the anomeric carbon atom is replaced by a phosphorus atom. Cyclic phosphonate analogs also know as phostones[1] have received continued attention in the literature mainly due to they are potential inhibitors of glycosidases. Phosphinosugars (phostines) are considered as mimes of glycosides and have shown anticancer activities. Of special interest are 2-deoxy-1-phospha-sugars that possess a certain structural relation with ß-KDO and the sialic acids in general.

Herein we report on a new general methodology for the synthesis of phostones and phostines that has been developed in only four steps starting from glycals. The alkoxyl radical fragmentation (ARF) reaction of carbohydrate anomeric alcohols with hypervalent iodine reagents in the presence of iodine has been investigated by this laboratory[2] and used as key step. Following the literature glycals **1** can be converted to 2,3-dideoxy-hexopyranoses **2**. The anomeric ARF of these compounds gave iodo compounds **3** in high yields. Organophosphorus **4** were obtained by Arbuzov[3] reaction that followed by saponification of the formyl ester and subsequent cyclization produce 1-phospha-sugars **5** in good yields.



These organophosphorus compounds may be powerful building blocks for the preparation of complex systems and scaffolds in biological chemistry.

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XI JORNADAS DE CARBOHIDRATOS

Exploring gold glyconanoparticles (GNPs) as carriers to prepare fully synthetic vaccines against HIV

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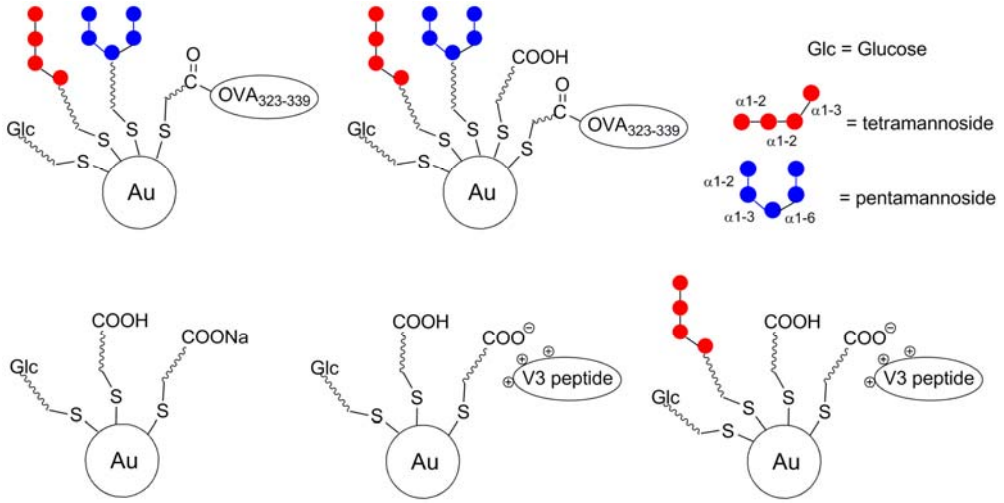
The infection by the human immunodeficiency virus (HIV) is the cause of AIDS and is one of the greatest infectious diseases ever seen. HIV infects cells of the human immune system such as T-helper cells (specifically CD4+ T cells), macrophages, and dendritic cells. After almost 30 years of reasearch, an effective vaccine against HIV is still a challenge.[1]

We have already demonstrated by surface plasmon resonance (SPR) and STD-NMR that gold glyconanoparticles (GNPs) coated with oligomannosides of HIV-1 glycoprotein gp120 are able to mimic the carbohydrate epitope of 2G12 antibody.[2]

Recently, we have also demonstrated that ~2nm GNPs are a suitable platform to construct a potential carbohydrate-based vaccine against *S. pneumoniae* type 14. The co-presence of the T cell-stimulating $OVA_{323–339}$ peptide and the tetrasaccharide antigen β-D-Gal*p*-(1–4)-β-D-Glc*p*-(1–6)-[β-D-Gal*p*-(1–4)-]β-D-Glc*p*NAc-(1–), which corresponds to a single epitope of the capsular polysaccharide allowed the induction of specific and functional IgG antibodies against this bacterium.[3]

We now explore the use of GNPs as a platform to prepare a fully synthetic vaccine against HIV. In these work we have synthesized two series of GNPs, one bearing oligomannosides and $OVA_{323–339}$ peptide and one with oligomannosides and the highly immunogenic gp120 V3 peptide (see figure).



Rabbit immunization experiments with oligomannosides/$OVA_{323–339}$ GNPs and oligomannosides/ V3 GNPs were performed and the results will be presented.

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XI CARBOHYDRATE SYMPOSIUM

Synthesis of fused tetrazolo iminosugars from azido monosaccharide derivatives

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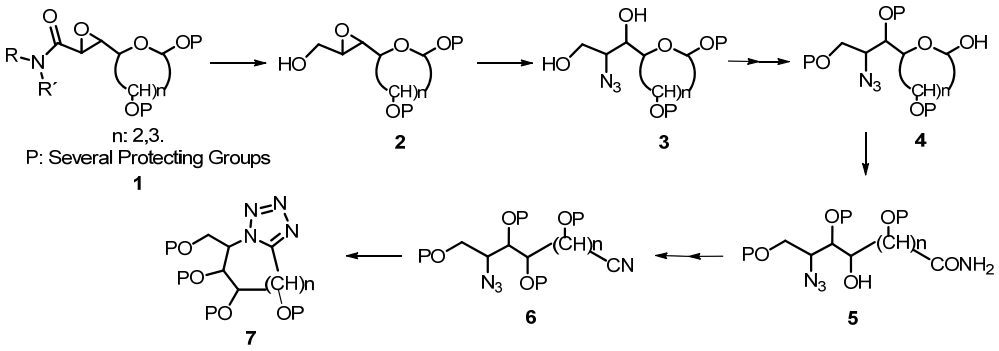
Iminosugars, by virtue of their structural resemblance to monosaccharides, are among the most potent inhibitors of glycosidases, mimicking the transition states of the sugars involved in processes of inhibition. Due to this fact, a variety of monocyclic and bicyclic iminosugars have been synthesized or isolated from natural sources over the years. As part of our ongoing work on the preparation of glycosidase inhibitors, we developed stereoselective methods for synthesizing iminosugars from 2,3-epoxyamides **1** obtained from monosaccharides.[1] Now our attention is focused on the syntheses of novel bicyclic tetrazoles **7**, by intramolecular cycloaddition, due to the possibility of combining azido and ciano groups in the same molecule. The tetrazole system is widely found in bioactive products but only a few examples of syntheses of fused pyrrolidines and piperidines with tetrazoles[2,3] have been reported to be evaluated as inhibitors.

