Precision Glycodendrimers for DC-SIGN Targeting


Dedicated to Professor Cesare Gennari on his 70th birthday.

Abstract: Multivalent ligands of the C-type lectin receptor DC-SIGN have emerged as effective antiadhesive agents against various pathogens. Some years ago, we described a hexavalent DC-SIGN ligand, Polygon-26, designed to bridge two of the four binding sites displayed by the receptor. Here we present our efforts to accomplish simultaneous coordination of all four carbohydrate binding sites of DC-SIGN through the synthesis of cross-shaped glycodendrimers. The tailored rigid scaffold allowed multivalent presentation of glycomimetics in a spatially defined fashion, while providing good water solubility to the constructs. Evaluation of the biological activity by SPR assay revealed strong binding avidity towards DC-SIGN and increased selectivity over langerin. Inhibition of DC-SIGN binding to SARS-CoV-2 spike protein and of DC-SIGN mediated Ebola virus trans-infection testifies for the glycodendrimers potential application in infection diseases. The tetravalent platform described here is easily accessible and can be used in modular fashion with different ligands, thus lending itself to multiple applications.

Introduction

Carbohydrate-protein interaction in living systems is an archetype of multivalency, where proteins (called lectins) presenting either multiple carbohydrate recognition domains (CRDs) or an oligomeric structure selectively recognize and bind to specific polyglycosylated targets.[1] This strategy takes advantage of the mechanisms governing multivalency, i.e. chelation, statistical rebinding and receptor clustering, to provide strong binding, while overcoming the intrinsic low affinity of the monovalent glycan ligands for their receptors.[2-4]

Following the very same approach, the past two decades have seen a prosperous generation of multivalent glycoconjugate antagonists able to interfere with such interactions.[5-9] Altogether, these studies revealed the complexity in designing effective antagonists, whose efficacy is determined by the nature of the ligand displayed, as well as by parameters difficult to predict, such as the architecture of the polyvalent scaffold, the valency, the ligand density, the kind of linker engaged and the flexibility of the construct.[10]

Lately, we have disclosed structure-based design as a guiding principle in the development of strong polyglycosylated antagonists for Dendritic Cell-Specific Intercellular adhesion molecule-3 (ICAM-3)-Grabbing Non-integrin (DC-SIGN),[11,14] a tetrameric transmembrane C-type lectin receptor (Figure 1) exploited by pathogens such as HIV, Ebola, Hepatitis C, to invade the host and propagate the infection.[15,16] While multiple ligand presentation on polyvalent scaffolds is generally the choice to reach high avidity towards DC-SIGN,[7-9] we showed that scaffold optimization plays a role in achieving high affinity levels with constructs of relatively low valency. Specifically, rigid rod-like scaffolds of controlled length were loaded with glycodendrons, giving access to hexavalent constructs (Polygon-31 PM31 and Polygon-26 PM26, depending on the monovalent ligand, Figure 2)[17] able to bridge two contiguous CRDs within the DC-SIGN tetramer, that are separated by ca. 4 nm. These constructs that combine chelation with a high local concentration of monovalent ligands in the proximity of the sugar binding site showed nanomolar activity in the inhibition of DC-SIGN mediated HIV[12,18] and SARS-CoV-2[19] infection, in sharp contrast with the low micromolar activity range of less preorganized structures of similar or even higher valency.[20]
The strong impact of chelation on the inhibition potency led us to consider whether stronger antagonists could be obtained by simultaneous binding of the four CRDs of DC-SIGN extra-cellular domain (ECD). In small-angle X-ray scattering (SAXS) derived models, they are arranged at the four corners of a square with 4 nm side and diagonals going from 5.2 to 6 nm (Figure 1), but the system is highly dynamic both within the single oligomer and in the tetramer. Thus a modular design, based on a rigid core of appropriate topology and low-valency ligand presentation is expected to provide optimal match.

