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Synthetic assembly of novel avidin-biotin-GlcNAc (ABG) complex as an attractive bio-probe and its interaction with wheat germ agglutinin (WGA)

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ABSTRACT

A tetravalent GlcNAc pendant glycocluster was constructed with terminal biotin through C_6 linker. To acquire the multivalent carbohydrate-protein interactions, we synthesized a glycopolymer of tetrameric structure using N-acetyl-D-glucosamine (GlcNAc) as the target carbohydrate by the use of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as coupling reagent, followed by biotin-avidin complexation leading to the formation of glycocluster of avidin-biotin-GlcNAc conjugate (ABG complex). The dynamic light scattering (DLS) system was implied for size detection and to check the binding affinity of GlcNAc conjugate with a WGA lectin we use fluorometric assay by means of specific excitation of tryptophan at λ_{ex} 295 nm and it was found to be very high $K_a \sim 1.39 \times 10^7\ M^{-1}$ in case of ABG complex as compared to GlcNAc only $K_a \sim 1.01 \times 10^4\ M^{-1}$ with the phenomenon proven to be due to glycocluster effect.

1. Introduction

Carbohydrate derivatives had become much more manipulatable and diverse in recent scenario. Interactions between carbohydrates and lectins have attracted great attention due to their extensive roles in normal biology, fertilization, immune function, cell signaling, translation, transcription, genomic activities, host-pathogen recognition or cancer metastasis [1,2].

Usually, the lectin-carbohydrate interaction is one of the weakest in nature, with values in the millmolar range [3,4], chemical modifications of the carbohydrates in order to obtain higher binding values have been widely employed approach that has led to new molecules endowed with enhanced binding affinity by means of the "cluster glycoside effect" [5–9]. Undoubtedly, analysis of biologically applicable molecular recognition episodes have generally centered on high affinity interactions. Here, we will focus on multivalent protein-carbohydrate recognition, a key mechanism that operates in fundamental physiological recognition processes.

To the best of our knowledge there are surprisingly few reports on the linear linkage of N-acetylglucosamine (GlcNAc) *via* biotin-avidin interactions. In this paper we describe the synthesis of biotinylated GlcNAc monomer-using 6-chlorohexanol as a linker and further modifications *via* organic synthesis followed by biotin coupling using DMT-MM [10] as a coupling reagent leads to the formation of unique bio-probe, and we also demonstrate the biological evaluation of synthesized tetravalent glycoconjugate having N-acetyl-D-glucosamine (GlcNAc) moieties for wheat germ agglutinin (WGA) as a model lectin.

Bioprobes are basically acted as an agent to give the information of cellular activities and these are the special tools for optical imaging and analytical sensing [11]. Biotin (244.31 Da) is known as a vitamin B7 and use as a bioprobe for avidin or streptavidin. Biotin and avidin have a high affinity, the dissociation constant is measured to be ($K_d \sim 10^{-15}$ M), which is 10^3–10^6 times higher than that of a typical antigen-antibody interaction [12,13]. This strong affinity has been employed to modify surfaces, nanoparticles and even nano- and micro-hydrogels for biological imaging, sensing and target drug delivery purpose [14,15]. Avidin (66–69 kDa) has four binding sites for biotin with a stoichiometry of 1:4 of avidin/biotin [16–18], which makes avidin a perfect glycopolymer to synthesize tetrameric multivalent glycoconjugate in this research.

Wheat germ agglutinin (WGA) from *Triticum vulgaris* is a stable homodimer protein (36 kDa) with eight specific binding sites for GlcNAc that are separated by a distance of 14 Å [19,20]. This dimeric lectin is made up of monomeric unit and these monomeric units organized into four domains with different affinities for GlcNAc [21]. Such structural characteristics and the compact contiguity of binding sites make this lectin an exemplary biological

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model to investigate multivalent carbohydrate-protein interactions and evaluate the influence of structural modifications of glycomonomers, glycopolymers, and glycoclusters. In that case the four molecule of biotinylated GlcNAc can bind simultaneously to the tetrameric avidin by so called glycocluster effect and was evaluated for its interaction against WGA on the basis of fluorescence change of tryptophan [22,23] in WGA at the wavelength of 292 nm.

2. Results and discussion

2.1. Synthesis of GlcNAc-glycoside via glycosylation

In this research N-acetyl-D-glucosamine (GlcNAc) is used as a target carbohydrate and its glycoside was derivatized using a C_6 linker (Scheme 1). The glycoside was derived from oxazoline derivative **1** which is readily prepared by the trimethylsilyl trifluoromethylsulfonate (TMSOTf) method [24] from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose, through glycosidation reaction with 6-chlorohexan-1-ol in the presence of camphorsulfonic acid (CSA) as the promoter. Subsequent replacement of chlorine atom at the ω-position of the side chain to azide by substitution followed by transesterification using the Zemplen method. Then reduction provided an amide by Staudinger reaction. Prior to the Staudinger reaction Zemplen deacetylation was necessary to avoid the inter- or intramolecular acyl migration. For instance, during intermolecular acyl migration, the nucleophile (i.e.; :NH_2) attack on one of the *O*-acetyl group present on the sugar moiety leads to the formation of unwanted material rather than the wanted one.

2.2. Synthesis of biotin-GlcNAc conjugate

Since an amine derivative was successfully prepared, our attention turned toward an amide coupling between biotin and synthesized compound **5**. We carried out two kinds of biotin-coupling reactions in respect of formation of targeted compound.

