Bachelor thesis
An approach to diketone-galectin-3 inhibitor

Sieu Hoang
Organic chemistry, Lund University, 14-10-2011
KEMK01
Examiner: Ulf Nilsson
Supervisor: Vishal Rajput

Contents

Abstract

In many biological mechanisms galectins play important roles. The galectins are important in inflammation, immunologic response and cancer. A key function of galectins is cross-linking of their glycoprotein ligands which are connected with the biological activities. Carbohydrate recognition domains (CRD) are responsible for the cross-linking. The galectin CRDs are divided into subsites A-E and it has been discovered that there is a binding affinity for Arg144 in subsite A-B of galectin-3. In a recent study they discovered that diacetyl is binding covalently to *N*-α-acetylarginine. It is interesting to synthesize a galectin inhibitor with diketone functionality and test if it binds to Arg144.

Introduction

Galectins are a family of β-galactoside-binding lectins that are important in many biological mechanisms[1]. Examples of mechanisms that are regulated by galectin activities are intracellular trafficking, cell signaling, apoptosis and cell adhesion. Galectins play many important regulatory roles in inflammation, immunologic response, and cancer. A key function 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 only one is galectin-3. Galectin-3 has a proline-rich collagen-like N-terminal linked to a C-terminal CRD. The N-terminal is responsible for galectin-3 multimerization therefor cross-linking properties.
Recent discoveries have led to better understanding of the key functions of galectins. Different cell surface protein glycosylation patterns on different regulatory T-cells control galectin-1-binding which result in T-cell apoptosis. Galectin-8 orchestrates intracellular targeting and galectin-3 regulates cell surface receptor localization. Therefore galectins are potential targets for novel anti-cancer and anti-inflammatory compounds.
In an early study, an X-ray structure of galection-3 co-crystallized with *N*-acetyllactoseamine (LacNAc) was achieved. The galectin CRDs are divides into subsites A-E. Subsite C is the galactose binding site and galectin inhibitors are categorized based on which subsite is targeted. A 3'-benzoamido-LacNAc derivative had been co-crystallized with galectin-3. The X-ray structure reveals binding affinity for arginine (Arg144) and Arg144 is in subsite A-B. In a recent study[2] a reaction of diacetyl with *N*-α-acetylarginine (AcArg) had been done. Diacetyl is binding covalently to AcArg. Therefor it is interesting to synthesize a galectin inhibitor with diketone functionality and test if it binds to Arg144.
Figure 1 shows the target molecule and figure 2 shows the starting molecules. The starting molecules were already synthesized in the group.

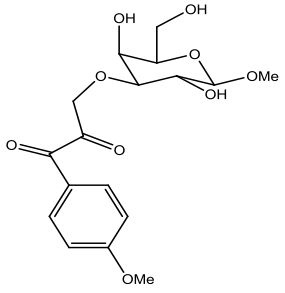
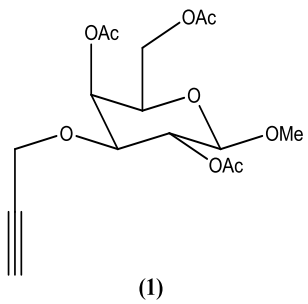
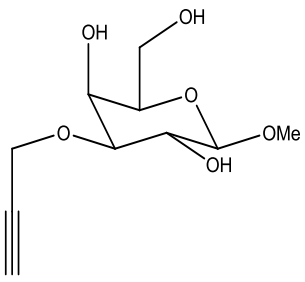


Figure 1. Target molecule



(1)