To test this hypothesis, we targeted the synthesis of cross-shaped glycodendrimers PM59 and PM58 (Figure 2). These compounds are characterized by a tetravalent rigid core of 22 Å diagonal length, which is prolonged by four copies of trivalent glycodendron moieties, resulting in an extended distance over 6 nm between two complexing units. As monovalent ligands, we selected the pseudo-disaccharide 1 and the corresponding more potent bis-p-hydroxyethylbenzylamide derivative 2, which we previously reported as effective and selective DC-SIGN antagonists. Ideally, the tailored geometry of the scaffold would confer optimal ligand presentation towards DC-SIGN, while disfavoring binding to C-type lectins characterized by a different spatial arrangement of their CRDs. Selectivity towards DC-SIGN is indeed crucial in order to inhibit its biological functions without interfering with the protective mechanisms provided by other C-type lectins.

Herein, we report the synthesis of compounds PM58,59 and the evaluation of their interaction with DC-SIGN by Surface Plasmon Resonance (SPR). Selectivity over langerin, a trimeric C-type lectin able to induce virus elimination and clearance in HIV infection, was also assessed. Moreover, we show that PM58,59 are effective at preventing DC-SIGN binding to SARS-CoV-2 spike protein and that, at 0.5 μM, they block DC-SIGN mediated trans-infection by Ebola virus.

**Results and Discussion**

**Synthesis of cross-shaped glycodendrimers.**

For the synthesis of glycodendrimers PM59 and PM58, we identified the tetravalent phenylene-ethynylene core 6 as a key intermediate, which enables for late-stage diversification at its four ends through copper catalyzed alkyne azide cycloaddition (CuAAC) (Scheme 1). From a retrosynthetic point of view, the central core 6 can originate from the iodoxyon 4 and the protected tetraalkynylbenzene unit 5, whose synthesis has been reported starting from 1,2,4,5-tetramethoxybenzene 3.

As the first step of the synthesis (Scheme 2), a Sonogashira coupling of 1,2,4,5-tetramethoxybenzene 3 with trimethylsilylacetylene afforded the desired protected tetraalkynyl 5 as a pure product, which was directly submitted to a deprotection reaction. Removal of the trimethylsilyl groups under basic conditions proceeded smoothly, yielding the tetraalkynyl central unit 5a with no need of further chromatographic purification. The selective formation of both 5 and 5a was confirmed by 1H NMR and electron impact (EI) MS analyses. A second Sonogashira coupling enabled connecting the central unit 5a to four copies of iodoxyon 4, finally providing the protected tetravalent scaffold 6. The formation of the product was monitored exploiting the intrinsic fluorescence of the construct, which allows
its detection by TLC analysis (365 nm irradiation), and by ESI-MS analysis. Purification by flash chromatography followed by size-exclusion chromatography (Sephadex LH-20 column) afforded the pure core 6 in 30% yield over three steps from 3.

With the phenylene-ethynylene core 6 in hand, the glycodendrimers PM58,59 were finally accessible (Scheme 2). In situ deprotection of the terminal alkyne moieties within 6 was accomplished upon treatment with a Bu4NF solution in THF for 1 h, and monitored by TLC analysis at 365 nm until full conversion was observed. A subsequent CuAAC step guaranteed efficient functionalization of the rigid tetravalent scaffold with four copies of either azido tethered glycodendrimer 7 or 8.[20] The reaction progression was assessed either by MALDI-TOF MS (DHB matrix) or HPLC analysis; purification by size-exclusion chromatography (Sephadex LH-20) afforded the final constructs PM59 and PM58 in very good yield (92% and 70% respectively). Pleasantly, the constructs showed good solubility in water (PM59, 2.5 mM) or water + 4% DMSO solution (PM58, 0.2 mM); they were fully characterized by NMR and HRMS analysis and their purity was assessed by HPLC analysis.
Surface plasmon resonance interaction studies: DC-SIGN vs Langerin selectivity.
The biological activity of glycodendrimers PM59 and PM58 towards DC-SIGN S-ECD and langerin S-ECD was assessed and compared with the corresponding linear constructs PM31 and PM26 by an established Surface Plasmon Resonance (SPR) direct interaction assay.[27] In this test, increasing concentrations of glycodendrimer solutions are flown over the surface of a sensor chip, functionalized with the immobilized targeted C-type lectins. Analysis of the assay sensorgrams provides the corresponding thermodynamic apparent dissociation constants $K_D$ (Table 1 and Figure 3).

