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Article

Glycodendritic structures: tools to interact with DC-SIGN

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The key role of carbohydrates in many biological events has attracted the interest of the scientific community. This fact has demanded the access to new tools necessary to understand this role and the interaction of carbohydrates with their corresponding receptors, lectins. Glycodendrimers and glycodendritic structures in general, have demonstrated to be very efficient and interesting tools to intervene in those processes where carbohydrates participate. In this review, we discuss the different glycodendritic structures that have been used to interfere with DC-SIGN, a very attractive lectin involved in infection processes and in the regulation of the immune response.

Uniterms: DC-SIGN. Ebola. Glycodendrimers. Glycofullerenes. HIV. Mannose.

O papel chave dos carboidratos em muitos eventos biológicos tem atraído interesse da comunidade científica. Este fato demonstrou o acesso de novas ferramentas para a compreensão da interação dos carboidratos com seus receptores correspondentes, lectinas. Glicodendrímeros e estruturas glicodendríticas, em geral, mostram-se como ferramentas muito eficientes e interessantes para intervir nos processos em que os carboidratos participam. Nesta revisão, discutimos diferentes estruturas glicodendríticas que têm sido úteis para interferir com DC-SIGN, uma lectina muito atraente envolvida em processos infecciosos e na regulação da resposta imune.

Unitermos: DC-SIGN. Ebola. Glicodendrímeros. Glicofulerenos. HIV. Manose.

INTRODUCTION

Glycans are not only an important source of metabolic energy; they are also widely expressed as glycoconjugates on the surface of cells where they play a key role in many important biological processes (Varki, 1993; Kamerling 2007; Werz, Seeberger, 2007). In the past decade, the increased appreciation for the ubiquity of glycans and their ability to encode biochemical information has given rise to the field of glycobiology (Varki, 1999; Bertozzi, Kiessling, 2001) with the aim to explain and understand how chemical information is encode in sugar structures and how this information is read out by sugar binding proteins (lectins) (Gabius *et al.*, 2011; Gupta, Surolia, 2012). Lectins are proteins that recognize glycans (glycolipids and glycoproteins) with high specificity, and this carbohydrate-lectin interaction is implicated in several important biological events such as cell-cell self-recognition processes, cell-extracellular matrix interaction, gamete fertilization, embryonic development, cell growth, cell differentiation, cell signaling, cell adhesion and migration, apoptosis, immuno-

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modulation and inflammation, host-pathogen interactions, glycoprotein folding and routing, mitogenic induction and homeostasis (Varki, 1993; Weis, 1998; Sharon, Lis, 2004; Ghazarian, 2011). Thus, in principle, lectins can clearly be considered as potential targets for the development of new drugs. However, they have rarely been exploited for the discovery of novel therapeutic opportunities. Frequently, medicinal chemists have mostly disregarded carbohydrates as a class of molecules for drug development, basically due to their poor drug-like properties including their low stability and their high polar character.

Carbohydrate-protein (carbohydrate-lectin) interactions are characterized by a high selectivity, metal ion dependence, and a low affinity compensated in nature by a multivalent presentation of glycans and receptors (Lee, Lee, 1995; Chabre, Roy, 2010). The carbohydrate recognition domains (CRD) of lectins are often able to recognize complex oligosaccharides in a selective manner; however, the recognized oligosaccharide determinants often consist of only one or two residues, usually located in the non-reducing end of the oligosaccharide structure, that appear to act as anchors, driving the entire glycoconjugate to interact with the protein. The most abundant animal lectins are the C-type lectins (CTL) (Kilpatrick, 2000), that in most of the cases are large, asymmetric trans-membrane glycoproteins, with one or more CRDs attached to a variable number of structurally and functionally different polypeptide domains.

The interaction between sugars and lectins is driven by hydrogen bonds, coordination of monosaccharide residues with metals (for C-type lectins and related calcium-dependent proteins), ionic and hydrophobic interactions, that all contribute to the binding affinity (Weiss, Drickamer, 1996). The energy associated with hydrogen bonding in sugar–protein interactions is significantly reduced by competition from bulk solvent and by the flexible nature of hydroxyl groups, which results in a considerable entropic penalty when they become constrained upon binding. Sugar CH bonds can engage in stacking interactions with protein aromatic side chains, but natural carbohydrates usually lack extended hydrophobic areas, often a dominant factor in high-affinity receptor–ligand interactions. Hence, the affinity of lectins for monovalent carbohydrates is typically weak (dissociation constants are in the mM to μM range). However, most lectins are multimeric and, therefore, polyvalent presentations of monosaccharides acting as binding determinants for a given lectin can be used for inhibition, increasing the affinity in comparison with the corresponding monovalent ligands (Mammen, 1998; Culf, 2006; Turnbull, Field, 2007). For this aim, a vast array of unnatural glycoconjugates (neoglycoconjugates) with different valencies and spatial arrangement of the ligand have been constructed as sugar binding proteins antagonist to prevent or treat diseases caused by pathogens (Bernardi *et al.*, 2013). Scaffolds based on peptides and proteins (Davis, 2002; Payne, Wong, 2010; Westerlind, 2012), polymers (Miura, 2012; Eissa, Cameron, 2013), calixarenes (Baldini, *et al*., 2007; Dondoni, Marra, 2010), dendrimers (Li, Cheng, Xu, 2007; Chabre, Roy, 2008), cyclodextrins (Fulton, Stoddart, 2001; Gomez-Garcia, 2012; Martínez, Ortíz Mellet, García Fernández, 2013), cyclopeptides (Galan, Dumy, Renaudet, 2013), fullerenes (Nierengarten, 2010), gold nanoparticles and quantum dots (El-Boubbou, 2011; Marradi *et al.*, 2013) provide nanoscale materials with anti-adhesive and cell targeting properties.

In our research group, we are interested in a specific lectin named DC-SIGN, a C-type lectin able to recognize and interact with a number of highly glycosylated proteins presented in the surface of a diverse group of pathogens (Van Kooyk, Geijtenbeek, 2003). The broad pathogen spectrum that could use DC-SIGN during the infection process places this lectin as an important target in infection diseases. In the present review, we will describe the approaches to design and synthesize glycodendritic antagonist for DC-SIGN.

DC-SIGN

DC-SIGN (Dendritic cell-specific ICAM-3 grabbing nonintegrin, CD209) was originally defined as an intercellular adhesion molecule-3 (ICAM-3) receptor that play an important role in establishing the first contacts between dendritic cells (DCs) and resting T cells (Geijtenbeek *et al.*, 2000). It is a type II trans-membrane C-type lectin with a single C-terminal Carbohydrate Recognition Domain (CRD). In the cellular membrane, DC-SIGN is assembled as a tetramer, due to an extended coiled-coil region that allows simultaneous presentation of four CRDs (Mitchell *et al*., 2001; Feinberg *et al*., 2005; Serrano-Sierra-Gomez *et al*., 2008; Tabarani *et al*., 2009).

