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FULL PAPER

Efficient Synthesis of Muramic and Glucuronic Acid Glycodendrimers as Dengue Virus Antagonists

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Abstract: Carbohydrates are involved in many important pathological processes, such as bacterial and viral infections, by means of carbohydrate-protein interactions. Glycoconjugates with multiple carbohydrates are involved in multivalent interactions, thus increasing their binding strengths to proteins. In this work, we report the efficient synthesis of novel muramic and glucuronic acid glycodendrimers as potential Dengue virus antagonists. Aromatic scaffolds functionalized with a terminal ethynyl groups were coupled to muramic and glucuronic acid azides by click chemistry through optimized synthetic strategies to afford the desired glycodendrimers with high yields. Surface Plasmon Resonance studies have demonstrated that the compounds reported bind efficiently to the Dengue virus envelope protein. Molecular modelling studies were carried out to simulate and explain the binding observed. These studies confirm that efficient chemical synthesis of glycodendrimers can be brought about easily offering a versatile strategy to find new active compounds against Dengue virus.

Introduction

Glycodendrimers are an important class of synthetic macromolecules that can be used to mimic many structural and functional features of cell surface glycoconjugates.[1] Glycoconjugates perform key important functions in many pathological processes, such as viral and bacterial infections,[2] often regulated by carbohydrate-protein interactions. With mounting evidence that not only the molecular structure but also the valency and spatial organization of carbohydrate epitopes in glycoconjugates influence the specificity and avidity of their interactions with cognate receptors,[3] glycodendrimers have increasingly served as a tool for probing the underlying mechanisms of these events.[1a, 1b, 1e, 1f, 2a] In addition, there has been much interest in using glycodendrimers mimetics of glycoconjugates found on cell membrane to control the presentation of carbohydrates in glycan microarrays,[4] which have emerged as a powerful platform for interrogating ligand specificities of carbohydrate-binding proteins.[4b-f]

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Supporting information for this article is given via a link at the end of the document.

As mentioned above, the significance of glycoconjugates, including glycolipids, glycoproteins and glycosaminoglycans (GAGs) play a major role in bacterial and viral infection.[2c, 5] On the one hand glucuronic acid (GlcA) plays an essential role in the structure of GAGs, polysaccharide chains with variable degrees of sulfation at different positions. Previous studies have demonstrated that a highly charged heparan sulfate, a GAG found on the cell surface, serves as a receptor for dengue virus by binding to its envelope protein.[2d, 6] The main driving force for interactions between the surface GAGs on the host cell and envelope protein of DENV and other pathogenic flaviviruses is electrostatic interaction between the negative charge of GAGs and positive regions on the envelope proteins.[6a, 6e] Interventions that disrupt this binding effectively inhibit infectivity.[7]

On the other hand, *N*-acetylmuramic acid (MurA) can be found naturally as peptidoglycans, the repeating disaccharide element β-D-GlcNAc-(1→4)-β-D-MurNAc composing bacterial cell walls.[8] Both monosaccharides (GlcA and MurA) with an acid group are involved in biological processes which are essential for the infection and survival of the infective agents. However, these structures are extremely difficult to synthesize, as can be seen in the scarcity of studies on the topic. The availability of the carbohydrates is a crucial step to simulate and understand their implication in these processes. Monosaccharides may not be enough by themselves to trigger a biological response due to the small unit of interaction. This problem could be solved by the preparation of dendrimers, consisting of a central core with a variable number of branches, which can be decorated with several saccharides, increasing the number of molecules exposed.[9]

All pathogenic flaviviruses, such as dengue virus (DENV),[10] yellow fever virus, West Nile virus, Japanese encephalitis virus, and Zika virus, bind to GAGs through the putative GAG binding sites within their envelope proteins to gain access to the surface of host cells. DENV, the world's most dangerous mosquito-borne flavivirus disease, is classified in four different serotypes,[11] places 2.5 billion at risk of infection and results in 20 million cases each year in 100 countries and to date there is no completely effective vaccine. Since a GAG is a putative receptor for DENV envelope protein, soluble GAGs or other GlcA or MurA glycodendrimers (monosaccharides with an acid group) could be effective inhibitors of viral infection.

In this study, we present for the first time an efficient synthesis of novel GlcA and MurA glycodendrimers as potential active compounds in the treatment of Dengue virus. Surface plasmon resonance (SPR) in conjunction with molecular modelling were used to investigate the interaction of the MurA and GlcA glycodendrimers with DENV envelope protein.