Table 1. Dissociation constants $K_D$ (nM) and selectivity factor $S$ of glycodendrimers PM31, PM26, PM59 and PM58 obtained for direct interaction with DC-SIGN and Langerin by SPR assays.

<table>
<thead>
<tr>
<th>Dendrimer</th>
<th>$K_D$ (nM)</th>
<th>DC-SIGN</th>
<th>Langerin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM31</td>
<td>27.3 ± 1</td>
<td>51.6</td>
<td>1.9</td>
</tr>
<tr>
<td>PM59</td>
<td>14.45 ± 0.85</td>
<td>40.6</td>
<td>2.8</td>
</tr>
<tr>
<td>PM26</td>
<td>6.6 ± 1.45</td>
<td>101</td>
<td>15</td>
</tr>
<tr>
<td>PM58</td>
<td>6.45 ± 0.3</td>
<td>142</td>
<td>22</td>
</tr>
</tbody>
</table>

These tests showed that the glycodendrimers PM58,59 strongly bind to DC-SIGN in comparable way with the previously reported linear PM31, PM26. The PM59 construct, loaded with 12 copies of the pseudo-1,2-mannobioside ligand 1, is almost twice more effective than its related hexavalent linear glycoconjugate PM31 ($K_D = 14.4$ nM and 27.3 nM respectively). On the other hand, the constructs carrying the more active and selective bis-amide monovalent ligand 2, i.e. the cross-shaped PM58 and linear PM26 glycodendrimers, exhibit exactly the same potency ($K_D = 6.45$ nM and 6.6 nM respectively), corresponding to a lower multivalency enhancement factor ($\beta$) for the higher valency PM58. Direct interaction studies with langerin ECD showed that selectivity depends mostly on the nature of the monovalent ligand: both the PM26 and PM58 constructs loaded with the intrinsically DC-SIGN selective ligand 2 discriminate effectively against langerin and for DC-SIGN. However, interestingly, the introduction of the tetravalent core within the dendrimer scaffold translates into an increased selectivity towards DC-SIGN, with PM58 reaching a factor of 22.

Inhibition of DC-SIGN binding to SARS-CoV-2 spike protein.
We have recently shown that DC-SIGN binds to immobilized SARS-CoV-2 spike protein and that PM26 inhibits this binding in an SPR competition experiment.[19] The inhibition curves of PM26, PM58 and PM59 in the same experiment are compared in Figure 4. These data confirm that the nature of the monovalent spearhead is the main determinant of activity for these ligands, as the cross-shaped ligand ($IC_{50}=5.9 \pm 0.6$ μM) is about 5 fold more active than PM59 ($IC_{50}=26.8 \pm 4$ μM), but, similar to PM26 ($IC_{50}=10.4 \pm 0.4$ μM), considering that PM58 has twice the valency of PM26.

Figure 3. Comparison of dissociation constant $K_D$ values of glycodendrimers PM31, PM26, PM59 and PM58 towards DC-SIGN (red bar) and langerin (yellow bar) obtained by direct interaction SPR assay. The intrinsic activity of the monovalent ligands 1 and 2, estimated by binding inhibition assays (SPR) are 0.9 and 0.3 mM, respectively.[11]

Inhibition of DC-SIGN mediated trans-infection by Ebola virus.
Finally, the antiviral activity of the cross-based systems was tested and compared to PM26 in a cellular model of Ebola virus infection. The model uses pseudotyped recombinant vesicular stomatitis virus-luciferase (rVSV-luc) viral particles presenting the Ebola envelope glycoprotein (EBOV), and a Jurkat cell line expressing DC-SIGN on the surface, which can transfer the virus to VeroE6 cells (trans-infection).[28] The results of the infection assays are represented as a percentage of infection inhibition compared with Jurkat DC-SIGN cells infected by the pseudovirus in the absence of the inhibitors (blank experiment). Both PM58 and PM59 at 500 nM block EBOV trans-infection by 88% and 96%, respectively, while PM26, at the same concentration is only 63% effective. In this case, it is possible that the increase in activity observed for the cross-shaped systems is related to the ability to cross-link different DC-SIGN tetramers, which are not immobilized on the Jurkat cells membrane, and are known to clusterize in response to binding stimuli.
Discussion.

