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2019

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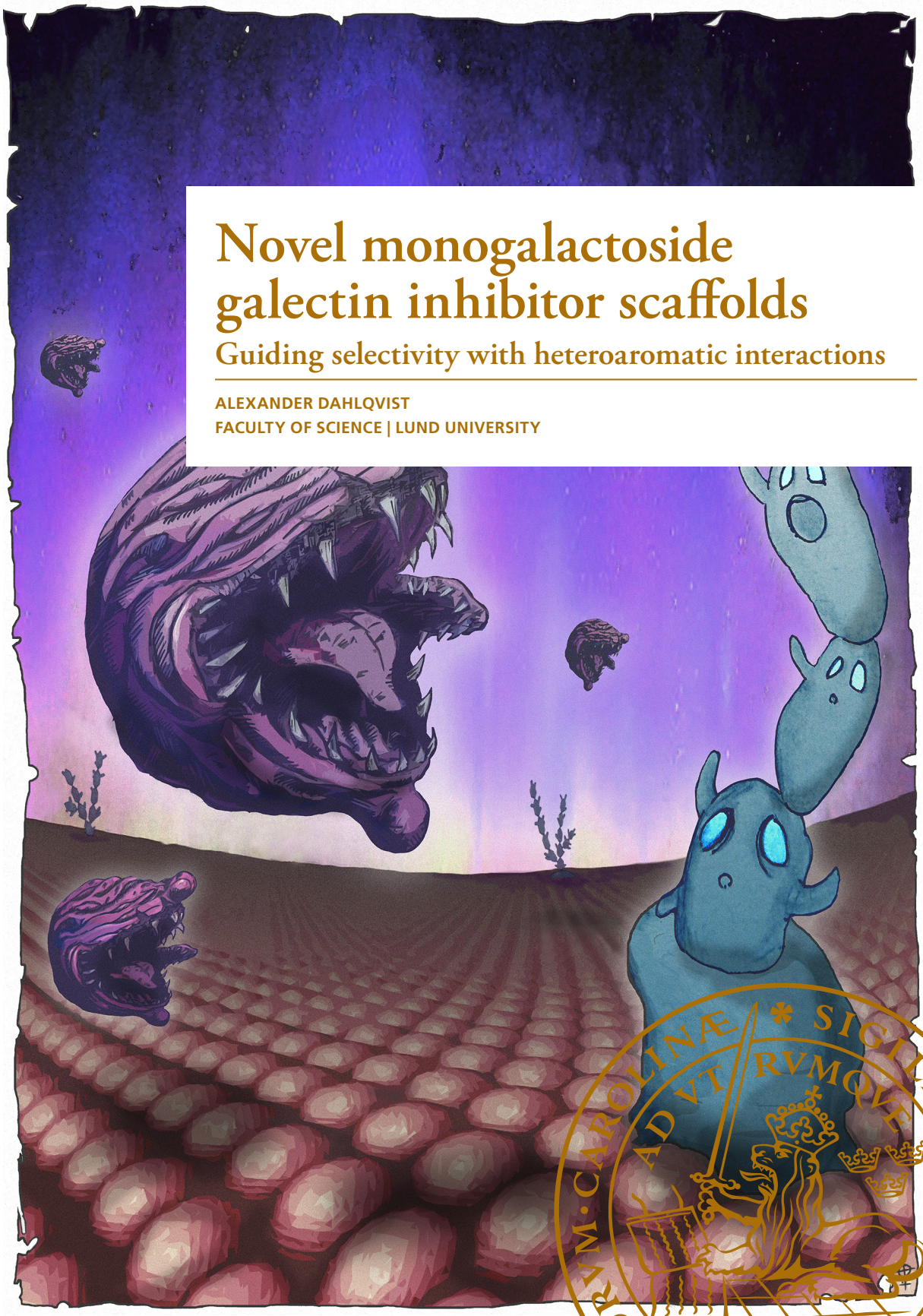

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DOCTORAL DISSERTATION

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To be defended at lecture hall K:A, Kemicentrum,
Wednesday the 29/5 2019 at 09:00

Faculty opponent
Prof. László Somsák, University of Debrecen

Organization	Document name
LUND UNIVERSITY Centre for Analysis and Synthesis Lund University, Box 124 SE-221 00 Lund, Sweden	DOCTORAL DISSERTATION
	Date of issue 2019-04-12
Author(s) Alexander Dahlqvist	Sponsoring organization

Title and subtitle: Novel monogalactoside galectin inhibitor scaffolds - Guiding selectivity with heteroaromatic interactions

Abstract: Carbohydrates are involved in many cellular processes, and most biomolecules are glycosylated. These modifications are used in biological systems as information carriers, helping regulate organization on the cell surface and interactions between cells and the environment. Galectins are a family of carbohydrate binding proteins that bind to polysaccharides containing a galactose. Galectins have the ability to crosslink glycosylated proteins – especially on the cell surface – giving galectins a role in modulating cell signalling and environmental interactions, influencing angiogenesis, immune regulation and cell adhesion. This implicates galectins in diseases like cancer and immune related disorders. Subsequently, many glycomimetics have been developed as galectin inhibitors, based on a variety of scaffolds, many with very high affinities, but selectivity between galectins remains a challenge. The galectin family of proteins has a very conserved binding motif, hence the differences in the binding pocket are small, making designing a selective inhibitor a challenge.

We investigated C1-galactosides as possible galectin inhibitor scaffolds, exploiting one of the few differences between galectin-1 and galectin-3 – histidine 52. We used C1-arylheterocycles to control the selectivity via the interaction between the anomeric heterocycle and the histidine, an approach which turned out to be fruitful resulting in the inhibitors 1-naphthyloxazole galactose, a galectin-3 selective inhibitor with 90μM affinity and 2-fluorophenyltriazole galactose, a galectin-1 selective inhibitor with a 170 μM affinity with fivefold and eightfold selectivity respectively. Extending the C1- system with a methylene linker resulted in the galectin-1 selective 4-fluorophenyltriazole 2-deoxygalactoheptulose, an inhibitor with 170 μM affinity and fourfold selectivity. In order to pursue these 2-deoxyheptulose scaffolds we developed a diastereoselective hydroboration method for C1-exomethylene glycopyranosides. Combining C1-substitutions with substitution in position three on galactose with a phenyltriazole motif did not increase affinities in a straightforward way; instead of increasing affinity and perserving selectivity patterns set by the C1-substitutents, the disubstituted molecules emerged as galectin-4 selective inhibitors with affinities down to 2.3 μM and up to thirty-eightfold or better selectivity for galectin-4. This shows that C1-galactosides can be selective galectin inhibitors with good affinities, but more work needs to be done to understand the interaction between substitution patterns. We also investigated aminpyrimidine substituted galactosides and identified compounds with a threehundred-fold selectivity for galectin-3 over galectin-1 and affinities down to 1.7 μM. These results show that careful selection of heterocycles with an aim towards exploiting even minute differences in the binding pocket can be effective in achieving selectivity.

Key words: Galectin, Galectin inhibitor, C-glycoside, C-galactoside, Aminopyrimidine, Hydroboration

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language: English**
ISSN and key title		**ISBN 978-91-7422-658-4**
Recipient's notes	**Number of pages: 106**	Price
	Security classification	

Signature  Date 2019-04-12

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LUND
UNIVERSITY

A doctoral dissertation at a university in Sweden is produced as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal dissertation, which summarizes the accompanying papers. These have already been published or are manuscripts at various stages.

Coverphoto by Olof Röckner

Illustrations on pages 18 & 20 by Mats Öberg

Faculty of Science
Department of Chemistry, Centre for Analysis and Synthesis

ISBN 978-91-7422-658-4
e-ISBN 978-91-7422-659-1

Printed in Sweden by Media-Tryck, Lund University
Lund 2019

To my wife who has endured my curiosity,
and my child whom I hope has inherited it

Table of Contents

Abstract

Carbohydrates are involved in many cellular processes, and most biomolecules are glycosylated. These modifications are used in biological systems as information carriers, helping regulate organization on the cell surface and interactions between cells and the environment. Galectins are a family of carbohydrate binding proteins that bind to polysaccharides containing a galactose. Galectins have the ability to crosslink glycosylated proteins – especially on the cell surface – giving galectins a role in modulating cell signalling and environmental interactions, influencing angiogenesis, immune regulation and cell adhesion. This implicates galectins in diseases like cancer and immune related disorders. Subsequently, many glycomimetics have been developed as galectin inhibitors, based on a variety of scaffolds, many with very high affinities, but selectivity between galectins remains a challenge. The galectin family of proteins has a very conserved binding motif, hence the differences in the binding pocket are small, making designing a selective inhibitor a challenge.

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List of papers

Paper I: Alexander Dahlqvist, Hakon Leffler, Ulf J. Nilsson

C1-Galactopyranosyl heterocycle structure guides selectivity: Triazoles prefer galectin-1 and oxazoles prefer galectin-3

ACS Omega, accepted

Contributions: Conceived of the project, synthesized and characterized the compounds. Analysed the results and wrote the first draft of the paper.

Paper II:

Alexander Dahlqvist, Axel Furevi, Niklas Warlin, Hakon Leffler, Ulf J. Nilsson

Stereo- and regioselective hydroboration of 1-exo-methylene pyranoses: Discovery of aryltriazolylmethyl C-galactopyranosides as selective galectin-1 inhibitors

Beilstein Journal of Organic Chemistry, accepted pending minor revision

Contributions: Conceived of the project, supervised AF and NW during the development of the synthesis methodology, synthesized and characterized the compounds. Analysed the results and wrote the first draft of the paper.

Paper III: Alexander Dahlqvist, Fredrik Zetterberg, Hakon Leffler, Ulf J. Nilsson Aminopyrimidine-galactose hybrids are highly selective galectin-3 inhibitors

MedChemComm, accepted pending minor revision

Contributions: Synthesized and characterized most of the compounds. Analysed the results and wrote the first draft of the paper.

Paper IV: Alexander Dahlqvist, Hakon Leffler, Ulf J. Nilsson

C1-Aryltriazolyl galactopyranosides are galectin-1-selective inhibitors

In manuscript

Contributions: Conceived of the project, synthesized and characterized the compounds. Analysed the results and wrote the first draft of the paper.

Paper V: Alexander Dahlqvist, Hakon Leffler, Ulf J. Nilsson

Unexpected substituent synergy in 3-aryltriazolyl-C1-galactosides give high-affinity galectin-4C selective inhibitors

In manuscript

Contributions: Conceived of the project, synthesized and characterized the compounds. Analysed the results and wrote the first draft of the paper.

Abbreviations

Ac	Acetyl
ACN	Acetonitrile
Ar	Aryl
9-BBN	Borabicyclo[3.3.1]nonane
Bn	Benzyl
CRD	Carbohydrate recognition domain
DCM	Dichloromethane
DMF	Dimethylformamide
Gal	Galactose
Glu	Glucose
HPLC	High pressure liquid chromatography
Lac	Lactose
LacNAc	N-Acetyllactosamine
Man	Mannose
Me	Methyl
Nap	Naphthyl
NOESY	Nuclear Overhauser Effect Spectroscopy
Ph	Phenyl
RT	Room temperature
TDG	Thiodigalactoside
THF	Tetrahydrofuran
TMS	Trimethylsilyl
Wt	Wild type

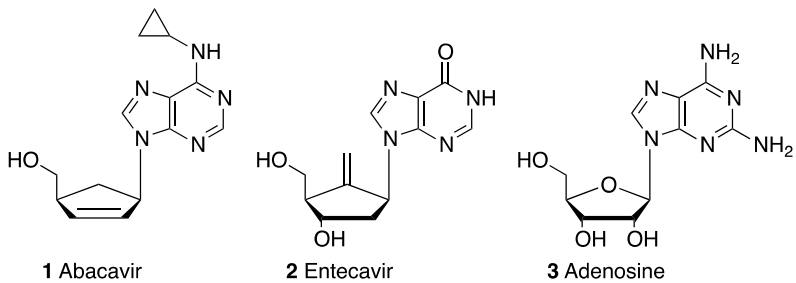
1. Introduction – C-glycosides, galectins and galectin inhibitors

"I'm not here to start trouble. I am just doing some research for my thesis."
– Jessica Fortunato

C-glycosides as drugs

When deciding to dedicate your research efforts towards making C1-glycoside based drugs, it pays to keep in mind that what you ultimately strive for is to no longer be a carbohydrate chemist. The IUPAC Gold Book definition of "carbohydrate" conspicuously fails to mention anything about C1-glycosides, defining a carbohydrate as such: "*The generic term carbohydrate includes monosaccharides, oligosaccharides and polysaccharides as well as substances derived from monosaccharides by reduction of the carbonyl group (alditols), by oxidation of one or more terminal groups to carboxylic acids, or by replacement of one or more hydroxy group(s) by a hydrogen atom, an amino group, thiol group or similar groups. It also includes derivatives of these compounds*."[1]. So what is a C-glycoside? It is simply a glycoside where one or more hydroxyl groups has been replaced with a carbon atom. In this thesis, we will mostly be looking at C-glycosides in the anomeric position, known as C1-glycosides. From the definition alone it is quite clear that C1-glycosides have received limited interest and enthusiasm compared to many other types of carbohydrate modification and not entirely without cause. Simply put, carbon-carbon bonds are often hard to make, and introducing a new carbon-carbon bond in a richly functionalized molecule where even regioselectivity, much less stereoselectivity, might already be a problem is not an easy feat. So why bother? In short, increased affinity and selectivity as well as improved pharmacokinetic properties, are very good reasons to bother, and on occasion, it might actually simplify the synthesis by simplifying the compound. Finally, on occasion solving a hard problem is a source of satisfaction in and of itself[2]. We will start our journey by looking at examples of successful uses of C-glycosides as drugs, before moving on to galectins and galectin inhibitors.

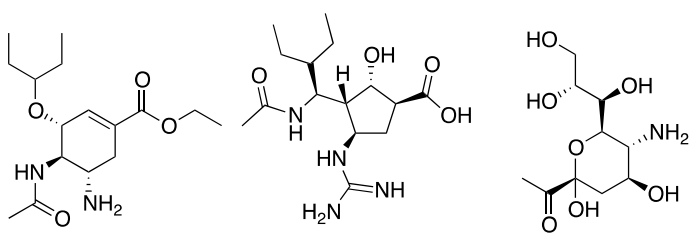
Classic examples of C-glycoside drugs are the antiretrovirals Abacavir and Entecavir, both HIV reverse transcriptase inhibitors (figure 1). Both are derived from ribonucleosides, where the ribose part of the molecule has undergone the most extensive modifications (compare with adenosine, **3**). If we look at Abacavir **1**, the ring oxygen and both the 2- and 3-hydroxy groups have been removed and replaced by a double bond (and for Entecavir **2** the 2-hydroxy group has been removed, the ring oxygen has been removed and replaced by a methylene group, making both drugs into endocyclic C-glycosides. In both cases these modifications were done to increase affinity and improve pharmacokinetic properties by removing non-interacting and non-essential parts of the carbohydrate scaffold[3,4].



1 Abacavir **2** Entecavir **3** Adenosine

Figure 1: Antiretroviral C-glycosides Abacavir 1 and Entecavir 2, and adenosine 3

Oseltamivir[5] **4** was a name heard around the world when the H1N1 influenza virus, more colloquially known as swine flu, loomed as a potential pandemic in 2009. Especially troubling was the reports of influenza strains resistant to this neuraminidase inhibitor, hinting that one of the few available remedies – not based on rest and chicken soup[6] – had perhaps run its course as a viable treatment[7]. This made Oseltamivir arguably the most famous C-glycoside in the world, together with its less known cousin Peramivir[8] **5**. Both are C-glycosidic simplifications of neuraminic acid or its N-acetylated variant sialic acid **6**, removing the ring oxygen and in the case of Peramivir reducing the ring size (figure 2). In both cases, these alterations improved both affinity and pharmacokinetic properties such as uptake.



4 Oseltamivir **5** Peramivir **6** Neuraminic acid

Figure 2: Neuraminidase inhibitors Oseltamivir (4) and Peramivir (5) with their parent, neuraminic acid (6).

Another example of increasing both affinity and metabolic stability through the introduction of a C-glycosidic motif is the case of C-α-glucosylceramide **7** (figure 3), a natural killer T-cell stimulant that helps activate the immune system and with therapeutic potential as an antimalarial and antimetastatic compound. Moving from an O-glycoside to a C-glycoside meant a 1000-fold improvement in antimalarial activity and a 100-fold improvement in antimetastatic activity, as well as increased metabolic stability[9–11].

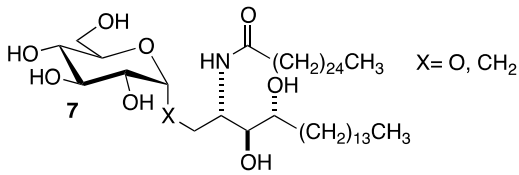


Figure 3: α-Glucosylceramide 7 in O- and C-glycosidic forms. Introduction of a C-glycoside leads to a 100- to 1000-fold increase in efficacy.

Another example of switching from an O-glycosyl to a C-glycosyl structure are C-glycoside castanospermine analogues used as α-glucosidase inhibitor antitumor compounds[12]. Even nature ventures into C-glycosides on occasion, such as with the antibiotics Vineomycinone B2[13], C-glucosyl juglone[14] and angucycline[15,16] or with the cytotoxic C1-indole glucosides from Isatis indigotica[17].

So far we have seen C-glycosides that are either small alterations of O- and N-glycosides found in nature or actual natural products, but now it is time to move on to C-glycosides that have not been designed as atom replacements or structure simplifications, but as the addition of completely new structural motifs. In figure 4 we see three such examples; one naphthyl C-xyloside (**8**) that is a tumour growth inhibitor[18] one C1-spiroheterocyclic glucose (**9**)[19] and a C1-napthyhylthiazolyl glucose (**10**)[20] that are both glycogen phosphorylase inhibitors. An astounding variety of different C1-spiroheterocylic and C1-heterocyclic glucose derivatives exist[21–24], showcasing the large number of C-heterocyclic glycosides that can be made and also illustrating how small alterations of the aglycon can have large effects on affinity, something we shall encounter again later. Other synthetic C-glycosides include the C1-arylglucosides Canagliflozin and Dapagliflozin, as well as the C1-spirocyclic Tofogliflozin, all renal glucose transporter inhibitors used in the treatment of diabetes[25,26]. We now have several examples of C-glycosides bearing an aromatic or heteroaromatic aglycon being powerful scaffolds for glycomimetics, setting the stage for investigating galectin inhibitors with these structural motifs.

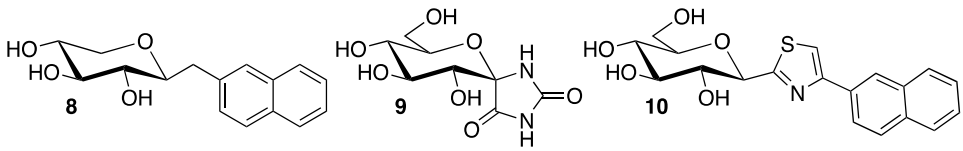


Figure 4: C1-naphthylxyloside 8, a tumour growth suppressor, C1-spiroheterocycle galactoside 9 and naphthylthiazolyl glucose 10, glycogen phosphorylase inhibitors; all examples of C-glycosides with structural motifs not inspired by natural compounds.

We are now in the position of being able to look at our list of examples and state a few things about glycomimetic design. The design principles in going from a natural carbohydrate structure to a functioning glycomimetic drug has been summed up in an excellent review by Ernst and Magnani[27] as:

- Identification of relevant interacting parts of the carbohydrate, using a combination of crystallography, NMR interaction studies and other structural biology techniques.
- Removal of oligosaccharide subunits that are non-interacting or contribute little to the interaction
- Removal of non-interacting hydroxyls in the remaining carbohydrate unit or units
- Addition of structural motifs that provide increased affinity and selectivity based on binding pockets and potential exploitable interactions identified in the structural biology stage
- Early screening of pharmacokinetic parameters such as distribution coefficient (logD), metabolic stability and cellular uptake.

In all, this is a lightly adapted variant of the standard workflow for drug development, with an emphasis on structural simplification.

As the synthesis of elaborately substituted glycosides can be quite involved, requiring large amounts of steps – especially sequences of protecting group pattern changes – it is often beneficial to attempt to first screen and optimize one or a few substituent patterns at a time and then combine them, in order to minimize the amount of synthesis involved in producing non-viable designs. This is a variant of fragment-based design[28–31], where instead of screening for fragments from a library, finding hits and then connecting them with a suitable linker, we design fragments connected to our carbohydrate scaffold and then "connect" them using our carbohydrate scaffold as a linker – reminiscent of the fragment merging approach. This creates a situation where the combination of fragments might be synergistic or antagonistic, something that is hard to predict from the outset. After so far having seen that a change from a regular glycoside to a C-glycoside might lead to higher

affinities and improved selectivities and pharmacokinetic properties, we will discuss the role of carbohydrate recognition in cellular biology briefly before we move on to the target proteins; the galectins.

Glycobiology – A brief introduction

To better appreciate the magnificence of the galectins, we need to lay down a foundation of the role of carbohydrate molecular recognition in biology. Carbohydrate recognition is fundamental in cell biology. Most, if not all, macromolecules in the cell are covered in carbohydrate structures and hence it is not surprising glycosylation of a protein being an almost ubiquitous posttranslational modification. The span of glycosylations go from single carbohydrates to large oligosaccharides that end up dwarfing the core protein[32]. Two basic types of protein oligosaccharide modification exist, N-glycans and O-glycans. N-Glycans are transferred as an oligosaccharide to the protein in the endoplasmatic reticulum (ER) during translation and guide the correct folding, transport in the cell and final destination of the protein through the ER, the Golgi apparatus and onwards. If the proteins leave the cell, this is termed the classical secretion pathway[33,34]. During the folding and transport through the cell, the N-glycan structure is modified through removal and addition of various saccharides, each modification acting as a quality control checkpoint[35–37]. The N-glycan therefore functions as a "ticket book", guiding the proteins path via the ripping and punching of tickets as it passes through the cells synthesis, modification and transport machinery. O-Linked glycans in contrast, are synthesized directly on the protein one sugar at a time and the exact structure is dependant on the exact levels of different glycosyltransferases and glycosidases in the cellular environment[38]. O-Linked glycans have a variety of different biological roles, from structural roles in the extracellular matrix to being the classical blood group markers[39,40]. The glycans are multifunctional; they do different things depending on where and when they are present and which carbohydrate recognition system they interact with. There are a myriad of carbohydrate recognition systems that read glycans, but one large superfamily of these are the lectins, which among other members include the families of C-type lectins which have calcium dependant oligosaccharide binding[41] and mannose-binding lectins[42], both of which serve important roles in cell-cell interactions in the immune system, recruitment of immune cells and innate immunity. Another lectin superfamily member are the galectins, our target family, which we will now look at in depth.

Galectins – The basics

Galectins are first and foremost defined by their ability to bind to and strict preference for galactose-containing oligosaccharides and a conserved galactose binding site, known as the carbohydrate recognition domain (CRD)[43–45]. The galectins are divided into three different subfamilies based on the number of CRDs and how they are connected. Prototype galectins are considered the oldest from an evolutionary standpoint and have one CRD, which can dimerize and crosslink. Prototype galectins include galectins -1, -2 and -7. Tandem repeat galectins have two dissimilar CRDs, one N-terminal and one C-terminal connected by a linker that can be spliced to a variable length, and include galectins -4, -8 and 9. The lone member of the chimera family is galectin-3, with one CRD and a collagen-like tail. Galectin-3 can form a variety of different oligomers, but the pentamer is one of the classical examples[46,47]. An illustration of the different subfamilies of galectins can be found in figure 5. The galectins are cytosolic proteins that are transported to the cell surface – where they have many of their biological roles – using a non-classical secretion pathway that doesn't include the Golgi apparatus sorting and secretion machinery[48].

