LUND UNIVERSITY

Synthesis of 3-C-(4-aryl-5-halo-1,2,3-triazol-1-yl)-galactosides: Probing halogen bonding in galectin-ligand complexes

by

Marcus Ljungberg

A thesis done at

Lunds University

Lund, Sweden

2011-08-19

Supervisor: Ulf Nilsson

Abstract

The continued search for compounds exhibiting a better inhibition of Galectin-3 and -1 has been performed in this thesis. It has been shown that the Galectins are over-expressed in cancer cells and play an important role in inflammatory conditions connected to apoptosis (cell death). The focus in this research project has been to explore if halogen-bonding, which gives a hydrogen-bond like character, can be used to improve the affinity for binding to the galectins. Activity was also found in synthesized monosaccharides but not nearly as good as previously tested disaccharides. But the results are a good foundation for future research on developing more drug candidates which are monosaccharides.

Contents

Abstract 1

Introduction 3

Results and discussion 6

Conclusions and future work 9

Experimental 9

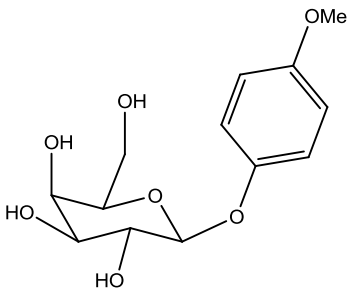
Acknowledgements 15

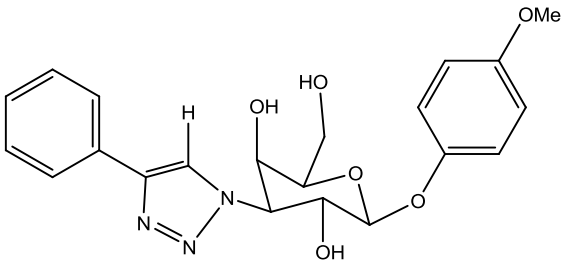
1. Introduction

1.1 Background

1.1.1 Galectins

Galectin is a family name for a group of animal lectins, sugar-binding proteins. Lectins having common properties, that is, specific for β- galactosides and requiring no metal ion for activity have been found in sponges to vertebrates[1]. The molecular function of galectins is not so complicated: they bind sugar chains which contains galactosides. Their biological role, however, cannot be easily specified because they appear to correspond to too many diverse biological processes. It is not possible to assume a one-to-one relationship between a galectin and biological function[1]. The Galectins are found, in vertebrates, in various different tissues and cells; *eg* skin, muscle, brain, intestine, liver, kidney and placenta. The galectin family is also characterized by its specific amino acid sequence motif for sugar binding (CRD, standing for carbohydrate-binding domain). The CRD allows the galectins to cross-link their glycoprotein ligands[2]. This is achieved in three principal ways ruled by galectin structural features. The so-called prototype Galectins that have one CRD within their polypeptide chain form non-covalent dimmers. The tandem-repeat Galectins have two CRD within their polypeptide chain. The only chimera-type galectin, galectin-3, carries a proline-rich collagen-like N-terminal linked to a C-terminal CRD[3]. Recent investigations have shown that metastasis of malign cells to be related to the presence of galectins on the cell surface. In the effected cells galectins are known to occur in local high concentrations[4]. Also that different cell surface protein glycosylation patterns on different regulatory T-cells control galectin-1-bindning and subsequent T-cell apoptosis[3]. More recently it has been shown that CD8-TCR co-localization was abolished by galectin-TCR lattice formation, which conferred anergy in tumor-infiltrating CD8+ lymphocytes[3]. All these observations have led to the hypothesis that galectins are potential targets for novel anti-cancer and anti-inflammatory compounds. High affinity inhibitors have been made, for galectin-3, using thiodigaloctoside derivaties[3,4] and protein crystallography of these inhibitors in complex with galectin-3 has shown a distinctive conformation. This has been the scaffold for computer simulations on monosaccharides which is more tightly bound to the protein (figure 1) and the orientation of the substituents on the galactoside.





