Master thesis: Synthesis of fluorescent carbohydrate coumarin hybrids via a three-component reaction: nanomolar galectin-3 inhibitor

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Abstract

Galectins play an important role in inflammation, immunity, cancer progression and are potential targets for novel anti-cancer and anti-inflammatory compounds. Coumarin thiodigalacoside hydrid had proved to be potent, nanomolar, and selective inhibitor of galectin-3. Three novel coumarin thiodigalactoside derivatives have been synthesized by copper-catalyzed multicomponent reaction. Two compounds exhibited better inhibition than an earlier coumarin thiodigalactoside hybrid and possessed potentially useful fluorescence properties.

Contents

1. Introduction

1.1 Coumarins

Coumarins are widely abundant in nature and used in many applications such as medicine, fluorescent indicators, dyes in laser technology and perfumery[1]. In medicine, coumarins have shown activities against fungal, cancer and HIV. Iminocoumarins had been reported to be potential protein tyrosine kinase (PTK) inhibitors, therefore attractive target for the treatment of diseases involving excess cell proliferation and antitumor processes.

1.2 Galectins

Galectins are a family of lectins defined by having specific binding affinity for β-galactosides[2][3]. In a wide range of biological mechanisms, galectins have an important role. Examples of mechanisms that are regulated by galectin activities are intracellular trafficking, cell signaling, apoptosis and cell adhesion. These functions are closely related to inflammation, immunity, and cancer progression. Galectins are widely spread throughout the nature in plants, vertebrates, invertebrates and even in sponges and fungi. They are synthesized in the cytosol and can reach the extracellular space or lumen of vesicles by a non-classical secretory pathway. A characteristic feature of galectins which is connected with the biological activities is multiple carbohydrate recognition domains (CRD). CRD allows galectins to cross-link their glycoprotein ligands. There are three ways for galectins to cross-link which are dependent on structural features. Prototype galectins which have one CRD within their polypeptide chain to form non-covalent dimers. Tandem-repeat galectins have two CRD within their polypeptide chain. Chimera galectin and the only one is galectin-3, carries a proline-rich collagen-like N-terminal linked to a C-terminal CRD. All these observations have led to the hypothesis that galectins are potential targets for novel anti-cancer and anti-inflammatory compounds.

1.3 Goal of project

The aim of this project was to synthesize galectin-3 ligands, hydroxycoumarin (umbelliferone) thiodigalactoside hybrid and other coumarin thiodigalactoside hybrids and to test if these compounds are both fluorescent and better inhibitors than unsubstituted coumarin thiodigalactoside hybrid earlier synthesized in the research group.

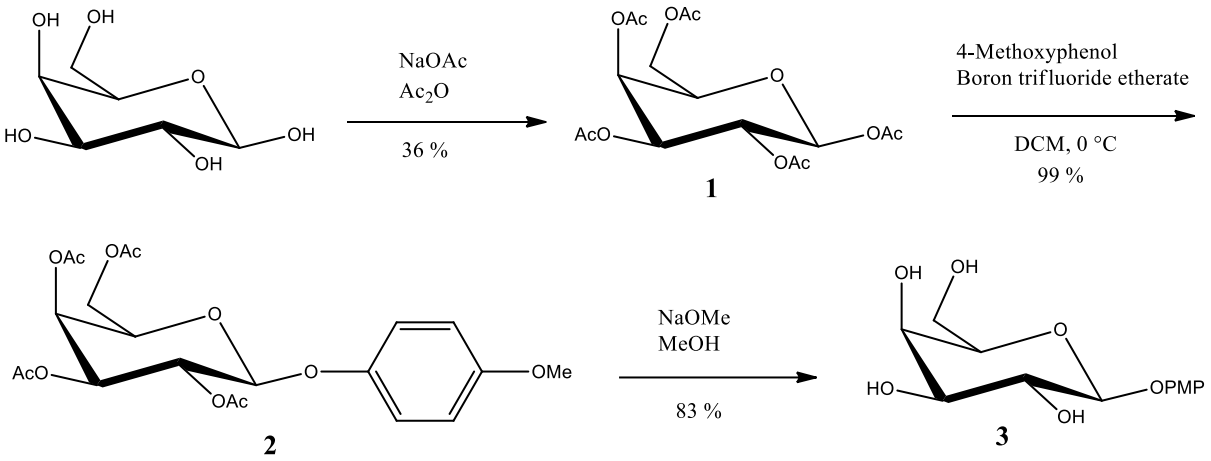
1.4 Competetive fluorescence polarization assay

To evaluate the inhibition of galectin-3 a fluorescence polarization assay[4] was used. In this method a fluorescent probe is excited with plane-polarized light and the degree of polarization remaining in the emitted light is measured. The remaining polarization decreases in relation to how much the fluorescent probe moves during the excited state. When the probe is bound to the protein it rotates slower than when free. From this measurement K_d values can be calculated.

2. Results and discussion

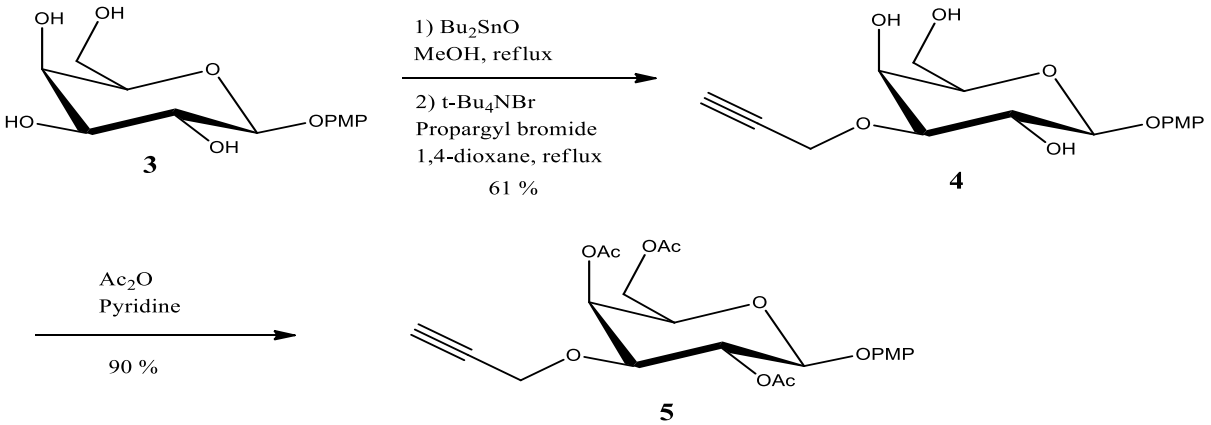
4-Methoxyphenyl β-D-galactopyranoside **3** was synthesized from D-galactose[5] (scheme 1). The first reaction was acetylation or esterification of D-galactose to form **1** and the yield was low, 36 %. After pouring the reaction solution on crushed ice, it was put in the fridge for three

nights. In water, acetic acid was formed and hydrolyzed the acetates and acid-catalyzed ester hydrolysis is reversible, but all product was not hydrolyzed. The solid had a brown color instead of white because the reaction was heated for too long time. But the product was pure according to ^{1}H-NMR. Next step was to transform the acetate at C1 to 4-methoxyphenyl to yield **2**. The reaction was done several times, sometime 1.5 equivalent boron trifluoride etherate was enough to complete the reaction. Other time the reaction was started with 1.5 equivalents and 1 more equivalent was added to complete reaction. The product and starting material had the same R_f in TLC and the reaction was analyzed by MS to see if completed. **3** was synthesized by deacetylation of **2** in base-catalyzed hydrolysis, which was irreversible. Sodium methoxide was used as a base and the reaction was stopped by ion-exchange resin.



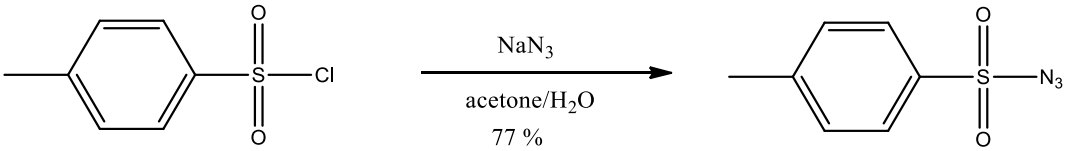
Scheme 1. Synthesis of 4-Methoxyphenyl β-D-galactopyranoside from D-galactose

Propargylation of **3** afforded product **4** (scheme 2). Two different procedures for the reaction were done, one by Zhang[6] and the other by Pera[7]. In the first article, toluene was used as solvent and 1.2 equivalent propargyl bromide. After stirring overnight at 60 °C, there was more starting material than product according to TLC. In the second paper, 1,4-dioxane was used as solvent and 5 equivalent propargyl bromide. After refluxing for 4.5 h the reaction was completed. The faster reaction may be due to higher temperature, the boiling point of 1,4-dioxane was 101 °C, and more reagent. Also the solubility of the reagents was different because toluene was not as polar as 1,4-dioxane. **4** was then acetylated in mild conditions to give **5**, scheme 2.



