Molecular recognition of galectins by different glycans: *in silico* comparative studies

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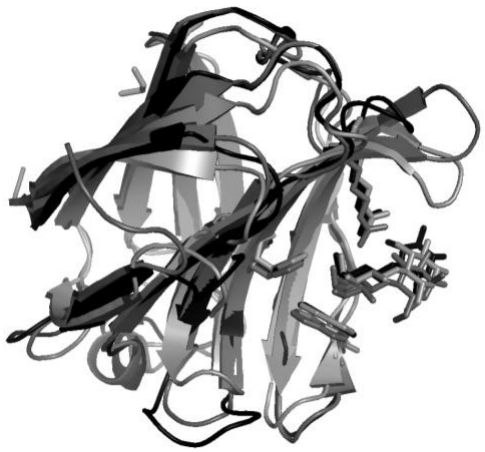
Carbohydrates are involved in a variety of physiological processes, acting as signals for cellular recognition.[1] Among these processes, immune and inflammatory responses, organogenesis, metastasis, and diverse infectious processes should be mentioned.[2] In this context, the elucidation of the mechanisms that govern how oligosaccharides are accommodated 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.



In particular human galectins 1, 3, and 7 (in figure complexed with lactose), present specific and selective recognition for different types of glycans, also exhibiting differencies in the recognition patterns. Taking advantage of experimental data, computational studies were performed [3]. By means of docking and MD simulations, it is possible to deepen into the specific recognition of different glycans, and into the key carbohydrate/protein interactions at atomic detail. These studies can assist in the design of novel ligands with potential therapeutic applications.

[1] Gabius, H.J.; André, S.; Jiménez-Barbero, J.; Romero, A.; Solis, D. *Trends Biochem. Sci.* **2011**, *36*, 298.
[2] Gabius, H.J. *The Sugar Code. Fundamentals of glycosciences;* Wiley-VCH: Weinheim, **2009**.
[3] Martin-Santamaria, S.; Gabius, H.J.; Jiménez-Barbero, J. *Pure Appl. Chem.* **2012**, *84*, 49.

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XI JORNADAS DE CARBOHIDRATOS

gem-Difluoro-carbasugars: the recovery of the exo-anomeric effect restrains the conformational space

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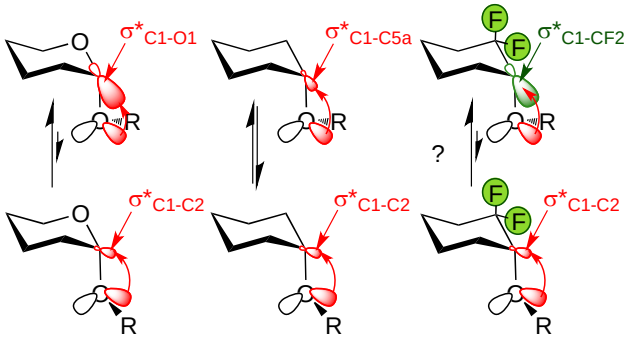
Molecular recognition of carbohydrates is at the heart of different events of paramount biomedical interest. In this context, the employment of sugar mimics as enzyme inhibitors or molecular probes has been widely developed in the last years [1]. Among them, we herein present our advances in the use of fluorine-containing carbasugars. The replacement of the ring-oxygen by a CHF or CF_2 group could somehow restore the typical stereo-electronic effects occurring in natural sugar as the anomeric effect [2].



The origin of the anomeric effects remains a topic of discussion [3]. Herein, the stereoelectonic properties of a novel type of sugar mimics whose endocyclic oxygen atom has been replaced by CF_2 (gem-difluorocarbasugars) have been explored by NMR spectroscopy and computational methods. Strikingly, these difluorinated pseudosugars retain the structural features of the exo-anomeric effect, a key factor for modulating the conformational preferences of these relevant biomolecules. The presence of the exo-anomeric effect is demonstrated both experimentally and theoretically.

