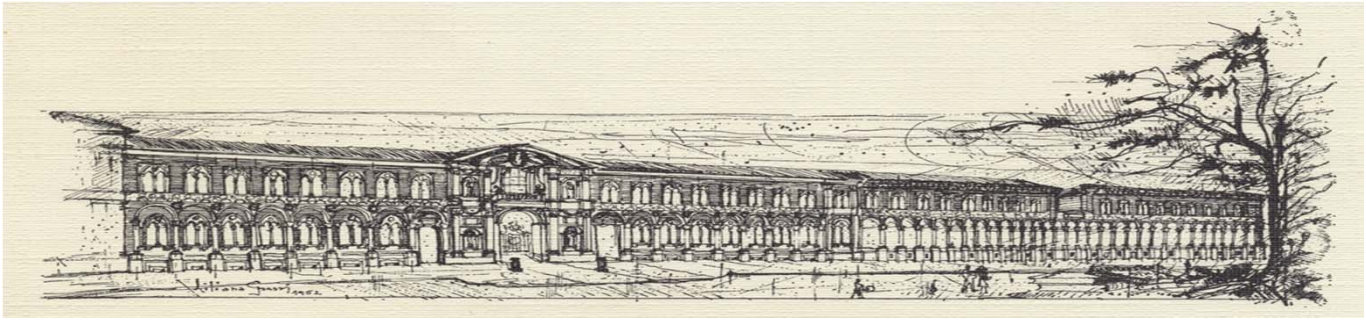
Disrupting Glycan-Protein Interactions with Glycomimetics

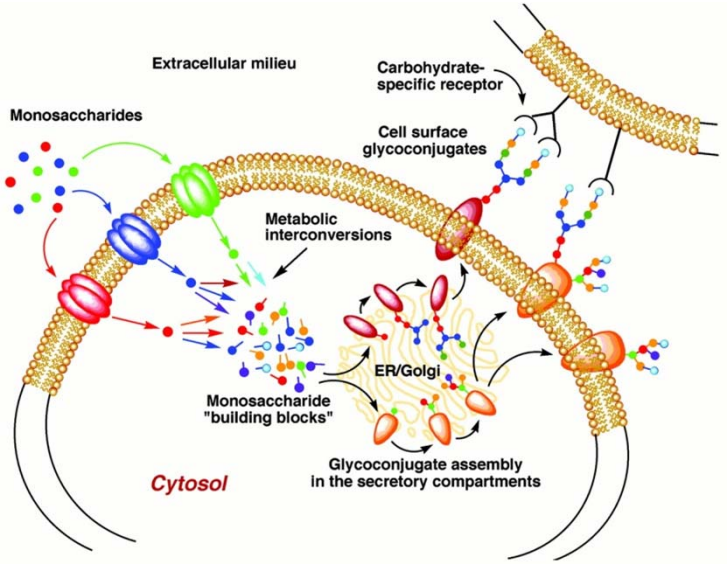
Anna Bernardi – Università degli Studi di Milano - Dipartimento di Chimica



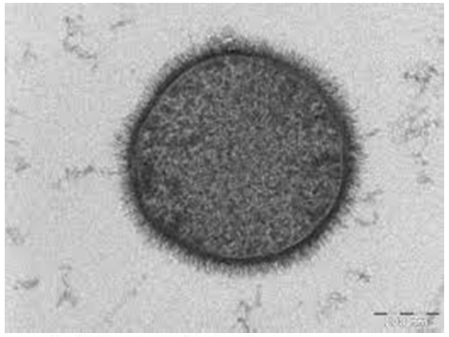
IASOC 2022 – Ischia 23-26 Sep 2022

Why glycan-protein interactions ?

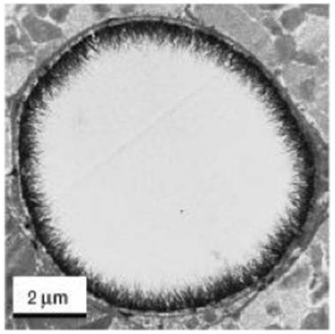
Carbohydrates

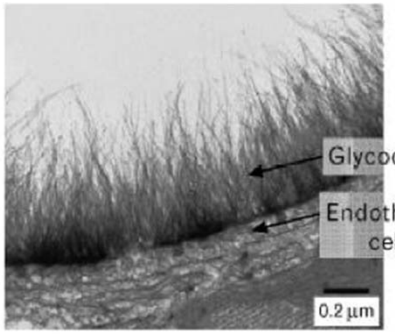


Glycocalyx



TEM micrograph of a B. subtilis cell

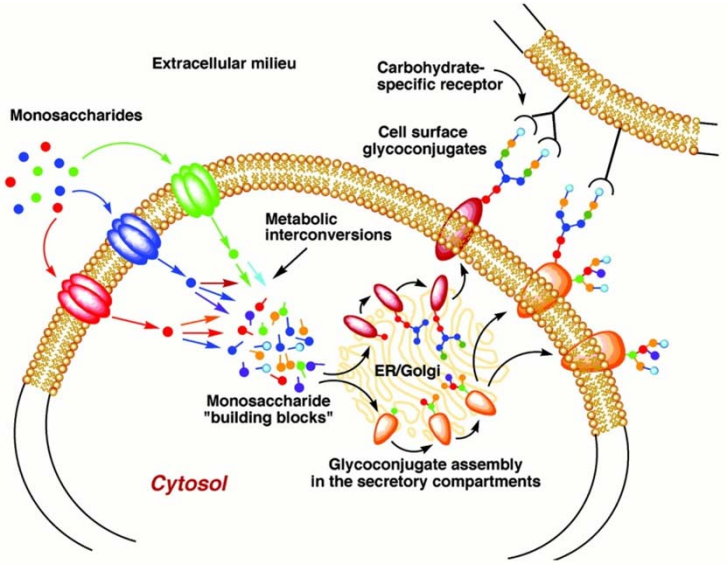


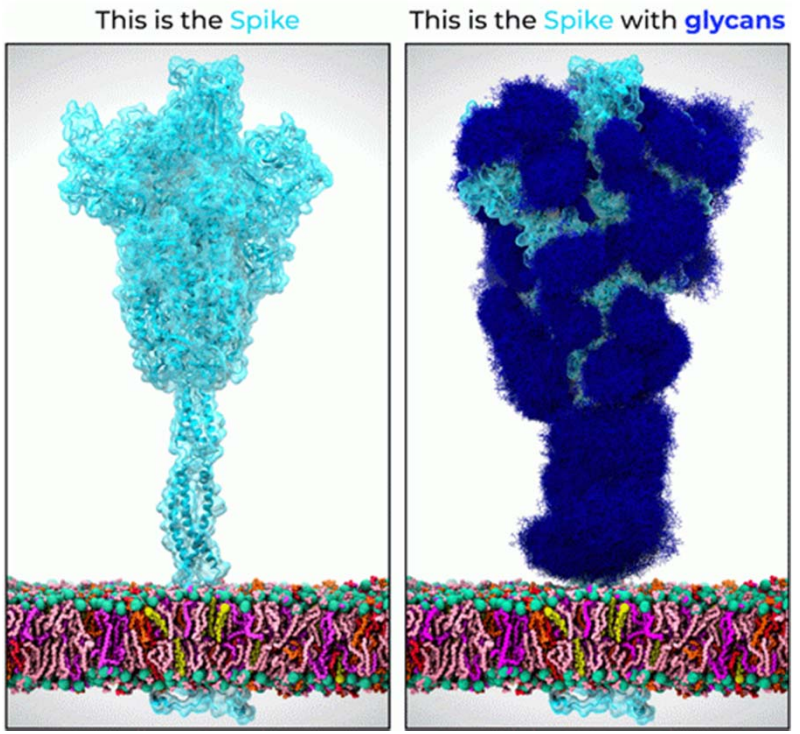


Blood vessel

Why glycan-protein interactions ?

Carbohydrates





Elisa Fadda et al bioRxiv (2020)

Glycomimetics: rationale and challenges

- **Sugar/protein interactions as target. Development of probes, diagnostics, delivery vectors and drugs**

  Glycomimetics can be

  ✓ more metabolically stable and bioavailable than oligosaccharides (more drug-like)

  ✓ easier to synthesize

  ✓ more active (higher affinity) and selective

- **Challenges**

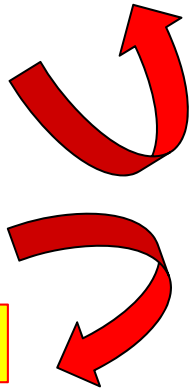
  Low affinity

  Large binding sites, solvent exposed

  Affinity dependent on solvation/desolvation

  Many lectins with overlapping specifity

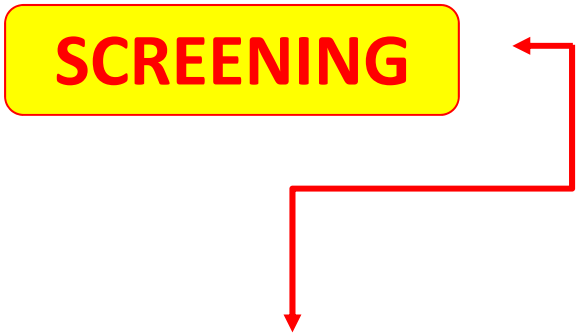
Hard to predict
Computational tools often fail



Selectivity ?

