Tetrahedron 68 (2012) 7148–7154

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

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

Yang Yang [a], Xiao-Chao Xue [a], Xiao-Feng Jin [a], Li-Jun Wang [b], Yin-Lin Sha [b], Zhong-Jun Li [a,*]

[a] *State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China*
[b] *Single-molecule & Nanobiology Laboratory, Department of Biophysics, School of Basic Medical Sciences and Biomed-X Center, Peking University, 38 Xueyuan Road, Beijing 100191, China*

ARTICLE INFO

Article history:
Received 26 February 2012
Received in revised form 30 May 2012
Accepted 8 June 2012
Available online 15 June 2012

Keywords:
Glyco-quantum dots
NMR
SPR
Galectin-3
N-Acetyl lactosamine
Cluster effect

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

* Corresponding author. Fax: +86 01 8280 5496; e-mail addresses: zjli@bjmu.edu.cn, zjli@mail.bjmu.edu.cn (Z.-J. Li).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.06.035

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.

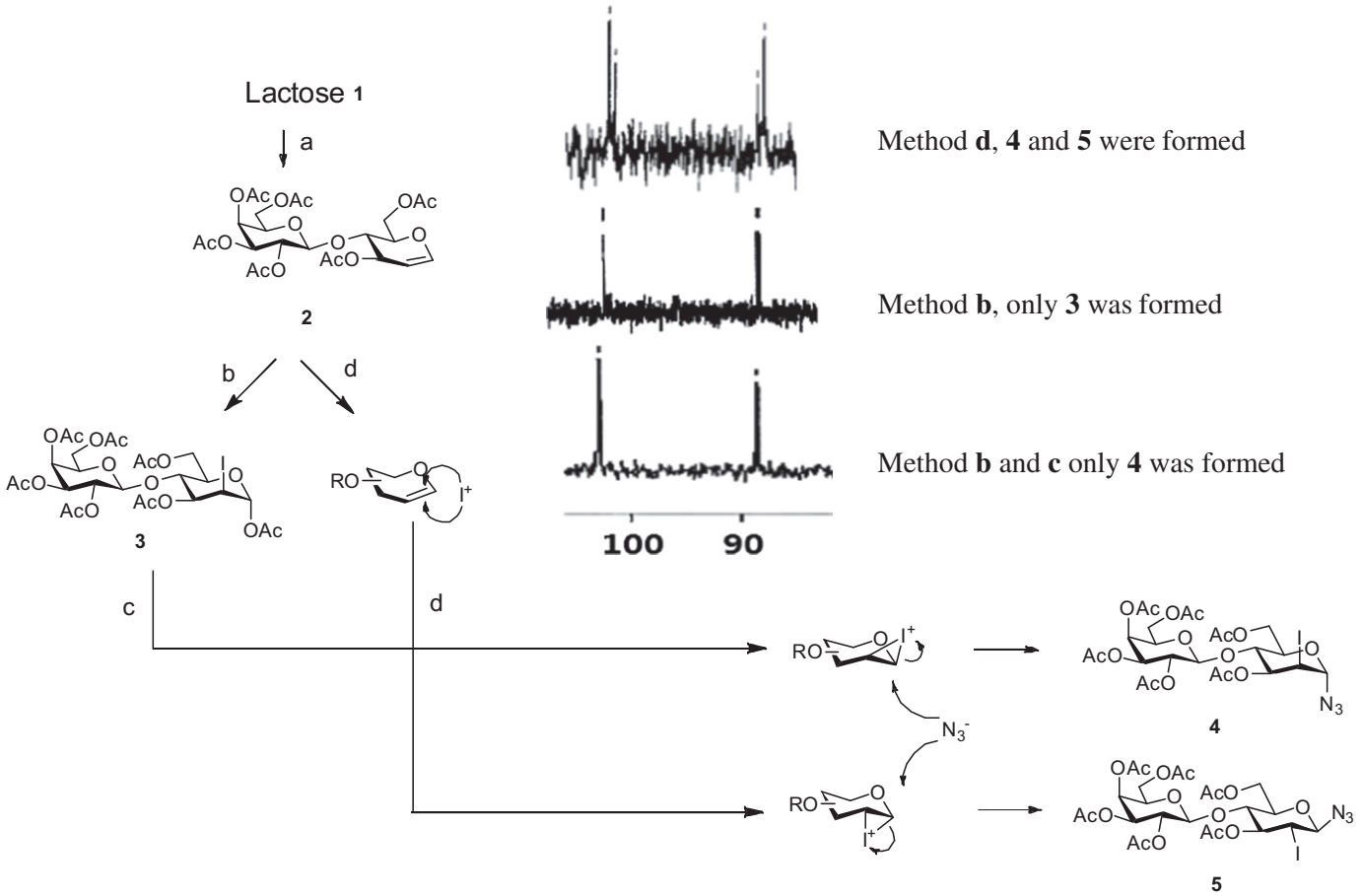
2. Results and discussion

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

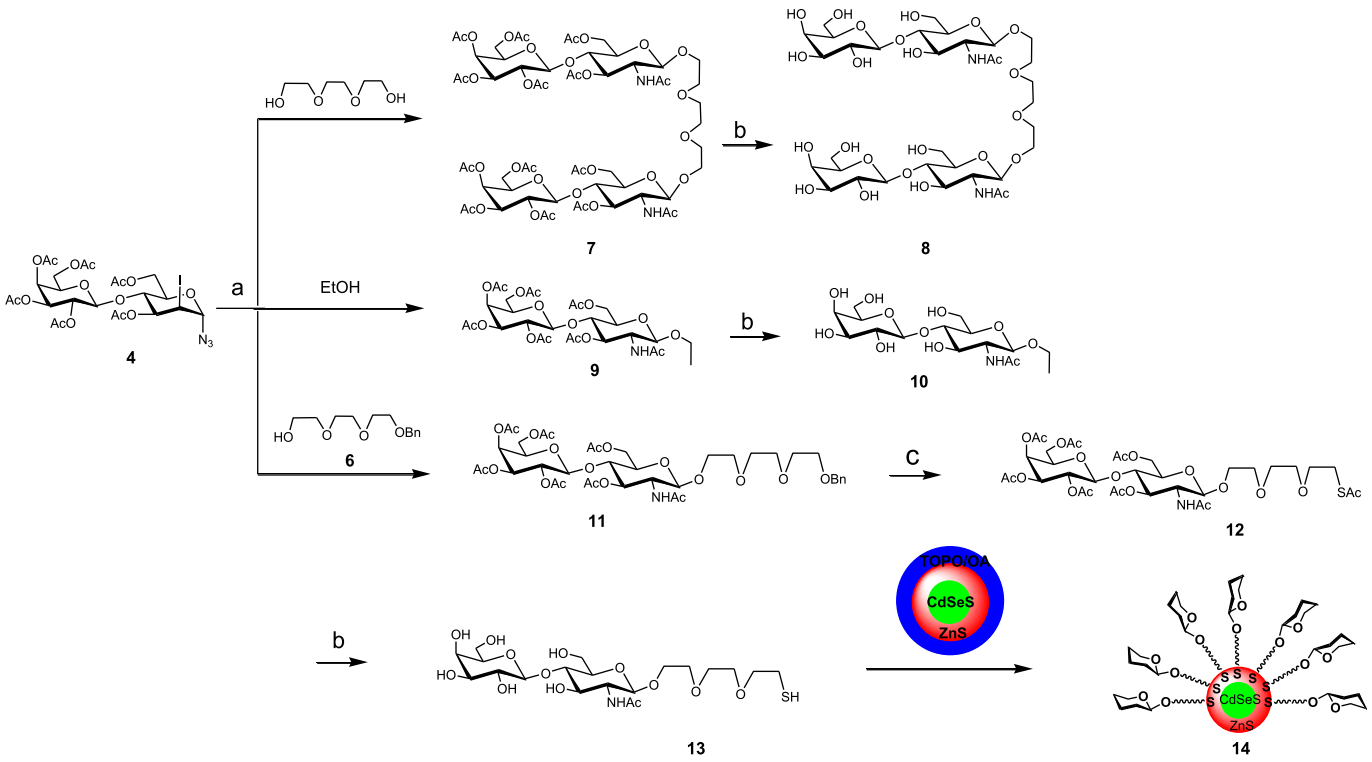
It is difficult to synthesize oligosaccharide fragments containing hexosamine because of the low activities and high steric hindrance induced by amine protecting groups. The yield and stereoselectivity of the glycosylation using 4-hydroxy group of *N*-acetyl glucosamine derivatives as acceptors are not 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%.



Scheme 2. Synthesis of the glyco-QDs and mono and bivalent LacNAc. (a) (i) CH_2Cl_2; (ii) Dowex OH-; (iii) CH_3ONa/CH_3OH; (iv) Ac_2O/Py; (b) CH_3ONa/CH_3OH; (c) (i) H_2, $Pd(OH)_2/C$; (ii) MsCl/Py; (iii) KSAc/butanone.

2.2. Synthesis of QDs

QDs ($\lambda_{em,\ max}$=534 nm) were prepared according to previous reports with minor modifications.[8d,e] The prepared CdSeS/ZnS-QDs displayed a photoluminescence emission ($\lambda_{em,\ max}$=534 nm) with the full width at the half maximum (FWHM) of less than 30 nm. The diameter of the QDs is 4.5±0.5 nm confirmed by Transmission electron microscopy (TEM) (see Supplementary data).

2.3. Synthesis of LacNAc-QDs