DC-SIGN is one of the dendritic cells specific pathogen-uptake receptors and recognizes glycoconjugates on the surface of several pathogens, including viruses (HIV, Ebola, Cytomegalovirus, Dengue, SARS), bacteria (*M. tuberculosis*, *S. pneumoniae*), fungi (*C. albicans*, *A. fumigatus*), and parasites (*Leishmania*, *S. mansoni*) (Van Kooyk, Geijtenbeek, 2003). It has been proven that this lectin plays a key role in the initial steps of infections caused by some of these pathogens. In particular, DC-SIGN was brought to attention by the group of van Kooyk, who reported that HIV-1 targets DC-SIGN, but escapes degradation in lytic

compartments, thus using DCs as a Trojan horse to invade the host organism (Geijtenbeek *et al.*, 2000a). Inhibition of DC-SIGN is currently considered as an interesting approach for the design of new anti-infective agents (Reina *et al.*, 2010; Sánchez-Navarro, Rojo, 2010; Reina, Bernardi, 2012). The detailed molecular mechanism by which this receptor operates is not fully understood, thus effective modulators of DC-SIGN are also needed to unravel the different biological processes in which this receptor is involved. The main carbohydrate ligand recognized by DC-SIGN is the high mannose glycan, $(Man)_9(GlcNAc)_2$, (Figure 1) a branched oligosaccharide presented in multiple copies by several pathogen glycoproteins and specifically by the gp120 envelope protein of HIV. DC-SIGN can also recognize branched fucosylated structures bearing terminal fucose residues, such as the Lewis antigens.

The primary interaction of oligosaccharides and DC-SIGN occurs by coordination of a residue of the oligosaccharide (often the non-reducing end) to a Ca^{2+} binding site exposed to the surface of the protein (Feinberg *et al.*, 2001; Guo *et al.*, 2004; Feinberg *et al.*, 2007). X-ray data are available for complexes of the DC-SIGN carbohydrate recognition domain (CRD) with both mannose oligosaccharides and Lewis-X (Feinberg *et al.*, 2001; Guo *et al.*, 2004; Feinberg *et al.*, 2007). Besides the role that DC-SIGN plays in infection processes, the recent discovery of DC-SIGN as a lectin involved in immunoregulation processes attracted the interest of many scientists due to the possibility to exploit this lectin as a new potential target in immunotherapy. This important discovery has opened the possibility to design specific carbohydrate multivalent systems to modulate the immune response through the interaction with DC-SIGN. This is a field of tremendous interest and very active research is ongoing in this area for the design of new glycodendritic structures targeting this receptor.

CARBOHYDRATES MULTIVALENT COMPOUNDS TARGETING DC-SIGN

Highly mannosylated polyvalent compounds have been developed in an effort to target DC-SIGN mediated viral transmission. This topic has been recently reviewed (Sánchez-Navarro, Rojo, 2010) and therefore, only two representative examples, one based on gold nanoparticles and another one based on a polymer scaffold have been selected to be highlighted in the present review.

Penadés and Alcami have reported a remarkable example using gold nanoparticles (GNP) displaying different mannosyl oligosaccharides (Manno-GNPs) as potent inhibitors of DC-SIGN mediated HIV trans-infection of human activated peripheral blood mono-nuclear cells (Martinez-Avila *et al.*, 2009). The selected ligands were linear and branched oligosaccharides containing one to seven mannose units. Using Biosensors with Surface Plasmon Resonance (SPR) detection it was demonstrated that these GNPs were very good inhibitors in a competitive study using DC-SIGN and gp120 (Hijazi, 2009). In particular, the GNPs functionalized with the linear disaccharide Manalpha1-2Man was the best inhibitor showed a

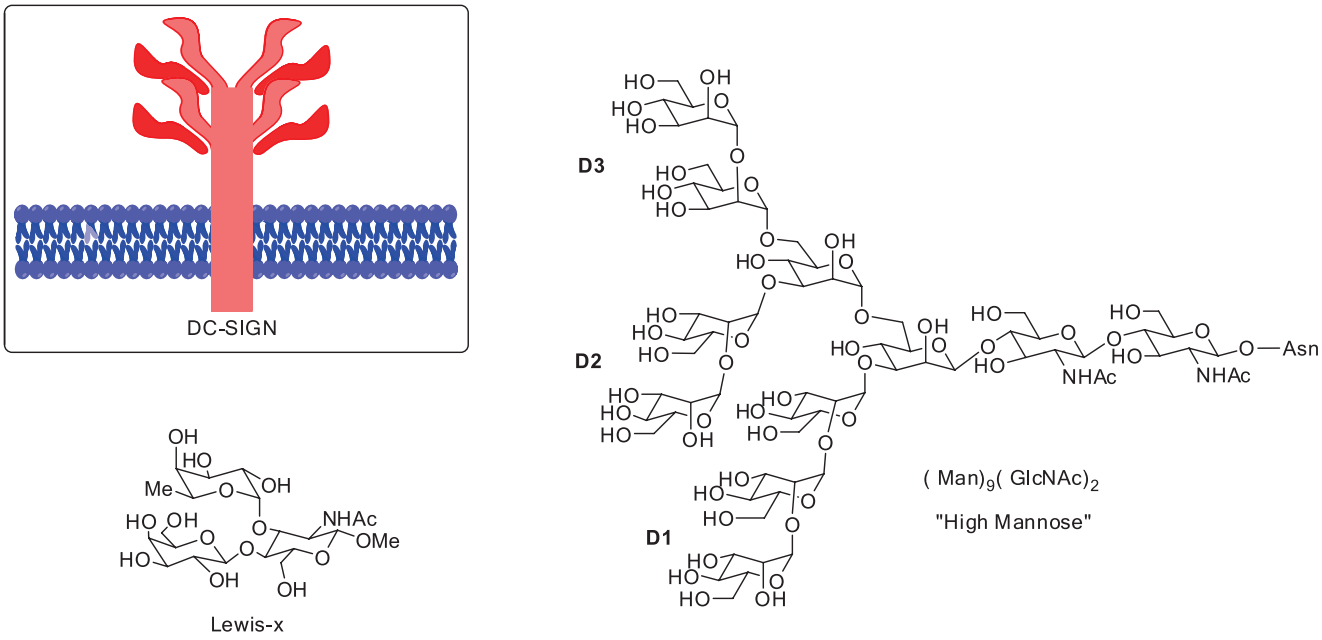

FIGURE 1 - Cartoon of DC-SIGN and chemical structure of Lewis X and $(Man)_9(GlcNAc)_2$ glycans.

20,000-fold increased activity in comparison with the corresponding monovalent ligand. It was also demonstrated that the maximum activity was achieved with 50% surface density. A selection of GNPs was tested in *in vitro* HIV-1 infection studies using viruses with different tropism (Martinez-Avila *et al.*, 2009). The experiments demonstrated that these GNPs were able to inhibit the trans-infection of T cells at very low concentrations. In particular, the GNP with 56 copies of a linear tetrasaccharide at a density of 50% (Figure 2) was the most potent inhibitor with an IC_{50} in the low nanomolar range. This is the first example where a carbohydrate multivalent system has been tested in *in vitro* HIV infection studies successfully.

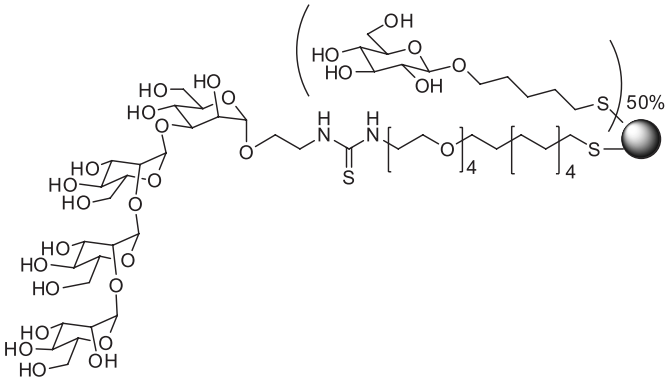

FIGURE 2 - Gold glyconanoparticle with 50% of glucose and 50% of a linear tetrasaccharide.