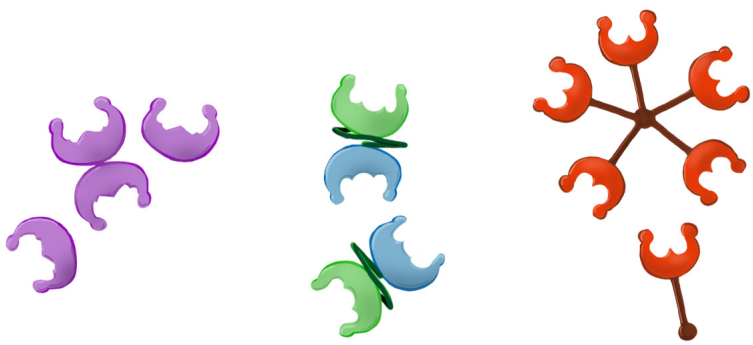


Figure 5: The different galectin types, from left to right. (1) Prototype galectins (including galectin-1, -2 and -7) which form homodimers. (2) Tandem galectins with two non-identical carbohydrate recognition domains connected by a linker (Including galectin-4, -8 and -9). (3) Galectin-3 the only member of the chimera family, with one CRD and a collagen-like tail, which can oligomerize. Illustration by Mats Öberg.

On the cell surface, galectins are involved in the cross-linking of all kinds of cell surface proteins, from receptors (including vascular endothelial growth factor – VEGFR –epidermal growth factor – EGFR – and transforming growth factor beta - TGFβR[49,50]) to environmental adhesion molecules (like integrins[51]). This crosslinking is also connected to endocytosis through the linking of glycosylated cargo proteins and glycolipids, resulting in increasing membrane curvature and the

induction of endocytosis[52,53]. Through these processes, the concentration of galectins in the extracellular space greatly influences the organization and residence time of membrane structures, in effect forming a galectin lattice[54], having a great impact on not only cell signalling but also cell-cell[55] and cell-extracellular matrix interactions[56] (figure 6). This influences, among other things, blood vessel[57] and lymphatic vessel[50] growth. Inside the cell, the galectins have functions in both the cytosol and the nucleus. Galectin-3 has been associated with both the cytoskeleton[58] and the mitotic spindle[59] giving it a role in cell division, one of the most fundamental workings of the cell. Intracellular signalling is another domain where we find galectins, such as in the Wnt/β-catenin pathway[60] and the Bcl-2 pathway[61] involving it in cell proliferation, stem cell differentiation, cell migration and apoptosis.

The galectins are not only of differing types and cellular location related complexity, they are also expressed differently in different tissues. Galectin-1 and galectin-3 are the most ubiquitous, expressed in most tissues[46]. Galectin-2 and galectin-4 are primarily in the gastrointestinal tract, with galectin-2 also being expressed in the placenta[62,63]. Galectin-7 finds its primary home in the gastrointestinal tract, epithelial cells in stratified epithelia – that is, the lining of mucosal membranes – and the skin[64]. Liver, kidney, cardiac muscle, lung and neuronal tissue play host to galectin-8[65], while the small intestine, various types of epithelium and T-lymphocytes express galectin-9[63]

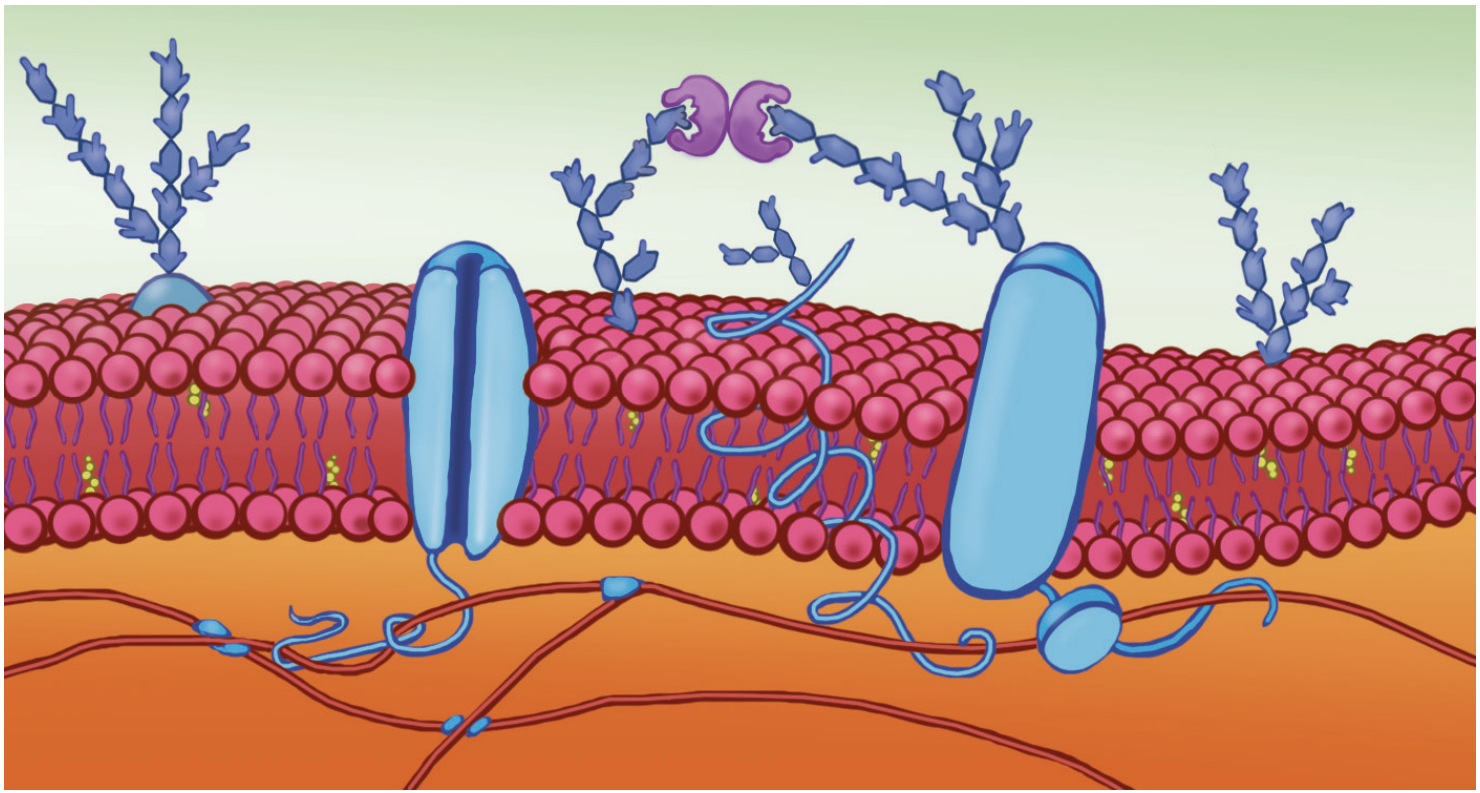
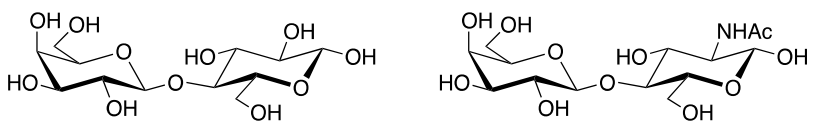


Figure 6: The galectin lattice on the cell surface. Most structures on the cell surface are covered in glycans, from glycoproteins to glycolipids. Galectins crosslink these structures by binding to lactose/N-Acetyllactosamine motifs in the glycans which leads to the formation of a lattice, which in turn influences the activity and residence time of the bound proteins. Proteins that are involved in lattices like this include receptors like VEGFR, EGFR and TGFβR and extracellular adhesion proteins like integrins. This affects cell signalling, cell adhesion and in general how the cell interacts with its environment. Illustration by Mats Öberg.

So what does this mean in the context of pathologies? It is known that galectin-3 overexpression contributes to cancer stemness[66], the loss of differentiation experienced by cells in a developing tumor. The connections to blood vessel growth (galectin-3) and lymphatic vessel growth (galectin-8) are also important, as these are ways for tumors to secure nutrients, oxygen and a pathway to spread through metastasis. In immune related pathologies, galectins are involved in several different ways. One of the best studied diseases with galectin involvement is idiopathic pulmonary fibrosis (galectin-3), one of the pathologies where galectin inhibition has an effect in animal models[67–69]. Likewise, in the case of pathological lymphangiogenesis, there is evidence in animal models that galectin inhibition can be an effective treatment[70]. Differing types of immune related intestinal disorders like inflammatory bowel disease in general and especially cholitis[71] are exacerbated by high galectin-4 expression but supressed by high expression of galectins -1 and -2[72], while there is evidence that some other inflammation related intestinal disorders[73] as well as colorectal cancer[74] are instead supressed by galectin-4, highlighting the complexity of the effects of galectins. The excellent review "Galectins at a glance" summarizing the vast trove of knowledge about galectin biological function by Johannes, Jacob and Leffler was recently published, and is a good place to start for a deeper dive into galectins[75] while a more light-hearted take on the vast variety of diseases with galectin involvement is "Galectin-3: One Molecule for an Alphabet of Diseases, from A to Z" by Sciacchitano et al[76]. So far we have the why, and it is time to start moving on to how.



11 Lactose (lac) **12** N-acetyllactosamine (lacNAc)

Figure 7: Natural ligands of galectins; lactose 11 and N-acetyllactosamine 12.

The galectins preference for linear β-galactoside containing oligosaccharides has already been mentioned, but it is time to dive deeper into the natural ligand preferences for galectins before looking at synthetic galectin inhibitors. The canonical binding motif for galectins is lactose **11** or its 2-acetamido variant N-acetyllactosamine **12**, seen in figure 7. In both cases the affinity is in the lower micromolar range. N-Acetyllactosamine has Kd of about 60 μM for both galectin-1 and -3, while lactose has an affinity of 187 μM for galectin-1 and 160 μM for galectin-3[77,78]. The galectins, as expected have a wide range of preferences when it comes to the glycan structures in the subsites on either side of the lac/lacNAc motif. Galectin-1 binds to a wide set of N-glycans with the lac/lacNAc motif present while galectin-3 primarily prefers poly-lacNAc N-glycans[79]. From this we can learn two

things; galectins were not intended to bind one single glycan with high specificity but rather to have a preference range, and there are few exploitable differences between the galectins close to the canonical binding motif[80,81]. Galactose is traditionally said to be located in subsite C of the galectin binding pocket, with the glucose of lactose in subsite D. On the further side of the glucose lies subsite E, while subsites A and B are found towards the 3-hydroxyl of the galactose. On galactose, hydroxyls 4 and 6 as well as the ring oxygen are crucial for binding, forming hydrogen bonds to a network of amino acids. In galectin-3 hydroxyl 4 binds to histidine 158, asparagine 160 and arginine 162, hydroxyl 6 binds to asparagine 174, glutamate 184 and the ring oxygen to Arginine 162. The hydrophobic α-face of the galactose rests on top of the flat, hydrophobic tryptophan 181, giving us a pocket subsite with a very polar β-face with lots of directional bonding and a non-polar α-face without directional bonding[82].

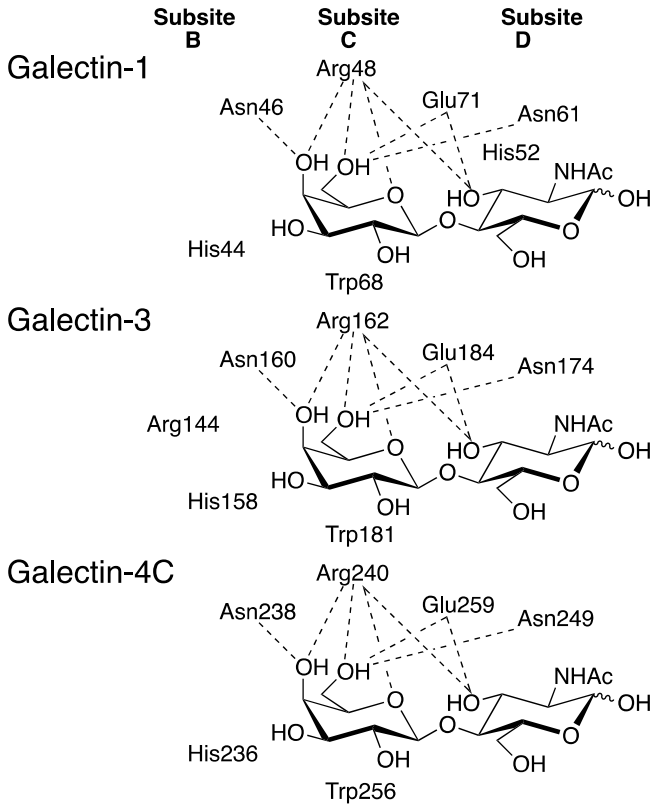
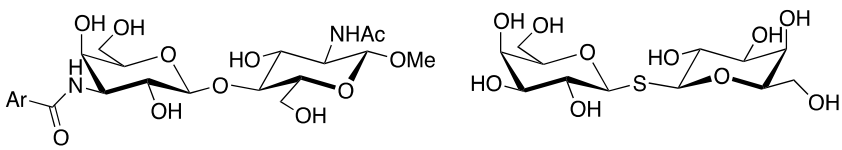


Figure 8: Comparison of the binding pockets of galectin-1, -3 and -4C with N-acetyllactosamine 12 in each pocket, a prototype galectin, chimera galectin and tandem repeat galectin respectively. The hydrogen bonding pattern interacting with lacNAc is highly conserved, especially the interactions with galactose, as is the tryptophan on which the hydrophobic α-side of galactose rests. The small differences make achieving selectivity a challenging prospect.

In other galectins, the core galactose bonding pattern is similar (figure 8). The only real difference between galectin-1 and galectin-3 in close proximity to the galactose binding subsite C is the presence of a histidine, His52, in subsite D. With this information in hand, we can see that we can substitute galactose at positions -1, -2 and -3, or lactose at position -3 on the galactose and positions -1, -2 and -6 on glucose.

Galectin inhibitors

Armed with an understanding of the natural ligands and their binding we are now prepared to move on to designed inhibitors. We will observe recurring iterations of addition of interacting motifs and subsequent structural simplification. Starting out with the 3-benzamido-N-acetyllactosamines **13** where the standard N-acetyllactosamine **12** has been substituted with an arylamide (figure 10)[78,83]. The best arylamido N-acetyllactosamine was 2-carboxynaphth-1-amide with a dissociation constant of 0.32 μM, an improvement over the unsubsituted on the order of three orders of magnitude. The interaction of the 3-arylamides and the galectin-3 is hypothesized to be a cation-pi stacking with arginine 144[84,85]. The thiodigalactoside (TDG) scaffold **14** is a simplification of the lactose scaffold, being composed of two symmetrical galactosides and connected by a sulphur bridge with increased metabolic stability compared to lactose based derivatives,[77] with an affinity towards galectin-1 approximately eight times better compared to that of lac and lacNAc and approximately threefold better towards galectin-3[86,87].



13 3-arylamido-N-acetyllactosamine **14** Thiodigalactoside (TDG)

Figure 9: 3-Arylamido-N-acetyllactosamines 13 and thiodigalactoside 14, two scaffolds for galectin inhibitors.

Elaborating the thiodigalactoside scaffold with either 3-arylamides or 3-(aryl]triazoles gives us structures like the (3-methoxy)benzamido) **15** and 3-(3-fluorophenyl)triazole TD139 **16**, which are galectin-3 selective and with very high galectin affinities. **15** has an affinity of 50 nM[86] while **16** is even better with an affinity of 2 nM[67,68] (figure 10). TD139 has been shown to reduce idiopathic lung fibrosis in murine model studies[68], and has successfully completed phase Ib/IIa clinical trials in IPF patients[88]. The thiodigalactoside scaffold of these inhibitors bind in a way similar to the 3-arylamido N-acetyllactosamines in the lac/lacNAc

subsite, while the aryltriazoles form dual cation-pi stacking interactions to arginine-144 and arginine-186[89]. The phenyl thiogalactoside scaffold **17** has been used as a testing platform to screen aryl substituents on the triazole, simplifying the effort in finding the best phenyl substitution pattern by obviating the need to synthesize the entire thiodigalactoside, and some have affinities that are impressive on their own. The best of these compounds was the 3,4,5-trifluorophenyl derivative **17** with a galectin-3 affinity of 5.2 μM[90].

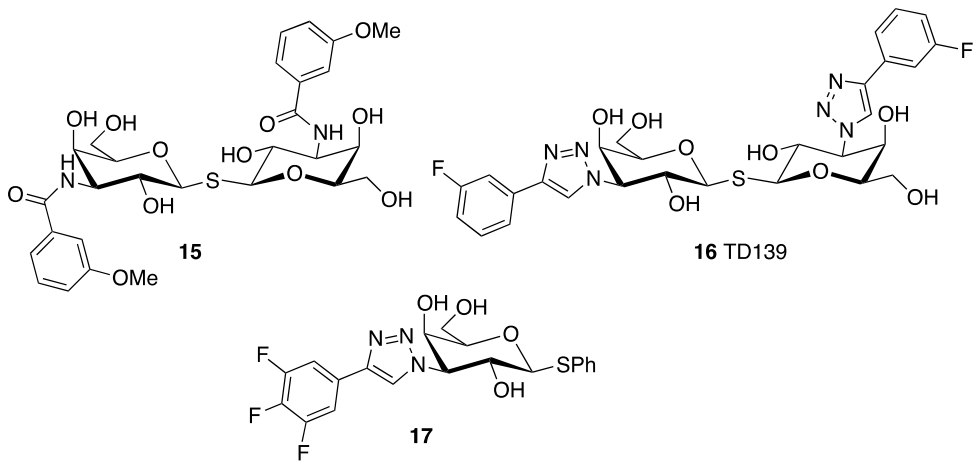


Figure 10: 3,3'-Bis-(3-methoxybenzamido) thiodigalactoside 15 and 3,3'-bis-(4-(3-fluorofenyl)-1H-1,2,3-triazolyl) thiodigalactoside 16, galectin-3 selective high-affinity disaccharide inhibitors as well as 3-((3,4,5-trifluorophenyl)-1H-1,2,3-triazolyl)-1-β-thiophenyl galactopyranoside 17, the best monosaccharide.

Another scaffold simplification takes us to cyclohexylthiogalactoside **18** in figure 11, where the non-interacting hydroxyls have been removed, a simplification reminiscent of Oseltamivir **4**. The affinity of **18** is 150 nM towards galectin-3, with a tenfold selectivity over galectin-1[91]. This is worse than the unsimplified compound **16** by about a factor of ten, which is somewhat unexpected. It might be the case that the cyclohexyl has an unfavourable conformation or there might be a solvation effect involved due to its considerably lower number of hydrogen bond donors and acceptors.

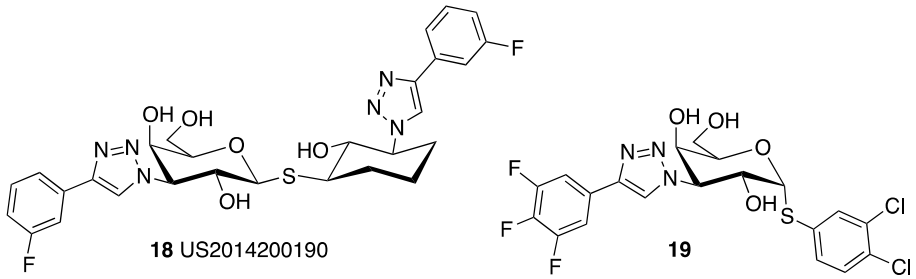


Figure 11: Cyclohexylgalactopyranoside US2014200190 18 and α-1,3-dideoxy-1-(3,4-dichlorothiophenol)-3-(4-(3-fluorophenyl)-1H-1,2,3-triazolyl) galactopyranoside 19, two replacements of the distal carbohydrate in subsite D.

The α-3,4-dichlorothiophenyl **19** is a successful monogalactoside galectin inhibitor, with an affinity of 37 nM, showing that it is possible to design high-affinity monogalactoside galectin inhibitors[90]. In **19** most of the selectivity is derived from the trifluorophenyl substituent, while the α-S-aryl on its own has a less marked effect on selectivity. Ideally we would want a substituent in the anomeric position that both increases affinity and has good selectivity for one particular galectin.

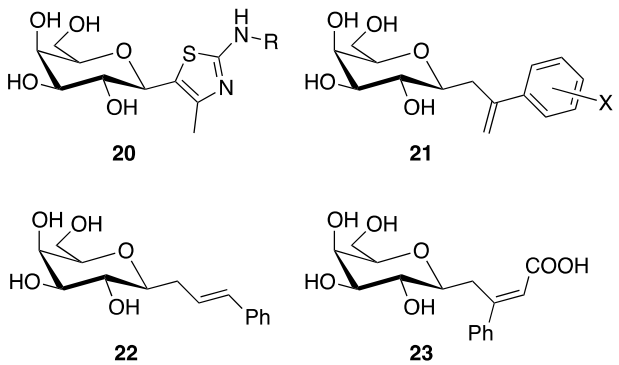


Figure 12: C-Galactoside galectin inhibitors by Giguére et al, most with millimolar affinities towards galectins -1 and -3 (20-22) except 23 with an affinity (IC50) of 313 μM towards galectin-1.

Finally, we will look at the state of art C1-galactoside galectin inhibitors. Giguére et al have published a series of papers[92–95] where they investigate C-galactoside based galectin inhibitors, albeit with limited success. Inhibitors **20-22** have millimolar affinities and rather lacklustre selectivities, which is initially discouraging. The star of the show however, is **23** with an affinity of 313 μM and good galectin-1 selectivity (figure 12). This shows the viability of C1-galactosides as galectin inhibitors, and sets a starting point for further investigations into C1-galactosides. The hypothesis thus becomes that it is possible to design and synthesize C1-galactosides that will be selective and metabolically stable high-

affinity galectin inhibitors. The inhibitors presented so far are of course a selection out of a vast number of different inhibitors in the existing literature, but represent a clear illustration of the considerations involved in designing high-affinity galectin inhibitors that are also selective. Small molecule galectin inhibitors are not the only road towards galectin inhibition. Large oligosaccharides like synthetic poly-lacNAc[96] or natural oligosaccharides like the 20.5 kDa arabinogalactan RN1[97] as well as synthetic peptides[98] and siRNA[99] are either not easily druggable or have met with limited success when evaluated as therapeutics.

Affinity measurements

If the final arbiter of success when designing a drug is activity *in vivo*, then the initial arbiter of success is good affinity *in vitro*. But what is affinity, and how do we measure it? Two common ways to report affinity is dissociation coefficient K_D and IC50. The dissociation coefficient is the equilibrium constant of the breakdown of the drug-protein complex, expressed this way because of the more convenient units. It is also directly related to the free energy ΔG. IC50 is the concentration at which 50% of the activity of a target protein is inhibited. Different affinity assays give different results, which are not always easy to compare. Isothermal titration calorimetry (ITC) gives a lot of useful information; not only free energy but also enthalpy and thus by calculation the entropy. This makes thermodynamic fingerprinting possible, helping guide selection of appropriate drug candidates[100]. ITC is often slow, and is not amenable to high throughput screening.

Two other assays that are frequently used for evaluating galectin inhibitors is a fluorescence polarization assay[101] and a hemagglutination assay[102]. The hemagglutination assay works by performing a dilution series of the molecule to be tested, and determining what concentration inhibits agglutination of red blood cells by galectins. The assay gives affinity as IC50, and requires no probe or sophisticated equipment and is therefore popular. Fluorescence polarization works by exposing a sample containing protein, a fluorescent probe and the molecule to be measured to plane polarized light. Low inhibitor concentration or a low-affinity inhibitor will leave most of the probe bound to the protein, where it does not undergo significant motion during the fluorescence measurement timescale due to the size of the protein it is bound to, returning a strong signal in the same plane as it was excited. A high-affinity inhibitor (or a sufficiently high concentration of a low affinity one) will exclude the probe from the binding pocket and extinguish the fluorescence polarization. We use single point measurements of the loss of fluorescence polarization done in replicates and at different concentrations to calculate the dissociation constant at each point from points that have – normally – between 20% and 80% inhibition, and then combined using a statistical calculation. The method

is described in greater detail in the excellent work by Sörme et al[101]. Fluorescence polarization is a fast and reliable method for acquiring affinity data, and is the assay of choice for the affinities measured in this thesis.