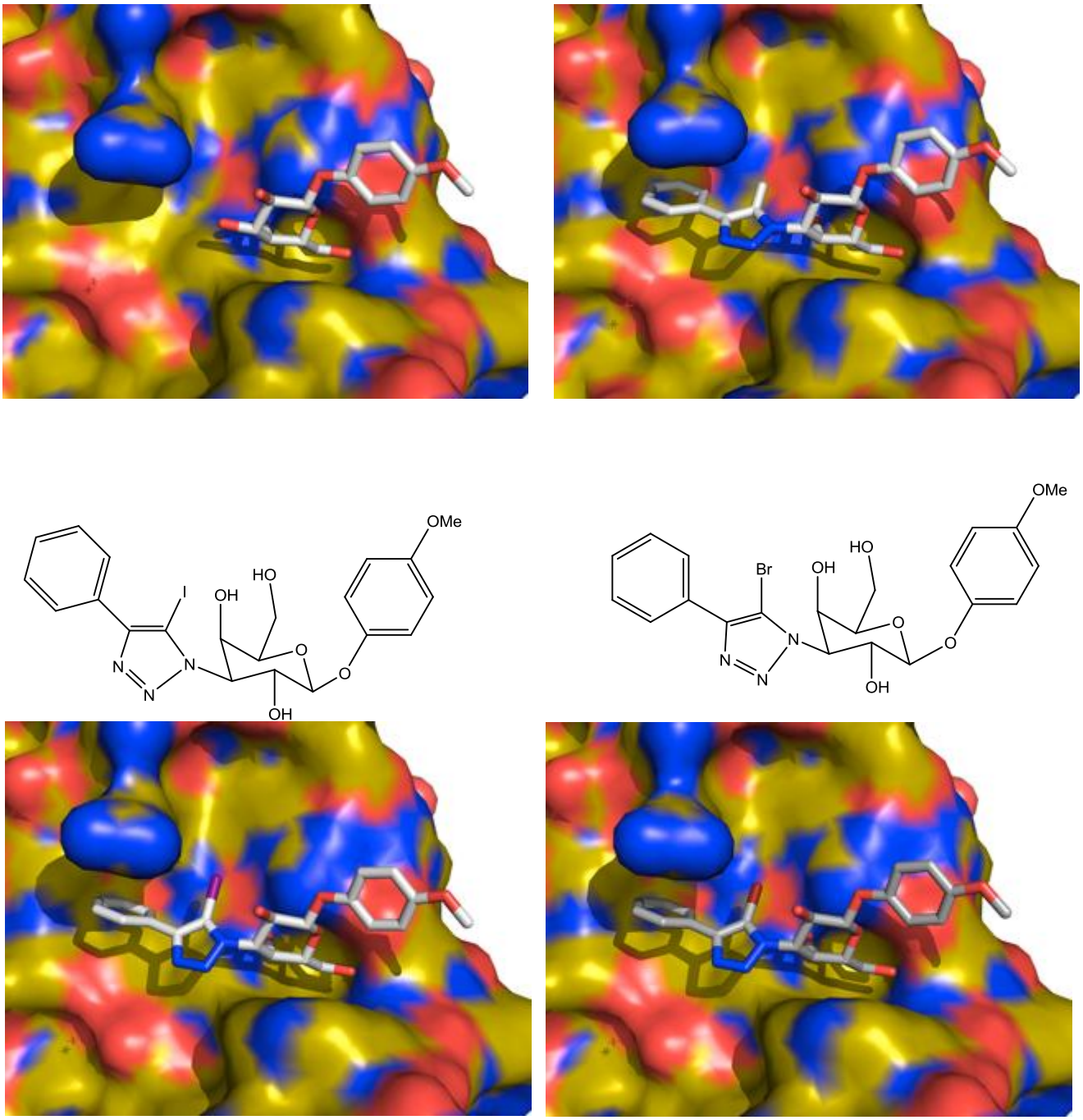


Figure 1. Computer simulations on docking with galectin-3 and tested inhibitors.

1.2 Goal of project

This project aims to find a compound which utilizes the concept of halogen bonding as means of inhibiting galectin-3. Manuscript done in the research group has shown that triazole compounds have a good affinity for inhibition if fluorine is present on the six membered ring[5]. To that end compounds analogues which have halogens on the five-membered ring has previously not been tested and computer simulations show that interaction is possible for additional inhibition (see figure 1).

1.2.1 Halogen bonding

Halogen bonding, a specific intermolecular interaction between a halogen atom and an electron rich partner (O, N, or S), has been studied extensively using computational

chemistry and has found a role in molecular engineering for material design. Recently, it has also received recognition in biological systems as a distinct class of hydrogen-bond-like interactions, holding vast potential for applications in biological engineering. Halogen bonding cannot be properly described by force field and scoring function, as halogen atoms are negatively charged as whole but positively charged at certain regions of their atomic surface. Consequently the interaction has not achieved a significant role in drug design and lead optimization. The fact that many drugs contain halogens in their molecular structure is not due to a rational computational approach but due to medicinal chemists past experience[6].
To this end this thesis wanted to try if halogen bonding in different positions could be utilized in a research project concerning inhibition of galectins.

1.3 Competitive fluorescence polarization assay

To evaluate the inhibition of galectin-3 effect of synthesized molecules *in vitro*, the competitive fluorescence polarization assay was used. Fluorescence polarization assay uses polarized light to detect if a molecule binds to receptor. A fluorophore whose absorption vector is aligned with polarized excitation light is selectively excited. If the fluorophore tumbles rapidly relative to its fluorescent lifetime then it will be randomly orientated prior to light emission and therefore will show a low polarization value (situation A below). However, if this fluorophore's rotation is slowed down so that it tumbles slowly with respect to the fluorescent lifetime (e.g. by binding to a large receptor as shown in B below) it will not rotate much before light emission and will show a high polarization value (see figure 1)[7]. From this *in situ* assay, a value for K_d (μM) to each galectin can be calculated from the polarization value. This has shown to work for the attended galectins[8]

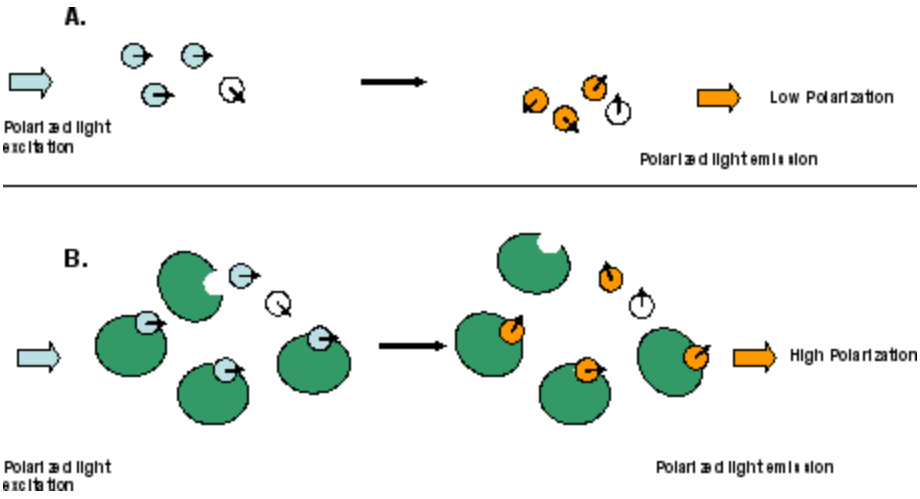


Figure 2. Principle of fluorescence polarization assay[7].

2. Results and discussion

2.1 Syntheses

The important goal of these compounds is not the yield, but rather the purity and activity. Accordingly, no extra work was done to optimize yields. In order to have enough

starting material for the novel research part previously done reactions in the research group were done.

2.1.1 Triazoles on β-D-galactose

Acetyl-protected starting material was made from 4,6-O-benzylidene-4-methoxyphenyl-β-D-galactopyranoside **4** by introducing the triflate group (at C3), protecting the oxygen with an acetate forming **5**. As the triflate is a good leaving group **6** is formed with tetrabutylammonium nitrite and inversion of the stereochemistry occurs on C3 (figure 3).

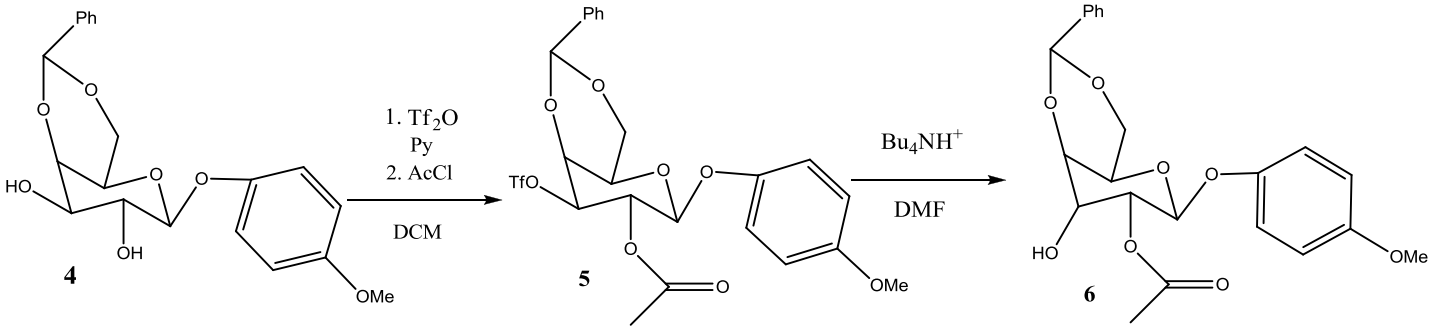


Figure 3. Introducing the triflate group to form **5** (99%) and the subsequent removal of the triflate group **6** (45%).