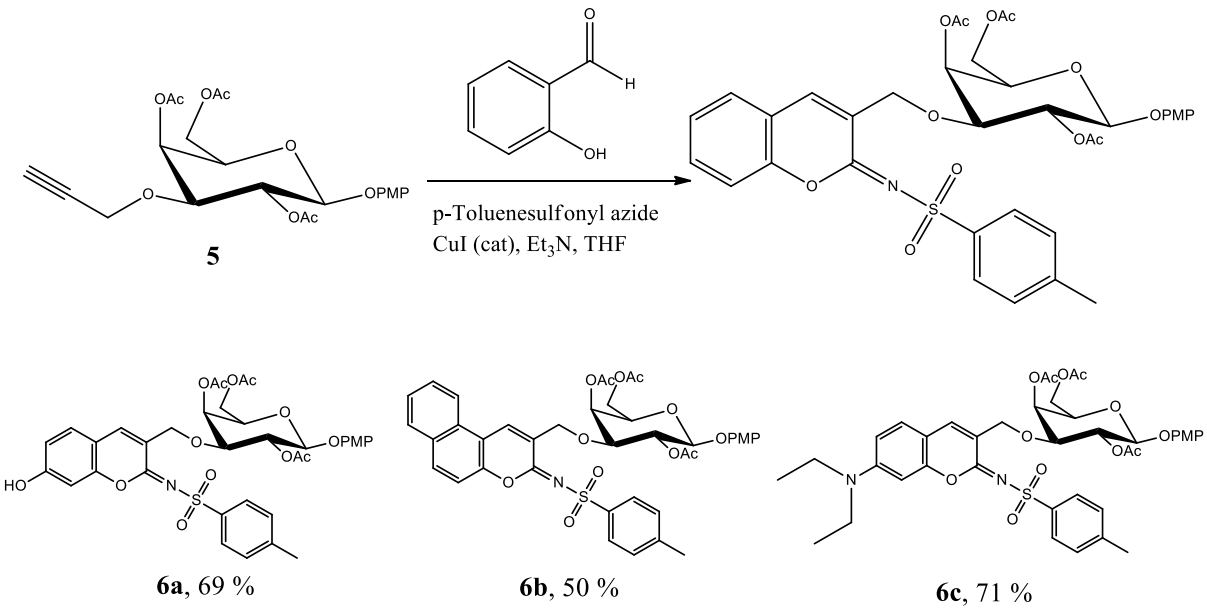
Scheme 2. Synthesis of 4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-propargyl-β-D-galactopyranoside

p-Toluenesulfonyl azide[8] (scheme 3) was synthesized and used as reagent in the synthesis of iminocoumarins via copper-catalyzed multicomponent reaction, MCR.



Scheme 3. Synthesis of *p*-Toluenesulfonyl azide

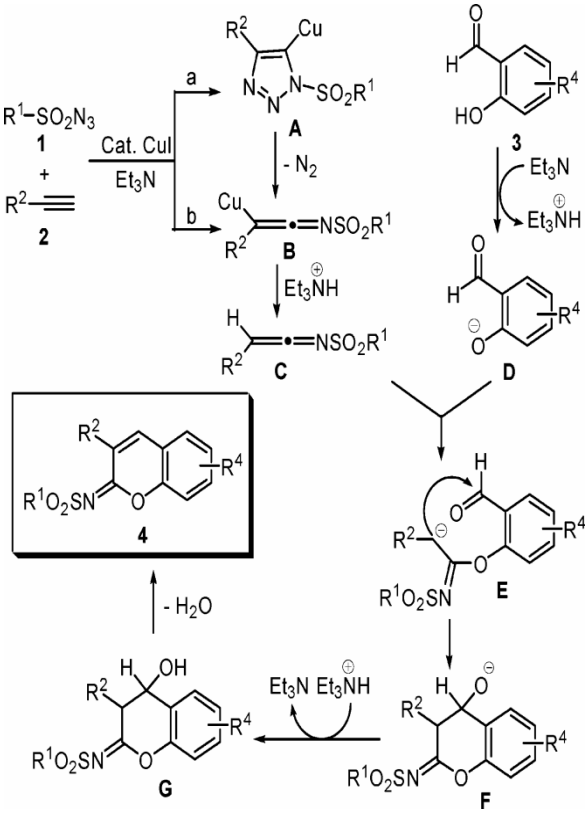
6a, **6b** and **6c** (scheme 4) were synthesized by MCRs[1]. In the synthesis of **6a**, 2,4-dihydroxybenzaldehyde was used and the product was difficult to purify. When DCM/MeOH was used in column chromatography, 2,4-dihydroxybenzaldehyde was co-eluted with the product. The impurities had to be eluted with EtOAc/Hep first, to elute the product DCM/MeOH had to be used. **6b** was synthesized easier than **6a**, because **6b** was a less polar compound than **6a** and EtOAc/Hep could be used to elute the product. In the synthesis of **6c**, the product was not formed after stirring the reaction overnight as for **6a** and **6b**. According to TLC, the starting material was the major spot, but there was a slower moving spot. It was purified and analyzed, according to MS and ^{1}H-NMR, the slower moving spot was the uncyclized molecule. For this reaction, 4-(diethylamino)salicylaldehyde was used. The electron pair of the nitrogen could be donated which would make the carbonyl carbon less electrophilic.



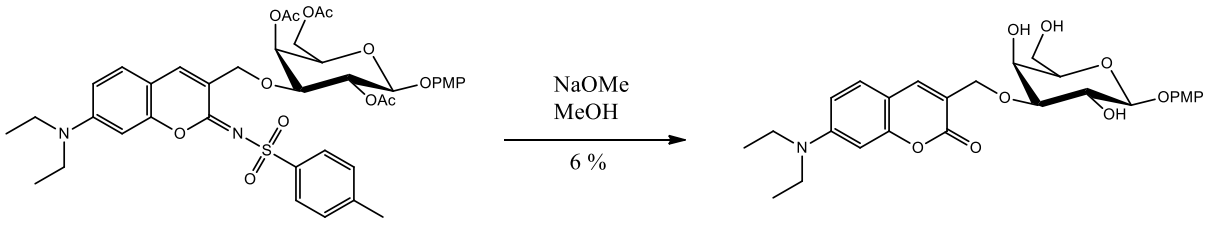
Scheme 4. General procedure for synthesis of 3-*O*-iminocoumarin

Scheme 5 showed the postulated mechanism for formation of iminocoumarins[9]. The intramolecular nucleophilic addition of the anionic intermediate E was one of critical steps for the domino process. The reaction towards **6c** was done again, but this time the reaction was stirred for six days. TLC showed no starting material left and the slower moving spot was much stronger than first time. It was purified and analyzed by MS and ^{1}H-NMR and it was the product in 71 %. The uncyclized molecule and the product had the same R_f. The reaction may have been completed earlier because the reaction was not followed day by day.

A reaction with 2,3,6,7-Tetrahydro-8-hydroxy-1H,5H-benzo[ij]quinolizine-9-carboxaldehyde was tried, but the reaction did not work. The reaction was only stirred overnight, if the reaction was stirred for longer time it may had worked, because this reagent should have similar properties as 4-(diethylamino)salicylaldehyde. A reaction using 2,5-dihydroxybenzaldehyde was done. The reaction was analyzed by MS and TLC, but the product was not purified. Next step was to remove the protective groups of **6a** by using sodium methoxide, but the reaction did not work. Under basic condition the hydroxyl could be deprotonated and elimination could occur. Deacetylation of **6b** and **6c** under basic conditions worked. The deacetylated product of **6b** was not purified, but confirmed by MS and impure ^{1}H-NMR. Deacetylation of **6c** afforded **7c** (scheme 6) and the pure product was isolated.