Whit the aim of obtaining new and more potent analogues, we studied the formation of tetrazolic systems fused to different heterocycles formed from monosaccharide derivatives. Epoxyamides **1** were sequentially transformed into epoxyalcohols **2** and azido alcohols **3**. Deprotection of the anomeric hydroxyl group after convenient functionalization gave **4**. Conversion to azidoamides **5** and further transformation into azidonitriles **6** gave tetrazolobicycles **7** by cycloaddition.



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XI JORNADAS DE CARBOHIDRATOS

A nitro sugar based route to branched-chain poly-hydroxylated octahydro-1*H*-indole-4,5,6-triols, as new potential glycosidase inhibitors

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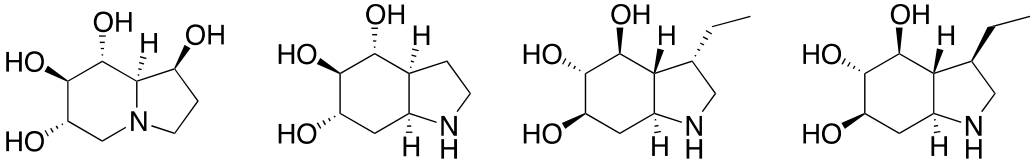
Glycosidases became an important class of targets for pharmaceutical research due to the roles they play on the control of the cellular oligosaccharide processing involved in digestion, biosynthesis of proteins and catabolism of glyconconjugates.[1] This is the reason why glycosidase inhibitors have been extensively studied over the last years, as potential terapeutic agents.[2] Specifically, castanospermine (**1**) is an indolizidine alkaloid first isolated from the seeds of *Castanospermum australe*, which shows to be a potent inhibitor of some glucosidase enzymes and has antiviral activity.[3]

Some castanospermine analogs, have been reported, including the poly-hydroxylated octahydro-1*H*-indole-4,5,6-triol **2**, which, as a rigidified mimic of disaccharides, is expected to exhibit significant antidiabetic properties.[4]

In connection with an ongoing project aimed at the development of new synthetic applications of nitro sugars, a synthesis of the branched-chain poly-hydroxylated octahydro-1*H*-indole-4,5,6-triols **3** and **4** has recently been developed.



Studies on the glycosidase inhibition properties for these novel compounds **3** and **4** have also been carried out.

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XI CARBOHYDRATE SYMPOSIUM

Glycosylation and lectin-binding properties of thiolated polymers

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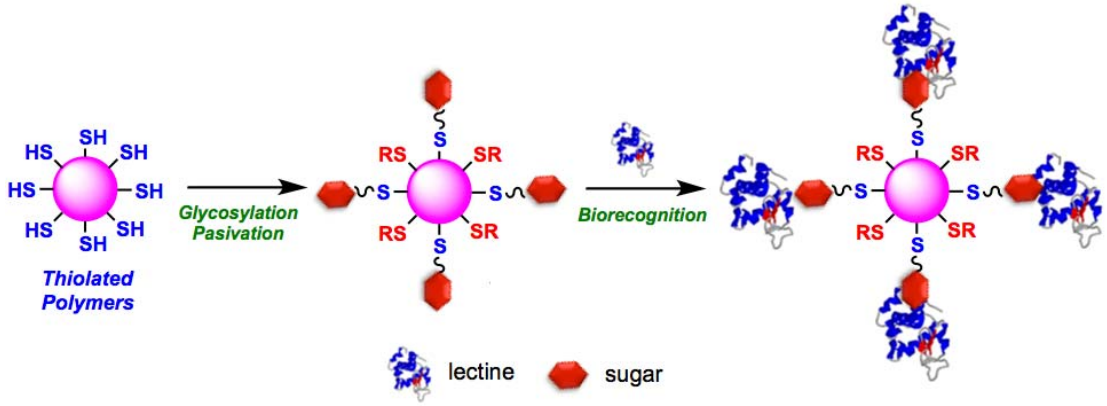
The importance of the glycosylation in Biology has led to the coining of terms such as *glycomics* and *sugar code* to express the perspective of a new function and *lectinomics* to forecast trends in lectin-biorecognition technology.[1,2] In this context, the search for new materials is a key element for the development of lectin related diagnostics and thiolated polymer are good candidates.

Immobilization of saccharides to the thiolated polymers was easily performed by a variety of efficient glycosylation techniques including both direct and indirect strategies. In the direct approach, halo and vinyl sulfone anomeric saccharide derivatives were used for the branching by means of the nucleophilic substitution or the Michael-type addition reaction, respectively. By contrast, in the indirect approach the thiolated polymers were first transformed in their corresponding azido and alkyne counterparts by exploiting the sulfur chemistry of these compounds and later conjugation with suitable clickable complementarily functionalized saccharides derivatives.