Glycomimetics: some solutions

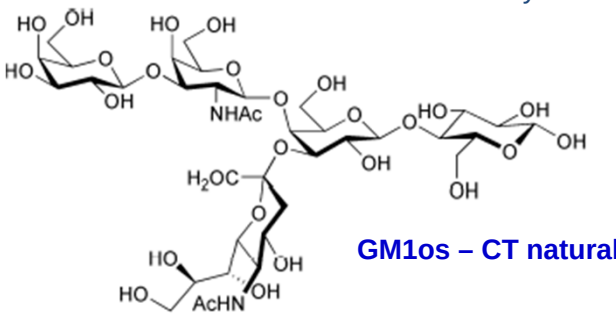
- DESIGN BASED ON LIGAND STRUCTURE
- THE SUGAR ANCHOR
- USE FRAGMENT-BASED DESIGN
- EXPLOIT MULTIVALENCY



✓ AVOID GLYCOSIDIC BONDS	✓ KEEP IT SIMPLE
✓ INCREASE LIPOPHILICITY	✓ EXPLOIT MULTIVALENCY

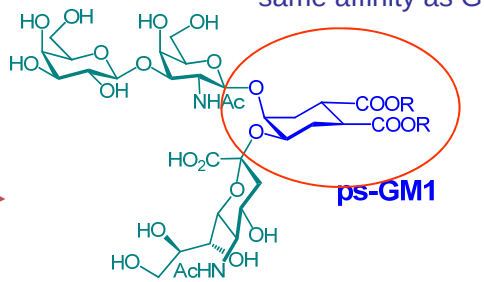
Design based on ligand structure: cholera toxin

GM1-os affinity 43 nM

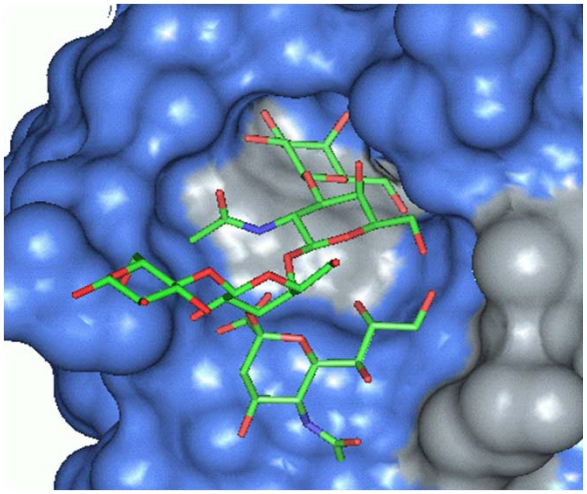


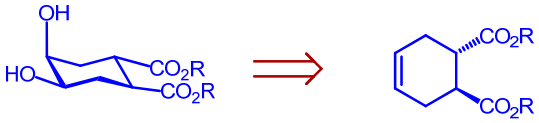
GM1os – CT natural ligand

same affinity as GM1-os



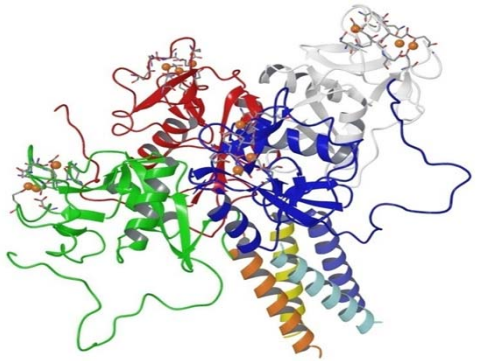
J. Am. Chem. Soc. **1999,** *121*, 2032

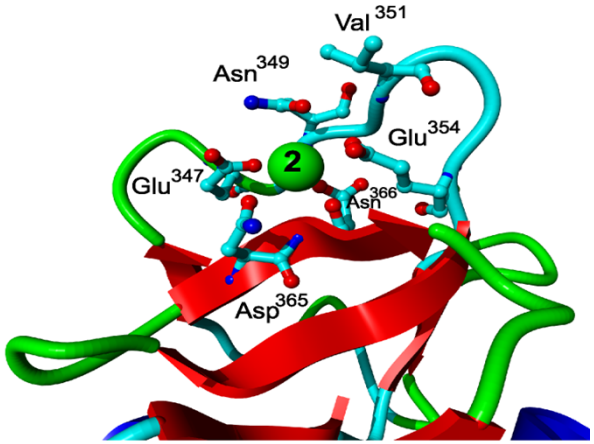




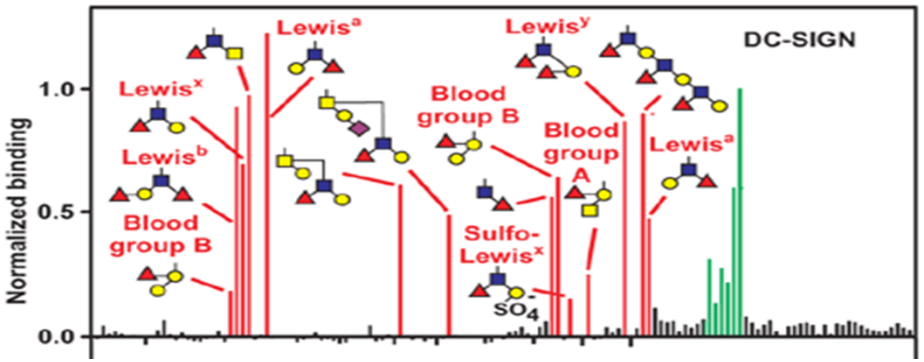
A. Bernardi, P. Cheshev *Chemistry Eur. J.* **2008**, *14*, 7434-7441

Design based on ligand structure: DC-SIGN



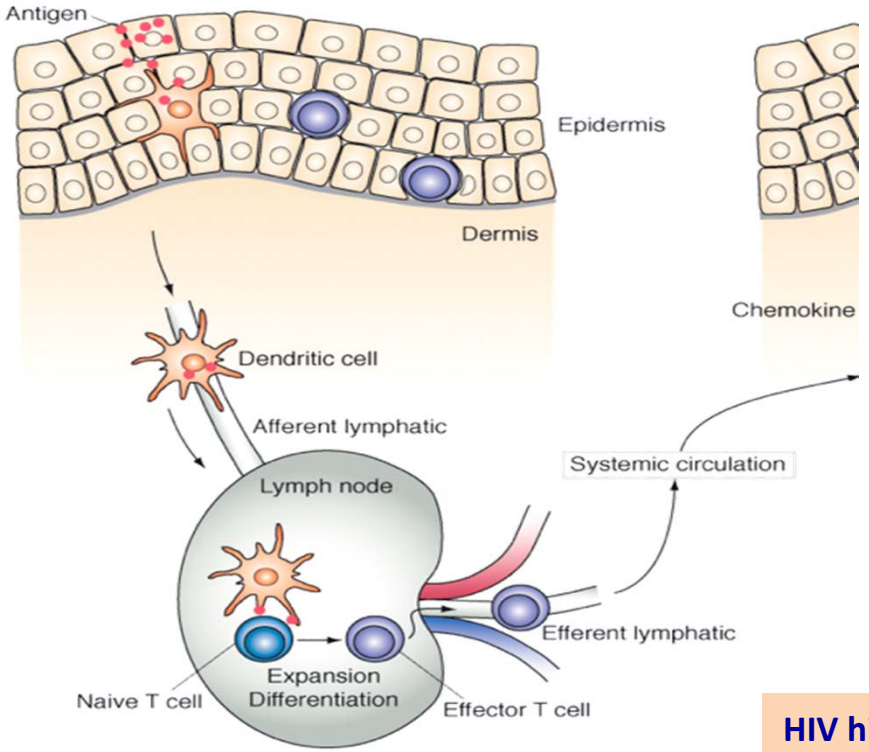


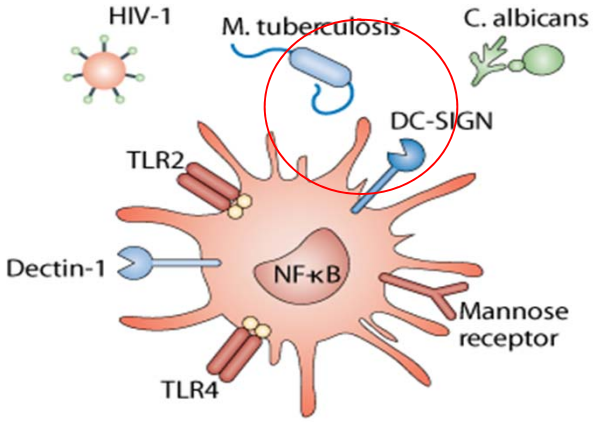
- Type-II transmembrane protein (C type – lectin Ca^{2+}-dependent interaction) mainly expressed by **Dendritic Cells**
- DC-SIGN recognizes many important pathogens among which: **HIV, Ebola, Dengue, Leishmania, M. Tuberculosis, Candida Albicans, Hepatitis C, SARS-CoV2**
- Generally presented as a **homotetramer**
- Binds **Mannose oligosaccharides** and **Fucosylated Lewis-type structures**

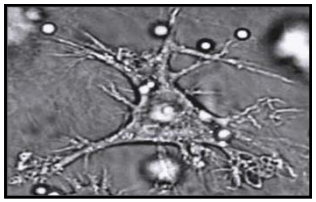


K. Drickamer *et al.*, Nat. Struct. Mol. Biol., **2004**, *11*, 591
Li Wu & Vineet N. KewalRamani, *Nat. Rev. Immunol*. **2006**, *6*, 859

Dendritic cells : immunity guard







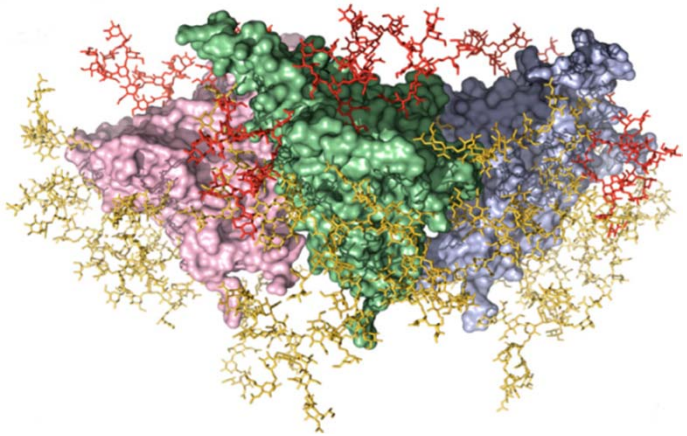
HIV hijacks dendritic cells and uses DC-SIGN to enhance infection

Antiadhesive prophylaxis can prevent HIV and Mycobacterium tuberculosis infection

- first encounter site with pathogens
- single cell able to activate naïve T lymphocytes
- initiate specific immune response

Courtesy of F. Fieschi and T. Geijtenbeek

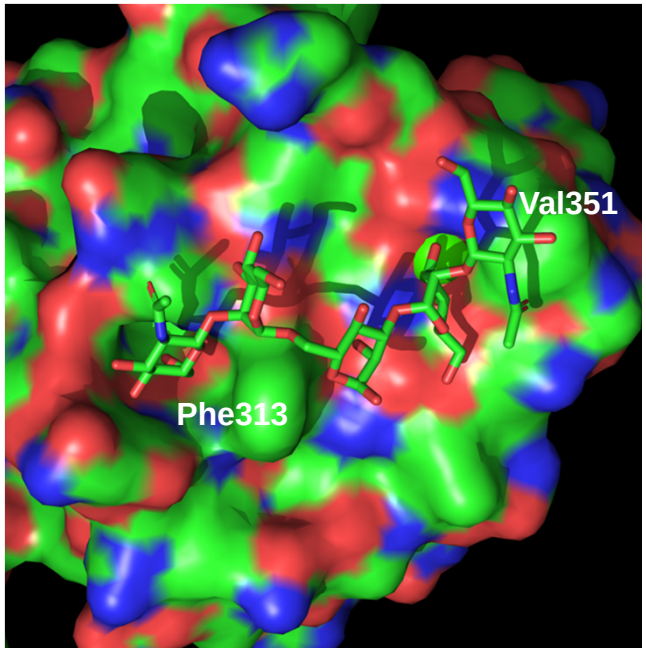
Design based on ligand structure: DC-SIGN