Summary and conclusions

In summary, we have seen that C-glycosides can be used to improve affinity, selectivity, and stability in drug scaffolds and are effective simplifications of the complex carbohydrate structures often involved when carbohydrate binding drug targets are concerned. Galectins are a family of proteins with a wide array of functions, both intracellular and extracellular. The galectins seem to prefer to do things their own way; being translated in the cytosol and not the ER, being excreted through a non-classical pathway and being recycled back into the cell using yet another non-standard pathway. One of the most well understood galectin functions is to crosslink glycoproteins on the cell surface. This effect can give rise to a galectin lattices, the dynamics of which greatly influence cell signalling and cell-environment interactions through governing the cell surface organization and residence time of receptors such as VEGFR and TGFβ, as well as adhesion proteins such as integrins. Through these routes, galectins are involved in apoptosis, angiogenesis, lymphangiogenesis and the activity level of the immune system. Overexpression of galectins and thus perturbations in these functions are associated with a wide variety of pathologies; certain cancers and immune related disorders like idiopathic pulmonary fibrosis are two of the best studied examples. There is evidence from animal models that pathological lymphangiogenesis and pulmonary fibrosis can be treated using galectin inhibitors, meaning galectin inhibitors are promising as drugs for a variety of diseases.

Galectin inhibitors have undergone an evolution from natural disaccharide ligands such as lactose and N-acetyllactosamine, which are metabolically labile and with fairly lacklustre affinity, through successive cycles of elaboration with affinity and selectivity increasing motifs followed by structural simplifications removing non-interacting parts of the structure, mainly hydroxyls. In the end this has taken us to monogalactosides with excellent affinities. In order to attempt to improve on this, the lessons learned about C-glycosides combined with a few known C1-galactosides from the literature point us towards introducing C1-substituents with the hope of achieving high-affinity galectin inhibitors with high selectivity and good pharmacokinetic properties.

2. C1-Heterocycles as galectin inhibitors

"It may not work, but if it does, it will be a footnote to a footnote in the history of chemistry."
– James Bryant Conant

In chapter 1 we saw that the differences between the galectins are minor, especially around the conserved subsite of the binding pocket galactose binds in. This is not surprising, after all galectins all favour the same main binding motif; lactose/N-acetyllactosamine. This means that if we want to design small monogalactoside galectin inhibitors with selectivities for specific galectins we will have to design unorthodox structures aimed at interactions with the differences there are. As we saw this. A key difference between galectin-1 and the other galectins is the presence of a histidine in the binding pocket close to the anomeric position of galactose, in the C/D portion of the binding pocket (figure 8). As we have seen, molecules retaining only the parts of the distal carbohydrate that are actually interacting with the CRD has been successful as simplifications of the scaffold[91], but there is potential in incorporating structural motifs that enhance affinity and selectivity even further. We therefore resolved to try something radically different – C1 arylheterocycle galactopyranosides (figure 13). As the histidine has the ability to be involved in hydrogen bonding to the sugar, either directly or through water networks, the introduction of a heterocycle with hydrogen bond donors and/or acceptors seems to be an attractive structural feature to explore. Heterocyclic aromatic systems also have the added ability to perhaps participate in aromatic stacking with the histidine. Giguère et al have made a few different heterocycles, most of them with limited success (see chapter 1)[92,94,103]. The best C-galactosyl inhibitor has an IC50 of 313 μM towards galectin-1, but is not a C1-heterocycle. Based on the known heterocycles, it is a prudent strategy to vary the heterocycle to find one that gives the desired affinity- and selectivity patterns before optimizing the substituent patterns on the heterocycle substituent.

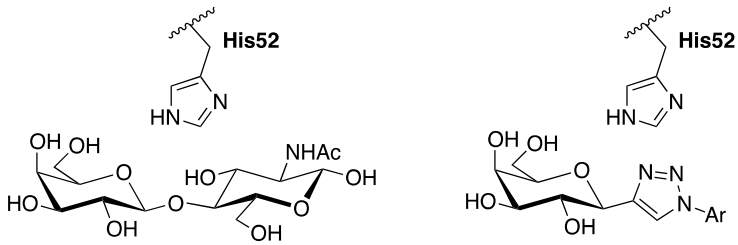


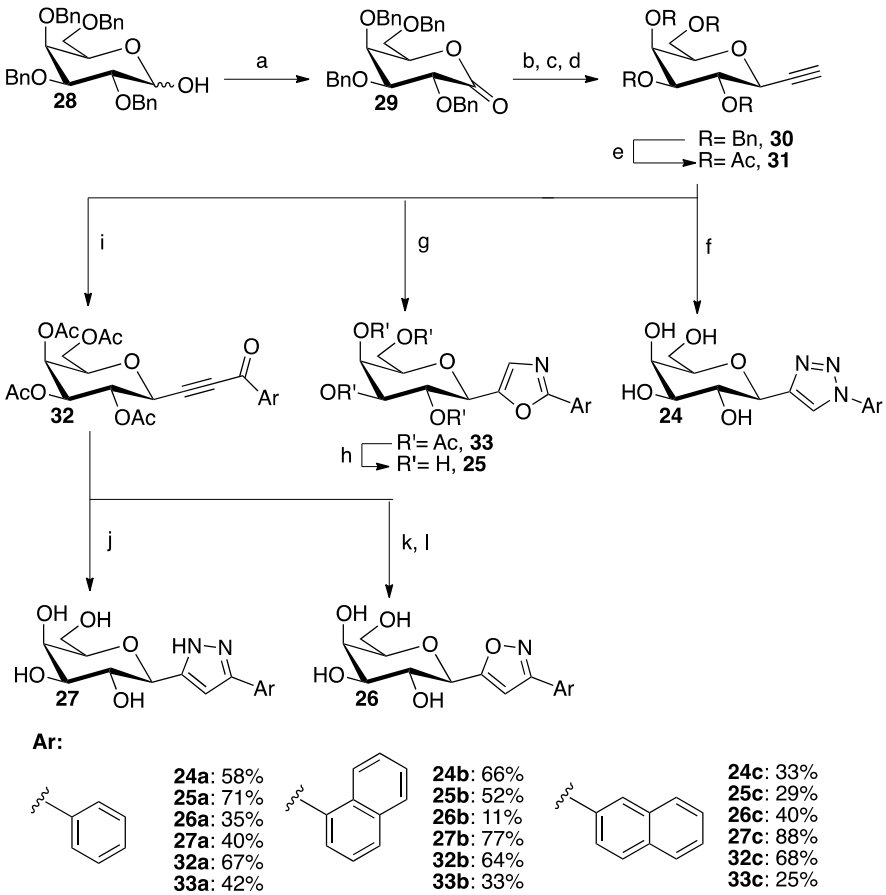
Figure 13: Design idea for C1-arylheterocycles; motifs interacting with histidine 52 in the galectin-1 binding pocket through aromatic stacking or hydrogen bonding.

Synthesis of C1-arylheterocycle galactopyranosides

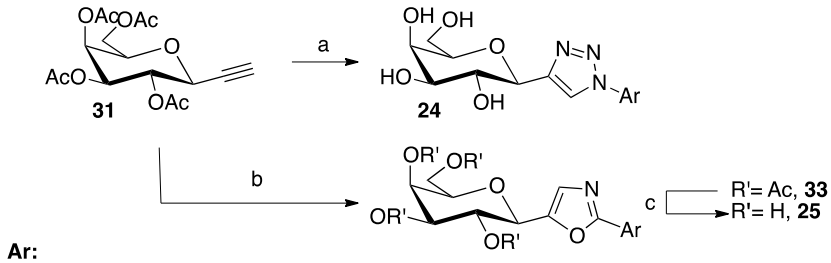
An easy entry to a variety of different heterocycles goes through alkynes, as a variety of different heterocycles can be synthesized starting from an alkyne. Hence, the ethynyl galactopyranoside like **31** (scheme 1) is a good late-branching starting point for our library. A convenient published synthesis by Yepremyan et al exists for obtaining alkyne **30**[17,104,105], starting with the commercially available 2,3,4,6-tetra-O-benzyl-galactopyranoside **28** that was oxidized to lactone **29** with Dess-Martin periodinane in dry dichloromethane, with a yield of 99%. Trimethylsilylethynyl cerium(III) dichloride generated in situ using a transmetallation from the corresponding lithium reagent was then used to perform an ethynylation on the lactone at -78°C in dry THF, the subsequently produced alkynylketose was then reduced to benzyl protected ethynyl derivative **30** using triethylsilane and boron trifluoride etherate in acetonitrile/dichloromethane at 0°C. Finally, the trimethylsilyl group was removed using sodium hydroxide in methanol/dichloromethane to give C1-alkynyl galactopyranoside **30**, in 68% yield over three steps (scheme 1, steps a-d). The use of the somewhat exotic organocerium organometallic reagent was necessary due to the unreactive nature of lactone **29**; regular organolithium reagents lead to no reaction, resulting in recovery of the starting material. Organocerium reagents are known for their exceptional nucleophilicty combined with an astounding level of non-basicity, something that gives them the ability to add selectively to a carbonyl carbon without enolization, properties that seemed to be needed for this reaction to proceed smoothly. Cerium reagents are also [104,106]. We could have proceeded to a library from **30**, but global benzyl protection was a less than optimal protecting group choice as the deprotection conditions were incompatible with some of the aryl substituent patterns in an extensive library, like halogens due to dehalogenation[107] (something which was exploited later) and thus we finished off the common synthesis pathway by switching the protecting groups to acetyl groups in a debenzylation/acetylation with

trimethylsilyl triflate in acetic anhydride to obtain the common intermediate **31** with a yield of 83% (scheme 1, step e).

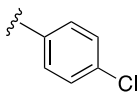
The stage was then set for the synthesis of the different C1-heteroaryls from **31**, We began with the C1-aryltriazolyl galactopyranosides **24a-ag** that were constructed and deprotected in a one-pot reaction with first aryl azide, copper(I) iodide and triethylamine in acetonitrile[108–110], followed by sodium methoxide in dry methanol[108] in yields between 3-68% over two steps (schemes 1, 2 and 3). The isoxazoles and pyrazoles ,**26** and **27**, respectively were synthesized through condensation reactions on common intermediates, the arylalkynones **32a-c**, which were synthesized from **31** using Sonogashira couplings[111,112] with aroyl chloride, bis(triphenylphosphine)palladium(II) dichloride, copper(I) iodide and triethylamine in tetrahydrofuran for 18 hours with yields of 67-69%. Isoxazoles **26a-c** were synthesized by refluxing **32a-c** in dry tetrahydrofuran with hydroxylamine hydrochloride and sodium carbonate, then deprotection with sodium methoxide in dry methanol with yields of 11-40%. A similar procedure where hydroxylamine hydrochloride is replaced with hydrazine was used to synthesize the pyrazoles **27a-c**[113–115] with yields of 40-88%. The oxazoles were synthesized using a novel gold catalysed method[116] utilizing alkyne **31**, (triphenylphosphine)gold(I) triflimide, 8-methylquinoline N-oxide and aryl cyanide in a neat melt to give **33a-i** in yields of 4-48%. The yields of these reations were less than optimal, something most likely owed to the difficulty to control the choice of reaction temperature when the reaction needs takes place in a neat melt. Reactions in solvent failed, both in the referenced paper and in our own attempts. The protected oxazoles were deprotected in the usual fashion with sodium methoxide in dry methanol at yields between 18-71% to give **25a-i** (scheme 1 & 2). The normal synthesis route to the triazoles failed when attemped for unsubstituted pyridyl azides **24ah-aj**, and an alternate route was used through the dechlorination of **34** by refluxing with palladium hydroxide on carbon in cyclohexene/ethanol and subsequent deprotection of **35** with sodium methoxide in dry methanol to give **24ah** (scheme 3). A working hypothesis on why the synthesis of 4-chloro substituted 2-pyridine **24af** worked while the unsubstituted pyridines were inaccessible through our regular synthesis route is that the 4-chloro substituent decreases the ability of the nitrogen lone pair to participate in complexation that prevents the reaction from going to completion. Introducing a chlorine substituent has a significant effect on the complexation ability of pyridines, as measured by the hydrogen-bond basicity difference[117].



Scheme 1: Synthesis of a library of four different types of C1-arylheterocycle galactopyranosides. (a) Dess-Martin periodinane. DCM (dry), r.t., 2h, 99%. (b) trimethylsilylethynyl cerium(III) dichloride, THF(dry), -78°C, 2h. (c) Triethylsilane, boron trifluoride etherate, ACN/DCM (dry), 0°C, 2h. (d) Sodium hydroxide, MeOH/DCM, r.t, 1h. 68% over 3 steps. (e) Trimethylsilyl triflate, acetic anhydride, 0°C, 48 h, 83%. (f) Aryl azide, Copper(I) iodide, triethylamine, ACN (dry), 18h, then sodium methoxide in MeOH (dry), 1h. 3-68%. (g) Aryl cyanide, (triphenylphosphine)gold(I) triflimide 8-methylquinoline N-oxide, neat melt. 72h, 4-48%. (h) Sodium methoxide, MeOH (dry), r.t. 2h, 18-71%. (i) Aroyl chloride, bis(triphenylphosphine)palladium(II) dichloride, copper(I) iodide, triethylamine, THF (dry), r.t, 18h, 67-69%. (j) Hydrazine, potassium carbonate, THF (dry), reflux, 18h, 40-88%. (k) Hydroxylamine hydrochloride, sodium carbonate, THF (dry), reflux, 18h. (l) Sodium methoxide, MeOH(dry), r.t, 2h, 11-40% over two steps.



Ar:



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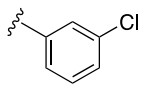
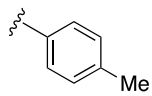
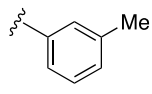
25d: 30%
33d: 19%



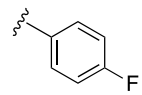
24e: 99%
25e: 30%
33e: 11%



24f: 16%
25f: 46%
33f: 4%



24g: 49%%
25g: 46%
33g:48%



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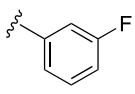
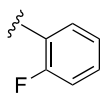
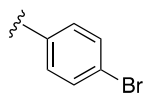
25h: 43%
33h: 11%



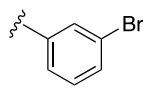
24i: 64%
25i: 18%
33i: 31%



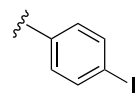
24j: 20%



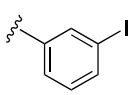
24k: 7%



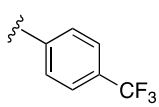
24l: 16%



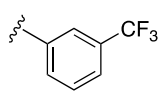
24m: 3%



24n: 39%



24o: 36%



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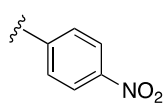

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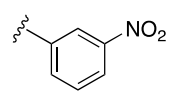

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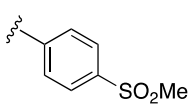

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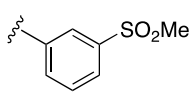

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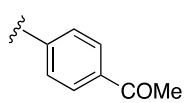
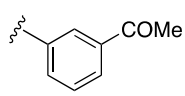


24u: 42%



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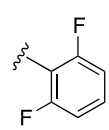
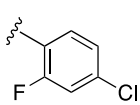
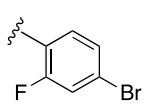


24w: 24%



24x: 47%



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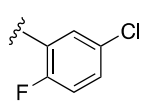
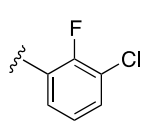
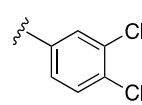


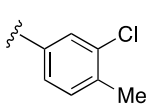
24z: 32%



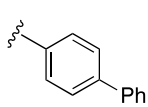
24aa: 24%



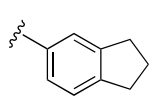
24ab: 68%



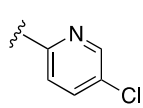
24ac: 66%



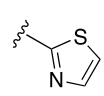
24ad: 8%



24ae: 36%

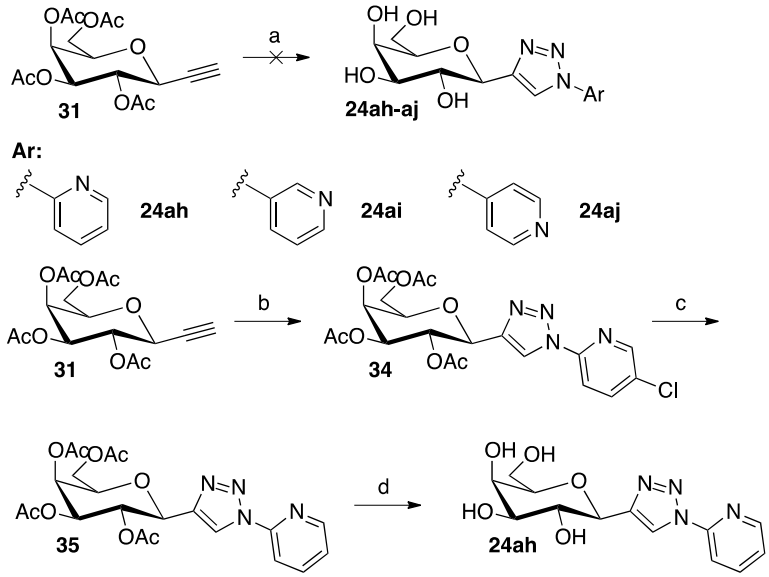


24af: 13%



24ag: 55%

Scheme 2: Larger library of oxazoles and isoxazoles. (a) Aryl azide, Copper(I) iodide, triethylamine, ACN (dry), 18h, then sodium methoxide in MeOH (dry), 1h. (b) Aryl cyanide, (triphenylphosphine)gold(I) triflimide, 8-methylquinoline N-oxide, neat melt. 72h. (c) Sodium methoxide, MeOH (dry), r.t. 2h.



Scheme 3: Failure to synthesize pyridinyltriazoles 24ah-aj, with regular conditions, and the alternate synthesis pathway through dechlorination of 5-chloro-pyridin-2-yltriazole 34. Conditions: (a): Copper (i) iodide triethylamine, appropriate aryl azide, ACN (dry), 40°C, 24 h. (b): Copper (i) iodide triethylamine, appropriate aryl azide, ACN (dry), r.t., 12 h. (c): Palladium hydroxide on carbon (20wt%) cyclohexene/ethanol 2:1 80°C, 12h. (d): Sodium methoxide in MeOH (dry), 2 h.

Affinity evaluations and structure-activity relationships

Affinities of all potential inhibitors **24-27** were evaluated using the previously mentioned fluorescence polarization assay were measured for galectins -1, -2, -3, -4C, -4N, 7, 8C, 8N, 9C and 9N. The affinities towards all galectins, except galectins -1 and -3, were in the millimolar range. The affinities for galectins-1 and -3 are presented in tables 1-3 below.

Table 1: Affinities (K_D) of C1-arylheterocycle galactopyranosides 24a-c, 25a-c, 26a-c and 27a-c calculated from 4-6 data points at different concentrations.

		Triazole (24)	**Oxazole (25)**	**Isoxazole (26)**	**Pyrazole (27)**
Phenyl (a)	Galectin-1	760±55	830±63	760±60	1000±140
	Galectin-3	1700±150	1000±300	1200±100	800±120
1-naphthyl (b)	Galectin-1	550±50	490±60	600±80	1600±390
	Galectin-3	1300±140	90±18	1500±65	1000±140
2-naphthyl (c)	Galectin-1	290±12	790±54	430±200	1400±310
	Galectin-3	1600±110	230±40	380±76	1300±74

Initially, we synthesized and evaluated three different aryl substituents (phenyl, 1-naphthyl, 2-naphthyl) for four different heterocycles (triazolyl, oxazolyl, isoxazolyl and pyrazolyl, scheme 1 and table 1). We see that the triazoles are generally galectin-1 selective, with 2-naphthyltriazolyl galactopyranoside **24c** having the best affinity of 290±12 μM and a fivefold selectivity for galectin-1 over galectin-3. The oxazoles on the other hand have a clear galectin-3 preference with 1-naphthyloxazolyl galactopyranoside **25b** having a galectin-3 affinity of 90±18 μM and a fivefold selectivity for galectin-3 over galectin-1. The isoxazoles **26a-c** have decent affinities with 380±76 μM for galectin-3 for **26c**, but have significantly worse selectivity. The pyrazoles **27a-c** have poor improvement in affinity over the tested series and poor selectivity. Based on this, we chose to proceed with varying the substituent patterns on phenyltriazoles and phenyloxazoles, the results of which are presented in tables 2 and 3 respectively.

Table 2: Affinities of C1-aryltriazolyl galactopyranosides 24d-j. K_D calculated from 4-6 data points at different concentrations.

		KD Galectin-1	**KD Galectin-3**
24d	*4-ClPh*	340±19 μM	1700±130 μM
24e	*3-ClPh*	400±50 μM	1500±300 μM
24f	*4-MePh*	1100±80 μM	2300±380 μM
24g	*3-MePh*	680±53 μM	2800±330 μM
24h	*4-FPh*	1800±220 μM	1800±170 μM
24i	*3-FPh*	540±80 μM	2000±310 μM
24j	*2-FPh*	170±28 μM	880±140 μM

Table 3: Affinities of C1-aryloxazolyl galactopyranosides 25d-i. K_D calculated from 4-6 data points at different concentrations.

		KD Galectin-1	KD Galectin-3
25d	*4-ClPh*	870±81 µM	280±28 µM
25e	*3-ClPh*	920±160 µM	670±3 µM
25f	*4-MePh*	790±39 µM	450±32 µM
25g	*3-MePh*	890±25 µM	350±17 µM
25h	*4-FPh*	1900±120 µM	600±130 µM
25i	*3-FPh*	900±85 µM	520±43 µM

Starting with the triazoles in table 2, we observe some interesting trends. The methyl derivatives **24f-g** have very poor affinity and selectivity. The 4-chlorophenyl **24d** has an affinity of 340±19 µM and a fivefold selectivity, so slightly poorer affinity that the 2-naphthyl **24c** but with a similar selectivity. The 3-chlorophenyl **24e** has a very similar affinity and selectivity, an interesting lack of selectivity. The fluorinated series is very interesting; 4-fluorophenyl **24h** has a very poor affinity, in the millimolar range, and no selectivity. 3-fluorophenyl **24i** is better, with a high micromolar affinity, while 2-fluorophenyl **24j** is the best triazole in this initial series with an affinity of 170±28 µM and a sixfold selectivity. Overall, we see the trend that the substituents – apart from affecting affinity – can counteract and even remove the selectivity between galectin-1 and galectin-3, but not reverse it. The oxazoles **25d-i** are more lacklustre; the affinity improvements over the unsubstituted phenyl are small, and the substituent pattern seems to affect affinity and selectivity to a smaller degree than in the triazoles. 3-Methylphenyl (**25g**) turns out to be the best one of the substituted phenyls with an affinity of 350±17 µM, but this is still significantly poorer than the 1-naphthyl **25b**. In this case also, the substituent patterns can influence affinity and selectivity to a certain degree, but not reverse selectivity. Overall, this tells us that the main structural motif setting the galectin selectivity is the choice of C1-heterocycle with substituent patterns reinforcing or counteracting this effect but not being able to completely reverse it.

The larger series of C1-aryltriazolyl galactopyranosides, **24k-ah** (scheme 3) represent a deeper dive into the structure-activity relationship with the aim to both test new substituent patterns and to evaluate the combination of successful substituent patterns. We start out with testing the rest of the halogens; bromo and iodo substituents, as well as the chlorine bioisostere trifluoromethyl[118] substituent, giving us inhibitors **24k-p**. The 4-bromophenyl **24k** is even better than the 4-chlorophenyl **24d**, with a 140±25µM galectin-1 affinity and a tenfold selectivity, about twofold better than the 4-chlorophenyl **24d** in both affinity and selectivity.