The resulting glycomaterials were assayed against commercial lectins and some of them were put to test with biological challenge: detection of lectins that are produced by plant as defense proteins against phytophagous insects.[3]



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Enantioselective synthesis of nectrisine

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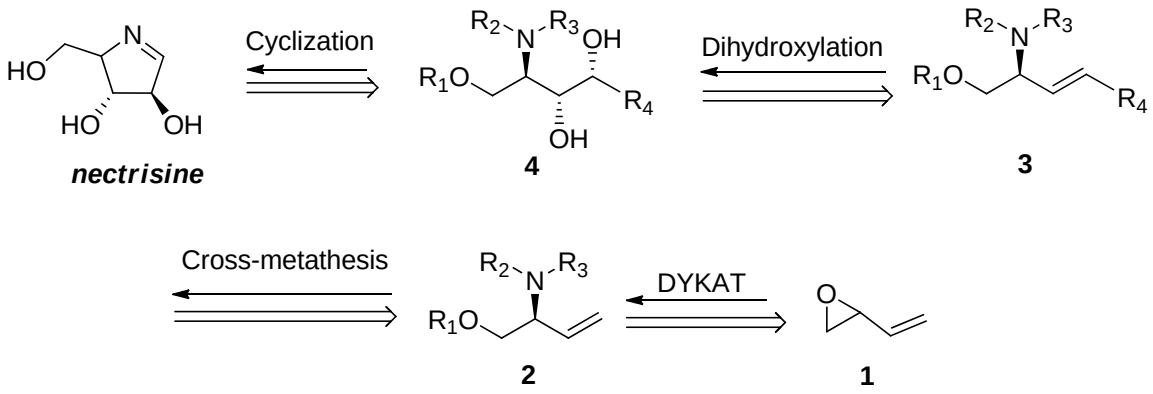
Nectrisine is an azasugar isolated from a strain of the fungus *Nectricine lucida* as immunomodulator FR-900483 and found to exhibit inhibitory activity on α–glycosidases.[1] Moreover, nectrisine is involved in the prevention of different diseases such as Newcastle disease virus. Due to this important biological activity many organic chemists are focused on the development of new methods to synthesize nectrisine.

We recently described that Trost's DYKAT process based on Pd-catalyzed asymmetric allylic amination in combination with cross-metathesis and dihydroxylation reactions is an efficient strategy for accessing important natural products such as Jaspine.[3] Here we explore an enantioselective synthesis of nectrisine based on Pd-catalyzed asymmetric allylic amination, cross-metathesis and dihydroxylation as key steps. The scheme below shows the retrosyntheis proposed, where the key synthon is the allylamine **2** which is obtained in high enantiomeric purity by a deracemization process using Pd/DACH as a catalytic system



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Influence of polysaccharide commercial product addition on volatile composition of white sparkling wines

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Natural sparkling wines are obtained after a second fermentation in closed bottles, and they remain in contact with the yeast lees for at least 9 months. During sparkling wine aging, different compounds such as polysaccharides can be released due to yeast autolysis that can cause important changes in wine composition, affecting the quality of sparkling wines. Yeast autolysis is a slow natural process that takes long time. Therefore, the aim of this work was to study the effect of the addition of several commercial products rich in polysaccharides and/or mannoproteins on the volatile composition of white sparkling wines elaborated from two white grape varieties (*Godello* and *Verdejo*), and aged for 9 months. The volatile compounds were analyzed by gas chromatography coupled to a mass detector, after a previous liquid-liquid extraction.[1] The polysaccharide and monosaccharide composition of the commercial preparations was determined by GC-MS of their trimethylsilyl-ester O-methyl glycosyl residues obtained after acidic methanolysis and derivatization.[2]

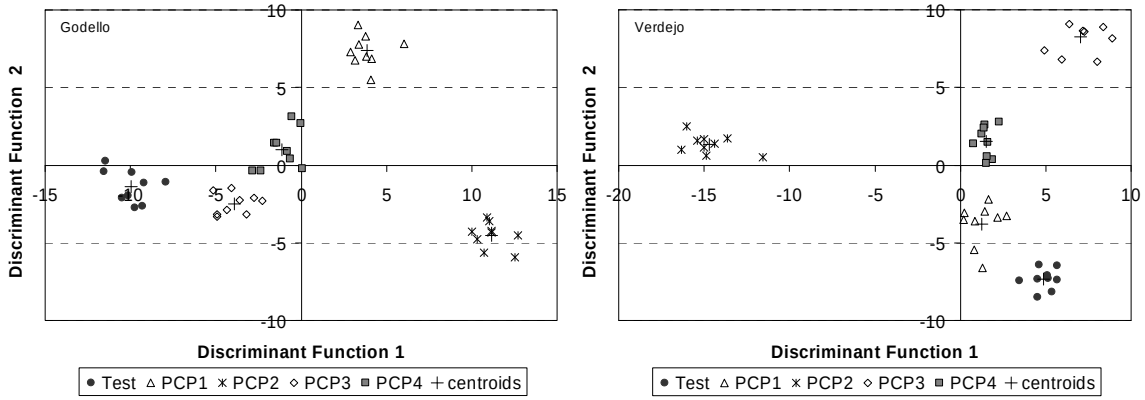

Figure 1. Distribution of wines in the plane defined by the first two discriminant functions. Test: control wines; PCP: wines treated with polysaccharide commercial products

The discriminant analysis indicated that the wines treated with PCP2 showed the highest differences in the volatile composition of both sparkling wines studied, being the ethyl esters, alcohol acetates and terpenes the compounds that were affected in a greater extent. PCP2 was the product with the highest percentage of mannoproteins.

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The authors thank the INIA for financing this study through the project RTA2009-029-C02.

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XI JORNADAS DE CARBOHIDRATOS

Applications of ^{1}H and ^{19}F NMR spectroscopy in combination with computational methods for the screening of fluorine-tagged oligosaccharides versus lectin receptors

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Lectins are ubiquitous proteins from non-immune origin that specifically bind saccharide epitopes showed by the cell surface and thus promote a wide array of physiological processes. In particular, plant lectins are known because of their series of potent biological activities, such as agglutination, toxicity, or anti-proliferation of cancer cells, as well as having anti-fungal and anti-bacterial action.[1] From this perspective, it can be ackwnoledged that the study of the rational behind lectin-carbohydrates interaction events represents a key step to design new molecular probes, which could lead to novel sugar-based therapeutic agents. Indeed, different experimental studies have allowed to get a deeper understanding of the molecular basis of this phenomenon.[2]

NMR spectroscopy has become an established tool to provide high-resolution molecular structures in solution and valuable informations on ligand binding. In particular, ^{19}F nuclei can act as reporter atoms for carbohydrate recognition by lectins. Thus, herein we present a combination of NMR-based protocols assisted by molecular modelling methods for unraveling the key features of the interaction between different glycomimetics with fluorine atoms and several lectins of biomedical interest.

