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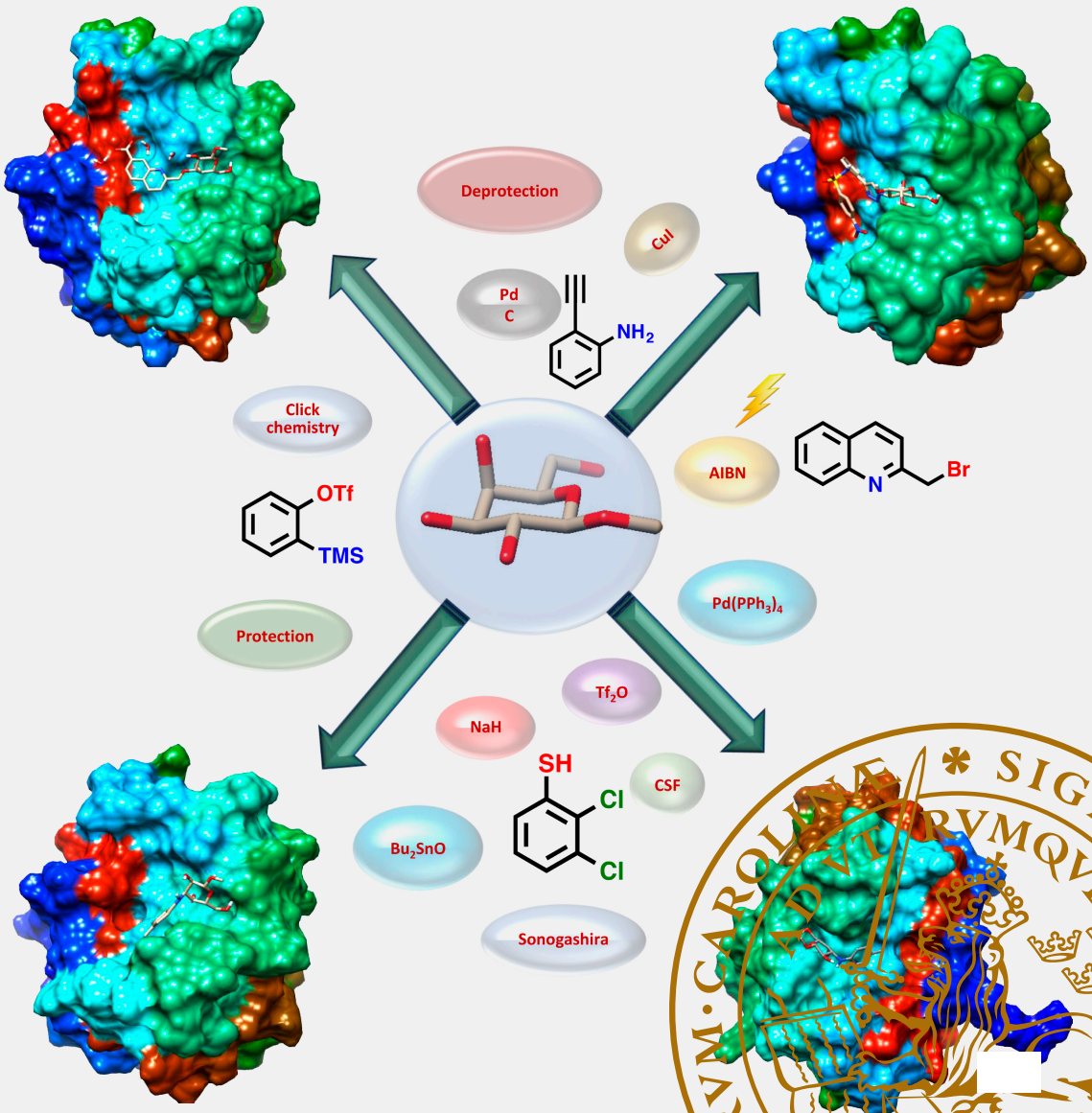

Design and Synthesis of Glycomimetics as Selective Galectin Inhibitors

Mukul Mahanti

LUND
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DOCTORAL DISSERTATION
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Abstract

In last couple of decades the field of glycobiology has developed rapidly due to the increased understanding of glycans abilities to encode biological information, which may have impact on biological event and disease, such as for example tumour growth and inflammation. This development has been accompanied by an increasing focus on drug discovery revolving around a category of carbohydrate-based drugs termed glycomimetics. The proteins of interest in this thesis are the galectins, a family of proteins with affinity towards β-D-galactopyranoside-containing glycans. Galectins have been found to be involved in various diseases, such as cancer, inflammation, fibrosis, heart failure. Hence, the discovery of the small molecule inhibitors will not only provide key tools to study their biological functions and galectin-ligand interactions on a atomic level, but also provide lead compounds for discovery of galectin-targeting drugs.

The overall aim of this thesis is divided into two parts, the first one is to develop organic synthetic methodology towards novel glycomimetic structures and to validate the new methodology by evaluating glycomimetic inhibitors fofor galectins and the second part is to develop galectin selective and high affinity inhibitors with novel glycomimetic structures targeting unexplored periferal galectin ligand subsites. The first part is presented in chapters 2-3, where I present in chapter 2 a new methodology to arylate amino sugars and in chapter 3 I used this method to develop *N*-aryl galactoside and guloside-based glycomimetics as domain selective inhibitors of human galectin-9 controlled by the galactose-gulose epimerization. This is the first report on domain selective inhibitors for any galectin. The impact of this discovery is that it opens a viable strategy for elucidating the particular roles of each domain of galectin-9 in different disease condition and as well in normal biological functions. Chapters 4 and 5 are dedicated to narrating the discovery of sulfonamide-derivatised galactoside as galectin-9N selective inhibitors and quinoline-derivatised galactosides as galectin-8N selective inhibitor, in which the sulfonamides and quinolines form specific and galectin-selective interactions with hitherto unexplored subsites periferal to the core galactopyranose subsite. These galectin-8N and 9N selective inhibitors are expected to be valuable in studies of biological functions of these galectins and as possible leads towards the discovery of galectin-8 or 9-targeting drugs.

Key words Galectin, Galectin inhibitors, Glycomimentics, Epimeric switch, MD simulation

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To my niece, Samyukta

Popular Summary

In the current era we cannot think of a single day without the use of medicines. If we hear the term “medicine” the instant images that form in our brain is those pills that cure us from many diseases. And that is indeed true, the miraculous power of medicine postpones the mortality or in other words help us to buy some more time in life.

There are different kinds of body disorders that can cause a disease. So, on the road to the discovery of medicines, the first and foremost thing is to understand that disorder. This disorder can be of several types and one of these types is overexpression of some substances inside our body such as proteins. Abnormal behavior of some proteins might cause or spread a disease. In this kind of scenario, one of the ways to fight against those diseases is to block the activities of such proteins. Here comes the understanding of how a medicine works in the molecular level. As we know that a medicine is nothing but a chemical compound, we need to design such chemical compound that can selectively bind with that particular protein and block its activity. That’s how we can prevent the disease progression and provide a cure to that.

In my project such proteins are from galectins. Different kinds of galectins can be found in our body across almost all tissues and organs. Researchers have found that in different disease condition, such as cancer, inflammation, heart failure, overexpression of different galectins has been observed. So, as a synthetic chemist my challenge is to design and synthesize such chemical compounds that can inhibit the activity of these galectins and thus may be a future medicine. In my thesis, across different projects, I have invested my time in synthesizing such chemical compounds, also called inhibitors, which not only bind with galectin(s) but also rather selectively bind to a specific galectin. These discoveries provide us with a better understanding of the function of individual galectins and also its role in a particular disease, as well as serve as starting points for medicine development.

List of Papers

1. Pal, K.B[†], **Mahanti, M**[†], Nilsson, U. J. Arynes in Transition-Metal-Free Monoarylation of Unprotected Carbohydrate Amines, *Org Lett*. **2018**, *20*, 616-619.
†These authors contributed equally
Contribution: I and Kumar Bhaskar Pal did all the reactions, purifications and the data analysis. I and Kumar Bhaskar Pal wrote the manuscript and the other author edited the manuscript.

2. **Mahanti, M**, Pal, K.B, Sundin, A.P, Leffler, H, Nilsson, U. J. An epimer switch for galectin-9 subunit selectivity: 3C-*N*-Aryl galactopyranosides bind the C-terminal and gulopyranosides bind the N-terminal domain.
Under Revision ACS Med. Chem. Lett.
Contribution: I and Kumar Bhaskar Pal did all the reactions, purification and the analysis of the data. Anders Sundin perfromed the MD simulation. I wrote the manuscript. Other author edited the manuscript.

3. **Mahanti, M**, Sundin, A.P, Leffler, H, Nilsson, U. J. Sulfonamide-derivatised galactosides target an unexplored binding site in galectin-9N
In Manuscript
Contribution: I synthesised all the compounds, performed the purifications and analysed the data. Anders Sundin performed MD simulations. I wrote the manuscript. Other authors edited the manuscript.

4. Pal, K.B[†], **Mahanti, M**[†], Hunang, X, Persson, S, Sundin, A. P, Zetterberg, F. R, Oredsson, S, Leffler, H, Nilsson, U. J, Quinoline–galactose hybrids bind selectively with high affinity to a galectin-8 N-terminal domain, *Org. Biomol. Chem.* **2018**, *16*, 6295.
†These authors have contributed equally
Contribution: I and Kumar Bhaskar Pal did all the reactions, purifications and data analysis. Xiaoli Huang performed the cell assays. Anders Sundin performed the MD simulations. I and Kumar Bhaskar Pal wrote the manuscript, Xiaoli Huang contributed to writing the cell assay part and Anders Sundin wrote the MD simulation part. All other authors contributed to editing the manuscript.

Paper not included in the thesis

5. **Mahanti, M**, Bhakat, S, Nilsson, U. J, Söderhjelm, P, Flap Dynamics in Aspartic Proteases: A Computational Perspective, *Chem Biol Drug Des*, **2016**, *88*, 159.

6. Pal, K. B†, **Mahanti, M**†, Leffler, H, Nilsson, U. J, A Galactoside-Binding Protein Tricked into Binding Unnatural Pyranose Derivatives: 3-Deoxy-3-Methyl-Gulosides Selectively Inhibit Galectin-1, *Int. J. Mol. Sci*. **2019**, *20*, 3786.
†These authors have contributed equally

7. **Mahanti, M**, Pal, K. B, Kumar, R, Leffler, H, Logan, D. T, Nilsson, U. J, Ligand sulfur oxidation states stepwise alter ligand-galectin-3 complex formations.
In Manuscript

Abbreviations

Ac	Acetyl
ACN	Acetonitrile
Ar	Aryl
Bn	Benzyl
Bz	Benzoyl
CRD	Carbohydrate Recognition Domain
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	*N,N*-dimethylaminopyridine
FP	Fluorescence Polarisation
K_d	Dissociation constant
HPLC	High Pressure Liquid Chromatography
LacNAc	*N*-acetyllactosamine
MD	Molecular Dynamics
NMR	Nuclear Magnetic Resonance
TBAF	Tetrabutylammonium fluoride
THF	Tetrahydrofuran
TMS	Trimethylsilyl

Table of Contents

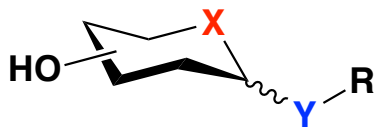
1.Introduction

Glycomimetics

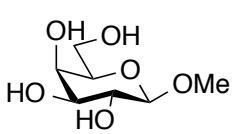
Carbohydrates are the group of biological compounds consisting of carbon hydrogen and oxygen (empirical formula $C_m(H_2O)_n$) and a main structural building block and source of energy for living organisms. Furthermore, complex carbohydrate structures can code biological information, the topic of this thesis, and influence cell biochemistry and signalling. Studies of the roles of complex carbohydrates in biological systems is the field of glycobiology. From our daily diet, in plants, and animals to bacterial cell walls they can be found almost everywhere as form of oligo- or polysaccharides. When a saccharide is linked to another type of biomolecule, such as a protein or lipid, the resulting molecules is called a glycoconjugate and the glycoconjugate saccharide is called the glycan.

Glycomimetics are molecules that resemble a carbohydrate by structure and by biological function(s). That means any modification at the anomeric position or in the ring such as oxygen is replaced by any other atom or any molecule that mimics the function of a carbohydrate can be termed as glycomimetic. Figure 1 exemplifies the concept of glycomimetics and the corresponding terminology with anomeric derivatization or different atoms in or on the carbohydrate ring.

(A)



O-glycoside[1] When X=O and Y=O



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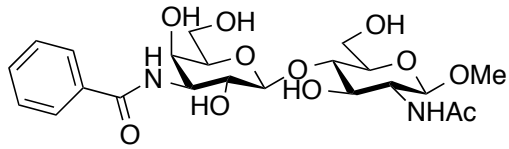

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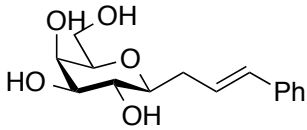
C-glycoside[2,3] When X=O and Y= C



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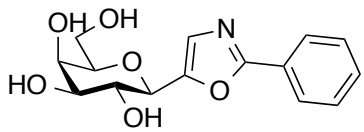

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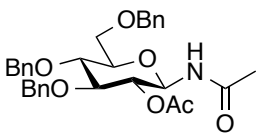
N-glycoside[4,5]: When X=O and Y= N



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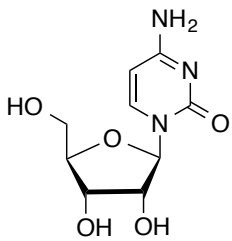
Se-glycoside[6]*:* When X=O and Y= Se



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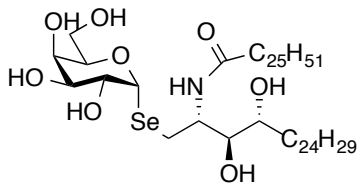

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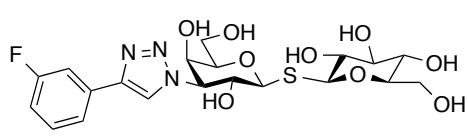
Figure 1 (A). General representation of selected glycomimetic structures and specific examples thereof.

Figure 1(**A**) continued.

S-glycoside[7]*:* When X=O and Y=S



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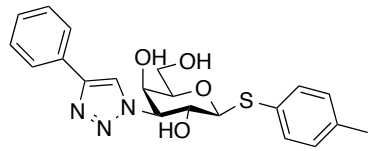

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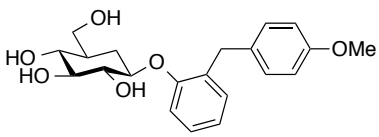
Carbasugar[8]: When X=C and Y=O or C or N



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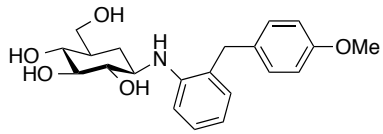

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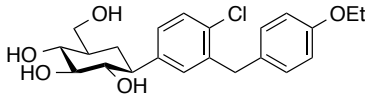

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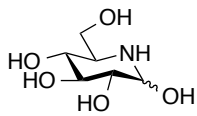
Iminosugar[9,10]: When X=N and Y=O or H



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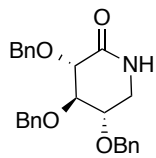

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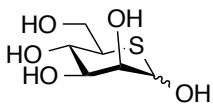
Thiosugar[11]*and phosphasugar*[12]: When X= S/P and Y= O



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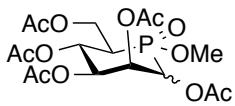

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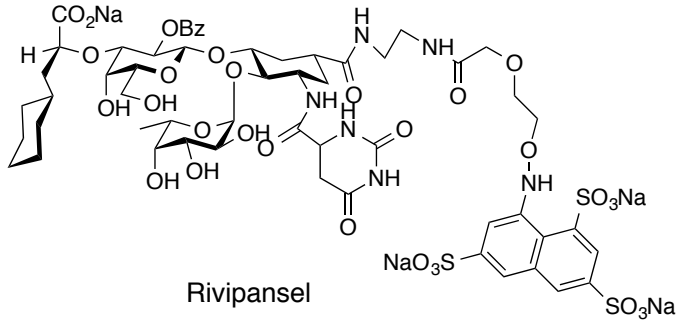
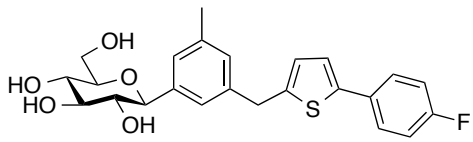
(**B**)



Rivipansel

17



Canagliflozin

18

Figure 1 (**B**)The structures of Rivipansel **17** and Canaglifozin **18**.

