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Communication

Multivalent neuraminidase hydrolysis resistant triazole-sialoside protein conjugates as influenza-adsorbents

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ABSTRACT

We report the synthesis of *pseudo* triazole-sialoside protein conjugates of various valency that are resistant to neuraminidase for the adsorption of influenza viruses. The glycotriazole monomer bearing an amine-functionalized linker was synthesized by click chemistry and grafted to the lysine residues of bovine serum albumin (BSA) or human serum albumin (HSA) *via* diethyl squarate and adipate-based strategy. The binding of hemagglutinin (HA) and neuraminidase (NA) on the virion surface by the synthetic neoglycoproteins were evaluated by hemagglutination and neuraminidase inhibition assay, respectively. The results demonstrated that these synthetic glycoproteins have significantly higher affinity with NA than HA. The interactions between these neoglycoproteins and intact influenza viruses were further investigated by Dynamic Light Scattering (DLS) technique. The pronounced agglutination indicated that these glycoconjugates can be used as adsorbents to prevent virus from invading host cells as well as the release of newly synthesized viral particles, which are crucial in the life cycle of the influenza virus. With the high binding affinity to intact influenza viruses, these neoglycoproteins can also be used as probe to elucidate the molecular mechanism of the sialic acid-influenza recognition and biosensors for influenza detection.

N-Acetyl neuraminic acid (Neu5Ac, also called sialic acid, SA) (Scheme 1), an acidic monosaccharide with nine-carbon backbone, is commonly found on the termini branches of *N*-glycans, *O*-glycans, glycosphingolipids and glycoproteins on the cell surface [1]. Due to its external position, SA is fully accesible to other biomolecules and further control cell-cell interactions [2]. It has been found that SA-protein interactions play vital roles in various biological and pathological processes including cancer [3,4], inflammation [5] and immunization [6]. Moreover, many viruses [7,8] and bacteria [9,10] also utilized this interaction at various stages in their life cycles for cell entry or release.

Influenza virus, a genus of the *Orthomyxoviridae* family that causes outbreaks of respiratory disease as annual epidemics and unpredictable pandemics remains a significant risk to global health and economy [11]. It has been widely accepted that two multivalent SA-protein interactions serve important roles in the initial and final steps of the replication cycles of influenza viruses [12]. Hemagglutinin (HA) is one of the major surface glycoprotein of the virus (80%, ~300 copies of trimer), which binds to α-SA to induce fusion between viral and cellular membranes [13]. Neuraminidase (NA) is another receptor-destroying surface glycoprotein (17%, ~50 copies of tetramer), it cleaves the residual SA to release the virus from infected cells [14]. Two FDA approved SA derivatives Zanamivir and Osetamivir as potent NA inhibitors were invented based on the elucidation of SA-NA interactions, which were effective measurements to prevent potential Flu pandemics [15]. However, the mutations lead to the drug resistance [16] have decreased the effectiveness of the two drugs, thus developing new anti-influenza agents are in great need.

An alternative strategy for the development of antiviral agents is inspired by the mucin [17], a heavily glycosylated protein secreted by epithelial tissues of organisms of mammal to trap viruses and expel the virions by mucocilliary transportation *via* multivalent SA-HA/NA interactions [18]. Native *O*-linked sialoside can be hydrolyzed by NA [19], which is the major drawback of using