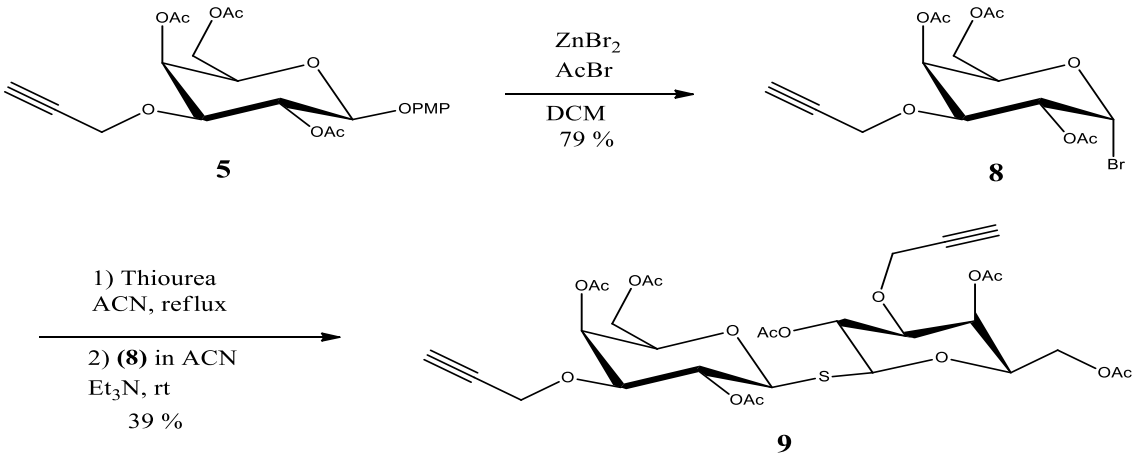
Scheme 5. Postulated Mechanism for Formation of Iminocoumarins[9]



6c **7c**

Scheme 6. Deacetylation of **6c**

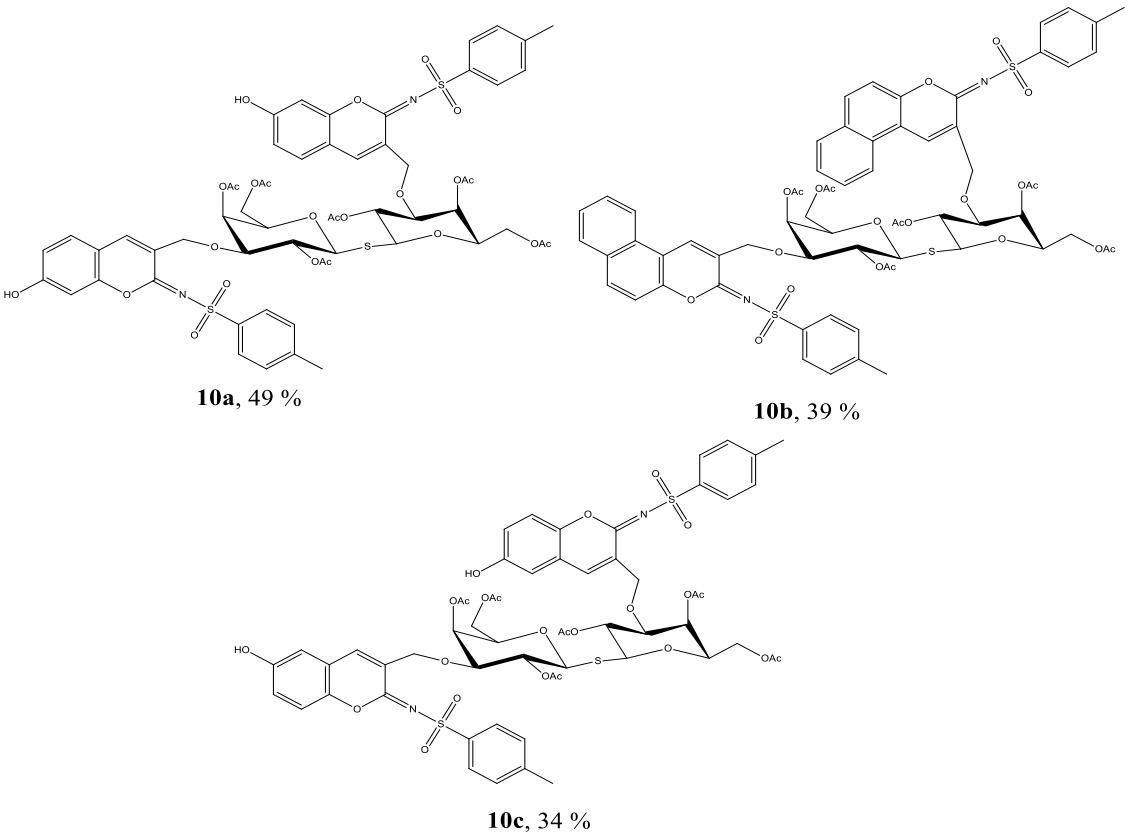
Instead of going further with monogalactoside, thiodigalactoside (tdg) was synthesized. Because tdg had shown similar binding mode and conformation as that in case of LacNAc. Attaching and fine-tuning aromatic amides to galactose C3 in LacNAc or tdg results in increase binding affinity of ligand against different galectins[1]. The synthesis of **9** was done in two steps (scheme 7) first step was conversion of 4-methoxyphenyl glycoside into corresponding glycosyl bromide[10]. Conversion of **5** afforded **8** in good yield. Next step was to convert **8** into **9**[1] and the yield was low. According to the procedure, catalytic amount of triethylamine should be enough. But 1 equivalent of triethylamine was needed for the reaction to finish.



Scheme 7. Synthesis of 3,3'-di-propargyl substituted thiodigalactoside

9 was used to synthesize **10a**, **10b** and **10c** (figure 1). In the synthesis of **10a** and **10c** the reactions were not completed after stirring overnight as for monogalactoside. Instead 72 h were needed and the purification was difficult due to that impurities were moving close to the product. The reaction to synthesize **10b** was cleaner because only one slower moving spot was observed.

The reactions of tdg with aminosalicylaldehyde, 4-(diethylamino)salicylaldehyde and 2,3,6,7-Tetrahydro-8-hydroxy-1H,5H-benzo[ij]quinolizine-9-carboxaldehyde, did not work. The reactions were stirred for seven days, heated at 50 °C for two days and even refluxed. But no product could be observed by MS and TLC. This problem may be solved by first synthesize iminocoumarin monogalactoside via MCR, and then do the thiodigalactoside coupling reaction.



10a, 49 %

10b, 39 %

10c, 34 %

Figure 1. 3,3'-di-substituted iminocoumarin thiodigalactosides

The final step was deacetylation of **10a**, **10b** and **10c** to afford products **11a**, **11b** and **11c** (figure 2). **10b** was deacetylated with sodium methoxide. As mentioned before basic conditions did not work with compounds with base-sensitive groups. **10a** and **10c** were deacetylated by acetyl chloride in methanol[11][12]. When catalytic amount, 0.50 mol%, of acetyl chloride was used, the reactions did not go forward. Acetyl chloride had to be in excess, at least 100 equivalent was used and this may be due to imine was stable against acid. The reaction was slow and excess of reagent was used to push the reaction towards formation of the product. The reactions were stopped by evaporation at room temperature. Before evaporation only one spot was observed on TLC and after evaporation there were more spots. Which can be explained by that acid-catalyzed ester hydrolysis is reversible and therefore the low yields. Different methods for work-up were tried, but without success. The reactions were washed with saturated $NaHCO_3$ solution and brine. Triethylamine and pyridine were used to neutralize the acid. Even ion-exchange resin, Amberlite IRA-400 (OH), was used but with no success.

Because of the low yields alternative methods for deacetylation were tried, the first one was using dibutyltin oxide[13]. Deacetylation of **6b** using dibutyltin oxide worked, the product was not purified, but instead compared with the MS and TLC of deacetylation of **6b** with sodium methoxide. When the reaction was tried with **10c**, it did not work. Another method was using methylamine in ethanol[3][14], but with no success.

