MANNOSE-BASED GLYCOMIMETICS ACTING AS SELECTIVE LIGANDS FOR L-SIGN

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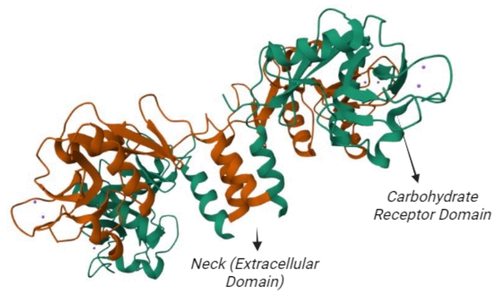
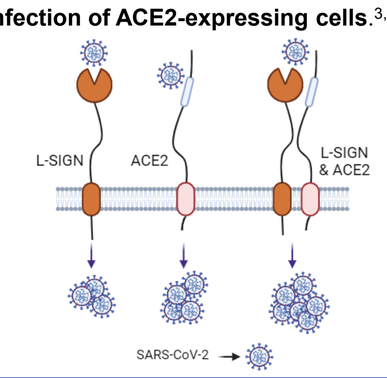
1. L-SIGN: IDENTIKIT

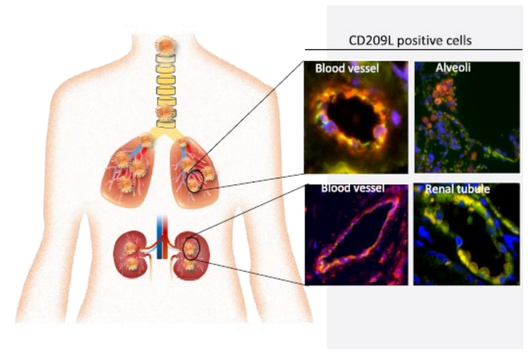
L-SIGN (DC-SIGNR) is a **C-type lectin receptor** (CLR), which has a similar structure to DC-SIGN. Both are transmembrane tetramers with four Carbohydrate Receptor Domains (CRDs). They share 77% of their sequence and show the same ability of binding glycan motifs expressed at the surface of different pathogens thanks to a Ca^{2+} ion in the binding site. Carbohydrate binding to DC-SIGN induces the **activation of the initial stages of adaptive immune response**. Differently from DC-SIGN (expressed by DCs on dermal and mucosal tissues), L-SIGN is specifically expressed by **liver sinusoidal endothelial cells** (LSEC), a liver-resident APC, by **endothelial cells in lymph nodes** and by placenta. Interestingly, L-SIGN is also **co-expressed with ACE2 on respiratory tract cells**.[1]



2. PROBLEM

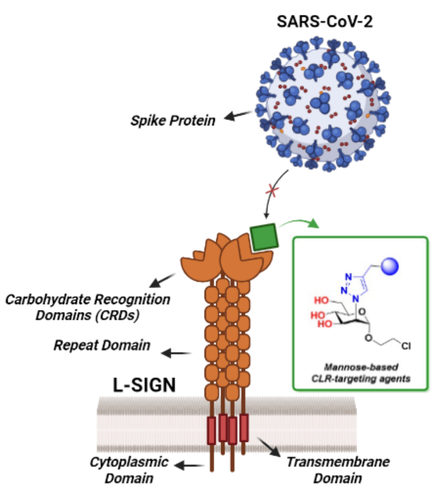
Despite their crucial role in the activation of immune response, it was found that several deadly viruses – such as HIV, Ebola, hepatitis C viruses, Dengue and West Nile virus – have developed strategies to subvert the function of CLRs to escape antiviral immunity and promote infection.[2] In particular, DC-SIGN and **L-SIGN** have been recently found to be **entry co-factors for SARS-CoV-2**, promoting **trans-infection of ACE2-expressing cells**.[3,4]





