Synthesis of a fluorescein-labelled *N*-acetyllactosamine derivative for use in fluorescence polarization studies with galectins.

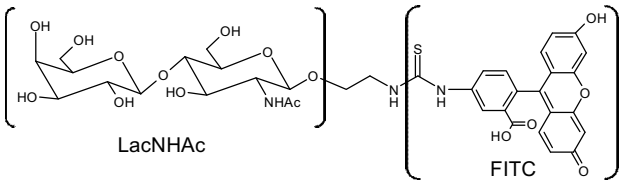
Thomas Andén, Pernilla Sörme and Ulf J. Nilsson*.

Department of Bioorganic Chemistry, Center of Chemical Engineering, Lund Institute of Technology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden.

RECEIVED DATE (automatically inserted by publisher); ulf.nilsson@bioorganic.lth.se

It is quite clear that carbohydrates play an important role inside the body. They are our main source of energy but are used in the body for much more than energy [1]. They can for instance act as recognition molecules for antibodies in the blood [2]. This project is connected to another type of proteins, namely galectins. Galectins are a class of proteins called lectins that recognize carbohydrates, more specifically β-galactosides. Galectins have been studied for a long time and they have been suggested to be involved in tumor growth and metastasis, as well as in regulating immune responses [3]. In this project a fluorescent derivative of *N*-acetyllactosamine (LacNHAc) has been synthesized in order to allow measurement of its binding to galectins. The fluorescent derivative was obtained by connecting the fluorophore fluorescein-5-isothiocyanate to the 2-aminoethyl glycoside of LacNHAc.

Figure 1. 2-(fluorescein-5-thiourea)-ethyl (β-D-galacto-pyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-gluco-pyranoside) (**1**).



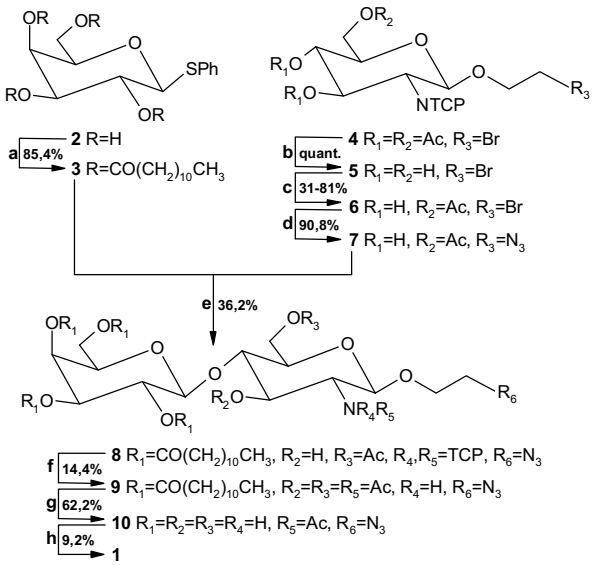
The starting materials for this molecule can be seen in scheme 1. **2** are a galactose and it is fitted with ester protective groups, which are lauroyl tails, to produce **3** (the donor). These tails were put there to be able to be used for C18 separation later in the synthesis [4]. **4** are a fully acetylated glucose derivative, which is deprotected to give **5** and then protected again but with different conditions give **6**. To be able to do this final step, *sym*-collidine was used to react with the acylchloride to form the hindered acylcollidinium chloride, which preferentially reacts with the primary alcohol. Finally, compound **6** were transformed from a bromide to the azide **7** (the acceptor).

In the next step, a glycosylation were made with **3** and **7** to produce the wanted disaccharide **8**. The β-configuration was obtained by the use of protection group participation [5]. The first thing to happen in this reaction, is that the thio group is directly activated by the iodonium ion generated from the promotor NIS, thereby forming an oxocarbenium ion. The lauroyl group then forms a complex with the oxocarbenium ion called an acyloxonium ion. This ion together with the steric hindrance of the 3-OH by the NTCP group on the acceptor guides the acceptor to form the wanted β-(1→4) coupling [6].

The next two steps in the synthesis consisted of removing the different protection groups. First the NTCP group was removed almost as described in the literature [7, 8]. The differences were that the temperature were lowered from 60-80ºC to rt and that the acetylation conditions were run in a solution of Pyridine:Ac_2O instead of MeOH:Ac_2O:H_2O. However, the NTCP group presumably only opened halfway [9] and the reaction therefore had to be remade at 74ºC and even though TLC showed at least six different products, the wanted **9** could be collected.

The second deprotection step could be done easily by treatment of metanolic sodium metoxide since all of the remaining protection groups were esters.

Scheme 1. The reaction sequence.