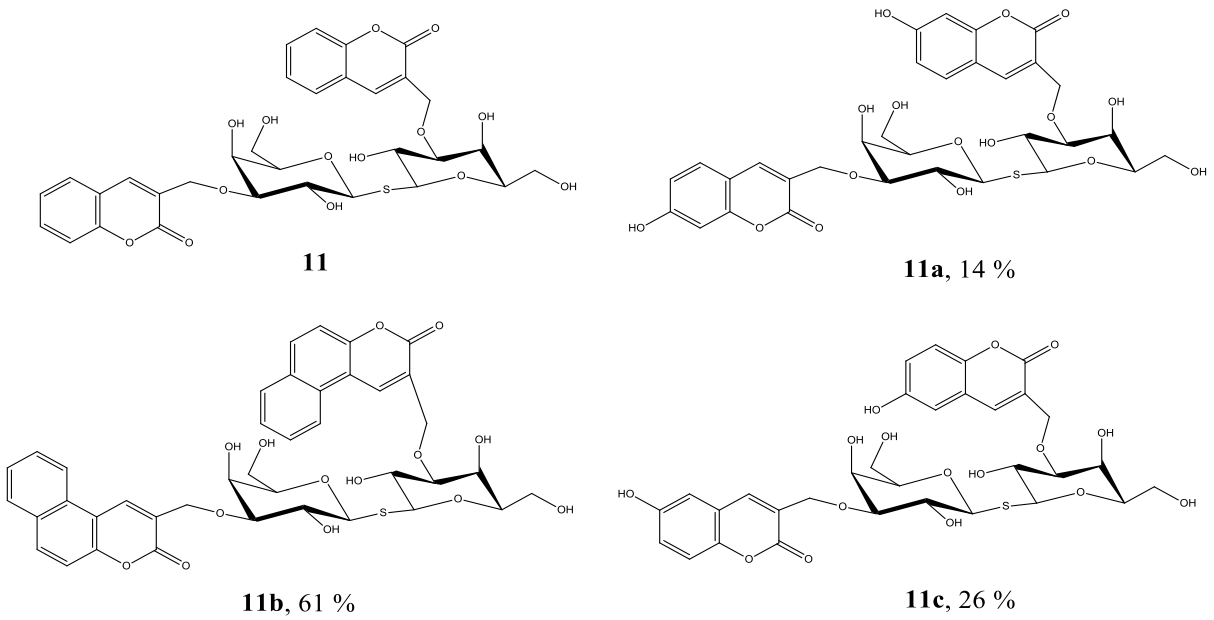


Figure 2. 3,3'-di-substituted coumarin thiodigalactosides

Compound **11** (figure 2) was synthesized by Mandal in the research group. Compounds **11**, **11a**, **11b** and **11c** were purified by HPLC and evaluated in vitro as galectin-3 inhibitors in a fluorescence polarization assay[4] (table 1). Compound **11** was used as reference to previous work and both **11a** and **11c** showed higher affinity. This may be due to additional hydrogen bonding at the binding site. The position of the hydroxyl in **11a** may be more favorable than in **11c**, therefore better inhibition. Compound **11b** had the lowest affinity, it may be too bulky and wrong size for the binding site.

Table 1. K_d-values (µM) of **11**, **11a**, **11b** and **11c** against human galectin-3 measured by a fluorescence polarization assay[4]

Compounds	Galectin-3
11	0.23±0.04
11a	0.079±0.02
11b	0.51±0.17
11c	0.15±0.07

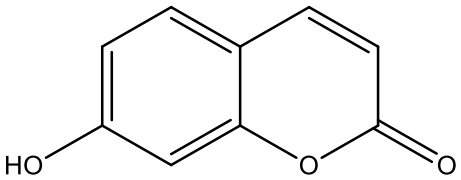


Figure 3. Umbelliferone

The compounds were also analyzed by UV-Vis- and fluorescence spectrophotometer (figure 4 and 5). From the UV-Vis and fluorescence measurements the absorption-, fluorescence intensity maximum, Stokes shift, normalized absorption and fluorescence intensity were determined for compounds **11**, **11a**, **11b** and **11c**. The absorption maximum, where the absorption was strongest, of **11a** was the same as umbelliferone[15] (figure 3), which meant that umbelliferyl was the absorbing moiety. It was compared to umbelliferone because the structure of umbelliferyl on **11a** was similar to umbelliferone and should therefore have similar fluorescence properties. The wavelength of absorption maximum, usually the same as excitation maximum, was used to determine the fluorescence intensity maximum. The fluorescence intensity maximum, where the fluorescence was strongest, is an important parameter in measurement of fluorescence lifetime. The lifetime indicates the average time the molecule stays in its excited state before emitting a photon and it is important in applications such as fluorescence resonance energy transfer and fluorescence-lifetime imaging microscopy[16]. Stokes shift (table 2) is the difference in wavelength between the maxima of the absorption and fluorescence intensity spectra. Stokes shift is important to determine how good a fluorophore is for fluorescence studies. It is easier to distinguish the light produced by emission from the light used for excitation with a large Stokes shift. The higher the fluorescence intensity is the larger numbers of photons are emitted and hence better fluorescence properties. To compare the fluorescence intensity, the normalized absorption and fluorescence intensity (figure 6) were calculated. The non-dashed lines are the absorbance and the dashed lines are the fluorescence intensity. In figure 4 one can see that compound **11a** had two absorbance peaks, one at 325 nm and one above 400 nm. During the measurements, **11a** was diluted many times because the fluorescence intensity was too high to be measured. When diluted only the peak at 325 nm was changing and **11a** was normalized regarding to it. Compound **11a** had the highest intensity and should be the most fluorescent compound. Also it was the only one that was fluorescent in the visible spectrum when dissolved in DMSO.

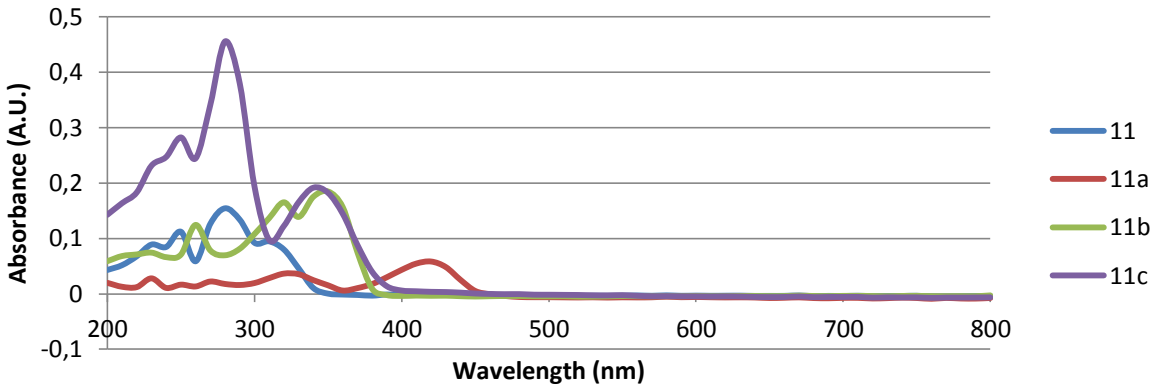


Figure 4. Absorbance vs. wavelength determined by UV-Vis spectrophotometer

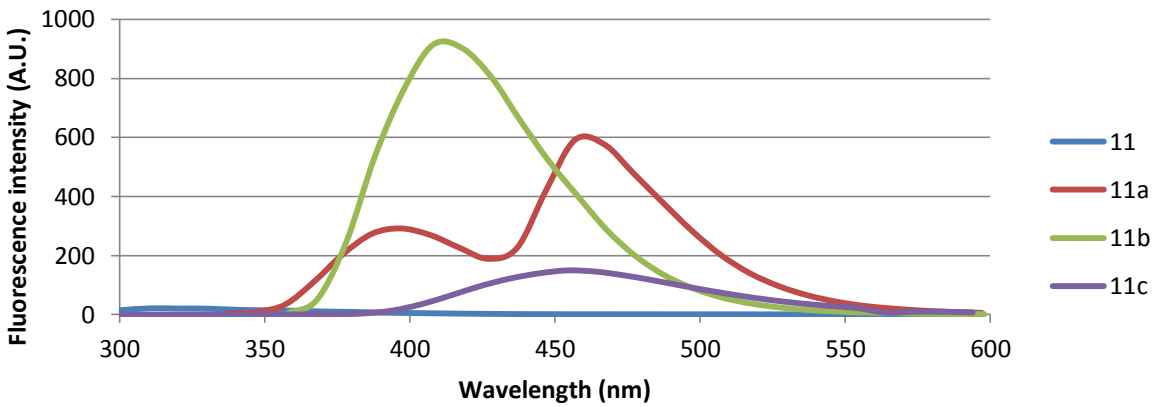


Figure 5. Fluorescence intensity vs. wavelength determined by fluorescence spectrophotometer

Table 2. Absorption-, fluorescence intensity maximum and Stokes shift of **11**, **11a**, **11b** and **11c**

Compounds	λ^{a}_{max} (nm)[a]	λ^{f}_{max} (nm)[a]	**Stokes shift** (nm)
11	278	-	-
11a	325	460	135
11b	346	412	66
11c	282	455	173

[a]Determined in DMSO

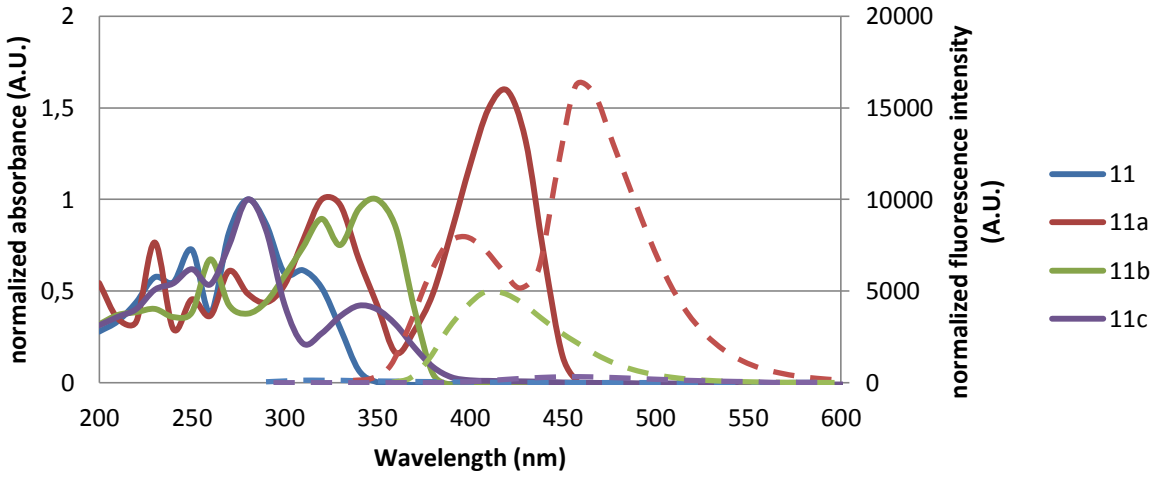


Figure 6. Normalized absorbance and fluorescence intensity vs. wavelength, the non-dashed lines are the absorbance and the dashed lines are the fluorescence intensity