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XI CARBOHYDRATE SYMPOSIUM

Carbohydrate-responsive gated silica mesoporous supports as controlled delivery systems

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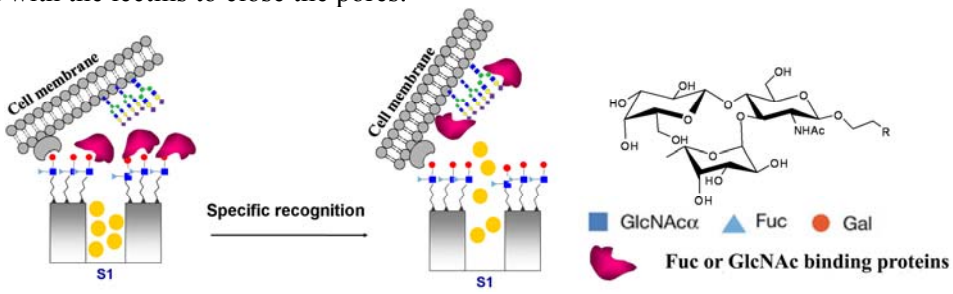
Colon cancer is one of the most prevalent cancers worldwide. Although early detection, increased awareness, and developments in treatment have increased complete cure rates especially in some advanced countries, distant metastasis is still a critical event that makes colon cancer a lethal disease. Drug delivery systems are being developed as novel therapeutic approaches to inhibit metastasis. Here we present mesoporous silica supports (SMPS) functionalized with Sialyl Lewis x (sLex) and sialyl Lewis a (sLea) antigens. sLex and sLea glycans are expressed on highly metastatic colon cancer cells.[1] They promote extravasation of cancer cells and tumor angiogenesis via interacting with E-selectin on endothelial cells.[2] High sLex/a expression levels in colon cancer patients are correlated with poor prognosis. Therefore, these glycans are frequently evaluated as tumor markers. Whereas the diagnostic utility of sLex/a has been well established, effective approaches targeting these glycans are not well developed for treatment the disease.

Gated silica mesoporous supports (SMPS) functionalized with molecules that act as "molecular gates" have demonstrated to have high potential in delivery applications.[3] In these hybrid systems, specific interactions trigger the opening of the "gate" allowing the delivery of the cargo in the desired cells/tissues. We have elaborated a new bio-gated support for controlled delivery of drugs against cancer colon cells. The gated SMPS is functionalized with the Lewis x antigen able to interact with fucose or glucosamine binding proteins that act as a cap for the gate of the SMPS. The isocyanate-functionalized solid was first loaded with ATO- 430LS dye, and then functionalized with ethylamino glycosidated Lewis x by a urea bond and finally was capped with the lectins to close the pores.



As a proof of concept and before cell studies, an opening protocol was performed. This was carried out by a displacement of the lectin by addition of specific carbohydrate ligands. Delivery of the dye has been studied in the presence of fucose in phosphate-buffered saline (PBS; pH 7.5, 1 mM $CaCl_2$, 2 mM $MgCl_2$). An increase in the fluorescence was observed in the solution.

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UNIVERSIDAD DE LA RIOJA

XI JORNADAS DE CARBOHIDRATOS

Synthesis and biological evaluation of furyl galactosides and non-carbohydrate multivalent ligands with affinity towards enterotoxins

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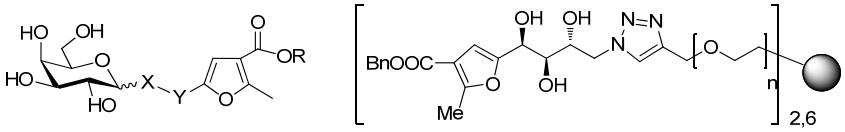
Vibrio cholerae (CT) toxin and the closely related heat-labile toxin of *Escherichia coli* (LT) are proteins that present heterophilic binding with their receptors.[1] They belong to the bacterial AB_5 holotoxin family where the B subunits are responsible for binding to ganglioside GM1.

Several glycomimetics have been reported presenting affinity towards CT and LT.[2,3] We have recently described the synthesis of a new type of non-hydrolyzable bidentate ligands featuring D-thiogalactose and polyhydroxyalkylfuroic esters as pharmacophoric residues. They constitute novel mimetics of GM1 ganglioside. STD-NMR experiments, have allowed the identification of the binding epitopes of the ligands interacting with the protein. The non-carbohydrate moiety based on polyhydroxyalkylfuroic ester structure showed the highest affinity.[4]

In this communication we present the synthesis of new *N*- and *O*-galactosides bearing substituted furoates as aglycones and the assembly of polyhydroxyalkyl furoates into multivalent structures.



X= α-*O*, β-N
Y= C=O, CH_2
R = Bn, Et

Biological evaluation of some of the conjugates will be presented.

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UNIVERSIDAD DE LA RIOJA

XI CARBOHYDRATE SYMPOSIUM

Divalent ligand targeting MMP12 and Gal3: studying interactions by NMR

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Matrix metalloproteinases (MMP) family is implicated in various pathologic states such as tumor invasion, diseases of central nervous system (CNS) and disorders of the immune system. MMPs are zinc dependent metalloproteins responsible of degradation of most extracellular matrix macromolecules in cell growth.[1] In particular, MMP12 plays important role in cancer growth and metastasis where is found to be overexpressed. In fact, MMP12s has been chosen as a versatile pharmaceutical target to design potent MMP inhibitors (MMPIs).

On the other hand, Galectin 3(Gal3) has been observed indeed related with MMPs in tumor progression. Gal3 is present in tumoral cells and regulates cell proliferation.[2] Particularly, cytoplasmic Gal3 has anti-apoptotic activity maintaining mitochondrial integrity being upregulated in tumoral events.

Exhaustive study of both proteins, MMP12 and Gal3, has allowed the design of potent inhibitors and potential drugs. Thus, compound **1** (fig.1) provides hydroxamic acid epitope for binding with MMP12 and lactose residue for the case of Gal3. To investigate the ability of compound 1 to bind MMP12 and Gal3, NMR techniques have been used.