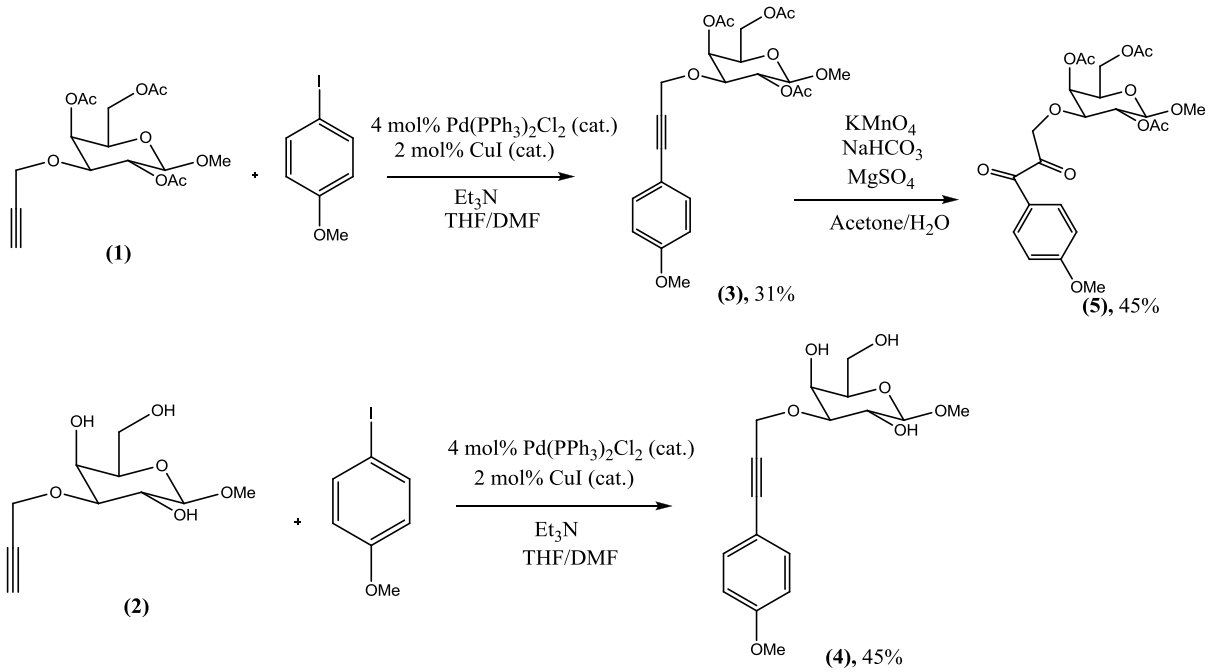


(2)

Figure 2. Starting molecules

General strategy

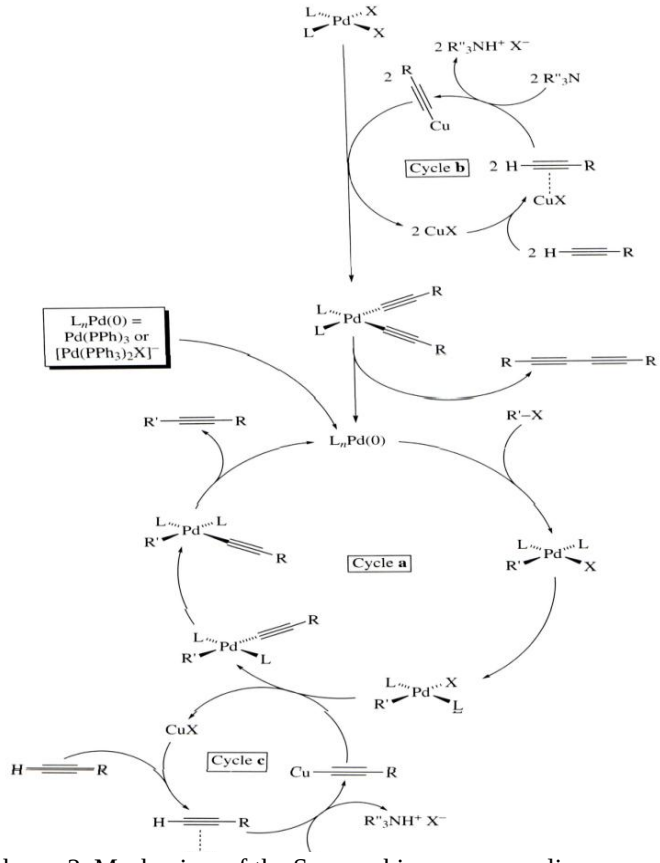
Scheme 1 summarizes the reactions that succeeded. The first reaction is Songashira cross-coupling, scheme 2, then alkyne oxidation by $KMnO_4$.