3. Conclusions and future work

Three carbohydrate coumarin hybrid molecules have been synthesized and tested against galectin-3. Two of the compounds show higher affinity than compound **11** and one compound shows lower affinity. It seems that having a hydroxyl group in the coumarin gives better inhibition and having a bulky group as in compound **11b** gives worse inhibition. Larger amounts of compounds **11**, **11a**, **11b** and **11c** will need to be synthesized to determine which is the most fluorescence one. In overall most synthetic steps have been simple and given good yields except for deacetylation of hydroxy-iminocoumarins. An alternative method for deacetylation is needed and one way is to protect the hydroxyl with a silyl group which is

stable towards acid and bases. The acetates can easily be hydrolyzed by a base and the silyl group can be removed with fluoride salts. The reaction of monogalactoside with aminosalicylaldehyde did work, but did not work with tdg. What can be done is to introduce an electron withdrawing group either before or after MCR. It will be more difficult for the nitrogen to donate its electron pair. Another way is first synthesize iminocoumarin monogalactoside via MCR, and then do the thiodigalactoside coupling reaction.

4. Experimental

4.1 General methods

All reagents and solvents were dried prior to use according to standard methods[17]. Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on silica gel 60-F_{254} (Merck) with detection by fluorescence and/or by charring following immersion in a 10 % sulfuric acid in ethanol solution. Column chromatography was performed with silica gel 40-60 µm, 60 Å. ^{1}H NMR spectra were recorded on a Bruker Advance spectrometer at 400 MHz. 1-D and 2-D spectra are processed using Bruker Topspin software. NMR characterization data are represented using peak-multiplicity abbreviations as s-singlet, d-doublet, t-triplet, q-quartet and m-multiplet. Coupling constants are given in Hertz (Hz). Low-resolution MS were obtained using ESI ionization. Absorption and fluorescent spectra were recorded on a Varian Cary-100 Bio UV-Visible Spectrophotometer and Varian Cary Eclipse Fluorescence Spectrophotometer.

4.2 Synthesis

1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranoside (1)

Acetic anhydride (400 ml, 4.26 mol) was added to sodium acetate (80 g, 0.98 mol) and the mixture was refluxed for 1 h. To the brown solution, but not clear, D-galactose (100 g, 0.56 mol) was added in small portions. After addition the solution turned to a clear dark brown solution and heated for another hour. A precipitation was formed while cooling to room temperature. The thick slurry solution was poured on crushed ice and put in the fridge for three nights, filtered and washed with water (8 x 250 ml). Recrystallization with EtOH/H_2O (2:1) afforded the product as a brown solid (90.9 g, 0.20 mol, 36 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.71 (d, 1H, *J* 8.4 Hz, H-1), 5.43 (dd, 1H, *J* 0.8 Hz, 3.2 Hz, H-4), 5.36 (dd, 1H, *J* 8.4 Hz, 10.4 Hz, H-2), 5.09 (dd, 1H, *J* 3.6 Hz, 10.4 Hz, H-3), 4.17 (m, 2H, H-6), 4.07 (t, 1H, *J* 0.8 Hz, H-5), 2.17 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.04 (s, 6H, 2xOAc), 1.99 (s, 3H, OAc).

4-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (2)

(1) (44 g 0.11 mol) and 4-methoxyphenol (16.8 g, 0.14 mol) were dissolved in dry DCM (200 ml) under nitrogen. The solution was cooled to 0 °C and boron trifluoride etherate (20.8 ml, 0.17 mol) was added dropwise. The reaction was stirred at 0°C for 4 h and the reaction was stopped by adding water (200 ml). The organic phase was washed with water and saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered and removal of solvent left a brown solid (51 g, 0.11 mol, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.97 (d, 2H, *J* 8.8 Hz, Ph), 6.83 (d, 2H, *J* 9.2 Hz, Ph), 5.48 (m, 2H, *J* 3.6 Hz, 8 Hz, 10.4 Hz, H-2, H-4), 5.11 (dd, 1H, *J* 3.6 Hz, 10.4 Hz, H-3), 4.93 (d, 1H, *J* 8 Hz, H-1), 4.26 (m, 2H, H-6), 4.02 (t, 1H, H-5),

3.78 (s, 3H, OMe), 2.18 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.01 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 477.1 $[M+Na]^+$.

4-Methoxyphenyl β-D-galactopyranoside (3)

(2) (51 g, 0.11 mol) was dissolved in MeOH (600 ml) and 1M NaOMe solution was added until the reaction mixture was basic. The reaction was stirred for 20 h and stopped by Amberlyst 15. The solution was filtered and removal of solvent left a white/brown solid. The solid was washed with DCM. The product yield was 26.8 g (0.094 mol, 83 %). ^{1}H NMR (400 MHz, MeOD) δ: 7.09 (d, 2H, *J* 9.2 Hz, Ph), 6.85 (d, 2H, *J* 9.2 Hz, Ph), 4.74 (d, 1H, *J* 7.6 Hz, H-1), 3.90 (dd, 1H, *J* 0.8 Hz, 3.2 Hz, H-4), 3.79 (m, 3H, H-2, H-6), 3.75 (s, 3H, OMe), 3.66 (t, 1H, *J* 0.8 Hz, H-5), 3.58 (dd, 1H, *J* 3.2 Hz, 9.6 Hz, H-3). LRMS (ESI) *m*/*z*: 309.1 $[M+Na]^+$.

4-Methoxyphenyl 3-*O*-propargyl-β-D-galactopyranoside (4)

(3) (26.3 g, 0.092 mol) was dissolved in dry MeOH (750 ml) under nitrogen. Dibutyltin oxide (25.2 g, 0.10 mol) was added and the reaction was stirred at 75°C for 23 h. The dark brown solution was concentrated and the residue was dissolved in dry 1,4-dioxane (800 ml) under nitrogen. Tetrabutylammonium bromide (29.6 g, 0.092 mol) and propargyl bromide (49.5 ml, 0.46 mol) were added and refluxed for 4.5 h. The solvent was concentrated and the residue was dissolved in MeOH and filtered. Removal of solvent left an dark brown oil and column chromatography, EtOAc/heptane (10:1), afforded pure product as a white/brown solid (18.2 g, 0.056 mol, 61 %). ^{1}H NMR (400 MHz, MeOD) δ: 7.07 (d, 2H, *J* 9.2 Hz, Ph), 6.84 (d, 2H, *J* 9.2 Hz, Ph), 4.77 (d, 1H, *J* 8 Hz, H-1), 4.42 (t, 2H, *J* 2.4 Hz, OC*H*$_2$), 4.13 (br d, 1H, *J* 3.2 Hz, H-4), 3.88 (dd, 1H, 8 Hz, 9.6 Hz, H-2), 3.78 (m, 5H, H-6, OMe), 3.64 (m, 2H, *J* 3.2 Hz, 9.6 Hz, H-3, H-5), 2.88 (t, 1H, *J* 2.4 Hz, C*H*). LRMS (ESI) *m*/*z*: 347.1 $[M+Na]^+$.

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-propargyl-β-D-galactopyranoside (5)

(4) (13 g, 0.040 mol) was dissolved in 150 ml pyridine/acetic anhydride (2:1) under nitrogen. The reaction was stirred for 22 h and then diluted with EtOAc and washed with 1M HCl (3 x 200 ml) and water (3 x 200 ml). The organic phase was dried over $MgSO_4$ and filtered. Removal of solvent left an brown oil and column chromatography, EtOAc, afforded the product as a yellow solid (16.1 g, 0.036 mol, 90 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.97 (d, 2H, *J* 9.2 Hz, Ph), 6.82 (d, 2H, *J* 9.2 Hz, Ph), 5.45 (br d, 1H, *J* 2.4 Hz, H-4), 5.34 (dd, 1H, *J* 8 Hz, 10 Hz, H-2), 4.92 (d, 1H, *J* 8 Hz), 4.22 (m, 4H, *J* 2.4 Hz, 6.4 Hz, H-6, OC*H*$_2$), 3.96 (t, 1H, *J* 6 Hz, H-5), 3.90 (dd, 1H, *J* 3.6 Hz, 10 Hz, H-3), 2.45 (t, 1H, *J* 2.4 Hz, C*H*), 2.17 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.08 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 473.3 $[M+Na]^+$.