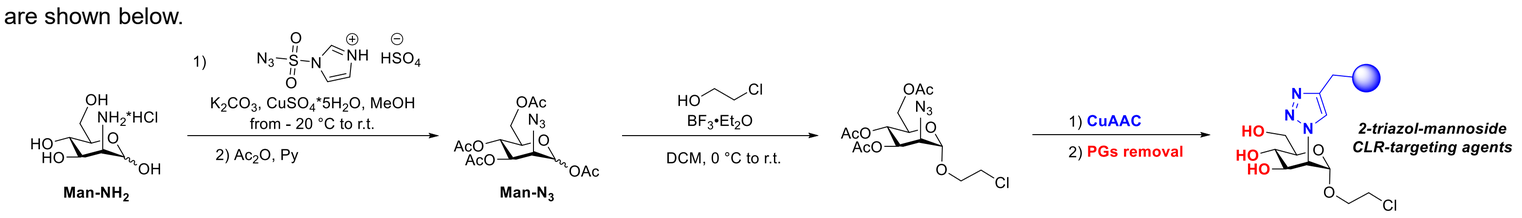
3. OUR APPROACH

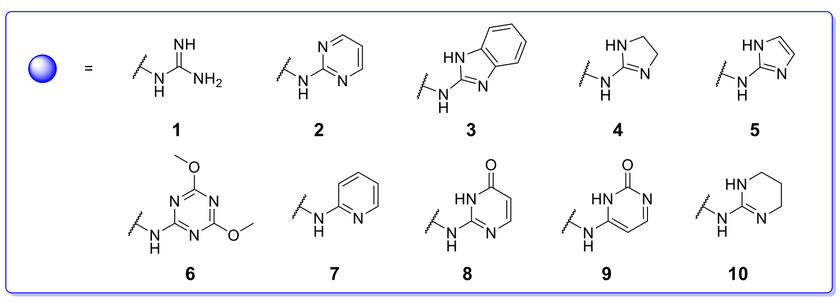
In this context, **adhesion phenomena and entry co-factors** have proven to be crucial elements in the infection process, being also an **interesting source of additional therapeutic strategies**. In particular, the **competitive inhibition of virus binding to host co-receptors** is a valid approach to design antiviral therapies which could avoid the increasing in the frequency of viral mutations.