The triflate group is once again introduced and acts as good leaving group for the reaction, a second inversion of stereochemistry, with the azide to form **7**. This can then react, called click- chemistry[9], with a desired ethynyl along with catalytically amount of copper iodine and base to form **8** (figure 4).

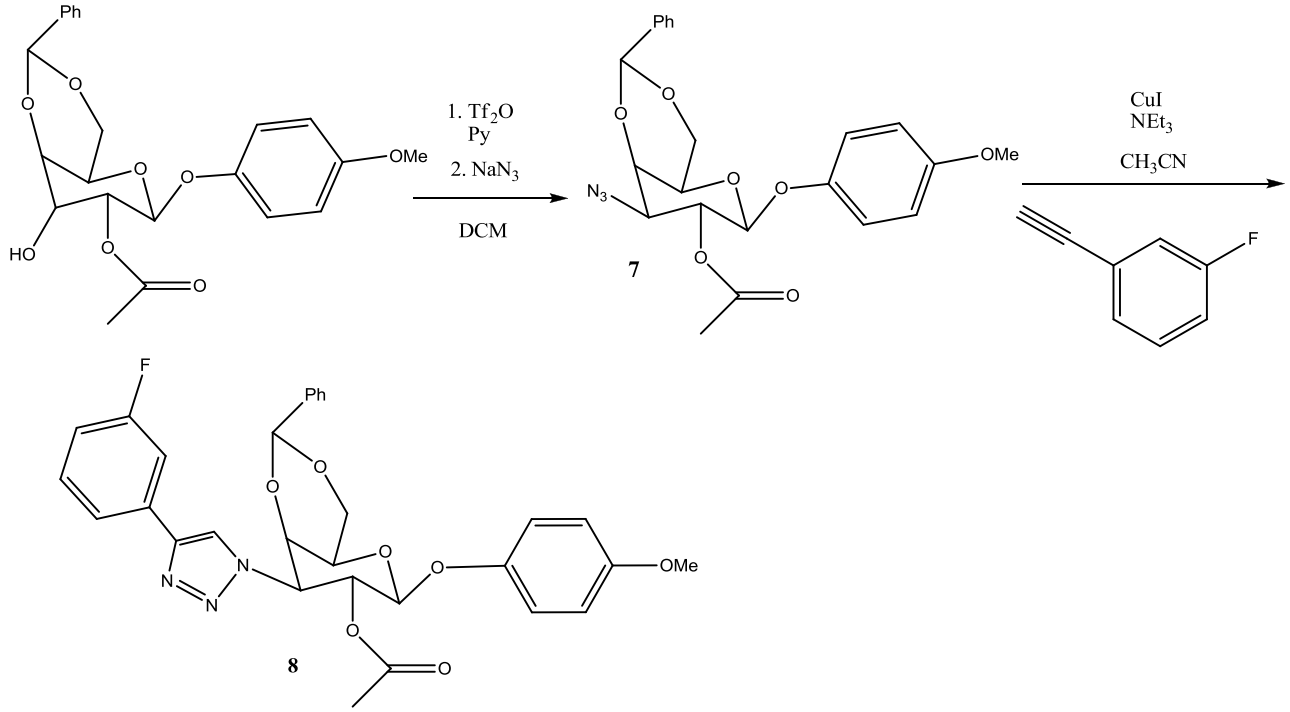


Figure 4. Nucleophilic attack from the azide to form **7** (40%) and then a so-called click reaction forming **8** (80%).

Again the azide product **7** was used to form triazole product with iodine on the five membered ring **10**. This reaction also caused a formation of a by-product with hydrogen instead of the iodine (figure 5).

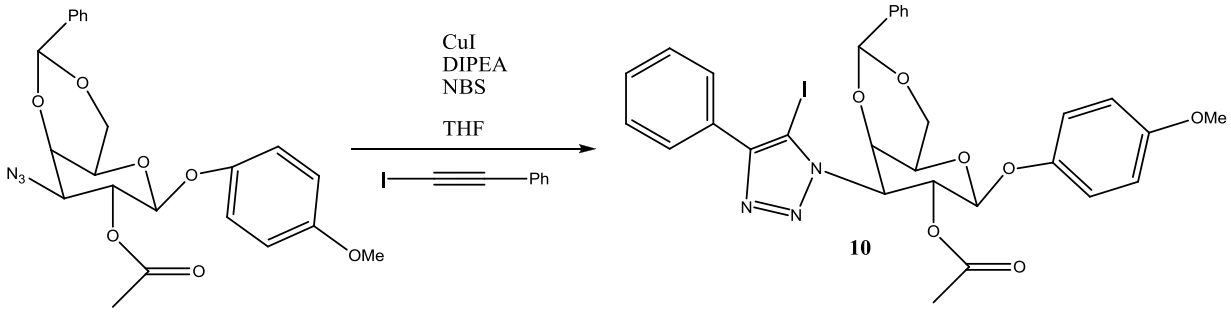


Figure 5. Copper catalyzed click reaction using (iodoethynyl)benzene to form **10** (42%).

The final triazole formed **13** was first tested by using tetrahydrofuran (THF) as solvent but did not work. Instead when using acetonitrile the reaction worked well giving quantatively yield. This was probably due to problems dissolving the copper bromide with the THF.