***p*-Toluenesulfonyl azide**

p-Toluenesulfonyl chloride (20 g, 0.10 mol) was dissolved in acetone (200 ml) and cooled to 0°C. Sodium azide (10.2 g, 0.16 mol) in water (100 ml) was added dropwise over 1h. The reaction was warmed to room temperature and stirred for 21 h. Acetone was removed under reduced pressure at 25°C and the reaction mixture was extracted with EtOAc (2 x 100 ml). The combined organic phases were washed with water (2 x 100 ml), 5 % Na_2CO_3 (2 x 100 ml) and water (2 x 100 ml), dried over Na_2SO_4 and removal of solvent left a colorless liquid (15.9 g, 0.081 mol, 77 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 (d, 2H, *J* 8.4 Hz, ArH), 7.42 (d, 2H, *J* 8 Hz, ArH), 2.49 (s, 3H, Me). LRMS (ESI) *m*/*z*: 219.9 $[M+Na]^+$.

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-{[2-(*p*-Tolylsulfonyl-imino)-7-hydroxy-2*H*-chromen-3-yl]-methyl}-β-D-galactopyranoside (6a)

(5) (0.22 g, 0.49 mmol), 2,4-dihydroxybenzaldehyde (0.075 g, 0.54 mmol), CuI (9.4 mg, 0.049 mmol) and *p*-Toluenesulfonyl azide (0.097 g, 0.49 mmol) were dissolved in dry THF (10 ml) under nitrogen and stirred for 1 h. Triethylamine (0.14 ml, 0.98 mmol) was added dropwise and stirred for 21 h. The solution was concentrated in vacuo and the residue was dissolved in DCM (10 ml) and washed with NH_4Cl solution (2 x 30 ml) and brine (2 x 30 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography using EtOAc/heptane (1:1) to elute impurities and increasing polarity with MeOH to elute the product, afforded the product as a yellow solid (0.25 g, 0.34 mmol, 69 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, 2H, *J* 8 Hz, SPh), 7.60 (s, 1H, ArH), 7.35 (d, 2H, *J* 8 Hz, ArH), 7.31(d, 1H, *J* 8.4 Hz, SPh), 6.97 (d, 2H, *J* 9.2 Hz, Ph), 6.88 (d, 1H, *J* 2 Hz, ArH), 6.84 (m, 3H, *J* 9.2 Hz, Ph, ArH), 5.55 (br d, 1H, *J* 3.2 Hz, H-4), 5.42 (dd, 1H, *J* 8.4 Hz, 10.4 Hz, H-2), 4.86 (d, 1H, *J* 8 Hz, H-1), 4.59, 4.39 (2d, 2H, *J* 15.2 Hz, C*H*$_2$Ar), 4.27 (m, 2H, H-6), 3.92 (t, 1H, H-5), 3.78 (m, 4H, H-3, OMe), 2.40 (s, 3H, SPhMe), 2.16 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.04 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 762.0 $[M+Na]^+$.

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-{[2-(*p*-Tolylsulfonyl-imino)-4,5-phenyl-2*H*-chromen-3-yl]-methyl}-β-D-galactopyranoside (6b)

(5) (50 mg, 0.11 mmol), 2-hydroxyl-1-naphthaldehyde (23 mg, 0.13 mmol), CuI (2 mg, 0.011 mmol) and *p*-Toluenesulfonyl azide (26 mg, 0.13 mmol) were dissolved in dry THF (3 ml) under nitrogen and stirred for 1 h. Triethylamine (31 µl, 0.22 mmol) was added dropwise and stirred for 12 h. The solution was concentrated in vacuo and the residue was dissolved in DCM (10 ml) and washed with NH_4Cl solution (2 x 10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography, EtOAc/heptane (1:1), afforded the product as a green solid (43 mg, 0.056 mmol, 50 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.55 (s, 1H, ArH), 8.45 (d, 1H, *J* 8.4 Hz, ArH), 8.05 (d, 2H, *J* 8.4 Hz, SPh), 8.02 (d, 1H, *J* 8.8 Hz ArH), 7.77 (t, 1H, *J* 7.2 Hz, ArH), 7.62 (t, 1H, *J* 7.2 Hz), 7.53 (d, 1H, *J* 9.2 Hz, ArH), 7.34 (d, 2H, *J* 9.2 Hz, SPh), 6.99 (d, 2H, *J* 9.2 Hz, Ph) 6.84 (d, 2H, *J* 8.8 Hz, Ph), 5.62 (br d, 1H, *J* 2.4 Hz, H-4), 5.53 (dd, 1H, *J* 8 Hz, 10 Hz, H-2), 4.92 (d, 1H, *J* 8 Hz, H-1), 4.79, 4.49 (2d, 2H, *J* 15.2 Hz, C*H*$_2$Ar), 4.30 (m, 2H, *J* 6.4 Hz, 6.8 Hz, H-6), 3.95 (t, 1H, *J* 6.4 Hz, 6.8 Hz, H-5), 3.79 (m, 4H, *J* 3.2 Hz, H-3, OMe), 2.41 (s, 3H, SPhMe), 2.18 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.09 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 796.2 $[M+Na]^+$.

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-{[2-(*p*-Tolylsulfonyl-imino)-7-diethylamino-2*H*-chromen-3-yl]-methyl}-β-D-galactopyranoside (6c)

(5) (0.60 g, 1.33 mmol), 4-(diethylamino)salicylaldehyde (0.31 g, 1.61 mmol), CuI (26 mg, 0.14 mmol) and *p*-Toluenesulfonyl azide (0.32 g, 1.62 mmol) were dissolved in dry THF (10 ml) under nitrogen and stirred for 1 h. Triethylamine (0.37 ml, 2.67 mmol) was added dropwise and stirred for 6 days. The solution was concentrated in vacuo and the residue was dissolved in DCM (20 ml) and washed with NH_4Cl solution (2 x 40 ml) and brine (2 x 40 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography, EtOAc/heptane (2:1), afforded the product as a green/yellow solid (0.75 g, 0.94 mmol, 71 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, 1H, *J* 8 Hz, SPh), 7.54 (s, 1H, ArH), 7.27 (m, 3H, SPh, ArH), 6.97 (d, 2H, *J* 9.2 Hz, Ph), 6.83 (d, 2H, *J* 9.2 Hz, Ph), 6.63 (dd, 1H, *J* 2.4 Hz, 8.8 Hz, ArH), 6.47 (d, 1H, *J* 2 Hz, ArH), 5.54 (br d, 1H, *J* 2.8 Hz, H-4),

5.39 (dd, 1H, *J* 8.4 Hz, 10.4 Hz, H-2), 4.81 (d, 1H, *J* 8 Hz, H-1), 4.52, 4.41 (2d, 2H, *J* 14.4 Hz, C*H*$_2$Ar), 4.25 (m, 2H, *J* 6.4 Hz, 6.8 Hz, H-6), 3.87 (t, 1H, *J* 6.4 Hz, 6.8 Hz, H-5), 3.78 (m, 4H, *J* 3.2 Hz, 9.2 Hz, H-3, OMe), 3.47 (q, 4H, *J* 6.8 Hz, 2xC*H*$_2$N), 2.39 (s, 3H, SPhMe), 2.14 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.24 (t, 6H, *J* 6.8 Hz, 2x*CH*$_3$CH$_2$N). LRMS (ESI) *m*/*z*: 817.2 $[M+Na]^+$.

4-Methoxyphenyl 3-*O*-(3-methylene-7-diethylamino-coumarin)-β-D-galactopyranoside (7c)

MeOH (4 ml) was added to **(6c)** (50 mg, 0.063 mmol) and DCM was added dropwise until **(10b)** had dissolved. 1 M NaOMe solution was added until the solution was basic and stirred overnight. The reaction was stopped by Amberlite 15, filtered and concentrated. Column chromatography, EtOAc/Hep (4:1 → 8:1), afforded the product as a green/yellow solid (2 mg, 0.0039 mmol, 6 %). ^{1}H NMR (400 MHz, MeOD) δ: 7.98 (s, 1H, ArH), 7.42 (d, 1H, *J* 8.8 Hz, ArH), 7.08 (d, 2H, *J* 9.2 Hz, Ph), 6.85 (d, 2H, *J* 9.2 Hz, Ph), 6.75 (dd, 1H, *J* 2.4 Hz, 8.8 Hz, ArH), 6.55 (d, 1H, *J* 2.4 Hz, ArH), 4.79 (d, 1H, *J* 8 Hz, H-1), 4.67, 4.51 (2d, 2H, *J* 13.6 Hz, C*H*$_2$Ar), 4.23 (br d, 1H, *J* 2.4 Hz, H-4), 3.92 (dd, 1H, *J* 7.6 Hz, 9.6 Hz, H-2), 3.81 (m, 2H, *J* 6.8 Hz, H-6), 3.75 (s, 3H, OMe), 3.67 (t, 1H, *J* 6.8 Hz, H-5), 3.54 (m, 5H, *J* 3.2 Hz, 9.6 Hz, 7.2 Hz, H-3, 2xC*H*$_2$N), 1.23 (t, 6H, *J* 7.2 Hz, 2x*CH*$_3$CH$_2$N). LRMS (ESI) *m*/*z*: 538.2 $[M+Na]^+$.