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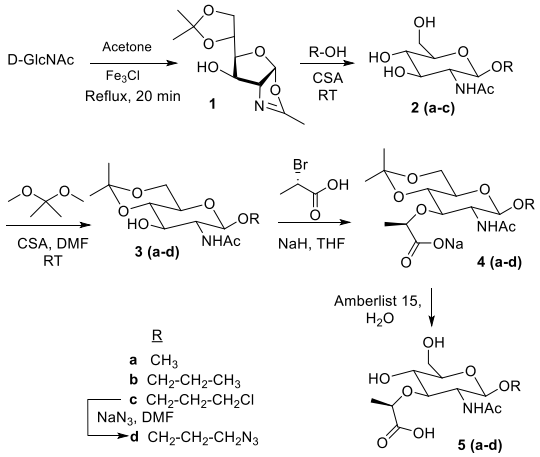
Results and Discussion

Synthesis of O-linked MurA β-glycosides

Four O-linked MurA β-glycosides having different linkers were prepared following a concise and efficient synthesis and using commercially available D-GlcNAc as starting material (Scheme 1). The synthetic strategy is shown in Scheme 1.



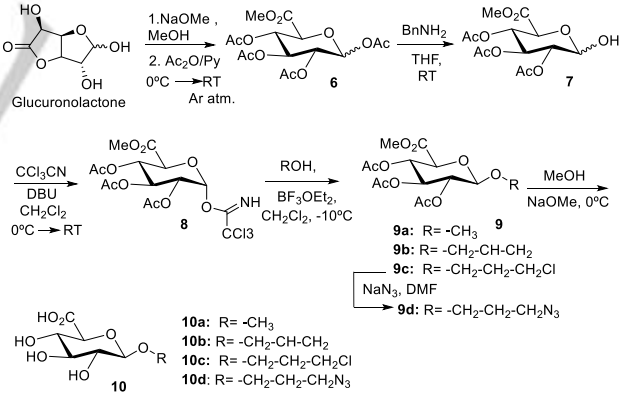
Scheme 1. Synthesis of O-linked MurA β-glycosides

In this strategy, first O-linked GlcNAc-β-glucopyranosides (compounds **2a-c**) via a furanosyl oxazoline **1** were synthesized (see Supporting Information for Experimental section), and then incorporated the acid moiety in C-3 (compounds **4a-d**). For the preparation of O-linked GlcNAc-β-glucopyranosides (**2a-c**) a protocol developed by Bundle and co-workers was followed.[12] The furanose Oxazoline **1** was prepared on a large scale from D-GlcNAc in anhydrous acetone and dry $FeCl_3$ as catalyst. The crude reaction was used without further purification. The presence of the product was confirmed by ^{1}H-NMR, showing a characteristic signal of anomeric carbon δ (ppm) 6.09 (d, 1H $J_{1,2}$=5 Hz). [13] Oxazoline **1** can be cleanly converted to the corresponding unprotected β-glucopyranosides **2** (**a-c**) in quantitative yield. The ring expansion from furanose to pyranose was detected at δ (ppm) 4.3 (d, 1H $J_{1,2}$= 8.5 Hz) .[14] This strategy simplifies the number of steps in comparison with other glycosylation alternatives, such as the imidate formation, the use of temporary protecting groups, or glycosylation of simple alcohols under Koenings-Knorr conditions[15] (employing heavy metal). Compound **2** (**a-c**) was obtained in high yield (80 %) by simply evaporating the reaction mixture after the neutralization with Et_3N, followed by multiple washing of the residue with CH_2Cl_2.[14b] Subsequent regioselective protection of the C-4 and C-6 positions of the obtained β-glucopyranoside was performed using 2,2-dimethoxypropane and catalytic amount of camphor-10-sulfonic acid (CSA) in anhydrous dimethylformamide (DMF) to produce the partially unprotected saccharides **3** (**a-c**) with a free 3-hydroxyl group.[14a] In order to obtain the conveniently functionalised carbohydrate ligand that displayed a suitable linker for further conjugation with alkyne-terminated dendritic cores, compound **3c** was treated with sodium azide to afford monosaccharide **3d.** With this approach one can take advantage of the presence of a single hydroxyl group at C-3 by coupling **3**(**a-d**) with (*S*)-2-bromopropanoic acid in the presence of sodium hydride, resulting in the formation of different muramic acids 4,6-O-protected and functionalized in the anomeric position **4(a-d)**.[16] The (S)-2-bromopropanoic acid was obtained by diazotization with sodium nitrite and KBr in acid pH and using natural L-Alanine as starting material.[17]

Only compound **4d** was purified and characterized, compounds **4** (**a-c**) were employed in the deprotection without further purification. Then deprotected O-linked MurA β-glycosides (**5a-d**) were also prepared as monovalent ligands. The dimethyl acetal was treated with Amberlyst in acid pH to provide the required deprotected O-linked MurA β-glycosides (**5a-d**).