In order to conjugate the sugar onto the QDs surface, a thiol group was attached through a PEG linker. Mono benzyl protected PEG (n=3) **6** was prepared first, which can be achieved in the presence of freshly prepared silver (I) oxide and benzyl bromide in excellent yield (94%).[15] Benzyl-protected PEGylated *N*-acetyl lactosamine **11** can also be synthesized through azidoiodoglycosylation reaction as described above. Introduction of a thiol group to **11** was achieved in four steps (Scheme 2), and the ligand **13** for the preparation of glyco-QDs was finally synthesized.

We have reported a facile method to fabricate glyco-QDs using two phase reaction system.[16] Typically, the CdSeS/ZnS-QDs (λ_{em}=534 nm) (13 mg) was first dispersed in chloroform (10 mL), followed by the addition of a solution of LacNAcPEGSH **13** (65 mg) in 50 mM phosphate buffered saline (PBS 30 mL, pH 8.0). Unlike 1-thiol sugar used in our previous reports,[10,11] **13** is very sensitive to the air and easily oxidized to form disulfide, which cannot be coated on QDs. Therefore, the coating of QDs must be operated under argon. After vigorously stirred for 2 h at room temperature, the lower layer became colorless and the upper became green, indicating the reaction was finished. The upper aqueous layer was subsequently collected and purified with Sephdex G-70, followed by lyophilization to afford a yellow powder.

2.4. Determination of the glyco-QDs formula

To quantitatively determine the number of sugar molecules on QDs surface, we have developed a facile strategy using NMR, which can provide more structural information and does not destroy the glyco-QDs.[10,11] In our previous works methanol and acetonitrile were used as internal standards (IS) to quantitatively determine the amount of the ligands on QDs and glyco-QDs, respectively. In this study, due to the NMR peak of methyl group in acetonitrile (singlet, δ near 2.0) overlap with the *N*-acetyl group (singlet, δ near 2.0), we used ethanol as IS, in which the methyl group (triplet, δ near 1.05) displays NMR signal different from **13** (Fig. 1).

When the thiol compound **13** attached on the surface of QDs, the NMR peak of the $-CH_2SH$ in the PEG linker is almost missing. Similar observation has been reported by Penadés, S. et al. on lactose and Le^x capped gold-nanoparticles.[17] In this experiment, the

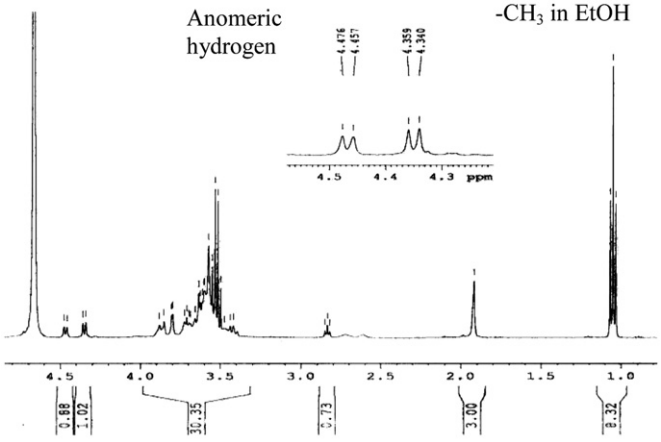


Fig. 1. ^1H NMR of LacNAcPEG-QDs with ethanol as IS.

accurate integration of the anomeric or the *N*-acetyl could be used to calculate the amount of **13** on the QDs surface.