2,4,6-tri-*O*-acetyl-3-*O*-propargyl-α-D-galactopyranosyl bromide (8)

(5) (4.78 g, 10.6 mmol) was dissolved in dry DCM (40 ml) under nitrogen. $ZnBr_2$ (0.12 g, 0.53 mmol) and AcBr (2.36 ml, 31.8 mmol) were added and stirred for 22 h. The reaction was diluted with DCM (50 ml) and washed with saturated $NaHCO_3$ solution (150 ml) and brine (150 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography, EtOAc/heptane (1:5 → 1:1), afforded the product as an yellow oil (3.86 g, 9.48 mmol, 79 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.69 (d, 1H, *J* 4 Hz, H-1), 5.54 (br d, 1H, *J* 2 Hz, H-4), 4.96 (dd, 1H, *J* 4 Hz, 10 Hz, H-2), 4.43 (t, 1H, H-5), 4.25 (t, 2H, *J* 2.4 Hz, OC*H*$_2$), 4.22 (m, 2H, H-3, H-6), 4.12 (dd, 1H, H-6), 2.51 (t, 1H, *J* 2.4 Hz, C*H*), 2.14 (s, 6H, 2xOAc), 2.08 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 429.0, 431.0 $[M+Na]^+$.

Di-(2,4,6-tri-*O*-acetyl-3-*O*-propargyl-β-D-galactopyranosyl)-sulfane (9)

Half amount of **(8)** (1.98 g, 4.86 mmol) was dissolved in dry acetonitrile (30 ml) under nitrogen. Thiourea (0.37 g, 4.86 mmol) was added and refluxed for 4 h. The reaction was cooled to room temperature and the other half of **(8)** in dry acetonitrile (20 ml) was added. Triethylamine (0.68 ml, 4.86 mmol) was added and stirred for 24 h. The solvent was concentrated and column chromatography, EtOAc/heptane (1:1), afforded the product as a white solid (1.29 g, 1.88 mmol, 39 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.45 (br d, 1H, *J* 2.4 Hz, H-4), 5.11 (t. 1H, *J* 10 Hz, H-2), 4.82 (d, 1H, *J* 10 Hz, H-1), 4.20 (m, 4H, H-6, OC*H*$_2$), 3.86 (m, 2H, H-3, H-5), 2.45 (t, 1H, *J* 2.4 Hz, C*H*), 2.15 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.08 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 709.1 $[M+Na]^+$.

Di-(2,4,6-tri-*O*-acetyl-3-*O*-{[2-(*p*-Tolylsulfonyl-imino)-7-hydroxy-2*H*-chromen-3-yl]-methyl}-β-D-galactopyranosyl)-sulfane (10a)

(9) (50 mg, 0.073 mmol), 2,4-dihydroxybenzaldehyde (24 mg, 0.18 mmol), CuI (3 mg, 0.015 mmol) and *p*-Toluenesulfonyl azide (35 mg, 0.18 mmol) were dissolved in dry THF (3 ml)

under nitrogen and stirred for 1 h. Triethylamine (41 µl, 0.29 mmol) was added dropwise and stirred for 72 h. The solution was concentrated in vacuo and the residue was dissolved in DCM (10 ml) and washed with NH_4Cl solution (2 x 10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography, EtOAc/heptane (2:1) → DCM/MeOH (20:1), afforded the product as a yellow solid (45 mg, 0.036 mmol, 49 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, 2H, *J* 8 Hz, SPh), 7.56 (s, 1H, ArH), 7.37 (d, 2H, *J* 8 Hz, SPh), 7.32 (d, 1H, *J* 10.8 Hz, ArH), 6.86 (d, 1H, *J* 2 Hz, ArH), 6.81 (dd, 1H, *J* 2 Hz, 10.8 Hz, ArH), 5.58 (d, 1H, *J* 3.2 Hz, H-4), 5.18 (t, 1H, *J* 9.6 Hz, 10 Hz, H-2), 4.82 (d, 1H, *J* 10 Hz, H-1), 4.57, 4.40 (2d, 2H, *J* 14 Hz, C*H*$_2$Ar), 4.23 (m, 2H, *J* 6.8 Hz, 7.2 Hz, H-6), 3.87 (t, 1H, , *J* 6.8 Hz, 7.2 Hz, H-5), 3.77 (dd, 1H, *J* 3.2 Hz, 9.6 Hz, H-3), 2.42 (s, 3H, SPhMe), 2.14 (s, 3H, OAc), 2.10 (s, 3H, OAc), 1.98 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 1287.0 $[M+Na]^+$.

Di-(2,4,6-tri-*O*-acetyl-3-*O*-{[2-(*p*-Tolylsulfonyl-imino)-4,5-phenyl-2*H*-chromen-3-yl]-methyl}-β-D-galactopyranosyl)-sulfane (10b)

(9) (30 mg, 0.044 mmol), 2-Hydroxy-1-naphthaldehyde (18 mg, 0.11 mmol), CuI (1.7 mg, mmol, 0.009 mmol) and *p*-Toluenesulfonyl azide (21 mg, 0.011 mmol) were dissolved in dry THF (3 ml) under nitrogen and stirred for 1 h. Triethylamine (24 µl, 0.18 mmol) was added dropwise and stirred for 17 h. The solution was concentrated in vacuo and the residue was dissolved in DCM (10 ml) and washed with NH_4Cl solution (2 x 10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography, EtOAc/heptane (2:1), afforded the product as a green solid (23 mg, 0.017 mmol, 39 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.53 (s, 1H, ArH), 8.44 (d, 1H, *J* 8.4 Hz, ArH), 8.05 (d, 2H, *J* 8 Hz, SPh), 8.02 (d, 1H, *J* 9.2 Hz, ArH), 7.93 (d, 1H, *J* 7.6 Hz, ArH), 7.76 (t, 1H, *J* 7.2 Hz, 8 Hz, ArH), 7.62 (t, 1H, *J* 7.2 Hz, ArH), 7.52 (d, 1H, *J* 9.2 Hz, ArH), 7.39 (d, 2H, *J* 7.6 Hz, SPh), 5.65 (br d, 1H, *J* 3.2 Hz, H-4), 5.33 (t, 1H, *J* 9.6 Hz, 10 Hz, H-2), 4.86 (d, 1H, *J* 10.4 Hz, H-1), 4.78, 4.49 (2d, 2H, *J* 13.6 Hz, C*H*$_2$Ar), 4.25 (m, 2H, H-6), 3.90 (t, 1H, *J* 6.8 Hz, H-5), 3.79 (dd, 1H, *J* 3.2 Hz, 9.6 Hz, H-3), 2.42 (s, 3H, SPhMe), 2.16 (s, 3H, OAc), 2.12 (s, 3H, OAc), 1.99 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 1355.2 $[M+Na]^+$.

Di-(2,4,6-tri-*O*-acetyl-3-*O*-{[2-(*p*-Tolylsulfonyl-imino)-6-hydroxy-2*H*-chromen-3-yl]-methyl}-β-D-galactopyranosyl)-sulfane (10c)

(9) (0.20 g, 0.29 mmol), 2,5-dihydroxybenzaldehyde (97 mg, 0.70 mmol), CuI (11 mg, 0.058 mmol) and *p*-Toluenesulfonyl azide (0.14 g, 0.70 mmol) were dissolved in dry THF (3 ml) under nitrogen and stirred for 1 h. Triethylamine (0.16 ml, 1.17 mmol) was added dropwise and stirred for 72 h. The solution was concentrated in vacuo and the residue was dissolved in DCM (10 ml) and washed with NH_4Cl solution (2 x 10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography, DCM/MeOH (20:1), afforded the product as a yellow solid (0.13 g, 0.10 mmol, 34 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, 2H, *J* 8.4 Hz, SPh), 7.52 (s, 1H, ArH), 7.34 (d, 2H, *J* 8 Hz, SPh), 7.19 (d, 1H, *J* 9.2 Hz, ArH), 7.01 (dd, 1H, *J* 3.2 Hz, 9.2 Hz, ArH), 6.84 (d, 1H, *J* 3.2 Hz, ArH), 5.55 (br d, 1H, *J* 2.4 Hz, H-4), 5.21 (t, 1H, *J* 9.6 Hz, 10 Hz, H-2), 4.81 (d, 1H, *J* 10 Hz, H-1), 4.61, 4.39 (2d, 2H, *J* 14.8 Hz, C*H*$_2$Ar), 4.25 (m, 2H, *J* 6.4 Hz, 7.2 Hz, H-6), 3.88 (t, 1H, *J* 6.8 Hz, 7.2 Hz, H-5), 3.76 (dd, 1H, *J* 3.2 Hz, 9.6 Hz, H-3), 2.41 (s, 3H, SPhMe), 2.14 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.03 (s, 3H, OAc). LRMS (ESI) *m*/*z*: 1287.5 $[M+Na]^+$.