Why we need glycomimetics

The field of studying biological functions of saccharides and glycoconjugates, glycobiology, has over recent decades developed rapidly. The main reason behind this is the increased understanding of glycans' (complex carbohydrates/oligosaccharides) and their abilities to encode diverse aspects of biological information[13]. The glycan-encoded information can control biological characteristics, signalling, and mechanisms in different stages of diseases such as cancer proliferation, inflammation, and infection[13,14]. It is not an exaggeration to say that our increased understanding of the biological importance of glycans to a large extent popularised the area of designing glycan-related drugs, including

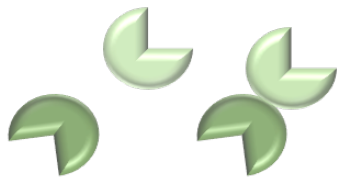
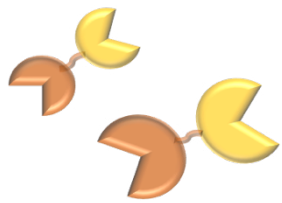
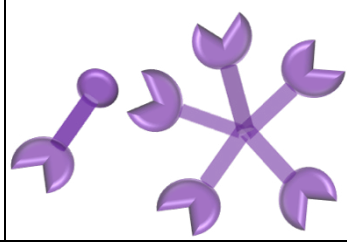
glycomimetics. When carbohydrate-based drugs and glycomimetics are discussed, one question pops up: Why can´t we directly use the naturally occurring carbohydrates as drugs, why we need to modify them? The major reasons are their insufficient metabolic stability, in many cases poor bioavailability, high polarity, and often compromised the potency. In order to circumvent these challenges, a glycomimetic of a certain biologically important glycan can designed and synthesised with chemical modifications that improve *e.g.* stability and bioavailability. Such glycomimetics structurally and functionally mimic the corresponding natural saccharide as mentioned above, but can possess enhanced chemical and enzymatic stability together with ideally better affinity or selectivity towards the target protein in comparison to their naturally occurring counterparts.

Till date, near a hundred carbohydrate binding proteins have been discovered in humans and the characteristics of their binding interactions has in many cases been elucidated[15]. These discoveries have opened various doors in the field of drug discovery, as well as in glycobiology. Even though the carbohydrates are important in biological processes and hold many pathological significances, the popularity of carbohydrates and carbohydrate-based drugs has been limited. This is due to the lesser understanding of the binding interactions between carbohydrates and proteins and that the binding sites are large, often polar, and mostly shallow and solvent exposed. The design and synthesis of the carbohydrate-based drugs are thus indeed challenging. Furthermore, as carbohydrates are typically polar, as are their protein binding partners, then ligands, such as glycomimetics, often tend to be polar as well and thus show limited passage through the gut enterocyte layer, This restricts their oral availability, a big drawback from a pharmacokinetic point of view[15]. Recently significant success in clinical trials of lectin inhibitors, such as TD139[16](**26**, Figure 3b) as topically administered galectin modulator, Rivipansel (GMI-1070)[17] as an intravenous selectin antagonist has drawn the interest of glycobiologists, as well as of pharmaceutical companies. Canaglifozin[18] a member of glifozin family and SGLT2 inhibitor is administered orally for the treatment type2 diabetes (Figure 1b). Glycomimetics have also found important applications as transition state analogues for enzyme inhibition[19,20].

Lectin proteins: The Galectins

The carbohydrate binding proteins are classified into two categories: Lectins and glycosaminoglycans binding proteins[15]. Lectins are typically highly specific for oligosaccharide structures and can play key roles in cellular and molecular recognition involving cells, proteins, and carbohydrates. Lectins are ubiquitous in nature and found in all vertebrates. Depending on where they are found, lectins are divided into two categories: Intracellular lectins and extracellular lectins[15]. Unlike many other lectins, galectins are not membrane bound, but are cytosolic proteins and can be transported out of cells and to the cell surface[21]. Typically, galectins are defined as a lectin having affinity towards β-D-galactopyranosides. Structurally, galectins can be classified into three categories as described in Table 1.

Table 1. Structural category classes of galectins[21,22] with structural description and pictorial representation.

Dimeric type: Oldest prototype according to an evolutionary perspective. Galectin-1,-2,-5,-7,-10,-11,-13,-14,-15 fall into this category. They have one CRD which can dimerise.	
Tandem type: Two different CRD, one in the N-terminal domain and one in the C-terminal domain, connected via a peptide linker. Galectin-4,-6,-8,-9,-12 belong to this subclass.	
Chimera type: This subclass comprises with only galectin-3. It has one CRD and long non-lectin domain. Galectin-3 can occur either as monomer or by association through non-lectin domain it can form oligomers.	

Galectins play different roles depending on where they are present, their ligand binding specificities, and on the glycoconjugate ligand aglycon structure, *i.e.* the cells glycosylation pattern. The latter is controlled by the cell's expression of glycan processing enzymes, such as glycosyl transferases and glycosidases. The

galectins are mainly cytosolic proteins and are synthesised on cytosolic ribosomes. From the cytosol they can be targeted to the nucleus or other subcellular sites. Inside the cell, the galectins are involved in many events including for example RNA splicing[23]. On the cell surface, galectins are typically involved in cross-linking of surface proteins or receptor by interacting with β-D-galactoside-containing glycans with its carbohydrate recognition domain. Thus, galectin can upon ligand (*e.g.* glycoprotein) binding form matrixes on cell surfaces or in vesicles, which can influence for example cell signalling and extracellular interactions between cells[24].

In humans galecin-1,.3,-4,-7,-8,-9, and 12 are mostly found[21]. Next question is in which organs are galectins found? Galecin-3 and 1 are ubiquitous in nature, they can be found almost everywhere in our body but the other galectins are mostly localised. See Table 2.

Table 2. Galectin and their distribution across different organs.

Galectin	Distribution in human body
Galectin-1	Can be found in various places, expressed in many tissues and cell[25]
Galectin-2	Gastrointestinal tract, placenta[26]
Gaectin-3	Ubiquitous in nature, can be found almost everywhere in our body[27].
Galectin-4	Intestine, stomach, blood vessels walls, uterine epithelial cells, hippocampal and cortical neurons[28,29]
Galectin-7	Gastrointestinal tract, skin, heart[30,31]
Galectin-8	Liver, kidney, cardiac muscle, lung, brain[32]
Galectin-9	T-Lymphocytes, small intestine, uterine epithelial cells, liver, skin epidermis, oesophageal epithelium[27,33]
Galectin-12	Adipose tissue[34,35]

Pathologies: The importance of developing inhibitors

This thesis work was dedicated to developing inhibitors for galectins. Now the question is why we need to block galectins to combat diseases? What role do galectins play in those disease conditions? According to PubMed publications 126 different diseases have been discovered where the overexpression of one or more galectins has been found[36]. Overexpression of galectin-1 and -3 has been observed in most cancers[37,38] and they also serve roles in tumour progression. Galectin-1, 3, and -9 are important for regulating chronic inflammatory responses, as well play roles in acute inflammation[39,40]. Furthermore, galectin-3 plays a crucial role in causing fibrosis[41,42] in many organs such as lung, kidney,

heart, pancreas. Galectin-3 also has significant contribution to heart failure[43,44]. Considering these facts, we have enough reason to invest our time in developing inhibitors for galectins.

The Galectin CRD

The knowledge of galectins involved in pathologies naturally leads to the importance of why we need to develop inhibitors to block galectin functions. In this regard, the natural saccharide ligands are not ideal as discussed above; high polarity, poor stability, and limited affinities and selectivities. Hence, in order to tackle this problem, we need to develop synthetic inhibitors that are easy to synthesize and handle, have improved pharmacokinetic/ADME properties, and are potent and selective towards a particular galectin; a task easier said than done. In order to develop galectin specific inhibitors, we need to study thoroughly the CRD of each galectin and bear in mind their high sequence similarities. In the coming chapters, the projects will be circulating around galectin-8N, -9N and -9C, so we will focus in the CRD of these galectins.

Galectin CRD is a β-sandwiched structure comprising about 135 amino acids. Six strands form a concave side (S1-S6) and 5 strands form a convex side (F1-F5)[22]. The concave side forms a groove-like structure that can hold up to a tetrasaccharide unit. Based on that, galectin CRDs have been compartmentalised into 4 defined subsites (A-D) and one less defined subsite E[22]. Here it is noteworthy to mention that C and D subsites are highly conserved in all galectins. In the following examples we will discuss galectin complexes with galactose and lactose. A ligand galactose unit is invariably occupying subsite C of galectin CRDs, while the *e.g.* glucose of lactose occupies subsite D.

Galectin-8N: The X-ray structure of galectin-8N complexed with lactose helps us to understand the binding site chemistry and the amino acid residues that are most essentials in binding. Interaction of 4OH and 6OH of galactose in C subsite is believed to be the most conserved interaction across the galectins and hence it is the most important one. For better understanding a two 2D simplistic visualisation has been presented (Figure 2a) where we can see that in case of galectin-8N, the 4OH of galactose is involved in H-bond interaction with 3 different amino acid residues Arg45, Hi 65, Asn67, and Arg69. Furthermore, the 6OH forms hydrogen bonds to Glu89 and Asn79. The ring oxygen in galactose interacts with His65 and Arg69. Finally, the C-H hydrogens (H1, H3, H4, H5 and H6) exposed on the α-face of the β-D-galactopyranose form C-H-π interactions with the indole rings of Trp86.

Galectin-9N: When we come to galectin-9N, breaking down the binding interactions in its lactose complex reveals that it is almost of replica of what we see for galectin-8N in complex with lactose (Figure 2b).

Galectin-9C: Galectin-9C is also similar in this perspective. The representation of the X-ray structure of a galectin-9C complex with an oligosaccharide (Figure 2c) shows that the amino acid residues involved in the core interaction with 4OH and 6OH of the galactose unit are essentially the same as in galectin-8N and 9N.

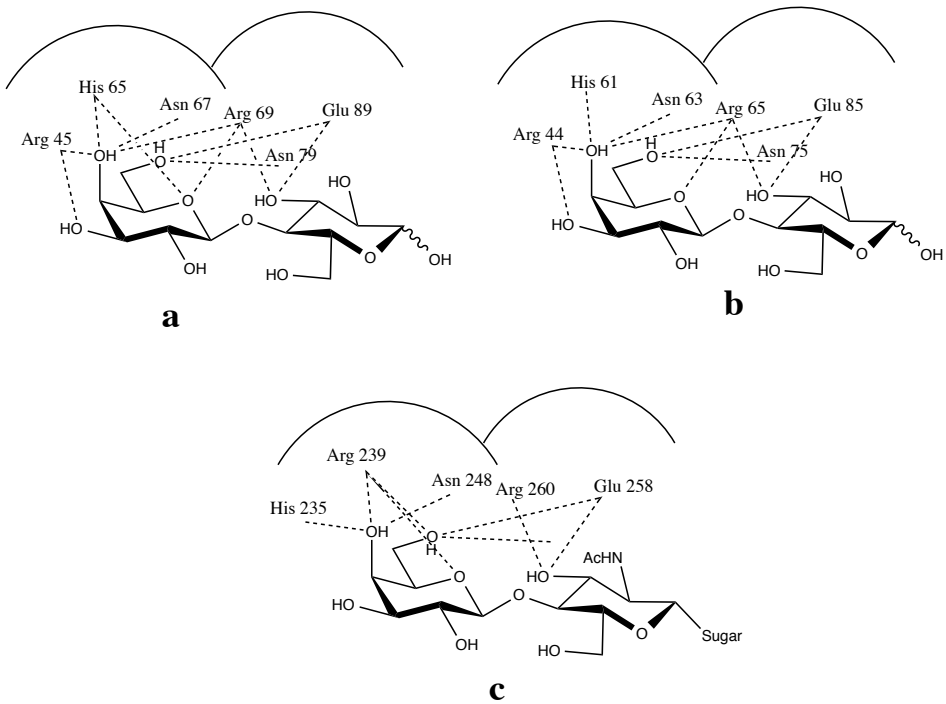


Figure 2. (**a)** Binding interaction representation of lactose in human galectin-8N CRD (pdb 5t7s). **(b)** Binding interaction representation of lactose in human galectin-9N (pdb 2eak) CRD. **(c)** Binding interaction representation of a LacNAc-based oligosaccharide in human galectin-9C CRD (pdb 3nv3).

Galectin inhibitors

Over last two decades, numerous inhibitors have been synthesised and tested against different galectins. As discussed above that galectins have nature affinity towards β-D-glactopyranosides. So, different modified galactose or glycomimetics of galactopyranosides would serve as inhibitor for galecins. In this journey, the most exploited galectin has been Galectin-3. As discussed earlier, each galectin´s CRD can be compartmentalised into 5 main subsites A-E[22]. In case of galectin-3, all these subsites have been strategically spanned by different inhibitors over the years bit by bit.

The stimulating journey along the path to develop inhibitors for galectins started with the natural ligands lactose and LacNAc. Lactose-OMe (methyl β-glycoside of lactose) has a K_d value of 220 μM for galectin-3[45], whereas LacNAc-OMe (methyl β-glycoside of *N*-acetyllactosamine) binds almost 4-fold better than lactose to galectin-3 (K_d=59 μM)[46] (Figure 3). As we discussed earlier. in case of lactose and LacNAc, the galactose unit occupies subsite C and the glucose/GlcNAc unit occupies subsite D. Initially, the LacNAc moiety was subjected to modification to target subsites A-B with 3-arylamido-*N*-acetyllactosamine derivatives **21** and **22** that bind with K_d of 820 nM and 320 nM, respectively, to galectin-3[47], which is more than 200-fold better than LacNAc itself. It was hypothesised that a cation-π interaction between the 3-arylamide and Arg144 was responsible for the increased affinity. Inhibitor development then continued with thiodigalactosides, which encompass two galactoside units connected by a sulfur linkage creating symmetrical structures. The thiodigalactosides are on one hand more simplified substitutes of lactose and the sulfur linkage provides more metabolic stability, while it retains almost the same affinity as LacNAc-OMe with galectin-3 (K_d=49 μM)[48,49]. Thiodigalactoside-based inhibitors, such as a symmetric 3-fluorophenyl-triazole **26**[16], was proven to have high affinity (K_d=2.3 nM) and selectivity towards galectin-1 and 3. The X-ray structure of **26** or TD139 with galectin-3 and how it spans all five defined subsites A-E is shown in figure 3a. It is worth mentioning that TD139 (**26**) reduces idiopathic pulmonary lung fibrosis in a mouse model and is currently in clinical trials. Recently, monosaccharide-based inhibitors, such as *p*-methylphenyl 3-deoxy-3-[4-(3,4,5-trifluorophenyl-1*H*-1,2,3- triazol-1-yl]-1-thio-β-D-galactopyranoside **23**[7], have been reported to have down to 5.2 μM affinity for galectin-3. From the X-ray structure of **23** in complex with galectin-3C (C-terminal domain) it was believed that multipolar fluorine-amide interactions with R-144 contributes to that high affinity. Later on it was suggested that the desolvation effect and London dispersion force are much more prominent than fluorine-amide interaction[50]. When the β-STol (4-methylphenyl) group of **23** was substituted with an α-3,4-dichlorophenyl group (**24**), the dissociation

constant dropped to 37 nM[51]. The chlorine at 3-position of the phenyl aglycon forms a halogen bond with the carbonyl oxygen of G182 (in subsite E). This halogen bond can be strengthened by increasing the size of the halide σ-hole[52], which may be done by placing an electron withdrawing group in the close vicinity, such as nitro or cyano group.

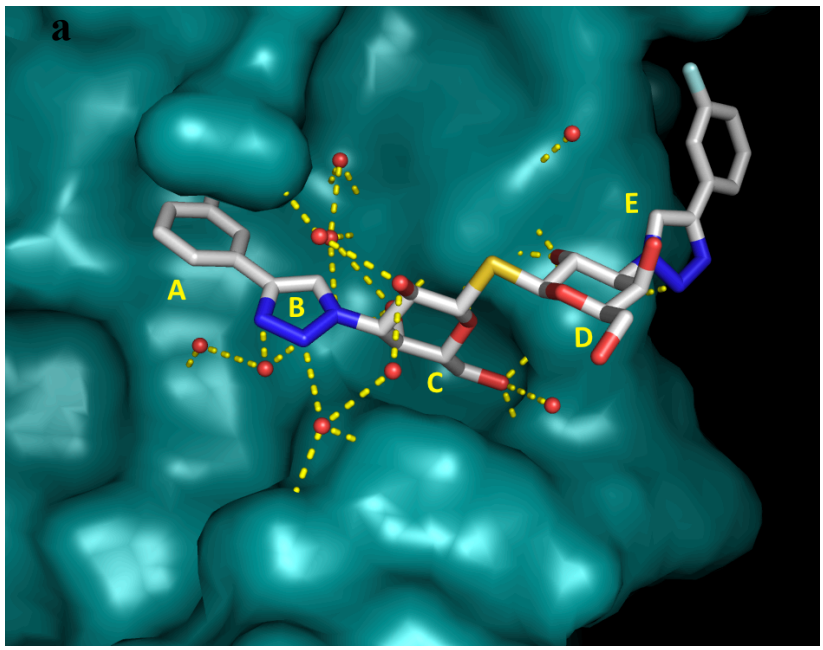


Figure 3. (**a**) X-ray structure of TD139 with galectin-3 showing the subsites A-E.