Scheme 1. Summary of the reactions

Songashira cross-coupling[3], scheme 2, is useful for transfer of terminal alkyne to sp^2 hybridized carbons. Because one of the reagents must be a terminal alkyne, Sonogashira cross-coupling is more limited than other cross-coupling reactions. R'-X is generally limited to R' = aryl, heteroaryl or vinyl and X = I, Br or OTf. Cycle b shows the conversion of Pd(II) to Pd(0). Cycle a is the heart of the mechanism. Oxidative addion of R'-X to Pd(0) followed by cis-trans isomerization. Cycle c connects with cycle a and shows how Cu catalyzes the formation of the Cu-alkyne, which then transmetalates with Pd. Then trans-cis isomerization occurs and the last step is reductive elimination of the product and regeneration of Pd(0).

The mechanism for oxidation of alkyne by $KMnO_4$ is similar to oxidation of alkene. A diketone is formed instead of two ketones.



Scheme 2. Mechanism of the Sonogashira cross-coupling reaction[3]

Experimental

(3): 4-Iodoanisole (65 mg, 0.28 mmol), $Pd(PPh_3)_2Cl_2$ (4 mg, 0.0056 mmol) and CuI (2 mg, 0.011 mmol) were dissolved in dry DMF (5 ml) under N_2. The solution was cooled to -20 °C with dry ice/acetone. **(1)** (0.10 g, 0.28 mmol) was dissolved in dry THF (5 ml) and added to the solution and stirred for 5 min. Et_3N (0.16 ml, 1.12 mmol) was added dropwise and the solution was heated to room temperature slowly. The yellow solution turned a yellow-brown color while stirring at room temperature. After 4 h the solution was quenched by H_2O (10 ml). Extraction with ethyl acetate (3x25 ml), dried over $MgSO_4$ and removal of solvent left a yellow-brown oil. Column chromatography on SiO_2 (ethyl acetate/heptane, 1:1) yielded yellow-brown solid (0.040 g, 0.086 mmol, 31 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.39 (d, 2H, *J* = 8.8 Hz), 6.86 (d, 2H, *J* = 9.2), 5.47 (dd, 1H, *J* = 2.4, 1.2 Hz), 5.09 (dd, 1H, *J* = 8, 1.6 Hz), 4.42 (d, 1H, *J* = 8 Hz), 4.38 (s, 2H), 4.22 (m, 2H), 3.92 (m, 2H, *J* = 1.2 Hz), 3.82 (s, 3H), 3.51 (s, 3H), 2.15 (s, 3H), 2.06 (s, 6H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 170.55, 170.49, 169.77, 159.86, 133.20, 114.47, 114.02, 102.05, 86.67, 83.08, 75.73, 70.83, 70.18, 65.77, 61.88, 57.51, 56.75, 55.32, 21.03, 20.85, 20.75.

(4): 4-Iodoanisole (0.40 g, 1.70 mmol), $Pd(PPh_3)_2Cl_2$ (22 mg, 0.031 mmol) and CuI (12 mg, 0.062 mmol) were dissolved in dry DMF (5 ml) under N_2. The solution was cooled to -20 °C with dry ice/acetone. **(2)** (0.36 g, 1.55 mmol) was dissolved in dry THF (5 ml) and added to the solution and stirred for 5 min. Et_3N (0.86 ml, 6.2 mmol) was added dropwise and the solution was heated to room temperature slowly. The yellow solution turned a yellow-brown color while stirring at room temperature. After 18 h the solution was quenched by H_2O (8 ml). Extraction with ethyl acetate (3x25 ml), dried over $MgSO_4$ and removal of solvent left a yellow-brown oil. Column chromatography on SiO_2 (ethyl acetate/heptane, 1:1) and (DCM/MeOH, 10:1) yielded yellow-brown oil (0.24 g, 0.69 mmol, 45 %). ^{1}H-NMR (400 MHz, MeOD): δ 7.40 (d, 2H, *J* = 8.8 Hz), 6.92 (d, 2H, *J* = 8.8 Hz), 4.39 (d, 2H, *J* = 2.4 Hz), 4.23 (d, 1H, *J* = 7.6 Hz), 4.17 (d, 1H *J* = 2 Hz), 3.83 (s, 3H), 3.80 (m, 2H), 3.68 (m, 3H), 3.56 (s, 3H). ^{13}C-NMR (400 MHz, MeOD): δ 160.00, 132.75, 114.58, 113.63, 104.52, 85.74, 83.38, 80.43, 75.07, 70.19, 65.81, 60.96, 57.13, 55.83, 54.36.