STD-NMR has been used to study interactions with Gal3 from ligand point of view providing information of epitope mapping. In the case of binding with MMP12, protein point of view techniques have been chosen such as HSQC titration with compound 1. HSQC requires labelled ^{15}N MMP12 and it is very sensitive to chemical environment change. During binding, signals of the aminoacids of binding site suffer a change on chemical environment resulting in a change of their chemical shifts. Moreover, ternary system was also studied by diffusion NMR (DOSY).

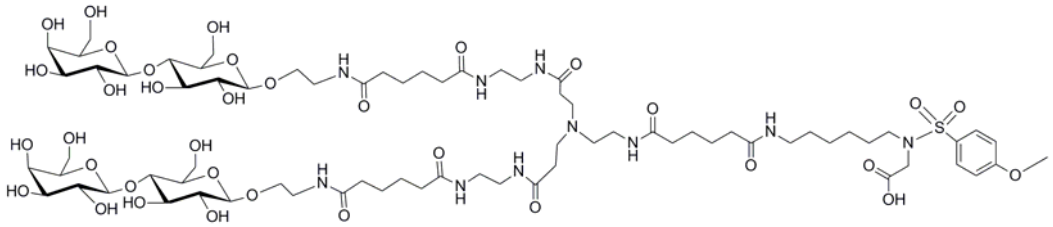

Figure 1. Compound **1.**

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UNIVERSIDAD DE LA RIOJA

XI JORNADAS DE CARBOHIDRATOS

Intramolecular 1,8-hydrogen atom transfer processes in cyclodextrin systems

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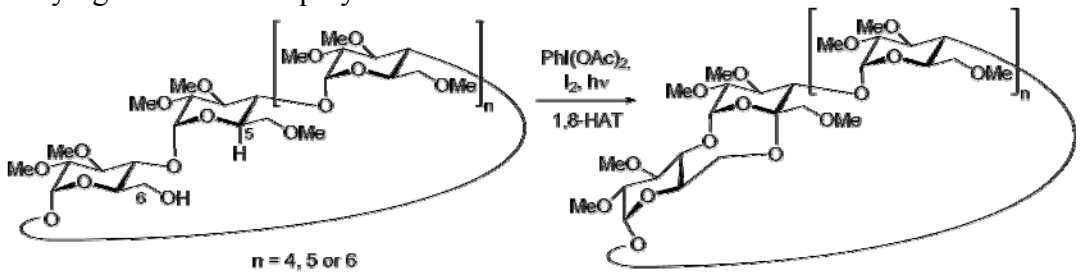
During the last decades cyclodextrins (CDs) and their derivatives have been widely investigated for their important applications such as catalysis, molecular receptors towards a great variety of guest molecules and building blocks in supramolecular chemistry.[1] Thus, an important amount of work has been made in an attempt to improve their functions. However, selective modifications in these structures are not easy to control because of steric factors derived from the torus shape and the large number of hydroxy groups.[2]

According to our previous studies based on intramolecular 1,8-hydrogen atom transfer (1,8-HAT) reaction between the two pyranose units of (1→4)-*O*-disaccharides promoted by 6-*O*-yl radical,[3] herein we show our lastest results by extension of this methodology of remote functionalization to more complex carbohydrates such as CDs. The glucose units in these systems present a proper spatial disposition giving abstraction exclusively at C-5 position of the vicinal unit in a regioselective manner and without modifying the rest of the polysaccharide.



Acknowledgements

We thank the Ministerio de Economía y Competitividad (CTQ2010-18244) and the Gobierno de Canarias (SolSubC200801000192) for financial support.

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UNIVERSIDAD DE LA RIOJA

XI CARBOHYDRATE SYMPOSIUM

Trifluoromethylation of 2-iodoglycals with fluoroform derived $CuCF_3$

Jordi Mestre,[a] Omar Boutureira,[a] Sergio Castillón,[a] Anton Lischynskyi,[b] and Vladimir Grushin[b]

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Fluorinated carbohydrates are used in many medical applications since they play important roles as enzyme inhibitors, non-invasive diagnostic agents, antiviral and antitumoral agents.[1] More recently they have been identified as ligands in proteincarbohydrate interactions and molecular recognition processes.

Despite recent efforts for the preparation of advanced fluoro sugars probes the incorporation of important CF_3 units is still scarce. Only few examples of trifluoromethylated carbohydrates are known and were prepared either starting from trifluoromethylated synthons (building block approach) or by the nucleophilic trifluoromethylation of oxosugars with the Ruppert's reagent.[2]

Here we describe a short and simple late-stage cross-coupling methodology for the synthesis of 2-trifluoromethyl-glycals with the $CuCF_3$ reagent derived from fluoroform developed by Grushin *et al*.[3] The method operates under mild conditions and has proven regioselective and tolerant to a wide range of protecting groups.

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XI JORNADAS DE CARBOHIDRATOS

Hyperbranched glycolipids as multivalent inhibitors of carbohydrate-lectin interactions

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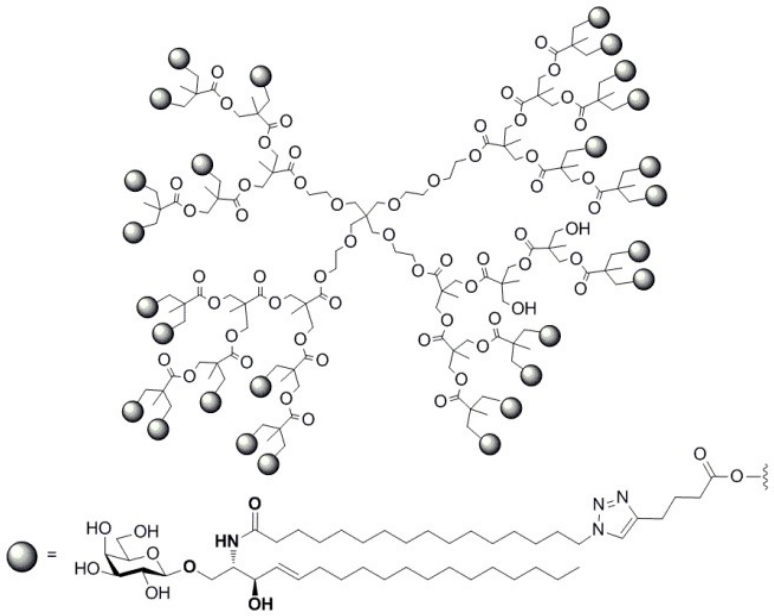
The synthesis of multivalent neoglycoconjugates is currently promoted by the extensive findings of multiple ligand-receptor interactions that occur in Nature and by the phenomenon generally referred to as the glycoside cluster effect. In this context, inhibition of pathogen binding by targeting surface protein receptors is a particularly attractive approach with therapeutic potential for the treatment of several diseases that utilize surface glycolipid receptors.[1]