The early involvement of DC-SIGN in the setting of viral infections makes it a promising target in the development of antiadhesive drugs. Most of the antagonists developed so far interact with DC-SIGN by mimicking the highly mannosesylated structure of the naturally occurring (Man)₃(GlcNAc)₂ (Man₆) ligand, which is often exposed in multiple presentation by several pathogenic proteins.

During the past years, we have disclosed multivalent presentations of glycomimetics as a successful strategy to access potent and selective DC-SIGN antagonists. Our endeavors have led to the pseudo-1,2-dimannobiosides 1,2, which mimic the Manα(1,2)Man terminal epitopes of Man₆, featuring increased potency, improved drug-like properties and higher stability towards glycosidases. Both mimics have been obtained replacing the reducing end mannose of the Manα(1,2)Man unit by a conformationally locked cyclohexanediol ring, with the bis-amido derivative 2 performing as the most potent and selective of the series. 

Multivalent presentation of mimics 1,2 with glycodendrimers was crucial to achieve high levels of avidity, which was boosted when the glycomimetics were loaded on the dendrimers, which may disfavor binding towards C-type lectins of the CRDs. Remarkably, despite the modest contribution to potency, the tetravalent rigid core positively affects the relative selectivity of the dendrimers, which increases by almost two-fold (Table 1 PM31 vs PM 26 and PM59 vs PM58). This enhancement might possibly arise from the square arrangement of the ligands imparted by the rigid planar core of the dendrimers, which may disfavor binding towards C-type lectins with different topology of the CRDs.

We have shown with PM26 that the combination of a rigid core with flexible trivalent ligands allows to exploit both chelation and statistical rebinding effects and to achieve high affinity with relatively low valency of the construct. The merit of this design was also noted in structurally different systems and has the additional advantage of being able to adapt to the dynamics of oligomeric targets, as well as to the intrinsic mobility of the single recognition domain. More generally, ligands consisting of geometrically matching cores connected by flexible linkers to monovalent ligand units have been demonstrated as a robust, modular and widely applicable design to target multivalent receptors. The extended core of the glycodendrimers here described allows multivalent display of ligands in a spatially defined fashion at the four corners of a square of 2.2 nm diagonal.

Direct interaction studies with DC-SIGN oriented surfaces performed by SPR assay (Figure 3) revealed that both PM59 and PM58 act as potent antagonists, binding DC-SIGN with nanomolar activity (Kᵦ = 14.4 nM and 6.4 nM respectively). As expected, higher potency was shown by dendrimer PM58, bearing multiple copies of the most performing monovalent bis-amido ligand 2. However, the increased valency of the cross-shaped PM59 and PM58, is not reflected in a significant gain of avidity, as confirmed by comparing the Kᵦ of these constructs with those of the respective linear constructs PM31, PM26. This observation suggests that while multivalent effects, comprising chelation of adjacent CRDs, are still operative, simultaneous coordination of the four CRDs of DC-SIGN may not be occurring, or may not have a significant effect in reducing the dissociation constant of the complex. This is also observed in SPR inhibition studies performed using the spike protein of SARS-CoV-2 as a reporter (Figure 4). Here PM58 inhibits DC-SIGN binding with an IC₅₀, which is 0.5. The results were analysed using GraphPad Prism v8.

Figure 5. Inhibition of trans-infection of Jurkat DC-SIGN with EBOV-pseudotyped VSV-luc. Results are presented as percentage of EBOV trans-infection in the presence of compounds: PM26, PM58 and PM59 as compared to trans-infection of EBOV in VeroE6 mediated by Jurkat DC-SIGN without addition of any compound. EBOV-pseudotyped vsV-luc was used at MOI: 0.5. The results were analysed using GraphPad Prism v8.
and should be applicable to a wide array of situations where binding to a tetravalent receptor is sought after.