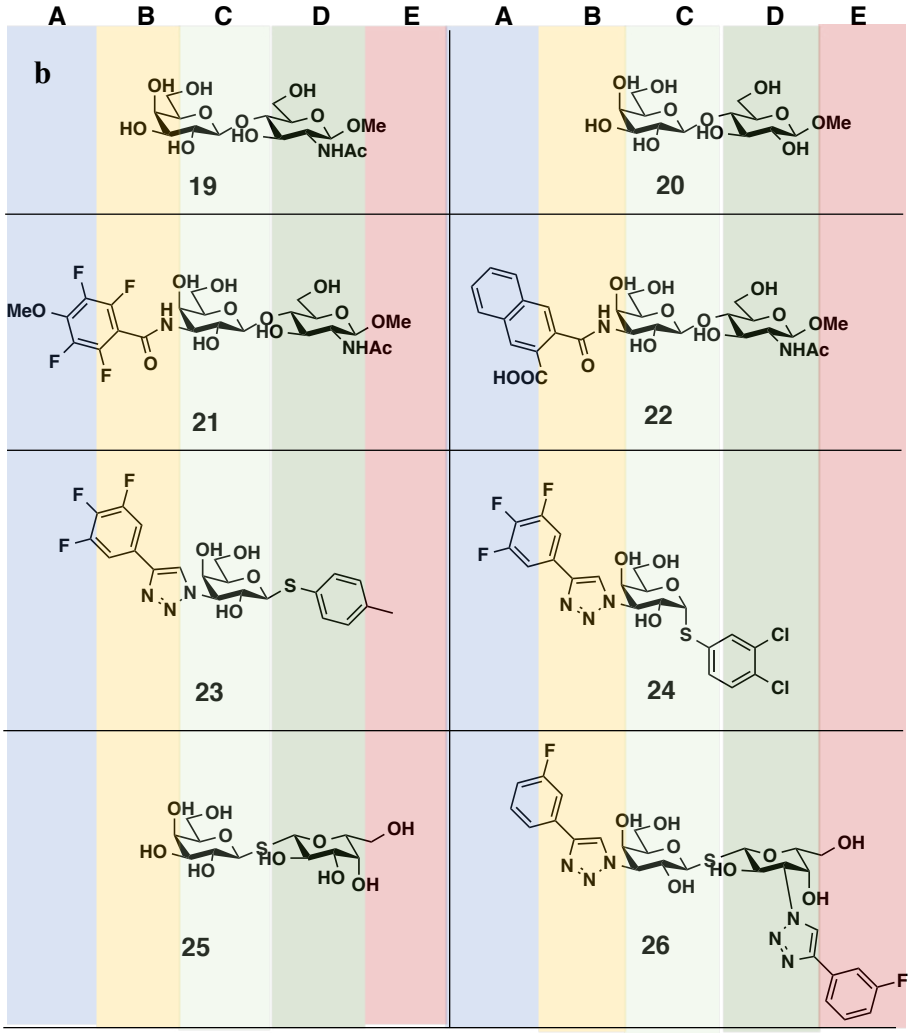


Figure 3b). Galactose-based compounds and their K_d values against human galectin-3 and representation of how they are spanning in the CRD subsites (A-E). Each color code designates one subsite.

Affinity Evaluation

There are numerous methods, from NMR, UV spectroscopy to isothermal titration calorimetry, to determine the dissociation constant of an inhibitor for a particular protein. In the projects of this thesis we have in-house access to NMR, ITC and fluorescence polarisation assay techniques to determine the K_d values of the inhibitors for galectins. ITC is a direct and more accurate method than FP, based on determined by the heat released during the titration of the inhibitor with the protein. This method is however time consuming and often requires high concentration of the inhibitors and the protein, whereas FP is a fast technique and requires very low amounts of the inhibitors and the protein. This is the reason why we primarily screen the inhibition potency of our inhibitors through FP technique[53].

Fluorescence polarisation assay

The Theory: In this measurement a fluorescent probe, which often is a fluorescein-tagged saccharide ligand, is excited with plane polarised light and the remaining degree of polarisation of the fluorescence is measured. When the probe is free in the solution the degree of rotation and tumbling is higher and hence the remaining fluorescence polarisation is less than the polarization of the excitation light. However, when probe is bound with the protein the size of the complex is much larger than the free probe and thus does not undergo as much rotation on the fluorescence time scale and hence the remaining polarisation higher than it is with a free probe. Now, as an inhibitor is introduced in this system and it replacing the probe binds with the protein and the concentration of the free probe in the solution increases and as a result the degree of remaining polarisation is also decreasing. From the change in polarisation upon inhibitor addition, the dissociation constant of the inhibitor is calculated.

The Method: Fluorescence polarization experiments were performed either with a POLARStar plate reader and FLUOstar Galaxy software or with a PheraStarFS plate reader and PHERAstar Mars version 2.10 R3 software (BMG, Offenburg, Germany) and fluorescence anisotropy of fluorescein-tagged probes (Table 3) measured with excitation at 485 nm and emission at 520 nm. K_d values were determined in PBS[54].

Table 3. List of the galectin specific probes and the concentration of the galectins during the dissociation constant measurement by fluorescence polarisation assay of the compounds described in this assay.

Galectin	Galectin concentration(μM)	Probe (0.1 μM)
1	0.5	3,3'-dideoxy-3-[4-(fluorescein-5-yl-carbonylaminomethyl)-1H-1,2,3-triazol-1-yl]-3'-(3,5-dimethoxybenzamido)-1,1'-sulfanediyl-di-β-D-galactopyranoside[55]
2	10	3,3'-dideoxy-3-[4-(fluorescein-5-yl-carbonylaminomethyl)-1H-1,2,3-triazol-1-yl]-3'-(3,5-dimethoxybenzamido)-1,1'-sulfanediyl-di-β-D-galactopyranoside[16]
3	1	2-(fluorescein-5/6-yl-carbonyl)aminoethyl 2-acetamido-2-deoxy-a-d-galactopyranosyl-(1–3)-[α-l-fucopyranosyl-(1–2)]-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside[54]
4N	3	3,3'-dideoxy-3-[4-(fluorescein-5-yl-carbonylaminomethyl)-1H-1,2,3-triazol-1-yl]-3'-(3,5-dimethoxybenzamido)-1,1'-sulfanediyl-di-β-D-galactopyranoside[16]
4C	0.5	2-(fluorescein-5/6-yl-carbonyl)aminoethyl 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1–3)-[a-l-fucopyranosyl-(1–2)]-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside[16]
7	2	β-D-galactopyranosyl(1–4)-2-acetamido-2-deoxy-β-D-glucopyranosyl(1–3)-b-d-galactopyranosyl(1–4)-(N1-fluorescein-5-yl-carbonylaminomethylcarbonyl)-β-D-glucopyranosylamine[56]
8N	0.4	β-D-galactopyranosyl(1-4)-2-acetamido-2-deoxy- β-d-glucopyranosyl(1–3)-β-D-galactopyranosyl(1–4)-β-D-glucopyranoside[57]
9N	1	2-(fluorescein-5-ylcarbonylamino)ethyl β-d-galactopyranosyl(1–4)-2-acetamido-2-deoxy- β-D-glucopyranosyl(1–3)-β-D-galactopyranosyl(1–4)- β-D-glucopyranoside[16]
9C	2	3,3'-dideoxy-3-[4-(fluorescein-5-yl-carbonylaminomethyl)-1H-1,2,3-triazol-1-yl]-3'-(3,5-dimethoxy-benzamido)-1,1'-sulfanediyl-di-β-D-galactopyranoside[16]

Objectives and Outlines

Overall objectives

The overall objective is based on that complex carbohydrates have important roles in biology and disease and this motivates further investigations into these roles. Studies of carbohydrate-protein interactions with specific small-molecule probes, such as glycomimetics, constitutes a powerful tool towards increased fundamental understanding and to find potential drug discovery leads. The broad objectives of this thesis were:

-To develop novel and broadly applicable organic synthesis methodology towards synthetic compounds that mimics structure and function of complex carbohydrates: novel glycomimetic structures

-To design and synthesize novel glycomimetics with high affinity and selectivity for individual members of the galectin family of proteins.

Specific objectives

In order to advance towards the overall objectives, the specific objectives of this thesis were:

-To investigate and optimize benzyne-mediate arylation of amino-sugars (chapter 2).

-To evaluate *N*-arylated amino-pyranoses as selective inhibitors of galectin proteins (chapter 3).

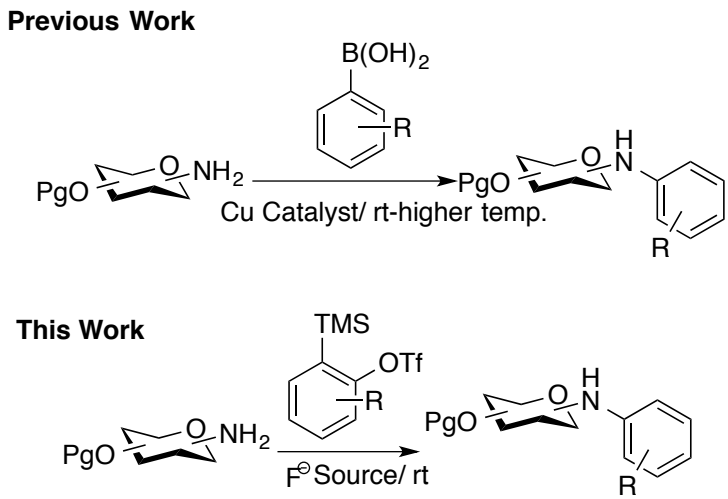
-To design, evaluate, and synthesize glycomimetics that interact with more peripheral unexplored subsites of galectins to discover selective inhibitors of galectin proteins (chapter 4).

-To combine known biologically active molecules such as quinoline, coumarin *etc*. with galactose and investigate their combined effect on galecins and on cells (chapter 5).

2. *N*-Arylation of Amino Sugars

Having one or more nitrogen atoms is a typical feature of numerous marketed drugs. However, when it comes to carbohydrates and glycomimetics, *N*-functionalization is less exploited though there is evidence of some natural occurrence of *N*-functionalised structures[58–61]. Most are *N*-acylated, *N*-alkylated, or *N*-sulfate derivatized structures. Synthetic carbohydrate *N*-arylation is much less developed. Hence, this inspired us to first develop *N*-arylation methodology for amino sugars and then evaluate this method for developing novel galectin inhibitors (see chapter 3).

Only one method (Scheme 1) for the synthesis of *N*-arylated carbohydrates is reported in the literature and it involved Cu-catalysed *N*-arylation of protected carbohydrate with arylboronic acids[62–65]. Arynes are known to react smoothly with amines[66,67,76,77,68–75], why we hypothesized that arynes could be efficient reagents for the *N*-arylation of carbohydrate amines (Scheme 1).

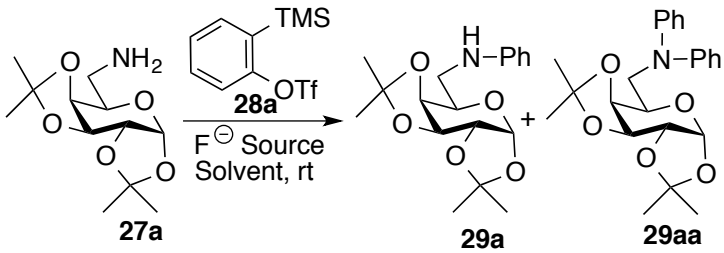


Scheme 1. The previously reported *N*-arylation with arylboronic acids and our aryne-mediated *N*-arylation of carbohydrate amines.

Reaction condition optimisation

Initially the reaction conditions were investigated by treating the amino sugar **27a** with the aryne precursor **28a** under different reaction conditions. Using CsF (2 eq) as the fluoride source, triflate reagent (0.98 eq) and acetonitrile as the solvent yielded mono *N*-arylated product **29a** at 90% yield in 2 hours (Table 4, entry 1). Prolonging the reaction up to 4 hours and increasing the amount of CsF up to 3 eq did not enhance the yield significantly (entry 2), but heating the reaction for one hour while keeping the other conditions unchanged, the yield of the mono *N*-arylated product **29a** decreased to 45%, while 22% di-*N*-arylated product **29aa** was formed (entry 4). Increasing the amount of benzyne precursor **28a** to 1 eq (entry 3) or 1.1 eq (entry 5) compromised the yield of mono-*N*-arylated product **29a** due to the competing formation of di-*N*-arylated product **29aa**. Changing the solvent to THF and having fluoride source to be CsF (entry 6) and KF/18-crown ether (entry 7) yielded virtually no product at all. However, TBAF and THF combination (entry 11) produced 72% of **29a** in 4 h. Almost same result was obtained when TBAF and acetonitrile was used (entry 10), while having KF/18-crown-6 (entry 8) or KF (entry 9) as the fluoride source and acetonitrile to be solvent again yielded nothing.

Table 4. Reaction optimization with amino sugar **27a** and benzyne precursor **28a**.

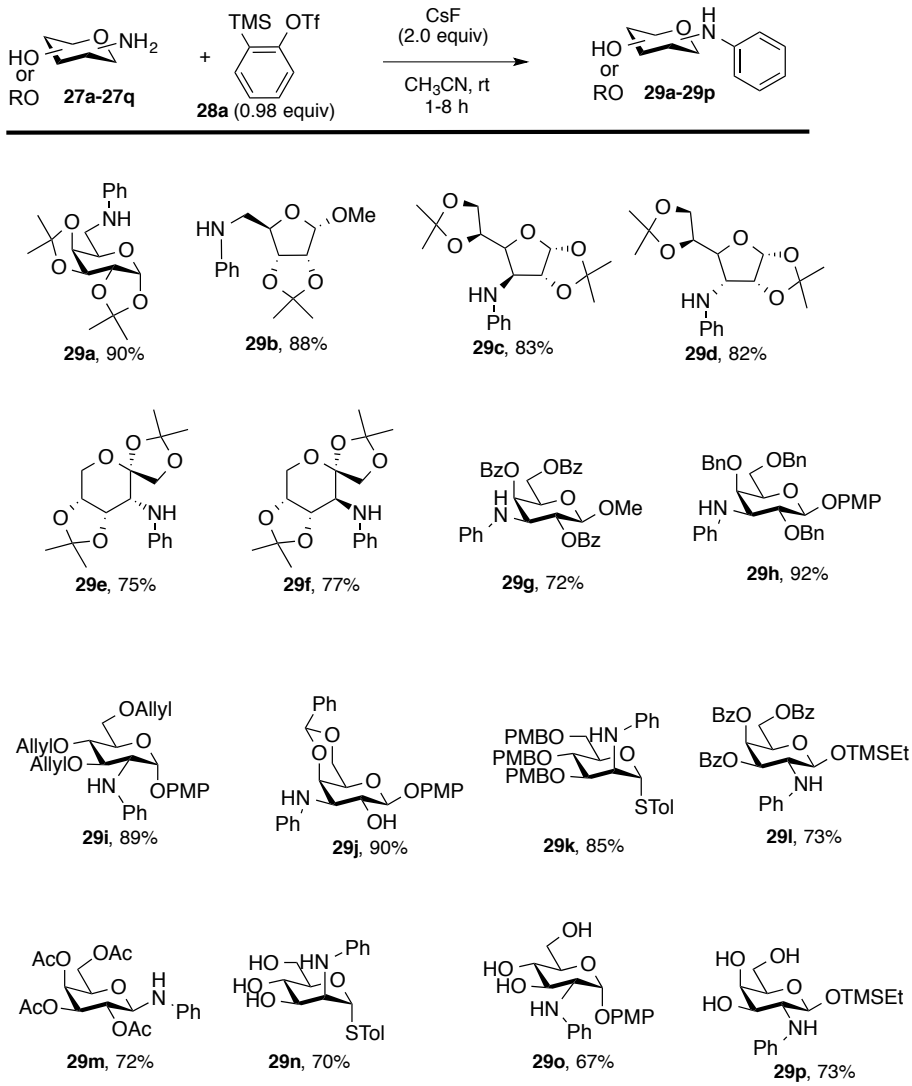


Entry	F⁻ source (eq.)	Solvent	28a (eq.)	Time (h)	Yield (%)	
					29aa	**29aaa**
1	CsF (2)	CH_3CN	0.98	2	90	NO[b]
2	CsF (3)	CH_3CN	0.98	4	94	NO[b]
3	CsF (2)	CH_3CN	1.0	2	80	4
4[a]	CsF (2)	CH_3CN	0.98	1	45	22
5	CsF (2)	CH_3CN	1.1	2	65	16
6	CsF (2)	THF	0.98	12	<10	NO[b]
7	KF/18-Crown-6 (2)	THF	0.98	24	NR[c]	NR[c]
8	KF/18-Crown-6 (2)	CH_3CN	0.98	24	NR[c]	NR[c]
9	KF (2)	CH_3CN	0.98	24	NR[c]	NR[c]
10	TBAF (2)	CH_3CN	0.98	4	70	NO[b]
11	TBAF (2)	THF	0.98	4	72	NO[b]

[a]Reaction at 60 °C. [b]Not observed. [c]No reaction.