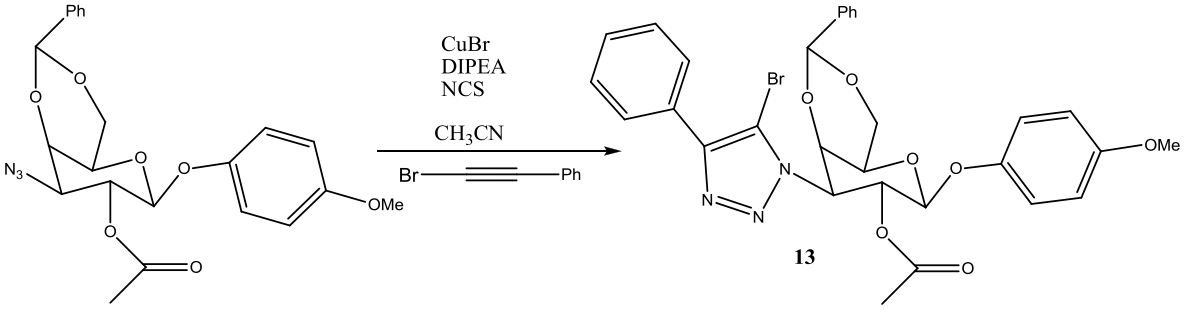
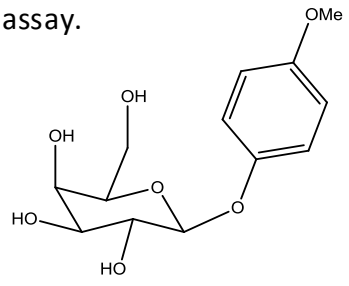
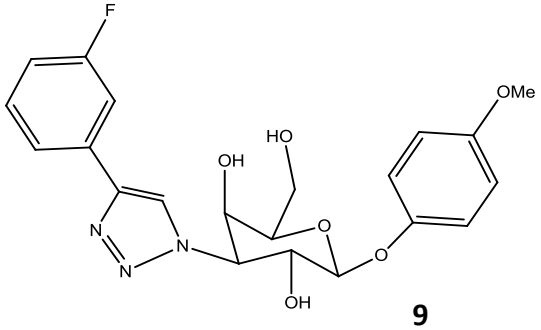


Figure 6. Copper catalyzed click reaction using (bromoethynyl)benzene to form **13** (quantative yield).

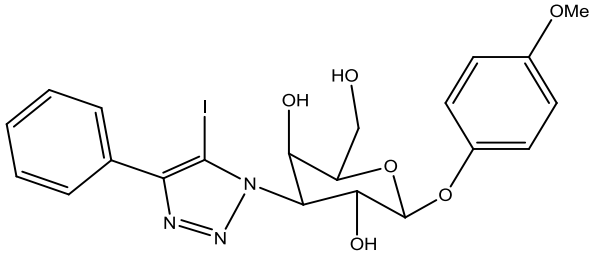
Finally the four triazole compounds underwent deprotection and deacetylation reactions to form **9** (75%), **11** (55%), **12** (45%) and **14** (82%). After HPLC-purification, the four compounds along with **3** was evaluated in vitro as galectin-3 inhibitors in a fluorescence polarization assay.



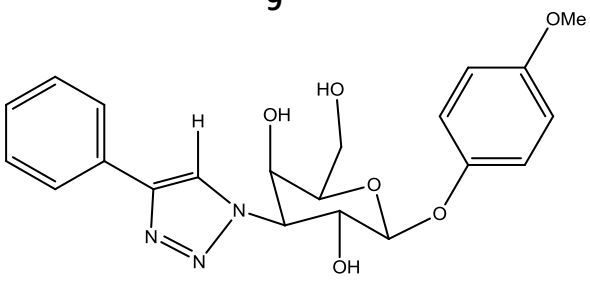
3



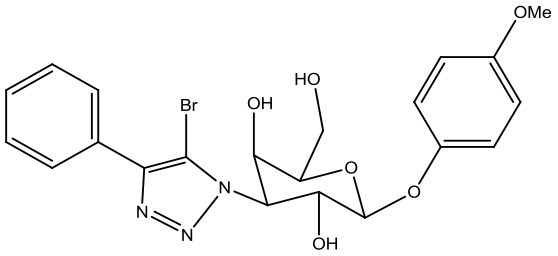
9



11



12



14

Figure 7. All compounds tested for biological activity

2.1.3 $ZnBr_2$ cleavage of triazole

As part of the continuation of the project new synthetic routes for making disaccharides were explored. There was in the research group of interest to cleave protection groups off at C1, C5 and C6 and to introduce a bromine atom at C1 and two acetates at C5 and C6 in one reaction step. It has been previously described in the literature[10] but not on the triazole **8** in question. This reaction did not work (figure 12) which would indicate that the mechanism of the cleavage is hindered in some way.

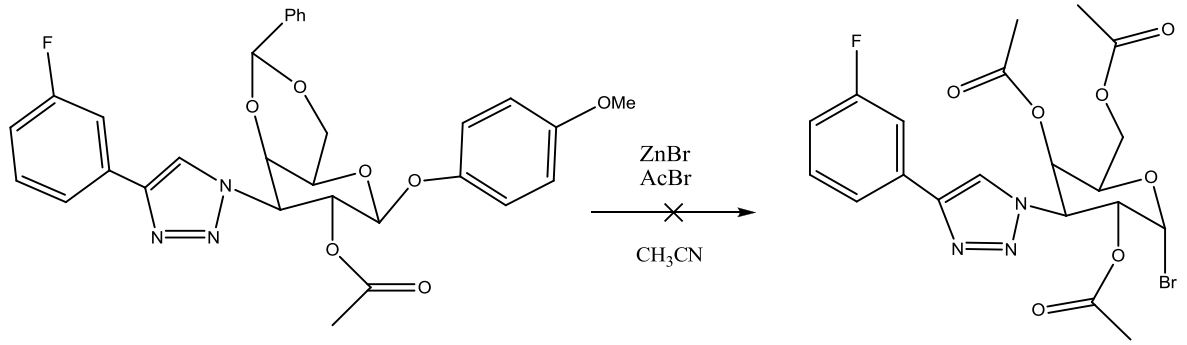


Figure 8. Zinc bromide assisted cleavage of triazole galactoside.

As the triazole might be the cause of the non working cleavage, the precursor **7** was tested to be cleaved with the $ZnBr_2$ (figure 13). This seemed to work, which would indicate that the reaction is sensitive to steric hindrance by the bulky triazole group or its electronic effects on the galactoside ring.

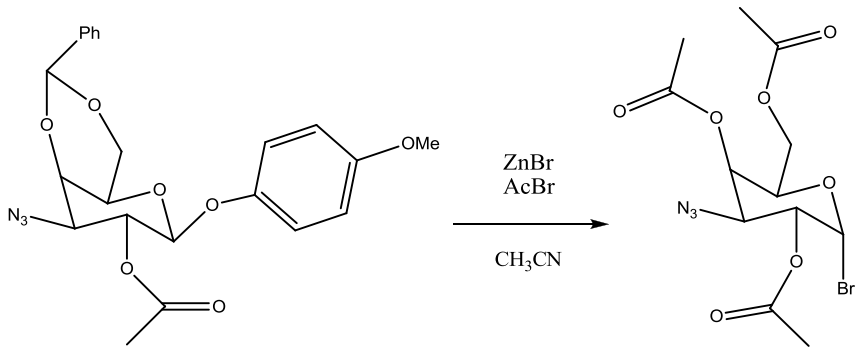


Figure 9. Zinc bromide assisted cleavage of azide galactoside.