[1] Deleuze, A., Menozzi, C., Sollogoub, M., & Sinay, P. *Angew. Chem.* 2004, **48**, 6848-6851
[2] Jiménez-Barbero, J., Sollogoub, M., et al. Chem. Asian J. 2008, **3**, 51-58
[3] Cocinero, E. J., Çarçabal, P., Vaden, T. D., Simons, J. P., and Davis, B. G. *Nature Letters, 2011, **469**, 76-80.*

Understanding of Toll-Like Receptor 4 recognition: searching for novel modulators and probes

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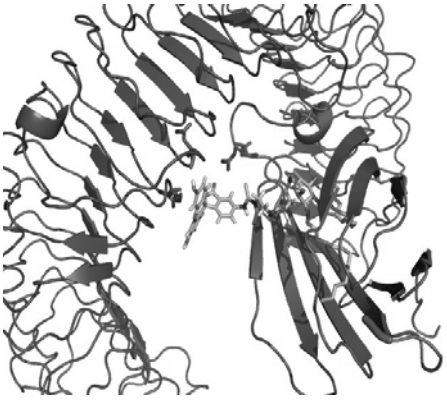
The family of Toll-like receptors (TLRs) are potential regulators and controllers of the immune response through their ability to recognize pathogen-associated molecular patterns. TLRs are involved in the modulation of the immune system, including the processes of inflammation and defence against cancer. In particular, TLR4 is located in the plasma membrane, where binds to lipopolysaccharides (LPSs), a membrane constituent of gram-negative bacteria, and together with MD-2, forms a heterodimeric complex which leads to the activation of the innate immune system response.[1] LPS interaction with MD-2/TLR4 involves at least two other proteins: the lipopolysaccharide binding protein, and CD14. The major role for CD14 is to enhance the sensitivity of the TLR4/MD-2 signaling complex, dropping the binding affinity for LPS to picomolar concentrations.



TLR4 activation has also been associated with certain autoimmune diseases, noninfectious inflammatory disorders, and neuropathic pain, suggesting a wide range of possible clinical settings for application of TLR4 antagonists.[2] However, agonists of TLR4 can also be useful as adjuvants in vaccine development and in cancer immunotherapy.

Since the direct interactions of these proteins with different ligands remain unclarified, we have undertaken computational studies in order to characterize, at atomic level, the involved molecular recognition processes, and to understand the key features of agonism/antagonism behaviour to propose new chemical scaffolds for the development of new TLR4 modulators and probes.[3]

[1] Park, B.S.; Song, D.H.; Kim, H.M.; Choi, B.S.; Lee, H.; Lee, J.O. *Nature*. **2009**, *458*(7242), 1191-5.
[2] Peri, F. Piazza, M. *Biotechnol. Adv.* **2012**, *30(1)*, 251.
[3] R. Cighetti, C. Ciaramelli, S. Enza Sestito, I. Zanoni, L. Kubik, A. Ardá-Freire, V. Calabrese, F. Granucci, R. Jerala, S. Martín-Santamaría, J. Jiménez-Barbero, F. Peri. *ChemBioChem*, **2014**, *15*, 250.

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XI JORNADAS DE CARBOHIDRATOS

Rapid discovery of potent α-L-fucosidase inhibitors by *in situ* screening of (pyrrolidin-2-yl)triazoles

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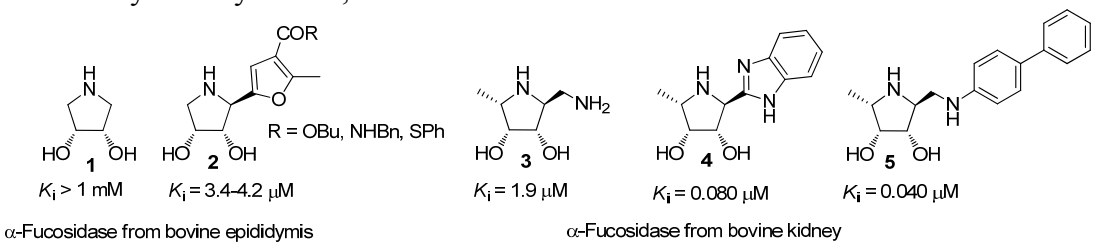
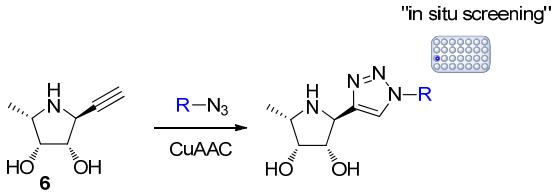
Over recent years we have been actively working on the development of new iminocyclitols with inhibitory activity towards α-L-fucosidases.[1-3] We have shown that the presence of an additional heteroaromatic (furyl, imidazolyl) or aromatic (phenyl, biphenyl) moiety, close to a five membered iminocyclitol framework, increases notably their inhibitory activity: **1** *vs* **2**, or **3** *vs* **4** and **5**.