Amino carbohydrate scope

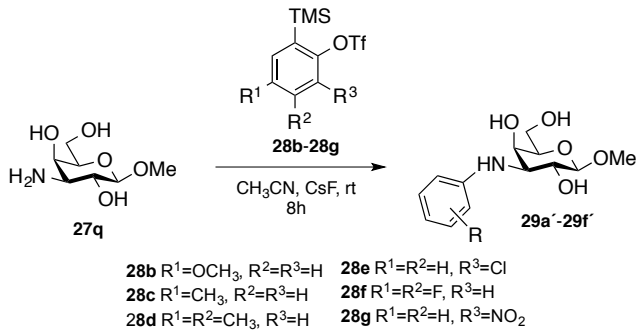
With optimised reaction conditions at hand, the method was extended to different amino sugars in order to determine the scope of the reaction. A series (**29a**-**p**) of furanose and pyranose structures bearing different common carbohydrate protecting groups and with different amine positions, including the anomeric position, were treated with the unsubstituted triflate reagent **28a** (Scheme 2). In each case, good to high yields were observed. A noteworthy observation was that in case of amino sugars carrying electron-donating protecting groups (**29h**, **29i**, and **29k**) yields were higher and reaction times were shorter compared to amines carrying electron withdrawing-protecting groups (**29g** and **29m**). The reason is likely that the amine nucleophilicity increases with electron-donating groups in contrary to electron-withdrawing groups. Quite satisfactory results were found with unprotected amino sugars (**29n**-**29p**), only the nitrogens were chemoselectively arylated with no trace of *O*-arylated products (Scheme 2). This clearly reflects that under the optimised conditions nitrogen is much more nucleophilic than oxygen and *N*-arylation advances faster than *O*-arylation. The promising results with unprotected amino sugars significantly broaden the scope of the method. Another interesting observation was that when *N*-arylation reactions were performed in presence of a 2-trimethylsilylethyl (TMSEt) anomeric protecting group (**29l** and **29p**), the TMSEt group remained intact despite the use of a fluoride source (CsF).

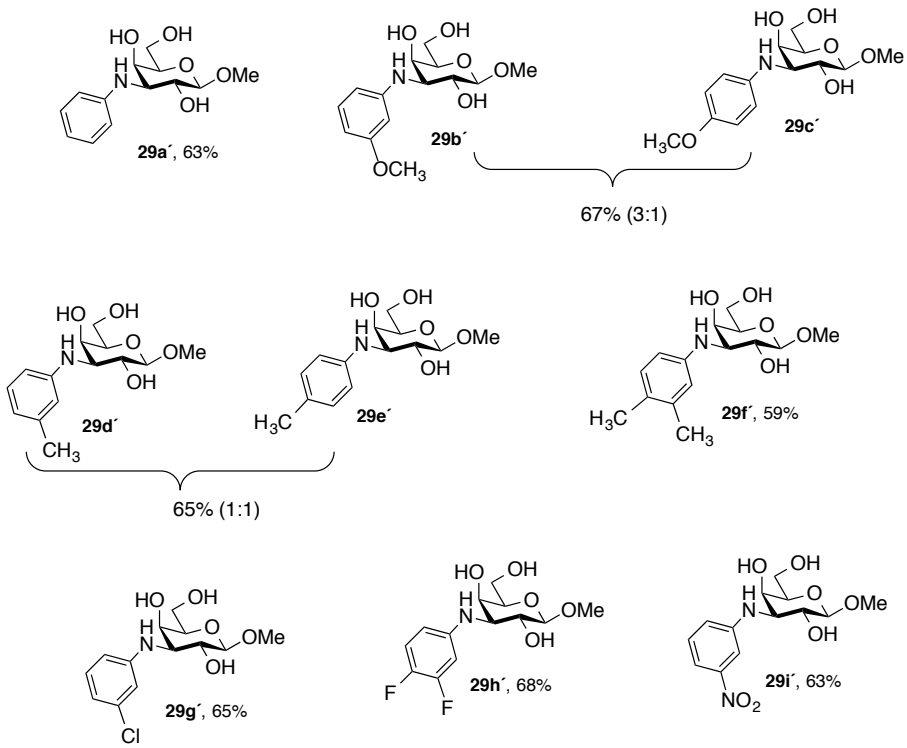


Scheme 2. Selective mono-*N*-arylation of different aminosugars (**29a**-**29p**) with the unsubstituted aryne precursor 2-(trimethylsilyl)aryl triflate **28a**.

Aryne scope

Subsequently, we investigated how the electronic and steric nature of the substituents on 2-(trimethylsilyl)-phenyl trifluoromethanesulfonate aryne precursors influence the reaction outcome. Different reagents with electron donating and electron withdrawing substituents were selected (**28b**-**28g**) and reacted with unprotected amine **27q** (Scheme 3). Arylation worked well with moderate yields, ranging from 59-68%, with different substituents. Symmetric arynes derived from **28d** and **28f** gave mono-arylated product **29f´** and **29h´,** respectively. On the other hand, the asymmetric arynes generated from **28b** and **28c** gave two regioisomers **29b´**+**29c´** and **29d´**+**29e´**, respectively. In case of **28e** and **28g**, only one regioisomer **29g´** and **29i´**, respectively, were formed. This observation can be explained by the electronic effect of the substituents. With both nitro and chloro being electron withdrawing groups and due to their negative inductive the aryne carbon closer to these groups becomes more electron deficient and hence it attracts π-electrons from the aryne bond. Therefore the nucleophile attacks on the carbon distant from the electron-withdrawing substituent leading to a single meta isomer[78].





Scheme 3. Regio-selective mono-*N*-arylation of **27q** with different 2-(trimethylsilyl)aryl triflates **28b**-**28g**.

Oligosaccharide and diamine scope

After obtaining convincing results with different monosaccharides, either by having different protecting groups on the sugar or with reagents bearing different substituents on the phenyl ring, the stage was set to evaluate the methodology on oligosaccharide derivatives. Hence, we tried on protected disaccharides (to give **30a**, **30b**, **30c**) and a trisaccharide (to give **30d**) and again the method resulted in excellent yields (Figure 4). Finally, we performed a selectivity test on a symmetric diamine, the benzyl protected diamine 3,3'-diamino-thiodigalactoside. With 1 equivalent of the phenyl triflate **28a** and 2 equivalents of CsF, 70% of mono *N*-arylated compound **30f** and 15% of symmetric di *N*-arylated **30ff** was formed. Furthermore, **30f** could also be converted in asymmetric di-*N*-aryl compound **30ff´** in a high yield upon performing a second *N*-arylation reaction with the fluorinated aryne precursor **28f**. On the other hand, when the diamine 3,3'-diamino-thiodigalactoside was treated with 2 equivalents of the aryl triflate **28a** or **28f** and 4 equivalents of CsF, the symmetric *N*,*N*-di-aryl compounds **30ff** or **30f´f´** were formed exclusively.

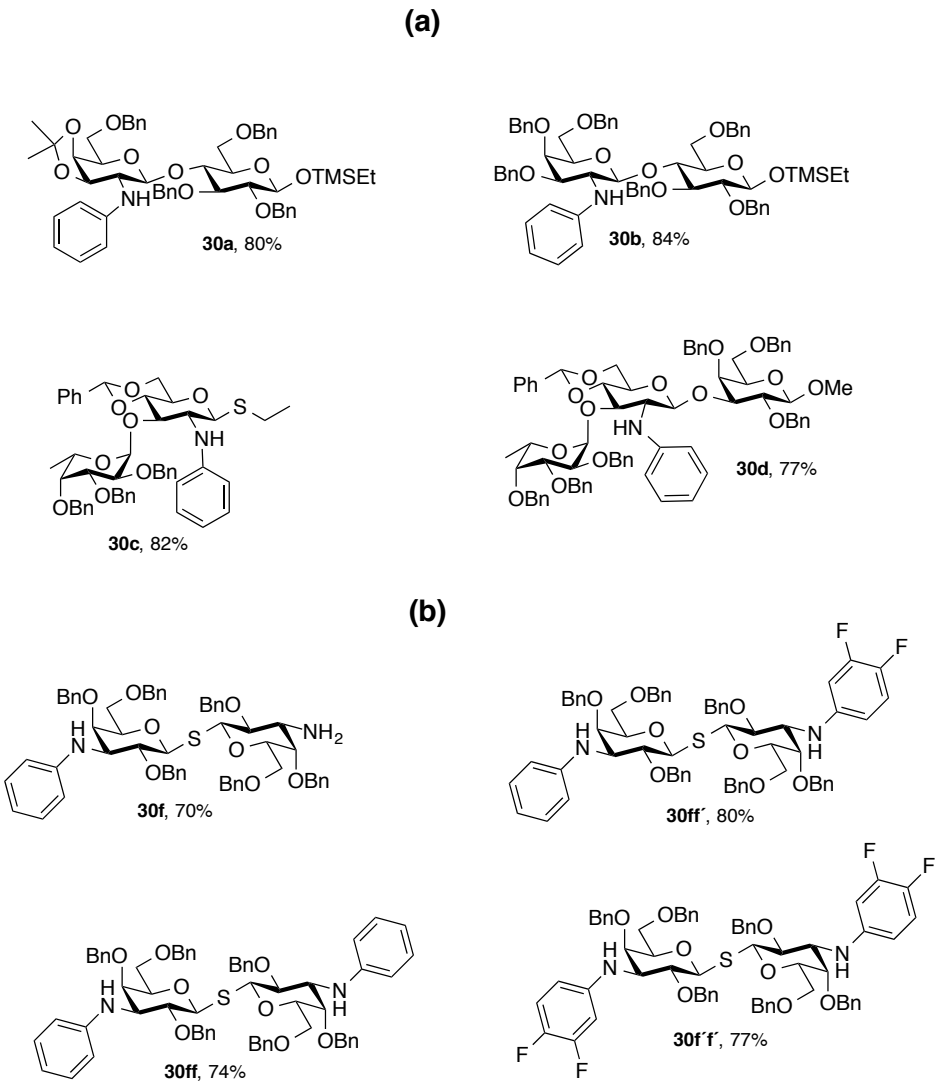


Figure 4. (**a**) Mono-*N*-arylation of di and trisaccharide amines. (**b**) Selectivity of mono- or di-*N*-arylation of a symmetric 3,3'-diamino-thiodigalactoside.

Conclusions

A novel method to mono- and di-*N*-arylate amino sugars was developed and the scope and viability of this method was been explored. This method comes with many advantages, such as it is high yielding, robust, proceeding at room temperature, and transition metal free. Furthermore, it has a broad amino carbohydrate scope allowing a wide choice of the carbohydrate. Aryl triflate precursors bearing both electron-donating and -withdrawing substituents resulted in products with high yields and most importantly tolerated by most of the common carbohydrate protecting groups. A most useful feature of the method is that it can be used to chemoselectively *N*-arylate unprotected amino carbohydrates leaving no traces of *O*-arylated products. The method was successfully extended to di- and trisaccharides, which demonstrates its usefulness on higher carbohydrate. Last, but not the least, one of the most important and exciting findings was that the method selectively mono-*N*-arylate symmetric diamino-sugars and, furthermore, simply by increasing the concentration of the triflate reagent a di-*N*,*N*-aryl product can be obtained.

Hence, this method opens up a new door to arylated amino sugar derivatives. We believe this will contribute to the toolbox for developing new glycomimetic structures, thus playing significant roles in future glycomimetic drug discovery.

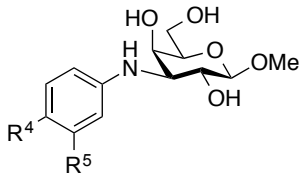
3. *N*-Aryl-Derivatised Galacto- and Gulosides as an Epimer Switch for Galectin-9 (Paper II)

The hypothesis behind synthesising *N*-arylated carbohydrate derivatives in chapter 2 was to establish a novel methodology that could be used towards the discovery of structurally novel glycomimetics binding galectins. The 3-*N*-arylated galactoside **29a´-29h´** (Scheme 3) were evaluated against different galectins and were found to be good inhibitors of galectin-9C both in terms of affinity and selectivity. In a recent article[79] we showed that 3C-methylene gulopyranoside derivatives have as galectin inhibitors. This discovery encouraged us to use the aryne-mediated *N*-arylation method to synthesise 3-*N*-arylated gulo derivatives. Hence, we synthesised 3-*N*-aryl gulopyranosides **35-42** and evaluation as inhibitors of galectins, which revealed that they were selective towards galectin-9N. The observation that 3-*N*-aryl-galactosides are selective for galectin-9C and 3-*N*-aryl-gulosides are selective for galectin-9N can be described as a inhibitor epimer switch controlling galectin-9 domain selectivity.

Organic synthesis and affinity evaluation of *N*-arylated methyl galacto- and guloside ligands

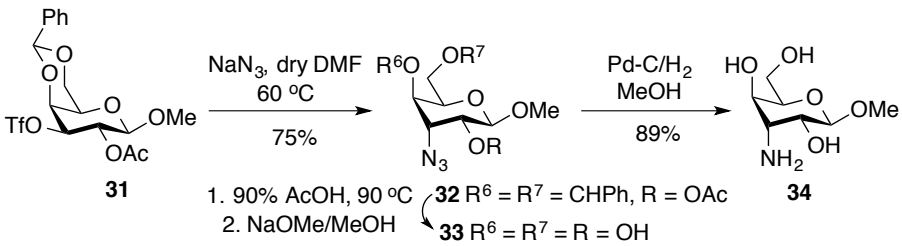
Synthesis of methyl 3-*N*-aryl-3-deoxy-β-D-galacto- and gulosides

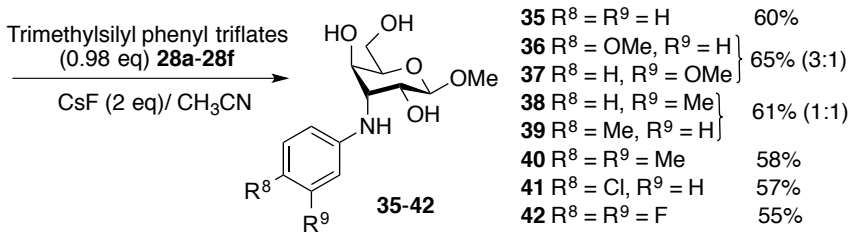
The synthesis of *N*-aryl galactosides **29a´-29h´** was discussed in chapter 2[80]. In order to obtain the *N*-aryl gulosides **35-42**, the galacto triflate **31**[81] was subjected to S_N2 azidation by heating with NaN_3 in DMF at 60 ^{0}C to afford the azide **32**. Removal of benzylidene protection of **32** by heating in 90% AcOH at 90 ^{o}C and followed by one pot de-*O*-acetylation provided **33**. Now, Pd catalysed hydrogenation of azide **33** gave the corresponding amine **34**, which was finally arylated by aryl triflate and CsF in acetonitrile to afford the *N*-arylated gulo derivatives **35-42**.



29a´-29h´

29a´ $R^4 = R^5 = H$
29b´ $R^4 = OMe, R^5 = H$ } 3:1
29c´ $R^4 = H, R^5 = OMe$ }
29d´ $R^4 = H, R^5 = Me$ } 1:1
29e´ $R^4 = Me, R^5 = H$ }
29f´ $R^4 = R^5 = Me$
29g´ $R^4 = Cl, R^5 = H$
29h´ $R^4 = R^5 = F$





Scheme 4. *N*-arylated methyl β-D-galctopyranosides **29a´-29h´** (above). Synthesis of of *N*-arylated methyl β-D-gulopyranosides **35-42** (below).