To our satisfaction and in agreement of previously done work and computer simulations, compound **9** shows high affinity for galectin-3 but compounds **11** and **14** shows a lower affinity for binding indicating that the iodo/bromo halogen interaction wanted seems lacking. The bromine compound **14** has also a much lower affinity than iodine compound **11**

indicating that there might be a different binding position of the **14** compound than the similar **11**. One might understand if the situation was reversed since compound **11** with the iodine is much bigger (electron cloud) than bromine and therefore might not "fit" into the binding pocket but this is not seen.

Table 1. Fluorscence polarization assay calculations for tested compounds, K_D is the measure, by which, the affinity for binding to the galectins is shown (STD= standard deviation)

	Galectin-3 inhibitor K_D (µM ± STD)	Galectin-1 inhibitor K_D (µM ± STD)
Compound **3**	3719±450	3509±194
Compound **9**	24±4	184±74
Compound **11**	450±175	582±82
Compound **12**	249±170	250±27
Compound **14**	7911±241	3513±291

3. Conclusions and future work

Compound **9** definitely shows that monosaccharides can have low affinity for galectin-3, but is still a long way from the affinity of disaccharides. This has also shown that halogens on the five-memered ring does not improve affinity but lowers it. The binding affinity for bromine is much lower than the corresponding compound with iodine, this might be a halogen bond formed but due to steric hindrance the affinity is still low. The higher affinity for the iodine product might also be due to possible a degradation of the product to hydrogen instead. Galectin-4C, -4N, -7, -8N, -9N, -9 was also tested but the affinity was much lower than that of the above data for the tested compounds. This is in accordance with other work previously done on the dissacharides and similar monosaccharides.

Overall, most synthetic steps have been simple and given good yields. Room for improvements in the click reactions is possible, for example to change solvent and/or other conditions. Furthermore, it would have been interesting to explore a compound which has the fluorine on the phenyl ring like compound **9** and an additional halogen on the triazole ring. Also to explore if a halogen on the triazole ring improves the affinity for a disaccharide.

4. Experimental

4.1 Synthesis

Dry solvents were dried on molecular sieve unless otherwise stated. Flash chromatography was performed on silica gel (Davisil 35-70 µm, 60Å). HPLC purification was made by RF-HPLC (Beckman, system gold).

^{1}H NMR were done on Bruker Ultra Shield 400+, 400 MHz using residual $CHCl_3$ (7.26 ppm) or CD_3OD (3.31 ppm) for calibration. NMR spectra are reported as follows (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz; integration. LRMS and HRMS were done on Micromass Q-tof micro

Reactions were monitored with thin layer chromatography (TLC), SiO_2 on aluminum plates, and visualization was done with ultra violet (UV) light or H_2SO_4/EtOH.

All reactions were done in a round bottom flask equipped with stirring bar unless otherwise specified. All tested compounds were after final purification, purified once more by preparative-HPLC.

4.1.1 β-D-galactose pentaacetate (1)

Acetic anhydride (400 ml, 3.62 mol) was mixed with sodium acetate (80.0 g, 0.97 mol) in an E-flask. The mixture was allowed to heat (~100°C) to reflux under one hour (solution was not dissolved, brown color) under heavy convection. To the solution an addition of D-galactose (100.0 g, 0.55 mol) was done in small portions. The solution became even darker brown but dissolved. After about one hour it was taken of heating and when it cooled a precipitation formed, the solution was then poured on crushed ice. The whole slurry was filtered and washed with water (200ml × 10). Purification was made by recrystallization (MeOH:H_2O, 2:1). The product was filtered and dried in vacuo. Product yield was quantitatively.

4.1.2 4-methoxyphenyl-β-D-galactose, tetraacetate (2)

β-D-galactose pentaacetate **1** (50.0 g, 0.13 mol) and 4-methoxyphenol (19.1 g, 0.15 mol) was dissolved in dry dichloromethane (150 ml) under nitrogen atmosphere. The solution was stirred and put on ice bath at 0°C. Boron trifluoride etherate (23.6 ml, 0.19 mol) was added dropwise and reaction was allowed to stir for three hours at 0°C. Reaction was stopped by washing with water and then subsequently with sodium bicarbonate (aq, saturated). Water phases were washed with dichloromethane and all organic phases were combined and dried with magnesium sulphate. No further purification was needed. The solution was concentrated in vacuo and formed crystals (white) were dried in vacuo. Product yield was quantitative. LRMS *m/z* (relative intensity) 477.0 (MNa^+,15).

4.1.3 4-methoxyphenyl-β-D-galactose (3)

4-methoxyphenyl-β-D-galactose, tetraacetate **2** (50.0 g, 0.11 mol) was dissolved in 500 ml of methanol under convection. Sodium methoxide was added until solution was basic and the reaction was allowed to run overnight (18 hours). The solution was concentrated in vacuo and the formed crystals were washed with dichloromethane to yield **3** (33.5 g, quantitative yield). Product was used as reference for fluorescence polarization assay, therefore HPLC purification was done.

^{1}H NMR (400 MHz, CD_3OD), δ (ppm); 3.56(dd, *J* = 4.5, 3.5 Hz 1H), 3.62-3.65(m, 1H), 4.14-4.09(m, 2H), 4.37(dd, *J* = 11.0, 1.0 Hz, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 5.57 (s, 1H), 6.83 (d, *J* = 9.0, 2H), 7.06 (d, *J* = 9.0 Hz, 2H), 7.37 (d, *J* =5.0, 3H), 7.50-7.53 (m, 2H); LRMS *m/z* (relative intensity) 309.1167 (MNa^+,100).

4.1.4 4,6-O-benzylidene-4-methoxyphenyl-β-D-galactopyranoside (4)

4-methoxyphenyl-β-D-galactose **3** (41.2 g, 0.144 mol) was mixed with acetonitrile (600 ml) under N_2 atmosphere with convection. Benzaldehyd-dimethylace1al (27.7 ml, 0.187 mol) was added along with catalytically amount of 10-camphorsulfonic acid (solution is acidic and becomes briefly dissolved). The reaction was allowed to run for one and half hours and was stopped by adding triethylamine (until solution was basic/neutral). The solution was concentrated in vacuo and formed crystals were dried in vacuo. Purification was made by recrystallization in Ethylacetate/n-heptane at 60°C. The white precipitation was filtered and dried in vacuo to yield **4** (32.96 g, 61 %).
^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm); 3.58(s, 1H), 3.75-3.80(m, 4H), 4.00(t, *J* = 8.0 Hz, 1H), 4.09-4.14(m, 2H), 3.74-3.79(m, 6H), 3.90 (d, *J* = 8.0 Hz, 1H), 4.73 (d, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 7.5 Hz, 2H), 7.07 (d, *J* = 7.0 Hz, 2H); LRMS *m/z* (relative intensity) 397.1 (MNa^+,100).

