Synthesis of a cluster-forming sialylthio-D-galactose fullerene conjugate and evaluation of its interaction with influenza virus hemagglutinin and neuraminidase

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ABSTRACT

In order to obtain self assembling, multivalent ligand for influenza virus hemagglutinin α-N-acetylneuraminyl-(2-6)-D-galactopyranose has been synthesized and bonded to a water soluble fullerene derivative using 1,3-dipolar cycloaddition click reaction. The aggregating amphiphilic compound did not inhibit the influenza virus hemagglutinin, but it proved to be an inhibitor of its neuraminidase with a 50% inhibitory concentration of 81 μM.

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Human influenza A and B viruses are highly contagious respiratory pathogens causing considerable morbidity and mortality, particularly in aged individuals.[1] Current influenza vaccines require annual updating and provide only partial protection in elderly people.[2] Currently available anti-influenza drugs include the M2 blockers amantadine and rimantadine, which nowadays are rarely used in clinical praxis because of widespread viral resistance to these agents, and the neuraminidase inhibitors oseltamivir and zanamivir.[3] Influenza viruses carry two surface glycoproteins with a sialic acid recognition site: the hemagglutinin (HA), that is responsible for initial virus attachment to host cell receptors, and the neuraminidase (NA) which releases the newly formed virions at the end of the virus life cycle.

Due to its crucial role in viral entry, the influenza virus HA represents[4,5] a suitable target for antiviral intervention. The HAs of human-adapted influenza viruses bind mainly to host cell surface glycans having sialyl-α(2-6)-galactosyl termini, while avian influenza viruses preferentially bind to glycans with sialyl-α(2-3)-galactosyl termini.[6,7] Since sialic acid binding is similar in all influenza virus (sub)types,[4] interfering with the HA–sialic acid interaction is a relevant antiviral strategy. According to the
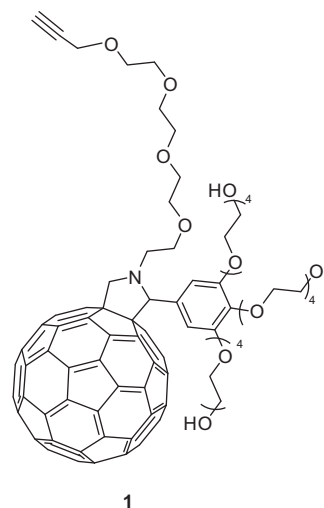

Figure 1. Structure of the fullerene–pyrrolydine carrier **1**.

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estimations of Whitesides et al.,[8] the association constant for inter-action of a single sialic acid with monomeric HA is 10^3 M^{-1}. How-ever, due to polyvalent virus–cell interactions, the binding affinity between multiple viral HA ligands and sialic acid receptors of an erythrocyte is estimated 10^{13} M^{-1}. This strong cooperative interaction inspired the pioneering works of the teams of Bovin,[9] Roy[10] and Whitesides[11] to synthesize multivalent sialic acid-containing polymers and study their effect on HA–sialic acid binding.

In the last decades, many sialoconjugates of polymer carrier molecules have been synthesized[12,13] and evaluated for their anti-influenza virus activity. However, the polymer backbone of these sialosides can be immunogenic, insoluble, cytotoxic and inconsistent. These disadvantages initiated the research of mono-meric sialosides that can form multivalent aggregates. One of the other approaches is the incorporation of monosialosides into lipo-somes.[14–16] The Bovin's group elaborated an ingenious HA-decoy method: they conjugated sialosides to oligoglycine chains that can form self-assembled nano-sheets through hydrogen bonding.[17–19]

In the framework of our systematic studies of glycopeptide anti-biotic aglycons, we previously prepared a series of ristocetin and teicoplanin derivatives bearing lipophilic side-chains,[20] several of which exhibited high antibacterial and anti-influenza virus activity. We postulated that the unusual/serendipotous antiviral activity originates from their ability to form nano-aggregates. Since it is known that fullerene (C_{60}) derivatives can form self-assembled supramolecular nanostructures in water,[21] we designed and pre-pared the fullerene derivative 1 as one of the lipophilic side-chains. Compound 1 was provided with tetraethyleneglycole chains for improving the water solubility and a propargyl ether residue allowing its functionalization with bioactive compounds via a 1,3-dipolar cycloaddition (click) reaction (Fig. 1).[22]