Galectin affinity of the *N*-arylated methyl galacto and gulosides

In table 5, the K_d values of the *N*-arylates methyl galactosides **29a´-29h´** and gulo sides **35-42** against galecin-9N (N-terminal doman) and -9C (C terminal domain) are listed. For the full table with affinities for other galectins; see paper II. The general observation was the galacto derivatives **29a´-29h´** bound galectin-9C with moderate to good affinity and also showed good selectivity over galectin-9N. The better inhibitors, such as *p*-OMe-substituted **29b´** bound galectin-9C with a K_d of 140 μM with high selectivity, while its meta-isomer **29c´** had near 3 times worse galectin-9N affinity and displayed no selectivity over galectin-9N in our assays. Dimethyl- **29f´** and chloro-substituents **29g´** lead to similar affinity (150 μM) towards galectin-9C and selectivity (10-fold) over galectin-9N. On the other hand, the *N*-aryl gulo derivatives **35-42** showed moderate affinity and not distinct SAR for galectin-9N (mid micromolar range), but high selectivity over galectin-9C.

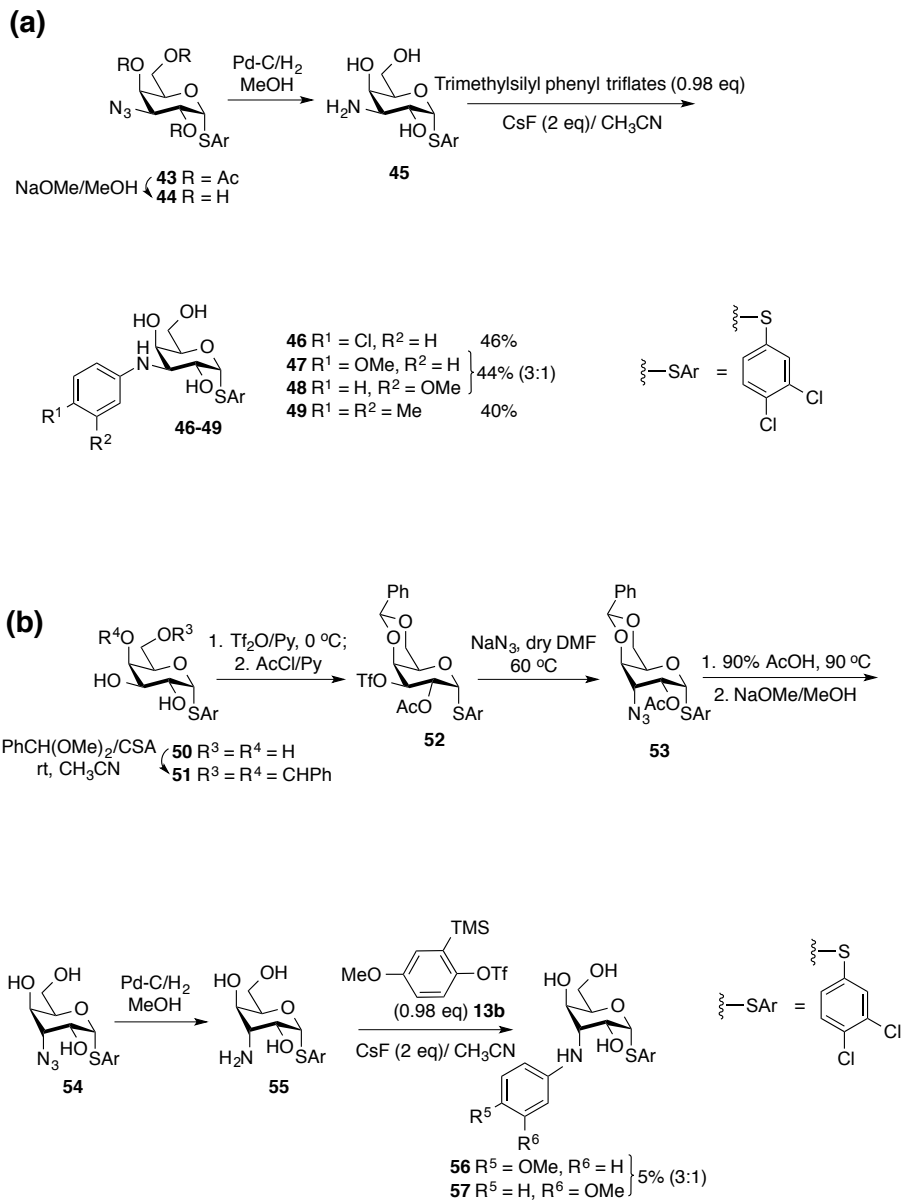
Table 5. K_d-values (μM)[a] of methyl 3-deoxy-3-*N*-aryl-β-D-galactopyranosides **29a´-29h´** and methyl 3-deoxy-3-*N*-aryl-β-D-gulopyranosides **35-42** against human galectin-9N, and 9C as measured by a fluorescence anisotropy assay.

Compound	Galectin-9N[b]	Galectin-9C[c]
29a´	NB	678±30
29b´	NB	140±16
29c´	600±26	440±22
29d´	NB	770±25
29e´	1700±20	670±23
29f´	1100±30	130±9.0
29g´	1500±300	160±6.0
29h´	NB	220±7.0
35	1200±70	NB
36	530±49	NB
37	580±61	NB
38	590±40	NB
39	980±55	NB
40	470±19	NB
41	670±26	NB
42	1100±150	NB

[a]The data are average and standard error mean of 4–8 single-double point measurements. [b]N-terminal domain. [c]C-terminal domain. [d]Not binding at the highest concentration tested: 2 mM.

Synthesis of 3,4-dichlorophenyl 3-*N*-aryl-3-deoxy-1-thio-α-D-galacto- and gulosides

In order to possibly reach a significant affinity enhancement of the *N*-aryl gulo- and galacto scaffolds towards galectin-9N and -9C, a recently reported strategy[51] to combine a 3,4-dichlorophenyl-α-thiophenyl aglycon with the *N*-aryl moieties was investigated. Hence, selected good inhibitors for galectin-9C and as galectin-9N were selected from the Table 5 and they were converted to their corresponding 3,4-dichlorophenyl-1-thio-α-D-galactopyranosides **46-49** and **56-57** (Scheme 5). The synthesis of 3-*N*-aryl-3-deoxy-1-thio-α-D-galctosides **46-49** started with deacetylation of the known compound **43**[51] with methanolic sodium methoxide solution to give deprotected azide **44**, which was further subjected to Pd-catalysed hydrogenation to afford the corresponding amine **45**. The amine **45** was arylated with aryl triflates and CsF in acetonitrile to yield compounds **46-49**. The synthesis of 3-*N*-aryl-3-deoxy-1-thio-α-D-gulosides was initiated with benzylidene-protection of the 4- and 6-hydroxyls of the galactoside **50**[51] were selectively protected by benzylidene group to give **51**. The 3-hydroxyl of **51** was selectively 3-*O*-triflated and directly 2-*O*-acetylated to give compound **52**. Compound **52** was subjected to S_N2 azidation with NaN_3 at 60°C to afford the azide **53**. The benzylidene and the acetate groups in **53** were deprotected sequentially as mentioned above to afford azide **54** carrying three unprotected hydroxyl groups. The azide **54** was reduced to amine **55** with $LiAlH_4$. Finally, the amine **55** was *N*-arylated a mentioned above to give α-thiogulosides **56** and **57**.



Scheme 5. (**a**) Synthesis of *N*-arylated 3,4-dichlorophenyl-1-thio-α-D-galactopyranosides **46**-**49**. (**b**) Synthesis of *N*-arylated 3,4-dichlorophenyl-1-thio-α-D-gulopyranosides **56** and **57**.

Galectin affinity of *N*-arylated 3,4-dichlorophenyl thio-galacto and -gulosides

The galacto derivates **46-49** showed almost 50-fold affinity enhancement over their corresponding methyl β-D-galactopyranosides **29b´, 29c´, 29f´ and 29g´** (Table 5), but at the same time the selectivity was largely compromised (Table 6). The *p*-OMe guloside derivative **56** retained a 5-fold selectivity for galectin-9N over galectin-9C, while the *m*-OMe **57** failed to contribute to any affinity enhancement over the corresponding methyl guloside derivative **37.** Nevertheless, **37** turned out to retain a high selectivity (Table 6).

Table 6: K_d-values (μM)[a] of 3,4-dichlorophenol 3-deoxy-3-*N*-aryl-1-thio-α-D-galactopyranosides **46-49** and 3,4-dichlorophenol 3-deoxy-3-*N*-aryl-1-thio-α-D-gulopyranosides **56** and **57** against human galectin-1,- 3, -7, -8N, -8C, -9N, and -9C as measured by a fluorescence polarization assay.

Compounds	Galectins						
	1	**3**	**7**	**8N[b]**	**8C[c]**	**9N**	**9C**
46	46±4.0	81±9.0	17±1.8	444±15	96±6.0	14±0.5	4.6±0.4
47	41.1±1.3	79±3.0	36±7.4	331±25	98±4.0	14±0.6	14.8±0.9
48	117±7.0	79±9.0	13±2.1	NB	235±25	6.1±0.1	2.8±0.3
49	43.5±6.0	170±6.0	94±20	493±16	135±6.0	12.2±1.3	5.8±0.3
56	NB[d]	NB	NB	NB	NB	40±0.9	207±5.6
57	NB	NB	ND[e]	ND	ND	260±12	NB

[a]The data are average and standard error mean of 4–8 single-double point measurements. [b]N-terminal domain. [c]C-terminal domain. [d]Not binding at the highest concentration tested: 2 mM. [e]Not determined.

Computational analysis

In order to understand the reason behind the domain selectivity of the epimer switch, the galacto and gulosides **29f´** and **40** were subjected to MD simulations in complex with both galectin-9N and 9C. All computations were performed with Schrödinger software suit 2018-3. The crystal structures of PDB 3WLU of galectin-9N and PDB 3NV3 of galectin-9C were prepared for molecular dynamics simulation using the Protein Preparation Wizard. MD simulations were performed using Desmond with default settings except for the duration, which was 120 ns. The carbohydrate O4 atom and all beta sheet backbone atoms were subjected to light position restraint with a force contestant of 1 kcal/mol/Å^2.

The galactoside **29f´** in complex with galectin-9C managed to find a stable pose throughout the MD simulation and by replacing water molecules in the subsite, the complex possibly gained some extra stability. Additionally, a T-stacking of

the *N*-aryl ring with His223 was observed, which can contribute to the stability and increased affinity of **29f´** for galectin-9C. In contrast, the corresponding gulo epimer **40** never found a stable pose in complex with galectin-9C for a considerable period of time during the simulation. However, compound **40** managed to find a stable pose in simulations with galectin-9N and, similar to **29f´** in complex with galectin-9C, the *N*-aryl of **40** was also involved into T-stacking with a binding site amino acid side chain; Trp82. Additionally, the *N*-aryl ring of **40** shielded one side of an arginine side chain (Arg77) from apparently poorly solvated water molecules[82], which may lead to a gain in complex stability. Compound **29f´** never reached a stable pose during MD simulations in complex with galectin-9N.

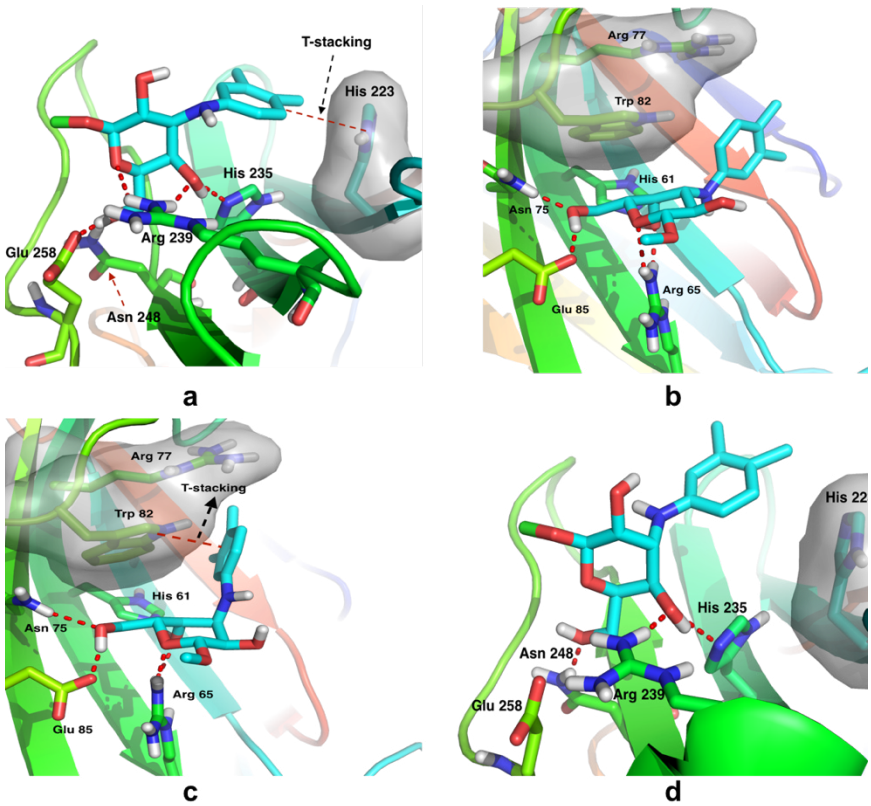


Figure 6. Representative molecular dynamics simulation snap-shots of **a**) compound **29f´** with galectin-9C (aryl-His223 stacking is indicated with a dashed orange line), **b**) compound **29f´** with galectin-9N, **c**) compound **40** with galectin-9N, (aryl-Trp 82 interactions are indicated with dashed. orange line) and **d**) compound **40** with galectin-9C.

Conclusions and future perspectives

Selectivity between the two domains of galectin-9 was achieved with *N*-aryl derivatised monosaccharides. Even more interesting, the selectivity was obtained only by reverting the stereochemistry at carbon 3 of galactose into gulose; the 3 epimer of galactose. We observed that methyl 3-deoxy-3-*N*-aryl-galactopyranosides were up to 30-fold selective for galectin-9C over 9N, whereas the methyl 3deoxy-3-*N*-aryl-gulopyranosides were 10-fold or more selective for galectin-9N over galectin-9C. Then, in quest of enhancing the affinity, 3,4-dichlorophenyl group was installed α at the anomeric position, which resulted in remarkable affinity increases, dissociation constant dropped to low micromolar, but the selectively was largely compromised for the galactosides. On the other hand, the corresponding 3,4-dichlorophenyl α-thiogulosides showed affinity enhancements for galectin-9N, together with some affinity for galectin-9C. Nevertheless, the gulosides were largely selective for galectin-9N. Furthermore, this selectivity switch by pyranose C3-epimerisation was supported by a computational study. MD simulations performed with a pair of methyl galacto and gulosides on both the galectin-9 domains, suggested that the galactoside found a stable pose throughout the simulation in complex galectin-9C, but failed to find such stable pose in complex with galectin-9N. The corresponding guloside gave opposite result in the MD simulations. Hence, our observation was backed by the computational study. In literature, domain selective inhibitors for galectins are rare, but would provide valuable tools for investigating the separate roles of galectin domains by means of domain-selective pharmacological intervention. Thus, the discovery of domain-selective *N*-arylated inhibitors can be expected to aid in advancing our understanding the biological functions of the two domains of galectin-9 separately.

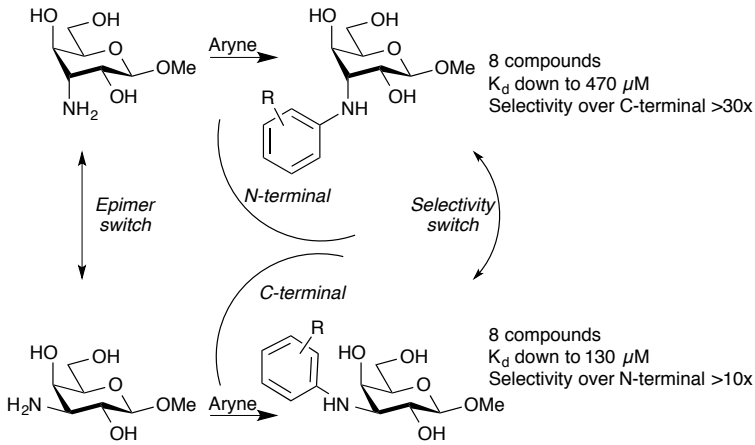


Figure 7. Pictorial representation of the ´epimer switch´ phenomenon and a short summary of what we saw in the chapter.

4. Sulfonamide-Derivatised Galactosides to Target Unexplored Subsites in Galectins (Paper III)

We start this chapter by discussing the "cloud theory" described by professor Uri Alon (https://www.youtube.com/watch?v=F1U26PLiXjM&t=403s) because in a true sense this explains the journey of this project. Cloud theory is really common in scientific research and a mundane thing when it comes to drug discovery. It is sometimes rightly said that a drug discovery project is nothing but serendipity: You are looking for something else but you find something wonderful that amazes you. However, the journey or the story is rarely discussed, we only get to see the end results. To narrate this, we often start at the origin X with a question (Figure 8) and target Y as a desired destination, but often in quest of finding Y we enter a cloud of failure and uncertainty. When the cloud goes away a different answer, or destination Z, which might be far away from Y is derived. This Z is often termed as serendipity, especially in medicinal chemistry.

