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Synthesis of multivalent *N*-acetyl lactosamine modified quantum dots for the study of carbohydrate and galectin-3 interactions

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ABSTRACT

Ternary core/shell CdSeS/ZnS-QDs coated with *N*-acetyl lactosamine was prepared as a fluorescent probe to study the interactions of *N*-acetyl lactosamine and galectin-3. The synthesis of *N*-acetyl lactosamine was achieved through the 'azidoiodoglycosylation' method. The amount of ligand coated on QDs was determined by ^{1}H NMR and ICP-OES. The interactions between carbohydrates and galectin-3 were measured using SPR. The results revealed that the affinity of galectin-3 with di- and multivalent *N*-acetyl lactosamine increased 20 and 184-fold, respectively. The prepared glyco-QDs could be used as an efficient fluorescent probe to study carbohydrates and galectin-3 interactions.

1. Introduction

Galectin-3 is a member of the β-galactose-binding protein family and plays a key role in initiating the adhesion of human breast and prostate cancer cells to the endothelium by specifically interacting with the cancer associated carbohydrate, T antigen.[1] Moreover, it was shown that galectin-3 possesses anti-apoptotic activity[2,3] in several tumor cell types. The mechanism of action of galectin-3 is not clear yet. And there is a high interest to discover high affinity inhibitors that could be used as tools for probing galectin-3 functions and as lead compounds in the development of therapeutics.[4c]

Lactose and *N*-acetyl lactosamine (LacNAc) are the natural ligands of galectin-3, but they have weak dissociation constants in the mM range. Two main approaches have been used to enhance their binding affinity. One is the structural modification of LacNAc or lactose. Nilsson et al. have reported that the 3-OH of galactoside is located in the extended groove of the protein and chemical modifications at this position can enhance the affinity greatly.[4] Pieters reported that thiodigalactoside also has strong affinity to galectin-3.[5] However, the synthesis of the high affinity free LacNAc or thiodigalactoside is tedious, which contain complicated protection, deprotection of the hydroxyl and conversion of the amine. The other approach is the preparation of multivalent compounds, which were designed to interact with more than one of the lectin's subunits. Pieters et al.[6] synthesized two, four, and eight valent lactose clusters using 3,5-di-(2-aminoethoxy) benzoic acid as the linking unit. The inhibition assays of protein and cell levels showed that galectin-3 was markedly sensitive to increased sugar valency. Compared to carrier-free lactose, the inhibitory potency of each lactose unit reached a maximum value of 144-fold. However, the valence of multivalent carbohydrates is determined by the limited branching of the linking scaffold. Moreover, fluorescence labeling is usually needed for the bioassay and organic dyes FITC[7a] and Rhodamine[7b] are often conjugated to the carbohydrates as the fluorophores.

Recently, the use of quantum dots (QDs)[8] to construct the glycoside cluster and as a fluorescent probe opens new opportunities for studying the carbohydrate–protein interactions.[9] Our previous work has demonstrated that binding of sugar molecules conjugated QDs to their target proteins can be dramatically enhanced.[10] It is easy to attach more than one hundred sugar ligands on the surface of the QDs due to its high surface/volume ratio. In addition, a new strategy with Nuclear Magnetic Resonance (NMR) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) has been used to analyze the coated polysaccharide molecules and the inner structure of the core and shell of the glyco-QDs.[10,11]

Herein we used the ternary core/shell CdSeS/ZnS-QDs to construct multivalent *N*-acetyl lactosamine clusters, which has

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stronger binding affinity with galectin-3 than lactose. An efficient azidoiodo-glycosylation reaction was used to synthesize LacNAc ligand. We expect the high affinity fluorescent probe will be useful in the study of carbohydrates and galectin-3 interactions.