Synthesis of O-linked GlcA β-glycosides

As illustrated in Scheme 2, a synthetic strategy was followed to obtain the key intermediate **8** from commercial available glucoronolactone (see Supporting information for Experimental section).[14b, 18] This was converted into the fully protected glucopyranuronate **6** by reaction with NaOH in MeOH at room temperature and acetic anhydride in pyridine from 0 ºC to room temperature. The anomeric position protected with the acetyl group could be deprotected using benzylamine to generate the intermediate **7** as an α-β mixture. Activation of anomeric position was carried out with trichloroacetonitrile and 1,8-diazabicyclo[5.4-0]undec-7-ene (DBU) in THF to give the activated trichloroacetimidate **8** that was efficiently glycosylated with different alcohol to afford monosaccharides **9(a-c)** that beared different linkers in the anomeric position. Compound **9c** was treated with sodium azide to obtain compound **9d** that displayed a suitable linker for further conjugation with alkyne-terminated dendritic cores.



Scheme 2. Synthesis of O-linked GlcA β-glycosides

Glycodendrimers synthesis

Several studies have reported the synthesis of glycodendrimers with peripheral propargyl groups to enable a 1,3-dipolar cycloaddition ("click") reaction with carbohydrate derived azides.[19] Three different cores (Scheme 3) bearing 2, 3 and 4 alkyne groups respectively (compounds **11**, **12** and **13**), were

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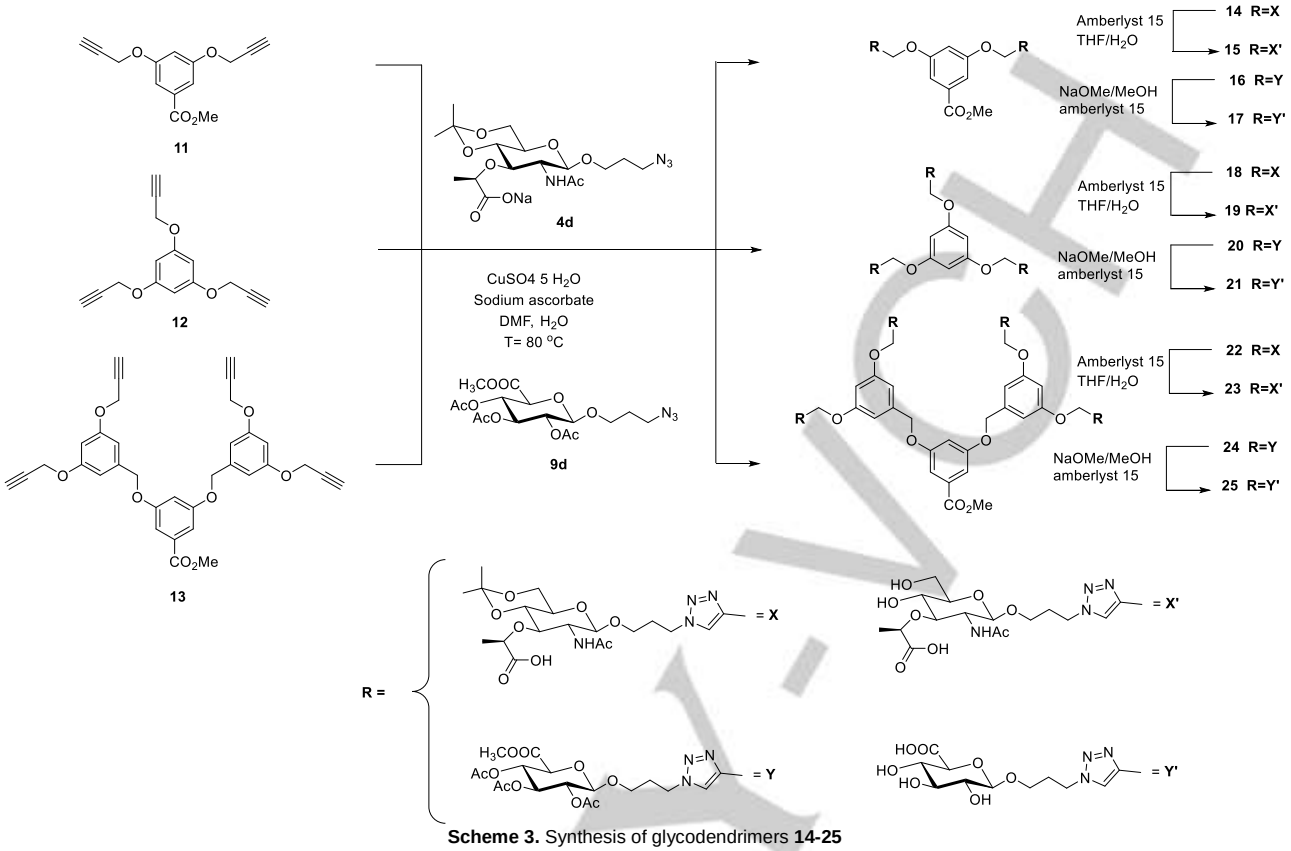

Scheme 3. Synthesis of glycodendrimers **14-25**

selected for the conjugation step in order to study the influence of valency on the molecular recognition of these multivalent systems. Multivalent core scaffolds were prepared as previously described in the literature from commercially available starting materials.[19a, 20] Once the ester-protected β-glycosyl azides (compounds **4d** and **9d**) were obtained, preparation of the corresponding glycodendrimers was undertaken (Scheme 3).