(5): (3) (0.13 g, 0.28 mmol) was dissolved in acetone (10 ml). $NaHCO_3$ (14 mg, 0.17 mmol) and $MgSO_4$ (68 mg, 0.56 mmol) in H_2O was added. $KMnO_4$ (0.13 g, 0.85 mmol) was added and the reaction mixture was stirred for 2.5 h. Then the mixture was poured into H_2O and extracted with DCM (3x60 ml). The organic phase was washed with H_2O then dried over $MgSO_4$. Removal of solvent left an yellow oil and column chromatography on SiO_2 (ethyl acetate/heptane, 1:1) yielded yellow oil (63 mg, 0.12 mmol, 45 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.98 (d, 2H, *J* = 8.8 Hz), 6.97 (d, 2H, *J* = 9.2 Hz), 5.47 (d, 1H, *J* = 2.4 Hz),
5.13 (dd, 1H, *J* = 8, 1.6 Hz), 4.73 (AB_q, 2H), 4.38 (d, 1H, *J* = 8 Hz), 4.22 (m, 3H), 3.89 (s, 3H), 3.65 (dd, 1H, *J* = 6.4, 3.2 Hz), 3.50 (s, 3H), 2.11 (s, 3H), 2.08 (s, 6H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 198.82, 189.92, 170.51, 170.41, 169.77, 165.13, 132.74, 124.82, 114.33, 101.92, 79.25, 72.23, 70.61, 70.13, 65.83, 61.66, 56.84, 55.66, 20.91, 20.76.

Results & Discussion

Figure 3 shows the molecules that were synthesized. **3** and **4** were synthesized by Pd catalyzed cross coupling (Songashira coupling)[4] with 4-iodoanisole from **1** and **2**. The yields were low, 31 and 45 %. Before the reaction was succeeded a reaction between **1** and bromobenzene[5] was done, but no product was formed. In the beginning it was a clear red reaction mixture and after stirring overnight a black-brown precipitate was formed. Then a reaction between **1** and 4-bromoanisole was done. Instead of acetonitrile, THF was used as

solvent. After addition of triethylamine the yellow solution turned black-brown and the result was the same as previous reaction. A reaction between 4-bromoanisole and propargyl alcohol[4] was performed, but no product was formed. In the next reaction 4-iodoanisole was used instead of 4-bromoanisole and the reaction succeeded, 4-bromoanisole was coupled to propargyl alcohol. The product was only confirmed by MS and not NMR. 4-iodoanisole is more reactive than 4-bromoanisole because C-Br bond is stronger than C-I bond. In the synthesis of **3**, the product was purified easily, but **4** was more difficult. Impurities and the product co-eluted when DCM/MeOH, 10:1, was used. Impurities were eluted with ethyl acetate/heptane, 1:1. Then the product was eluted with DCM/MeOH, 10:1.
5 was synthesized by oxidation of alkyne with $KMnO_4$[6] and the yield was quite low, 45 %. **5** was purified easily. The oxidation of **4** was tried out and product was confirmed by MS. But other impurities were formed which could be seen on TLC. The product could not be isolated. **4** contains three hydroxyl groups, which means high solubility in water. Instead of pouring the reaction mixture in water, the solvent was removed and column chromatography directly on the reaction mixture. But the product could not be isolated.
The last step was deacetylation of **5** to get the target molecule. There were three positions to be deacetylated. It was difficult to follow the reaction by TLC because more than one spot could be seen. MS was used instead and after one hour, one and two deacetylated groups were formed. After four hours the target molecule was formed but the other byproducts were still present. The reaction mixture was stirred overnight and only product peak was seen on MS. The product could not be isolated and the reaction was done in a small scale, 10 mg. Same reaction was performed and HPLC was used for purification. Two peaks could be seen on the chromatogram and the first peak was smaller than the second peak. The first peak had the mass of the product and it was difficult to see if product was presence by proton NMR. The large peak was also analyzed by MS and NMR. It seemed like instead of forming the product, 4-methoxybenzoic acid was formed instead.