In the present work, we present the synthesis and characterization of a series of water soluble glycoclusters consisting in Boltorn H30 hyperbranched polymers functionalized with β-neoglycolipids ligands[2] that could potentially mimic those natural glycolipidenriched domains. These functionalized dendritic polymers were evaluated against Cholera toxin (CTB5) and other lectins using Surface Plasmon Resonance (SPR).



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XI CARBOHYDRATE SYMPOSIUM

Application of microarrays containing parasitic N-glycans

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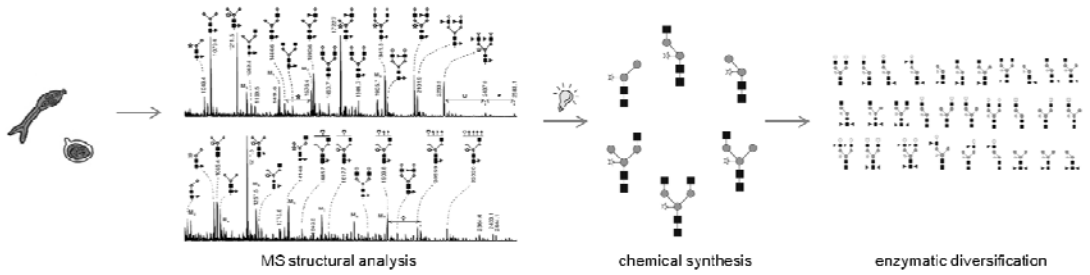
Helminth infections such as *S.mansoni* (snail fever, bilharzia) are the second after malaria major health problem caused by parasites. Even though 200 million people are affected worldwide, a diagnostic test or vaccine candidate is still missing on the market.[1]

Knowing that important immunogenic responses in mammalians infected with helminth are directed towards glycans and based on glycomics analysis of most infectious stages of *S.mansoni* lifecycle,[2] we had chosen and synthesised six xylosylated structures of N- glycans[3] which after further diversification with glycosyltransferases gave us library of compounds decorated with core fucose, Lewis X, LDN and LDNF epitopes.



Newly synthesized xylosylated, parasitic structures were combined with compounds available in our laboratory for the preparation of glycan microarray. We have found core xylosylation, next to other core modifications to have significant impact on the antibodies responses developed in human populations living in schistosomiasis endemic areas, as well as on the interactions with certain C-type lectins receptors.

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XI JORNADAS DE CARBOHIDRATOS

Synthesis, conformational analysis and biological evaluation of an antitumor vaccine derived from a non-natural MUC1 fragment

Nuria Martínez-Sáez,[a] Nitin Supekar,[b] Margreet A. Wolfert,[b] Juan L. Asensio,[c] Jesús Jiménez-Barbero,[d] Jesús H. Busto,[a] Alberto Avenoza,[a] Francisco Corzana,[a] Jesús M. Peregrina,[a] and Geert J. Boons[b]

a) Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, E-26006 Logroño, Spain; b) Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, Georgia 30602, United States; c) Instituto de Química Orgánica General, CSIC. Juan de la Cierva 3, 28006 Madrid. Spain; d) Centro de Investigaciones Biológicas, CSIC. Ramiro de Maetzu 9, 28040 Madrid. Spain; e-mail: nuria.martinez@unirioja.es

A Tn antigen (αGalNAc-Thr) mimic, based on the replacement of Thr by the non-natural quaternary amino acid α-methylserine (MeSer),[1] has been incorporated in the tandem repeat sequence (AHGVTSAPDT[10]RPAPGSTAPPA) of MUC1 mucin glycoprotein at 10-position. In addition, a conformational analysis, combining NMR data with molecular dynamics, has been carried out on the small glycopeptidic sequence Pro-Asp-MeSer(αGalNAc)-Arg in water solution indicating that the main backbone conformation obtained matches a X-ray structure corresponding to a small peptide bound to a monoclonal anti-MUC1 antibody (SM3). Once demonstrated by ELISA tests that this monoclonal antibody recognized the above-cited 21-mer fragment, a tripartite vaccine[2] containing this Tn antigen mimic at the most immunogenic domain PDTR has been synthesized and tested on mice. This novel vaccine elicits immune response, recognizing both glycosylated and unglycosylated tumor-associated MUC1 derivatives.[3]

[1] Lakshminarayanan, V.; Thompson, P.; Wolfert, M. A.; Buskas, T.; Bradley, J. M.; Pathangey, L. B.; Madsen, C. S.; Cohen, P. A.; Gendler, S. J.; Boons, G. J. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 261.

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UNIVERSIDAD DE LA RIOJA

XI CARBOHYDRATE SYMPOSIUM

Designing non-natural cancer vaccines by replacing the methyl group of Thr-10 in MUC1 derivatives

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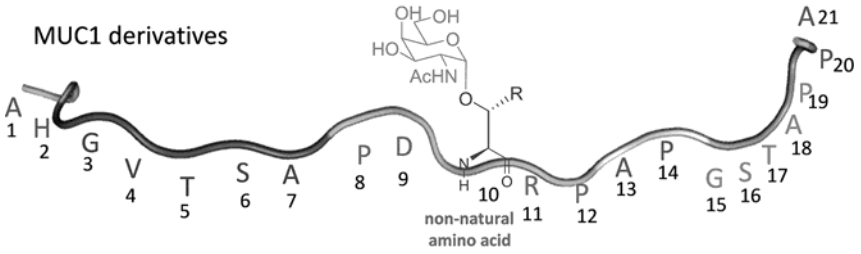
MUC1 mucin is an *O*-glycoprotein which is being widely studied as a potential cancer vaccine.[1] Its importance lies in the fact that in tumor cells these proteins are overexpressed and their carbohydrates are pretty simple due to incomplete glycosylation. As a consequence, different tumor-associated carbohydrate antigens (TACAs), such as the Tn determinant (α-*O*-GalNAc-Ser/Thr), are exposed to the immune system. Unfortunatelly, most TACAs are self-antigens, hence tolerated by the immune system. Our research group has a great deal of experience in organic synthesis of amino acids. Particularly, we are focused on the synthesis of the non-natural ones,[2] which presumably can overcome the aforementioned issue.