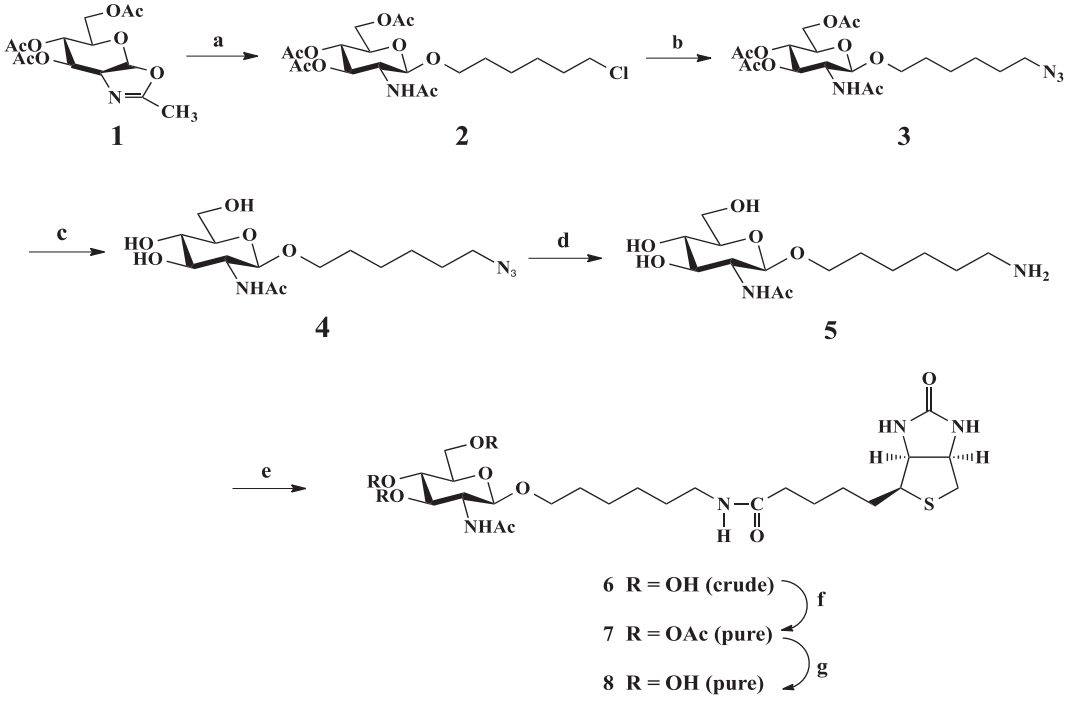
2.2.1. DMAP-DIC coupling

Amide coupling of carboxylic acids with primary aliphatic amines employing diisopropyl carbodiimide (DIC) [25] as a coupling reagent and 4-dimethylamino pyridine (DMAP) [26] as a catalyst when performed in polar solvent dimethyl formamide (DMF) leads to the formation of null target material (Scheme 1). Nevertheless, a remarkable acceleration is observed, if catalysts as DMAP are present, forming highly reactive intermediates. The reaction mixture was analyzed by mass spectroscopy but the signal for target material was not at all appeared.

2.2.2. DMT-MM coupling

As a consequence of DMAP-DIC coupling failure, our attention turned towards another coupling reagent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) in DMF which proceeded with good yield. To auspiciously aid a one-step condensation of acids and amines to give amides, the condensing agent must be both stable and soluble in the solvent. Reaction of **5** with biotin in DMF, in which DMT-MM is simply added to, without requirement of any catalyst to give **7** with 63% yield, as shown in Scheme 1. Finally, the structure of the **7** and **8** were confirmed by ^1H and ^{13}C NMR spectroscopy. The assigned ^1H NMR spectrum of **7** and **8** are shown in Fig. 1.

The structure of the product was elucidated as follows. Glycosidic anomeric proton signals were clearly observed in the lower field with larger coupling constants (δ 4.96 ppm, d, 1 H, $J_{1,2}$ = 8.48 Hz, H-1) in **7** and (δ 4.63 ppm, d, 1 H, $J_{1,2}$ = 8.28 Hz, H-1) in **8**. The amide linkage proton is clearly estimated in the range of (δ 8.10 ppm, t, 1 H, J = 5.50 Hz, g) and the two singlet signals from ureido ring of biotin came in the range of (δ 6.76 ppm, s, 1 H, 2′ and δ 6.70 ppm, s, 1 H, 1′) and counter due to mutual diffusion. In the ^{13}C NMR spectrum of **7** the anomeric signal was also observed in the lower field (δ 100.3 ppm, C-1) and the same with compound **8** (δ 101.5 ppm, C-1). In addition to the results of ^1H NMR and ^{13}C NMR, HMQC experiments were also supported the adduct **7** and **8**. The simplicity of the spectra suggests that the



Scheme 1. (a) CSA/6-Chlorohexan-1-ol, DCE, 90 °C, 3 h; (b) NaN_3, DMF, 80 °C, 20 h; (c) NaOMe, MeOH, rt, 2 h; (d) TPP·H_2O/THF, 0 °C, 15 min, rt, 24 h; (e) Biotin/DMT-MM, DMF, 0 °C, 15 min, rt, 10 h; (f) Ac_2O, Py, 0 °C, 15 min, rt, 15 h; (g) NaOMe, MeOH, rt, 3 h.