4.1.5 2-O-acetyl-3-trifluoromethylsulfonyloxy-3-deoxy-4,6-O-benzylidene-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (5)

4 (0.500 g, 1.335 mmol) was stirred with dry dichloromethane (75 ml) along with pyridine (0.43 ml, 5.34 mmol) giving a slightly dissolved solution under N_2 atmosphere. The solution was allowed to cool to -10° C. Triflouromethanesulfonic anhydride (0.24 ml, 1.40 mmol) was slowly added dropwise to the solution (completely dissolved after addition) under heavy convection. The reaction was allowed to run until the starting material was consumed (1.5 hour) and then acetyl chloride (0.19 ml, 2.67 mmol) was slowly added under icebath. Reaction was stopped (two hours) by washing with diluted hydrochloric acid (100 ml × 2, 5 %) and subsequently with sodium bicarbonate (100 ml × 2, saturated aq). Water phases were washed with dichloromethane (400 ml) and all organic phases were combined and dried with magnesium sulphate. No further purification was needed. Solution was concentrated in vacuo and the yellow crude was dried in vacuo to yield **5** (0.73 g, 99%).
HRMS *m/z* (relative intensity) 571.0879 (MNa^+,100).

4.1.6 (4aR,6S,7R,8R,8aR)-8-hydroxy-6-(4-methoxyphenoxy)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl acetate (6)

5 (0.73 g, 1.33 mmol) was dissolved in dry dimethylformamide (50 ml) along with tetrabutylammonium nitrite (0.77 g, 2.67 mmol) under N_2 atmosphere. The amber/brown solution was heated to 50°C and was allowed to react for 18 hours. The residue was flash chromatographed (SiO_2, Ethyl acetate: n-heptane (1:1)) to yield **6** (0.49 g, 88 %) as crystals. Some impurities still remained, so further purification was made by recrystallization in Ethylacetate/n-heptane at 60°C. The white precipitation was filtered and dried in vacuo to yield **6** (0.25 g, 45 %).
^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm); 2.15 (s, 3H), 3.77(s, 3H), 3.95(s, 1H), 4.12(s, 1H), 4.09(d, *J* = 2.0 Hz, 1H), 4.31(s, 1H), 4.38 (d, *J* = 11.0 Hz, 1H), 5.34-5.40 (m, 2H), 5.57(s, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 7.37 (d, *J* =4.0 Hz, 3H), 7.52 (d, *J* = 7.0 Hz, 2H); HRMS *m/z* (relative intensity) 439.1353 (MNa^+,100).

4.1.7 2-O-acetyl-3-azido-3-deoxy-4,6-O-benzylidene-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (7)

6 (0.15 g, 0.36 mmol) was dissolved in dry dichloromethane (10 ml, slight yellow) along with pyridine (0.15 ml, 1.80 mmol) under N_2 atmosphere. The solution was put on icebath and allowed to cool to 0°C. Triflouromethanesulfonic anhydride (0.07 ml, 0.43 mmol) was slowly added dropwise to the solution. Reaction was allowed to heat to room temperature and the reaction was allowed to run until starting material was consumed (3 hour). Reaction was stopped by washing with diluted hydrochloric acid (50 ml × 2, 5 %). The water phase was washed with dichloromethane and all organic phases were combined and dried in magnesium sulphate. Solution was concentrated and dried in vacuo. The product was dissolved in dry dimethylformamide (10 ml) under N_2 atmosphere along with sodium azide (0.12 g, 1.80 mmol). The reaction mixture was heated to 50°C and was allowed to react for 19 hours. The formed solid was dissolved in dichloromethane (10 ml). The solution was washed with water (20 ml × 3) and the water phase was washed with dichloromethane (10 ml × 2). All organic phases were combined and dried with magnesium sulphate. The solution was concentrated and dried in vacuo. Purification was made by recrystallization in Ethylacetate/n-heptane. The brown precipitation was filtered and dried in vacuo to yield **7** (0.06 g, 40 %).
^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm); 2.16 (s, 3H), 3.39(dd, *J* = 7.0 Hz, 3.0 Hz, 1H), 3.76(s, 1H), 3.78(s, 4H), 3.79 (s, 1H), 4.13(dd, *J* = 11.0, 2.0 Hz, 1H), 4.37 (s, 1H), 4.40 (s, 1H), 4.94 (d, *J* = 4.0 Hz, 1H),5.59 (d, *J* = 11.0 Hz, 1H), 5.63 (s, 1H), 6.81 (d, *J* = 7.0 Hz, 2H), 6.99 (d, *J* = 9.0 Hz, 2H), 7.36 (d, *J* = 4.0 Hz, 3H), 7.53 (d, *J* = 7.0 Hz, 2H); HRMS *m/z* (relative intensity) 464.1423 (MNa^+,100).

4.1.8 2-O-acetyl-3-(4-(3-fluorophenyl)-1H-1,2,3-triazol-1-yl)-3-deoxy-4,6-O-benzylidene-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (8)

7 (0.500 g, 1.13 mmol) was dissolved in dry acetonitrile (25 ml) under N_2 atmosphere. To the solution copper (I) iodine (0.022g, 0.113 mmol) was added along with triethylamine (0.19 ml, 1.36 mmol) and the solution was stirred for 10 minutes. Finally 1-ethynyl-3-flourobenzene (0.131 ml, 1.113 mmol) was added (yellow precipitation formed initially then amber solution). The reaction was allowed to run for 5.5 hours and was then concentrated and dried in vacuo. The yellow crude was dissolved in dichloromethane and washed with water (30 ml × 3). Water phases were washed with dichloromethane (40 ml × 2) and all organic phases were combined and dried with magnesium sulphate. The solution was concentrated and dried in vacuo. Purification was made by recrystallization in dichloromethane/n-heptane. The brown precipitation was filtered and dried in vacuo to yield **8** (0.51 g, 80 %).
^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm); 1.16 (s, 3H), 3.76(s, 3H), 3.84(s, 1H), 4.15(dd, *J* = 11.0, 2.0 Hz, 1H), 4.60 (s, 1H), 4.49 (s, 1H), 5.21 (d, *J* = 7.5 Hz, 1H),5.25 (dd, *J* = 8.0, 3.0 Hz, 1H), 5.51 (s, 1H), 5.90 (dd, *J* = 11.0, 3.0 Hz, 1H), 6.83 (d, *J* = 7.0 Hz, 2H), 7.03 (d, *J* = 9.0 Hz, 3H), 7.34-7.53(m, 8H), 8.09(s, 1H); HRMS *m/z* (relative intensity) 562.2009 (MH^+,100).