In this regard, we are currently developing non-natural mucins based on the replacement of the methyl group of Thr by different substituents. The sudy involves the synthesis, using conventional and SPPS methodology, conformational analysis, supported by molecular dynamics simulations, and biological evaluation to different anti-MUC1 antibodies with ELISA tests. As a future prospective, the best candidates will be selected to be assembled to a TLR2 antagonist and a T_{helper} fragment to give a three-component vaccine, which has been proved to elicit strong immune response.[3] The novel vaccines will be tested *in vivo* studies.

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UNIVERSIDAD DE LA RIOJA

XI JORNADAS DE CARBOHIDRATOS

S-Glycosylated mixed α,β-peptides stabilized by CH/π interactions

Iván García-González, Nuria Mazo, Fernando Rodríguez, Alberto Avenoza, Jesús H. Busto, Francisco Corzana, and Jesús M. Peregrina

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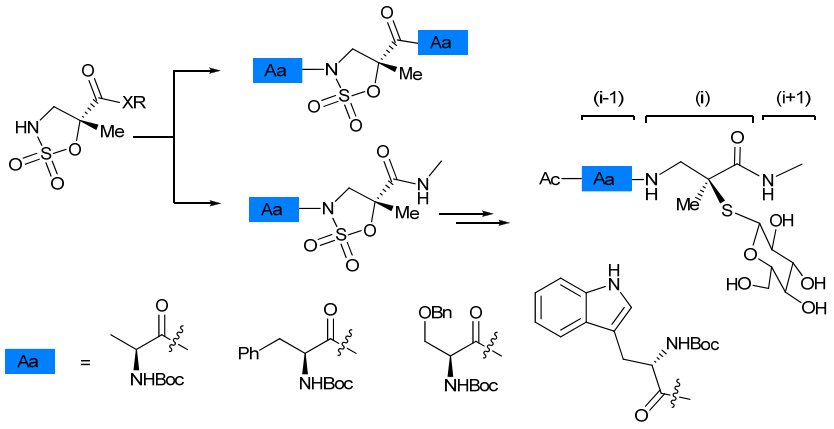
It is well-known that the incorporation of β-amino acids into peptides provides a high degree of stability.[1] In this context, the combination of proteinogenic α-amino acid residues and β-amino acids opens the door to a larger pool of accessible structures with potential applications. A lot of studies exists in this field, but there are few analyzed cases on $\beta^{2,2}$-amino acids incorporated in peptide chains.[2] Our research group has developed an efficient strategy to the synthesis of $\beta^{2,2}$-amino acids through the ring opening of cyclic sulfamidates.[3]

Additionally, it is clear that a variety of weak hydrogen bonds play crucial roles in the behavior of molecules and molecular assemblies. In this context, the CH/π interactions[4] make an important contribution to understanding the crystal packing, structures of biological molecules and molecular recognition processes.

In this work, we present the synthesis of several mixed peptides by the incorporation of $\beta^{2,2}$-amino acids in different peptides through ring opening of cyclic sulfamidates with thioglucose. Conformational studies of these compounds were obtained by combination of NMR experiments and molecular dynamics calculations. Remarkably, in the cases of Phe and Trp, a CH/π interaction between the aromatic ring of these (i-1) α-amino acids and the α-methyl group of $\beta^{2,2}$-amino acid (i) contributes to stabilize the backbone conformation.



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Glycopeptides incorporating sulfur-based Tn antigen mimics

Carlos Aydillo, Victor Rojas, Ismael Compañón, Marta I. Gutiérrez-Jiménez, Miguel Ayala, Francisco Corzana, María del Mar Zurbano, Jesús H. Busto, Alberto Avenoza, and Jesús M. Peregrina

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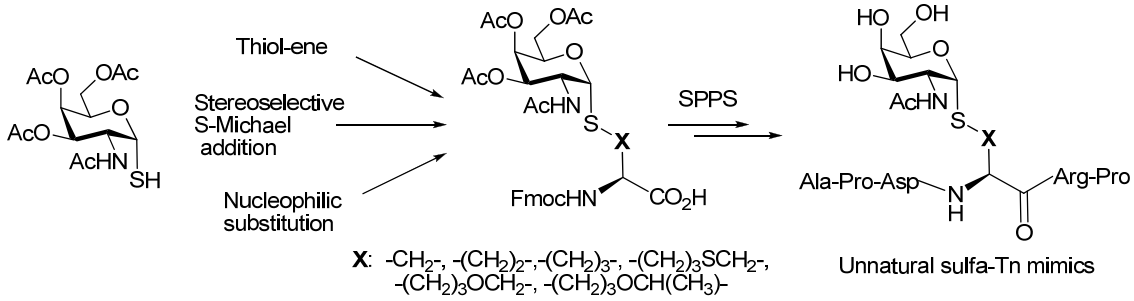
Tn antigen (α-*O*-GalNAc-Ser/Thr) is involved in the recognition processes of cancerous cells. This simple structure is found in a great number of recognition epitopes of glycoproteins, i.e., MUC1 mucin.[1] For this reason, the synthesis of new Tn mimics has been atracting the interest of carbohydrate chemists. In this sense, we have been carrying out the synthesis of different sulfur-based Tn antigen mimics. Changing oxygen by sulfur can provide different affinities of the Tn antigen when including in epitope glycopeptides. The synthesis of different sulfa-Tn building blocks were achieved through three strategies starting from tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-galactose: radical thiol-ene reaction over alkenes, nucleophilic substitution over bromo-compounds and stereoselective *S*-Michael additions to chiral dehydroalanines.[2]