Cu (I) catalyzed cycloaddition reaction between fully protected β-glycosyl azides (compounds **4d** and **9d**) and the dendritic cores (**11**, **12** and **13**) was first performed. Global deprotection would afford the final water-soluble MurA and GlcA dendrimers.

We envisioned that the use of **4d** and **9d** , instead of the highly polar unprotected monosaccharides (**5d** and **10d**), would facilitate the monitoring and the purification of the click chemistry reaction products. Thus, **4d** and **9d** were coupled to compounds **11**, **12** and **13** using $CuSO_4$ as copper source and sodium ascorbate to reduce in situ Cu (II) to Cu (I). Di, Tri and tetravalent glycodendrimers (**14**, **16**, **18**, **20**, **22** and **24**) were obtained in excellent yield after microwave heating at 80ºC for 45 min. The compounds were purified by flash chromatography. The complete absence of propargylic $C{\equiv}CH$ ^1H resonance at δ 2.55 ppm and appearance of a new triazole singlet δ 8.03 ppm suggested successful completion of the reaction.

Deprotection of *N*-acetylmuramic glycodendrimers (**14**, **18** and **22**) was then attempted by treatment with Amberlist H 15 in a THF: H_2O (1:1) mixture obtaining the desired compounds in quantitative yields (**15**, **19** and **23**). For the deprotection of glucuronic acid derivatives (**16, 20** and **24**), the compounds were treated with NaOMe/MeOH and the final products were neutralized with amberlist H^+ obtaining the desired compound in quantitative yields (**17**, **21** and **24**). All six individual deprotected glycodendrimers were fully characterized by one- and two-dimensional NMR spectroscopy (^1H, ^{13}C, HSQC) to verify their structures as shown in Scheme 3.

Surface Resonance Plasmon studies

In order to evaluate the ability of the glycodendrimers **15**, **17**, **19**, **21**, **23** and **25** to interact with Dengue virus envelope protein 2 (DENV2) a SPR binding study was carried out. As mentioned above, previous studies have demonstrated that a heparin-like glycosaminoglycan found on the cell surface, where GlcA plays an essential role, serves as a receptor for Dengue virus by binding to its envelope protein.[2d, 6a]

Compounds **15**, **17**, **19**, **21**, **23** and **25** were flowed on the immobilized DENV2 at a 175 μm concentration (Fig 1). As can be seen in Fig. 1, the glycodendrimers binding to DENV2 depends on the type of carbohydrate presence and the valency. MurA compounds **15** and **19** showed negligible binding response with DENV2 envelope protein whereas compound **23** displayed a higher response (45 RU).

On the other hand, a clear effect was observed with GlcA compounds **21** and **25**, especially with compound **25**. Thus, compounds with a GlcA showed stronger binding than the MurA derivatives of equal core structure and valency. More significant was the effect due to the GlcA glycodendrimer with higher valency, compound **25**. The four GlcA had a stronger interaction with DENV2 than their corresponding MurA glycodendrimer **23**. Compound **25**, with four GlcA acid blocks, showed a substantial increase in the interaction, supporting the positive correlation between valency and interaction intensity. Then glycodendrimer **25** was flowed over the chip containing immobilized DENV2. Sensograms obtained showed increased interaction profiles with

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the GlcA tetravalent glycodendrimer (Fig. 2). Our results indicated a strong interaction between DENV 2 and glycodendrimer **25** with four GlcA with a K_D value in μM range. Using steady-state analysis of SPR measurements for the interaction between **25** and DENV2, the dissociation constant (K_D) was found to be 22 μM (Fig S1 in Supporting Information). These results demonstrate that the presentation of GlcA ligands on dendritic scaffolds strongly increase their interaction with DENV2 due to the multivalent effect. Previous studies showed that DENV2 bound to heparin with a K_D of 31 nM.[21] Our studies assessing the interaction of the DENV2 with glycodendrimer **25** demonstrated binding (K_D of 22 μM) that was weaker than that observed with heparin. Nevertheless, high affinities can be achieved if the sugar residues are presented with higher valency. This compound represents an excellent candidate for structural optimization to achieve higher affinity and to expect anti-adhesive properties against Dengue virus.