The 4-iodophenyl **24m** is worse than both the 4-chlorophenyl **24d** and 4-bromophenyl **24k**, while the 4-trifluoromethylphenyl **24o** has millimolar affinity and no selectivity between galectins, illustrating that the trifluoromethyl group is not a universal bioisostere for chloro substituents. The 3-bromophenyl **24l**, 3-iodophenyl **24n** and 3-trifluoromethylphenyl **24p** are all millimolar in affinity and with poor selectivity, an interesting fact considering the relative similarity in affinity between 4-chlorophenyl **24d** and 3-chlorophenyl **24e**. The nitrophenyls, sulfonylphenyls and acetylphenyls 2**4q-v** are a mixed bag. Most have poor galectin-1 affinities, like the 3-nitrophenyl **24r**, the 4-methylsulfonylphenyl **24s** and the 3-acetylphenyl **24v** that all have millimolar galectin-1 affinity and no selectivity, but the 4-nitrophenyl **24q**, the 3-methylsulfonylphenyl **24t** and 4-acetylphenyl **24u** all have affinities similar to the 4-chlorophenyl and 3-chlorophenyl derivatives **24d-e** with the 3-methylsulfonylphenyltriazolyl galactopyranoside having the best affinity with 300±20 μM.

Table 4: Affinities of C1-aryltriazolyl galactopyranosides 24k-ah. K_D calculated from 4-6 points at different concentrations.

		KD Galectin-1	**KD Galectin-3**
24k	*4-BrPh*	140±25 μM	1400±340 μM
24l	*3-BrPh*	1400±95 μM	1600±30 μM
24m	*4-IPh*	460±50 μM	720±67 μM
24n	*3-IPh*	1300±220 μM	>1600 μM
24o	*4-CF3Ph*	1300±310 μM	1200±31 μM
24p	*3-CF3Ph*	1500±62 μM	1300±69 μM
24q	*4-NO2Ph*	400±5 μM	1500±67 μM
24r	*3-NO2Ph*	1800±64 μM	1000±23 μM
24s	*4-MeSO2Ph*	1300±190 μM	1200±90μM
24t	*3-MeSO2Ph*	300±20 μM	1200±110 μM
24u	*4-MeCOPh*	580±12 μM	870±140 μM
24v	*3-MeCOPh*	1100±48 μM	<600*
24w	*2,6-F2Ph*	310±29 μM	770±89 μM
24x	*2-F,4-ClPh*	1300±200 μM	660±53 μM
24y	*2-F,4-BrPh*	1200±240 μM	1400±38 μM
24z	*2-F,5-ClPh*	650±3 μM	490±6 μM
24aa	*2-F,3-ClPh*	980±7 μM	910±92 μM
24ab	*3,4-Cl2Ph*	970±25 μM	2000±270 μM
24ac	*3-Cl,4-MePh*	2100±73 μM	1400±160 μM
24ad	*4BiPh*	780±43 μM	690±70 μM
24ae	*5-indanyl*	1400±85 μM	1400±70 μM
24af	*5-Cl-2-pyridyl*	970±65 μM	1200±83 μM
24ag	*2-thiazolyl*	1300±260 μM	1000±200 μM
24ah	*2-pyridyl*	150±17 μM	880±100 μM

If we move to combinations of substituent patterns and start with the 2,6-difluorophenyl **24w** we can see that double ortho fluorination is not a productive substitution, with an affinity comparable to the chlorinated derivatives **24d-e**, but

with worse selectivity. The 4-chloro-2-fluorophenyl **24x** and 4-bromo-2-fluorophenyl derivatives combine the 2-fluoro and 4-halogen substituents that were favourable on their own, but turn out to have a counteracting effect, having no selectivity and millimolar affinity, except **24y** that is actually slightly selective for galectin-3 with an affinity towards galectin-3 of 660±53 μM, a reversal of the selectivity set by the heterocycle! The 5-chloro-2-fluorophenyl **24z** and the 3-chloro-2-fluorophenyl **24aa** have high-micromolar affinities and marginal selectivity. **24z** has the selectivity reversed from **24y**, even if the reversal is slight. The 3,4-dichlorophenyl derivative **24ab**, combining the two monochloro substitutions, and the 3-chloro-4-methylphenyl derivative **24ac** both have poor affinity. Overall, no substituent combinations were favourable; they are almost universally of poor affinity and on occasion they even manage to reverse the galectin-1 selectivity normally induced by the triazole.

When investigating variations of the 2-naphthyl derivative **24c**, we have the larger, more flexible, 4-biphenyl **24ad** and the smaller and partially aliphatic 5-indanyl **24ae** that shed some light on other hydrophobic substituents that are quite different from the original 2-naphthyl **24c**. They both turn out to completely lack selectivity, and the indane has a millimolar affinity towards both galectin-1 and galectin-3. The 4-biphenyl has a galectin-1 affinity of 780±43 μM, which is a worse affinity than the 2-naphthyl has by a factor of three. The reason for the selectivity loss when going from 2-naphthyl **24c** to 4-biphenyl **24ad** is not easily explained, but might be related to the loss of planarity in the biphenyl system compared to the 2-naphthyl.

We wished to investigate the affinities of 2-pyridyltriazolyl compounds, due to the good affinity and selectivity of 2-fluorophenyl derivative **24j**, and especially to see if we could combine this with the halogen substitution patterns we established as effective. The 4-chloro-2-pyridiyl derivative **24af** repeats what we have already seen so many times, affinity in or near the millimolar range and poor selectivity. Being the only five membered ring we investigated, thiazole **24ag** unfortunately has a millimolar affinity and no or poor selectivity between galectins -1 and -3. Finishing off the heterocycles is 2-pyridyltriazolyl galactopyranoside **24ah**, which has a galectin-1 affinity of 150±17 μM and a galectin-3 affinity of 880±100 μM, giving it a comparable affinity and selectivity profile to the 2-fluorophenyl derivative **24j** which is not unsurprising, as fluorines are nitrogen lone pair isosteres[119]. One glance at the difference between **24af** and **24ah** tells us how finely tuned the interactions are; the addition of the 4-chloro substituent leads to a large recovery of affinity towards galectin-3 and a large loss of affinity towards galectin-1.

Binding interactions – a computational hypothesis

In order to understand the binding interactions of **24j** and **25b** we decided to pursue modelling using molecular dynamic simulations of their galectin complexes. Each of the ligands **24j** and **25b** were placed in galectin-1 (PDB-ID 1GWZ) and galectin-3 (PDB-ID 1KJL) respectively by superimposing the galactosyl ring of the ligands with the galactose residue of the lactose bound in the crystal structure. We then ran molecular dynamics simulations using the OPLS3 force field and the SPC solvation model, running for 200 ns. Representative snapshots of the MD simulations are shown in figures 14 and 15.

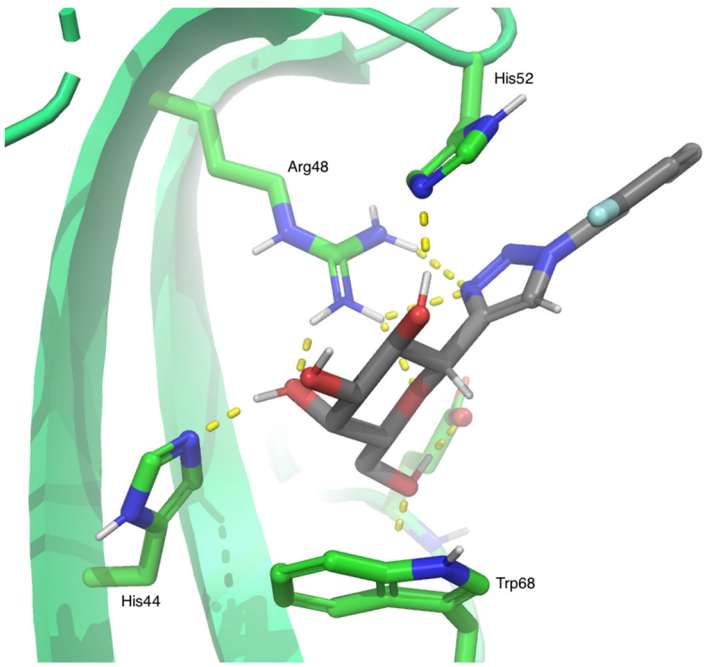


Figure 14: Snapshot from the MD simulation of 2-fluorophenyl triazole 24j in galectin-1 with PDB-ID 1GWZ. The galactoside sits in the usual position while the triazole sits stacked against histidine 52. On occasion the triazole flips, orienting the hydrogen towards histidine 52, but the majority of time is spent in the conformation shown.The view is down the binding pocket groove.

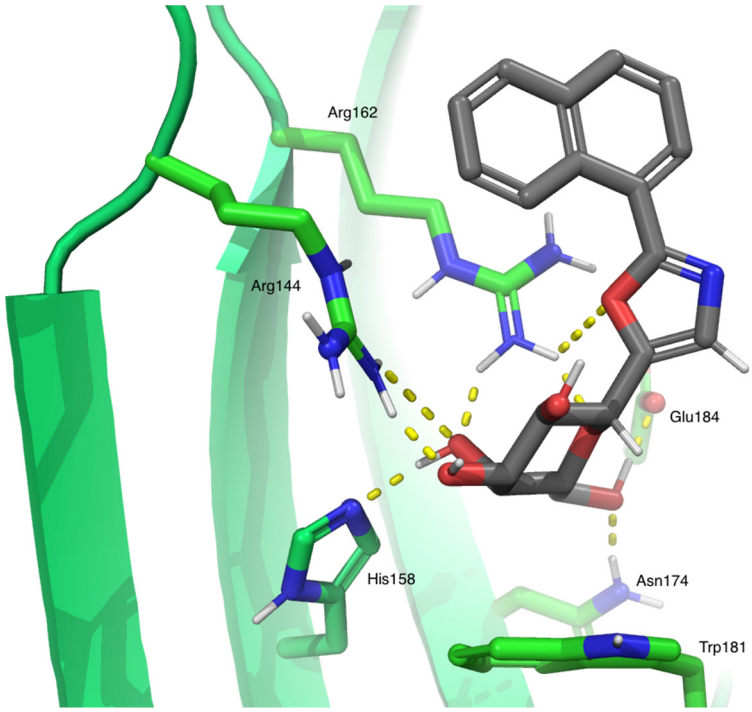


Figure 15: Snapshot from the MD simulation of 1-naphthoyl oxazole 25b in galectin-3 with PDB-ID 1KJL. The oxazole sits in a favourable orientation and distance to interact with arginine 162, while the naphthyl stacks against the side of arginine 162 in subsite C-prime, located above the galactose binding site. The view is down the binding pocket groove, towards subsites C-E.

We can now get some idea of the interactions in the binding pocket. The triazole in **24j** stacks against histidine 52, in an interaction that has the possibility to explain the selectivity differences between the heterocycles as different heterocycles will have different stacking abilities. The oxazole in **25b** interacts with arginine 162, as does the naphthyl which stacks against it in subsite C-prime, a small binding pocket located above the galactose binding subsite, C, unique to galectin-3. The choice of heterocycle influences the conformational preferences of the aryl substituent[120] and determines not only the dihedral angle between the aromatic systems, but also between the heterocycle and the sugar. This explains why 1-naphthyl oxazole **25b** can exploit subsite C-prime while 1-naphthyl triazole **24b** cannot, as is seen by its millimolar affinity towards galectin-3 – the 1-naphthyl cannot reach the C-prime pocket. Thus, the modelling seems to indicate that we have succeeded in exploiting the difference in the binding pocket between galectin-1 and -3, histidine 52.

Summary and conclusions

In summary, we have learnt that C1-arylheterocycle galactopyranosides are potential galectin-1 and galectin-3 inhibitors. The galectin selectivity is set by the heterocycle choice, with aryloxazolyls being galectin-3 selective and aryltriazolyls being galectin-1 selective. For most substituent patterns on the aryl, the heterocycle galectin preference is the selectivity-driving element with substituent patterns being able to remove selectivity only at the cost of affinity. For more complex substitution patterns the selectivity is occasionally reversed, but not by much and not with good affinity. The resulting galectin-3 inhibitor, 1-naphthyloxazolyl galactopyranoside **25b** has an affinity of 90±18 μM and fivefold selectivity for galectin-3 over galectin-1. Our three best galectin-1 inhibitors are 2-fluorophenyltriazolyl galactopyranoside **24j** with an affinity of 170±28 μM and fivefold galectin-1 selectivity, 4-bromophenyltriazolyl galactopyranoside **24k** with an affinity of 140±25 μM and tenfold galectin-1 selectivity and finally 2-pyridyltriazolyl galactopyranoside **24ah** with an affinity of 150±17 μM and sixfold selectivity for galectin-1 over galectin-3.

This means we have replaced the distal saccharide (*e.g.* glc or glcNAc) from the standard ligand lactose/N-acetyllactosamine with a structural motif that is entirely different and with a tunable selectivity influencing element built in. Our inhibitors also outperform the known C-galactosyl inhibitors from literature, the best of which has an affinity of 313 μM. A summary of the structure-activity relationships observed can be found in figure 16. The resultant inhibitors are comparable to both lactose and N-acetyllactosamine[121] while being about twice as good as already reported C1-galactosides by Giguére et al, but with better selectivity[93,103]. Modelling supports that interaction with histidine 52 in galectin-1 by the triazole drives selectivity in galectin-1, while selectivity in galectin-3 is driven by the oxazoles conformational preferences and potentially by hydrogen bonding interactions, making occupation of the C-prime subpocket located above the galactose binding site by a 1-naphthyl possible.

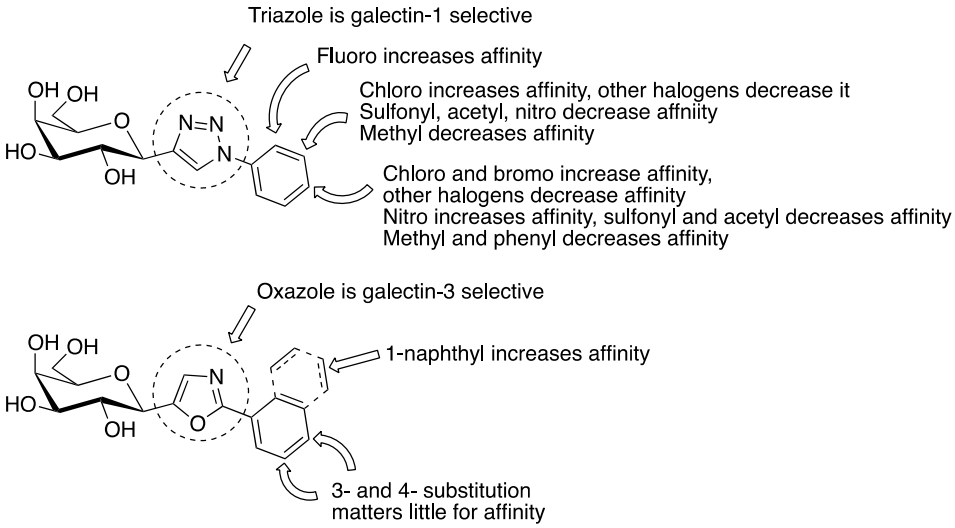


Figure 16: Summary of the structure-activity relationships of C1-arylheterocycles

3. Hydroboration of C1-exomethylene ethers and C1-(aryltriazolyl)methyl galactopyranosyls as galectin inhibitors

"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny...'"
– Isaac Asimov

After the success with C1-arylheterocycle galactopyranosyls, we decided to try moving the heterocycle one step out from the anomeric position and make C1-(arylheterocycle)methyl galactopyranosyls (figure 17). The methylene bridge gives these compounds more flexibility and puts interacting motifs further down into the binding pocket, reaching further subsites D and E. This gives the triazole the ability to explore more binding interactions, hopefully leading to better selectivities and affinities.

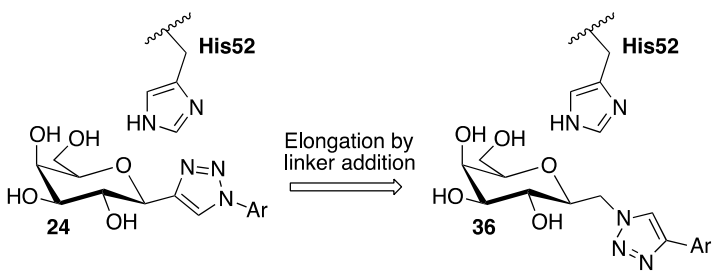


Figure 17: C1-(Aryltriazolyl)methyl galactopyranosyls are interesting extensions of the C1-arylheterocycle library with more flexibility and the ability to reach further parts of the binding pocket.

C1-Methylene pyranoside synthesis

A common entry to C1-methylene carbohydrates is through C-glycosylation with allyl acceptors, generally allyl trimethylsilane, and a carbohydrate donor[9,122–125]. Another similar entry is an allylation using allyl bromide and tin or indium, which – spectacularily enough – does not require any protecting groups and proceeds in water![126,127] The pathway starting from C1-allylated galactose is a complicated and long route to 2-deoxyheptuloses due to the need for subsequent ozonolysis and reduction of the C1-allyl carbohydrates. One alternative is for reductive 2-deoxygenation of ketoheptuloses[128], which introduces additional problems with diastereoselectivity and a small pool of starting materials as few ketoheptuloses are commercially available and synthesizing them is a fairly involved procedure. Another alternative is using the Ramberg-Bäcklund methodology on a 1-thioether glycoside and then diastereoselectively reducing the resulting 1-exomethylene enol ether in a process that usually involves a significant amount of steps with occasionally harsh conditions[11,129]. Anomeric glycosyl fluorides are also entry points to various exomethylene C-glycosides, for example through the same C1-allyl carbohydrates mentioned above[12,130]. The one thing all of these synthesis routes have in common are that they involve several steps and less than mild conditions, restricting their utility.

Hydroboration of C1-exomethylene pyranosides

To avoid these problems, we decided to go through the hydroboration of a C1-exomethylene enol ether to simplify the synthesis. Hydroborations of exomethylene ethers has been known to have excellent regioselectivities in a variety of different synthetic applications, and often good to excellent stereoselectivities as well[131–134]. While hydroborations on C1-exomethylene glucose with 9-BBN are known and proceeds with excellent yield (94%), regioselectivity and stereoselectivity (only β)[135,136], it turned out to not work for C1-exomethylene galactose. Therefore, we started screening hydroboration conditions, mainly by screening various reagents using the hydroboration of C1-exomethylene galactopyranosyl **37** as a model reaction (figure 18 and table 5).

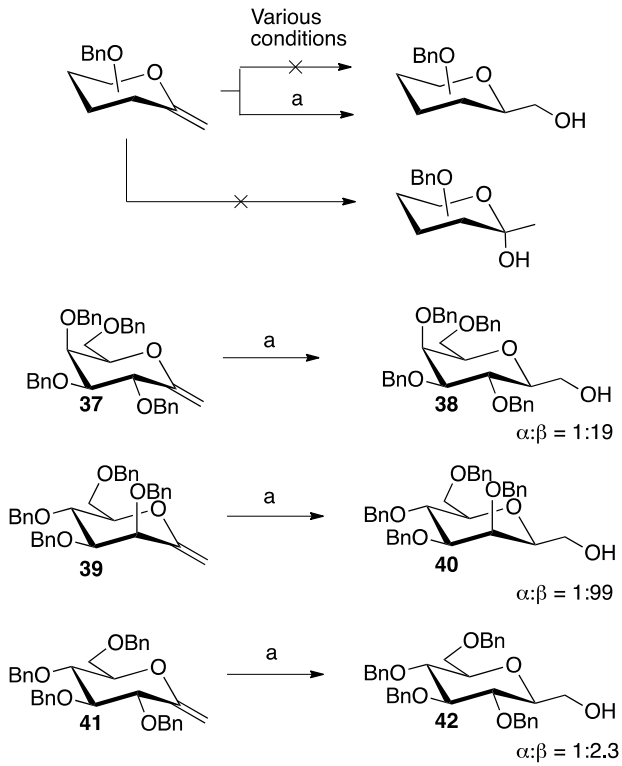


Figure 18: Hydroboration of C1-exomethylene glycopyranosyl ethers. Various conditions screened, see table 5, (a) Boron dimethyl sulfide in THF (dry), 0°C, 2h then hydrogen peroxide, sodium hydroxide in THF/water. No 1-methyl glycopyranoside was formed for any of the sugars galactose 38, mannose 40 and glucose 42. The diastereoselectivity was good to excellent in the synthesis of 2-deoxygalactoheptulose 3.3 (19:1 beta preference) and 2-deoxymannoheptulose 40 (99:1 beta preference) but significantly poorer for 2-deoxyglucoheptulose (2.3:1 beta preference).

Table 5: Hydroboration reagent screening, 2-deoxygalactoheptulose synthesis as the model system.

	Reagent	**Yield 38**
1	9-Borabicyclo[3.3.1]nonane	0%
2	Pinacol borane	0%
3	Borane pyridine complex	0%
4	Borane dimethyl sulfide complex	89%

After a few failed attempts with reagents such as 9-BBN and pinacol borane, borane dimethyl sulfide[134] turned out to be an effective reagent for the hydroboration with a yield of 89%. The reaction displayed excellent regioselectivity, no 1-methyl

galactopyranosyl was formed in the model reaction and the diastereoselectivity turned out to be α:β 1:19. Diastereomer ratios were measured using HPLC, and stereochemistry was assigned by NOESY. After trying the established reaction conditions on C1-exomethylene mannopyranosyl **39** and C1-exomethylene glucopyranosyl **41**, the diastereomer ratios turned out to be α:β 1:99 and α:β 1:2.3, respectively, and the yields 78% **39**, 43% **41-β** and 18% **41-α**. The diastereoselectivity for the pyranoses with an axial substituent turned out to be excellent, while the diastereoselectivity for the hydroboration of C1-exomethylene glucopyranosyl **41** turned out to be poor, in accordance with the results in literature for THF-borane complex (1:1 α:β mixture reported[136]) There are two main different explanations for these observations, a kinetic model based on the steric hindrance by the axial substituents limiting the approach direction of the borane and a thermodynamic model based on minimizing the steric clashes through equilibration between the pseudo-anomers (figure 19 and 20 respectively).

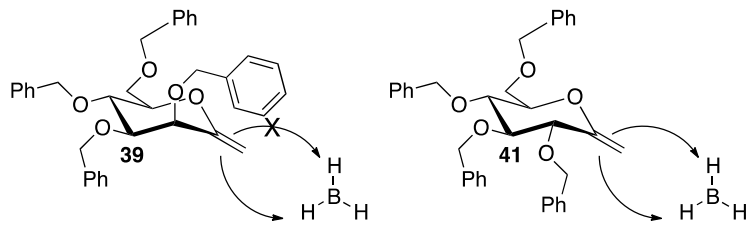


Figure 19: Kinetic diastereoselectivity control model. Steric hindrance in C1-exomethylene pyranosides with an axial substituent directs the hydroboration towards the beta pseudoanomer. In C1-exomethylene glucopyranosyl the lack of an axial substituent and subsequent lack of steric hindrance leads to a mixture of pseudoanomers.

In the kinetic diastereoselectivity control model (figure 19) the approach by the borane is hindered by axial substituents, making a nucleophilic attack by the enol ether on the borane from above – leading to the α-diastereomer – significantly disfavoured due to steric hindrance. This is in accordance with the fact that hydroborations proceed mainly with attack from the less hindered side[137]. In C1-exomethylene mannopyranosyl **39** where the axial substituent is right next to the anomeric carbon the steric shielding is very strong, leading to the high degree of stereoselectivity. C1-exomethylene glucopyranosyl **42** has no axial substituents and thus has no strong stereoinduction leading to a poor diastereoselectivity. The galactopyranosyl **37** has an axial substituent farther away from the anomeric centre, giving it a less outstanding, but still clear diastereoselectivity.