Doores *et al PNAS* **2010**, 17107

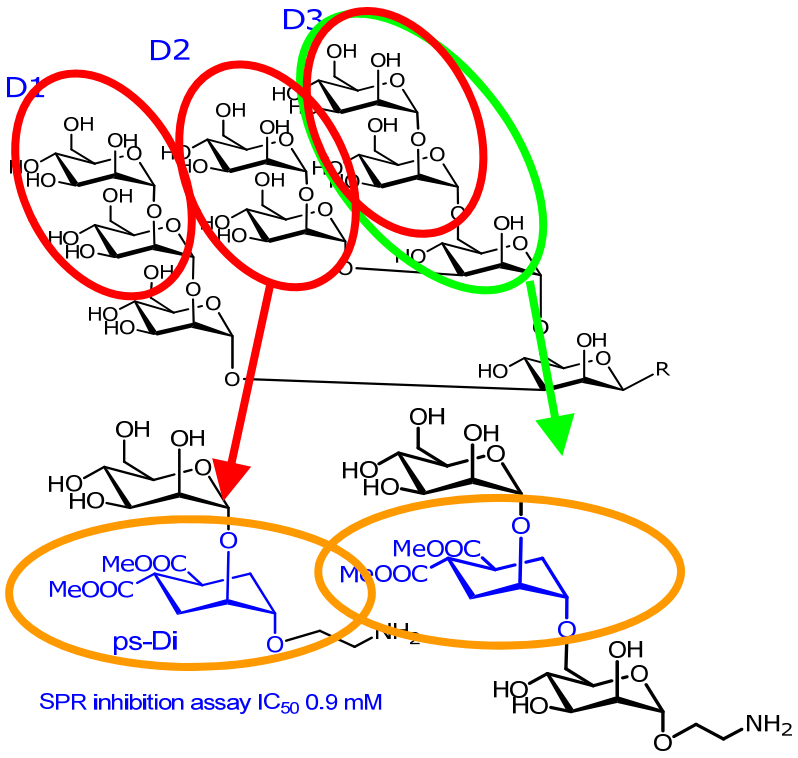
Man_9
Estimated affinity 0.21 mM

Kiessling *et al Chem. Commun*. **2010**, 46, 6747

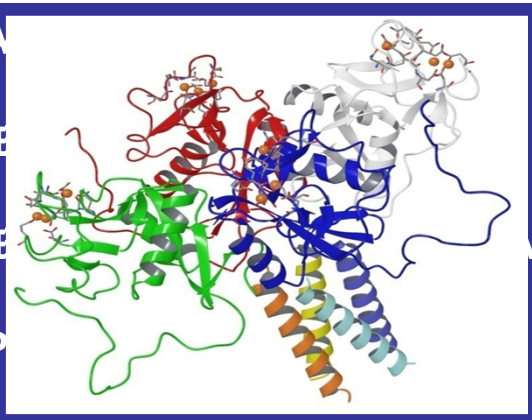


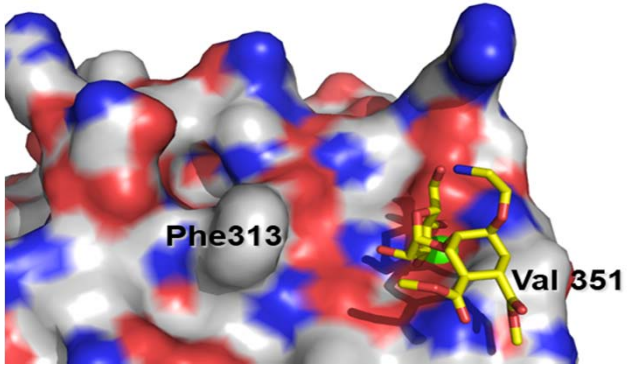
1K9I
$GlcNAc_2Man_3$
Science **2001**, *294*, 2163-2166

Design based on ligand structure: DC-SIGN



- ✓ UNNA
- ✓ INCRE
- ✓ INCRE ABILITY
- ✓ EQUIP
- ✓ SIMPLE SYNTHESIS

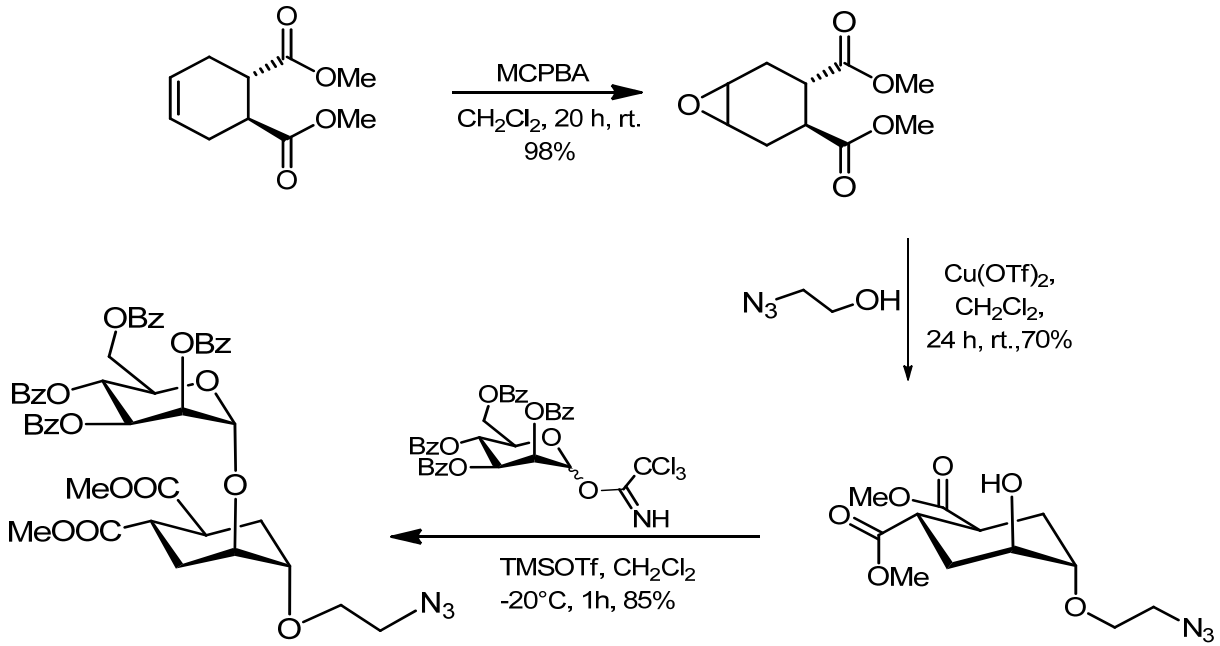




Thépaut et al *J. Am. Chem. Soc.* **2013**, 2518

Ps-diMan : the synthesis

Sara Sattin

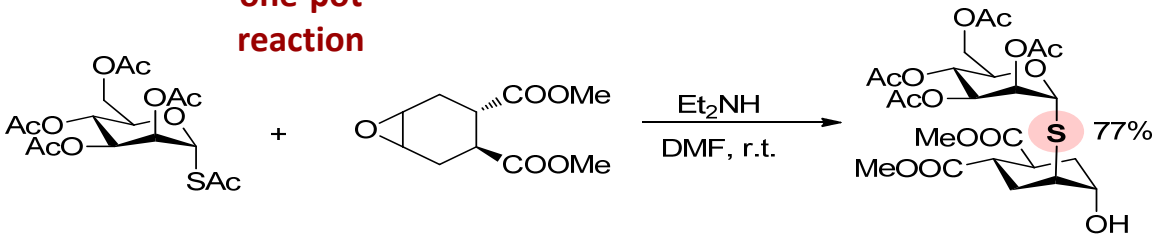


Sattin et al *ACS Chem. Biol.* **2010**, *5*, 301

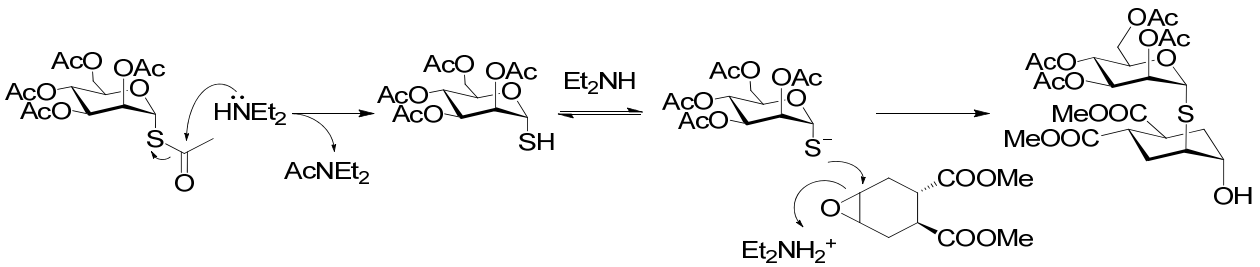
Thioglycosides: Faster synthesis – More stability