Another approach to prepare DC-SIGN inhibitors was described by the group of Kiessling, who used a shikimic acid-derived glycomimetic scaffold of general formula **A** (Schuster *et al.*, 2003; Gaber *et al.*, 2010). (Figure 3). The shikimic acid derivative prepared by Kiessling and co-workers shared the same hydroxyl arrangement as mannose at positions 2, 3 and 4, and could be considered as a good mimetic of mannose for lectin recognition. In addition to the hydroxyl groups that mimic mannose, this shikimic acid derivative presented two possible points of diversification, a carboxylic group and a thiol that were exploited to introduce different substituents and to synthesize a library of 192 compounds. All these compounds were tested using a fluorescence-based high-throughput competition assay that assessed their ability to compete with immobilized mannan for binding the fluorophore-labeled extracellular domain of DC-SIGN. The best compound of the library was **B**, (Figure 3) which had an IC_{50} of 11.2 mM, but was found to be more selective for DC-SIGN than for mannose-binding protein A (MBP-A). A multivalent presentation of compound **B** was prepared by ring-opening metathesis polymerization (ROMP) (Bielawski, Grubbs, 2007). The final glycopolymer was 1000-fold more potent than the monomeric inhibitor, the compound **B**.

GLYCODENDRIMERS TARGETING DC-SIGN

There are several precedents where dendrimers have been used as scaffolds to achieve a multivalent presentation of carbohydrates, the so called glycodendrimers. Furthemore, it have been observed that these kind of

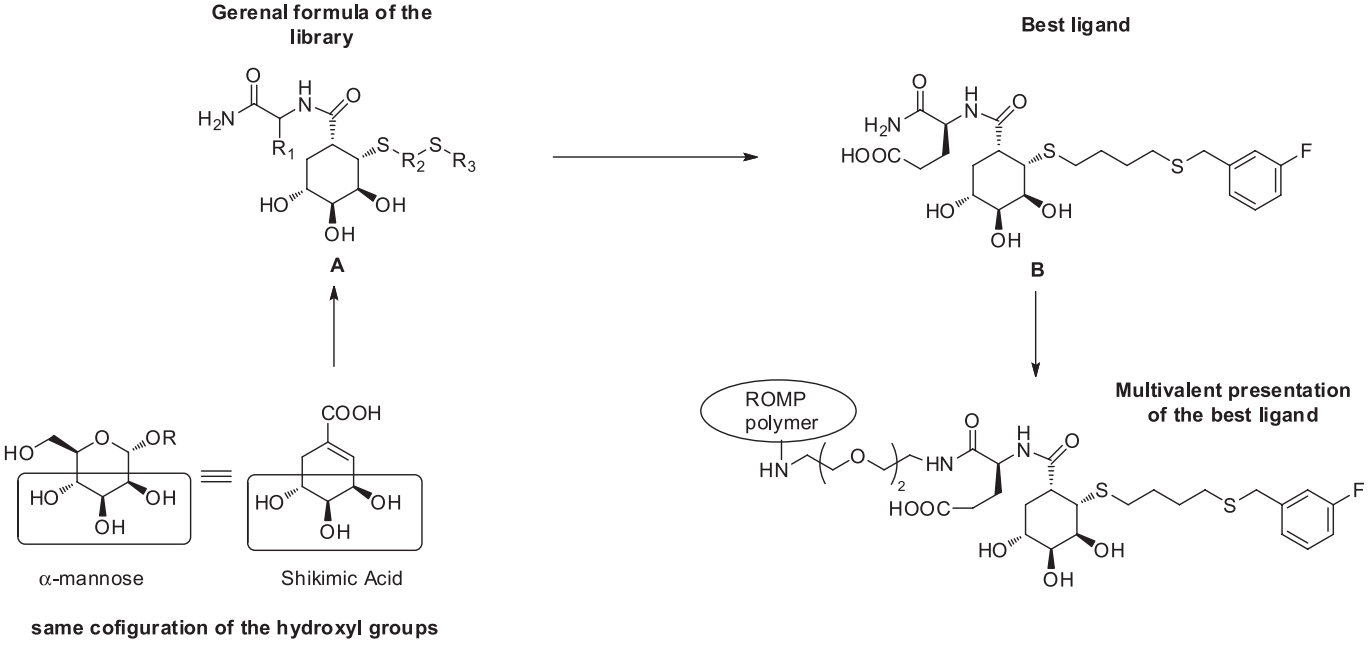

FIGURE 3 - Shikimic-based derivatives as ligands for DC-SIGN.

carbohydrates multivalent systems are important tools to interfere in biological processes where lectins or sugar binding proteins are implicated (Roy, 1996; Turnbull, Stoddart, 2002; von der Lieth, Frank, Lindhorst, 2002; Roy, Baek, 2002; Bezouška, 2002; Roy, 2003; Li, Cheng, Xu, 2007; Chabre, Roy, 2008, 2010; Sánchez-Navarro, Rojo, 2010).

Among them, a recent interesting example where a glycodendron was used to interact with DC-SIGN was described by Wong and co-workers. They have reported oligomannose dendrons which display 3, 9 and 27 copies of complex oligomannoses (Figure 4) in high density as inhibitors of gp120 binding to recombinant dimeric DC-SIGN (Wang *et al.*, 2008). The selected carbohydrates were a linear tetramannosyl ligand (Man_4) corresponding to the D1 arm of high mannose and a branched nanomannosyl oligosaccharide that represented the high mannose glycan (Figure 1). In a competition experiment using gp120 as a ligand for DC-SIGN, it was demonstrated that these glycodendrons inhibited the binding between gp120 and DC-SIGN with an IC_{50} of 20 and 8 nM for compounds with nine copies of Man_4 and nine copies of Man_9, respectively.

Since almost a decade ago, our group is interested in the design and synthesis of different multivalent compounds based on mannose and glycomimetics of high mannose oligosaccharides and Lewis-X, as potential molecules able to block the binding of pathogen envelope glycoproteins to DC-SIGN. For this aim, we focused on dendrimers and dendritic structures as appropriate scaffolds for a multivalent presentation of carbohydrates trying to achieve binding affinities to DC-SIGN in the μM to nM range, a task without precedents at that time. In this revision article, we will give and overview of the work performed in our group related with glycodendritic structures as DC-SIGN antagonist.

Boltorn-type glycodendritic structures

As a proof of concept, we addressed a divergent approach (Fréchet, Tomalia, 2001; Newkome, Moorefield, Vögtle, 2001; Sánchez-Navarro, Rojo, 2012) to synthesize glycodendrimers using very simple and accessible molecules. We selected a Boltorn hyperbranched dendritic polymer, commercially available at a very low price, as scaffold and a simple monosaccharide, mannose, as ligand to DC-SIGN to be conjugated on it. These hyperbranched polymers have been constructed using bis-hydroxymethylpropionic acid (Bis-MPA) as building block and pentaerythritol as core. The glycodendritic polymers were prepared using the 2nd and 3rd generation of Boltorn dendritic polymers conjugated with 16 and 32 mannoses, respectively. (Figure 5). We demonstrated that these glycodendritic structures were perfectly soluble in physiological conditions, non-toxic against several cell lines and easy to be prepared (Arce *et al.*, 2003).