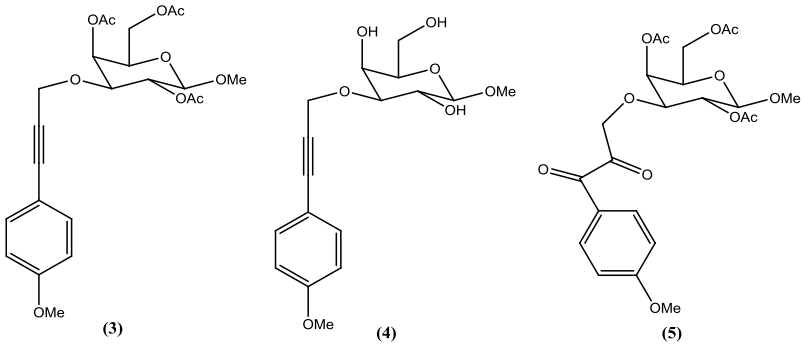


Figure 3. Synthesized molecules

Conclusions

Unfortunately the target molecule could not be synthesized. But it is only one reaction from it, either by deacetylation of **5** or oxidation of **4**. It is better to find a way to oxidize **4**, because then deacetylation is not needed. Instead of using $KMnO_4$ other methods can be tried. Like ozonolysis, oxidation by $RuCl_3$[7], I_2/DMSO[8], methylrhenium trioxide[9] and diphenyl diselenide[10]. Also the yields of **3**, **4** and **5** are quite low, optimizing the methods can help because working in small scale was difficult.

References

[1] C. T. Öberg, H. Leffler, U. J. Nilsson, *Inhibition of Galectins with Small Molecules*, CHIMIA, **2011**, 65, 1-6.

[2] J. M. Mathews, S. L. Watson, *Reaction of the Butter Flavorant Diacetyl (2,3-Butanedione) with N-α-Acetylarginine: A Model for Epitope Formation with Pulmonary Proteins in the Etiology of Obliterative Bronchiolitis*, J. Agric, Food Chem., **2010**, 58, 12761-12768.

[3] G. O. Spessard, G. L. Miessler, *Organometallic Chemistry,* 2:nd edition, Oxford University Press, **2010**, 601-607.

[4] M. A. Franks, E. A. Schrader, *Oxathiolene oxide synthesis via chelation-controlled addition of organometallic reagents to alkynols followed by addition of sulfur electrophiles and evaluation of oxathiolene oxides as anticarcinogenic enzyme inducers*, Bioorganic & Medicinal Chemistry, **2005**, 13, 2221-2233.

[5] S. Nishikawa, M. Sato, *Convenient Synthesis and Cytokinin Activity of β-Substituted 4-Styrylpyridines, the Simplest Cytokinin Analogs with a Moderate Cell Division-Promoting Activity,* J. Agric. Food Chem., **1996**, 44, 1337-1342.

[6] J. H. Tatlock, *Oxidation of Alkynyl Ethers with Potassium Permanganate. A New Acyl Anion Equivalent for the Preparation of α-Keto Esters,* J. Org. Chem., **1995**, 60, 6221-6223.

[7] J. Tummatorn, P. Khorphueng, *Convenient synthetic route to a dehydrorotenoid via selective intramolecular aldol condensation of 1,2-diaryl diketone*, Tetrahedron, **2007**, 63, 11878-11885.

[8] M. L. Keshtov, N. M. Belomoina, *Synthesis of new 4,4'-bis(arylethynyl)benzophenones and 4-4'-bis(arylglyoxalyl)benzophenones based on chloral*, Russian Chemical Bulletin, **1996**, 45, 630-632.

[9] Z. Zhu, J. H. Espenson, *Oxidation of Alkynes by Hydrogen Peroxide Catalyzed by Methylrhenium Trioxide,* J. Org. Chem., **1995**, 60, 7728-7732.

[10] M. Tingoli, M, Mazzella, *Elemental Iodine or Diphenyl Diselenide in the [Bis(trifluoroacetoxy)-iodo]benzene-Mediated Conversion of Alkynes into 1,2-Diketones,* Eur. J. Org. Chem., **2011**, 399-404.