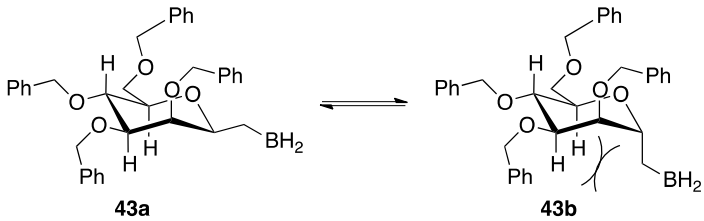
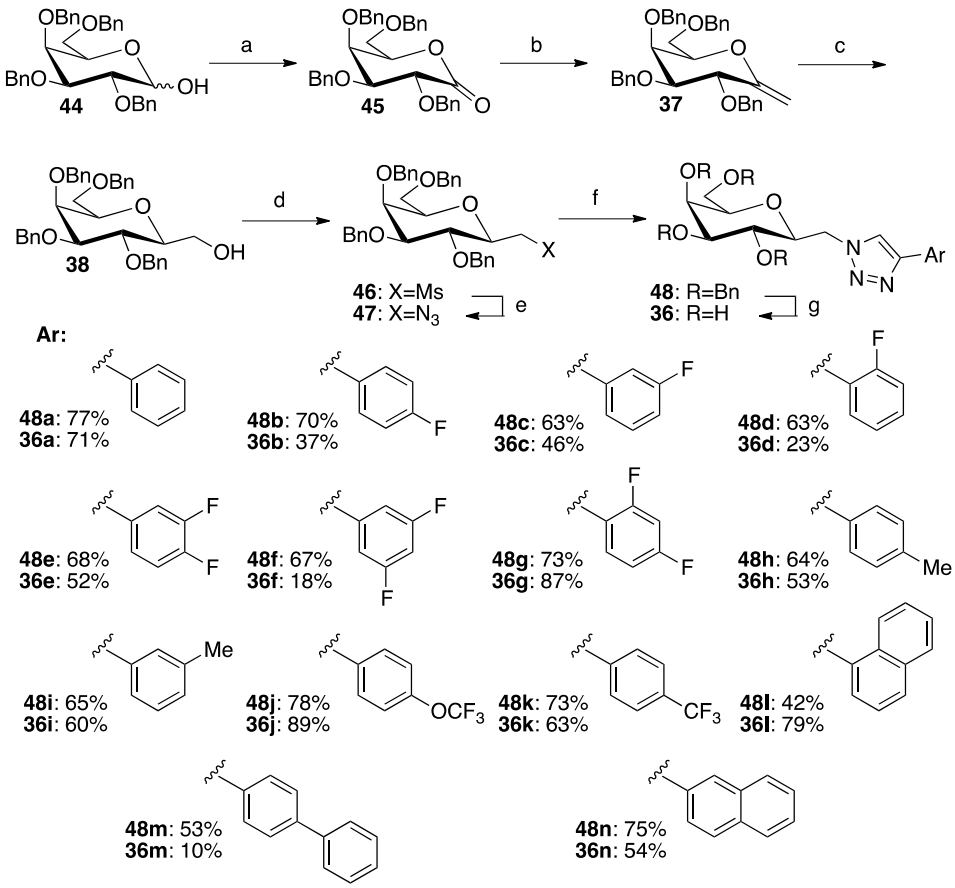


Figure 20: Thermodynamic diastereoselectivity control model. Reversibility of the hydroboration leads to an equilibration between pseudoanomers where the minimization of 1,3-diaxial strain leads to a preference for the β-pseudoanomer 43b.

An alternative explanation model is a thermodynamic diastereocontrol model (figure 20). In this model the reversibility of the hydroboration leads to equilibration between the different diastereomers and what sets the diastereoselectivity is the minimization of 1,3-diaxial strain. In mannose and galactose, there is already some 1,3-diaxial strain in the system thanks to the axial substituent in positions 2 and 4 respectively which glucose lacks. If we look at the pair of mannosyls **43a-b** in figure 20, we see that there is a 1,3-diaxial interaction between the axial 2-benzyloxy group and the axial hydrogen in position 4 in both structures while only **43b** has two additional 1,3-diaxial interactions between the axial methylborane group in the pseudoanomeric position and the axial hydrogens in positions 3 and 5, favouring the β-pseudoanomer due to three 1,3-diaxial interactions heavily destabilizing the α-pseudoanomer. Similar interactions are taking place in galactose, leading to a similar marked preference for the β-pseudoanomer. Glucose has no axial substituent and through that has the ability to tolerate the introduction of more 1,3-diaxial strain, which would explain the low diastereoselectivity of the hydroboration of C1-exomethylene glucopyranosyl. One of the problems with this explanation model is the dramatic difference between mannose and galactose, which is not easy to explain using only 1,3-diaxial strain on the α-side of the ring.

Synthesis of C1-(aryltriazolyl)methyl galactopyranosyls

The synthesis of C1-(aryltriazolyl)methyl galactopyranosyls **36a-n** is shown in scheme 4.



Scheme 4: Synthesis of a library of (aryltriazolyl)methyl galactopyranosyls. (a) Dess-Martin periodinane, DCM(dry), r.t, 2h. (b) Bis(cyclopentadienyl)dimethyltitanium, toluene(dry), 70°C, 48h. (c) Borane dimethyl sulfide, THF(dry), 0°C, 2h, then hydrogen peroxide, sodium hydroxide in THF/water, rt, 2h., 89%, (d) Mesyl chloride, pyridine(dry), 0°C, 90%. (e) Sodium azide, DMF(dry), 95°C, 91%. (f) Aryl acetylene, copper(I) iodide, triethylamine, ACN(dry), rt, 18h. (g) Palladium hydroxide on carbon, cyclohexene/ethanol, 80°C.

The synthesis started from the commercially available tetrabenzyl galactopyranosyl **44**, which was oxidized with Dess-Martin periodinane to give the galactonolactone **45**. We proceeded with a methylenation using bis(cyclopentadienyl) dimethyltitanium (Petasis reagent), both reactions which are firmly established procedures from literature[128,138]. Employing our previously described hydroboration, we got 2-deoxygalactoheptulose **38**, which was subsequently

mesylated using mesyl chloride in dry pyridine to give the 2-deoxy-1-mesylgalactoheptulose **46** in a yield of 91%. The mesylate was reacted with sodium azide in DMF to give 1-azido-2-deoxygalactoheptulose **47** in yields of up to 90%, which was the common intermediate of this set of compounds. Using a copper catalysed azide-alkyne Huisgen cycloaddition with arylacetylenes, copper(I) iodide and triethylamine in acetonitrile[108,110], the benzyl protected (aryltriazolyl)methyl galactopyranosyls **48a-n** were synthesized from **47** in yields of 53-78%. The benzyls were removed by refluxing **48a-n** in cyclohexene/ethanol with palladium hydroxide on carbon in yields of 10-89% to give our final compounds **36a-n**. All the final compounds were purified with preparative HPLC to purities above 95%, determined by analytical HPLC.

Synthesis of (aryloxazolyl)methyl galactopyranosyls

Inspired by the success we had with C1-arylheterocycles, investigating (aryloxazolyl)methyl galactopyranosyls seemed like an attractive idea, using the same gold catalysed [2+2+1] cycloaddition[116] that worked well to synthesise C1-aryloxazolyl galactopyranosyls from exomethylene cyanide **49** (figure 21). Despite repeated attempts however, no product **50** was ever recovered or detected.

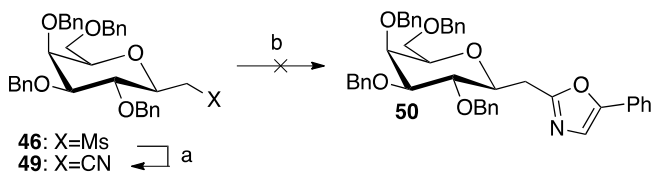


Figure 21: Attempted synthesis of (aryloxazolyl)methyl galactopyranosyls. (a) Sodium cyanide, DMF(dry), 95°C, 18h. (b) Benzonitrile, (triphenylphoshpine)gold(I) triflimide, 8-methylquinoline N-oxide, 55°C.

Affinity evaluations and structure-activity relationships

The affinities of **36a-n** were tested for galectins -1, -3, -4C, -4N, 7, 8C, 8N, 9C and 9N. Affinities for all galectins, except galectin-1 and -3, were found to be in the millimolar range and their affinities have been omitted for clarity. The results for galectin-1 and -3 are presented in table 6.

Table 6: Affinities of (aryltriazolyl)methyl galactopyranosyls 36a-n towards galectin-1 and -3 determined using fluorescence polarization assay. Calculated from 4-6 points at different concentrations.

		KD Galectin-1	**KD Galectin-3**
36a	*Ph*	600±48 µM	870±37 µM
36b	*4-FPh*	170±2 µM	710±36 µM
36c	*3-FPh*	1200±120 µM	720±64 µM
36d	*2-FPh*	390±38 µM	500±23 µM
36e	*3,4-F2Ph*	860±150 µM	610±160 µM
36f	*3,5-F2Ph*	1100±43 µM	790±48 µM
36g	*2,4-F2Ph*	1100±16 µM	490±55 µM
36h	*4-MePh*	1000±130 µM	>3000
36i	*3-MePh*	990±23 µM	>3000
36j	*4-OCF3*	500±59 µM	700±52 µM
36k	*4-CF3*	240±61 µM	830±38 µM
36l	*1-nap*	180±20 µM	470±95 µM
36m	*4-BiPh*	710±12 µM	1200±11 µM
36n	*2-nap*	1500±290 µM	1700±190 µM

Starting with the monofluorinated series of compounds **36b-d**, we see that the 4-fluorophenyl **36b** has a good affinity of 170±2 µM and a fourfold selectivity for galectin-1 over galectin-3. The 3-fluorophenyl **36c** has a millimolar galectin-1 affinity, but has almost exactly the same affinity for galectin-3 as **36b** (720±64 µM versus 710±36 µM respectively). The 2-fluorophenyl **36d** is better than the 3-fluorophenyl **36c**, but worse than the 4-fluorophenyl **36b** and with worse selectivity. Difluorinated compounds **36e-g** have generally quite poor performance with high micromolar affinities and no selectivity between galectin-1 and galectin-3. 2,4-Difluorophenyl **36g**, for example is significantly worse than both **36b** and **36d** and even galectin-3 selective by a twofold factor, meaning that their substituent effects are not additive. The methylphenyls **36h-i** are selective for galectin-1 only by virtue of being very poor binders to galectin-3, having an affinity that we cannot measure. However, this is not an attractive approach to selectivity as both the methylphenyls both have millimolar galectin-1 affinities. 4-Trifluoromethoxyphenyl **36j** has bad galectin-1 affinity and selectivity, while 4-trifluoromethylphenyl **36k** has an affinity of 240±61 µM and fourfold galectin-1 selectivity, and is thus another promising lead. Moving on to the larger aryls, we find that 1-naphthyl **36l** is an inhibitor almost as promising as **36b** with an affinity of 180±20 µM, but with a selectivity that is

worse than both **36b** and **36k**. 4-Biphenyl **36m** has poor affinity and selectivity and so does the 2-naphthyl **36n**. In all we can draw the conclusion that (aryltriazolyl)methyl galactopyranosyls are promising scaffolds for galectin inhibitors, with good affinities and selectivities for galectin-1. It is also another example of a molecule in which the distal carbohydrate normally found in subsite D to E has been replaced with a wholly different structural motif that confers higher selectivity and slightly better affinity.

Computational studies

Again, as in chapter 2, we turn to molecular dynamic simulations in order to understand the binding better. The ligand **36b** was placed into the binding pocket of galectin-1 (PDB-ID 1GWZ) in several different starting conformations and molecular dynamics simulations were run with the OPLS3 force field using the SPC solvation model, calculating the dynamics for 200 ns. The simulations converged to stable poses and a representative snapshot is shown in figure 22.

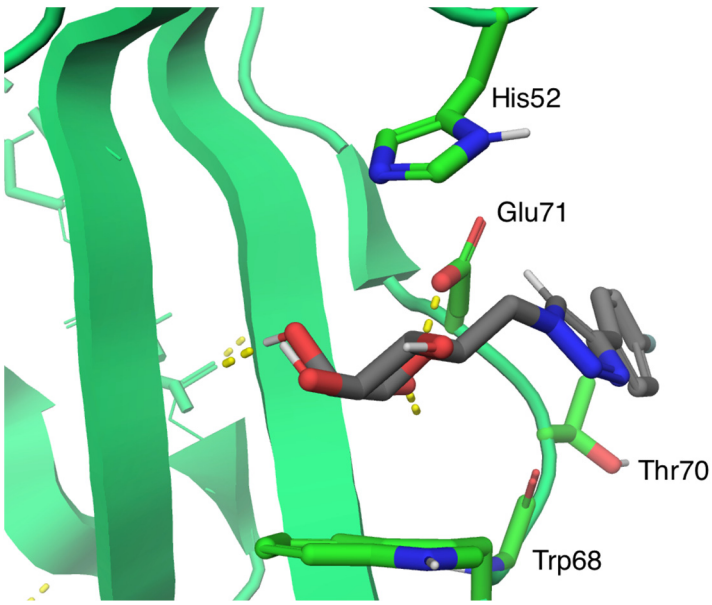


Figure 22: A representative MD snapshot of 36b in the binding pocket of galectin-1. The carbohydrate part of the molecule is situated in the regular carbohydrate binding site, but rather than stacking with histidine 52 like the triazoles (24), the hydrogen on the triazole points towards the carbonyl of glutamate 71. The phenyl system points down the lower side binding pocket towards threonine 70. The threonine-fluorine distance and angle are not optimal for any type of interaction. The view is down along the groove of the binding pocket.

The molecular dynamics simulation reveals that moving the triazole away from the anomeric position with a methylene linker has a dramatic effect on the possible binding interactions. If we compare with **24j** in figure 14, a clear difference is that the triazole not stacking with histidine 52, but rather the triazole hydrogen seemingly binding to the carbonyls of glutamate 71. This leaves the rest of the aryl system pointing away from the main carbohydrate binding pocket down towards threonine 70, however not in close enough proximity to have any meaningful interaction. To understand the steric of the protein better we take a look at the surface, seen in figure 23.

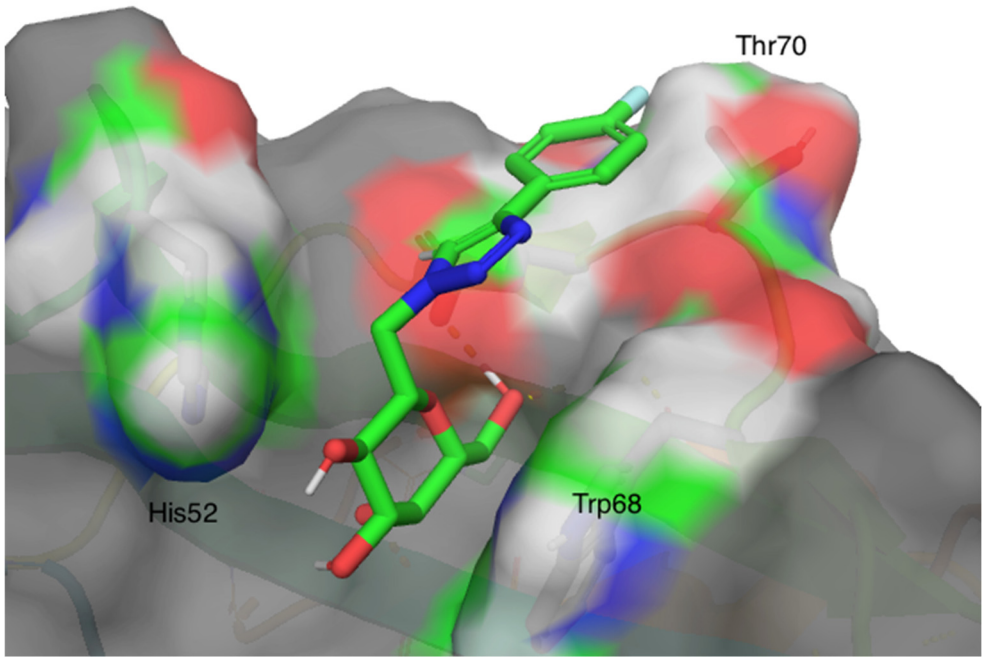


Figure 23: Surface of galectin-1 binding pocket with 36b, from the MD simulation. The ligands aryl system clearly points down, toward threonine 70, and not towards the regular carbohydrate binding pocket groove, seen continuing to the left behind histidine 52.

Using our understanding of the surface sterics (figure 21) and the interaction with Glu71 (figure 22) we can attempt to explain the structure-activity relationship in the para position by the inductive effect they have; depleting the electron density of the aromatic system and by extension the triazole of electron density may strengthen the triazole-glutamate 71 interaction. Electron donating phenyl-substituents will have the opposite effect. Substituents in the ortho and meta positions will potentially experience steric clash with the protein, and ortho substituents also have the potential to force the biaryl system into less favourable conformations through

conformational locking effects similar to those discussed in chapter 2. As for the bigger aryl systems, the extended systems of **36m-n** point out of the binding pocket while the 1-naphthyl **36l** can extend over the surface of the protein, explaining why **36l** is good while the other systems result in poor binding.

Summary and conclusions

A new hydroboration of C1-exomethylene hexopyranoses gives 2-deoxy-β-heptuloses in good yields and with excellent diastereoselectivity for 2-deoxymannoheptulose **40** (1:99 α:β) and 2-deoxygalactoheptulose **38** (1:19 α:β). The hydroboration of C1-exomethylene glucopyranosyl **41** results in a 1:2.3 α:β mixture of 2-deoxy-glucoheptulose **42**. Using this novel hydroboration methodology a library of 1-(aryltriazolyl)methyl-β-galactopyranosyls was synthesized and evaluated as galectin inhibitors. 1-((4-Fluorophenyl)triazolyl)methyl-β-galactopyranosyl **36b** was found to be galectin-1 selective with an affinity of 170±2 μM and a fourfould preference for galectin-1 over galectin-3. Molecular dynamics simulations indicate that the triazole – unlike the triazole in **24j** – does not interact with histidine 52. Rather the triazole hydrogen forms a hydrogen bond with glutamate 71, while the rest of the aryl system points out of the main carbohydrate binding pocket. A summary of the structure-activity relationships of this library can be found in figure 24. This gives us another scaffold where one unit of the lactose/N-acetyllactosamine disaccharide has been replaced with a structural motif that is entirely dissimilar to carbohydrates, while improving selectivity and affinity over *e.g.* lactose.

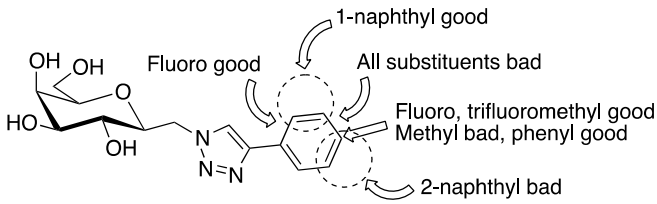


Figure 24: Summary of the structure activity relationships of the exomethylene aryl triazoles.

4. Disubstituted C-galactosides: 3-Phenyltriazolyl-C1-galactopyranosides as galectin inhibitors

"Task failed successfully"
–Windows XP

In chapters 2 and 3 we identified a set of different C1-arylheterocycle galactopyranosides and C1-(aryltriazolyl)methyl galactopyranosides that were selective for galectin-1 or galectin-3, a selection of the most promising compounds are shown in figure 25. C1-Aryltriazolyl galactopyranosides **24j** and **24ah** are galectin-1 selective, while C1-naphthoyloxazolyl galactopyranoside **25b** is galectin-3 selective. Finally, we have the C1-(aryltriazolyl)methyl galactopyranosides **36b** and **36k** that are galectin-1 selective.

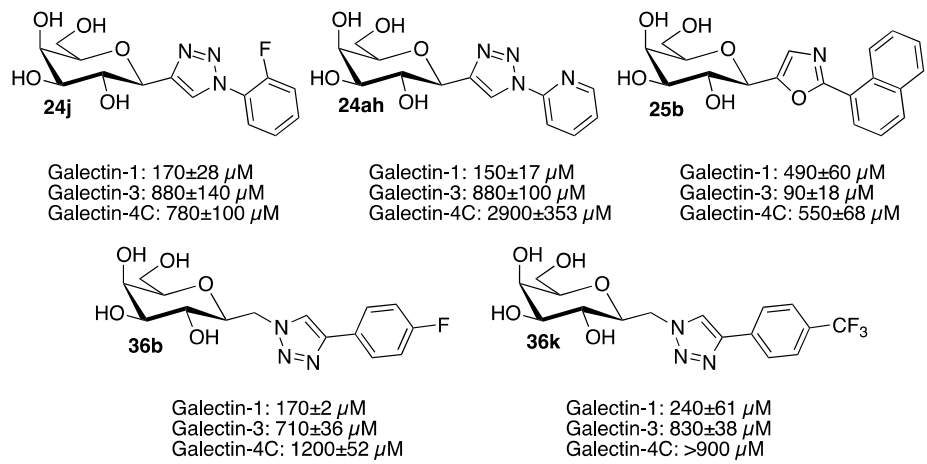
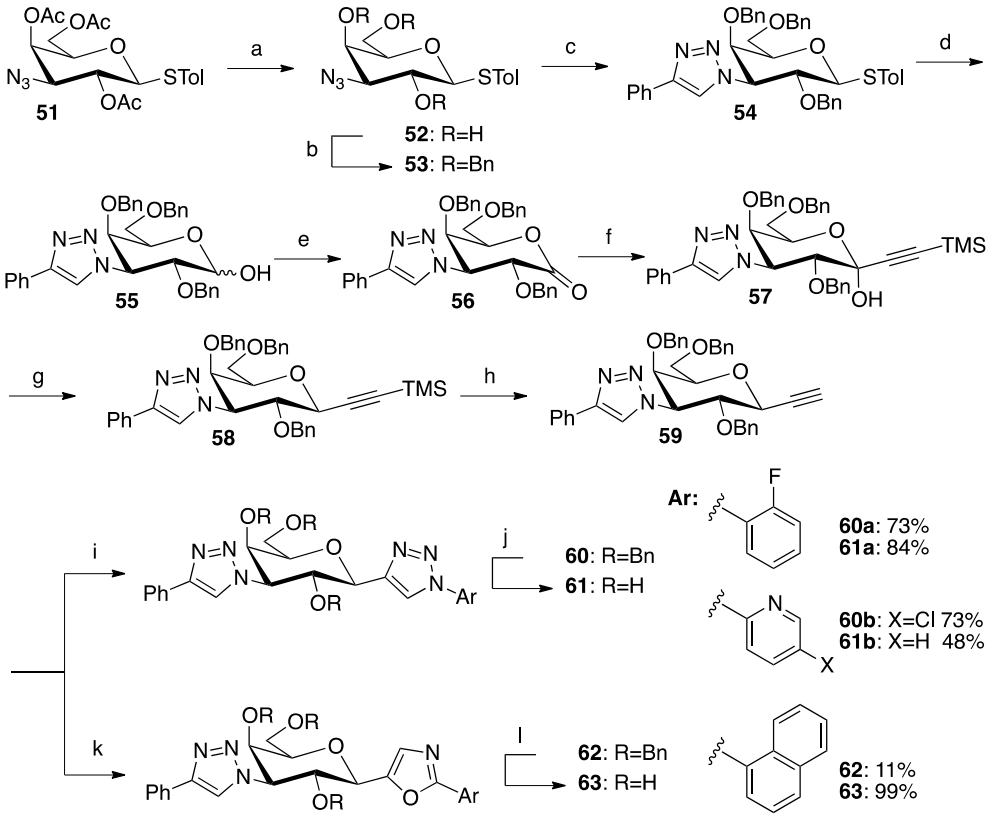


Figure 25: Different C1-galactopyranosides with galectin-1 or galectin-3 selectivity.