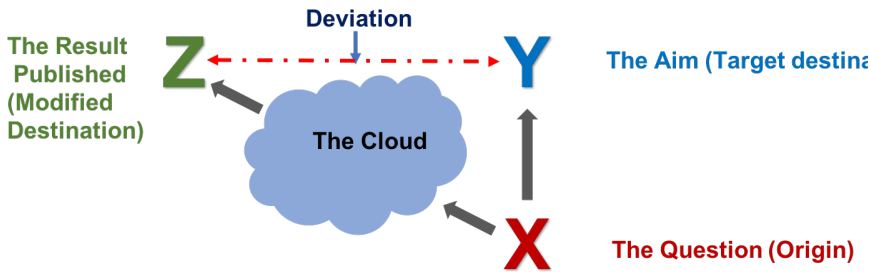


Figure 8. Philosophical representation of the journey of a project and where it reaches at the end deviating from the actual destination, the "cloud theory".

Galectin inhibitors targeting subsites adjacent to subsites A-B

This project started with the aim to design novel inhibitors that potentially could explore an unexplored binding site perpendicular to the A subsite in galectins, with the initial target being galectin-3. Hence, unexplored parts of the galectin-3 subsite A represented the original target destination Y in figure 8 above. The phenyl ring of 3-deoxy-3-(4-phenyl-1*H*-triazolyl)-galactosides has been shown to interact with subsite A according to X-ray analysis of a complex with galectin-3 (Figure 9)[7]. Here the question X was to find a proper functionality or design which can lead us to or near to the target point Y. Hence, the initial aim was to install a functionality at the phenyl ring of a C3-phenyltriazolyl galactoside that could protrude about 90^0 from the phenyl ring required to reach the unexplored binding pocket (A´) perpendicular to subsite A (Figure 9). Sulfur-derived substituents, such as sulfonamides, were a good bet as bond angles of sulfur-containing functionalities are near 90° and, hence, sulfonamides were considered as a viable option for anchoring moieties extending into galectin-3 subsite A'. Sulfonamide-carrying phenyltriazolyl galactosides could be accessible via Cu(I)-catalysed 1,3-dipolar cycloaddition of known 3-azido-galactosides with sulfonamidophenylacetylenes, why the initial task was to synthesise a collection of four sulfonamidophenylacetylenes in which the position (ortho and meta) and directionality (N-S and S-N) of the sulfonamide functionality were varied.

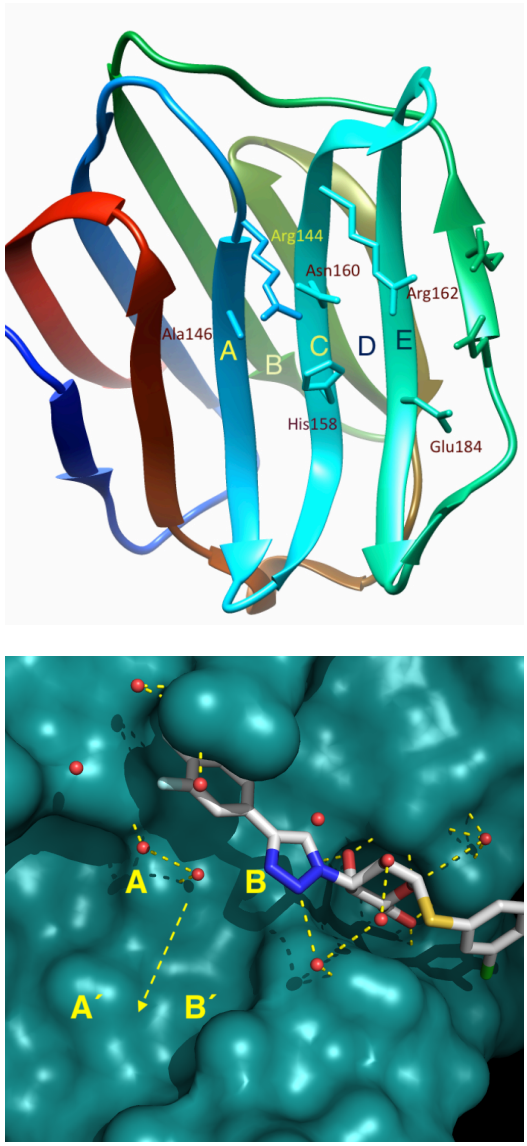
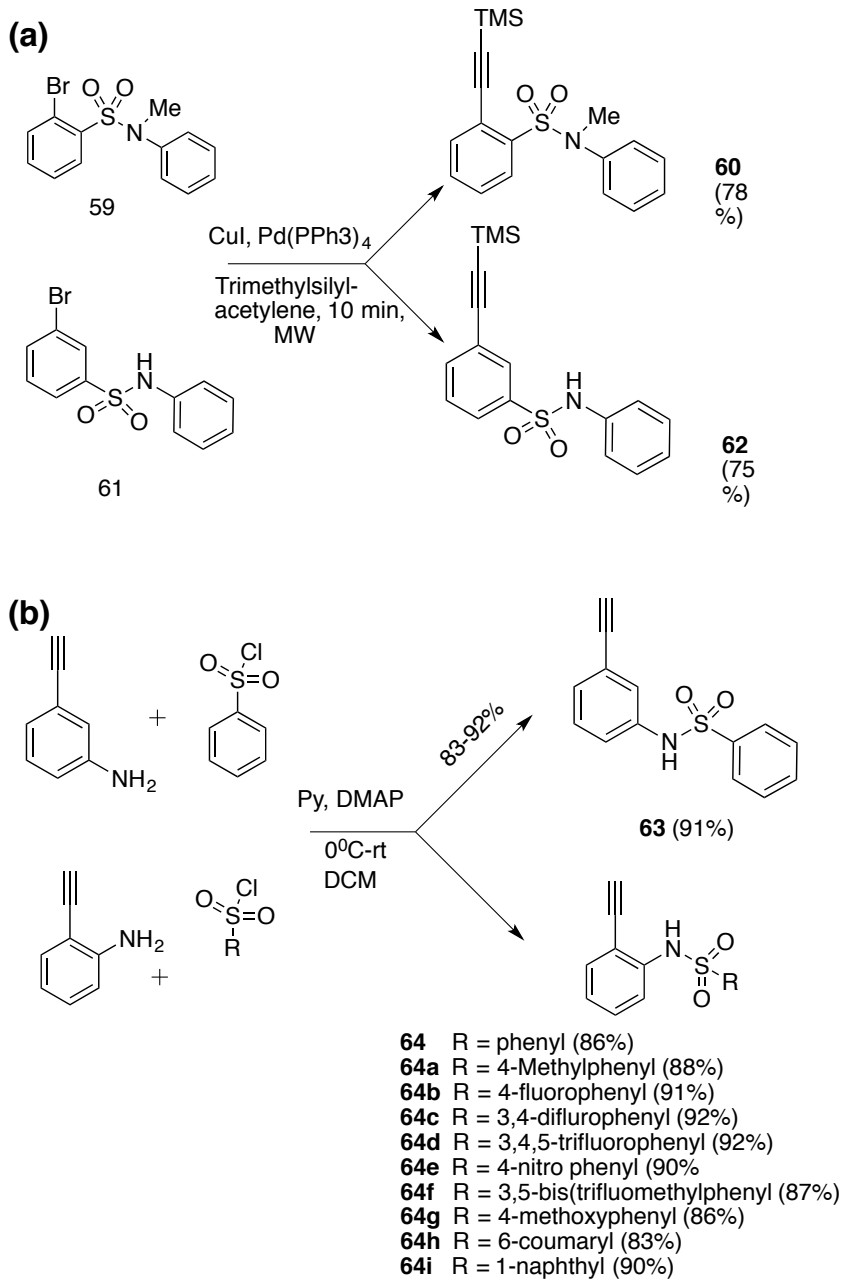


Figure 9. A) ribbon representation of the CRD in structure of galectin-3 (pdb id 1kjr) with the designation of the subsites A-E and the proposed targeted subsite A´ located below A. **B**) X-ray structure (pdb id 6eol) of 3-deoxy-3-(4-phenyl-1*H*-triazolyl)-galactosides with galectin-3[7] .

Synthesis of sulfonamide alkynes

In order to synthesise the alkyne **60**, the sulfonamide **59**[83] was subjected to Sonogashira coupling with TMS-acetylene, catalysed by CuI and $PdCl_2(PPh_3)_4$ in MW for 10 min to afford **60** at 78% yield. The choice of the *N*-methylated sulfonamide **59** was critical as is has been reported that the presence of a sulfonamide N-H in a Sonogashira reaction leads to a cycloaddition reaction with the alkyne to form a six membered ring[84]. In order to avoid this cycloaddition, sulfonamide *N*-methylation was chosen. Similarly, **62** was synthesised starting from sulfonamide **61**[85] at 72% yield, however without the need for *N*-methylation as the *m*-sulfonamide-position in this case prevented cyclisation. The sulfonamide alkyne **63** was synthesised by reacting 3-ethynylaniline with benzenesulfonyl chloride in presence of pyridine and DMAP in DCM. By following the same procedure, alkynes **64** and **64a-64i** were synthesised from 2-ethynylaniline and different aromatic and heteroaromatic sulfonyl chlorides.



Scheme 6. (**a**) Synthesis of silylethynyl-*N*-phenyl benzenesulfonamide **60** and **62**. (**b**) Synthesis of *N*-(ethynylphenyl) benzenesulfonamide **63**, and **64a**-**64i**.

Synthesis and evaluation of the sulfonamide-derivatised methyl α-D-galactopyranosides

Sulfonamide position and directionality optimisation

Initially, four different sulfonamide-based methyl galactosides **65**, **66**, **67**, **68,** and the reference compound **69** were synthesised by subjecting azide **58** to a Cu(I)-catalysed 1,3-dipolar cycloaddition with sulfonamide alkynes **60**, **62**, **63**, **64** and phenylacetylene, respectively, in the presence of DIPEA in DCM as the solvent at room temperature (Scheme 7). Compounds **65**, **66**, **67**, and **68** were evaluated against galectin-3 in the fluorescence polarization assay and **65** and **66** were found to be inactive up to 2 mM concentrations, whereas, **67** and **68** were worse than the reference compound unsubstituted phenyltriazolyl **69** against galectin-3. Furthermore, compounds **65** and **66** were still virtually inactive against most other galectins. Compound **67** showed some binding affinity towards most of the galectins, but comparing to the reference compound **69** those values were either similar or worse than **69** (Table 7). Interestingly, **68** displayed some promising affinity towards galectin-9N and galectin-4C, but, however, for galectin-4C the affinity was similar to the reference compound **69**. Hence, the phenylsulfonamide group of **68** did not contribute to galectin-4C binding. In contrast, **68** showed indeed 6-fold better affinity for galectin-9N than **69** did (Table 7). This observation was a first hint that exploring the extended subsite A and/or B in galectin-9N could lead to inhibitors with improved selectivity. Consequently, optimising the structure **68** through various substituents on the phenylsulfonamide moiety could provide a path to discovery not originally aimed at; that is a path through the cloud in figure 8 towards a new destination Z (galectin-9N) instead of galectin-3, destination Y. To conclude, we identified a new starting point, compound **68**, towards the discovery of compounds interacting with a hitherto unexplored binding pocket in galectin-9N.

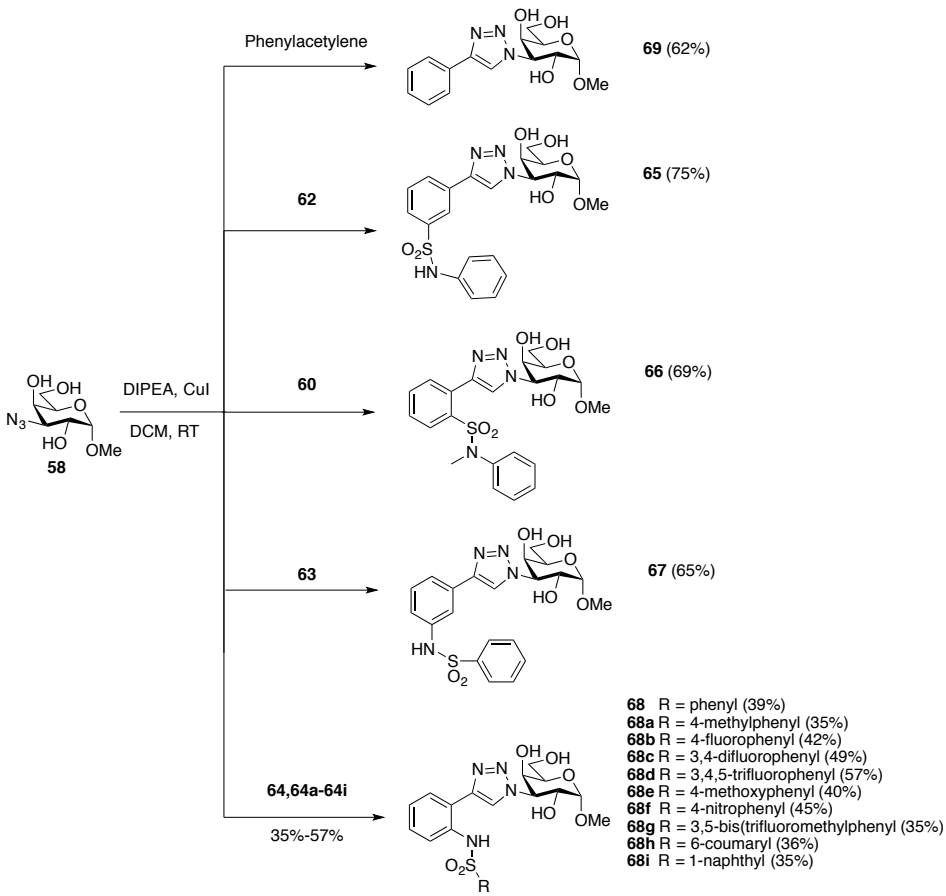
Table 7. K_d-values (μM)[a] of compounds **65-69** against human galectin-1, -3, -4N, -8N, -8C, -9N, -9C as measured with the fluorescence polarisation assay.

Compounds	Galectins								
	1	**3**	**4N**[b]	**4C**	**7**	**8N**	**8C**[c]	**9N**	**9C**
65	NB[d]	NB	NB	NB	NB	NB	2800±150	NB	NB
66	NB	NB	NB	NB	NB	NB	1900±250	NB	NB
67	430±17	200±29	NB	67±15	NB	1500±142	2400±377	270±22	670±24
68	510±21	320±6.0	NB	22±4.0	1100±90	2500±320	NB	60±4.0	480±30
69	320±15	150±13	440±32	32±6.0	600±35	2200±71	3100±327	390±42	1900±190

[a]The data are averages and standard error mean of 4–8 single-double point measurements. [b]*N*-terminal domain. [c]*C*-terminal domain. [d]Not binding at the highest concentration tested: 2 mM.

Benzensulfonamide substitution optimisation

As a lead molecule was at hand (**68**), the next step was to optimise the affinity by varying the benzenesulfonamide substituents. In this quest, a set of compounds **68a-68i**, with electron-donating or -withdrawing groups on the benzenesulfonamide ring, heteroaromatics, naphthyls, and biphenyls were synthesised from azide **58** in a similar manner as described above. (Figure 9). Here, one thing we should mention is the different reaction times for **65**, **66**, **67**, and **68**. For instance, the formation of **65** was the fastest, it took about 12 hours, whereas **66** took 18 hours to proceed to near completion. This could be due to the electronic effect of the sulfonamide group of **65** making the alkyne more electron deficient and facilitate the reaction, while in **66** with the sulfonamide group being ortho to the TMS-acetylene, the steric factor is likely slowing the reaction. On the other hand, **67**, the directional isomer of **65**, took about 24 hours to finish. Compounds **68** and **68a-68i** needed the longest reaction times with average times of 36-48 h accompanied with poorer yields (35-57%). In case of **68** and **68a-68i**, the sulfonamide directionality probably attributes less to an electron-withdrawing effect on the alkyne group and hence the reaction rate is slower and yields are poorer. This effect is then even worse for the ortho analogues **68** and **68a-68i** due to the additional steric factor.



Scheme 7. Synthesis of the reference phenyl triazole **69** and the methyl galactoside sulfonamides **65**, **66, 67**, **68** and **68a**-**68i**.