Alice Tamburrini

one-pot reaction



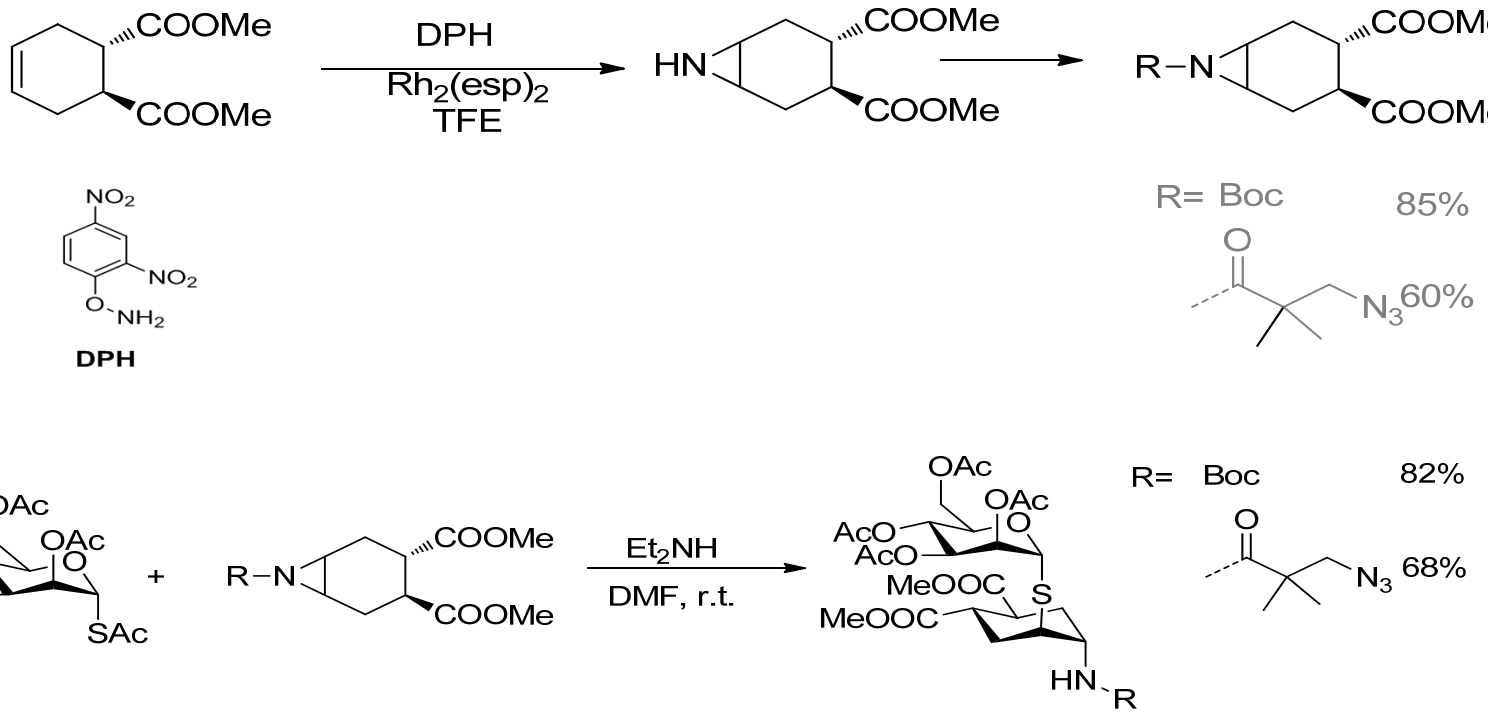
- ✓ **Same DC-SIGN affinity as ps-di**
- ✓ **Stable to jack-bean mannosidase for over 24 h**