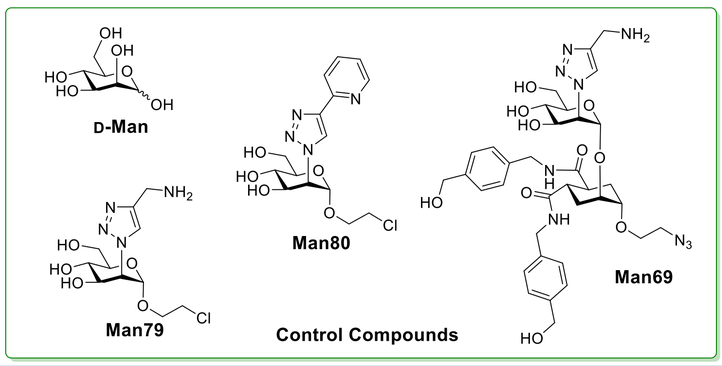


4. SYNTHETIC PATHWAY

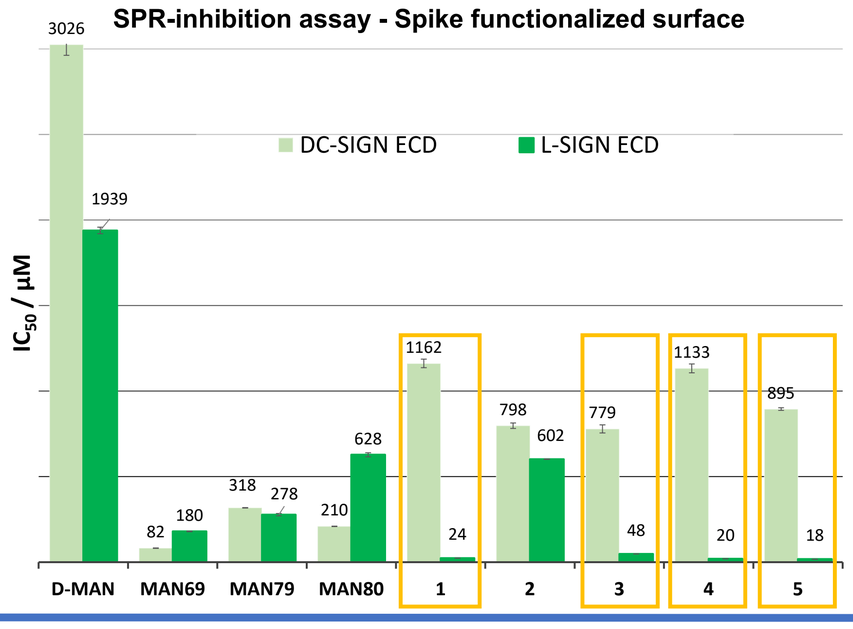
Due to the crucial role of L-SIGN in SARS-CoV-2 trans-infection process, we aimed to design and synthesize the first set of C2-modified monomannoside glycomimetics[5] showing high selectivity towards L-SIGN against DC-SIGN. The synthetic pathway and the structures of the ligands are shown below.







5. RESULTS

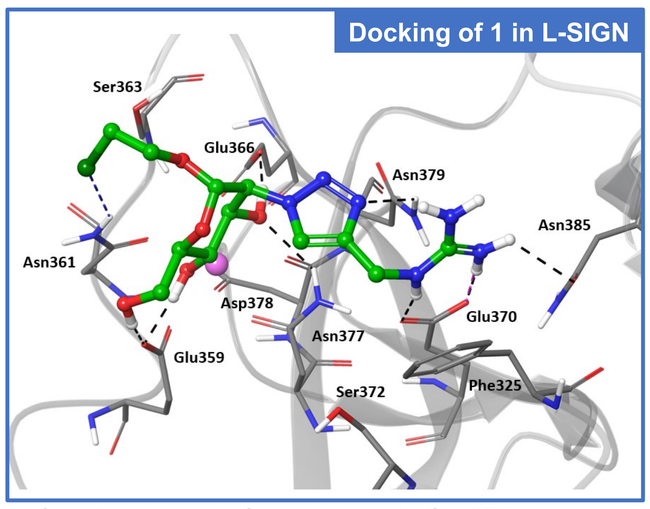


- C2-functionalization generally enhances ligand affinity towards both DC and L-SIGN relative to control compound **D-Man**;
- Pseudo-dimannoside **Man69** shows a very good affinity for DC-SIGN while the selectivity is poor (factor of 2);
- Removal of the cyclohexane moiety from **Man69** to **Man79** induces just a moderate loss in affinity (2-fold decrease), with obvious synthetic advantages;
- The monomannoside bearing a triazolyl-pyridine moiety (**Man80**) displays a moderate selectivity (3-fold) and a strong affinity for DC-SIGN;
- The introduction of a triazolyl-guanidine moiety as in **1** dramatically enhances the selectivity towards L-SIGN over DC-SIGN (48-fold);
- Strong selectivity for L-SIGN is shown for 1, 3, 4 and 5: in particular, the triazolyl-2-aminoimidazole-functionalized monomannoside **5** shows the lowest IC_{50} value in the series (IC_{50} (**5**) = 17,9 ± 0,1 µM). The monomannoside **2**, bearing a triazolyl-aminopyrimidine moiety, displays a moderate selectivity and a low affinity for L-SIGN;
- The common feature of the best L-SIGN ligands **1**, **3**, **4** and **5** is the presence of a "guanidine-type" cation at physiological pH. This is probably able to establish the same set of hydrogen bonding interactions with Glu370/Asn379/Asn385 residues in the binding pocket of the lectin, as shown below in the docking of **1** in L-SIGN (Glide XP V7.8, Schrödinger);
- The crystal structure of **1** in L-SIGN (not shown) and the related docking studies suggest that DC/L-SIGN selectivity may arise from **different binding modes** for the guanidium ion in the two lectins.