The general trend in the evaluation of compounds **68a**-**68i** against galectin-9N was that electron-withdrawing groups increased the affinity (**68b**, **68c**, **68d**, **68f**, and **68g**) more than electron-donating groups on the outer phenyl ring (**68a** and **68e**) did (Table 8). No such trend against galectin-4C was observed, though a couple of inhibitors (**68a** and **68b**) showed better affinity towards galectin-4C than galectin-9N. Quite interestingly, bicyclic aromatic systems, such as naphthyl or coumaryl, enhanced the selectivity towards galectin-9; the naphthyl **68i** was highly selective for galectin-9 with K_d values of 30 and 37 μM towards galectin-9N and 9C, respectively, whereas the coumaryl **68g** was highly selective for galectin-9N with K_d=63 μM. The 3,5-bis(trifluoromethyl)phenylsulfonamide **68g** (K_d = 18 μM) and the *p*-nitro **68f** ((K_d= 21 μM) were two of better inhibitors of galectin-9N.

Table 8. K_d-values (μM)[a] of compounds 68 and 68a-68i against human galectin-1, -2, -3, -4N, -4C, -7, -8N, -8C, -9N, -9C as measured with the fluorescence polarisation assay.

Compounds	Galectins								
	1	**3**	**4N**[b]	**4C**[c]	**7**	**8N**	**8C**	**9N**	**9C**
68a	400±12	150±10	324±10	20±2.0	1200±100	1000±100	2900±150	51±3.0	325±15
68b	970±30	249±29	204±15	20±2.4	600±30	2100±230	29000±120	49±4.0	520±8.0
68c	250±22	130±7.0	1106±50	26±3.0	ND[e]	2900±340	NB[d]	34±2.2	220±19
68d	570±5.0	111±4.0	ND	ND	ND	3000±61	NB	29±1.8	330±44
68e	650±30	150±25	ND	ND	ND	NB	NB	55±4.0	230±30
68f	410±17	98±2.6	500±20	50±4.0	400±20	1900±70	2900±370	21±1.4	319±16
68g	780±20	200±15	NB	ND	ND	NB	NB	18±2.1	220±10
68h	NB	NB	NB	NB	NB	NB	NB	63±2.0	NB
68i	NB	NB	NB	NB	NB	NB	NB	37.8±2.4	30±3.0

[a]The data are averages and standard error mean of 4–8 single-double point measurements. [b]*N*-terminal domain. [c]*C*-terminal domain. [d]Not binding at the highest concentration tested: 2 mM. [e]Not determined.

Computational analysis

In order to understand whether or not the sulfonamide-derivatised galactosides were interacting with extended subsites in galectin-9N and to understand what leads to the enhanced affinity for galectin-9N, we studied the most potent inhibitor in terms of both affinity and selectivity *i.e.* compound **68f**, in MD simulations of its complex with galectin-9N. All computations were performed with Schrödinger software suit 2018-3. The crystal structure of galectin-9N (PDB 3WLU) was prepared for molecular dynamics simulation using the Protein Preparation Wizard. MD simulations were performed using Desmond with default settings except for the duration which was 300 ns. The ligand O4 atom and all β-sheet backbone atoms were subjected to light position restraint with a force contestant of 1 kcal mol^{-1} $Å^{-2}$. The ligand **68f** found a stable pose throughout the simulation (Figure 10). A surface representation of a snapshot of a stable pose during the MD simulation shows that the sulfonamide unit is going perpendicular down to the A-B subsite, more specifically it is directed at a near 90^0 angle to the B subsite towards what we named the B´ pocket). Furthermore, the ligand triazole ring involved in intramolecular π stacking with the aromatic ring attached to the sulfonamide group. This stacking may provide some conformational rigidity to **68f**. We suggest that the enthalpic gain by this intramolecular ligand stacking and accompanying favourable interactions with galectin-9N, was enough to

overcome a possible entropic loss by reduced ligand flexibility. Furthermore, the MD simulations suggested that the **68f** nitro substituent was playing a role in enhancing the binding. The nitro group involved in H-bonding with Trp82 in the above-mentioned B´ pocket and replaces the poorly solvated water molecules close to Trp82 (Figure 10B).

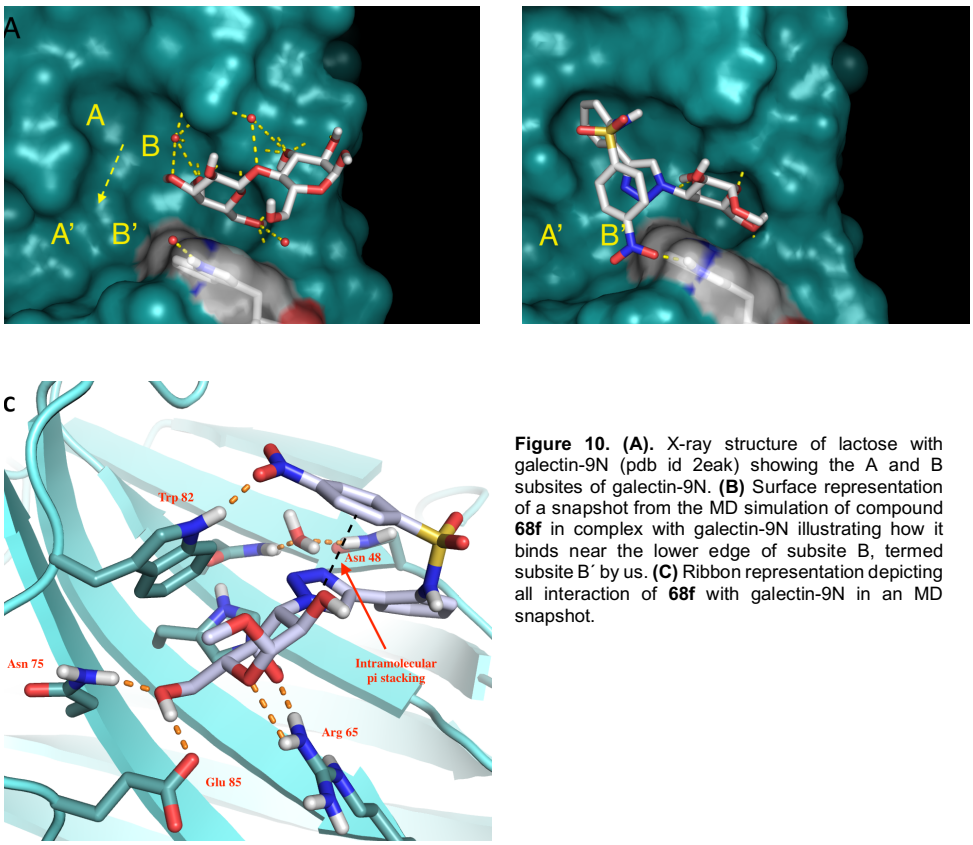


Figure 10. (A). X-ray structure of lactose with galectin-9N (pdb id 2eak) showing the A and B subsites of galectin-9N. **(B)** Surface representation of a snapshot from the MD simulation of compound **68f** in complex with galectin-9N illustrating how it binds near the lower edge of subsite B, termed subsite B´ by us. **(C)** Ribbon representation depicting all interaction of **68f** with galectin-9N in an MD snapshot.

Conclusion

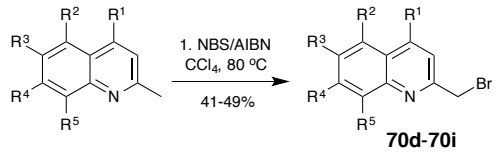
In summary, we propose that the sulfonamide-derivatised galactosides interacted with a hitherto unexplored binding subsite in galectin-9N. They were found to bind with galectin-9N with almost 6-15 fold better affinity than the reference phenyl triazole **69**. The computational modelling supported the hypothesis of interactions with an earlier unexplored subsite near the rim of Trp82 in galectin-9N. Subsequently, an inhibitor collection was synthesized in which the sulfonamide aromatic ring was varied or substituted. The resulting SAR showed that electron-withdrawing substituents on the sulfonamide aromatic ring led to better affinity. Furthermore, MD simulations of **68f** in complex with galectin-9N suggested that a stable intramolecular π-stacking between the ligand triazole ring and the sulfonyl aryl group may explain the observed affinity enhancements, which also correlates with the SAR analysis. Additionally, the nitro group in **68f** replaces a poorly solvated water near Trp82 and involves in hydrogen bonding with the Trp82 indole N-H in a subsite B´ that is perpendicular to the subsite B.

5. Quinoline-Derivatised Galactosides as Galectin Inhibitors (Paper IV)

The project was designed based on the notion that coumarins, quinolines, and indolizines are known structural elements in many biologically active molecules with medicinal potential[86–89], which made them attractive structures for galactose derivatization towards novel galectin inhibitors. Furthermore, as cancer is a disease where galectins in many cases are involved and as heterocycles based on coumarin, quinoline, and indolizines are known to have anti-cancer effects, a logical hypothesis would to combine galactose with these heterocycles may result in a multi-target drug. Hence, we synthesised a series of indolizine, coumarin and quinoline based galactoside derivatives and evaluated them for galectin binding. Eventually, we found that the quinoline-derivatised galactosides proved to be selective and better inhibitors over the other compounds against galectin-8N. Hence, in this chapter will focus our discussions on quinoline-derivatised galactosides. For further information about the coumarin- and indolizine-derivatised galactosides, see paper IV.

Inhibitor synthesis

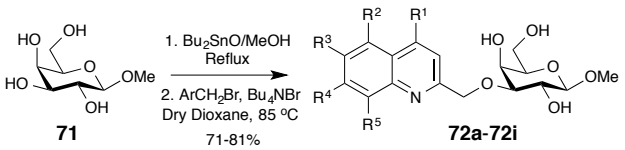
In order to have the desired quinoline synthons, we mono-brominated the 2-methyl group of substituted quinolines **69a-69i** in presence of AIBN and NBS to afford **70a-70i**. Thereafter, compounds **72a-72i** were obtained through stannylidene-mediated regioselective etherfication at 3-OH of methyl β-D-galactopyranoside **71** with the quinoline bromides **70a-70i**. Compounds **72j** and **72k** were obtained by the hydrolysis of the corresponding methyl esters **72d** and **72e** under basic conditions (K_2CO_3-EtOH) in 73–78% yield.



69a-69i

70d-70i

70a $R^1 = R^2 = R^3 = R^4 = R^5 = H$
70b $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^3 = F$
70c $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^3 = Cl$
70d $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^3 = CO_2CH_3$
70e $R^1 = R^2 = R^3 = R^3 = R^5 = H$, $R^4 = CO_2CH_3$
70f $R^1 = R^2 = R^5 = H$, $R^3 = R^4 = F$
70g $R^1 = Cl$, $R^2 = R^4 = R^5 = H$, $R^3 = CF_3$
70h $R^1 = Cl$, $R^2 = R^3 = R^5 = H$, $R^4 = CF_3$
70i $R^1 = Cl$, $R^2 = R^3 = R^4 = H$, $R^5 = F$

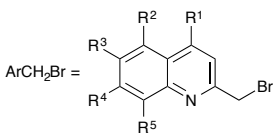


71

72a-72i

K_2CO_3/EtOH, 50 °C, 66% → **72d** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = CO_2CH_3$ / **72j** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = CO_2H$

K_2CO_3/EtOH, 50 °C, 63% → **72e** $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CO_2CH_3$ / **72k** $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CO_2H$



70a-70i

72a $R^1 = R^2 = R^3 = R^4 = R^5 = H$
72b $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^3 = F$
72c $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^3 = Cl$
72d $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = CO_2CH_3$
72e $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CO_2CH_3$
72f $R^1 = R^2 = R^5 = H$, $R^3 = R^4 = F$
72g $R^1 = Cl$, $R^2 = R^4 = R^5 = H$, $R^3 = CF_3$
72h $R^1 = Cl$, $R^2 = R^3 = R^5 = H$, $R^4 = CF_3$
72i $R^1 = Cl$, $R^2 = R^3 = R^4 = H$, $R^5 = F$
72j $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = CO_2H$
72k $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CO_2H$

Scheme 9. Synthesis of quinoline-derivatised methyl β-D-galactopyranosides **72a**-**72k**.

Affinity evaluation against galectins

Table 9 presents a selection of data from the affinity evaluations against galectins (for the full Table, see paper IV). Compounds **72a**-**72j** bound galectin-3, -8N, and, to some extent, galectin-9N, thus showing moderate and partial selectivity. However, with **72k** both selectivity and affinity were increased for galectin-8N. It bound with galectin-8N with K_d 110 μM which is somewhat better than the natural disaccharide fragment sialyl-α-(2-3)-galactoside **73**, which was included in the evaluations as a natural ligand fragment (Figure 11) and almost 60-fold more potent that methyl β-D-galactopyranoside. Compound **71** was selected as a relevant natural ligand disaccharide fragment reference, because sialyl-a-(2-3)-lactose has been identified as most potent lactose-containing trisaccharide ligand for galectin-8N[57,90]. An important observation was that **72j**, the 6-regioisomer of the 7-carboxylate **72k**, was almost 2.5 times worse binding towards galectin-8N and with almost no selectivity. Another interesting observation was the unsubstituted quinoline **72a** was 6-fold more potent towards galectin-8N than corresponding naphthalene **74**, which evidences the importance of nitrogen in galectin-8N binding. Prior to this work, a 2-*O*-acetyl-3-*O*-iminocoumarylmethyl galactoside derivative[45] was the best methyl galactoside inhibitor for galectin-8N reported in the literature with K_d=170 μM, albeit with no selectivity as it had higher affinities for several other galectins.

Table 9. K_d-values (μM)[a] of compounds **72a**-**72k**, **74** for human galectin-1, 3, 8N, 8C, 9N and 9C as measured by a fluorescence polarization assay.

Compounds	Galectins					
	1	**3**	**8N**	**8C**	**9N**	**9C**
72a	1700±100	620±50	700±41	2900±38	470±71	2200±110
72b	1300±130	580±22	700±50	3100±340	510±88	NB[d]
72c	1900±70	510±83	440±46	2800±21	550±110	2400±770
72d	1200±20	710±170	520±38	4600±760	1300±270	NB
72e	1700±20	610±2.0	630±9.0	3100±670	550±53	1900±340
72f	1200±15	410±53	1400±60	2700±14	580±86	NB[d]
72g	NB	820±33	1000±25	3500±440	1100±10	1300±13
72h	1300±90	NB	3500±650	NB	720±7.0	NB
72i	1400±130	450±84	640±80	2400±590	390±29	1700±78
72j	690±8.0	250±11	250±10	3100±41	1500±100	930±8.0
72k	880±25	380±11	110±6.0	3200±121	300±16	3900±150
71[48,91]	>10000	4400	6300	>30000	3300	8600±730
73	670±125	ND[e]	150±12	ND	ND	ND
74	>4000	730±80	>4000	>4000	1700±110	>3500

[a]The data are averages and standard error of the mean of 4–8 single-double point measurements. [b]*N*-terminal domain. [c]*C*-terminal do-main. [d]Not binding at the highest concentration tested: 2 mM. [e]Not determined

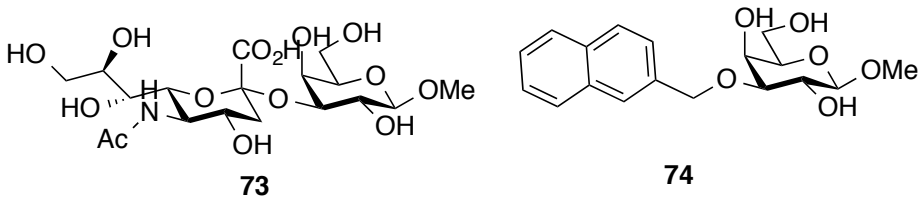
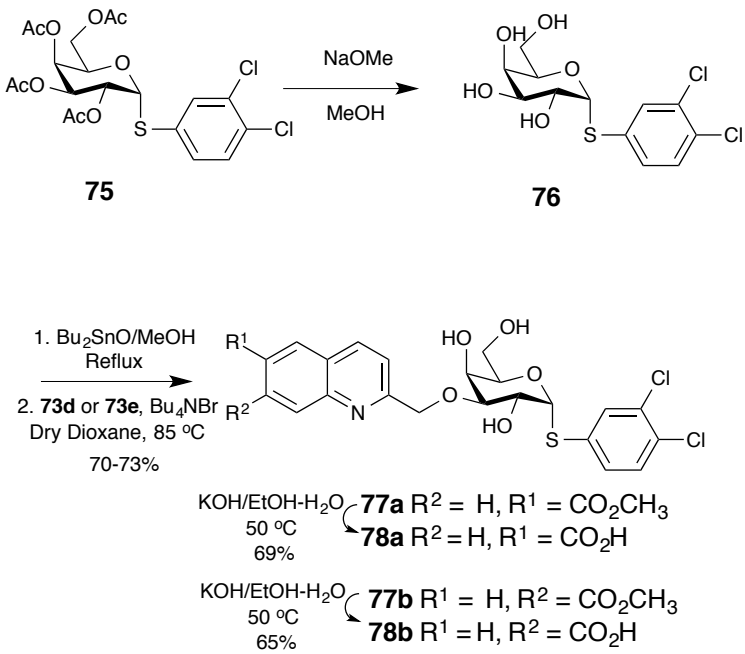


Figure 11. Reference compound methyl sialyl-α-(2-3)-galactopyranoside **73** and methyl 3-*O*-naphth-2-yl-β-D-methylgalactoside **77**.