**Conclusion**

We were able to study the interaction between DC-SIGN and two glycodendrimer antagonists possessing the structural requirements to simultaneously reach the four CRDs exposed by the target lectin. The novel constructs are characterized by a rigid cross-shaped scaffold, which pre-organizes and directs the ligands to fit the CRDs arrangement of DC-SIGN, and by the presence of PEG pendants, which confer water solubility to the dendrimers. This central property allowed us to evaluate the biological activity of the dendrimers by SPR assays as well as their selectivity over langerin. Moreover, the glycodendrimers were able to inhibit DC-SIGN binding to SARS-CoV-2 spike protein and DC-SIGN mediated trans-infection by Ebola virus. Altogether these studies demonstrated that both PM59 and PM58 act as potent antagonists of DC-SIGN. The results suggest that while the constructs are probably able to chelate two adjacent CRDs, a fine tuning for a better compromise between rigidity and flexibility is likely necessary to accomplish a tetracoordination of the tetramer. Importantly, the improved selectivity displayed by the cross-shaped glycodendrimers PM59 and PM58, compared to linear analogs PM31, PM26, confirms structure-based design as a powerful approach for planning and developing multivalent antagonists with increased DC-SIGN targeting. Finally, straightness and modularity are remarkable characteristics of the synthetic route that we adopted. Analogous elaboration of scaffolds with proper geometry could enable the generation of multivalent antagonists selective for a variety of pattern-recognition receptors.

**Experimental Section**

**General methods**

Chemicals were purchased from commercial sources and used without further purification, unless otherwise indicated. When anhydrous conditions were required, the reactions were performed under nitrogen atmosphere. Anhydrous solvents were purchased from Sigma-Aldrich® and Aldrich® (matrix DHB). EI MS (MALDI) spectroscopic data are in accordance with those previously reported in the literature.

**Synthesis of compound 6**

Crude 6 (50.6 mg, 0.109 mmol) was dissolved in freshly distilled THF (105 µL) under nitrogen atmosphere. Bu4NF (5 mL) to remove a white precipitate. The reaction was stirred at 50 °C for 19 h, TLC analysis showed complete conversion (eluent: n-hexane, Rf = 0.08). The mixture was filtered over a celite pad and washed with EtOAc. Evaporation of the solvent afforded crude 5 that was pure enough to be used in the next synthetic step without further purification. The spectroscopic data are in accordance with those previously reported in the literature.

**Synthesis of compound 5**

Crude 5 (50.6 mg, 0.109 mmol) was dissolved in water-soluble dry CH2Cl2 (900 µL). Then a NaOH solution in MeOH (45.2 mg in 700 µL) was added and the reaction was stirred at room temperature for 5 h, monitoring by TLC (eluent: n-hexane - EtOAc, 20:1, Rf = 0.33). The solvent was evaporated, the crude was dissolved in CH2Cl2 (5 mL) and filtered washing with fresh CH2Cl2 (5 mL) to remove a white precipitate. The organic phase was washed with brine (2x5 mL) and dried over anhydrous Na2SO4. Evaporation of the solvent afforded crude 5a that was pure enough to be used in the next synthetic step without further purification. The spectroscopic data are in accordance with those previously reported in the literature.

**Synthesis of compound 2**

Crude 2a (2.7 mg, 0.012 mmol) was dissolved in water-soluble dry THF (70 µL) and (Ph2P)3PdCl2 (1.3 mg, 0.002 mmol), CuI (1.5 mg, 0.008 mmol), distilled DIFPeA (12 µL, 0.069 mmol) were added in the order. Finally, the allyl iodide 4 (40 mg, 0.068) was added as a solution in dry THF (84 µL). The reaction was stirred at 50 °C for 3 h and complete conversion was assessed by TLC analysis (eluent: CHCl3 - MeOH, 9:1, Rf = 0.61) monitoring at 365 nm. The solvent was evaporated and the product isolated by flash chromatography (eluent: CHCl3 - MeOH, 20:1 for 6 fractions) then CHCl3 - MeOH (1:1). A further purification was performed by size-exclusion chromatography using a Sephadex LH-20 column (D = 3 cm, height = 50 cm; eluent: MeOH; ACD/NG) to afford pure 6 (7.4 mg, 30% over three steps from 3).