Various sulfa-Tn building blocks were incoporated in the Ala-Pro-Asp-Xaa*-Arg-Pro sequence. Competitive enzyme-linked lectin assays (ELLA) were carried out to evaluate the affinities with *Soybean agglutinin* (SBA) lectin. We demonstrated that non-natural sulfa-Tn antigen mimics show comparable affinity to α-*O*-GalNAc-Ser for this lectin.[2]

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XI JORNADAS DE CARBOHIDRATOS

A double diastereoselective Michael-type addition as an entry to Tn antigen mimics and analogs of Thiamet-G

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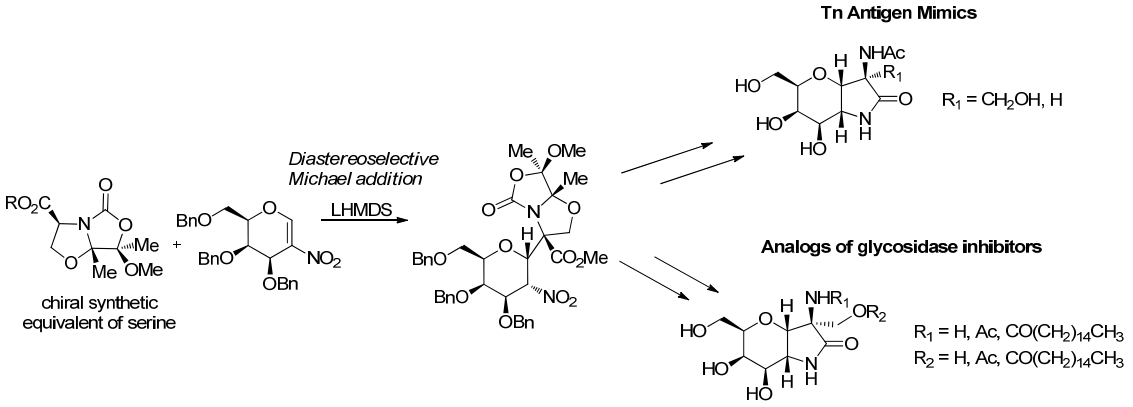
Our research group has recently reported a double diastereoselective Michael-type addition between chiral synthetic equivalents of serine, bicyclic *N*,*O*-acetals in this case, and a chiral Michael acceptor, such as tri-*O*-benzyl-2-nitro-D-galactal, affording a unique diastereoisomer of the eight possible ones.[1]



Using this adduct as starting point, we have been able to synthesize neoglycoamino acids, analogs of Tn antigen (α-*O*-GalNAc-Ser/Thr), which is recently attracting a deal of interest for the development of vaccines for cancer treatment.[2] We have carried out the conformational analysis in aqueous solution of these compounds, as well as *enzyme-linked lectin assays* (ELLA) to determine their behavior as Tn antigen mimics.

Following this synthetic strategy, a battery of different compounds structurally analogs of Thiamet-G is also presented here. Thiamet-G has been described as a potent inhibitor of 2-acetamido-2-desoxy-β-D-glucopyranosidase and it is being investigated as therapeutic potential for the treatment of Alzheimer.[3] Some of these compounds have shown interesting results as inhibitors.

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XI CARBOHYDRATE SYMPOSIUM

A study of CH/π interactions and their role in the stabilization of carbohydrate-receptor complexes by using model systems

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Carbohydrates play an important role in numerous biological routes.[1,2,3] Besides being considered a natural storage of energy in Nature, it is known that the specific interaction between sugars and their natural receptors (i.e. lectins) triggers many crucial biological processes. Thus, knowledge of both the chemical and structural factors that are decisive for an effective interaction is of great interest for the development of novel sugar-based drugs. Hence, biophysical techniques such as X-Ray crystallography or NMR spectroscopy, among others, usually assisted by molecular modeling, have been extensively used in this field to confirm conformational requirements that favour the formation of sugar-protein complexes.

Among the stabilizing factors that may contribute for an effective ligand-protein interaction into the receptor active site, we are particularly interested in the so-called "CH/π interaction" between carbohydrates and aromatic amino acid side chains[4,5,6] In this context, herein we present our investigations on the interaction of simple carbohydrates or analogous with aromatic moieties by NMR spectroscopy and molecular modeling. We have analyzed the variations in the chemical shifts of several sugar-like ligands upon addition of aromatic entities which have been correlated with specific interaction geometries.

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XI JORNADAS DE CARBOHIDRATOS

Targeting galectins. Virtual screening of fragment libraries for design of novel glycans

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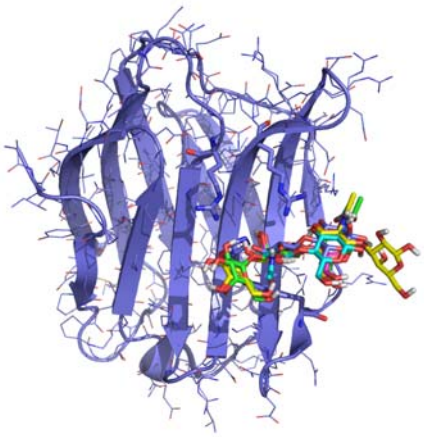
Carbohydrates are involved in a variety of physiological processes, acting as signals for cellular recognition.[1] Among these processes, immune and inflammatory responses, organogenesis, metastasis, and diverse infectious processes should be mentioned.[2] In this context, the search of novel ligands able to mimic oligosaccharides in the binding sites of lectins (such as galectins), antibodies, and enzymes is currently a topic of major interest because of its long-range potential for clinical applications.[3] In particular, we are interested in the identification of novel scaffolds able to lead to second generation of LacNAc derivatives with putative modulating activity on human galectins 1, 3, and 7. We have undertaken docking and virtual screening studies to identify moieties which can be accommodated in the secondary pockets close to the carbohydrate recognition domains of galectins. These studies can assist in the design of synthetic glycans with potential therapeutic applications.[4,5]



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