The affinity of these glycodendritic polymers to DC-SIGN was evaluated using a SPR biosensor (Tabarani *et al.*, 2006). Experiments clearly shown that the binding of

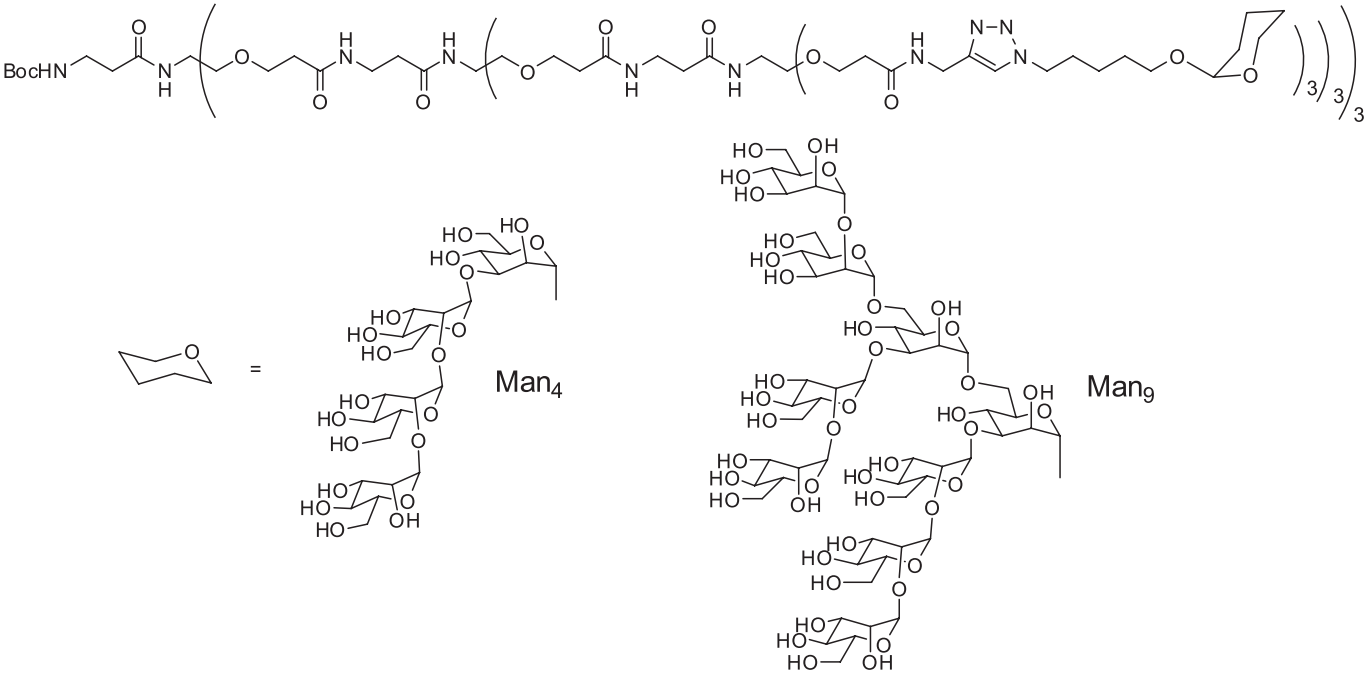

FIGURE 4 - Glycodendrons with 27 copies of Man_4 and Man_9.

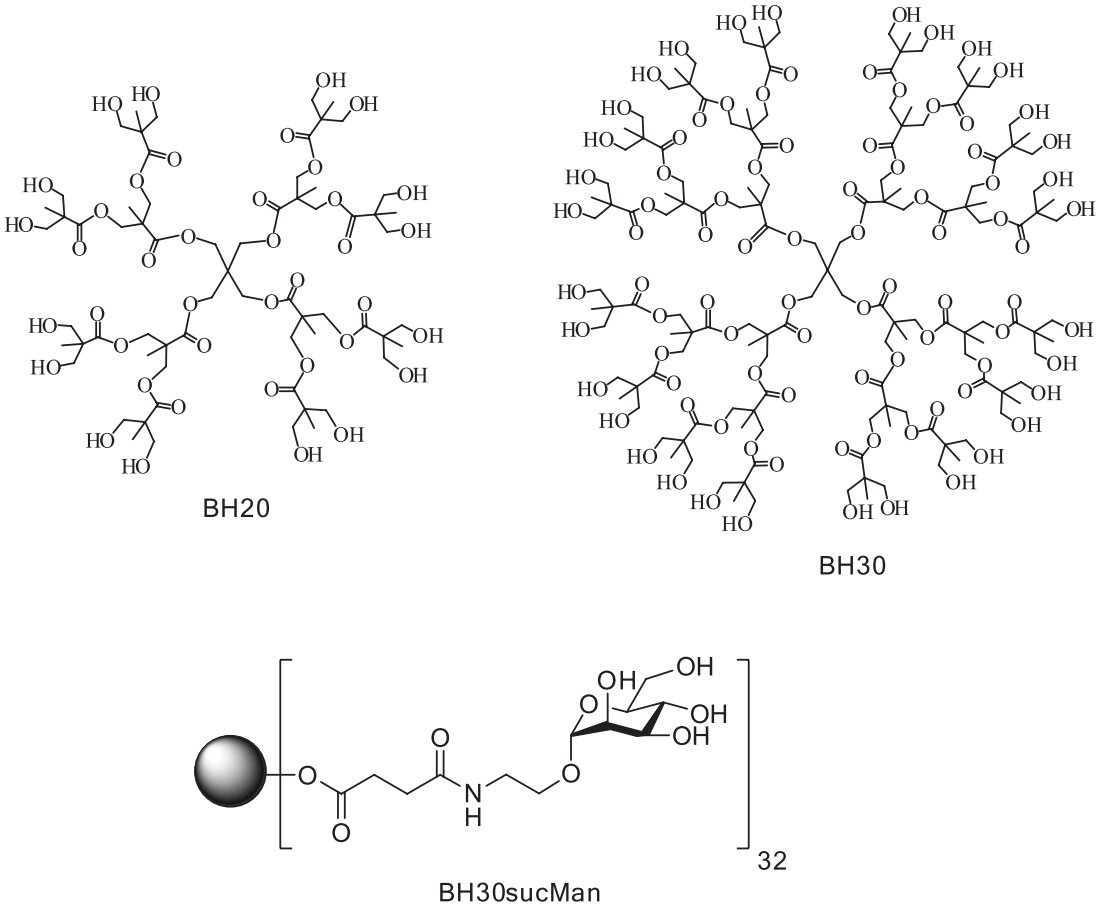

FIGURE 5 - Boltorn hyperbranched structures of 2nd (BH20) and 3rd (BH30) generation and the glycodendrimer BH30sucMan.