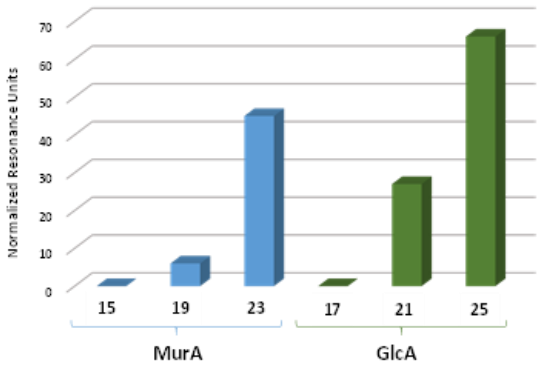

Figure 1. SPR measurement of MurA (**15**, **19** and **23**) and GlcA (**17**, **21** and **25**) glycodendrimers binding to immobilized DENV2 at 175 μm

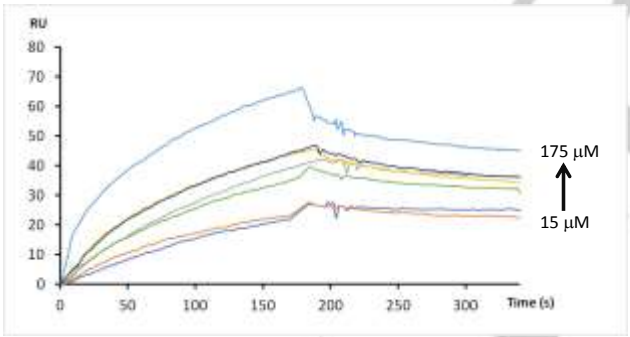

Figure 2. Binding responses of different concentrations of glycodendrimers **25** with DENV2 immobilized on the CM4 chip, showing association and dissociation phases. Responses were reference substracted and blank corrected.

Molecular modelling studies

In order to explain the strong interaction observed with the tetravalent GlcA glycodendrimer (compound **25**), we set out to characterize properties of DENV2−glycodendrimer **25** complex using molecular modeling. Crystal structure of DENV2 (PDB: 3C5X) was refined by homology modelling. Protein-glycodendrimers **25** interactions were studied performing a docking simulation in GAG-binding domains.

The Cartesian coordinates for a 3D model of the apo form of envelope protein (E) Dengue Virus 2 Thailand 16681/84 were taken directly from the PDB (accession code 3C5X),[22] internal missing string of six residues was modelled using Swiss-Model web server, the 3D model was further minimized with the same force field. 3D structure for dendrimer was built using glycam carbohydrate builder web server and the interactive molecular graphics program PyMOL.[23] Finally the 3D geometry of the complex formed by the DENV2 with glycodendrimer **25** was deduced by using AutoDock Vina.[24] Stability of the predicted DENV2-glycodendrimer **25** complex was studied by MD simulation (20ns). Structure stability of the complex was evaluated by calculating the root-mean-square-deviation (RMSD) of the Cα atoms along the trajectory. Global RMSD values of the protein were lower than 3Å, indicating high stability of the protein (Fig. S2 in Supporting Information). Regarding the ligand, after the first 2ns of simulation it changed the pose with respect to the docked structure, reaching values between 10-15 Å RMSD. This new pose was very stable, and no changes were observed in RMSD values after 8 ns of the MD, which means that the rest of the simulation the ligand remained in that position. The effective binding free energies between the glycodendrimer **25** and the more relevant residues in the binding site were qualitatively estimated using the program MM-ISMSA.[25]

DENV2-glycodendrimer **25** interaction energy was measured during the simulation and it remained nearly constant around -110 kcal/mol, indicating the pose of the dendrimer in the protein was energetically favourable (Table 1).

Table 1. Description of the interactions in complex DENV2-glycodencrimer **25**

DENV2	Glycodendrimer 25	E (kcal/mol)	Type of Interaction*
LYS-299	Glucuronic acid moiety	-12,85	qq+ HB+vdW
GLN-297	Glucuronic acid moiety	-5,91	HB+vdW
ASN-359	Glucuronic acid moiety	-5,38	qq+ HB
TYR-182	Glucuronic acid moiety	-5,21	qq
SER-302	Glucuronic acid moiety	-5,09	HB
GLY-181	Triazol and phenyl moieties (aromatic rings)	-5,05	HB
THR-180	Glucuronic acid and phenyl (aromatic ring) moieties	-4,89	HB
GLY-300	Triazol moiety (aromatic rings)	-4,63	HB
ILE-361	Glucuronic acid moiety	-4,53	HB+vdW
MET-301	Glucuronic acid moiety	-4,36	HB
LYS-295	Glucuronic acid moiety	-4,32	HB+vdW
TYR-303	Triazol and phenyl moieties (aromatic rings)	-3,91	vdW
SER-149	Glucuronic acid moiety	-3,52	HB+vdW
LYS-161	Triazol moiety (aromatic rings)	-3,33	vdW
LEU-298	Glucuronic acid moiety	-2,80	HB
LYS-42	Glucuronic acid moiety	-2,14	qq+ HB
GLY-183	Glucuronic acid moiety	-2,13	HB
LYS-164	Glucuronic acid moiety	-2,07	qq+ HB
LYS-338	Glucuronic acid moiety	-1,57	qq+ HB
PRO-360	Glucuronic acid moiety	-1,54	HB
THR-184	Glucuronic acid moiety	-1,45	HB
THR-363	Triazol moiety (aromatic rings)	-1,45	vdW
GLU-151	Glucuronic acid moiety	-1,31	vdW
LEU-179	Glucuronic acid moiety	-1,26	vdW
HIP-148	Glucuronic acid moiety	-1,24	HB
LEU-296	Glucuronic acid moiety	-1,09	HB
THR-159	Glucuronic acid moiety	-1,07	HB
HIE-162	Glucuronic acid moiety	-0,98	HB
ARG-292	Glucuronic acid moiety	-0,92	HB