**Synthesis of compound 4**

Crude 4a (2.7 mg, 0.012 mmol) was dissolved in water-soluble dry THF (70 µL) and (Ph2P)3PdCl2 (1.3 mg, 0.002 mmol), CuI (1.5 mg, 0.008 mmol), distilled DIFPeA (12 µL, 0.069 mmol) were added in the order. Finally, the allyl iodide 4 (40 mg, 0.068) was added as a solution in dry THF (84 µL). The reaction was stirred at 50 °C for 3 h and complete conversion was assessed by TLC analysis (eluent: CHCl3 - MeOH, 9:1, Rf = 0.61) monitoring at 365 nm. The solvent was evaporated and the product isolated by flash chromatography (eluent: CHCl3 - MeOH, 20:1 for 6 fractions) then CHCl3 - MeOH (1:1). A further purification was performed by size-exclusion chromatography using a Sephadex LH-20 column (D = 3 cm, height = 50 cm; eluent: MeOH; ACD/NG) to afford pure 4 (7.4 mg, 30% over three steps from 3).

**Synthesis of compound 1**

Crude 1a (2.7 mg, 0.012 mmol) was dissolved in water-soluble dry THF (70 µL) and (Ph2P)3PdCl2 (1.3 mg, 0.002 mmol), CuI (1.5 mg, 0.008 mmol), distilled DIFPeA (12 µL, 0.069 mmol) were added in the order. Finally, the allyl iodide 4 (40 mg, 0.068) was added as a solution in dry THF (84 µL). The reaction was stirred at 50 °C for 3 h and complete conversion was assessed by TLC analysis (eluent: CHCl3 - MeOH, 9:1, Rf = 0.61) monitoring at 365 nm. The solvent was evaporated and the product isolated by flash chromatography (eluent: CHCl3 - MeOH, 20:1 for 6 fractions) then CHCl3 - MeOH (1:1). A further purification was performed by size-exclusion chromatography using a Sephadex LH-20 column (D = 3 cm, height = 50 cm; eluent: MeOH; ACD/NG) to afford pure 1 (7.4 mg, 30% over three steps from 3).
 temperature for 1 h. Complete deprotection was assessed by TLC analysis (eluent: CHCl₃ - MeOH, 9:1, R₁ = 0.29) monitoring at 365 nm. A solution of TBTA (280 µg, 0.53 µmol) in freshly distilled THF (38 µL) was added, followed by 13 µL of a solution of CuSO₄·5H₂O (60 µg, 0.24 µmol) and 17 µL of a solution of sodium ascorbate (210 µg, 1.06 µmol) both in degassed H₂O (purred with nitrogen). Finally, dendor 7 (20 mg, 11.4 µmol) was added followed by THF (94 µL) and H₂O (102 µL) to reach a 2:1 THF:H₂O mixture. The reaction was stirred at room temperature, under nitrogen atmosphere, shielded from light for 15 h. The complete conversion into the desired product was assessed by TLC analysis (eluent: CHCl₃ - MeOH, 7:3 + 0.5 H₂O, R₁ = 0.22) monitoring at 365 nm and by MALDI-TOF MS (matrix DHB, HCCA). The copper scavenger QuadraSil MP was added to the solution which was stirred for 15 min. After filtering, the crude was finally purified by size-exclusion chromatography using a Sephadex LH-20 column (Ø = 3 cm, height = 50 cm; eluent: MeOH) and monitoring by TLC (eluent: CHCl₃ - MeOH, 7:3 + 0.5 H₂O). Dendorimer PM59 was recovered as a bright yellow oil (20.3 mg, 92%). The purity was confirmed by HPLC analysis of an analytical sample by a Waters Atlantis T3 5 µm 4.6x100 mm column, plateau at 90% (H₂O + 0.1% TFA) - 10% (CH₃CN + 0.1% TFA) for 1 min followed by a gradient to 100% (CH₃CN + 0.1% TFA) in 10 min, followed by a plateau for 1 min, flow rate 1 mL/min, λ = 254 nm, t₀ (product) = 7.0 min. [α]D + 28.5 (c = 0.49 in MeOH).