BH30sucMan based on BH30 (3rd generation of Boltorn) to DC-SIGN was calcium and carbohydrate dependent and therefore should take place through the carbohydrate recognition domain of the lectin. An apparent affinity in the sub microMolar range was found (3 orders of magnitude increase in comparison with the monovalent mannose). Using SPR, we also set up a competition experiment assay in which different glycodendritic structures were analyzed for their ability to inhibit the gp120/DC-SIGN interaction, finding an IC_{50} of 50 μM for BH30sucMan. Additionally, it was demonstrated the antiviral properties of the mannosyl glycodendritic structure BH30sucMan using an Ebola Virus infection model. This glycodendritic hyperbranched polymer inhibited DC-SIGN-mediated Ebola Virus infection *in cis* and *in trans* (Lasala *et al.*, 2003; Rojo, Delgado, 2004). The experiment showed that BH30sucMan was able to selectively inhibit direct DC-SIGN-mediated Ebola infection in an efficient dose-dependent manner (IC_{50} 337 nM). In the experiment *in trans*, in which a more complex series of events such as internalization and presentation of the viral particle to susceptible cells can take place, BH30sucMan also showed a significant reduction of DC-SIGN-mediated infection *in trans* at levels comparable to the inhibition shown *in cis*. These glycodendritic polymers were the first example of a multivalent DC-SIGN antagonist describe in the literature.

After this proof of concept where sub micromolar apparent affinity and good antiviral activity have been reached presenting a simple carbohydrate (a mannose monosaccharide) as ligand for DC-SIGN, an improvement of these activities could be envisaged using more complex and specific carbohydrate ligands. Moreover, Boltorn hyperbranched dendritic polymers are polydisperse materials and the control of the synthesis and the reproducibility of biological assays could be compromised by the presence of a mixture of compounds. A monodisperse Boltorn type dendrimers and dendrons were prepared using a step-wise synthesis avoiding these potential problems. In this way, a new set of carbohydrate multivalent compounds were synthesized using these monodisperse multivalent scaffolds.

From the point of view of the ligand moiety, it has been reported the design and synthesis of two glycomimetic compounds that bind to DC-SIGN. A pseudo-1,2-mannobioside **1** (Figure 6), which contains a mannose unit

connected to a conformationally locked cyclohexanediol to mimic 1,2-mannobioside (Manα (1,2)Man) (Reina *et al.*, 2007). The pseudo-trisaccharide **2** mimicking the linear Manα (1,2)Manα (1,6)Man trisaccharide of the D3 arm of Man_9, (Figure 6) was designed following the same concept (Mari *et al.*, 2007). The affinity for DC-SIGN of both monovalent ligands was too weak (mM range) to be considered as effective inhibitors of DC-SIGN-mediated infections and their therapeutic potential was limited. However, this low affinity could be overcome when ligands were presented in a multimeric form.

The multivalent presentation of glycomimetics **1** and **2** was achieved by conjugation of the monovalent ligands to tetra- and multivalent scaffolds based on Bis-MPA as building block to generate dendritic compounds with Boltorn-type structures. Two scaffolds with different valency were synthesized: a tetravalent dendron and a 3rd generation Boltorn-type dendrimer, both being monodisperse compounds. The corresponding tetravalent glycodendrons **3** and **4**, and the multivalent glycodendrimers **5** and **6** (Figure 7) were evaluated as potential inhibitors of DC-SIGN (Sattin *et al.*, 2010).

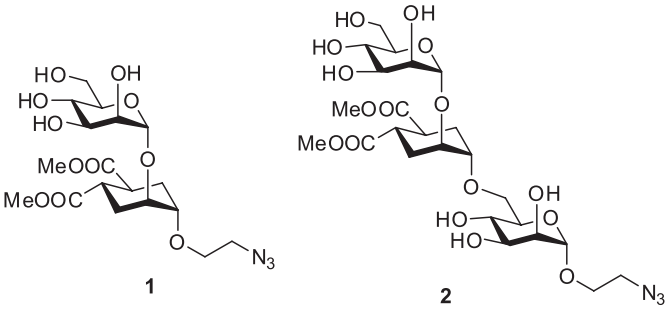

FIGURE 6 – pseudo-disaccharide **1** and pseudo-trisaccharide **2**.

The tetravalent dendron **4** with four copies of pseudo-trisaccharide **2** was shown to inhibit the *trans* infection of T lymphocytes by HIV. Infection was abrogated almost totally by **4** at 100 μM concentration, and an IC_{50} ca. 10 μM could be estimated (Sattin *et al.*, 2010), a very promising value as starting point to define better antiviral compounds. Furthermore, dendron **4** was evaluated as a suitable candidate for the development as topical HIV microbicide (Berzi *et al.*, 2012). This compound was tested as inhibitor in an experimental model based on HIV infection of explant tissues taken from human uterine cervix. This experiment was the previous step to test the inhibitors in *in vivo* models. It was demonstrated that dendron **4** inhibited 80-90% of cervical explant HIV infection.

Additionally, the tetra- and multivalent systems were tested *in vitro* using an infection model based on pseudotyped viral particles with the Ebola virus envelope glycoprotein GP1. In these experiments, the tetravalent

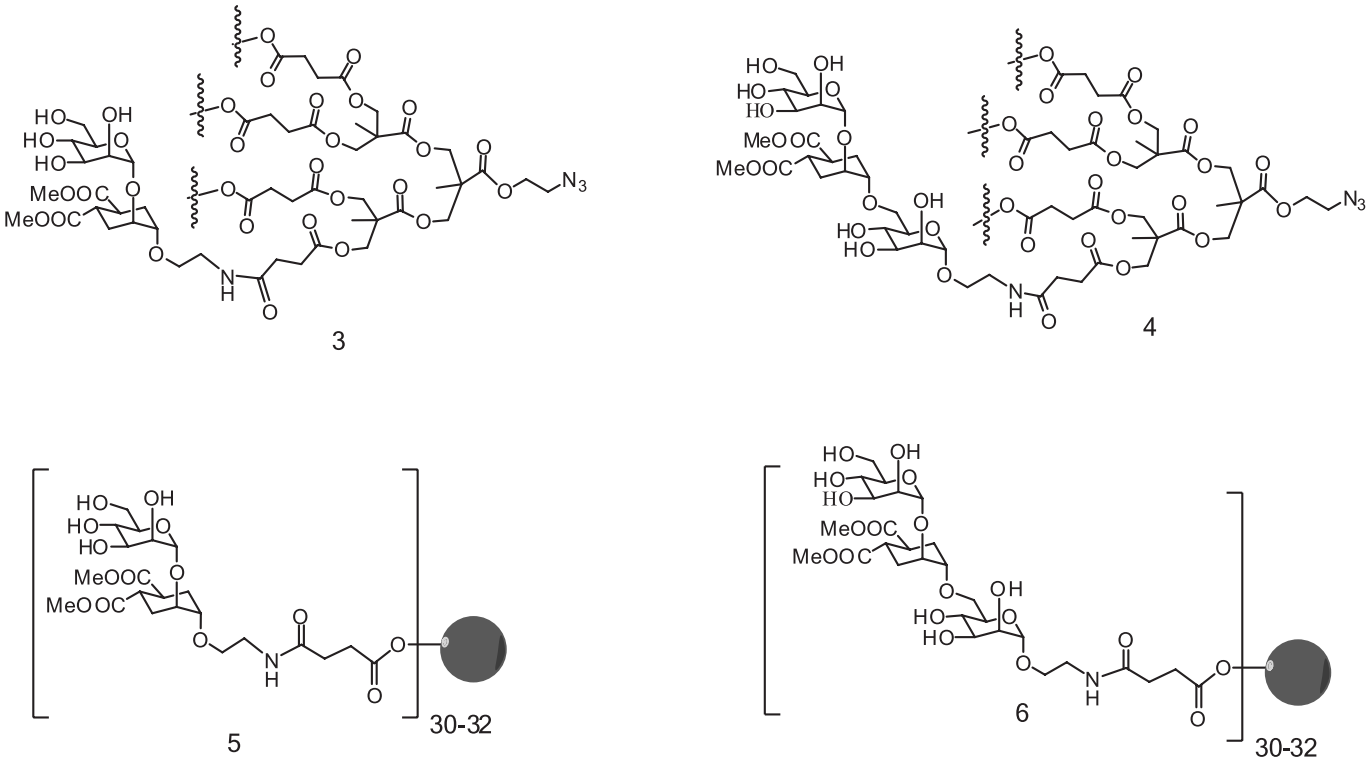

FIGURE 7 - Structure of tetravalent glycodendrons **3** and **4** and glycodendrimers **5** and **6**.

systems **3** and **4** were very active in the low micromolar range, and the multivalent systems **5** and **6** showed a very strong inhibition effect with IC_{50} in the nanomolar range (Luczkowiak *et al.*, 2011).