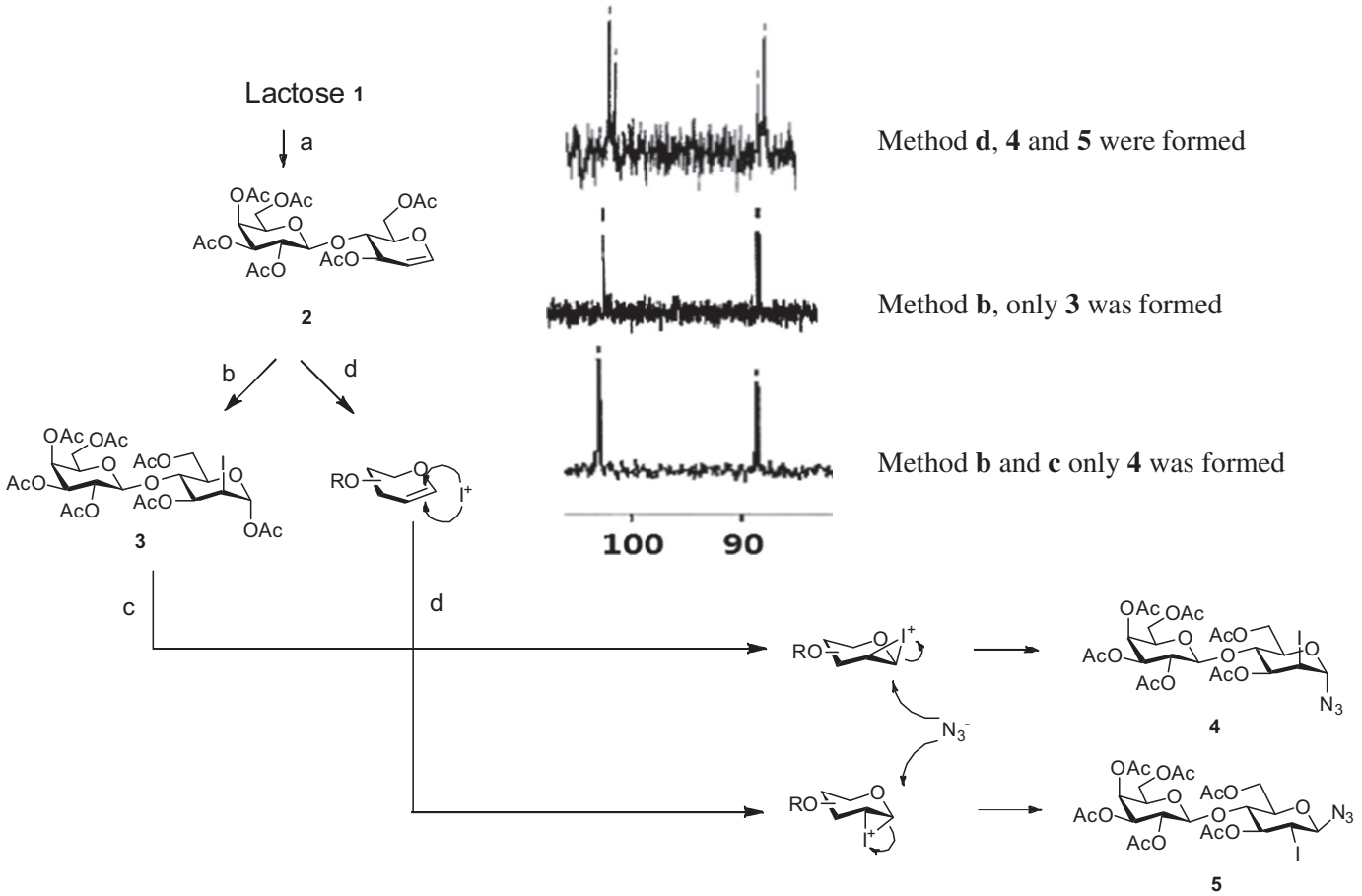
2. Results and discussion

2.1. Synthesis of mono and bivalent *N*-acetyl lactosamine

It is difficult to synthesize oligosaccharide fragments containing hexosamine because of the low activities and high steric hindrance induced by amine protecting groups. The yield and stereoselectivity of the glycosylation using 4-hydroxy group of *N*-acetyl glucosamine derivatives as acceptors are not satisfactory.[12] Therefore, lactal is often used as the starting material in the synthesis of LacNAc. We have reported a synthesis of divalent *N*-acetyl lactosamine using lactal as the starting material, which needs much more changing of the amino protecting groups and the α,β selectivity was influenced by the strength of the promotor.[13] In order to improve the selectivity and synthesis route, Lafont et al.[14] reported a convenient method to synthesize the β-glycosides of *N*-acetyl lactosamine through an azidoiodo-glycosylation reaction. In this method 2-iodo lactosyl azide **4** was used as the donor and PPh_3 as Staudinger reducer and Lewis acid. This methodology is easier to carry out and more efficient. Herein we used this method to prepare ligands containing *N*-acetyl lactosamine.

In order to obtain β glycosylation product, intermediate 1,2-*trans*-2-deoxy-2-iodo lactosyl azides with α-D-*manno* configuration **4** was needed. Under the condition **d** (Scheme 1), two configurations **4** and **5** (β-D-*gluco* configuration) were obtained. Unfortunately they were inseparable by column chromatography, even after they were reacted with the acceptor. Finally, another intermediate **3** was synthesized first. Iodoacetoxylation of acetylated lactal **2** was achieved by reaction of iodine in the presence of cupric acetate monohydrate in HOAc at 80 °C. This reaction showed high stereoselectivity, only **3** can be obtained by recrystallization with ethanol or purification by column chromatography after the reaction. Treatment of **3** with trimethylsilyl azide ($TMSN_3$) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) afforded the donor **4** in high yield (Scheme 1). The configuration and structure of **4** were confirmed by ^1H NMR ($J_{1,2}$=2.7 Hz), ^{13}C NMR (δ 90.71, C-1), and IR (ν_{N3} 2119 cm^{-1}).

After the synthesis of the donor **4**, azidoiodo-glycosylation reaction was used to synthesize the fully protected mono **9** and bivalent **7** LacNAc (Scheme 2). Typically, **4** and the alcohol were dissolved in CH_2Cl_2 and triphenylphosphine in CH_2Cl_2 was added dropwise. The reaction mixture was stirred overnight at room temperature. Exchange with anion resin (Dowex 2X8 (OH^-)) and treatment with catalytic amount of sodium methoxide in methanol afforded the amino compound. Acetylation under classical conditions (Ac_2O/Py) yielded the fully protected compound. Compared with other strategies, this method achieved glycosylation, azido reduction, and amine acetylation in one pot and no purification was needed. Moreover, this glycosylation reaction has high stereoselectivity and only β product was obtained. Deacetylation of **7** and **9** with sodium methoxide in methanol afforded mono **10** and bivalent LacNAc **8**, respectively.



Scheme 1. Synthesis of the donor. (a) (i) CH_3COBr/MeOH, 95%; (ii) Zn/50% HOAc, 71%; (b) I_2, $CuAc_2 \cdot H_2O$, HOAc, 80 °C, overnight, 77.2%; (c) $(CH_3)_3SiN_3$, TMSOTf, CH_2Cl_2, 4 Å MS, Ar, rt 52 h, 87.5%; (d) NaN_3, ICl, CH_3CN, 0 °C, 30 min, 76%.