In order to look into chemical diversity on the above 5-membered iminocyclitols by the systematic variation of the aromatic group in a quick and easy way, we have applied the *in situ* screening towards α-L-fucosidases by means of copper catalyzed click chemistry, CuACC.[4] Click reaction between unprotected alkynyl iminocyclitol **6** with synthetic or commercial azides afforded a small library of (pyrrolidin-2-yl)triazoles. This aproximation has allowed the discovery of an α-L-fucosidase inhibitor in the low nanomolar range.



The synthesis of lead compound **6** as well as the biological results of the library will be reported.

[1] Moreno-Vargas, A. J.; Robina, I.; Demange, R.; Vogel, P. *Helv. Chim. Acta*, **2003**, *86*, 1894.
[2] Moreno-Vargas, A. J.; Carmona, A. T.; Mora, F.; Vogel, P.; Robina, I. *Chem. Commun.* **2005**, 4949.
[3] Moreno-Clavijo, E.; Carmona, A. T.; Vera-Ayoso,Y.; Moreno-Vargas, A. J.; Bello, C.; Vogel, P.; Robina, I. *Org. Biomol. Chem.* **2009**, *7*, 1192.
[4] Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.

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XI CARBOHYDRATE SYMPOSIUM

Serine *versus* Threonine Glycosylation with α-*O*-GalNAc: Implications for the molecular recognition

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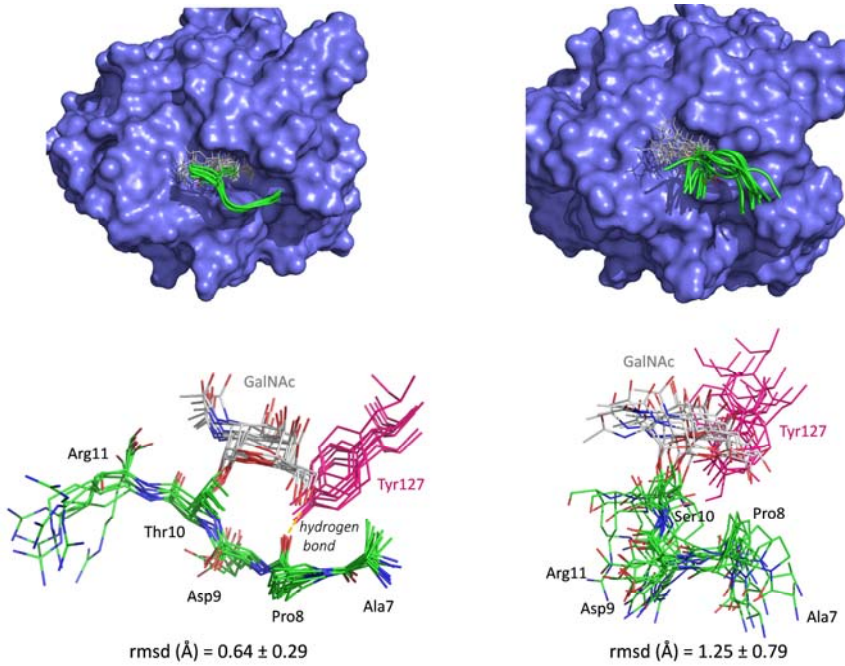
Tn antigen is one of the most specific human tumor-associated structures.[1] Although its structure is referred as α-*O*-GalNAc-Ser/Thr, not discriminating the amino acid to which GalNAc is linked, this work yields unexpected results for molecular recognition of this antigen by three lectins (*Soybean agglutinin* –SBA– , *Vicia Villosa agglutinin* –VVA– and Helix Poamatia agglutinin –HPA–). The study reveals that the aglyconic part of Tn (serine or threonine) plays a subtle but interesting role in the protein recognition. This feature has been tested using different glycopeptides that include Tn antigen (α-*O*-GalNAc-Ser or α-*O*-GalNAc-Thr). For SBA and VVA lectins, Tn antigen bearing threonine exhibits higher affinity than its serine homologue. In contrast, HPA lectin shows a clear preference for the serine-Tn antigen. The origin of the selectivity of the proteins studied here for Tn antigen is explained by the different presentation of the sugar moiety in the bound state.[2]



[1] Ju, T., Otto, V. I., Cummings, R. D. (). *Angew. Chem. Int. Ed.* **2011**, *50*, 1770.
[2] Corzana, F., Busto, J. H., Jiménez-Osés, G., García de Luis, M., Asensio, J. L., Jiménez-Barbero, J., Peregrina, J. M., Avenoza, A. *J. Am. Chem. Soc.* **2007**, *129*, 9458.