**1H NMR** (600 MHz, CD₃OD) δ ppm: 8.39 (bs, 4 H), 7.94 (s, 12 H), 7.80 (bs, 2 H), 7.73 (bs, 4 H), 7.07 (bs, 4 H), 4.96 (s, 12 H), 4.59 (bs, 32 H), 4.46 (bs, 24 H), 4.28 (bs, 8 H), 4.15 (d, J = 4.3 Hz, 8 H), 7.03 (bs, 8 H), 3.72 (m, 20 H), 3.72 (m, 20 H), 3.36 (m, 120 H), 3.36 (m, 120 H), 3.22 (td, J = 4.3 Hz, 8 H). HRMS (ESI): calcd for [M+Na]+ 2820.36951 [M+4Na]+ 5340.73902; found 2820.36945 [M+4Na]+ 5340.73902.

**2H NMR** (100 MHz, D₂O) δ ppm: 176.9 (C), 176.6 (C), 153.7 (C), 148.7 (C), 144.4 (CH), 143.5 (CH), 125.8 (CH), 125.6 (CH), 125.0 (CH), 120.9 (CH), 117.4 (CH), 112.2 (CH), 111.8 (CH), 98.8 (CH), 92.5 (C), 84.8 (C), 74.3 (CH), 73.4 (CH), 72.6 (CH), 71.8 (CH), 70.9 (CH₂), 70.7 (CH), 70.6 (CH), 69.8 (3xCH₂), 69.4 (CH₂), 68.8 (CH₂), 68.1 (4xCH₂), 66.8 (CH₂), 66.7 (CH₂), 63.7 (CH₃), 61.0 (CH₃), 60.6 (2xCH₂), 52.6 (2xCH₂), 50.1 (2xCH₂), 44.9 (CH), 38.7 (CH₂), 27.2 (CH₂), 26.2 (CH₂). HRMS (ESI) m/z: calcd for [M+Na]+ 2482.44331; found 2482.56764 [M+Na]+, 2482.56764 as control, while other flow cells were, after a second round of activation, functionalized with 49 µg/mL and 55.9 µg/mL of DC-SIGN S- and langerin S-ECT, respectively, up to a final density ranging between 2000 and 3000 RU, via tag specific capture and linkage by amine coupling chemistry simultaneously. The compounds were injected in running buffer of 25 mM Tris pH 8, 150 mM NaCl, 0.05% Tween 20 onto the surface at increasing concentrations with a flow rate of 30 µL/min. The ligand titration led to the determination of an apparent Kᵅ value. The data was analysed in BiAcore BIASolution software for steady state affinity calculations assuming that the Kᵅ will reflect the affinity of the ligands (glycoclusters) with the DC-SIGN oriented surface.

**Inhibition of DC-SIGN binding to SARS-CoV-2 spike protein**

Inhibition experiments on the SAR-CoV-2 spike surface were performed as previously described.[9]

**Production of EBOV-pseudotyped rVSV-luc and inhibition assays**

Inhibition property of PM26, PM58 and PM59 was tested by using an EBOV-pseudotyped recombinant vesicular stomatitis virus-luciferase (rVSV-luc) system. rVSV-luc was produced following previously published protocols.[28] The expression vector encoding EBOglycoprotein (strain Makona, GenBank accession no. M523102.1) was synthesized and cloned into pcDNA3.1 by GeneArt technology (Thermo Fisher Scientific). Pseudotyped viruses were normalized for infectivity to a multiplicity of infection of 0.5 and the inhibitory effect of the glycomimetic compounds: PM26, PM58, PM59 was evaluated on DC-SIGN-mediated trans-infection by Jurkat DC-SIGN to susceptible Vero E6 cells.[29] Jurkat DC-SIGN cells were first pre-incubated 20 min with the corresponding concentration of the compounds before being challenged with EBOV-VSV-luc. PM26, PM58 and PM59 were tested at 2 different concentrations: 5 µM and 500 nM. Cells were then incubated with the EBOV-VSV-luc during 2 h at room temperature with rotation. Cells were then centrifuged at 1200 rpm for 5 minutes and washed with PBS supplemented with 0.5% BSA and 1mM CaCl₂ three times. Cells were then resuspended in RPMI medium and cocultivated with adherent VeroE6 cells. After 24 h, the supernatant was removed and monolayer of VeroE6 was washed with PBS three times. Cells were then lysed and assayed for luciferase expression (GloMax Navigator, Promega).
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