Tamburrini et al *Bioorg&Med Chem.* **2017**, *25*, 5142

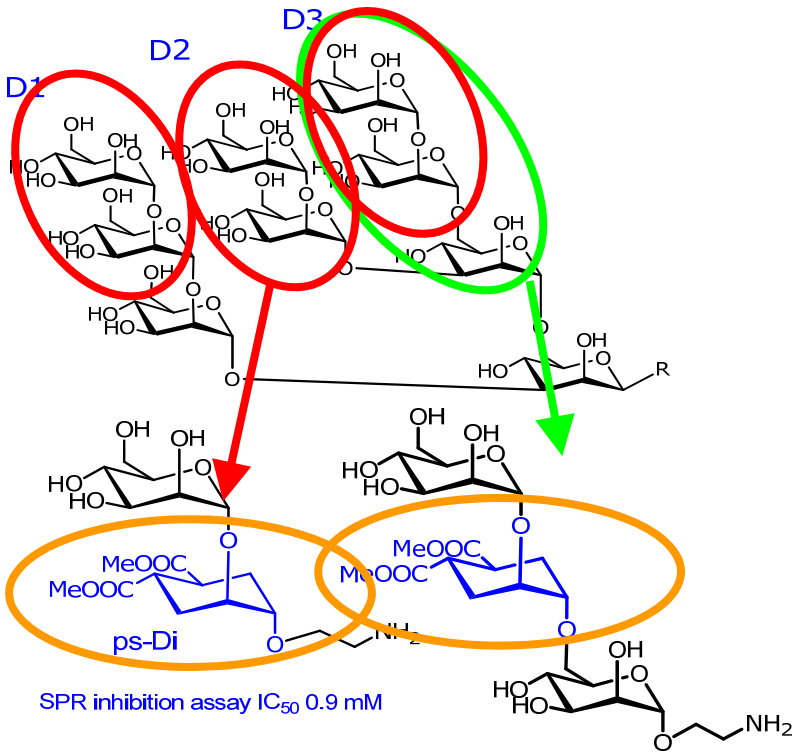
…or with an aziridine…

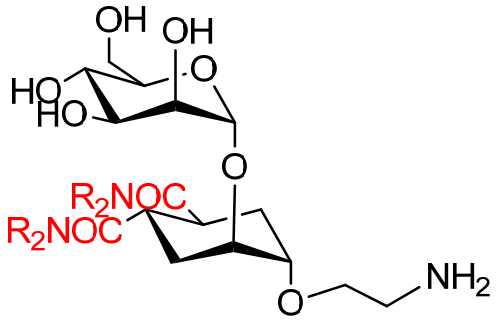
Nives Hribernik

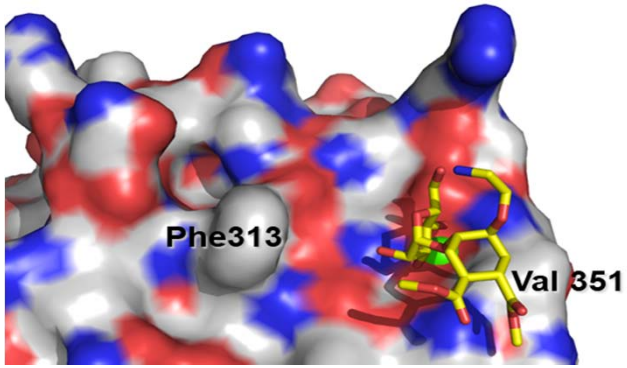


Hribernik *et al* *Org. &Biomol.Chem*. **2021**, 233

Design based on ligand structure: DC-SIGN





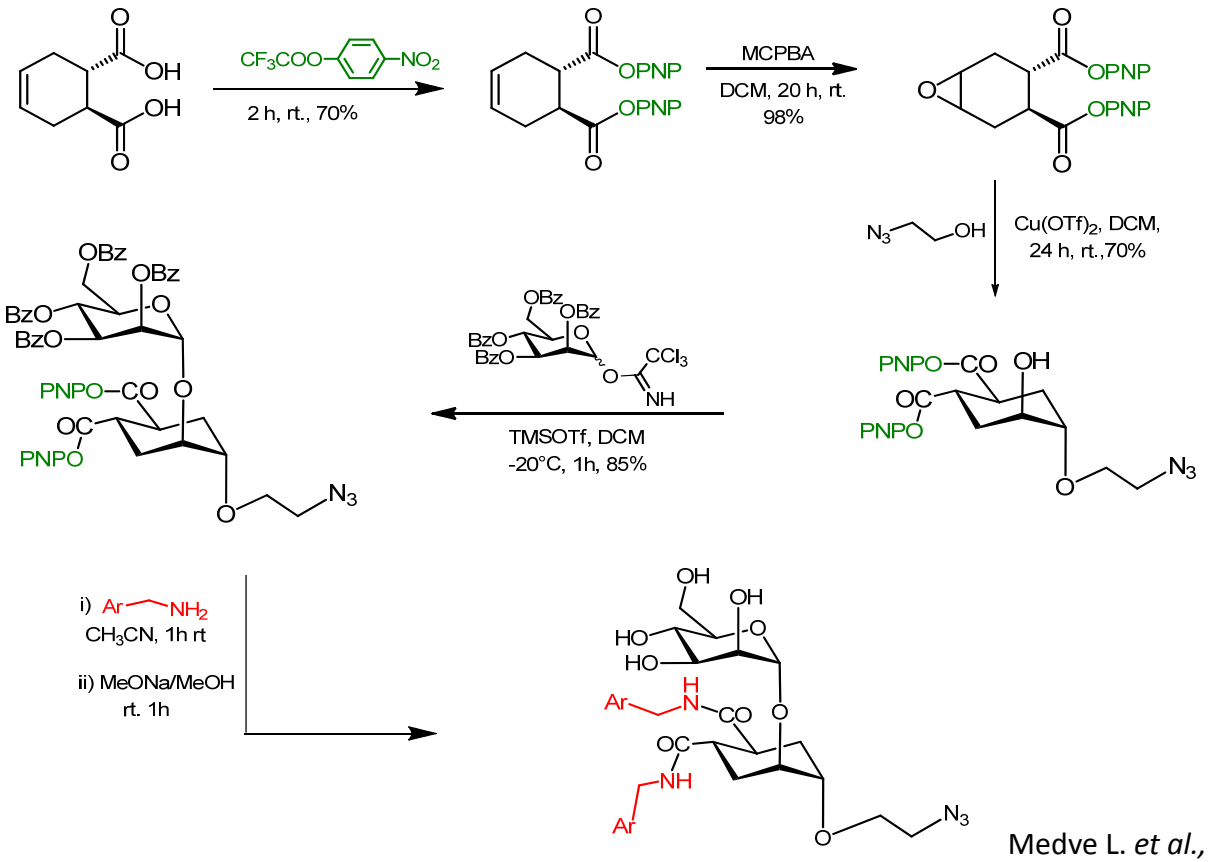


Thépaut et al *J. Am. Chem. Soc.* **2013**, 2518

Man-based antagonists: the synthesis

Norbert Varga

Laura Medve

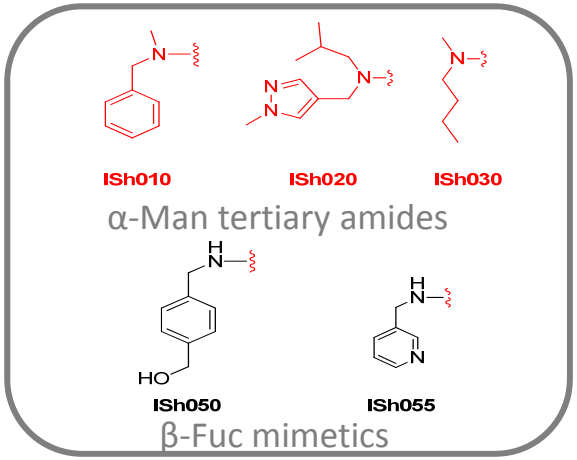


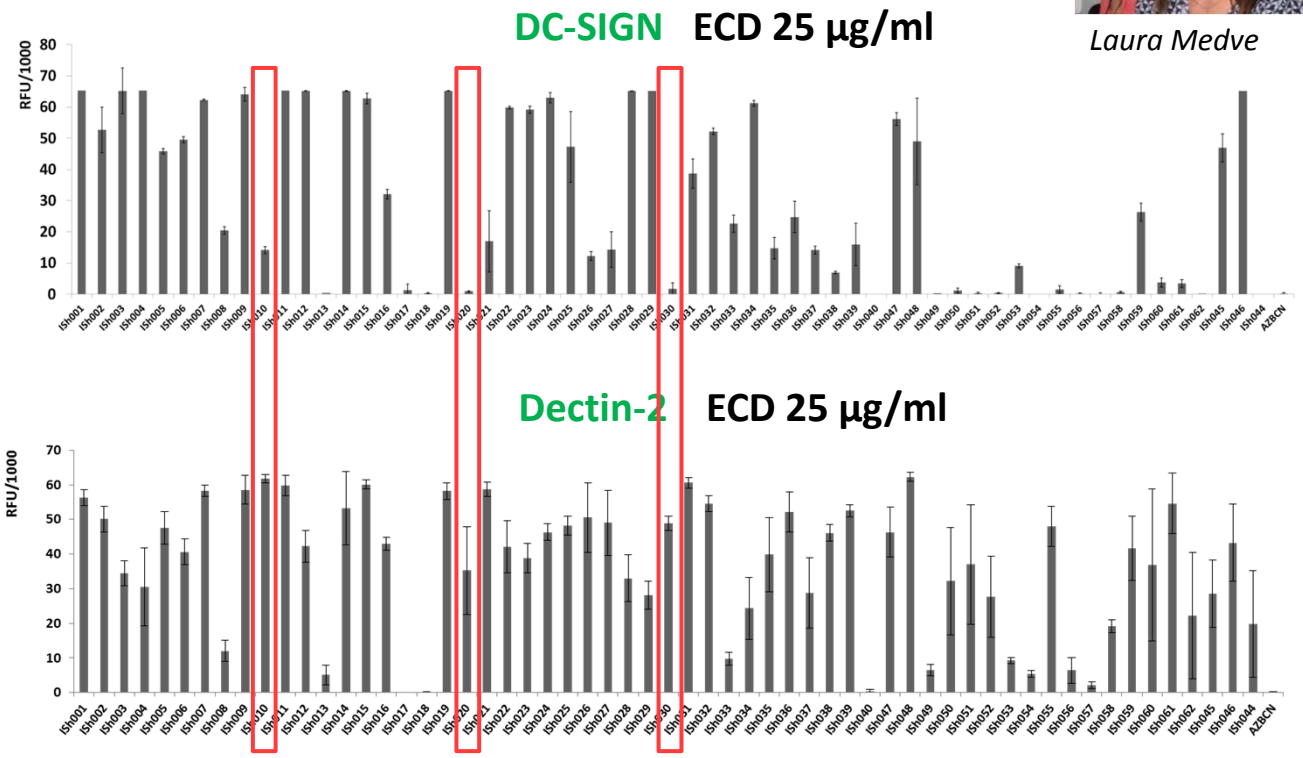
Medve L. *et al., Chem. Eur. J.* ***2019,*** *25,* 14659

Microarray Screening

Laura Medve

Tertiary amides of ps-Di **selective for Dectin-2 over DC-SIGN**

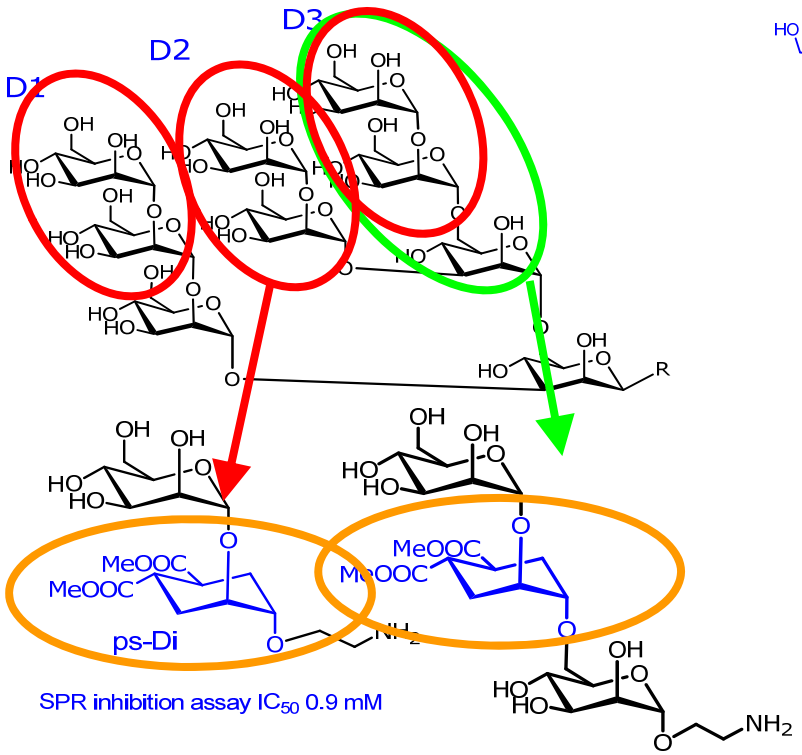


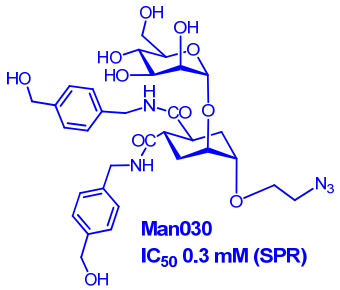


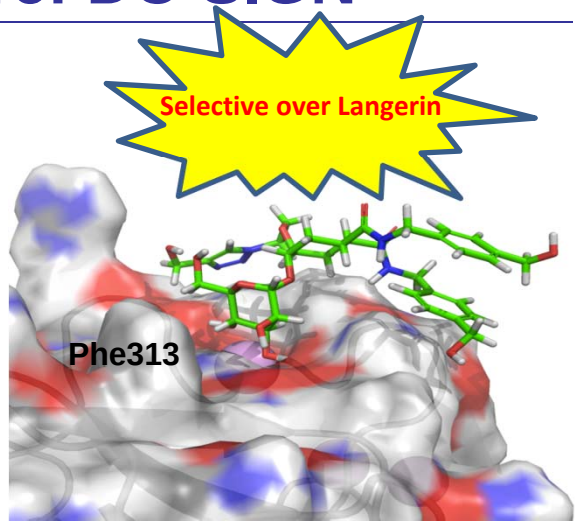
CIC biomaGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

Medve *et al Chem. Eur. J.* **2018**, *24,* 14448

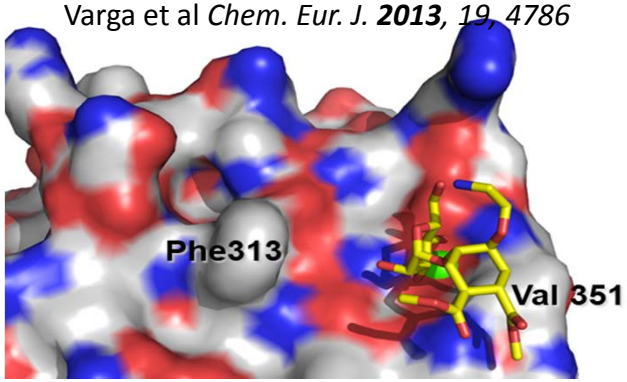
Design based on ligand structure: DC-SIGN







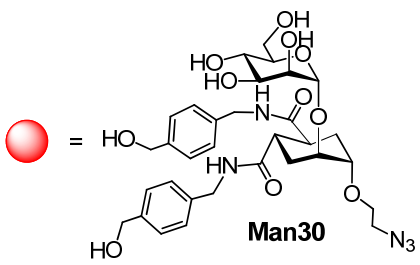
Varga et al *Chem. Eur. J.* ***2013***, *19, 4786*



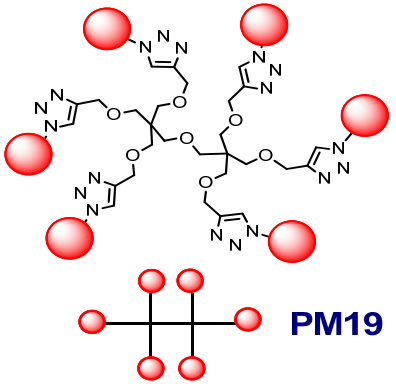
Thépaut et al *J. Am. Chem. Soc.* **2013**, 2518

DESIGNING MULTIVALENCY: Rod-based Dendrimers

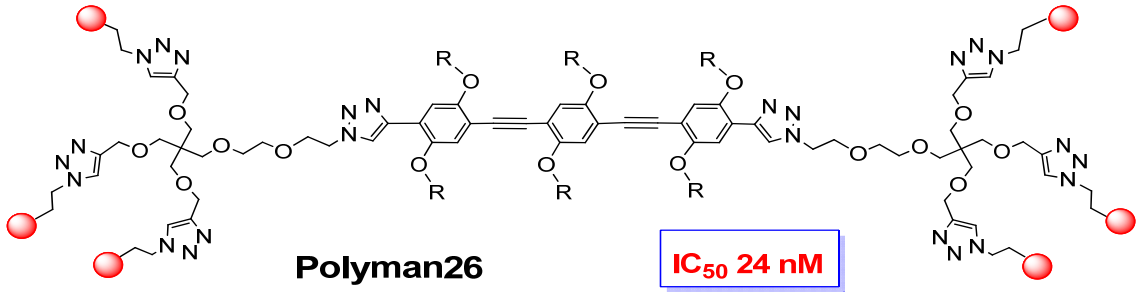
PM19 inhibits DC-SIGN mediated trans-infection by HIV and Dengue with IC_{50} 5 μM



N. Varga *et al* *Biomaterials* **2014,** 4175



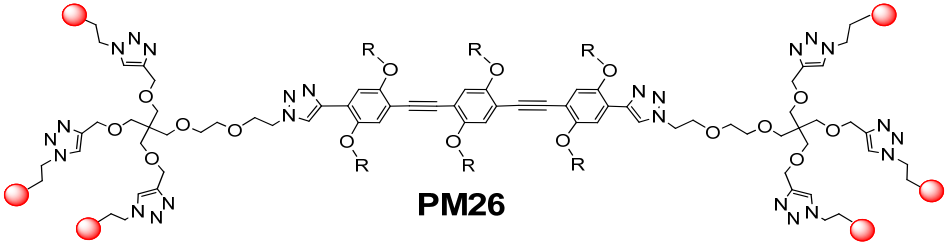
PM26 inhibits DC-SIGN mediated trans-infection by HIV with IC_{50} 24 nM