4.1.9 2-O-acetyl-3-(4-(3-fluorophenyl)-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (9)

8 (0.05 g, 0.089 mmol) and acetic acid (80%, 10 ml) was stirred at 90°C. The reaction was allowed to run 3 hours. The solution was concentrated and dried in vacuo. The crude was continued on deacetylation and no further purification was made. HRMS *m/z* (relative intensity) 474.1692 (MH^+,100).

4.1.10 3-(4-(3-fluorophenyl)-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (10)

9 was dissolved with methanol (5 ml) through heavy convection and sodium methoxide was added until solution was basic (pH 8-9). The reaction was allowed to run for 72 hours and was stopped by adding Amberlite 15 to the reaction mixture until pH was neutral/acidic. The Amberlite was filtered off and washed with methanol (10 ml × 3) and dichloromethane (10 ml). Solution was concentrated and dried in vacuo. The crude was purified by flash chromatography (SiO_2, Dichloromethane: methanol (30:1)) to yield 0.029 g (75%).
^{1}H NMR (400 MHz, CD_3OD), δ (ppm); 3.76 (m, 5H), 3.93 (t, *J* = 5.5 Hz, 1H), 4.14 (d, *J* = 2.5 Hz, 1H), 4.45 (dd, *J* = 7.5, 3.5 Hz, 1H), 5.01 (d, *J* = 7.5 Hz, 2H), 6.85 (d, *J* = 6.5 Hz, 2H), 7.10 (d, *J* = 9.0 Hz, 3H), 7.45 (q, *J* = 8.0, 6.0 Hz, 1H), 7.62 (d, *J* = 9.0 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 8.52(s, 1H); HRMS *m/z* (relative intensity) 432.0990 (MH^+,100)

4.1.11 2-O-acetyl-3-(5-iodo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-4,6-O-benzylidene-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (11)

Copper (I) iodine (0.021g, 0.113 mmol) and *N*,*N*-diisopropylethylamine (0.019 ml, 0.113 mmol) was mixed with dry tetrahydrofuran (3 ml) under nitrogen atmosphere. The solution (brown) was stirred for 10 minutes and subsequently **7** (0.05 g, 0.113 mmol) was added to the solution along with (iodoethynyl)benzene (0.026 g, 0.113 mmol) dissolved in 2 ml tetrahydrofuran and *N*-bromosuccinimide (0.022 g, 0.124 mmol). The reaction was allowed to run for 23 hours and was stopped by evaporating the tetrahydrofuran and subsequently dried in vacuo. The brown crude was dissolved in dichloromethane (10 ml) and washed with water (10 ml × 2) and subsequently with sodium chloride (20 ml × 2, saturated aq). Water phases were washed with dichloromethane (10 ml × 2) and all organic phases were combined and dried with magnesium sulphate. Solution was concentrated in vacuo and the crude was dried in vacuo. Purification was made by recrystallization in dichloromethane/n-heptane. The brown precipitation was filtered and dried in vacuo (0.049 g, 64%). NMR shows that by-product (see appendix 1) had formed, flash chromatography (SiO_2, Dichloromethane: methanol (50:1)) was used to separate the two similar compounds, which failed, removing some impurities (0.032 g, 42 %). The product mixture was instead continued to deprotection/deacetylation reactions. HRMS *m/z* (relative intensity) 670.1063 (MH^+,100).

4.1.12 2-O-acetyl-3-(5-iodo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (12)
(2-O-acetyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (13)

11 (product mixture) (0.032 g, 0.048 mmol) and acetic acid (80%, 10 ml) was stirred at 90°C. The reaction was allowed to run one hour. Solution was concentrated in vacuo and dried in vacuo (0.03 g). Flash chromatography (SiO_2, Dichloromethane: methanol (20:1)) was used again to separate the two compounds, partial separation was achieved. The two compounds were obtained in 0.016 g (**12**, 57%) and 0.010 g (**13**, 46%).

4.1.13 3-(5-iodo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (14)
3-(4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (15)

12 and **13** were mixed separately with methanol (5 ml). After dissolving through heavy convection sodium methoxide was added until solution was basic (pH 8-9). The reaction was allowed to run for 18 hours and the reaction was stopped by adding Amberlite 15 to the reaction mixture until pH was neutral/acidic. The Amberlite was filtered off and washed with methanol (10 ml × 3) and dichloromethane (10 ml). The solutions were concentrated and dried in vacuo to yield **14** (0.014 g, 55%) and **15** (0.009 g, 45%)
14 ^{1}H NMR (400 MHz, CD_3OD), δ (ppm); 3.35 (s, 1H), 3.81 (m, 5H), 3.98 (t, *J* = 6.5 Hz, 1H), 4.21 (d, *J* = 2.5 Hz, 1H), 4.82 (m, 1H), 5.00 (m, 2H), 5.02 (d, *J* = 7.5 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 2H), 7.13 (d, *J* = 9.0 Hz, 2H), 7.42 (m, 1H), 7.46 (t, *J* = 10.0 Hz, 2H), 7.86 (d, *J* = 7.0 Hz, 2H); HRMS *m/z* (relative intensity) 540.0631 (MH^+,100).
15 ^{1}H NMR (400 MHz, CD_3OD), δ (ppm); 3.80 (m, 5H), 3.94 (t, *J* = 7.0 Hz, 1H), 4.15 (d, *J* = 2.5 Hz, 1H), 4.45 (dd, *J* = 7.5, 3.5 Hz, 1H), 5.01 (d, *J* = 7.5 Hz, 2H), 6.87 (d, *J* = 6.5 Hz, 2H), 7.11 (d, *J* = 9.0 Hz, 2H), 7.35 (m, 1H), 7.46 (t, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.0 Hz, 2H), 8.45 (s, 1H); HRMS *m/z* (relative intensity) 414.1686 (MH^+,100).