We anticipated that conjugation of 1 with an appropriate sia-lodisaccharide ligand of the HA receptor would result in a sialo-cluster-forming fullerene derivative with HA-decoy properties. The thiosialoside analog of N-acetylneuraminyl-α(2,6)-D-galactose has been chosen for the conjugation, because this is the terminal structure of human cell surface glycoproteins recognized by the
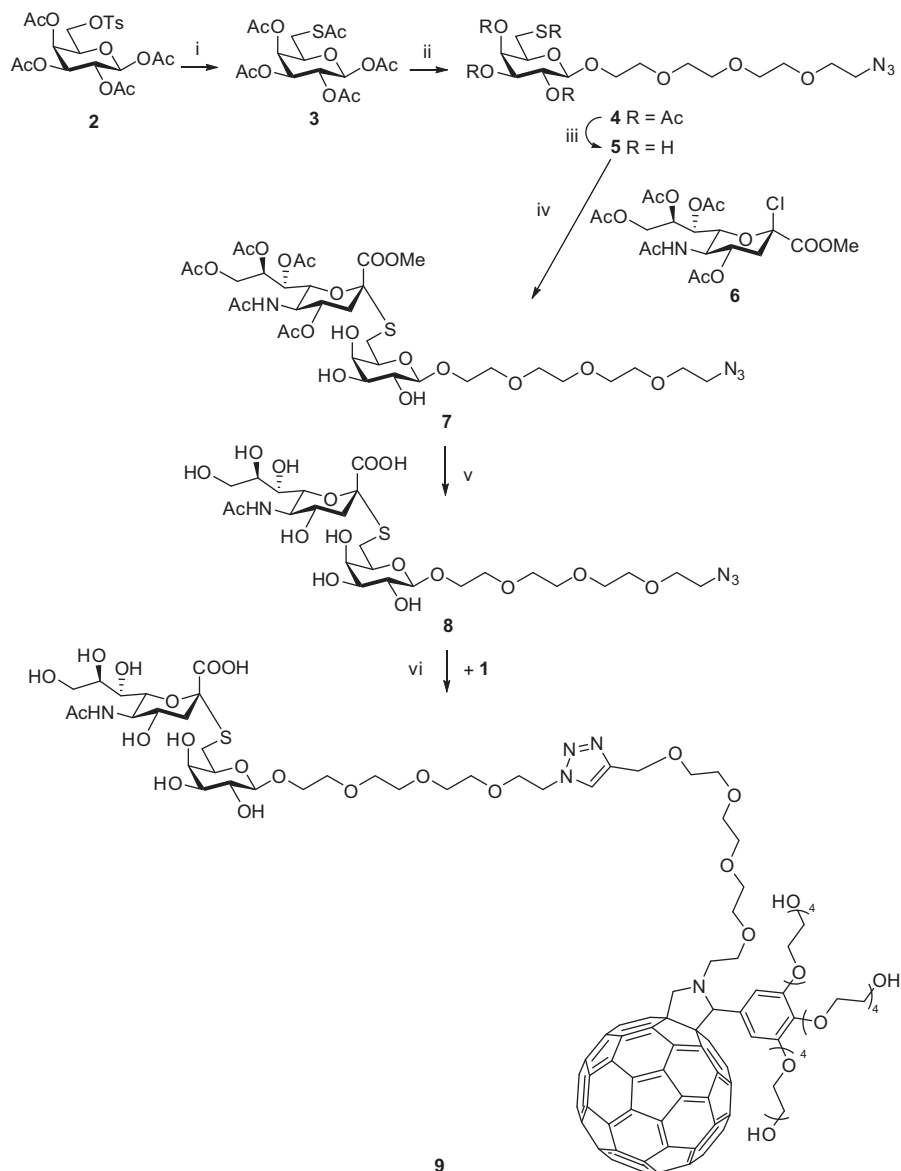
Scheme 1. Synthesis of the thiosialoside-functionalized fullerene 9. Reagents and conditions: (i) KSAc, dry DMF, 90 °C, 2 h, 70%; (ii) HO-TEG-N$_3$, BF$_3$·Et$_2$O, dry CH$_2$Cl$_2$, rt, 4 h, 33%; (iii) (1) NaOMe, dry MeOH, (2) Serdolite Red (H$^+$); (iv) Na$_2$CO$_3$, H$_2$O, Bu$_4$NHSO$_4$, EtOAc, 40% for 7 over two steps; (v) (1) NaOMe, dry MeOH, 2. Serdolite Red (H$^+$), (3) KOH, H$_2$O, MeOH, (4) Amberlite IR 120 (H$^+$), 51%; (vi) Et$_3$N, Cu(I)I, Ar, rt, 24 h, dry CH$_2$Cl$_2$, dry MeOH, 60%.
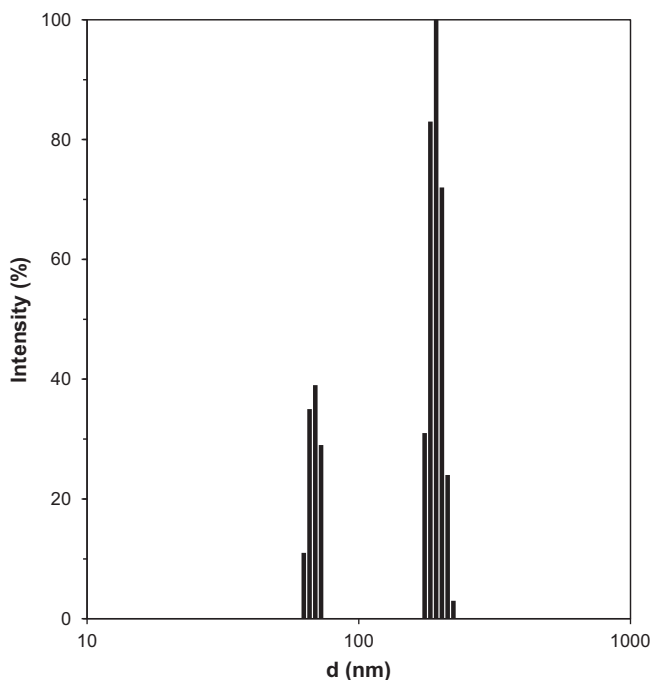
Figure 2. Size distribution of aggregates of compound **9** in water.