Glycodendritic systems based on the Cu (I) catalyzed azide-alkyne cycloaddition (CuAAC)

In the previous section, it has been described an approach to create carbohydrates multivalent systems based on Boltorn type structures. The synthesis of these kinds of polyesters-based glycodendrons and glycodendrimers presented some disadvantages. The divergent synthetic approach entailed the attachment of the saccharidic units to the multivalent scaffold in the last stage, generating several covalent bonds simultaneously in a single synthetic step. For this reason, this approach can generate structural defects due to an incomplete conjugation giving rise to molecules with similar structure, and physical and chemical properties and therefore, being very difficult the purification step to get pure and monodisperse glycodendrimers. A more efficient strategy should be addressed to achieve the preparation of this kind of molecules.

In this context, our group designed new multivalent systems based on a convergent strategy using a very efficient type of click reaction (Kolb, Finn, Sharples, 2001), in particular, the Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) (Rostovtsev *et al.*, 2002; Tornøe, Christensen, Medal, 2002), as an alternative to the preparation of glycodendrons and glycodendrimer. The click chemistry reaction CuAAC is a very efficient, versatile, and popular reaction that has found several applications in many fields to achieve the preparation of very diverse molecules ranging from small compounds to polymers, hydrogel and other larges entities, including dendrimers (Wu *et al.*, 2004; Joralemon *et al.*, 2005; Malkoch *et al.*, 2005; Fernández-Megia *et al.*, 2006; Franc, Kakkar, 2010). Following a convergent strategy based on CuAAC reaction, the synthesis of a new class of glycodendrons with full control of the structure was developed. In the new design, it was considered the presence at the focal position of an appropriated functional group permitting the conjugation of biomolecules of interest (peptides, nucleic acids, etc.) using again a CuAAC reaction. Glycodendrons, containing up to nine copies of carbohydrate ligands (mannose or fucose) (Figure 8), showed an efficient binding with the DC-SIGN receptor at the surface of DCs (Ribeiro-Viana *et al.*, 2012). The functional group at the focal position was used to conjugate a fluorophore (a BODIPY derivative) to study the DC-SIGN-mediated internalization of these glycodendrons after the binding event at the surface of DCs.

These glycodendrons internalized efficiently into monocyte derived DCs in a receptor-dependent manner as it was demonstrated using imaging flow cytometry. Additional experiments performed to determine the intercellular routing of these molecules into DCs showed that glycodendrons were co-localized within lysosomes. Finally, it was evaluated the capacity of glycodendrons to induce the maturation of DCs. None of the glycodendron showed any activity to induce DCs maturation neither expression of cytokines at the concentration tested. Besides this fact, they can be considered as interesting vectors to internalize into target cells biomolecules of interest such as immunogenic peptides, conjugated to the focal position of these glycodendrons, opening the door for their use to develop synthetic vaccines.

This convergent click chemistry approach to prepare glycodendritic structures could be applied to different scaffolds generating interesting carbohydrate multivalent systems.

Glycodendrofullerenes

Fullerene could be a very attractive core with a rigid spherical shape for a unique globular carbohydrate multivalent presentation. However, its low solubility in polar solvents is one of the major drawbacks to address the preparation of biological active compounds. On the other hand, fullerenes allow the possibility to functionalize its 3D structure in different positions of the C60 cage in a controlled fashion. Initially, the approaches reported in the literature to synthesize fullerenes derivatives bearing carbohydrates were based on a mono-addition pattern (Nishida *et al.*, 2004; Enes *et al.* 2005) on the fullerene sphere or on a "one side" multi-addition pattern (Cardullo *et al.*, 1998; Ito *et al*., 2003; Kato *et al*., 2005; Isobe *et al*., 2007). In all these cases, fullerene derivatives showed an amphiphilic character with the fullerene being the lipophilic part of the structures. Recently, it has been shown that this amphiphilic character of sugar fullerene conjugates can be avoided by obtaining hexakis-adducts of [60]fullerene in which the C60 sphere could be completely surrounded by the sugar moieties (mannose, glucose, galactose or fucose) in a *T*-symmetrical octahedral addition pattern (Nierengarten *et al.*, 2010). The conjugation of sugar residues to an alkyne-substituted Bingel-Hirsch hexakis-adducts was performed by employing the CuACC methodology. So, it was obtained the first example of a globular polytopic ligand named glycofulleres or "sugar balls" (Figure 9).

To increase the valency of these systems, a divalent dendron with mannose was used to obtain the first glycodendrofullerene with 24 sugars moieties on the periphery