Based on the NMR data, we calculated the amount of the sugar ligand on QDs was 44.7% of total weight. Together with ICP-OES data and the size of the sugar and QDs, the formula of the glyco-QDs was determined to be $[Cd_{171}Se_{12}S_{356}\text{-}(ZnS)_9]$@$(LacNAc\text{-}PEGS)_{108}$. The number of the sugar molecules on the QDs surface was in agreement with previous reports for the carbohydrate-encapsulated QDs with 5 nm diameter,[18] 6 nm gold nanoparticles[19] and our 4.5 nm lactose and galactose coated QDs.[11]

2.5. Affinity study of glyco-QDs with galectin-3

The binding of galectin-3 to glyco-QDs was measured using a BIACORE 3000 instrument (Biacore AB, Uppsala). Two *N*-acetyl lactosamine derivatives, LacNAcOEt **10** and $(LacNAc)_2$PEG **8**, were tested as mono and bivalent ligands, respectively. Although Surface Plasmon Resonance (SPR) has been used for the investigation of galectin–carbohydrate binding, lectin was often immobilized to the sensor surface rather than the glycan moiety,[20] because of the low sensitivity induced by the low molecular weight of the glycan. In order to compare the binding of different carbohydrates containing *N*-acetyl lactosamine to galectin-3, we immobilized the lectin to the sensor chip.

Fig. 2 showed the kinetic binding curves of the three sugar conjugates **8**, **10**, and **14** with galectin-3. All three compounds displayed binding to galectin-3. Fig. 2a showed that the bound amount 'Diff. Response (RU)' of **10** was small even at high concentration of 50 mM, because of weak binding. However, the binding of bivalent carbohydrate **8** was approximately five times higher than that of **10** at the same concentration (Fig. 2b), which can be explained by the cluster effect.[21] This result also showed that the galectin-3 was sensitive to the sugar valency.[6] Indeed, the largest amount of sugar binding on the sensor was observed for the glyco-QDs, even at lower concentration (<0.1 μM). Moreover, the glyco-QDs cannot be removed unless using the regeneration buffer (0.05 M NaOH), indicating a strong binding. Furthermore, the data indicated that *N*-acetyl lactosamine retained its specific binding to galectin-3 after conjugating onto the QDs surface.

In order to further quantitatively study the interactions of glycoconjugates and galectin-3, the K_D of each ligand binding to galectin-3 were calculated (Table 1). The sensorgrams were analyzed with the software provided by the instrument manufacturer (BIAEVAL 4.1). It was found *N*-acetyl lactosamine **10** having K_D=5.26 μM, a value consistent with that obtained by Murakami et al. (2.20 μM).[22] It should be noted that they immobilized the sugar on the chip, and the galectin-3 was flown over the sensor. Due to the high molecular weight and the aggregation behavior[23] of the galectin-3, a higher RU could be observed. Nilsson et al.[4c] used isothermal titration microcalorimetry (ITC) to investigate the interaction between galectin-3 and LacNAc. The K_d they reported was 82 μM. It should be emphasized that the ITC method measures the solution–solution carbohydrate and protein interactions, which is different from the solution-solid interactions measured by SPR.[24] The different phase interactions may affect the kinetic and dynamic properties of the carbohydrate–protein interactions.

Because the precise number of the ligand in target interactions in SPR experiment is unknown, an enhancement factor β ($K_{mono}/K_{multivalent}$),[25] which defines the relative affinity of a multivalent interaction is introduced. The bivalent $(LacNAc)_2$PEG **8** showed 20-fold higher affinity than the mono LacNAc **10**, and glyco-QDs **14** showed 184-fold higher affinity than the mono LacNAc **10**.

It should be also noticed that, it is very hard to determinate the formula of the glyco-QDs. Although we have given an approximate formula, it is only estimations. Therefore, the molar concentration could not be accurately calculated. On the other hand, different

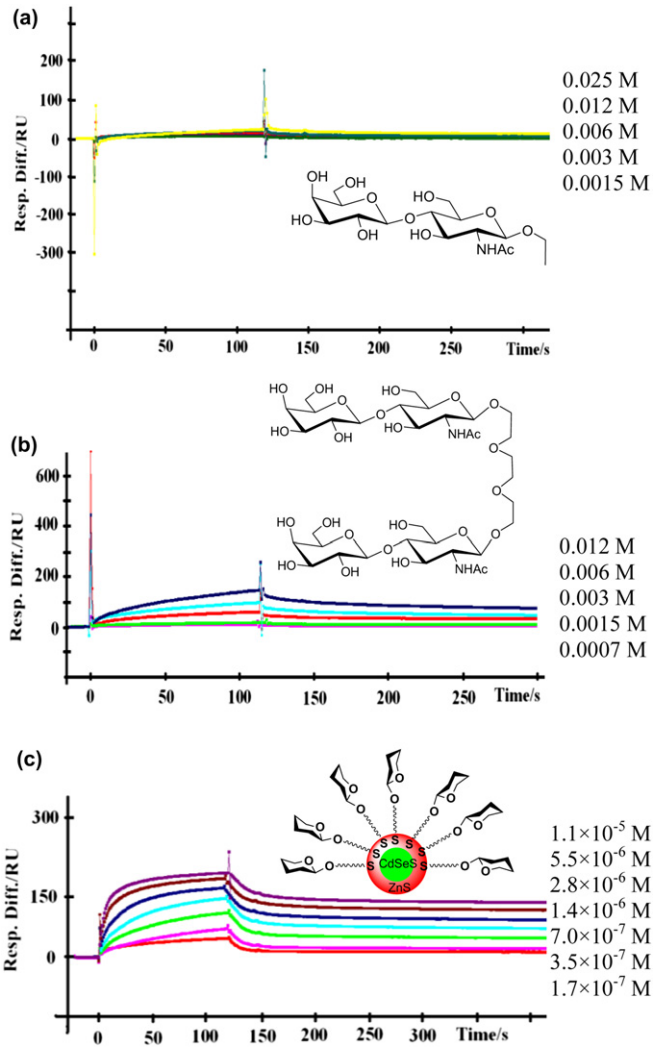


Fig. 2. SPR sensorgrams of three glycoconjugates binding to galectin-3 presenting surface.

method measure different carbohydrate–protein interaction phenomena, the enhancement of the cluster glycoside effect can also be different.[21] Our data showed the binding affinity of *N*-acetyl lactosamine to galectin-3 can be dramatically enhanced by cluster effect.

2.6. Fluorescent properties of the glyco-QDs

QDs have attractive optical properties, such as precisely tunable and sharp photoluminescence (PL), broad absorption cross section, high quantum yield (QY), and good photostability.[26] As shown in Fig. 3, the fluorescence emission peak of QDs was obtained at 534 nm. After being coated with carbohydrates, the maximum wavelength of the fluorescence emission exhibited small red shift. The full width at half maximum (FWHM) of the QDs and the glyco-QDs is less than 30 nm, which is much narrower than the organic dyes.