influenza virus HA. As thioglycosides display significantly lower susceptibility to enzymatic and acid hydrolysis,[23] and thiosialo-sides are not cleaved by neuraminidase enzyme,[24] the planned thio analog must be stable under physiological conditions.

The synthesis commenced with the conversion of the known galactose derivative **2**[25] into the 6-S-acetyl-containing compound **3** (Scheme 1). The tosyloxy group of **2** was displaced by potassium thiolacetate to afford **3**. Lewis acid mediated glycosylation of the monoazido-tetraethylene-glycol with **3** resulted in the β-galactoside **4**. After deacetylation, **5** was reacted with acetochloro neuraminic acid methyl ester **6**[26] under phase transfer conditions, to give the disaccharide **7**. Removal of the acetyl groups by the Zemplén method followed by hydrolysis of the methyl ester residue resulted in compound **8**. Copper(I) catalyzed click reaction of **8** with **1** gave the fullerene sialoconjugate **9**. To the best of our knowledge this is the first sialic acid containing fullerene derivative.

The cluster formation properties of the obtained sialic acid-functionalized amphiphile were studied by dynamic light scattering.[20a] According to the studies, compound **9** forms 70-nm and 190-nm sized aggregates in water with a bimodal distribution (Fig. 2). Next, we determined the cell culture antiviral activity of **9** against several influenza virus strains, that is, A/H1N1 (A/PR/8/34 and A/Virginia/ATCC3/2009); A/H3N2 (A/HK/7/87); and influenza B (B/HK/5/72) using the cytopathic effect reduction assay described in Vanderlinden et al.[20b] Compound **9** did not show anti-influenza virus activity in Madin–Darby canine kidney cells at 100 μM concentration. Furthermore, **9** did not inhibit influenza virus-induced hemagglutination of erythrocytes (highest concentration tested: 50 μM).[27] It was also inactive, at a concentration of 100 μM, in a cytopathic effect reduction assay in African green monkey Vero cells infected with parainfluenza type 3 virus, which alike influenza virus carries an HA activity. Finally, when we tested **9** for its inhibitory effect on influenza virus neuraminidase, the compound was found to moderately inhibit the viral neuraminidase (of strain A/Virginia/ATCC/3/2009) with an IC_{50} value of 81 μM. This is reminiscent of the recent data of Sakamoto et al.,[28] who reported multivalent thiosialoside dendrimers with millimolar activity against influenza virus neuraminidase.

In conclusion, compound **9**, a fullerene conjugate containing a thiosialosyl-α(2,6)-galactose disaccharide has been prepared, in order to study the multimeric interaction of a sialocluster with influenza virus HA and NA. This amphiphilic conjugate formed nano-sized aggregates in water but did not show anti-influenza virus activity, nor did it inhibit influenza virus-induced hemagglutination. It did show moderate inhibitory activity towards the influenza virus neuraminidase. We assume that the numerous tetraethyleneglycol chains applied in the molecule to improve its water solubility, probably shielded the active disaccharide part, preventing its multimeric interaction with viral hemagglutinin. On the other hand, interestingly, this shielding did not seem to disturb the interaction of **9** with the viral neuraminidase.

Acknowledgments

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014. 04.032.

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