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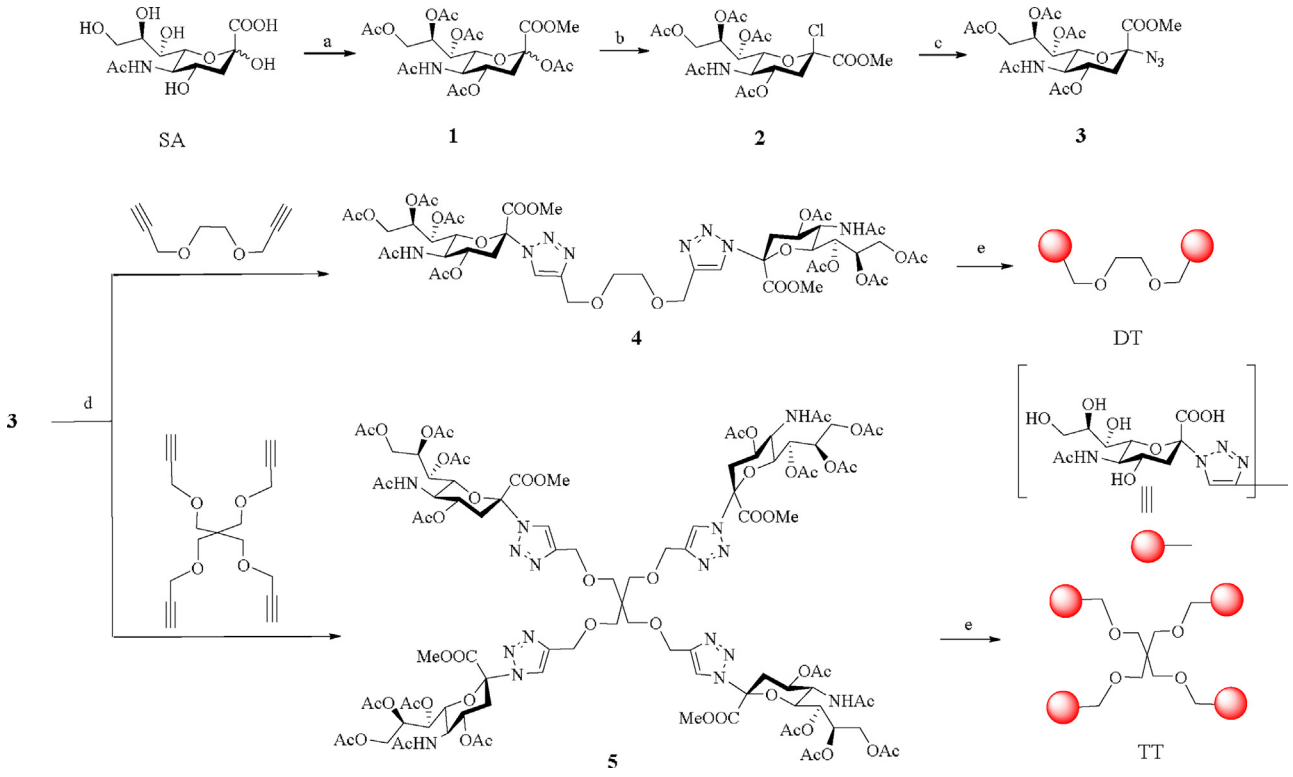
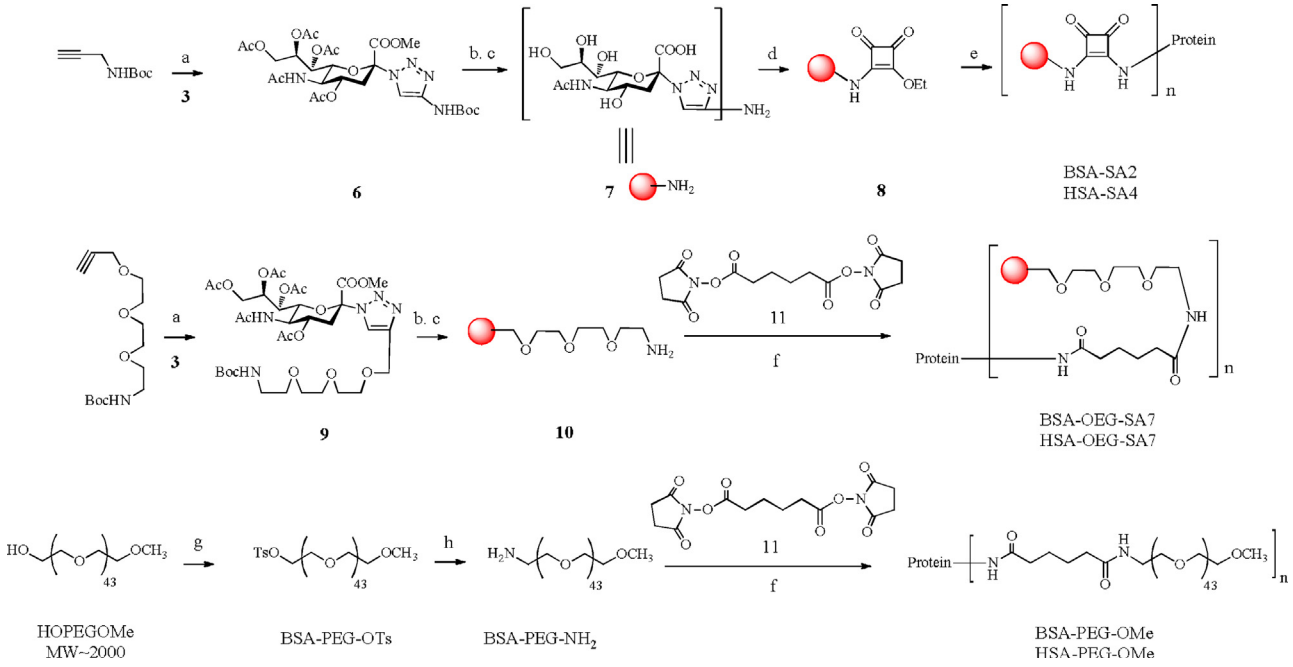


Scheme 1. Synthesis of di- and *tetra*-valent triazole-sialoside. (a) (i) H^+ resine/MeOH, (ii) Ac_2O/Py. (b) $BiCl_3/CH_3SiCl_3$, CH_2Cl_2, 90%. (c) NaN_3/nBu_4HSO_4, CH_2Cl_2/H_2O. (d) $CuSO_4{\cdot}5H_2O$, VcNa, THF/H_2O, alkynyl scaffold. (e) (i) CH_3ONa/CH_3OH, (ii) NaOH, H_2O/MeOH.

it directly as virus inhibitor. Alternatively, presenting multivalent NA resistant *pseudo*-SA on different scaffolds including polymer [20], liposome [21], dendrimers [22] and nanoparticles [23] as mucin mimic to bound to both HA and NA have been developed as the virus adsorbents to prevent the infection. Our previous work [24] has also demonstrated that unlike natural *O*-sialylated complex-type glycan protein conjugates [25], NA resistant *S*-sialosides protein conjugates can bind not only to HA with high affinity resulting in the inhibition of the viral adhesion to erythrocytes, but also NA with moderate affinity resulting in the prevention of the hydrolysis of the SA to reduce virus propagation. Compared with other SA modified macromolecules, the sialyl



Scheme 2. Synthesis of multivalent triazole-sialoside and PEG protein conjugates. (a) $CuSO_4{\cdot}5H_2O$, VcNa, THF/H_2O. (b) (i) CH_3ONa/CH_3OH, (ii) NaOH, H_2O/MeOH. (c) TFA/CH_2Cl_2. (d) Squaric acid diethyl esters, phosphate buffer saline (pH 7.0). (e) BSA/HSA, borate buffer buffer (pH 9.0). (f) (i) Et_3N, DMSO, (ii) sodium phosphate buffer (pH 7.5), overnight. (g) *p*-Toluensulfonyl chloride, TosCl/Et_3N. (h) (i) NaN_3/DMF, (ii) H_2, $Pd(OH)_2$/C.

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