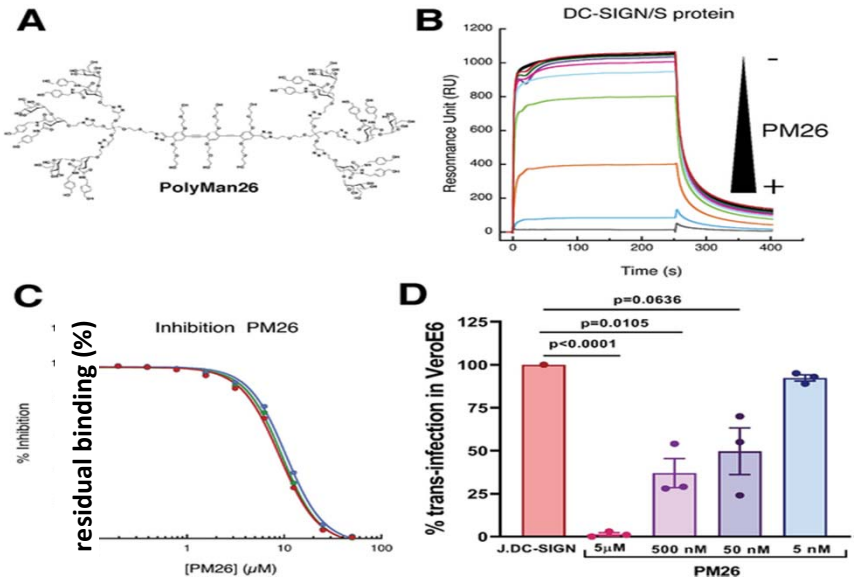


S. Ordanini *et al.* *Chem Comm* **2015**, *51*, 3816; *Sci. Rep.* **2016**, 35373

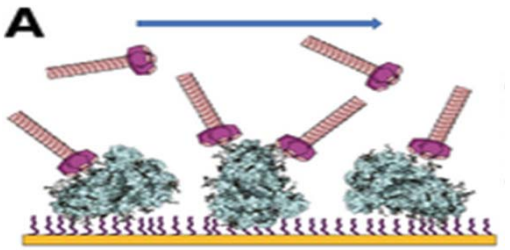
DC-SIGN and SARS-CoV-2

Franck Fieschi





- DC-SIGN binds to the Spike
- **PM26** blocks the interaction (SPR)
- No direct infection occurs
- DCs exposed to the virus promote *trans* infection of competent cells
- **PM26** blocks the *trans* infection



Rafael Delgado

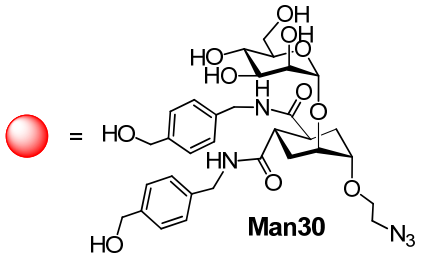
M. Thépaut *et al PLOS Pathogens* **2021**, 17(5):e1009576

Design of multivalent structures

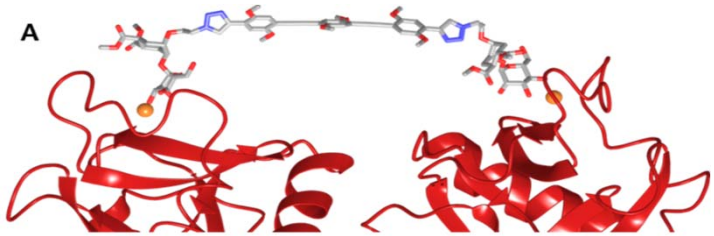
Stefania Ordanini

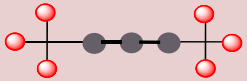
SPR: direct interaction assay

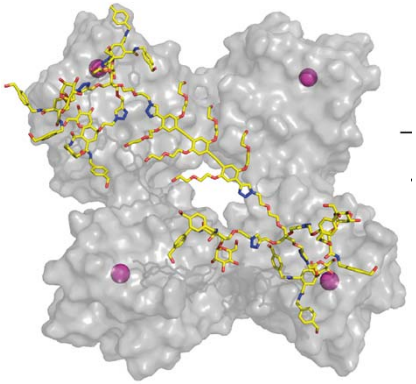
V. Porkolab and F. Fieschi



IC_{50} = 300 μM



Compound	K_D (μM)
	0.0115 ± 0.0023
	2.445 ± 0.25
	39.3 ± 3.9



Chelation and statistical rebinding amplify one another

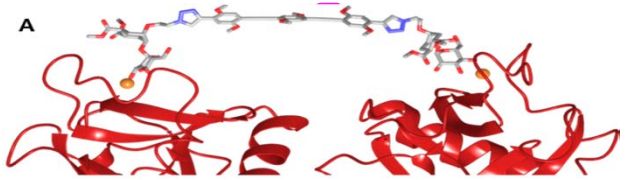
Franck Fieschi, Vanessa Porkolab

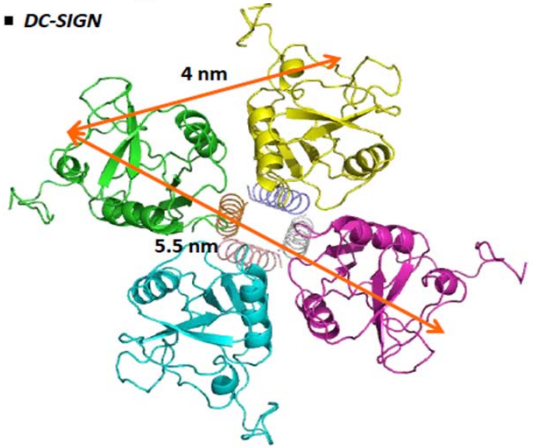
Porkolab *et al ChemRxiv* **2022** 10.26434/chemrxiv-2022-4n79q-v2