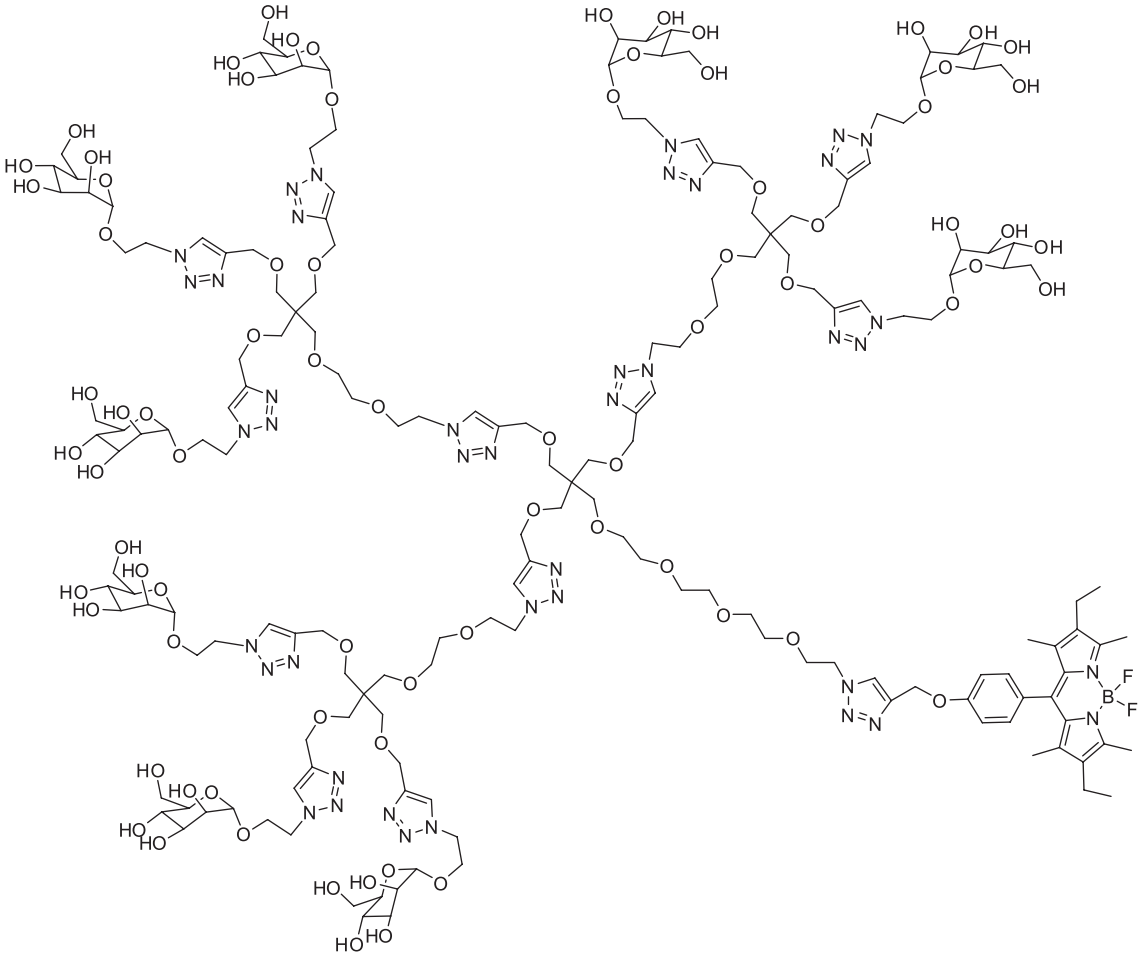

FIGURE 8 - Mannosyl nonavalent dendron fluorescence labeled with BODIPY.

of C60 (Sánchez-Navarro *et al*., 2011) (Figure 9). These compounds were water soluble and interact with Concanavalin A in a multivalent manner demonstrating the accessibility of the sugars on the fullerene surface to be recognized by a lectin. Moreover, the compounds showed a good stability and low toxicity which confirmed the appropriate features to be used in cellular assays.

Globular glycofullerenes with higher valency were prepared by a convergent strategy combining trimannosylated glycodendrons with a Bingel-Hirsch hexakis-adduct [60]fullerene to obtain glycodendrofullerenes with 36 mannoses (Figure 9) and two different spacers. Also, it was introduced for the first time the biological activity of these compounds in a cellular infection model providing important information about the potential use of these new glycomimetics as viral inhibitors. These glycodendrofullerenes act as antiviral agents in a DC-SIGN dependent Ebola pseudotype infection model (Luczkowiak *et al*., 2013). Glycofullerene with 12 mannoses showed an IC_{50} of 2 μM, a very promising data; however, an unexpected result was obtained for the activity of the glycodendrofullerene with 36 mannoses. The increase of valency induced a loss of antiviral effect (IC_{50} c.a. 70 μM). This could be probably related to the steric congestion of sugars at the surface of fullerene. Furthermore, one important factor to achieve high affinity in binding processes was not only the spatial presentation of ligands but also the adequate accessibility of these ligands to interact with the receptor, DC-SIGN in this case. Using a glycodendrofullerene with 32 mannose (same valency) but including a longer spacer, was observed a remarkably increase of the inhibitory activity of this compound (IC_{50} 0.3 μM), probably due to a more efficient interaction of the mannose ligand with DC-SIGN. This result highlights the importance to combine an adequate scaffold to achieve the multivalency (the spherical fullerene) with the right ligand accessibility and flexibility. The valency of the compound is an important factor to obtain good affinities in a carbohydrate-lectin interaction but as it has been shown in these assays, it is not the only factor to be taken into account. Based on these results, fullerenes appear as very attractive scaffolds for a globular multivalent presentation of sugars.

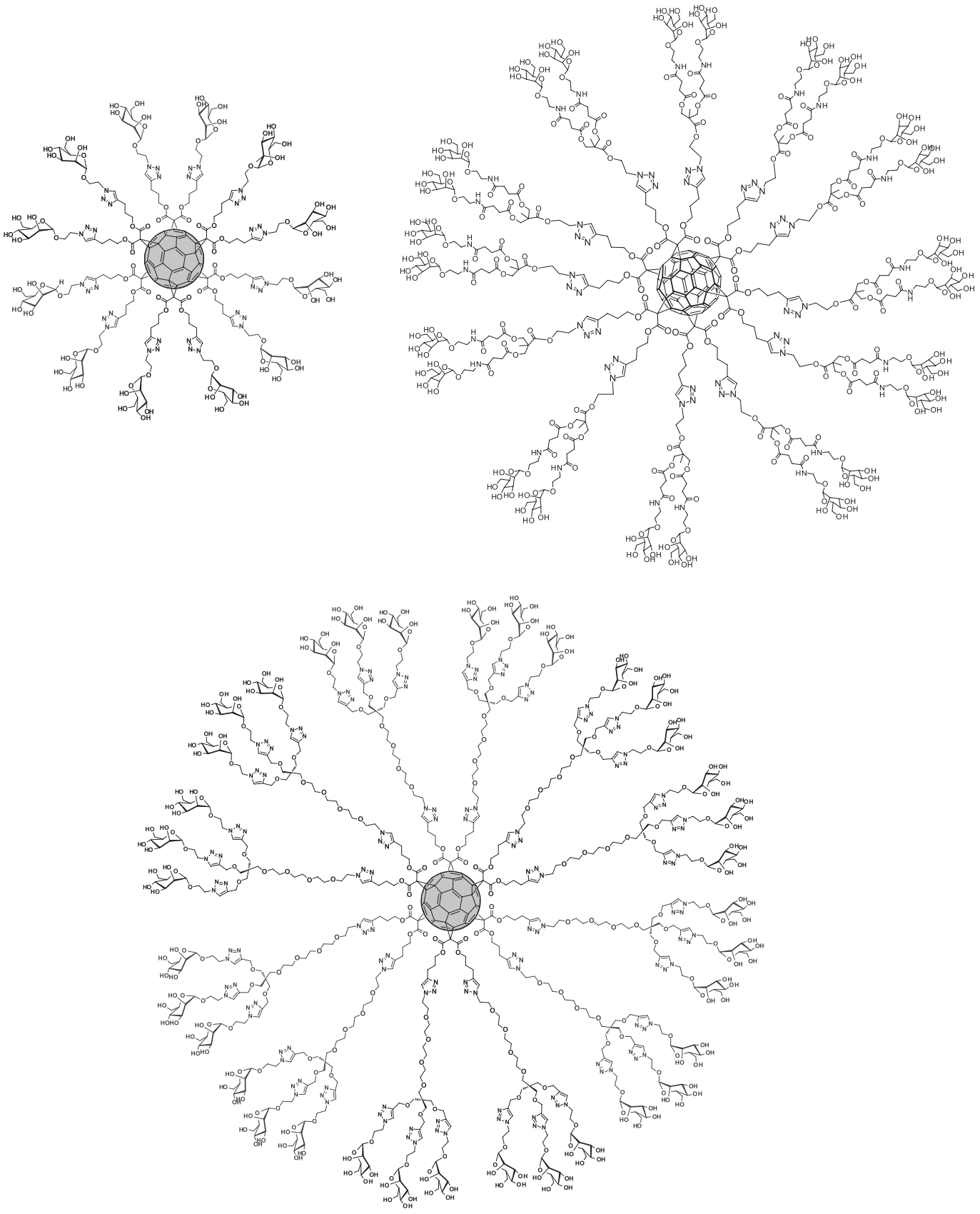

FIGURE 9 - Glycofullerenes with 12, 24 and 36 mannoses.