In chapter 1 we saw that the addition of an aryltriazolyl substituent at galactose C3 (structures **16-19**) is a powerful way of increasing affinity towards galectin-1 and galectin-3. Thus introducing this substituent pattern on our C1-galactopyranosyls from chapters 2-3 would be a possible way to increase affinity, while hopefully preserving the selectivity patterns we have seen in chapters 2-3. Choosing an aryl that mainly increases affinity and not selectivity to a too large degree and keeping it the same between the structures makes comparisons possible, introduction of specific selectivity inducing aryls can be done at a later stage.

Synthesis of 1,3-substituted C1-galactosides

The synthesis starts with the synthesis of the common intermediate for all final compounds, lactone **56**, after which the synthesis branches. Introduction of an alkyne fragment gives the common intermediate for the C1-arylheterocycle galactopyranosides **59**, while alkenylation and hydroboration takes us towards the common intermediate for the (aryltriazolyl)methyl galactopyranosides **69a-b**, the azide **67**. The synthesis of lactone **56** and on to ditriazoles **61a-b** and oxazole **63** is shown in scheme 5, while the synthesis of the exomethylenes **69a-b** from lactone **56** is shown in scheme 6.



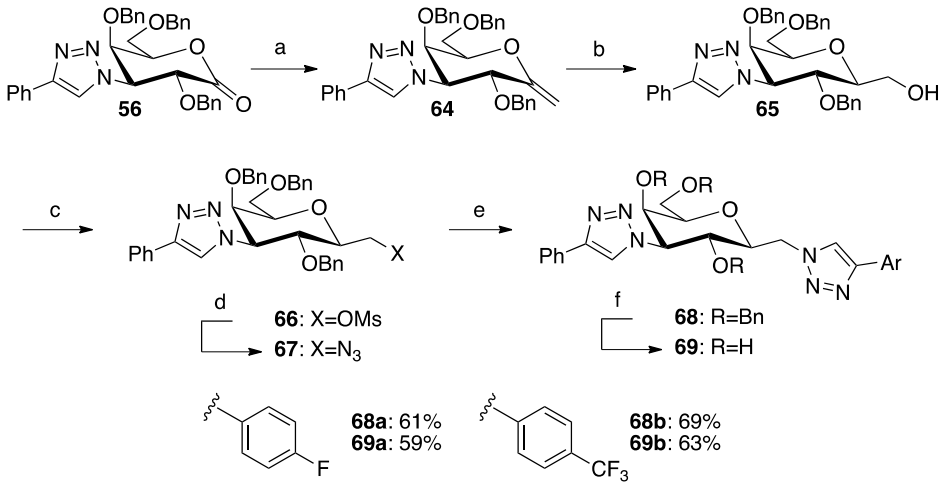
Scheme 5: Synthesis of 3-phenyltriazolyl substituted C1-arylheterocycle galactopyranosides 61a-b and 63. (a) Sodium methoxide, MeOH(dry), rt, 2h, 95%. (b) Sodium hydride, benzyl bromide, THF(dry), reflux, 18h, 84%. (c) Phenyl acetylene, copper(I) iodide, triethylamine, ACN(dry), rt, 84%. (d) N-bromosuccinimide, acetone/water, rt, 10 min, 92%. (e) Dess-Martin periodinane, DCM(dry), r.t, 18h, 99%. (f) trimethylsilylethynyl cerium(III) dichloride, THF(dry), -78°C to rt, 6h, 78%. (g) Triethylsilane, boron trifluoride etherate, ACN/DCM(dry), 0°C to rt, 8h, 60% (h) Tetrabutylammonium fluoride, THF, rt, 30 min, 91% (i) Aryl azide, copper(I) iodide, triethylamine, ACN(dry), rt., 18h (j) Palladium hydroxide on carbon, cyclohexene/EtOH, reflux, 18h. (k) 1-cyanonaphthalene, (triphenylphosphine)gold(I) triflimide, 8-methylquinoline N-oxide, neat melt, 55°C, 48h. (l) Palladium hydroxide on carbon, cyclohexene/ethanol, reflux, 18h.

The synthesis of lactone **56** started from the known 2,4,6-O-triacetyl-3-azido-1-thio(4-methylphenyl) galactopyranoside[108] **51**; removing the acetates using sodium methoxide in dry methanol with a yield of 95% of **52** and then reprotection with benzyl groups by refluxing **52** in THF with sodium hydride and benzyl bromide to give **53** with a yield of 84%. The necessity of refluxing the reaction is a good indication on how deactivating galactose 3-azido group is to the rest of the molecule. A copper catalysed azide-alkyne Huisgen cycloaddition reaction with phenylacetylene, copper(I) iodide and triethylamine in acetonitrile to introduce the 3-phenyltriazolyl substituent gave **54** in a yield of 84% . Removal of the thiocresol anomeric protecting group on **54** was done with N-bromosuccinimide in

acetone/water for 10 minutes with a yield of 92%[139,140] to set the stage for oxidation of the newly formed anomeric hydroxyl of **55** by Dess-Martin periodinane with a 99% yield to give **56**. At this point the synthesis branches, with the introduction of an 1-ethynyl group by a nucleophilic attack using an organocerium reagent in THF (78% yield) followed by reductive removal of the anomeric hydroxyl of **57** with triethylsilane and boron trifluoride etherate in acetonitrile/DCM to give **58** (60% yield) and removal of the trimethylsilyl group with tetrabutylammonium fluoride to give ethynyl derivative **59** (91% yield)[17,104]. The higher temperatures and longer reaction times necessary to achieve these reactions again tell of how deactivating the 3-phenyltriazolyl substituent is in the galactose system, reactions normally proceeding at -78°C need to be warmed to room temperature and kept there for hours to achieve complete conversion.

The triazoles were made by a second Huisgen cycloaddition with aryl azides, copper(I) iodide and triethylamine in acetonitrile to give **60a-b** in yields of 73% for both reactions. The ditriazoles **60a-b** were then deprotected with palladium hydroxide on carbon in cyclohexene/ethanol to give the deprotected ditriazoles **61a-b** in 48-84%. For **61b** the deprotection is also a dechlorination. The 4-chloro substituent is necessary to make the Huisgen cycloaddition leading to **60b** work, but has a detrimental effect on affinity and selectivity in the 3-unsubstituted case, see chapter 2 for a more thorough discussion.

Oxazole **62** was made by the same gold catalysed [2+2+1] cycloaddition used in chapter 2,[116] using 1-cyanonaphthalene, (triphenylphosphine)gold(I) triflimide and 8-methylquinoline N-oxide in a neat melt, with a yield of 11%. The deprotection proceeded smoothly by refluxing **62** with palladium hydroxide on carbon in cyclohexene/ethanol to give **63** in 99% yield. Most of the yields are excellent, except for some steps involving the anomeric position directly, namely the organocerium addition of the ethynyltrimethylsilane group and the subsequent reduction of the anomeric hydroxyl as well as the oxazole formation which has problematic yields even for galactopyranosides not substituted in the C-3 position as seen in chapter 2.



Scheme 6: Synthesis of the exomethylene derivatives 69a-b. (a) Bis(cyclopentadienyl)dimethyl titanium, toluene, 70°C, 48h, 56%. (b) Borane dimethyl sulfide complex, THF(dry), 0°C to rt, 16h, then hydrogen peroxide, sodium hydroxide, THF/water, rt, 2h, 58%. (c) Mesyl chloride, pyridine, 0°C, 1h, 95%. (d) Sodium azide, DMF(dry), 95°C, 18h, 85%. (e) Aryl acetylene, copper(I) iodide, triethylamine, ACN(dry), rt, 18h. (f) Palladium hydroxide on carbon, cyclohexene/EtOH, reflux, 18h.

Moving on to the synthesis of the (aryltriazolyl)methyl galactopyranosides **69a-b**, we start with an alkenylation of lactone **56** with bis(cyclopentadienyl) dimethyltitanium (also known as Petasis reagent) in toluene to give C1-exomethylene **64** in a yield of 56%[128,138]. Hydroboration of **65** with our previously described hydroboration method (chapter 3) using borane dimethyl sulfide in dry THF followed by hydrogen peroxide and sodium hydroxide in THF/water gave 2-deoxyheptulose **65** in a yield of 58%. This reaction is also significantly slower on the 3-phenyltriazolyl substituted galactose **65** thanon the unsubstituted **37**, again highlighting the deactivating effect of the phenyltriazole. A mesylation with mesyl chloride in pyridine to give **66** in 95% yield was followed by a substitution using sodium azide in dry DMF with a yield of 85% to give **67**, the common intermediate for the (aryltriazolyl)methyl galactopyranosides. Proceeding with yet another Huisgen cycloaddition with arylacetylenes, copper(I) iodide and triethylamine in acetonitrile gave the protected exomethylenetriazoles **68a-b** in yields of 61-69%. Debenzylation with palladium hydroxide on carbon in cyclohexene/ethanol with yields of 59-63% gave final products **69a-b**. All final compounds were purified with preparative HPLC to a purity of 95% or higher, determined by analytical HPLC.

Galectin affinities of 1,3-disubstituted C1-galactosyls

Galectin affinity was determined using a fluorescence polarization exclusion assay. Affinities were determined for galectins -1, -3, -4C, -4N, -7, -8C, 8N, 9C and 9N. The results in table 7 are for galectins -1, -3, -4C and -4N, affinities towards other galectins are higher than any of the reported affinities (Kd>600 μM).

Table 7: Measurements of affinities towards galectins -1, -3, -4C and -4N for final compounds 61a-b, 63 and 69a-b. Determined through fluorescence polarization assay, affinities calculated from 4-6 points at different concentrations.

		Galectin-1 Kd μM	Galectin-3 Kd μM	Galectin-4C Kd μM	Galectin-4N Kd μM
61a	2-FPh-triazole	280±39	34±1	18±2.0	90±17
61b	2-Pyr-triazole	180±1	34±3	9.6±2.3	240±54
63	2-Nap-oxazole	530±73	8.6±0.4	2.3±0.4	44±13
69a	4-F-exomet	220±10	200±32	9.5±1.3	130±20
69b	4-CF3-exomet	440±68	720±78	12±1.7	760±130

The C1-aryltriazoles **61a-b** were both designed to be selective for galectin-1, with **61a** being based on **24j** with a sixfold galectin-1 preference and **61b** being based on **24ah**, also galectin-1 selective, but we see that not only has there been a significant recovery of galectin-3 affinity – both of these compounds now have a much higher affinity for galectin-3 than for galectin-1! Compound **61b** shows that the 2-pyridyl motif has gone from having an almost millimolar galectin-3 affinity (**24ah**) to a 34±1 μM affinity through the introduction of the phenyltriazole. The fact that both **61a** and **61b** have the same galectin-3 affinity could be explained by the phenyltriazole substituent at galactose C3 dominating the interaction with galectin-3. Of note is also the massive shift in affinity towards galectin-4C: The 780±100 μM of **24j** improves to 18±2 μM for **61a**, a difference of forty-threefold improvement in affinity. The change for **61b** is even more dramatic, the 2900±350 μM **24ah** to 9.6±2.3 μM for **61b**, a change by a factor of three hundred, a truly astounding shift. If not for the poor selectivity, twofold to threefold over galectin-3, it would be a promising galectin-4 inhibitor.

The 1-naphthyloxazole **63** works more as intended in the design, showing a tenfold affinity improvement from 90±18 μM (**25b**) to 8.6±0.4 μM (**63**) for galectin-3, while keeping the galectin-1 affinity practically the same at 490±60 μM and 530±73 μM, respectively. This results in a decent selectivity increase from fivefold to sixty-twofold. This is however where surprise strikes again, the galectin-4C affinity increase is even more dramatic going from 550±65 μM for **25b** to only 2.3±0.4 μM for **63**. This is a twohundred-fortyfold increase in affinity, resulting in the galectin-4C inhibitor with the best affinity among all the compounds **61a-b**, **69a-b** and **63**. The selectivity for galectin-4C over galectin-3 is only slightly lower than fourfold.

The most interesting results are seen for the (aryltriazolyl)methyl galactopyranosides **69a-b**. For the 4-fluorophenyl **69a**, galectin-4C affinity goes from 1200±52 μM to 9.5±1.3 μM, a 126-fold affinity increase. The affinities towards galectin-1 and galectin-3 are not changing much, except to eliminate any selectivity between the galectins. Both affinities being around 200 μM, giving **69a** a 21-fold or better selectivity for galectin-4C over other galectins. The galectin-4C affinity of 4-trifluoromethyl **69b** goes from so low it is unmeasurable to 12±1.7 μM and with a selectivity that is 37-fold or better for galectin-4C over other galectins. This means that **69a-b** are galectin-4C inhibitors with good affinities and excellent selectivities, even viable starting points for the development of galectin-4 targeting compounds.

If we take a brief look at the galectin-4N affinities we see that they are always worse than the galectin-4C affinities, even if they are always on par with the affinities toward galectin-1 or galectin-3, except for **63**. Of special note is **69b**, the (4-trifluorophenyltriazolyl)methyl galactopyranoside where the affinity is suddenly in the high micromolar range, highlighting the distinct selectivity pattern of this inhibitor. Inhibition via binding to both binding pockets in the tandem repeat galectin-4 may not needed for the inhibitor to function, as the crosslinking action that is hypothesized to be behind galectin biological function is likely inhibited by blocking of one of the binding pockets. It is however not necessarily a disadvantage that the inhibitors have some affinity towards both binding sites of the same galectin.

So why this sudden massive increase in galectin-4C affinity across the board and why the massive recovery of affinity towards galectin-3 for the ditriazoles **61a-b**? As it has already been mentioned, the phenyltriazolyl at galactose C3 of the molecule seems to dominate the galectin-3 interaction while the anomeric 1-aryltriazolyl part of the molecule seems to dominate the galectin-1 interaction. This means that the interaction of one part of the molecule with the galectin binding pocket may place the rest of the molecule in a unfavourable position for productive interactions. Elucidating the exact nature of this interaction would require crystal structures or modelling, but it is entirely possible for the inhibitor to "slide" or move a bit in the binding pocket, maintaining its position in subsite C and not changing the bonding of the galactose by much, but perhaps moving the binding motifs enough to change the interaction. What is clear is that whatever process is taking place leads to a cooperative effect in galectin-4C, while the effect is non-cooperative or even counteracting for other galectins.

Pharmacokinetic properties

With our affinities in hand, we are now ready to take a look at the pharmacokinetic properties of some of our compounds. We saw in chapter 1 that C-glycoside based glycomimetics possess increased metabolic stability and improved oral availability, so what about our inhibitors? Inhibitors **61b** and **69a-b** were chosen to have their properties investigated further; **69a-b** as they have the interesting galectin-4C selectivity and **61b** in order to compare the C1-exomethylene and C1-heterocycle scaffolds. We begin by looking at our inhibitors through the lens of Lipinski's rule of five[141], a set of rules of thumb for assessing oral availability in drug structures which are as follows:

- Five or fewer hydrogen bond donors (i.e hydrogens on electronegative atoms)
- Ten or fewer hydrogen bond acceptors (i.e electronegative atoms)
- Molecular weight lower than 500 g/mol
- Partition and/or distribution coefficient (logP/logD) below 5.

If we look at our compounds we find that **61b** has three hydrogen bond donors and a molecular weight comfortably under the limits. It does however have eleven hydrogen bond acceptors. **69a** fulfils all the criteria beautifully, while **69b** only fails by virtue of a slightly too high molecular weight, so there is good hope for oral uptake of our inhibitors. Already on the design stage we have removed the most metabolically susceptible feature of carbohydrate drugs, which is the hemiacetal functionality, and the molecules lack functionalities like esters, amides and other metabolically labile structural motifs which should give us compounds with half lives that are – at least – not too short.

So, apart from the obvious distribution coefficient, what other pharmacokinetic parameters should we measure? We chose to measure the metabolic stability in liver microsomes and the potential oral uptake in a CACO-2 assay. So what are these parameters and tests? What do they tell us about our molecules, and what do they predict should we choose to move into clinical trials with these compounds?

The distribution coefficient is defined as follows:

$$logD = log\left([solute]_{octanol}^{ionized} + [solute]_{octanol}^{unionized} / [solute]_{water}^{ionized} + [solute]_{water}^{unionized}\right)$$

The measurement is buffered to physiological pH and the distribution of dissolved compound – both ionized and unionized – between water and octanol is measured. This is essentially a measure of the lipophilicity of a compound, and for compounds that do not ionize the distribution coefficient is equal to the partition coefficient (logP). A compound must balance lipophilicity and water solubility, not being so polar that it cannot pass the cell membrane whilst not being so nonpolar that it has

solubility problems or an unfavourable physiological distribution. Normally a logD/P between 2 and 3 is considered as the optimum, but the scale is not absolute[141]. A way to put lipophilicity into context is lipophilic efficiency, which is defined as follows:

$$LiPE = (-\log IC50) - \log D$$

It is a combined measure of affinity and lipophilicity, and a good drug candidate has a lipophilic efficiency greater than 6[119]. This being due to low lipophilicity and high affinity being associated with less unwanted interactions and thus less side effects in later clinical trials.

Metabolic stability in liver microsomes (microsomes being hepatic cell organelles filled with metabolic enzymes) measures the resistance of a compound to degradation by the liver, the most important results being the half life of the compound and the clearance rate[142,143]. A few bench marks; Oseltamivir (**4**) has a microsomal stability half life of 4.0 hours[5] while the well known painkiller ibuprofen has a half life of 1.6 hours and the notoriously tricky to administer anticoagulant warfarin (administering warfarin requires a careful dose regimen and frequent blood tests to monitor blood concentration levels) has a half life of 29.0 hours[144] We can look at these as sort of upper and lower bounds for our compounds, a successful drug will not have a half life that leads to an unreasonable dose regimen, even though modern drug formulation techniques means the range of acceptable half lives is quite wide.

Finally, the CACO-2 assay is used to predict oral uptake. A monolayer of colon carcinoma cells is grown in a well plate. These cells are essentially a sturdier analogue of the gut epithelium, and as such the monolayer has a distinct directionality similar to the wall of the gut. The experiment involves adding compound on both the "outer" side (usually called "A") and the "inner" side (usually called "B") in separate wells and then measuring the concentration curve in the receiving end of the plate. Values above $5x10^{-6}$ cm/s are usually considered to be indicative of good membrane permeability while values significantly higher, above $10\text{-}15x10^{-6}$ cm/s, usually indicate some kind of active transport. A significant difference between the A->B and the B->A direction usually indicates active uptake if the A->B value is significantly higher while the reverse situation indicates efflux, active transport outwards. Thus, both the absolute rates and the rate ratios yield important information about the oral uptake. There is a good correlation between in vitro CACO-2 diffusion rates and successful oral uptake, making it a powerful predictor[145–148].

Measuring these parameters we get a good idea about stability, uptake, and potential dose regimen for our compounds, a firm foundation on which to make the decision if we succeeded in our aim to improve not only pharmacodynamics properties but

also pharmacokinetic properties. We proceeded to measure these parameters, which are summarized in table 8.

Table 8: Pharmacokinetic parameters logD, half life ($T_{1/2}$), in vitro clearance rate (Cl_{int}) and CACO-2 diffusion rates for 61b and 69a-b.

Compound	**61b**	**69a**	**69b**
Structure	2-pyridyl	4-F-exomet	4-CF3-exomet
LogD	1.39±0.01	2.77±0.02	3.85±0.01
T1/2 min (h)	3212.1 (53.5)	526.7 (8.8)	88.8 (1.5)
Clint ml/min/kg	0.54	3.30	19.57
CACO-2 A->B 10-6 cm/s	<0.94	<1.15	<0.30
CACO-2 B->A 10-6 cm/s	15.29±0.06	25.95±0.07	48.63±0.03
CACO-2 B->A/A->B	>16.3	>22.6	>162.1

We immediately see that the distribution coefficient values of all compounds are in a good range if we look to the rule of five, the optimum being a logD between 2 and 3. The 2-pyridyltriazole **61b** has a lower logD than both of the C1-exomethylenes, probably due to its pyridyl substituent. If we take a look at the lipophilic efficiency of these compounds (table 9), we get a more nuanced picture. The lipophilic efficiency lies between 0.93 for the 4-trifluorophenyl exomethylene **69b** and 3.52 for the 2-pyridyltriazole **61b**, values that are on the poor end of the scale, mostly due to affinity which is not that surprising. This means affinity improvement while keeping the lipophilicy similar or slightly lower is the route forward in order to improve the overall drug properties of these compounds. A quick look at the logP of galactose (-2.9) and lactose (-4.7) tells us why it is an uphill battle to start with a carbohydrate scaffold, they are inherenly hydrophilic.

Table 9: Lipophilic efficiency (LiPE) of compounds 61b and 69a-b.

Compound	**61b**	**69a**	**69b**
LogD	1.39±0.01	2.77±0.02	3.85±0.01
IC50 (μM)	12 μM	13 μM	17 μM
LiPE	3.52	2.11	0.93

The half lives are also of note, **61b** is apparently more or less indestructible with a half life of almost 54 hours, almost twice that of the notoriously long-lived warfarin! This is approaching the concept of a “hard drug” which in this case means a drug that is not altered by metabolism[149]. It is worth taking this figure as approximate though, as the long half life means that the observed decay rate is liable to errors due to the comparatively small difference between initial and final concentration of the compound. The C1-exomethylenes have half lives closer to typical

pharmaceuticals – 8.8 hours for **69a** and 1.5 hours for **69b** respectively – but most importantly none of them are uncomfortably low. A prudent guess about which metabolic transformations are taking place on the C1-exomethylenes would probably be oxidation of the methylene carbon or oxidation of the former anomeric carbon, as shown in figure 26.[143,149,150]. The C1-pyridyltriazole has the same anomeric oxidation option, and also an opportunity to oxidize the position next to the pyridyl nitrogen[151].

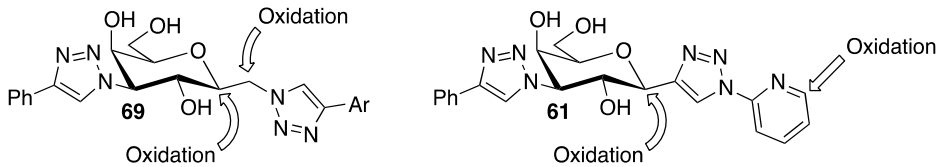


Figure 26: Likely sites for phase-1 metabolism in the C1-exomethylenes and C1-triazoles respectively.

The CACO-2 results are unfortunately where things take a turn for the worse. None of our compounds show any significant uptake, but all seem to be effluxed with great efficiency. All three compounds have efflux rates well into the range that indicates an active transport process, and the ratio is very high, confirming this. This is gloomy news for any hope for our compounds as systemically active orally available galectin inhibitors, but thanks to especially the metabolic stability there is still hope for an intravenously active compound, or one that has a local effect in the gut, which after all is where galectin-4 is located. These compounds might however be a starting point, especially if structural modifications that increase the lipophilic efficiency and decrease the efflux can be found.

Summary and conclusions

Out of a large library of different C1-galactoside based galectin inhibitors, five compounds based on three scaffolds were selected for combination with galactose C-3 substitution, two C1-aryltriazolyl galactopyranosides **24j** and **24ah**, with good galectin-1 selectivity and affinities down to 150 μM, one C1-naphthyloxazole galactopyranoside **25b** with good galectin-3 selectivity and an affinity of 90 μM and two C1-(aryltriazolyl)methyl galactopyranosides **36b** and **36k** with affinities down to 170 μM and good galectin-1 selectivity. Phenyltriazolyl substituted variants of these were synthesized and evaluated with the hypothesis that a phenyltriazolyl substituent at galactose C3 would increase affinity while maintaining some or all of the selectivity of the C1-galactosides. It turned out that there was little to no cooperativity between the substituent patterns for galectin-1 for the 3-substituted

C1-aryltriazolyl galactopyranosides **61a-b** and the C1-(aryltriazolyl)methyl galactopyranosides **69a-b**, leading to a loss of selectivity between galectin-1 and galectin-3. The C1-naphthyloxazolyl galactopyranoside **63** works more as intended, achieving a low micromolar affinity for galectin-3 with enhanced selectivity. However, it turned out all compounds exhibit a marked positive cooperativity effect while binding to galectin-4C making all of our inhibitors more or less promising inhibitors of galectin-4C, with (4-trifluorophenyltriazolyl)methyl galactopyranoside **69b** achieving a galectin-4C affinity of 12 μM and a 37-fold or better selectivity over other galectins.