Multivalency : Scaffold Design


Cinzia Colombo

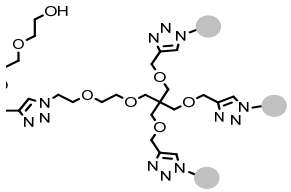

Giulio Goti

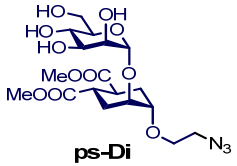


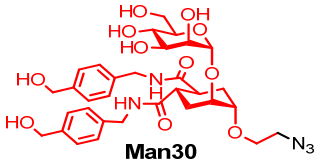


DC-SIGN Glycodendrimer Antagonists

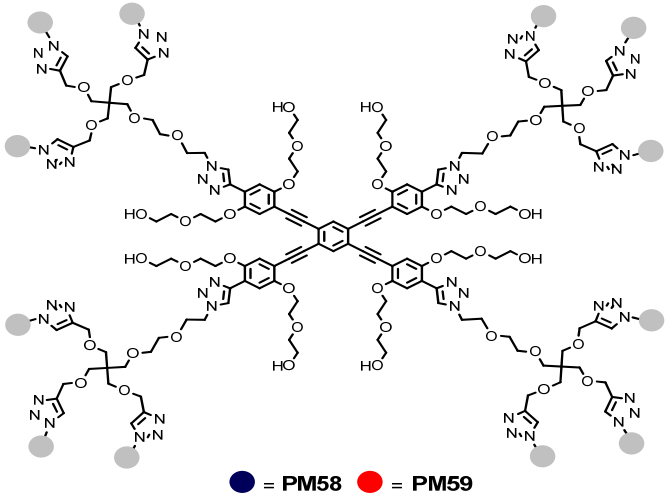
ligands







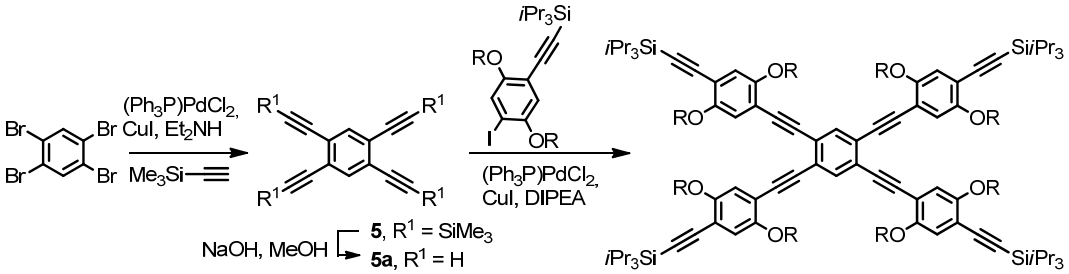
CROSS-shaped dendrimers

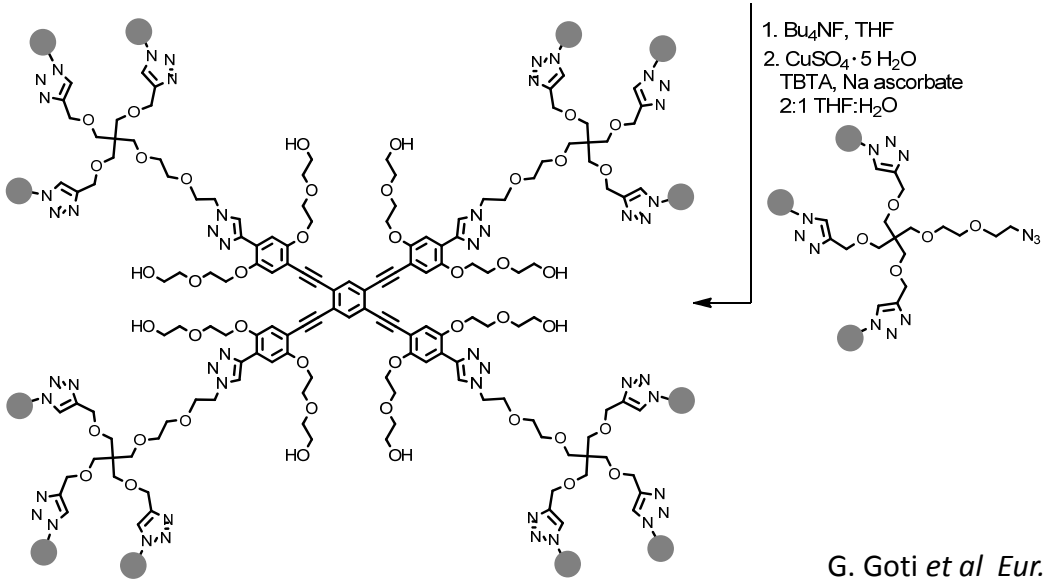


Multivalency : Scaffold Synthesis

Cinzia Colombo

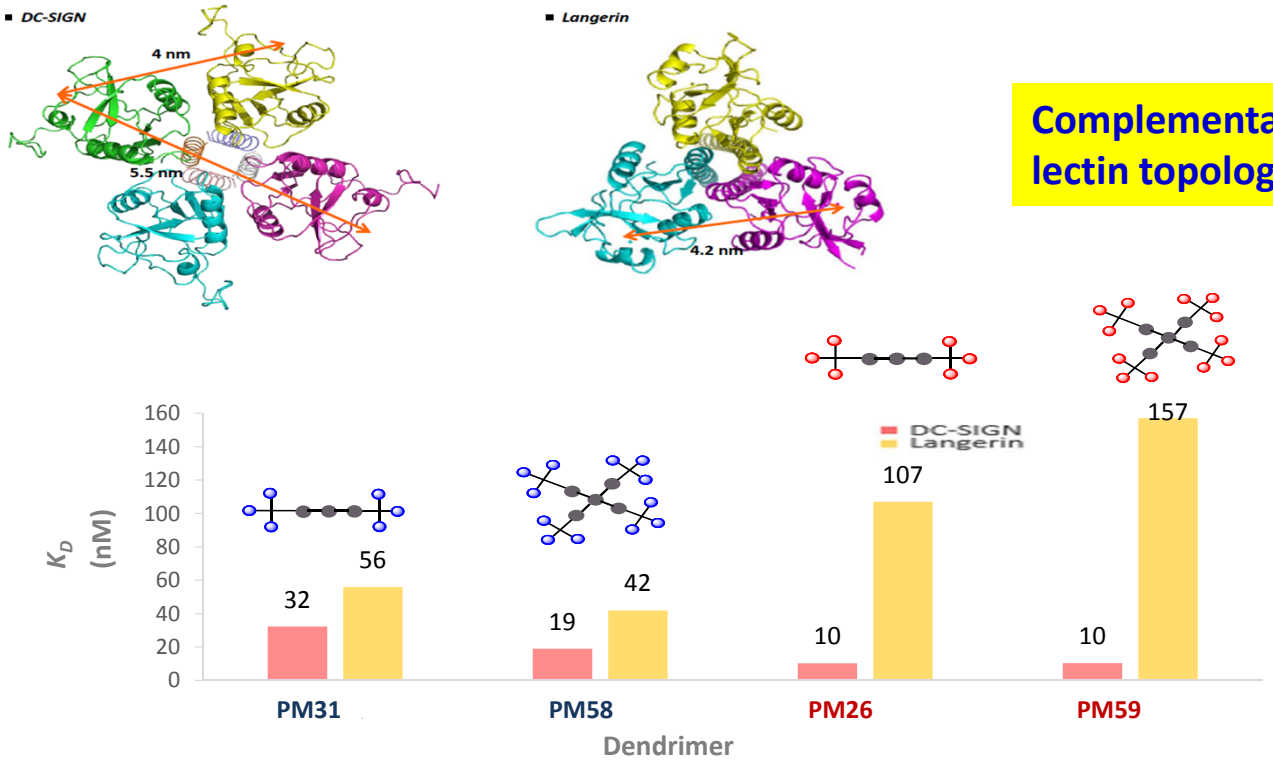
Giulio Goti





G. Goti *et al* *Eur. J. Org.Chem.* **2022**, e202200113

Multivalency: Rod vs. Cross

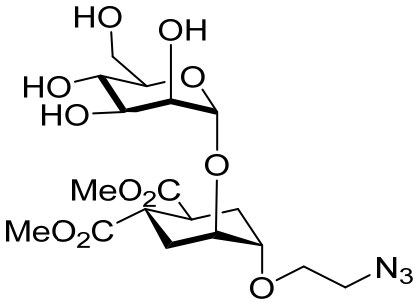


Complementarity of scaffold and lectin topology adds to selectivity

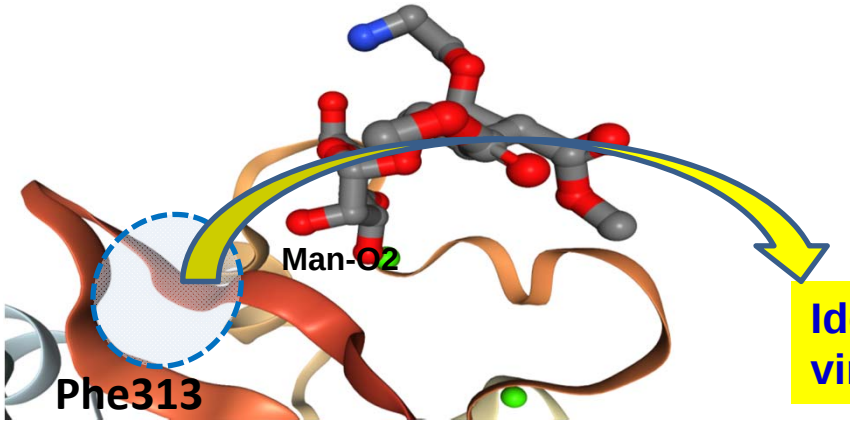
G. Goti *et al* *Eur. J. Org.Chem.* **2022**, e202200113

SPR direct interaction assay

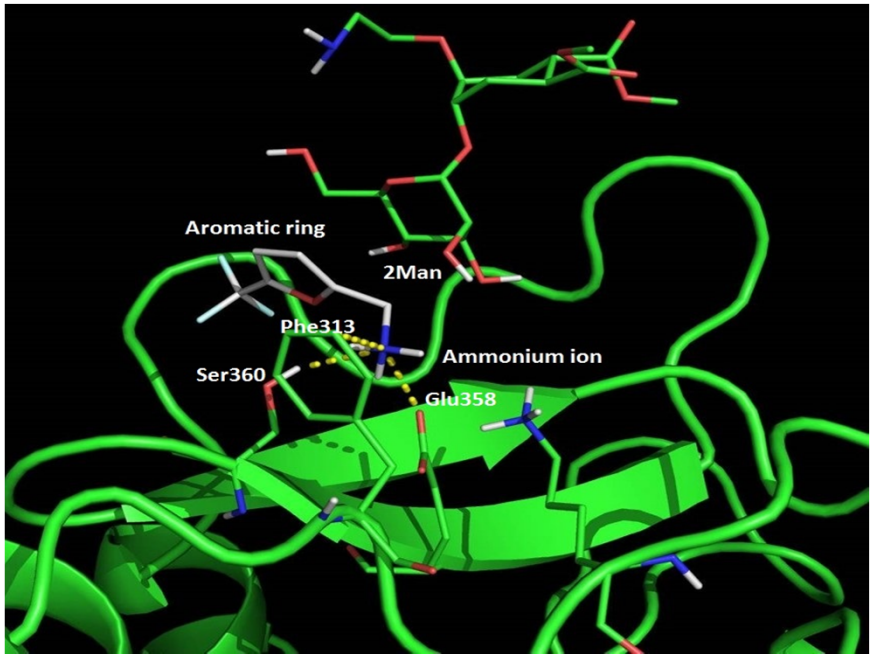
DC-SIGN antagonists – Fragment-based design



ps-di Man



PDB 2XR5

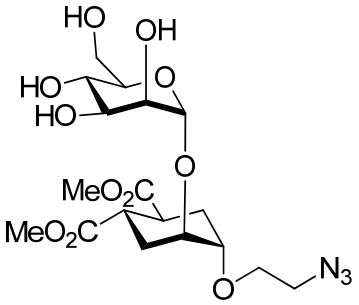


Identified an ammonium ion binding region by virtual screening (Sonsoles Martin-Santamaria)

Medve, Achilli *et al Chem. Eur. J.* **2019**, *25*, 14659

DC-SIGN antagonists – Fragment-based design

Laura Medve



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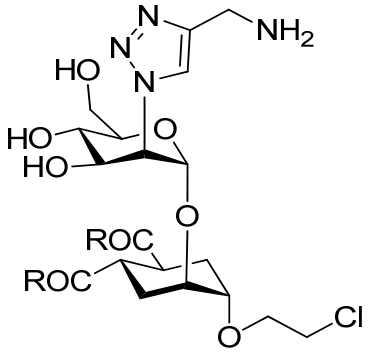

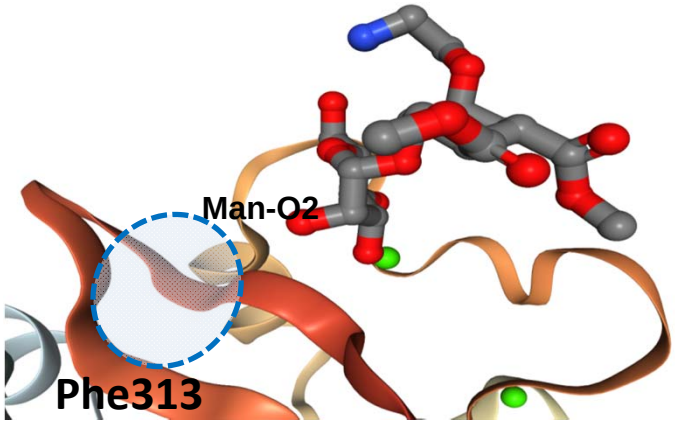

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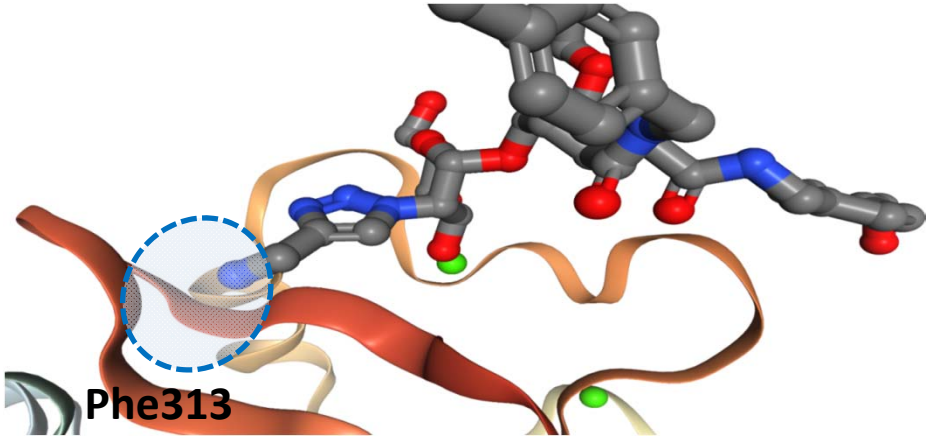
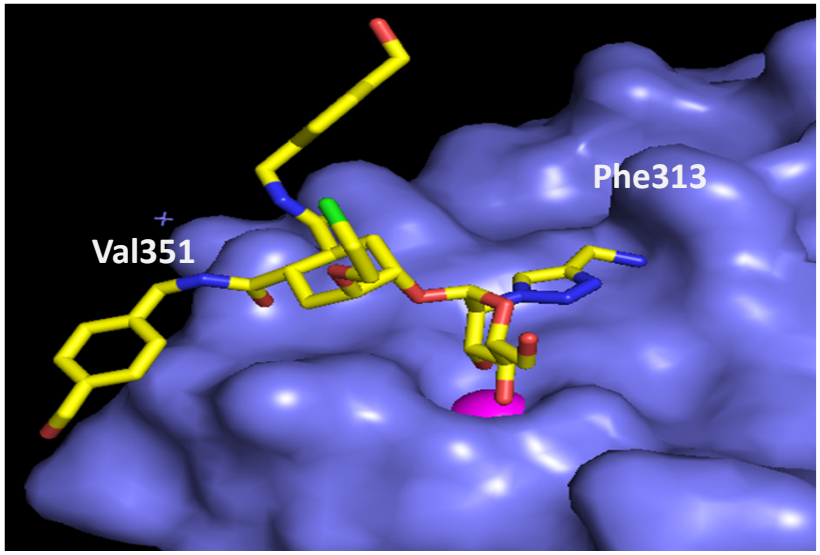