Table 1
Dissociation constants (K_D[a]) of three sugar ligands

Entry	K_D (μM)	β ($K_{free}/K_{multivalent}$)
LacNAcOEt	5.26	1
$(LacNAc)_2$PEG	0.25	20
LacNAcPEG-QDs	5.7×10^{-2}	184

[a] $K_D=k_d/k_a$.

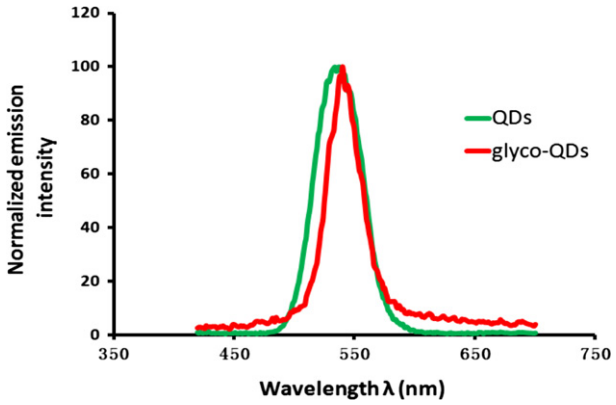


Fig. 3. The fluorescence spectrum of QDs and glyco-QDs.

3. Conclusion

We have prepared *N*-acetyl lactosamine, bivalent *N*-acetyl lactosamine, and *N*-acetyl lactosamine coated QDs. The bioassay using SPR showed that coating of *N*-acetyl lactosamine on QDs surface can dramatically enhance its binding to galectin-3 through cluster effect. The *N*-acetyl lactosamine coated QDs could be used as an efficient fluorescent probe to assess the carbohydrate–protein interactions and possibly to image the glycoproteins and their cellular activities.

4. Experimental section

4.1. General information

All solvents were dried prior to use. When dry conditions were required, the reactions were performed under an argon atmosphere. Thin-layer chromatography (TLC) was purchased from Liangchen Chemical Engineering Co. Ltd. (Anhui Province). All compounds were stained with 5% H_2SO_4 in ethanol followed by heating. Detection with UV light was employed when possible. Flash column chromatography was performed on silica gel 200–300 mesh. The boiling range of petroleum ether used as fluent in column chromatography is 60–90 °C. NMR spectra were recorded on a JEOL-300 (300 MHz) or Bruker AMX-400 (400 MHz) instruments. Chemical shifts (δ) were reported in parts per million downfield from TMS, the internal standard; *J* values were given in Hertz. Mass spectra were recorded on a Bruker Apex IV FTMS instrument. Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) was recorded on Thermo-iCAP 6000. Surface Plasmon Resonance (SPR) measurements were carried out with BIAcore 3000 instrument using carboxymethylated sensor chips (CM5, BIAcore). Galectin-3 was purchased from Sigma–Aldrich (G5170).

4.2. Synthesis of mono, bivalent LacNAc and other sugar ligand

*4.2.1. 3,6,2′,3′,4′,6′-Hexa-O-acetyl-D-lactal (**2**).* Acetyl bromide (0.92 mL, 12.3 mmol) and CH_3OH (0.5 mL, 12.30 mmol) were added to AcOH (10 mL) and stirred at room temperature (protected from light) for 15 min, followed by addition of lactose (1.0 g, 3.08 mmol) and Ac_2O (3.4 mL, 35.97 mmol). The reaction mixture was further stirred overnight at room temperature and the solution was diluted with chloroform, washed with saturated solution of $NaHCO_3$, and dried with Na_2SO_4. The solvent was removed, and cold ether was added to give 2,3,6,2′,3′,4′,6′-sept-*O*-acetyl-α-D-lactosyl bromide (2.15 g, yield: 95%) as a white solid. Then the product was dissolved in 50% AcOH/H_2O (25 mL), zinc dust (2.04 g, 31.4 mmol) was added at 0 °C. The reaction mixture was stirred for 1.5 h at 0 °C when TLC analysis showed the reaction was complete. The mixture was filtered and extracted with CH_2Cl_2. The combined organic extracts were washed successively with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), filtered, and concentrated. Purification by column chromatography (petroleum ether/EtOAc 2.5:1, v/v) afforded hexa-*O*-acetyl-D-lactal (**2**) (0.74 g, 71%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 3.91 (ddd, 1H, *J*=10.0, 4.2, 2.0 Hz, H-5′), 4.00 (dd, 1H, *J*=5.4, 7.2 Hz, H-5), 4.08 (dd, 1H, *J*=2.0, 12.4 Hz, H-6b′), 4.15 (dd, *J*=12.4, 4.2 Hz, H-6a′), 4.18 (dd, 1H, *J*=2.4, 11.6 Hz, H-6b), 4.21 (dd, 1H, *J*=6.0, 5.4 Hz, H-4), 4.44 (dd, 1H, *J*=2.4, 11.4 Hz, H-6a), 4.66 (d, 1H, *J*=8.0 Hz, H-1′), 4.84 (dd, 1H, *J*=6.4, 3.2 Hz, H-2), 5.01 (dd, 1H, *J*=3.6, 10.4 Hz, H-3′), 5.19 (dd, 1H, *J*=10.4, 8.0 Hz, H-2′), 5.37 (dd, 1H, *J*=9.6, 9.2 Hz, H-4′), 5.41 (dd, H, *J*=4.0, 4.4 Hz, H-3), 6.42 (d, 1H, *J*=6.0 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6, 20.8, 20.9 (6× $COCH_3$), 60.9, 61.8, 66.7, 68.8, 70.7, 74.1, 74.7, 99.0, 101.0 (C-1′), 145.4 (C-1), 169.2, 169.9, 170.0, 170.1, 170.3, 170.4 (6× $COCH_3$).