6. CONCLUSIONS

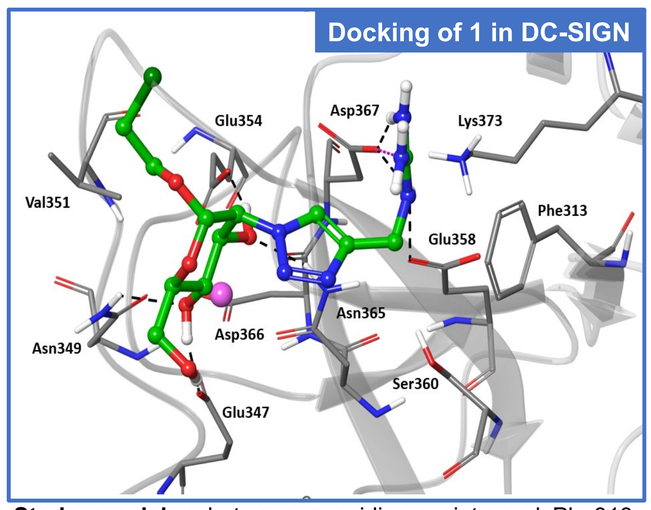
- We have here reported the first set of glycomimetic ligands showing a high selectivity towards L-SIGN against DC-SIGN;
- The activity of the studied compounds was determined through an inhibition SPR experiment, based on the use of immobilized SARS-CoV-2 spike protein as a reporter;
- Nine ligands were synthesized, five (**1-5**) have been tested, so far: they all show a higher selectivity for L-SIGN relative to DC-SIGN;
- Compound **5** is the **most potent binder known for L-SIGN**. It was found to inhibit L-SIGN binding to the SPR surface 108 times better than control compound, **D-Man** (IC_{50} (**5**) = 17,9 ± 0,1 µM Vs IC_{50} (**D-Man**) = 1939 ± 19 µM) and has a 50-fold selectivity against DC-SIGN;
- Compound **4** has a 58-fold selectivity for L-SIGN (IC_{50} = 19,5 ± 0,1 µM) over DC-SIGN (IC_{50} = 1133 ± 26 µM).

Docking of 1 in L-SIGN



Bifurcated H-bond of guanidine with Glu-370**. Favorable cation-π interactions** with Phe325 (PDB to be deposited).

Docking of 1 in DC-SIGN



Steric repulsion between guanidine moiety and Phe313 forces a **non-favorable binding mode of the guanidine** which disrupts Asp367-Lys373 electrostatic interaction (PDB 6GHV).

7. REFERENCES

[1] R.K. Gupta and G.S. Gupta, *Animal Lectins: Form, Function and Clinical Applications*, **2012**, 773–798 (Springer Vienna); [2] P.P. Avdonin, E.Y. Rybakova, S.K. Trufanov and P.V. Avdonin, *Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology*, **2023**, 17, 1–11; [3] M.Thépaut, J.Luczkowiak, C.Vives, N.Labiod, I.Bally, F.Lasala, Y.Grimoire, D.Fenel, S.Sattin, N.Thielens, G.Schoehn, A.Bernardi, R.Delgado and F. Fieschi *PLoSPathogen*, **2021**,17, 1-27; [4] S. Pollastri, C. Delaunay, M. Thépaut, F. Fieschi and A. Bernardi *Chem. Commun.* **2022**, *58*, 5136–5139; [5] A.Bernardi., S.Pollastri, C.Delaunay, M.Thépaut and F.Fieschi Patent application nr102022000008474; PCT/IB2023/054291