*Type of interaction: charge-charge (qq), hydrogen bond (HB), van der Waals (vdW)

The ligand was anchored by important electrostatic interactions including multiple hydrogen bonding and van der Waals (Figs. 3 and 4).

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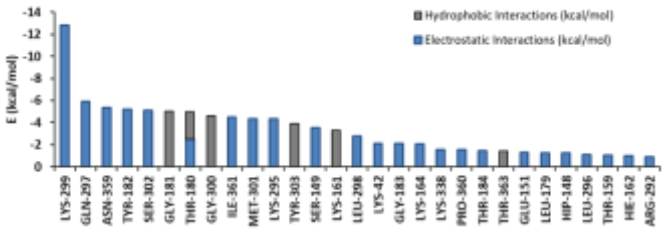

Figure 3. 2D free energy per-residue interaction plot of DENV2-glycodendrimer **25** complex. X axis shows the interaction energies, and Y axis the name and number of DENV2 residues.

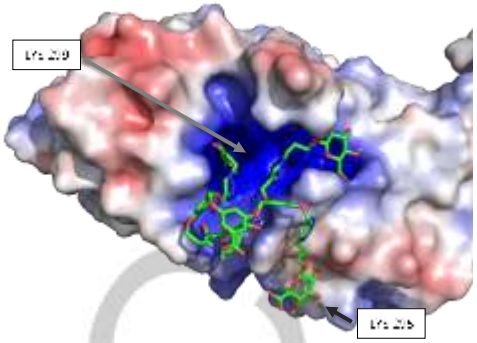

Figure 4. a) Final MD pose of GlcA glycodendrimer **25** in DENV2. Dendrimer **25** is shown as green sticks and protein is shown as electrostatic surface calculated by APBS (negative charged surface in red; positive charged surface in blue; hydrophobic surface in white)

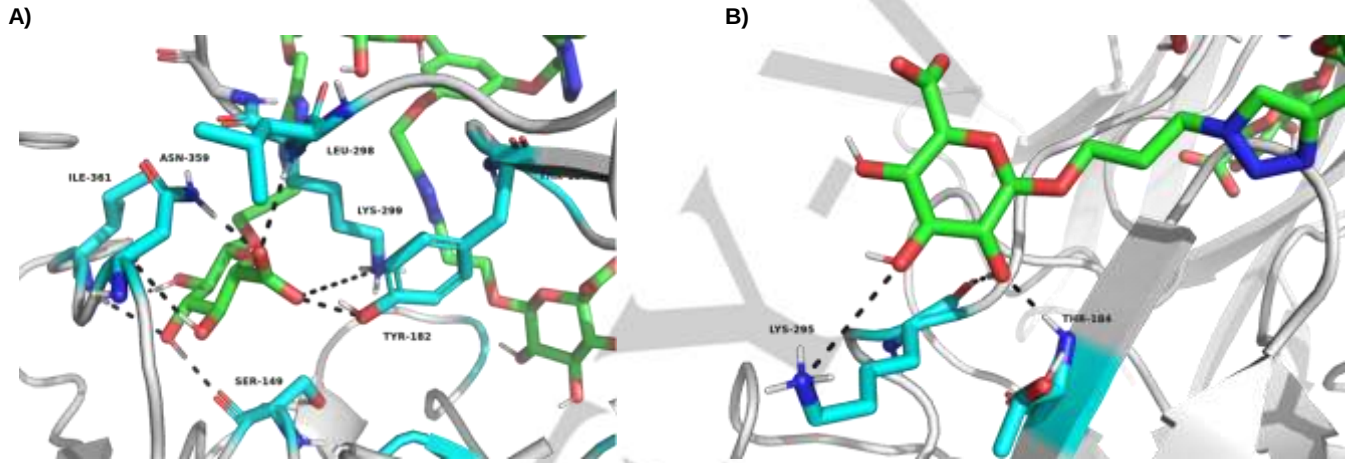

Figure 5. A) 3D view of the interaction between LYS-299 and final MD pose of GlcA glycodendrimer **25** in DENV2. **B)** 3D view of the interaction between LYS-295 and final MD pose of GlcA glycodendrimer **25** in DENV2. Dendrimer **25** is shown as green sticks and protein as white cartoon. Main residues of the protein are highlighted in blue. Electrostatic interactions are depicted as black dash lines.