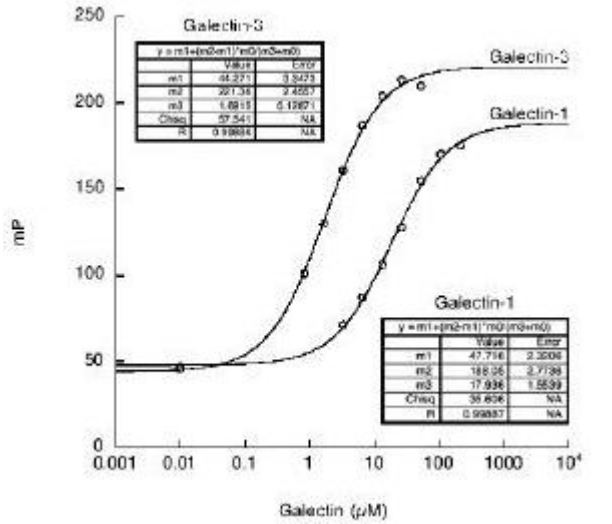
Conditions: a) Heptane, DMAP, pyridine, lauroylchloride, 0ºC→rt, 64 h. b) MeOH, AcCl, rt, 18.5 h, Ar. c) CH_2Cl_2, *sym*-collidine, AcCl, Ar, -26ºC, 1.5 h. d) DMF, 15-crown-5, NaN_3, 24 h. e) CH_2Cl_2, NIS, TfOH, 4Å MS, Ar, 2 h at -45ºC, then allowed to reach rt f) i) EtOH, 1,2-diaminoethane, Ar, 25 h, then Pyridine:Ac_2O 23.5 h; ii) EtOH, 1,2-diaminoethane, Ar, 21 h at 74ºC, then Pyridine:Ac_2O, 24 h. g) MeOH, MeONa-MeOH, over night, then Duolite C436. h) i) EtOH, HCl, Pd/C, H_2, 2 h, then filtered through Celite; ii) aq. 0.1 M $NaHCO_3$ with 0.9% NaCl (pH 9.3), FITC-DMSO.

In the final step of this synthesis, FITC was to be coupled with the LacNHAc derivative, thereby forming compound **1**. This was done in two steps. In the first the azide of **10** were transformed to an amine by catalytical hydrogenation at rt and at a low pressure. In the second step, the produced amine could be coupled with FITC with conditions quite similar to those described in the literature [10]. After three different separations, 0,59 mg of **1** was obtained and even though the amount is quite small, it can be

used to run about 44435 different tests or about 463 microwell plates since each test requires approximately 16 pmole/well.

Compound **1**, later prepared on a larger scale by Pernilla Sörme, was tested against galectin-1 and -3 by fluorescence polarization [11] and the resulting curves can be seen in figure 2. The regression results provide the dissociation constant K_d (K_d=m3).

Figure 2. Fluorescence polarization measurements with compound against galectin-1 and -3.



These results show that galectin-3 (K_d=1.69 ìM) has a higher affinity for the fluorescein-labeled LacNHAc, 2-(fluorescein-5-thiourea)-ethyl (β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranoside) **(1)**, than galectin-1 (K_d=17,9 ìM).

In conclusion, although several synthetic and practical problems were encountered, the wanted fluorescence-labelled ligand was obtained and could indeed be used for monitoring galectin-1 and -3 binding activities.

References

(1) Stryer, Lubert, *Biochemistry, Fourth Edition*, W. H. Freeman and Company, 1995.
(2) Vander, Arthur, Sherman, James, Luciano, Dorothy, *Human Physiology, the Mechanisms of Body Function, Seventh Edition*, The McGraw-Hill Companies, Inc., 1998, pg. 718.
(3) Sörme, Pernilla, Qian, Yuning, Nyholm, Per-Georg, Leffler; Hakon, Nilsson, Ulf J. (2002) *ChemBioChem*, 3, 183-189.
(4) Nilsson, Ulf J., Fournier, Eric J.-L., Hindsgaul, Ole (1998) *Bioorg. Med. Chem.* 6:1563-1575.
(5) Binkley, Roger W., *Modern Carbohydrate Chemistry*, Plenum Press, 1998, pgs. 307-311.
(6) Ellervik, Ulf (1998), *Synthetic Analogs of Sialyl Lewis X*, doctoral dissertation, Lund University, pg. 70.
(7) Debenham, J. S., Madsen, R., Roberts, C., Fraser-Reid, B. (1995), *J. Am. Chem. Soc.* 117, 3302-3303.
(8) Castro-Palomino, J. C., Schmidt, R. R. (1995), *Tetrahedron Lett.* 36, 5343-5346.
(9) Ellervik, Ulf, personal communication.
(10) Oda, Yasuo, Kinoshita, Mitsuhiro, Nakayama, Katsuyoshi, Kakehi, Kazuaki (1998), *Biol. Pharm. Bull.* 21:1215-1217.
(11) *Fluorescence Polarization, Technical Resource Guide, Third Edition*, PanVera, The Protein Company, www.panvera.com.