PDB 6GHV

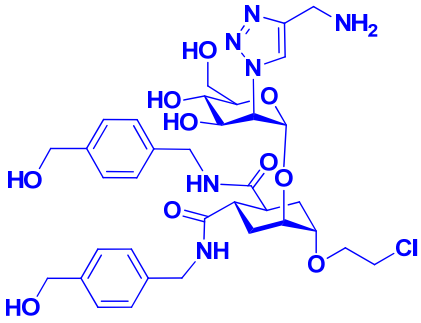
Medve, Achilli *et al Chem. Eur. J.* **2019**, *25*, 14659

Glycomimetics: the sugar anchor

DC-SIGN complex of Man69: X-ray



K_d 52 µM
(ITC)



Man069

- ✓ The sugar moiety anchors the sugar to the lectin
- ✓ The aglycone and the additional functionalities interact with secondary sites
- ✓ VS of fragments can be used for design

HIGHER AFFINITY
INCREASED SELECTIVITY

Medve, Achilli *et al Chem. Eur. J.* **2019**, *25*, 14659

Fragment screening: BC2L-C

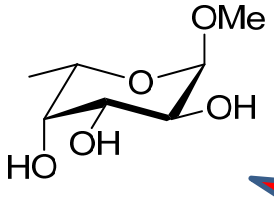

Rafael Bermeo *Kanhaya Lal*

One order of magnitude gain

Bifunctional ligands

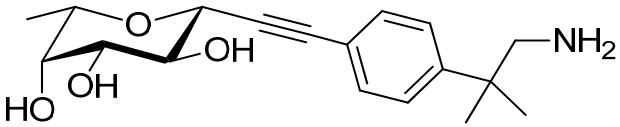
K_d , ITC

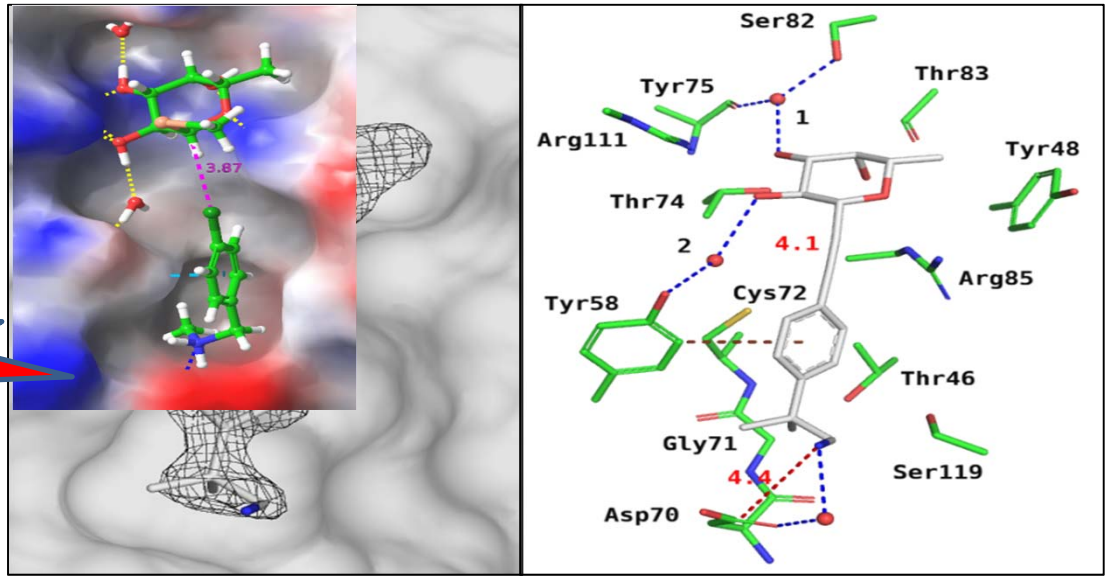
2431 µM



Sarah Mazzotta
Poster session

281 µM

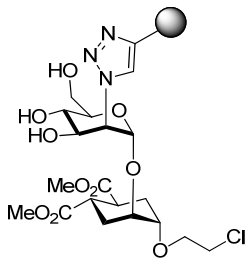


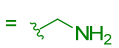


PDB: 7OLU

Bermeo *et al ACS Chem Biol* **2022**

DC-SIGN antagonists – Fragment-based design

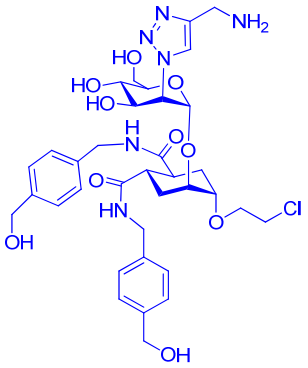


= 

Man062
108 μM

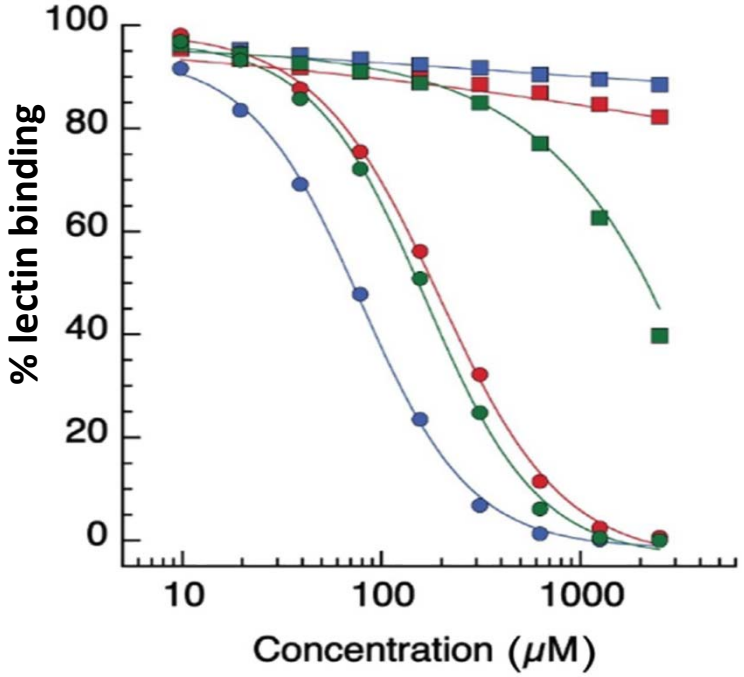
= 

Man065
180 μM



Man069
76 μM (IC_{50}, SPR)
52 μM (K_d, ITC)

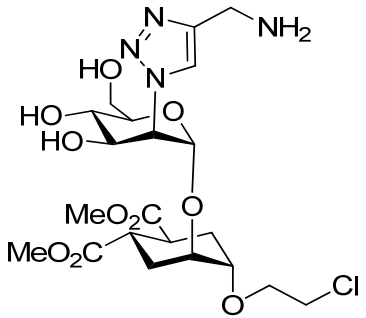
Inhibition curves of DC-SIGN (circle) and Langerin (square)



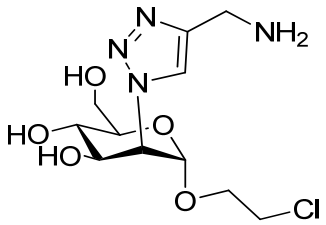
Medve, Achilli *et al Chem. Eur. J.* **2019**, *25*, 14659

The sugar anchor: towards small molecules

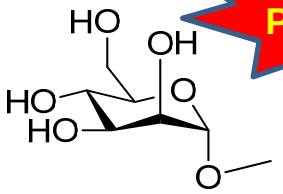
Sara Pollastri



IC_{50} 108 µM

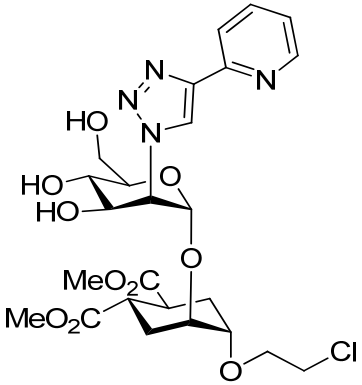


IC_{50} 232 µM

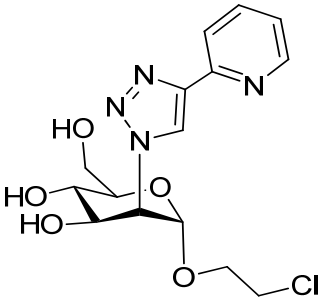


3-10 mM

Sara Pollastri
Poster session



IC_{50} 180 µM



IC_{50} 163 µM

✓ Synthetically less demanding

✓ Higher water solubility

Glycomimetics: Conclusions

- Glycomimetics using ligand-structure design successful
- Sugar anchor: design by fragment screening on monosaccharide complexes viable
- Selectivity: by differential design (different features of carbohydrate binding regions) or by serendipity (screening)
- In multivalent constructs the amplification factor depends on the affinity of the monovalent ligand: multivalency amplifies affinity differences
- The combination of multiple multivalent mechanisms increases affinity: the rebinding effect can be exploited to amplify the chelation effect
- Complementarity of the multivalent scaffold and the receptor contributes both to activity and selectivity