The main charge-charge interactions took place between the carboxylate groups of dendrimer and LYS-299 (-13 kcal/mol) and LYS-295 (-5 kcal/mol), that are the key residues involved in heparin binding[6d] (Fig. 5) (DENV2 ^{294}D**K**LQL**K**GMSYSMCTGKFKVVKEIAET^{319}).[6d]
As can be seen in Table 1 most of the residues of the DENV2 GAG binding region (23 residues) provide hydrogen bond and charge-charge interactions with the glucuronic acid moiety, while only few provide van der Waals interactions with this moiety (Glu151 and Leu179). No major contacts between the triazol and phenyl moieties (aromatic rings) could be highlight only Gly181, forms hydrogen bonds with this aromatic rings and Tyr303, Lys161 and Thr363 show van der Waals interactions. Therefore we can say that the core architecture of glycodendrimer **25** does not have a significant effect on the binding affinity These results are consistent with previous carbohydrate-protein studies carried out with similar glycodendrimer where the carbohydrate region of the ligand (sialic acid and lactose moieties) was in close contact with the Viscumin protein and played a significant role in the binding affinity. In the same study the effect of the triazol and phenyl moieties was lower.[19a]

Conclusions

MurA and GlcA acids are both monosaccharides that play a key role in biological processes such as bacterial or viral infection. It is well known that Dengue virus binds to GAGs through the putative GAG binding sites within their envelope proteins to gain access to the surface of host cells. These interactions are predominantly ionic where positively charged basic aminoacid residues in the GAG binding site of DENV2 interact with negatively charged residues in the cell receptor, a highly sulfated form of the GAG heparan sulfate. Since heparan sulfate GAG is a putative receptor for DENV2, one can envisage that a good strategy to develop viral infectivity inhibitors is the use soluble multivalent highly charged glycodendrimers. Multivalency is a very attractive approach to improve the affinity of carbohydrates. Therefore, conjugation of several copies of MurA or GlcA to a multivalent scaffold will certainly result in new multivalent glycostructures with good binding affinities to their receptors.

Due to the complexity of the synthetic route leading to MurA and GlcA acid derivatives, new strategies have to be developed.

A series of O-linked MurA and GlcA β-glycosides functionalized with a suitable linker for further conjugation with alkyne-terminated scaffolds were prepared. These compounds are readily obtained through a synthetic route that has been optimized by choosing appropriate protecting groups and reaction conditions. This methodology is mild and compatible with a range of functional linkers and has become the method of choice in our group.

MurA and GlcA derivatives bearing a short linker with a terminal azide group were coupled with alkyne-terminated scaffolds by using a simple, and efficient Cu(I) catalyzed cycloaddition reaction resulting in the desired MurA and GlcA glycodendrimers with excellent yields. The success in applying this methodology to MurA and GlcA chemistry should provide abundant opportunities to expand and apply these O-linked MurA or GlcA glycosides bearing an azide group for preparation of challenging new and different multivalent system related to bacterial or viral infections and investigations into mechanism of action.

MurA and GlcA glycodendrimers were evaluated in their ability to interact with DENV2 assayed. The binding capacity of MurA glycodendrimers (compounds **15**, **19** and **23**) is relatively low compared to GlcA glycodendrimers (compounds **17**, **21** and **25**) which confirms the selectivity of the DENV2 towards GAGs receptors on the cell surface. At the same time, multivalency is another important factor in the interactions between MurA and GlcA glycodendrimers to DENV, no binding was observed for the monovalent ligands, either MurA or GlcA. However, glycodendrimers with higher valency show strong binding, especially GlcA glycodendrimer **25** with four GlcA acid blocks.

Molecular modeling provided an explanation for the binding observed between compound **25** and the GAG-binding region of DENV2, which is a basic-residue rich region of DENV2. The main type of interactions are electrostatic interactions between the carboxylate groups of glycodendrimers **25** and Lys-299 and Lys-295 in the GAG-binding region of DENV2, which are the key residues involved in the heparin binding region. The evaluation of the triazol and phenyl moieties showed that the core architecture does not have a significant effect on the interactiob with DENV2. Binding mode of compound **25** in the GAG-binding region of DENV2 will form the basis for future structure-activity relationship optimization.

This novel multivalent approach using a simple monosaccharide (GlcA or MurA) as ligand has shown to be a viable strategy to develop compounds that interact with DENV2 and work is in progress to further increase these valuable properties.