*4.2.2. 1,3,6-Tri-O-acetyl-4-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-iodo-α-D-manno-pyranose (**3**).* Hexa-*O*-acetyl-lactal **2** (1.0 g, 1.78 mmol), cupric acetate hydrate (0.39 g, 1.95 mmol), and iodine (0.55 g, 2.17 mmol) were successively added to AcOH (10 mL). The mixture was stirred overnight at 80 °C, and cooled to room temperature. CH_2Cl_2 (20 mL) was added to the mixture and the organic layer was washed with saturated aqueous $NaHCO_3$ until neutral, saturated aqueous $Na_2S_2O_3$ (15 mL), and water (10 mL). The clear solution was dried with Na_2SO_4 and evaporated. The crude product was purified by recrystallization from ethanol as a yellow syrup (1.03 g, yield: 77.2%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.34 (d, 1H, *J*=2.1 Hz, H-1), 5.38 (d, 1H, *J*=3.0 Hz, H-4′), 5.16 (dd, 1H, *J*=8.1, 10.5 Hz, H-2′), 5.00 (dd, 1H, *J*=3.6, 10.5 Hz, H-3′), 4.68 (dd, 1H, *J*=4.2, 7.2 Hz, H-3), 4.62 (d, 1H, *J*=8.1 Hz, H-1′), 4.52 (dd, 1H, *J*=2.1, 3.9 Hz, H-2), 4.44 (d, 1H, *J*=13.5 Hz, H-6a), 4.21–4.03 (m, 5H, H-6a′,4,5,6b,6b′), 3.95 (m, 1H, H-5′), 2.17–1.98 (7s, 21H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 170.0, 169.9, 169.4, 169.30, 168.4 (6× $COCH_3$), 101.3 (C-1′), 94.3 (C-1), 75.3 (C-4), 71.6 (C-5), 70.8 (C-3′), 70.6 (C-5′), 69.2 (C-2′), 69.0 (C-3), 66.6 (C-4′), 61.6, 61.0 (C-6,6′), 27.3 (C-2), 20.9, 20.8, 20.7, 20.6, 20.5, 20.4 (6× $COCH_3$).

*4.2.3. 3,6-Di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-iodo-α-D-manno-pyranosylazide (**4**).* To a solution of **3** (1.0 g, 1.34 mmol) in anhydrous CH_2Cl_2 (10 mL) were added $TMSN_3$ (0.33 mL, 2.50 mmol) and TMSOTf (0.06 mL, 0.22 mmol) under argon. Stirring was continued until complete disappearance of the starting material on TLC. The solution was then neutralized by careful addition of saturated aqueous $NaHCO_3$ and the organic layer was dried with Na_2SO_4, concentrated, and purified by column chromatography (petroleum ether/EtOAc 2:1, v/v) to give **4** (0.86 g, yield: 87.5%) as syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.64 (d, 1H, *J*=2.7 Hz, H-1), 5.38 (d, 1H, *J*=3.9 Hz, H-4′), 5.16 (dd, 1H, *J*=9.9, 9.2 Hz, H-2′), 5.00 (dd, 1H, *J*=2.4, 10.5 Hz, H-3′), 4.68 (dd, 1H, *J*=3.6, 6.9 Hz, H-3), 4.59 (d, 1H, *J*=8.0 Hz, H-1′), 4.45 (m, 2H, H-6a, 2), 4.22–4.06 (m, 4H, H-5,6b,6′a,6′b), 3.95 (m, 2H, H-5′,4), 2.17–1.98 (6s, 18H, 6× $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.1, 170.0, 169.3, 169.2, 101.3 (C-1′), 90.7 (C-1), 75.5 (C-4), 71.5 (C-5), 70.8 (C-5′), 70.7 (C-3′), 69.5 (C-2′), 68.9 (C-3), 66.7 (C-4′), 61.6 (C-6), 61.1 (C-6′), 27.5 (C-2), 20.9, 20.8, 20.7, 20.6, 20.5; IR (film KBr): ν_{N3} 2119 cm^{-1}.

*4.2.4. 2-{2-[2-(Benzyloxy)ethoxy]ethoxy}ethan-1-ol (**6**).* Ag_2O (6.9 g, 30 mmol) was added to a solution of triethylene glycol (3.0 g, 20 mmol) in dry CH_2Cl_2. BnBr (2.6 mL, 22 mmol) was then added dropwise over 5 min, and the mixture was stirred for 2 h. After this time, the suspension was filtered through a pad of Celite, which was

thoroughly washed with CH_2Cl_2. The combined filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography to yield a yellow oil (4.46 g, 92.9%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.35 (m, 5H, Ph), 4.56 (s, 2H, $-CH_2$Ph), 3.73–3.58 (m, 12H, $-OCH_2CH_2O-$), 2.80 (br, 1H, –OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.0, 128.2, 127.6, 127.5, 73.1, 72.4, 70.5, 70.4, 70.2, 69.2, 61.5.

*4.2.5. 3,6-Dioxaoct-1,8-diyl-bis[2,3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-β-D-galactopyranosyl)-β-D-glucoside]dimer (**7**).* Under argon atmosphere, compound **4** (466 mg, 0.639 mmol) and polyethylene glycol (32 mg, 0.213 mmol) was dissolved in dry CH_2Cl_2 (10 mL), then 4 Å MS was added. The mixture was stirred at 0 °C for 30 min. PPh_3 (201 mg, 0.767 mmol) was dissolved in dry CH_2Cl_2 and added dropwise to the mixture above at 0 °C. The mixture was allowed to warm to room temperature and then stirred overnight. 4 Å MS was removed by filtration, and the filtrate was concentrated to dryness. The residue was dissolved in ethanol. Dowex OH^-/EtOH exchange resin was added and the mixture was stirred at room temperature for 6 h before filtration. The filtrate was concentrated to dryness and the residue was dissolved in methanol. A catalytic amount of sodium was added to the solution and the mixture was stirred overnight at room temperature. The mixture was concentrated to dryness and Ac_2O/Py (10 mL, 1:2 v/v) was added. The mixture was stirred overnight at room temperature, followed by cooling to 0 °C. Methanol was added dropwise to terminate the reaction. The mixture was concentrated to dryness and then diluted with CH_2Cl_2. The organic solution was washed successively with 1 M HCl, saturated aqueous $NaHCO_3$ and water, dried over Na_2SO_4, filtrated, and concentrated. The crude was purified by silica gel column chromatography (35:1 CH_2Cl_2/methanol) to give **4** (227 mg, 81% yield) as a pale yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.65 (d, 2H, –N*H*), 5.35 (d, J=2.8 Hz, 2H, H-4′), 5.08 (m, 2H), 5.04–4.96 (m, 6H), 4.64 (dd, 2H), 4.53–4.45 (m, 2H), 4.21–4.04 (m, 8H), 3.93–3.90 (m, 4H), 3.81–3.77 (m, 4H), 3.68–3.61 (m, 10H), 2.18–1.95 (7s, 42H, 6× OAc, CH_3CONH–); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.4, 170.3, 170.3, 170.2, 170.0, 169.9, 169.2, 101.4 (C-1′), 101.0 (C-1), 76.2, 73.4, 72.4, 70.8, 70.5, 69.0, 68.7, 66.5, 62.3, 60.7, 53.3, 30.8, 22.9, 22.8, 20.7, 20.5, 20.5, 20.4; HRMS m/z calcd for $C_9H_{16}N_2O_4Na$ $[M+Na]^+$: 239.1002. Found 239.0997.