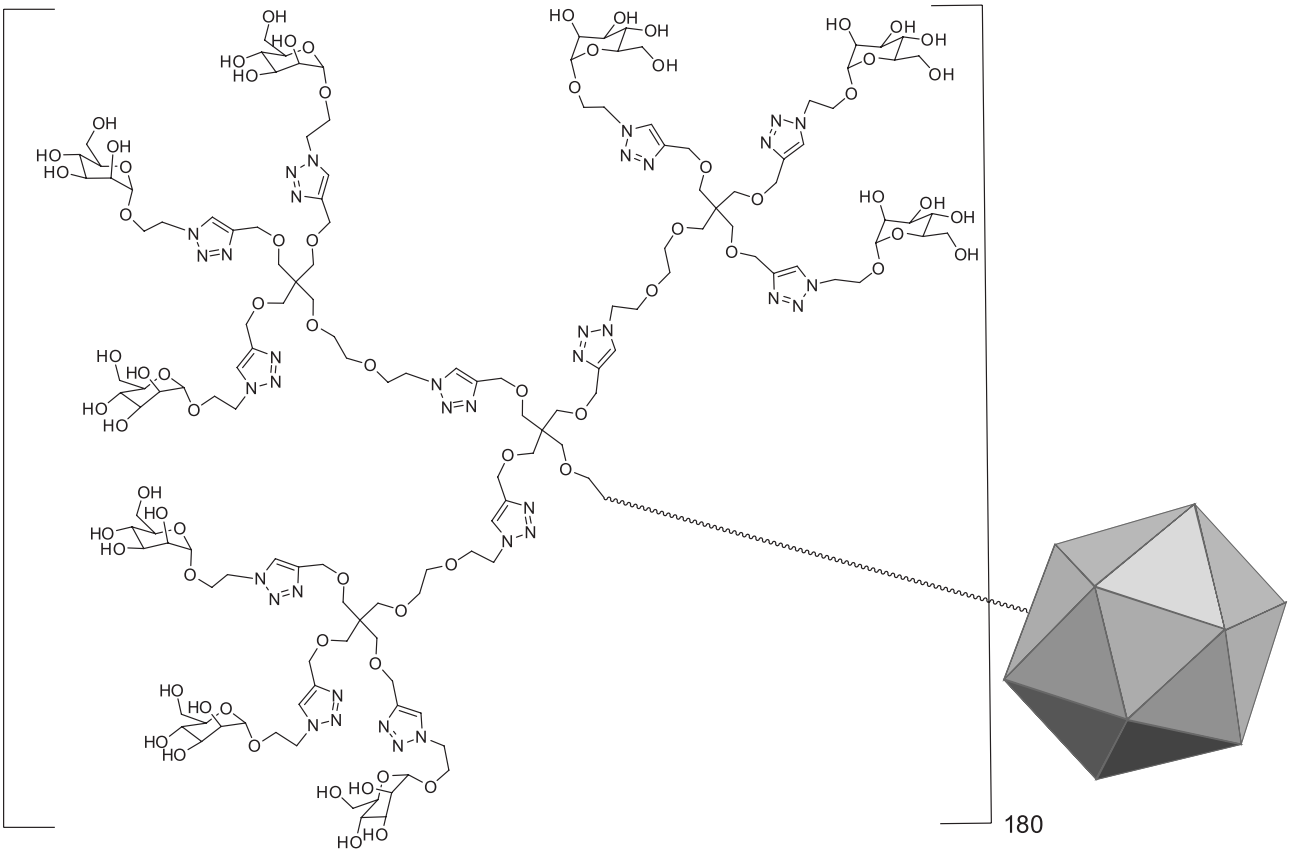

FIGURE 10 - Qβ particle bearing 1620 mannose units.

Virus-like glycodendrinanoparticles

Despite their elegant design, none of the artificial carbohydrates multivalent systems described (including the glycodendrofullerenes) have achieved the size and the valency to mimic properly natural systems implicated in DC-SIGN dependent infection processes such as viruses or other pathogens while maintaining full control of shape and structure. In the particular cases of glycodendritic structures, valency up to 100 are very rare and, in most of the cases, without full control of shape. Recently, a novel strategy of "nested polyvalency" was reported to achieve a controlled design of highly polyvalent glycodendriproteins. This approach was performed through the multivalent assembly of protein monomers themselves carrying polyvalent glycan display motifs (glycodendrons) (Ribeiro-Viana *et al.*, 2012a). These synthetic protein assemblies display the highest known number of glycans (n = 1620) yet presented in a homogeneous manner (Figure 10). To perform the construction of glycodendriprotein nanoparticles trying to mimic the display in target pathogens, the self-assembled protein sphere-like icosahedron Qβ (Kozlovska *et al.*, 1993) was used. The monomer of this protein can assemble into a 180 copy multimer. Using a tag-and modify strategy (Chalker, Bernardes, Davis, 2011), a non-natural alkyne containing amino acid (tag), the L-homopropargyl glycine (Hpg), was introduced in the Qβ monomer. This tag was used for the attachment of glycodendrons with three and nine mannose units appropriately functionalized at the focal position with and azide group. The well-defined polyvalent glycoprotein assemblies presenting on their surface 1620 copies of mannose was tested in the DC-SIGN dependent Ebola pseudotype infection model.

The glycoprotein particles showed exciting antiviral activity, preventing dendritic cells infection by Ebola pseudotyped virus through competitive blockage of the DC-SIGN receptor in the nanomolar to picomolar range (Ribeiro-Viana *et al.*, 2012a). This result clearly indicates the efficiency of these systems to interact with this pattern recognition receptor and to compete with pathogens during their entry into target cells.

CONCLUSIONS

The field of dendrimers is in its maturity and many achievements and goals have been reached since the first publication describing the synthesis of a dendritic molecule in 1978 (Buhleier, Wehner, Vögtle, 1978). One of the most

interesting areas of research where dendrimers start to have an important contribution is nanomedicine. Taking advantage of the possibilities that offer the dendritic structures and the opportunities to combine biomolecules or any kind of ligands with these platforms for a multivalent presentation, many different applications have been described for these so beautiful and interesting molecules. The combination of carbohydrates with dendrimers allows the creation of glycodendrimers, very powerful tools to be exploited in the field of glycoscience mimicking the presentation of carbohydrates in nature. DC-SIGN, a very important and attractive receptor with relevant biological implications, has been one of the targets for these glycodendrimers. Several examples have been described in the literature that demonstrate the potential applications of glycodendritic structures to interact efficiently with DC-SIGN inhibiting infection processes mediated by this lectin. From these examples, important lessons came out respect to the necessary relevant issues to have access to effective glycodendritic molecules. Efficient chemistries, valency, size, spatial presentation, flexibility and accessibility of the ligands are among the important issues to be considered. Still, much work remains be done in the near future and novel scaffolds for multivalent presentations will be explored to achieve new goals and applications.

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