4.1.14 2-O-acetyl-3-(5-bromo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-4,6-O-benzylidene-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (13)

Copper (I) bromide (0.049 g, 0.339 mmol) along with *N*,*N*-Diisopropylethylamine (0.056 ml, 0.339 mmol) and *N*-chlorosuccinimide (0.045 g, 0.339 mmol) was mixed with acetonitrile (2.5 ml) under nitrogen atmosphere. The solution (brown/green) was stirred for 10 minutes and subsequently **7** (0.05 g, 0.113 mmol) was added to the solution along with (bromoethynyl)benzene (0.020 ml, 0.113 mmol) dissolved in 2.5 ml acetonitrile. The reaction was allowed to run for 48 hours (additional (bromoethynyl)benzene was added after 18 hours (0.007 ml, 0.056 mmol)) and was concentrated and dried in vacuo. The crude was dissolved in dichloromethane (10 ml) and washed with water (10 ml × 2) and subsequently with sodium chloride (10 ml × 2, saturated aq). Water phases were washed with dichloromethane (10 ml × 3) and all organic phases were combined and dried with magnesium sulphate. Solution was concentrated and dried in vacuo, purification was made by flash chromatography (SiO_2, Ethyl acetate: n-heptane (1:1)) to yield **13** (0.079 g, 112%).
^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm); 1.95 (s, 3H), 3.77 (s, 5H), 3.91 (s, 1H), 4.17 (d, *J* = 11.5 Hz, 1H), 4.40 (d, *J* = 12.5 Hz, 1H), 72 h, 4.49 (d, *J* = 2.5 Hz, 1H), 5.15 (d, *J* = 3.0 Hz, 1H), 5.18 (d, *J* = 8.0 Hz, 2H), 5.29 (s, 2H), 6.55 (dd, *J* = 8.0, 3.0 Hz, 1H), 6.81 (d, *J* = 9.0 Hz, 2H), 7.00 (d, *J* = 9.0 Hz, 3H), 7.23-7.46 (m, 16H), 7.53 (d, *J* = 6.0 Hz, 3H), 8.04 (d, *J* = 8.0 Hz, 2H); HRMS *m/z* (relative intensity) 622.1204 (MH^+,100).

4.1.15 2-O-acetyl-3-(5-bromo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (14)

13 (0.079 g, 0.127 mmol) and acetic acid (80%, 10 ml) and was stirred at 90°C. The reaction was allowed to run 2 hours. Solution was concentrated and dried in vacuo (0.068 g, 100%). No further purification was done and the crude was continued on deacetylation.

4.1.16 3-(5-bromo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-deoxy-1-O-(4-Methoxyphenyl)-beta-D-galactopyranoside (15)

14 was mixed with dry methanol (10 ml). After dissolving through heavy convection sodium methoxide was added until solution was basic (pH 8-9). The reaction was allowed to run for 23 hours and the reaction was stopped by adding Amberlite 15 to the reaction mixture until pH was neutral/acidic. The Amberlite was filtered off and washed with methanol (10 ml × 3) and dichloromethane (10 ml). The solution was concentrated and dried in vacuo. The crude was purified by flash chromatography (SiO_2, Dichloromethane: methanol (40:1)) to yield **15** (0.046 g, 82%).
^{1}H NMR (400 MHz, CD_3OD), δ (ppm); 3.80 (m, 5H), 3.97 (t, *J* = 6.5 Hz, 1H), 4.19 (d, *J* = 2.5 Hz, 1H), 4.84 (m, 1H), 4.95 (m, 2H), 5.06 (d, *J* = 7.5 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 2H), 7.13 (d, *J* = 9.0 Hz, 2H), 7.45 (m, 1H), 7.49 (t, *J* = 7.0 Hz, 2H), 7.92 (d, *J* = 7.0 Hz, 2H); HRMS *m/z* (relative intensity) 492.0779 (MH^+,100).

Acknowledgments

I would like to thank my supervisor Ulf Nilsson for his insights along the way.
For the help in and outside the lab Ann-Louise Noresson
In the research group, a special thanks to Vishal Rajput, Alexander
For flourscence polarization assay meausurements, a thanks to Barbro Kahl-Knutsson
Also for the high-res mass studies, Einar Nilsson

[1] Kasai K.I, Hirabayashi J, Galectins: A Family of Animal Lectins That Decipher Glycocodes, *J. Biochem.* 119 (1996), 1-8.
[2] Salameh B.A, Leffler H, Nilsson U.J, 3-(1,2,3-Triazol-1-yl)-1-thio-galactosides as small, efficient, and hydrolytically stable inhibitors of galectin-3, *Bioorg. & Med. Chem. Lett.* 15 (2005) 3344-3346
[3] Öberg C.T, Leffler H, Nilsson U.J, Inhibition of Galectins with Small Molecules, *Glycochemistry today* Chimia 65 (2011) 1–6
[4] Salameh B, Cumpstey I, Sundin A, Leffler H, Nilsson U, 1H-1,2,3-Triazol-1-yl thiodigalactoside derivatives as high affinity galectin-3 inhibitors, *Bioorg. & Med. Chem.* 18 (2010) 5367-5378.
[5] Delaine T, Collins P, Demotte N, Wieers G, Rajput V, Cumpstey I, Larumbe A, Salameh B. A, von Wachenfeldt H, Blanchard H, van der Bruggen P, Salomonsson E, Leffler H, Nilsson U. J, Small-molecule galectin inhibitors reverse anergy in tumor-infiltrating lymphocytes: Synthetic, structural, and biological studies
[6] Xu Z, Liu Z, Chen T, Chen T.T, Wang Z, Tian G, Shi J, Wang X, Lu Y, Yan X, Wang G, Jiang H, Chen K, Wang S, Xu Y, Shen J, Zhu W, Utilization of Halogen Bond in Lead Optimization: A Case Study of Rational Design of Potent Phosphodiesterase Type 5 (PDE5) Inhibitors, *J. Med. Chem.* 54 (2011) 5607-5611.
[7] Picture and summary taken from:
http://assay.nih.gov/assay/index.php/Section5:Practical_Use_of_Fluorescence_Polarization_in_Competitive_Receptor_Binding_Assays
[8] Sörme P, Kahl-Knutsson B, Hufljet M, Nilsson U.J, Leffler H, Fluorescence polarization as an analytical tool to evaluate galectin–ligand interactions, *Analytical Biochemistry* 334 (2004) 36-47.
[9] Ackermann L, Potukuchi H.K, Landsberg D, Vicente R, Copper-Catalyzed "Click" Reaction/Direct Arylation Sequence: Modular Syntheses of 1,2,3-Triazoles, *Org. Lett.* 10:14 (2008) 3081-3084.
[10] Goto K, Mizuno M, Synthesis of monosaccharide units using fluorous method, *Tetrahedon Letters* 48 (2007) 5605-5608.