*4.2.6. Ethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl (1→4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-β-D-gluco-pyranoside (**9**).* The title compound was obtained as syrup in 73.9% yield from the reaction of **4** with ethanol using the general procedure as described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (d, 1H, N*H*CO), 5.85 (dd, 1H, H-4′), 5.34 (dd, 1H, H-2′), 5.08 (m, 2H, H-3, 3′), 4.95 (d, 1H, J=7.9 Hz, H-1), 4.50 (d, 1H, J=7.9 Hz, H-1′), 4.48, 4.15–4.11 (m, 4H, H-6a,6b, H-6a′,6b′), 4.08 (dd, 1H, H-2), 3.99 (dd, 2H, $-OCH_2CH_3$), 3.76 (m, 1H, H-5), 3.62 (m, 1H, H-5′), 3.50 (m, 1H, H-4), 2.15–1.97 (7s, 21H, 6× CH_3CO, $-CH_3CONH$); 1.16 (t, 3H, $-OCH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6–169.3 (7C, 6× $COCH_3$, CONH), 100.9 (C-1), 100.5 (C-1′), 77.4, 77.1, 76.7, 75.8, 72.5, 72.4, 70.8, 70.7, 69.1, 66.6, 65.0, 62.4, 60.8, 53.2, 23.2, 20.9, 20.6, 20.5, 15.0.

*4.2.7. 3,6-Dioxaoct-1,8-diyl bis[β-D-galactopyranosyl-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranoside] (**8**).* A catalytic amount of sodium was added to a solution of compound **7** (100 mg) in methanol (5 mL). The mixture was stirred at room temperature for 1 h, and then neutralized with H^+ cation-exchange resin. The solution was filtered and concentrated and the residue was dissolved in 10 mL water and freeze-dried to give **5** as a white solid (61 mg, 96%). ^{1}H NMR (400 MHz, D_2O): δ 4.50 (d, 2H, J=7.6 Hz, H-1), 4.38 (d, 2H, J=7.6 Hz, H-1′), 3.91–3.89 (m, 4H), 3.84–3.83 (m, 2H), 3.76–3.56 (m, 26H), 3.50–3.43 (m, 4H), 1.95 (s, 6H, 2× OAc); ^{13}C NMR (100 MHz, D_2O): δ 174.4, 102.8 (C-1′), 100.9 (C-1), 78.4, 75.3, 74.7, 72.4, 70.9, 69.7, 69.6, 68.9, 68.5, 61.0, 60.0, 55.0, 22.2; HRMS m/z calcd for $C_9H_{16}N_2O_4Na$ $[M+Na]^+$: 239.1002. Found: 239.0997.

*4.2.8. Ethyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**10**).* The title compound was obtained as a white solid in 95% yield using the general procedure as described above. ^{1}H NMR (400 MHz, D_2O): δ 4.42 (d, 1H, J=7.6 Hz, H-1), 4.33 (d, 1H, J=7.6 Hz, H-1′), 3.86–3.38 (m, 14H, H-2,3,4,5,6a,6b, H-2′,3′,4′,5′,6a′,6b′, $-OCH_2CH_3$), 1.90 (s, 3H, $-CH_3CO$), 1.04 (t, 3H, J=4.0 Hz, $-OCH_2CH_3$); ^{13}C NMR (100 MHz, D_2O): δ 174.6 (NHCO), 102.9 (C-1′), 100.6 (C-1), 78.5, 75.3, 74.8, 72.5, 70.9, 68.5, 66.2, 61.0, 60.1, 55.1, 22.1, 14.2.

*4.2.9. 8-Phenylmethyloxy-3,6-dioxaoctyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl (1→4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside (**11**).* The title compound was obtained as oil in 56.2% yield from the reaction of **3** and **5** using the general procedure as described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 5H, Ph), 6.67 (d, 1H, J=9.6 Hz, N*H*CO-), 5.34 (d, 1H, J=4.2 Hz, H-4′), 5.14–4.94 (m, 3H, H-2′,3′,3), 4.69 (d, 1H, J=8.1 Hz, H-1), 4.57 (s, 2H, PhCH_2–), 4.47 (d, 1H, J=8.0 Hz, H-1′), 4.37 (d, 1H, J=11.7 Hz, H-2), 4.10 (m, 4H, H-6a,6b,6′a,6′b), 3.60–3.85 (m, 15H, H-4,5,5′, CH_2O),1.91–2.13 (7s, 21H, 7× $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (NHCO–), 170.4–169.2 (6C, CH_3CO), 138.2, 128.4, 127.7, 127.6 (Ph–), 101.9 (C-1), 101.2 (C-1′), 73.7 (C-4), 72.9 (CH_2O), 72.4 (C-3), 71.7 (CH_2O), 71.0 (C-3′), 70.7 (C-5′), 70.6 (CH_2O), 70.5 (CH_2O), 70.4 (C-2′), 69.4 (C-5), 69.1 (CH_2O), 68.7 (C-4′), 66.6 (C-6), 63.2 (CH_2O), 62.4 (C-6′), 61.6 (C-2), 60.7 (PhCH_2–), 23.0 (CH_3CONH), 20.5–21.0 (6C, CH_3CO).