The pharmacokinetic parameters of the C1-pyridyltriazolyl **61b** and C1-exomethylenes **69a-b** were measured. They all have acceptable distribution coefficients (logD 1.39±0.01 for **61b**, 2.77±0.02 for **69a** and 3.85±0.01 for **69b**) and good to excellent metabolic stabilities (half lives 53.5 h, 8.8 h and 1.5 h respectively). Unfortunately, they have low membrane permeability and experience efflux, as shown by the CACO-2 assay.

5. Aminopyrimdine galactopyranosides

"A truly happy person is one who can enjoy the scenery on a detour."
– Gregory Benford

As we saw in chapter 1, a variety of aromatic substitution patterns in position 3 on galactose have been successful in increasing the affinity of galectin inhibitors through optimization of the interaction with arginine 144. There is also a difference in the binding pockets of galectins, where galectin-3 has more space to accommodate larger substituents than some of the other galectins, especially galectin-1. Based on this, we hypothesized that substitution in position 3 with larger aromatic systems extending further into subsites A/B compared to the phenyltriazoles should preserve the arginine 144 interaction while improving galectin-3 selectivity over galectin-1 due to steric reasons (figure 27). There is also the opportunity for the aminopyrimidine ring to participate in hydrogen bonding.

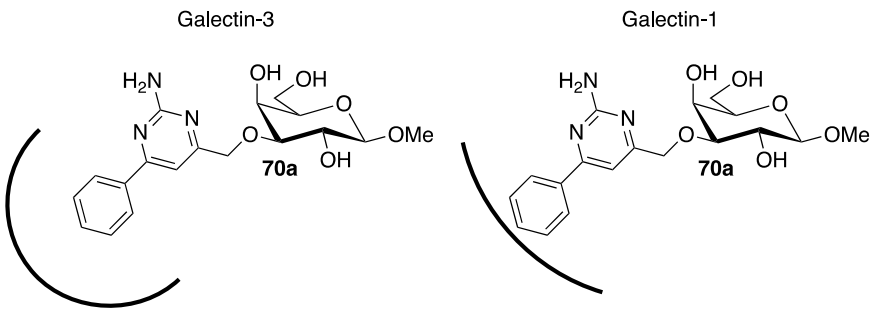
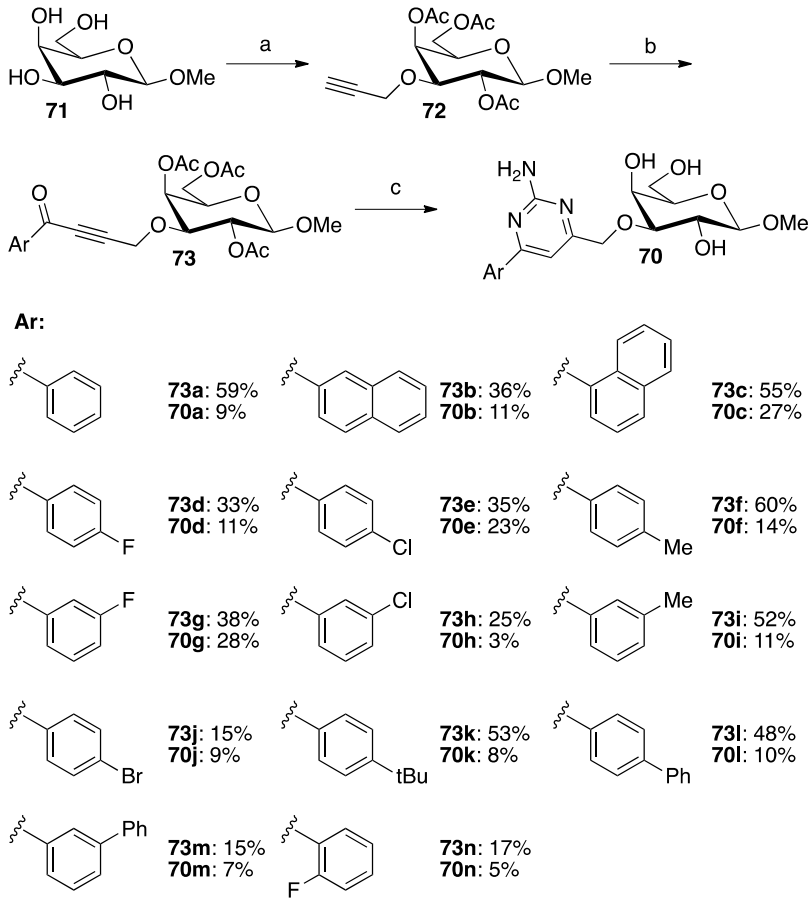


Figure 27: Methyl 3-(arylaminopyrimidinyl)methyl-β-D-galactopyranosides, a novel galectin inhibitor scaffold. The binding pocket of galectin-3 extends further than the pocket of galectin-1, opening the door to improved selectivity for galectin-3 over galectin-1.

Synthesis



Scheme 7: Synthesis of 3-(arylaminopyrimidine)methyl galactopyranosides 70a-n. (a) Dibutyltin oxide, MeOH (dry), reflux, 2h, then propargyl bromide, tetrabutylammonium bromide, DMF(dry), 80°C, 18h, then acetic anhydride/pyridine, 73% yield. (b) Aroyl chloride, bis(triphenylphosphine)palladium(II) dichloride, copper(I) iodide, triethylamine, THF(dry), rt, 18h, 15-60%. (c) Guanidinium hydrochloride, potassium carbonate, THF(dry), reflux, 18h, then sodium methoxide, MeOH (dry), 2h, 5-28%.

The synthesis started from the commercially available methyl β-D-galactopyranoside **71**, which was refluxed in dry methanol with dibutyltin oxide, then treated with propargyl bromide and tetrabutylammonium bromide in DMF before finally being subjected to an acetylation by a mixture of acetic anhydride/pyridine to give 3-propargyl **72**, all in a yield of 73%[92,152]. From this common intermediate, the synthesis branches via a Sonogashira coupling with aroyl chloride, bis(triphenylphosphine)palladium(II) dichloride, copper(I) iodide and triethylamine in tetrahydrofuran, to give alkynones **73a-n** in yields of 15-

60%[111,112,153]. The alkynones were then refluxed in tetrahydrofuran with guanidinium hydrochloride and potassium carbonate and finally deprotected with sodium methoxide in dry methanol[154] in yields of 5-28% to give aminopyrimdines **70a-n** (Scheme 7). The yields for the aminopyrimidine formation via a cyclisation reaction and subsequent deprotection are poor, probably due to a partial deprotection taking place during the reaction giving rise to a lot of side products, none of which were present in large enough amounts to allow for proper characterization.

Galectin affinities and galectin mutant affinities

Affinities of inhibitors **70a-n** were evaluated using the previously mentioned fluorescence polarization assay[101] for galectins -1, -2, -3, -4C, -4N, -7, -8C, -8N, -9C and -9N. The affinities towards all galectins, except galectins -1 and -3, were in the high micromolar to millimolar range and are omitted for clarity. The affinities for galectins-1 and -3 are presented in table 10 below.

Table 10: Affinities of aminopyrimidines 70a-n towards galectin-1 and galectin-3 as measured by fluorescence polarization assay. Kd calculated from 4-6 data points at different concentrations.

		KD Galectin-1	**KD Galectin-3**
70a	*Ph*	1400±62 µM	260±5 µM
70b	*2-Nap*	>900 µM	83±11 µM
70c	*1-Nap*	1300±160 µM	340±52 µM
70d	*4-FPh*	1100±230 µM	320±39 µM
70e	*4-ClPh*	>900 µM	260±15 µM
70f	*4-MePh*	>900 µM	270±27 µM
70g	*3-FPh*	1800±330 µM	230±10 µM
70h	*3-ClPh*	1900±130 µM	190±23 µM
70i	*3-MePh*	200±33 µM	200±19 µM
70j	*4-BrPh*	770±28 µM	310±10 µM
70k	*4-tBuPh*	1400±360 µM	300±37 µM
70l	*4-BiPh*	>600 µM	191±25 µM
70m	*3-BiPh*	1000±440 µM	280±41 µM
70n	*2-FPh*	430±45 µM	260±25 µM

The affinities in table 10 show that our design has largely been successful; most of the inhibitors have good galectin-3 affinity and poor or no measurable affinity towards galectin-1. A few exceptions exist; 3-methylphenyl **70i** has no selectivity between galectins, while the 4-methylphenyl **70f** is one of the better inhibitors with a good affinity and complete selectivity. Other 3-substituted compounds such as 3-fluorophenyl **70g** with a 230±10 μM galectin-3 affinity and eightfold selectivity and 3-chlorophenyl **70h** with an affinity of 190±23 μM and a tenfold selectivity are also more in line with the general trend. If we compare the halogen series fluorine-chlorine-bromine in **70d-e** and **70j**, we see no big differences in galectin-3 affinity pattern, all lie around 300 μM in affinity but with different selectivities; **70d** has threefold selectivity, **70e** has excellent selectivity and **70j** again has just over twofold selectivity. Moving on to other aryls, we see that the 2-naphthyl **70b** is the star of the show with an affinity of 83±11 μM and good selectivity for galectin-3 over galectin-1, while 1-naphthyl **70c** is a more modest inhibitor with an affinity of 340±52 μM and only threefold selectivity.

Looking at the more flexible biphenyls, 4-biphenyl **70l** has the second best affinity at 191±25 μM and good selectivity, while 3-biphenyl **70m** has an affinity of 280±41 μM and about fourfold selectivity. It is clear that of the larger aromatic systems, aryl systems extended along the axis of the binding pocket (like 4-biphenyl **70l** and 2-naphthyl **70b**) seems to be linked to higher affinity and selectivity. If we compare the larger aryls with the large aliphatic 4-tertbutylphenyl **70k** we see an affinity of 300±37 μM and fourfold selectivity meaning it is worse than the smaller aliphatic 4-methylphenyl **70f** and both the 2-naphthyl **70b** and the 4-biphenyl **70l**. Clearly nothing seems to be gained from larger aliphatic substituents. The 2-fluorophenyl **70n** is also interesting in that it has very poor selectivity, less than twofold. It is possible that this is due to the conformational restriction introduced by the ortho substituent due to the interactions between the lone pair of the aminopyrimidine nitrogen on the phenyl side and the 2-fluorine[120]. In all, apart from the larger linear aromatic systems the substituent effects seem to be larger on the galectin-1 side than the galectin-3 side.

Mutants of galectin-3 where arginine 144 has been replaced by lysine (R144K) and serine (R144S) respectively have been used to elucidate the importance of substituents in position 3 on galactose for several different galectin inhibitors[67,155]. This can be thought of as a kind of reversal of the regular structure-activity relationship process; instead of changing the ligand structure, we change the protein structure to reveal more about the interactions. The interaction aromatic systems substituted in position 3 has with arginine 144 is fairly well understood and can be used to map the interaction of new substituents in this position in greater detail. Based on the affinity and selectivity patterns in table 9, three different inhibitors were selected; phenyl **70a** as the unsubstituted base case, 2-naphthyl **70b** as the best inhibitor and 4-chlorophenyl **70e** due to having one of the better affinities of the

substituted phenyls, while also being completely selective for galectin-3 over galectin-1. These were tested for affinity towards galectin-3 R144K and R144S, these results are shown in table 11.

Table 11: Affinities of Galectin-3 mutants towards aminopyrimidines 70a-b,e. The mutants have had arginine 144 replaced with a lysine (R144K) and a serine (R144S) respectively. Affinities measured via fluorescence polarization assay, calculated from 4-6 points at different concentrations.

	KD Galectin-3 R144wt	**KD Galectin-3 R144K**	**KD Galectin-3 R144S**
70a	260±5 μM	840±230 μM	1200±580 μM
70b	83±11 μM	280±20 μM	1000±44 μM
70e	260±15 μM	1400±90	1200±450 μM

The results of the mutants reveal a few interesting patterns. Starting with the R144S mutant, we see that replacing the basic arginine residue with the non-basic serine, we have a loss of cation-pi stacking and a concomitant loss of both affinity and difference between the different inhibitors as they all jump up in affinity to a low millimolar and virtually identical level. This illustrates – with emphasis – the importance of the interaction with the arginine; for example 2-naphthyl **70b** experiences a twelvefold loss in affinity. Moving on to the lysine mutant R144K we see some loss of affinity, but crucially the 2-naphthyl **70b** does not experience an affinity loss as dramatic as the other inhibitors. The lysine is still capable of cation-pi stacking, albeit less efficiently so than the arginine. This may be interpreted as that the 2-naphthyl **70b** has a large enough aromatic system to successfully interact with the less efficient partner lysine, preserving some of the cation-pi interaction and thus some of the affinity, only experiencing about a threefold drop in affinity. As arginine 144 normally participates in hydrogen bonding with the galactose, and it is possible it is still able to do this while participating in cation-pi stacking with the aminopyrimidines **70**, due to the double-pronged nature of the multi-amino functionalized guanidine. The lysine might not have the reach to do both, despite its longer carbon chain, as it has a single amino group. The recovery of affinity for **70b** of the lysine mutant and the increased affinity of the wild type galectin for **70b** might then have a similar explanation – reach of the aromatic system. The hypothesis then becomes that the recovery of dual hydrogen bonding/pi stacking ability through the increased size of the aromatic system is responsible for the affinity recovery of the lysine.This is summarized in figure 28.

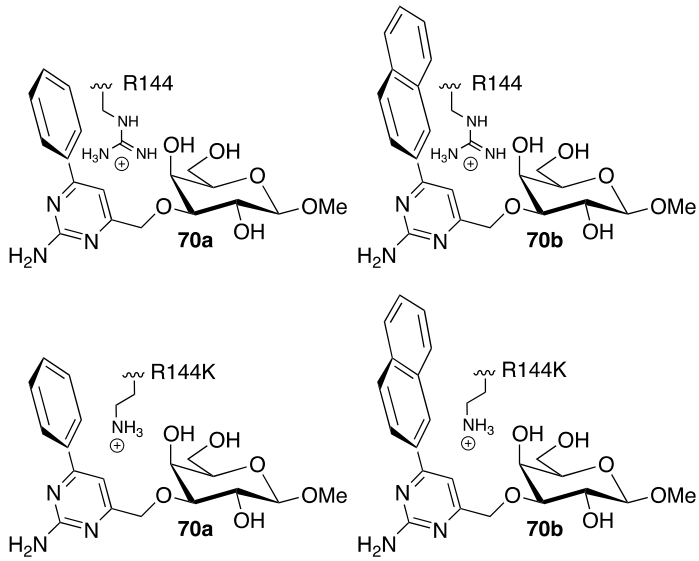
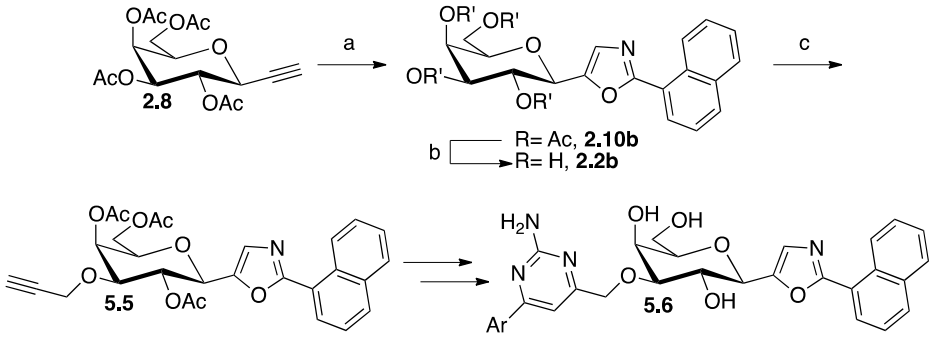


Figure 28: Binding interaction hypothesis: Arginine 144 overlaps with the aryl system interacting through a cation-pi interaction, while also interacting with parts of the carbohydrate through hydrogen bonding. Lysine cannot participate in both of these interactions, leading to a loss of affinity towards phenyl derivatives like 70a. Larger aromatic systems like 70b partially bridge this gap, making it possible for the lysine to again participate in both cation-pi stacking and hydrogen bonding.

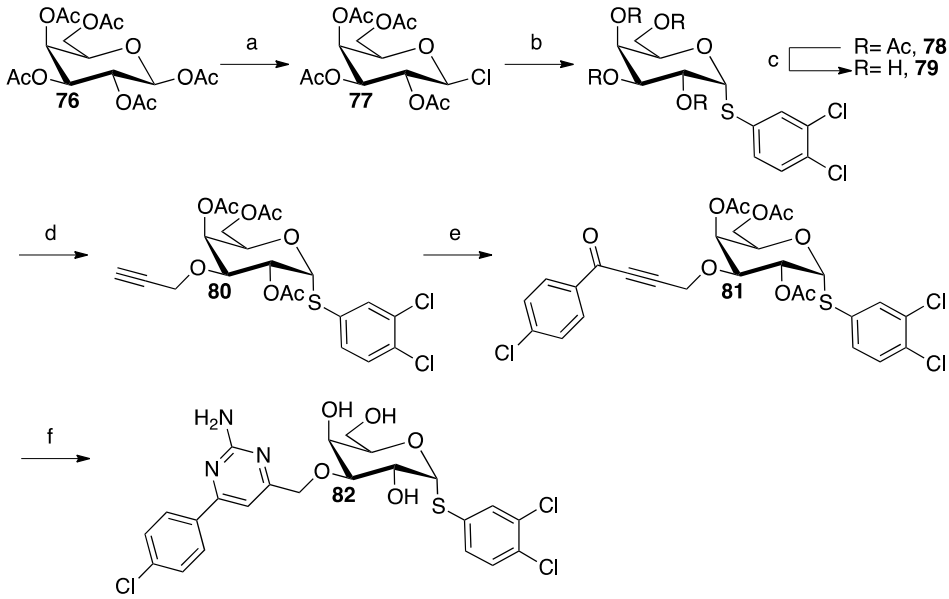
Combination of aminopyrimidines with 1-substituents

Aminopyrimidines can be combined with galectin-3 selective substituents in position one and the 1-naphthyloxazole **25b** from chapter 2 immediately comes to mind. If we start the synthesis from the **25b** and introduce a propargyl in position three through a tin acetal mediated propargylation, followed by the Sonogashira coupling and finally the cyclization/deprotection we have our combined aminopyrimidine/naphtyloxazole **75**, as seen in scheme 8.



Scheme 8: Synthesis of the disubstituted aminopyrimidine 75. (a) 1-cyanonaphthalene, (triphenylphosphine)gold(I) triflimide 8-methylquinoline N-oxide, neat melt. 72h. (b) Sodium methoxide, MeOH (dry), r.t. 2h. (c) Dibutyltin oxide, MeOH(dry), reflux, 2h, then propargyl bromide, tetrabutylammonium bromide, DMF(dry), 80°C, 18h, then acetic anhydride/pyridine, 6% yield.

Unfortunately, the yield for the propargylation is very low, 6%. Combined with the already moderate yield (33%) for the synthesis of **33b** and the less than excellent yields in the upcoming Sonogashira coupling and cyclization/deprotection we came to the decision to abandon this synthetic endeavour. If not the 1-naphthyloxazole, then what? In the introduction we saw that the 3,4-dichlorothiophenyl substituent (exemplified by **19**, chapter 1) is a good driver of galectin-3 affinity[90], so a compound with both an aminopyrimidine and a 3,4-dichlorothiophenyl seemed like a viable substituent combination.



Scheme 9: Synthesis of 3,4-dichlorothiophenyl/4-chloroaminopyrimidine disubstituted galactopyranoside 82. a) Phosphorous pentachloride,$BF_3 \cdot OEt_2$, DCM, 20min. b) 3,4-dichlorothiophenol cesium carbonate, DMF, 50°C c) Sodium methoxide, dry methanol d) Dibutyltin oxide, MeOH(dry), reflux then propargyl bromide, tetrabutylammonium iodide, DMF(dry) e) 4-chlorobenzoyl chloride, bis(triphenylphosphine) palladium(II) dichloride, copper(I) iodide, triethylamine, THF f) guanidinium hydrochloride, potassium carbonate, THF, reflux then sodium methoxide in MeOH(dry).

The synthesis begins with an anomeric chlorination with phosphorous pentachloride and boron trifluoride etherate in dichloromethane to give **77**, which is then reacted with 3,4-dichlorothiophenol and cesium carbonate in DMF at 50°C, giving **78** (Scheme 9). The thiophenyl galactoside is then deacetylated with sodium methoxide in dry methanol at room temperature to give **79**. A propargylation using first dibutyltin oxide refluxed in dry methanol to give a tin acetal which is then heated in DMF with propargyl bromide and tetrabutylammonium iodide to give propargylated thiophenyl galactoside **80**. Alkynone **81** is made with a Sonogashira coupling using 4-chlorobenzoyl chloride, bis(triphenylphosphine)palladium(II) dichloride, copper(I) iodide and triethylamine in THF. The alkynone is the cyclized to aminopyrimidine **82** with guanidium hydrochloride and potassium carbonate in THF, followed by deprotection with sodium methoxide in dry methanol. 4-Chlorophenyl was chosen due to its excellent selectivity for galectin-3 over galectin-1.

Affinity of **82** towards galectins -1, -3, -4N, -4C, -7, -8N, -8C, -9N, -9C was measured using the usual fluorescence polarization assay and the results can be found in table 12.

Table 12: Galectin affinity measurements of 82 towards galectins -1, -3, -4N, -4C, measured using fluorescence polarization assay. Kd calculated from 4-6 points at different concentrations.

Galectin	Affinity of 82
1	>500 µM
3	1.7±0.21 µM
4N	>500 µM
4C	380±90 µM
7	58±32 µM
8N	170±25 µM
8C	>500 µM
9N	73±11 µM
9C	>100 µM

The data tells us that 3,4-dichlorothiophenyl aminopyrimidine **82** retains its selectivity towards galectin-3 over most other galectins, with an affinity of 1.7±0.21 µM and a 294X or better selectivity for galectin-3 over galectin-1. Affinities towards galectins -4C, -8N and -9N are 380±90 µM, 170±25 µM and 73±11 µM respectively, with selectivities that are 223X, 100X and 43X respectively, which is respectable. The best affinity towards a galectin other than galectin-3 is towards galectin-7, with an affinity of 58±32 µM and a 34X selectivity which is still very good. We therefore succeeded in our aim to improve affinity whilst retaining the high selectivity imparted by the aminopyrimidine substituent pattern. In our aminopyrimidine paper we also sought computational insights in to binding and it supports our arginine 144 interaction hypothesis. The amino group of the aminopyrimidine also seems form a bridging interaction between asparagine 148 and serine 237 in subsite A/B.

Summary and conclusions

3-(Arylaminopyrimidine)methyl galactopyranosides are designed to be highly selective for galectin-3, exploiting the larger space available in the binding pocket. This design strategy turned out to be fruitful, as inhibitors with good selectivity and affinites down to 83±11 µM (2-naphthyl **70b**) were found. The reason for the good affinity of the 2-naphthyl derivative **70b** was proposed to be maximization of a favourable cation-pi interaction of the more polarizable naphthyl group with

arginine 144 in the galectin-3 binding pocket, as determined by arginine 144 mutants. Combining the aminopyrimidine motif with affinity- and selectivity driving substituents in position 1 turned out to be a challenge when it came to the 1-naphthyloxazole from chapter 2 due to several steps with very low yields in the synthesis making it unworkable. Introducing a 3,4-dichlorothiophenyl substituent seen in chapter 1 worked much better, resulting in inhibitor **82**, with an affinity of 1.7±0.21 μM towards galectin-3, a 294X selectivity for galectin-3 over galectin-1 and a 43X or better selectivity for galectin-3 over other galectins.