Di-(3-*O*-(3-methylene-7-hydroxy-coumarin)-β-D-galactopyranosyl)-sulfane (11a)

(10a) (25 mg, 0.020 mmol) was dissolved in MeOH/DCM (1:1) and AcCl (0.11 ml, 1.54 mmol) was added slowly. The solution was stirred for 2 days and concentrated at room temperature. Column chromatography, DCM/MeOH (5:1), afforded the product as a white solid (2 mg, 0.0028 mmol, 14 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.49 (s, 1H, HO-Ph), 8.11 (s, 1H, ArH), 7.50 (d, 1H, *J* 8 Hz, ArH), 6.82 (dd, 1H, *J* 2.4 Hz, 8.4 Hz, ArH), 6.74 (d, 1H, *J* 2 Hz, ArH), 5.20 (d, 1H, *J* 6 Hz, HO-2), 4.62 (m, 3H, H-1, HO-4, HO-6), 4.54 (2d, 2H, *J* 14.4 Hz, C*H*$_2$Ar), 4.02 (br s, 1H, H-4), 3.60 (m, 3H, H-2, H-6), 3.39 (m, 2H, H-3, H-5). LRMS (ESI) *m*/*z*: 729.1 $[M+Na]^+$.

Di-(3-*O*-(3-methylene-4,5-phenyl-coumarin)-β-D-galactopyranosyl)-sulfane (11b)

MeOH (10 ml) was added to **(10b)** (28 mg, 0.021 mmol) and DCM was added dropwise until (**10b)** had dissolved. 1 M NaOMe solution was added until the solution was basic and stirred for 2 h. Three drops of water was added and stirred overnight. The reaction was stopped by Amberlite 15, filtered and concentrated. Column chromatography, DCM/MeOH (10:1), afforded the product as a white solid (10 mg, 0.013 mmol, 61 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.09 (s, 1H, ArH), 8.61 (d, 1H, *J* 8 Hz, ArH), 8.18 (d, 1H, *J* 8.8 Hz, ArH), 8.08 (d, 1H, *J* 8 Hz, ArH), 7.79 (m, 1H, *J* 8 Hz, ArH), 7.65 (m, 1H, *J* 8 Hz, ArH), 7.62 (d, 1H, *J* 8.8 Hz, ArH), 5.60 (d, 1H, *J* 5.6 Hz, HO-2), 4.79 (d, 1H, *J* 4.8 Hz, HO-4), 4.67 (m, 4H, H-1, C*H*$_2$Ar, HO-6), 4.09 (br s, 1H, H-4), 3.76 (m, 1H, H-2), 3.58 (m, 2H, H-6), 3.47 (m, 2H, H-3, H-5). LRMS (ESI) *m*/*z*: 796.9 $[M+Na]^+$.

Di-(3-*O*-(3-methylene-6-hydroxy-coumarin)-β-D-galactopyranosyl)-sulfane (11c)

(10c) (14 mg, 0.011 mmol) was dissolved in MeOH/DCM (1:1) and AcCl (0.16 ml, 2.21 mmol) was added slowly. The solution was stirred for 6 days and concentrated at room temperature. Column chromatography, DCM/MeOH (5:1), afforded the product as a white solid (2 mg, 0.0028 mmol, 26 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (s, 1H, HO-Ph), 8.14 (s, 1H, ArH), 7.27 (d, 1H, *J* 8.8 Hz, ArH), 7.02 (m, 2H, *J* 2.8 Hz, 8.8 Hz, ArH), 5.26 (d, 1H, *J* 5.6 Hz, HO-2), 4.64 (m, 3H, H-1, HO-4, HO-6), 4.58 (2d, 2H, *J* 15.2 Hz, C*H*$_2$Ar), 4.02 (br s, 1H, H-4), 3.65 (m, 1H, H-2), 3.52 (m, 2H, H-6), 3.39 (m, 2H, H-3, H-5). LRMS (ESI) *m*/*z*: 729.2 $[M+Na]^+$.

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6. References

[1] V. K. Rajput, H. Leffler, U. J. Nilsson, B. Mukhopadhyay, Synthesis of carbohydrate coumarine N-sulfonyl Iminocoumarine hybrids: Discovery of selective nanomolar galectin-3 inhibitors, Lund University, **2011**.

[2] C. T. Öberg, H. Leffler, U. J. Nilsson, Inhibition of Galectins with Small Molecules, *Chimia*, **2011**, 65, 18-23.

[3] B. Salameh, I. Cumpstey, A. Sundin, H. Leffler, U. J. Nilsson, 1H-1,2,3-Triazol-1-yl thiogalactoside derivatives as high affinity galectin-3 inhibitors, *Bioorganic & Medicinal Chemistry*, **2010**, 18, 5367-5378.

[4] P. Sörme, B. Kahl-Knutsson, M. Huflejt, U. J. Nilsson, H. Leffler, Fluorescence polarization as an analytical tool to evaluate galectin-ligand interactions, *Analytical Biochemistry*, **2004**, 334, 36-47.

[5] M. Ljungberg, Synthesis of 3-C-(4-aryl-5-halo-1,2,3-triazol-1-yl)-galactosides: Probing halogen bonding in galectin ligand complexes, Lund University, **2011**.

[6] L. Zhang, G. Wei, Y. Du, Synthesis of Neu5Ac-Gal-functionalized gold glyconanoparticles, *Carbohydrate Research*, **2009**, 344, 2083-2087.

[7] N. P. Pera, U. J. Nilsson, N. Kann, Intermolecular Pauson-Khand reactions on a galactose scaffold, *Tetrahedron Letters*, **2008**, 49, 2820-2823.

[8] J. Waser, B. Gaspar, H. Nambu, E. M. Carreira, Hydrazines and Azides via the Metal-Catalyzed Hydrohydrazination and Hydroazidation of Olefins, *J. Am. Chem. Soc*., **2006**, 128, 11693-11712.

[9] S.-L. Cui, X.-F. Lin, Y.-G. Wang, Novel and Efficient Synthesis of Iminocoumarins via Copper-Catalyzed Multicomponent Reaction, *Organic Letters*, **2006**, 8, 4517-4520.

[10] Z. Zhang, G. Magnusson, Conversion of p-methoxyphenyl glycosides into the corresponding glycosyl chlorides and bromides, and into thiophenyl glycosides, *Carbohydrates Research*, **1996**, 925, 41-55.

[11] C.-E. Yeom, S. Y. Lee, Y. J. Kim, B. M. Kim, Mild and Chemoselective Deacetylation Method Using a Catalytic Amount of Acetyl Chloride in Methanol, *Synlett*, **2005**, 10, 1527-1530.

[12] N. Tewari, H. Nizar, A. Mane, V. George, M. Prasad, Deacetylation of Thioacetate using Acetyl Chloride in Methanol, *Synthetic Communications*, **2006**, 36, 1911-1914.

[13] H-M. Liu, X. Yan, W. Li, C. Huang, A mild and selective method for cleavage of *O*-acetyl groups with dibutyltin oxide, *Carbohydrate Research*, **2002**, 337, 1763-1767.

[14] J. Teljer, B. Salameh, H. Leffler, U. J. Nilsson, Fragment-based development of triazole-substituted *O*-galactosyl aldoximes with fragment-induced affinity and selectivity for galectin-3, *Organic & Biomolecular Chemistry*, **2009**, 7, 3982-3990.

[15] N. Nizomov, A. U. Kholov, A. A. Ishchenko, V. V. Ishchenko, V. P. Khilya, Electronic structure and spectral fluorescence properties of umbelliferone and herniarin, *Journal of Applied Spectroscopy*, **2007**, 74, 626-634.

[16] H. Wallrabe, A. Periasamy, Imaging protein molecules using FRET and FLIM microscopy, *Current Opinion in Biotechnology*, **2005**, 16, 19-27.

[17] D. D. Perrin, W. L. Amarego, D. R. Perrin, Purification of Laboratory Chemicals, Pergamon: London, **1996**.