*4.2.10. (8-Acetylthio-3,6-dioxaoctyl) 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl (1→4)-3,6-di-O-acetyl-2-acet-amido-2-deoxy-β-D-glucopyranoside (**12**).* $Pd(OH)_2$/C was added to a solution of **11** in methanol. The reaction mixture was stirred under hydrogen atmosphere (4 atm) for 4 h when TLC analysis showed the reaction was complete. The mixture was filtered over Celite and concentrated to afford the alcohol, which was directly used in the next step. To a solution of alcohol (0.4 g, 0.52 mmol) in pyridine (5 mL) was added methanesulfonyl chloride (0.1 mL, 0.148 g, 1.3 mmol). The resulting solution was stirred at room temperature for 1 h when TLC indicated complete conversion of the starting alcohol into the methanesulfonate, CH_2Cl_2 (25 mL) was added and the organic solution was washed sequentially with 1 M hydrochloric acid (100 mL), $NaHCO_3$ (aq) (50 mL), and water (50 mL) and then dried with Na_2SO_4. The solvent was evaporated to afford yellow oil (0.42 g, 85%), which was used directly in the next step. A mixture of the methanesulfonate (0.38 g, 0.63 mmol) and potassium thioacetate (0.144 g, 1.26 mmol) in butanone (25 mL) was heated under reflux for 2 h, when TLC showed that the starting material had been replaced with the thioacetate. The solvent was removed under reduced pressure, and the residue was distributed between CH_2Cl_2 (25 mL) and water. The separated organic layer was dried and concentrated, and the product was subjected to column chromatography (EtOAc/Petrol ether, 1:1) to give thioacetate **12** (0.26 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97–2.34 (m, 24H, 8× OAc); 3.09 (t, 2H, J=6.8 Hz, $-CH_2$ in $-CH_2SAc$); 3.57–3.64 (m, 8H, $-OCH_2$ in PEG); 3.69–3.74 (m, 1H); 3.79 (t, 1H, J=9.9, 9.2 Hz); 3.86–3.92 (m, 2H); 4.05–4.15 (m, 3H); 4.48–4.58 (m, 3H); 4.89 (dd, 1H, J=8, 9.2 Hz); 4.94 (dd, 1H, J=3.2, 10.4 Hz); 5.10 (dd, 1H, J=8, 10.4 Hz); 5.19 (t, 1H, J=9.2, 9.2 Hz); 5.10 (d, 1H, J=2.8 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6, 20.7, 20.7, 28.7, 29.6, 30.4, 60.7, 61.9, 66.5, 69.0, 69.0, 69.7, 70.2, 70.5, 70.6, 70.9, 71.6, 72.5, 72.7, 76.2, 100.5, 101.0, 168.9, 169.5, 169.6, 169.9, 170.0, 170.2, 170.2, 195.4.

*4.2.11. (8-Thiol-3,6-dioxaoctyl)-β-D-galactopyranosyl (1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**13**).* A solution of **13** (100 mg) in dry MeOH (25 mL) was treated with 0.05 M methanolic solution

of NaOMe. The reaction was stirred under an argon atmosphere. After 1 h, the solution was acidified with DOWEX 50WX4-100 ion-exchange resin (Sigma–Aldrich) to pH 7.0. Evaporation of the solution under reduced pressure produced a syrup. Because of the instability of the intermediate, it was immediately used in the following step without further characterization ^{1}H NMR (400 MHz, D_2O): δ 1.89 (s, 3H, –NHAc); 2.87 (t, 2H, J=6.4 Hz, $-CH_2$ in $-CH_2SH$); 3.36–3.93 (m, 20H); 4.29 (d, 1H, J=7.6 Hz); 4.36 (d, 1H, J=8 Hz); ^{13}C NMR (100 MHz, D_2O): δ 7.1, 60.0, 60.0, 60.9, 68.2, 68.3, 68.5, 68.7, 69.2, 9.4, 69.6, 70.9, 72.4, 72.7, 74.2, 74.7, 75.3, 78.3, 02.0, 10.8.

4.3. Preparation of CdSeS/ZnS-QDs (λ_{em}=534 nm)

CdO (0.26 g), oleic acid (OA, 2.5 mL), and tri-*n*-octylamine (TOA, 15 mL) were mixed in a three-necked flask and heated to 300 °C under an argon atmosphere to obtain a clear solution. A stock solution of Se (6.4 mg) and S (0.192 g) in 3.0 mL trioctylphosphine (TOP) was swiftly injected into the hot solution, and the reaction was allowed to proceed at 280 °C for 1 min. ZnS stock solution (a mixture of 0.2 mM ZnO powder in 1.0 mL OA and 0.2 mM S powder in 1.0 mL TOP) was then added dropwise (1 drop per second) at 220–240 °C. CdSeS/ZnS-QDs were obtained with ethanol sedimentation and repeatedly washed with ethanol.

4.4. NMR data of glyco-QDs 14

^{1}H NMR of LacNAcPEG-QDs (400 MHz, 5 mg in D_2O with 1 μL EtOH as IS): δ 4.47 (d, J=7.6 Hz, 1H, H-1), 4.35 (d, J=7.6 Hz, 1H, H-1′), 3.88–3.41 (m, 30H), 1.91 (s, 3H, $-CH_3$ in CH_3CO), 1.05 (t, 8.32H, J=7.2, 7.2 Hz, $-CH_3$ in CH_3CH_2OH).

4.5. Surface plasmon resonance analysis

The SPR results were obtained on the BIAcore 3000 instrument (BIAcore, Uppsala, Sweden). Lectin-functionalized BIAcore sensor chips were prepared from carboxymethylated sensor chips (CM5, BIAcore) by NHS/EDC activation followed by injection of galectin-3 in acetate buffer (10 mM, pH 4.5). Galectin-3 was immobilized on CM5 sensor chip at 4000 RU. 70 μL solutions of glyco-QDs, mono and divalent LacNAc in buffer were flown over the sensor chip with a rate of 35 μL/min.

Acknowledgements

This work was financially supported by the Natural Science Foundation of China (Grant Nos. 90713004, 20732001), the State New Drug Innovation (Grant Nos. 2009ZX09103-044, 2009ZX09501-011), the National Basic Research Program of China (Grant No. 2012CB822100), and the State Key Laboratory of Natural and Biomimetic Drugs and Peking University.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tet.2012.06.035.