6. Conclusions and future prospects

"The time is gone, the song is over, thought I'd something more to say."
– Pink Floyd, "Time"

Conclusions – What have we learnt?

In the course of our endeavours to investigate the C1-galactosides as galectin inhibitors we have learnt a great deal, succinctly summed up below. Among the C1-arylheterocycles from chapter 2 (exemplified by **24j**, **24ah** and **25b**, figure 29) the heterocycle sets the galectin preference, with oxazoles giving galectin-3 selectivity and triazoles giving galectin-1 selectivity. Molecular dynamics simulations indicate that stacking between histidine 52 and the C1-triazole gives rise to the galectin-1 selectivity of the triazoles, while favourable rotational conformation preference giving the ability to reach a unique subpocket with the 1-naphthyl system gives rise to the galectin-3 selectivity of the oxazoles.

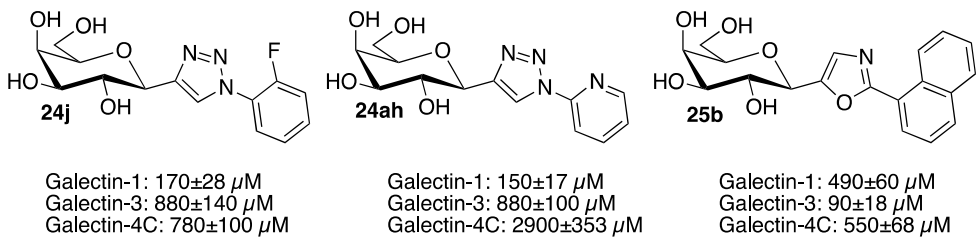


Figure 29: Galectin selectivity is set by the heterocycle in C1-arylheterocycle galectin inhibitors. Affinities of 150 μM and complete selectivity for galectin-1 is achievable, and an affinity of 90 μM and sixfold selectivity for galectin-3 is also achievable.

C1-Exomethylenes from chapter 3 (exemplified by **36b** and **36k**, figure 30) are galectin-1 selective with an affinity down to 170 μM and a sixfold galectin-1 selectivity.

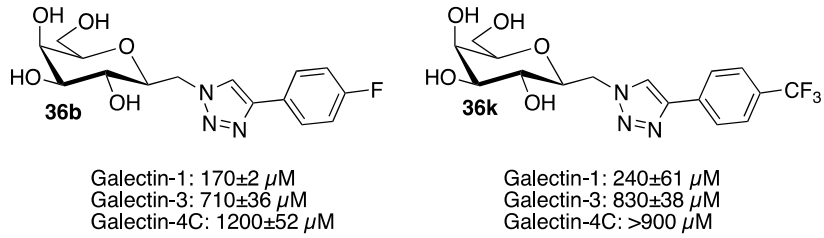


Figure 30: Galectin-1 selective C1-exomethylene galectin inhibitors with affinities of 170 μM and sixfold selectivity.

Hydroboration of C1-exomethylenes is a viable route to 2-deoxyheptuloses with good yields and with good diastereoselectivity, at least for galactose and mannose. This is in our opinion due to a kinetic effect, the approach angle from above the ring leading to alpha products is blocked by axial substituents.

Substitution of C1-arylheterocycles and C1-exomethylenes with a 3-phenyltriazolyl motif which in previous galectin inhibitor types have increased affinity were investigated in chapter 4 and turned out to undo the selectivity induced by the C1-substituents in the unsubstituted case. There is an "anti-cooperative" effect between the 1- and 3- substituents for the galecin-1 selective motifs leading to a loss of selectivity between galectin-1 and galectin-3, as seen for the triazoles **61a-b** and the exomethylene triazoles **69a-b**. The oxazole **63** retains its cooperativity and has an increased galectin-3 affinity preserving the galectin-3 selectivity as predicted (figures 31 and 32).

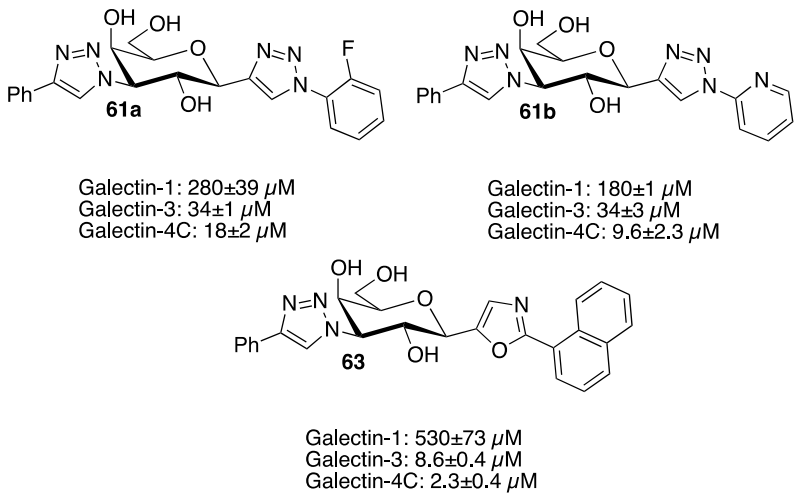
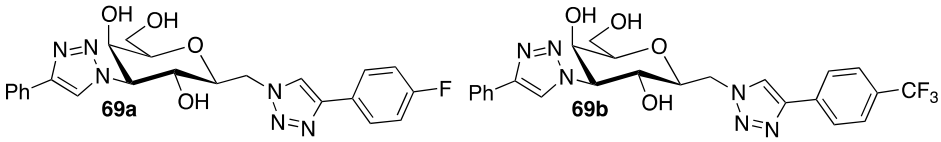


Figure 31: Disubstituted C1-heterocycles with unexpected antagonism between structural motifs leading to a loss of galectin selectivity for inhibitors intended to be galectin-1 selective. Unexpected synergy between binding motifs lead to an unexpectedly high galectin-4C affinity.

This all leads to a galectin-4C selective inhibitor, in the case of the C1-exomethylenes **69a-b** which have affinities down to 9.5 µM and a thirty-eightfold or better selectivity for galectin-4C. In addition to the affinity studies, a number of pharmacokinetic parameters were evaluated for **69a-b**. They all have decent logD: 2.77±0.02 for **69a** and 3.85±0.01 for **69b** – and metabolic half lives in human liver microsomes were 8.8 h and 1.5 h respectively. Unfortunately, they have low membrane permeability and are effluxed heavily, as shown in the CACO-2 assay.

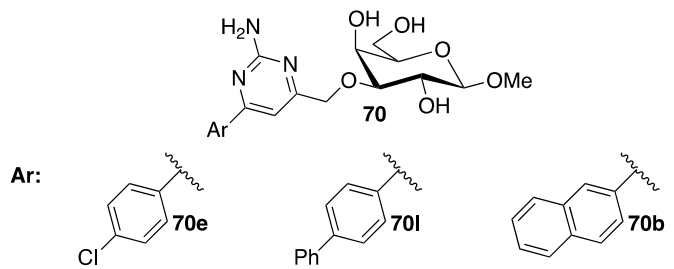


Galectin-1: 220±10 μM
Galectin-3: 200±32 μM
Galectin-4C: 9.5±1.3 μM

Galectin-1: 440±68 μM
Galectin-3: 720±78 μM
Galectin-4C: 12±1.7 μM

Figure 32: Similar problems as for the disubstituted C1-triazoles are encountered for disubstituted C1-exomethylenes; loss of selectivity for galectin-1 but an unexpected high affinity for galectin-4C. In contrast to the C1-triazoles the galectin-3 affinity remains low leading to a galectin-4C inhibitor with low micromolar affinity and thirty-eightfold or better selectivity.

In summary, we started out with galectin-1 and galectin-3 inhibitors and ended up with galectin-4C inhibitors. Moving on to the aminopyrimidines from chapter 5, we managed to achieve good selectivity for galectin-3 over galectin-1 while maintaining poor affinity towards other galectins with affinities of 83 µM (**70b**, **70e** and **70l**, figure 33), a task that was accomplished by exploiting the larger binding pocket of galectin-3 compared to galectin-1.



Galectin-3: 260±15 μM
Galectin-1: >900 μM

Galectin-3: 190±25 μM
Galectin-1: >900 μM

Galectin-3: 83±11μM
Galectin-1: >900 μM

Figure 33: Arylaminopyrimidines achieve complete selectivity towards galectin-3 over galectin-1 and affinities down to 83 µM.

With these results in hand, we look to the future.

Future prospects – Where should we go?

To start with, X-ray structures of the better of the C1-aryltriazoles with galectin-1, the C1-aryloxazoles with galectin-3 and the 1,3-substituted compounds with galectin-4C would be of immense help to understand the binding interactions and corroborate the molecular modelling. Calorimetry to obtain information on the thermodynamics of the binding would also be very useful to guide further synthesis and structural optimization. Thermodynamic fingerprinting, for example, is a useful tool to aid in design, deepening the understanding of what takes place on the molecular recognition level of binding[100]. A good beginning of deeper investigations would be to evaluate a larger panel of C1-heterocycles to identify better heterocycles for the selectivity patterns we already see, or perhaps find new ones. It is also possible that we might encounter C1-heterocycles that do not have the antagonism between 1- and 3- substitution patterns that hampered our efforts in chapter 4. A panel of suggestions can be seen in figure 34.

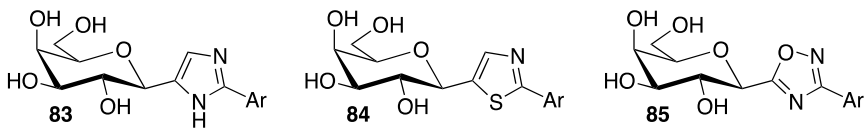


Figure 34: Suggested novel C1-arylheterocycles to investigate

Structures **83** and **84** retain the oxazoles spacing between the heteroatoms but with the oxygen replaced. **85** is a hybrid between the successful oxazole and the affinity increasing but non-selective isoxazoles from chapter 2. A myriad of other heterocycles are possible, but **83-85** are at least a starting point.

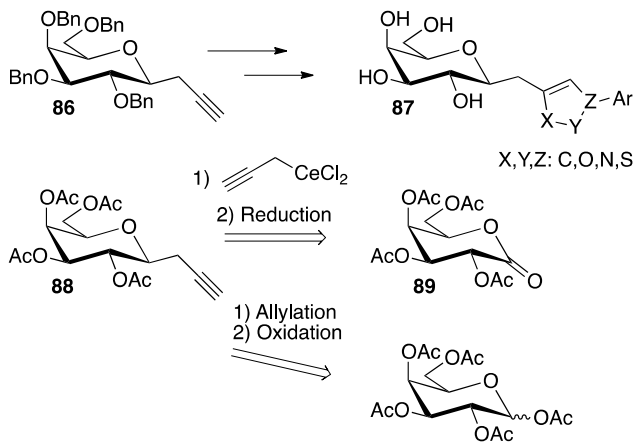


Figure 35: Exomethylene analogues of the C1-arylheterocycles, a simple retrosynthesis.

Another idea would be to synthesize a series of homologs to the C1-arylheterocycles with an exomethylene linker, as exemplified in figure 35. One way to synthesize these would be through a C1-propargyl galactose made by a propargyl cerium(III) dichloride addition and subsequent reduction on the δ-galactonolactone **29** that is the entry point for practically all the synthesis in this thesis. This should not present an insurmountable, as the organocerium reagent is known[106,156]. If this is undesirable, a route exists involving an allylation and subsequent ozone based oxidation to an alkyne[92], but the ozone conditions are quite harsh and might not be well tolerated by other substituents we might wish to introduce. Worth noting is that the triazole variant of **87** was actually synthesized by Gigueré et al[157], and was found to have poor galectin affinities. It would still be of interest to evaluate other possible heterocycles accessible from **86** with methods developed in this thesis.

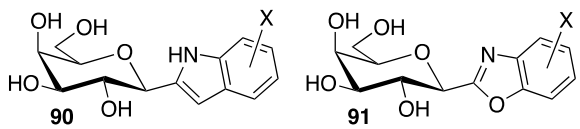


Figure 29: Bicyclic C!-heterocyclic systems.

Bicyclic systems would also be interesting to investigate (see figure 36), being rigid and with a more extended C1-aryl system compared to our investigated C1-aryls. Direct equivalent scaffolds to **90** have been made by Yepremyan et al on glucose, and thus **90** should be to obtainable with relative ease[17]. Benzoxazole **91** is a close analog to the oxazoles and can be obtained from an orthoester cyclization which can also give access to the benzothiazoles and benzimidazoles [158]. The orthoester can be obtained from an anomeric cyanide using a Pinner reaction[159].

Yet another idea would be to use silicon, an idea that has received some interest in medicinal chemistry for more than three decades[160–162]. Sila substitution has been shown to enhance affinity of antituberculosis diaryl pyrazole drugs, while still keeping the pharmacokinetic properties within acceptable limits[163]. Synthesis could be accomplished through hydrosilylation of the enol ether from chapter 3, using modern catalytic methods (see figure 37)[164–166].

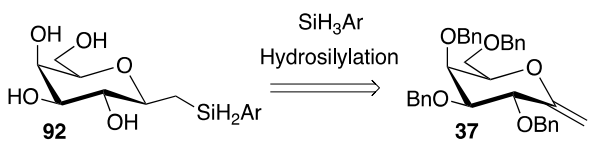


Figure 37: Sila substitution of exomethylene carbon, synthesis through hydrosilylation.

Some work that is already ongoing is comparing the binding of C1-naphthyloxazole **63** and C1-naphthoyl amide **93 (**figure 38). Naphthoyl amide **93** has an affinity towards galectin-3 of 4.7±0.7 μM and a sixfold selectivity for galectin-3 over galectin-1, while – as we saw in chapter 4 – naphthyl oxazoles **63** has an affinity of 8.6±0.4 μM and a sixty-twofold selectivity for galectin-1 over galectin-3. Preliminary X-ray crystallography results indicate that the amide and the oxazoles have differing rotational orientations while the naphthyl substituents both go in subsite C. In order to explain the selectivity differences and compare the oxazoles and amide motifs neutron crystallography will be used to obtain structures with a resolution where hydrogens are visible. This should help us to understand both the binding interactions between the ligands and the protein, and how binding influences the water hydrogen bonding network in the binding pocket. Coupled with calorimetry measurements to determine and compare the binding enthalpy and entropy we will be in a position to better understand the interplay between galectin C-glycoside recognition and C-glycoside structure, similar to how Verteramo et al investigated the effect of stereoisomerism on galectin recognition[167].

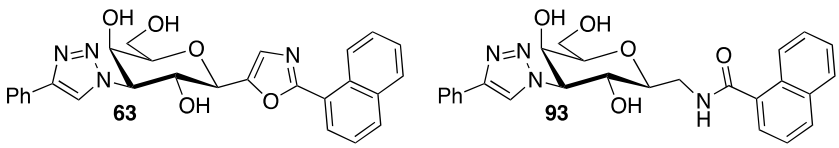


Figure 38: Comparing the binding of C1-naphthyloxazole 63 and C1-naphthoylamide 93 using neutron crystallography.

These ideas ought to be enough work for at least an additional thesis, but unfortunately, time has run out and all I can hope for is that these ideas inspire someone to strive to join me in no longer being a carbohydrate chemist and dive into the rich world of C1-glycosides and the opportunities they present as scaffolds for galectin inhibitors.

Populärvetenskaplig sammanfattning

Nästan alla större molekyler (som till exempel proteiner) i en cell är täckta av kolhydrater – socker. I det här fallet är sockret inte näring, utan används istället av cellerna som signalflaggor för att organisera var proteiner är och hur de är utspridda. Det finns särskilda proteiner vars jobb det är att känna igen de här kolhydraterna, och målproteinerna i vår forskning är en av dessa familjer av kolhydratigenkännande proteiner – galektiner.

Galektiner känner igen kolhydrater som innehåller en galaktosenhet (galaktos är en av sockren i disackariden laktos som finns i mjölk) genom att binda till den, och när det händer samlas proteinerna som har galaktosinnehållande kolhydrater på sig ihop i kluster. Om det till exempel är receptorer (signaleringsproteiner) som sitter i kluster signalerar de starkare än om de sitter långt isär. På så sätt reglerar galektinerna omfattningen av cellsignaleringen, lite på samma sätt som volymkontrollen på en megafon. I vissa sjukdomar – till exempel immunförsvarsrelaterade sjukdomar som idiopatisk lungfibros, inflammatoriska tarmsjukdomar och vissa former av cancer – tillverkar cellerna för mycket galektiner och volymratten i cellsignaleringen har med andra ord fastnat på max. Ett tänkbart läkemedel mot dessa sjukdomar skulle då vara en konstgjord kolhydrat som binder bättre till galektinerna än de naturliga kolhydraterna gör, för att lösa upp de onaturliga klustren – sänka volymen på cellsignaleringen. Eftersom olika medlemmar i galektinfamiljen föredrar att skapa kluster med olika receptorer och dessutom finns i olika delar av kroppen är det viktigt om man ska göra ett läkemedel att det i mycket stor utsträckning föredrar att binda till en enda av medlemmarna i familjen. Detta kan vara en utmaning eftersom de är så pass lika.

Den här avhandlingen handlar om hur man gör ett antal olika konstgjorda kolhydrater som är designade för att föredra en enda galektin, och hur bra de binder. Vi lyckades göra ett par konstgjorda kolhydrater som föredrar galektin-1 och ett par som föredrar galektin-3. När vi kombinerade strukturerna vi designat med ett par redan existerande strukturer som ska göra dem ännu bättre upptäckte vi att dock att de nya molekylerna plötsligt föredrog galektin-4. Våra upptäckter är viktiga pusselbitar inte bara när det gäller läkemedel mot galektinrelaterade sjukdomar, men också förståelsen av hur man designar läkemedel med utgångspunkt i kolhydrater.

Acknowledgements

"No man is an island"
– John Donne

Several people have helped me along the way to writing these words, and I am grateful to all of them. I would like to especially thank:

Professor Ulf Nilsson, my supervisor. Apart from sharing his deep knowledge in synthetic chemistry and medicinal chemistry, he spreads enthusiasm and joy for chemistry like few others I have ever met. His ability to always leave me inspired and his trust in letting me try my own ideas has been key in my development as a scientist.

Professor Ola Wendt has been my assistant supervisor, and has always been the best moral support I could have asked for. I am also grateful to him for teaching me everything I know (and most of what I have forgotten) about metal organic chemistry.

Dr Fredrik Zetterberg, my other assistant supervisor and a constant source of knowledge and ideas about medicinal chemistry – quite a few of which are lurking in the preceeding pages. Fredrik's ability to ask precisely the right question at the right time is a skill I am looking forward to learn one day.

Professor Ulf Ellervik has always been a willing discussion partner on subjects ranging from the proper nomenclature ("Treat the 2-deoxyheptulose as a substituted methanol. If nothing else it will check if people reading are awake.") to conference presentation attendance tactics and the finer points of scuba diving.

Professor Kenneth Wärnmark has always taken the time to discuss both chemistry and teaching with me, and always has a book recommendation ready if you should ask.

Professor Hakon Leffler, for providing all the knowledge about galectin biology I could possible want, and then some.

All the members of group Nilsson, past and present but especially **Dr** (soon) **Maria Luisa Verteramo** (willpower incarnate). **Dr Kristoffer Peterson**, **Tiago Bozzola** (ninja in training), **Mukul Mahanti**, **Mujtaba Hassan**, **Ankita Mitra**, **Floriane Baussière** and **Veronika Chadimová**. An all-star crew if there ever was one. Thank you for all the fun, but also all that I have learnt because of you.

All of my students, who have taught me so much about chemistry. Special thanks to **Dr Maëlle Vallade**, **Axel Furevi** and **Niklas Warlin**.

Daniel Willén (Lisbon Dream Team 2018), **Dr Kirill Popov** (The all-knowing all-seeing), **Dr Jakob Danielsson**, **Dr Anita Hoang**, **Dr Maitham Majeed** (KEMA12 Dream Team), **Dr Björn Borgström**, **Dr Karin Holmqvist**, **Valtýr Freyr Hlynsson**, **Lisa de Groot**, **Anna Lidskog**, **Olga Gordivska**, **Vicky Li**, **Dr Ian George**, **Niels Koning**, **Magdalena Tasic**, **Joel Olsson** and all my other CAS coworkers past and present. The opportunity to have conversations with the smartest people in the world on a daily basis is truly a privilege.

Barbro Kahl Knutsson, for being the fluorescence polarization assay guru who considers a two day turnaround on affinity measurements to be a long time.

Dr Sofia Essén, for purity analysis, high-resolution mass spectrometry, diastereomer ratio determinations and captivating discussions on analytical chemistry and most other things under the sun.

Dr Sophie Manner, for all the advice on life, chemistry and teaching, and for being excellent conference company.

Maria Levin, thanks to whom I can sleep easy knowing all is right in the world of administrative affairs.

Katarina Fredriksson, without whom I would not have any chemicals, solvents or glassware to put them in.

Bodil Eliasson, for all the help but mostly for the moral support when times were tough.

Dr Karl-Erik Bergquist, for NMR spectroscopy knowledge in general, but especially for helping to figure out how to assign stereochemistry when your sugar protons are a multiplet that integrates to seven.

Professor Marie Wahlgren, for teaching me about pharmaceutical technology and for being a rock to lean against during my days in student society leadership.

The instructors at the Ranger Company Officer School, K3, Karlsborg, especially **Kn Donat Hullman** and **Lt Joachim Axelsson** for teaching me that the only way out of hardship is to go through the hardship – Pergite!

My highschool chemistry teacher **Mårten Persson**, who rescued me from any notion I had that studying physics and set me on this path by being the best teacher one could wish for.

Alexandra Hermelin, for her laser focused ability to find numbering errors and typos, a skill she could – and should – charge for.

Mats Öberg, for the very nice illustrations in chapter 1, a work of true talent.

Olof Röckner, designer of my jaw-droppingly awesome front cover, my brother from another mother and my oldest friend.

Dr Henrik Jonsson, **Martin Blom** and **Ingrid Blom**, who from my time as an undergraduate up until today deserve the credit for teaching me many things I did not know I wanted to know.

Per Ekholm and **Dr Josef Berge, MD** for being the kind of friends who are practically family by now.

Mikael, **Hannele** and **Anders Dahlqvist**, my family who have been along for the journey the longest of all.

Dr Viveca Missler Dahlqvist, MD. Our relationship began with long walks home, talking about everything between heaven and earth, and so it has continued for eleven years now. It is a privilege to get to come home to someone who always makes you feel safe and loved, because only love could make a medical doctor listen to as much organic chemistry talk as she has.

Baby Dahlqvist for putting all of this in second place out of things to look forward to this spring.

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”A couple of months in the laboratory can frequently save a couple of hours in the library.”
– Frank Westheimer

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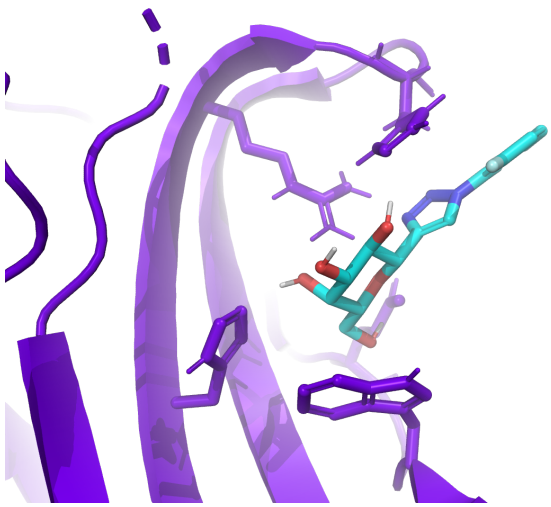
Printed by Media-Tryck, Lund 2019 NORDIC SWAN ECOLABEL 3041 0903



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Department of Chemistry,
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ISBN 978-91-7422-658-4
ISSN 1652-8220

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