Next, the strategy to increase the affinity analogous to the one presented in chapters 2 was attempted by combining the quinoline-derivatised structures with the anomeric α-3,4-dichlorophenyl group[51] Hence, we selected two of the best inhibitors, **72j** and **72k**, and installed the 3,4-dichlorophenyl α-aglycon to obtain 7**8a**-7**8b** (Scheme10). To our satisfaction, an almost 50-fold affinity enhancement was observed for both the inhibitors 7**8a** and 7**8b**. The dissociation constant of 7**8a** came down to 1.9 μM, whereas for 7**8b**, it was 1.5 μM (Table 10), which are till date the most potent monosaccharide inhibitors for human galectin-8N, but unfortunately the selectivity over galectin-3 was compromised.



Scheme 10 Synthesis of quinoline-derivatized α-thiogalactosides **81a** and **81b**

Table 10. K_d-values (μM)[a] of compounds **78a** and **78b** for human galectin-1, 2, 3, 4N, 4C, 7, 8N, 8C, 9N and 9C as measured by a fluorescence polarization assay.

Compounds	Galectins									
	1	**2**	**3**	**4N**	**4C**	**7**	**8N**	**8C**	**9N**	**9C**
78a	100±5.0	110±12	1.2±0.02	110±14	43±8	32±5.0	1.9±0.1	330±48	8.8±0.5	27±7.0
78b	48±4.4	59±4.0	1.3±0.07	43±7.1	43±5.7	ND[b]	1.5±0.08	240±15	2.1±0.09	14±1.3

[a]The data are average and standard error of the mean of 5-12 single point measurements. [b] Not determined

Understanding the binding through MD simulations

We performed an MD simulation of **72k** with galectin-8N in order to understand its interaction with galectin-8N. Low energy conformations of **72k** in complex with galectin-8N (pdb id 3VKO), with the galactose unit placed in the galectin core galactose binding site C in the same pose as natural galactose-containing ligands, were generated by rotating the three bonds between the galactose C-3 atom and the quinoline ring, followed by energy minimization with the OPLS3 force field and the GB/SA solvation method for water.

Initially, short MD simulations were run with these low energy conformations in order to have an initial understanding on how the ligand is interacting in the binding site. In all of the short MD simulations we found that **72k** drifted towards a conformation with the quinoline ring oriented near Arg45. Next, we kept this quinoline position as the starting pose in a 1000 ns MD simulation. The simulation converged after 300 ns into a stable complex of **72k** with galectin-8N. In this complex geometry, the amino acid Gly142 was buried under the **72k** quinoline rings and **72k** formed hydrogen bonds with Arg59, Asp49, His65, Gln147, and a buried water molecule. Furthermore, presumably poorly solvated water molecules were removed from the hydrophobic face of the side chain of Arg59, which likely contributes to the binding. The side chain of Arg59 adopted an altered position in the complex as compared to the apo structure (2YV8) or the corresponding lactose complexes (2YXS, 3AP4, 3VKL, and 3VKM). Furthermore, the 7-carboxylate of **72k** formed a water-mediated hydrogen bond framework with Arg45, Gln47, and Gly142. These hydrogen bonds may be less favourable in the case of the isomer **72j**, which could explain why it binds weaker than **72k** to galectin-8N. Last, but not least, the quinoline nitrogen is also involved in a water mediated hydrogen bond with the OH-2 of galactose; this conformation provides an ideal steric and electronic fit of both the quinoline and galactose moieties in **72k** in the galectin-8N binding pocket.

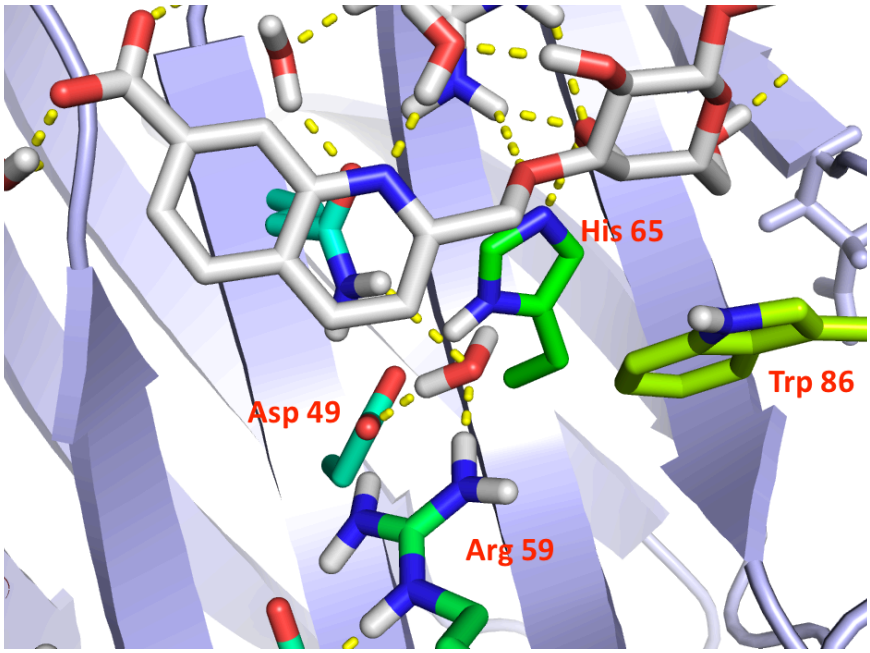


Figure 11. An MD simulation snapshot of the complex between **72k** and galectin-8N and polar inyeractions are shown with yellow dashed lines.

Cell assays

The quinolines **72a-72k** were evaluated for cytotoxicity on human cancer cell lines JIMT-1 and MCF-7, as well as on a normal human cell line MCF-10A as a control, in an MTT assay at a concentration range from 0.05 mM to 50 mM. The MTT assay is a colorimetric assay that is based on the reduction of the water-soluble tetrazolium salt MTT to an insoluble purple formazan in the mitochondria of viable cells. Thus, the MTT reduction is used to reflect the viable cell number. None of the compounds affected cell viabilities in any of the three cell lines. Although the inhibitors did not affect cancer cell viability, the positive outcome was that they did not affect the normal cells either. Hence, this indicated low toxicity, which is advantageous if the compounds are intended to have other effects than cytotoxicity in a biological system, as can be anticipated for galectin-8N inhibition.

Conclusions

In summary quinoline (also coumarin, and indolizine, paper IV)-derivatised galactosides were synthesised by robust and viable methods. Quinoline compounds, especially the 3-*O*-(carboxyquinoline)-derivatized methyl galactosides (**72j**, **72k**) showed good affinity as well selectivity towards galectin-8N. MD simulations of **72k** with galectin-8N showed that during the simulation Gly142 was buried under the ligand and the carboxylic acid was involved into water mediated hydrogen bonding interactions with Arg45, Glu47 and Gly142. In addition, the quinoline nitrogen also formed water mediated hydrogen bonding with OH-2 of galactose. Furthermore, when 3-*O*-carboxyquinoline derivative was combined with α-3,4-dichlorophenylthio aglycon part affinity was increased by almost 70-fold against galectin-8N (to K_d=1.5 μM), but at the same time selectivity was heavily compromised. Furthermore, these compounds were tested with both normal and tumour cell lines and they proved to be non-toxic against both cell types. These results support the hypothesis that 3-*O*-carboxyquinoline-derivatised galactosides might be a novel class of non-toxic and selective lead compounds towards the development of galectin-8N inhibitors.

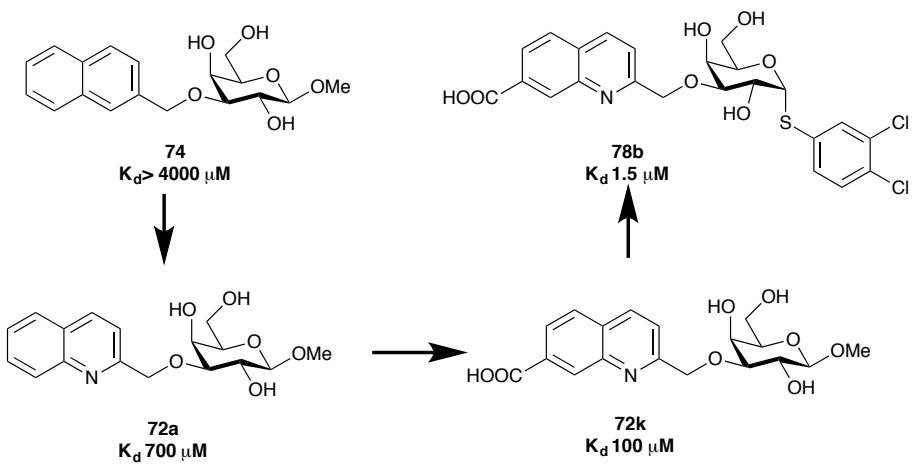


Figure 12. An summary of the structural alterations from the naphthyl **74** and via the quinolines **72a** and **72k** towards the high-affinity galectin-8N inhibitor **78b**.

6. Concluding Remarks and Future Perspectives

Though in an overall perspective this is a medicinal chemistry thesis, which mostly surrounds design and synthesis of galectin selective inhibitors, chapter 2 presents a synthesis method development carbohydrate amine arylations. This method is robust, transition metal free, and well tolerated by a majority of protecting groups used in carbohydrate chemistry. On top of this, the method is completely selective for the amino group over carbohydrate hydroxyls. Continuing with a medicinal chemistry application of this method in chapter 3, I showed that 3-*N*-arylated galactose derivatives selectively bind with galectin-9C, whereas its 3-epimer, 3-*N*-arylated gulosides, binds selectively to galectin-9N. In the literature of galectin inhibitors, such a domain selectivity is rare and achieving it via an "epimer switch" illustrates how minor changes in a ligand structure can have a profound influence on selectivity.

In chapter 4 we discovered a new class of sulfonamide-derivatised galactosides as high affinity and selective inhibitors of galectin-9N. Furthermore, chapter 5 concerned quinoline-derivatised galactosides as selective inhibitors of galectin-8N. We also proposed an importance of the quinoline nitrogen for binding as it during MD simulations of a quinoline-derivatised galactoside in complex with galectin-8N formed a water-mediated hydrogen bond with OH2 of galactose.

Galectin selective inhibitors have a clear significance as tools in experiments aiming at improving our understanding of biological functions of a particular galectin. For instance, the domain selective inhibitors, *N*-arylated galacto and gulo derivatives, can help us to separate the functions of *N*-terminal and C-terminal domains of galectin-9 from each other if they are different. The quinoline-derivatised galactosides were not cytotoxic for cancer or normal cells and are thus promising as lead compounds towards selective galectin-8N inhibitors.

In the introduction of chapter 2, I mentioned that natural glycans never or rarely are *N*-arylated, why *N*-arylated glycomimetics would clearly be worthwhile investigating in design of glycmimetics. As discussed in chapter 3, high domain selectivity in galectin-9 was discovered for *N*-arylated galacto- and gulosides. In

the future, the *N*-arylation method could be used on other carbohydrate structures aiming at discovering glycomimetics binding to other proteins.

The discoveries of sulfonamide-derivatised galactosides as galectin-9N inhibitors in chapter 4 also points to interesting future developments. The immediate follow up work would be to enhance affinity and selectivity even further with aid from the MD simulations. The MD simulations suggested that a nitro group is interacting with the Trp82 and one strategy could be to vary different substituents to replace the nitro group with functional groups that can involve in hydrogen bonding with Trp82 (Figure 13). The choice could be an acid, ester, or cyano group *etc*. Another possibility could to vary the position of a second substituent on the phenylsulfonamide, including substituents meta with respect to the sulfonamide group. This might open up the possibility to explore the A´ subsite even further. In order to do so, we would initially perform a MD simulation with galectin-9N in complex with **68f** carrying varying different *m*-substituent (R^2 in Figure 13). Synthesis and evaluation of selected *m*-substituted variants could lead to improved galectin-9N inhibitors.

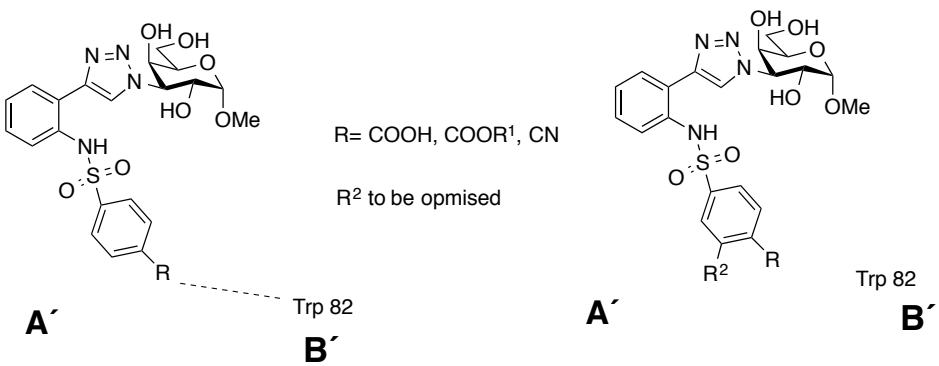


Figure 13. Proposition of different functional groups (R or R^1) and varying their position to find better interaction in A´ or/and B´ pocket.

In chapter 5, we presented quinoline-derivatised galactosides as galectin-8N inhibitors in which binding data and MD simulations suggested that quinoline nitrogen played a role for affinity and thus galectin-8N selectivity. A further investigation would be to change the quinolone ring system with other nitrogen containing heretoaromatic units (Figure 14). In this heterocycle ring variation, it wold be of interest to systematically investigate the influence of the heterocycle ring nitrogen electron density. Once the heteroaromatic ring is optimised then there will be an ample scope to study the effect of different substituent on that optimised heteroaromatic ring.

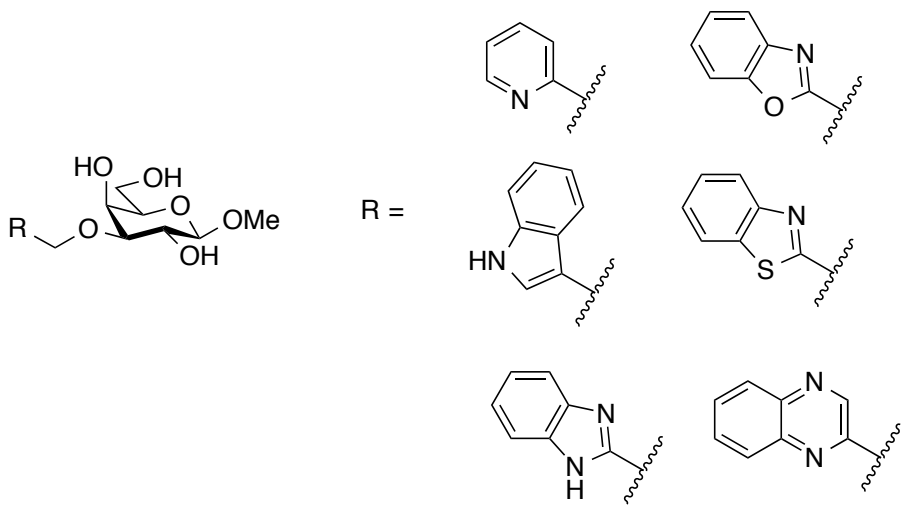


Figure 14. Proposition of different heteroaromatic groups in order to further optimise the affinity towards galactin-8N

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Almost 20% of my life I have spent in Lund doing PhD. So indeed, it's a quite a journey and along the journey we meet many people who one way or other brings forth changes in life. They say time is an illusion and, in the space-time, coordinate we are some small pieces that are all connected to each other. We all are meant to be present at a certain time and place and with a single missing piece our life could have been slightly or significantly different.

Talking about that piece, with that being taken out from my life it will be significantly different is my supervisor Ulf J Nilsson. I owe a big thank you to him. This is such a privilege to have him as my supervisor, it cannot be better. Thanks to Fredrik Zetterberg for all those important ideas and constant feedback. Thanks to Hakon Leffler for providing the help whenever needed. A huge thanks to Barbro, for always delivering the data no matter how short the time window is given. Thanks to Sofia Essen for all those help in mass sample and Maria, Katrina for administrative helps. Karl Erik for the help in NMR whenever I asked for you were right there. Paula for all the help in printing of my thesis.

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