References and notes

1. Khaldoyanidi, S. K.; Glinsky, V. V.; Sikora, L.; Glinskii, A. B.; Mossine, V. V.; Quinn, T. P.; Glinsky, G. V.; Sriramarao, P. *J. Biol. Chem.* **2003**, *278*, 4127–4134.
2. Matarrese, P.; Tinari, N.; Semeraro, M. L.; Natoli, C.; Iacobelli, S.; Malorni, W. *FEBS Lett.* **2000**, *473*, 311–315.
3. Liu, F. T.; Rabinovich, G. A. *Nat. Rev. Cancer* **2005**, *5*, 29–41.
4. (a) Cumpstey, I.; Sundin, A.; Leffler, H.; Nilsson, U. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 5110–5112; (b) Cumpstey, I.; Sundin, A.; Leffler, H.; Nilsson, U. J. *Angew. Chem., Int. Ed.* **2005**, *117*, 5240–5242; (c) Sörme, P.; Arnoux, P.; Kahl-Knutsson, B.; Leffler, H.; Rini, J. M.; Nilsson, U. J. *J. Am. Chem. Soc.* **2005**, *127*, 1737–1743; (d) Pieters, R. J. *ChemBioChem* **2006**, *7*, 721–728.
5. van Scherpenzeel, M.; Moret, E. E.; Ballell, L.; Liskamp, R. M. J.; Nilsson, U. J.; Leffler, H.; Pieters, R. J. *ChemBioChem* **2009**, *10*, 1724–1733.
6. Pieters, R. J.; Vrasidas, I.; Kaltner, H.; Kuwabara, I.; Liu, F. T.; Liskamp, R. M. J.; Gabius, H. J. *ChemBioChem* **2001**, *2*, 822–830.
7. (a) Ballell, L.; Alink, K. J.; Slijper, M.; Versluis, C.; Liskamp, R. M. J.; Pieters, R. J. *ChemBioChem* **2005**, *6*, 291–295; (b) Ballell, L.; van Scherpenzeel, M.; Buchalova, K.; Liskamp, R. M. J.; Pieters, R. J. *Org. Biomol. Chem.* **2006**, *4*, 4387–4394.
8. (a) Medintz, I. L.; Tetsuouyeda, H.; Goldman, E. R.; Mattoussi, H. *Nature Mater.* **2005**, *4*, 435–446; (b) Miyawaki, A. *Dev. Cell* **2003**, *4*, 295–305; (c) Schrock, E.; Manoir, S. D.; Veldman, T.; Schoell, B.; Wienberg, J.; Ferguson-Smith, M. A.; Ning, Y.; Ledbetter, D. H.; Bar-Am, I.; Soenksen, D.; Garini, Y.; Ried, T. *Science* **1996**, *273*, 494–497; (d) Pellegrino, T.; Manna, L.; Kudera, S.; Liedl, T.; Koktysh, D.; Rogach, A. L.; Keller, S.; Radler, J.; Natile, G.; Parak, W. J. *Nano Lett.* **2004**, *4*, 703–707; (e) Jang, E.; Jun, S.; Pu, L. *Chem. Commun.* **2003**, 2964–2965.
9. (a) de la Fuente, J. M.; Penades, S. *Biochim. Biophys. Acta* **2006**, *1760*, 636–651; (b) Mukhopadhyay, B.; Martins, B. M.; Karamanska, R.; Russell, D. A.; Field, R. A. *Tetrahedron Lett.* **2009**, *50*, 886–889; (c) Kikkeri, R.; Lepenies, B.; Adibekian, A.; Laurino, P.; Seeberger, P. H. *J. Am. Chem. Soc.* **2009**, *131*, 2110–2112; (d) Kenichi, N.; Kuniharu, I.; Shota, S. *Trends Glycosci. Glyctechnol.* **2009**, *21*, 335–344; (e) Coulon, J.; Thouvenin, I.; Aldeek, F.; Balan, L.; Schneider, R. *J. Fluoresc.* **2010**, *20*, 591–597; (f) Ohyanagi, T.; Nagahori, N.; Shimawaki, K.; Hinou, H.; Yamashita, T.; Sasaki, A.; Jin, T.; Iwanaga, T.; Kinjo, M.; Nishimura, S. I. *J. Am. Chem. Soc.* **2011**, *133*, 12507–12517.
10. Yang, Y.; Yu, M.; Yan, T. T.; Zhao, Z. H.; Sha, Y. L.; Li, Z. J. *Bioorg. Med. Chem.* **2010**, *18*, 5234–5240.
11. Yang, Y.; Zhao, Y. T.; Yan, T. T.; Yu, M.; Sha, Y. L.; Zhao, Z. H.; Li, Z. J. *Tetrahedron Lett.* **2010**, *51*, 4182–4185.
12. (a) Bovin, N. V.; Zurabyan, S. E.; Khorlin, A. Y. *Carbohydr. Res.* **1981**, *98*, 25–35; (b) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155–173; (c) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819–6825; (d) Clinch, K.; Evans, G. B.; Furneaux, R. H.; Rendle, P. M.; Rhodes, P. L.; Roberton, A. M.; Rosendale, D. I.; Tyler, P. C.; Wright, D. P. *Carbohydr. Res.* **2002**, *337*, 1095–1111.
13. Li, Q.; Su, B.; Li, H.; Meng, X. B.; Cai, M. S.; Li, Z. J.; Zhou, R. L.; Suo, T. L. *Carbohydr. Res.* **2003**, *338*, 207–217.
14. (a) Lafont, D.; Guilloux, P.; Descotes, G. *Carbohydr. Res.* **1989**, *193*, 61–74; (b) Lafont, D.; Boullander, P.; Carvalho, F.; Vottero, P. *Carbohydr. Res.* **1997**, *297*, 117–126; (c) Lafont, D.; Gross, B.; Kleinegesse, R.; Dumoulin, F.; Boullanger, P. *Carbohydr. Res.* **2001**, *331*, 107–118; (d) Lafont, D.; Boullanger, P. *Tetrahedron: Asymmetry* **2006**, *17*, 3368–3379.
15. Loiseau, F. A.; Hii, K. K.; Hill, A. M. *J. Org. Chem.* **2004**, *69*, 639–647.
16. Yu, M.; Yang, Y.; Han, R. C.; Zheng, Q.; Wang, L. J.; Hong, Y. K.; Li, Z. J.; Sha, Y. L. *Langmuir* **2010**, *26*, 8534–8539.
17. Barrientos, Á. G.; de la Fuente, J. M.; Rojas, T. C.; Fernández, A.; Penadés, S. *Chem.—Eur. J.* **2003**, *9*, 1909–1921.
18. Sun, X. L.; Cui, W.; Haller, C.; Chaikof, E. L. *ChemBioChem* **2004**, *5*, 1593–1596.
19. Lin, C. C.; Yeh, Y. C.; Yang, C. Y.; Chen, C. L.; Chen, G. F.; Chen, C. C.; Wu, Y. C. *J. Am. Chem. Soc.* **2002**, *124*, 3508–3509.
20. Haseley, S. R.; Talaga, P.; Kamerling, J. P.; Vliegenthart, J. F. *Anal. Biochem.* **1999**, *274*, 203–210.
21. Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.
22. Murakami, T.; Yoshioka, K.; Sato, Y.; Tanaka, M.; Niwa, O.; Yabuki, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1265–1269.
23. Barboni, E. A. M.; Bawumia, S.; Hughes, R. C. *Glycoconjugate J.* **1999**, *16*, 365–373.
24. Kulkarni, A. A.; Weiss, A. A.; Iyer, S. S. *Med. Res. Rev.* **2010**, *30*, 327–393.
25. Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794.
26. (a) Alivisatos, P. *Nat. Biotechnol.* **2004**, *22*, 47–52; (b) Klostranec, J. M.; Chan, W. C. W. *Adv. Mater.* **2006**, *18*, 1953–1964.