Experimental Section

All reagents were commercially available and used without further purification. A CEM-Discover focused microwave synthesizer with microwave power maximum level of 300 W and microwave frequency of 2455 MHz was employed for the microwave-assisted reactions,

NMR spectra were recorded at 293 K, with a 500 MHz spectrometer (Bruker AC) and 300 MHz spectrometer (Bruker Avance). Shifts are referenced relative to deuterated solvent residual peaks. Complete signal assignments from 1D and 2D NMR spectroscopy were based on COSY, HSQC, and HMBC correlation experiments. Mass spectra were recorded with an Applied Biosystems QSTAR XL. Quantitative elemental analysis by combustion of carbon, hydrogen, nitrogen and sulfur were carried out in Servicio de Microanálisis Elemental from Universidad Complutense (Madrid, Spain), using a Leco CHNS 932 Elemental Analyzer. The purity of new compounds was assessed by CHNS elemental analysis, and all values were verified to be within ±0.4% of the theoretical values. Thin layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F254 (Merck). TLC plates were inspected under UV light (l=254 nm) and developed by treatment with phosphomolybdic acid hydrate in ethanol (10 %), followed by heating. Silica gel column chromatography was performed with silica gel (230–400 mesh).

Dengue virus envelope protein 2 (DENV2) was purchased from The Native Antigen Company.

SPR sensor chips CM4 (carboxymethylated dextran) and other reagents used in SPR experiments were purchased from GE Healthcare. SPR experiments were performed with a Biacore 3000.

General procedure for click reaction, synthesis of compounds 14, 16, 18, 20, 22 and 24

Scaffold **11**, **12** or **13** (1 equiv.) was dissolved in a DMF:H_2O (4:1) mixture, $CuSO_4{\cdot}5H_2O$ (0.05 equiv./alkyne), sodium ascorbate (0.1 equiv./alkyne) and compound **4d** or **9d** (1.2eq/alkyne) were added to the solution. The resulting mixture was stirred at 60 °C overnight.[26] Undissolved compounds were removed by filtration and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (AcOEt: MeOH: NH_3 25 % 2:1:0.2).

The reaction mixture was carried out in a microwave reactor (CEM-Discover), Scaffolds **11**, **12** or **13** (1 equiv.) was dissolved in a DMF:H_2O (98:2) mixture, $CuSO_4{\cdot}5H_2O$ (0.2 equiv./alkyne), sodium ascorbate (0.3 equiv./alkyne) and compound **4d** or **9d** (1.2eq/alkyne) were added to the solution. The resulting mixture was heated at 80°C for 60 min.[19a] After cooling to RT, the mixture was dissolved in ethyl acetate and washed with a solution of 1% EDTA in water (2x10 mL) and brine (2x10 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (AcOEt: MeOH: NH_3 25 % 2:1:0.2).

General method for acetal group deprotection: Synthesis of compounds 15, 17, 19, 21, 23 and 25.

Compound **14, 18 and 22** (40 mg) was dissolved in 1 mL of a mixture THF:H_2O (1:1) and Amberlyst® 15 was added at room temperature to adjust the solution to acid pH. The mixture was stirred overnight. The solid of the mixture was removed by filtration and the solvent was evaporated under reduced pressure affording **15, 19** and **23** as a white solids.

Peracetylated compounds (**16, 20 and 24**) was dissolved in anhydrous methanol and NaOMe previously suspended in methanol was added dropwise (4 equiv). The mixture was stirred at 0°C until the consumption of the substrate. Then the reaction was quenched by the addition of Amberlite IRA-15 to pH 3. The solution was filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_3 (25%) 6:1:0,2) affording **17, 21** and **25** as a white solid.

Compound 14 (82 % yield)

^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 8.03 (s, 2H, H-11), 7.22 (d, *J*=2.3 Hz, 2H; H-2, H-6), 6.90 (t, *J*=2.3 Hz, 1H, H-4), 5.17 (s, 4H, H-9), 4.51 – 4.40 (m, 4H, H-12), 4.36 (d, *J*=8.2 Hz, 2H, H-1'), 4.26 (q, *J*=6.8 Hz, 2H, H-12'), 3.86 (s, 3H, H-8), 3.84 – 3.74 (m, 4H, H-6'$_{ax}$, H-6'$_{eq}$), 3.74 – 3.71 (m, 2H, H-14a), 3.71 – 3.65 (m, 2H, H-2'), 3.68 – 3.49 (m, 4H, H-4', H-3'), 3.40 (dt, *J*=10.0, 5.8 Hz, 2H, H-14b), 3.20 (ddd, *J*=9.6, 9.6 y 5.5 Hz, 2H, H-5'), 2.14

– 2.03 (m, 4H, H-13), 1.99 (s, 6H, H-11'), 1.47 (s, 6H, H-8'), 1.33 (s, 6H, H-9'), 1.29 (d, *J*=6.8 Hz, 6H, H-14').
^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 179.8 (C-13'), 174.5 (C-10'), 167.9 (C-7), 160.8 (C-3, C-5), 144.4 (C-10), 133.3 (C-1), 125.8 (C-11), 109.7 (C-2,C-6), 108.1 (C-4), 104.0 (C-1'), 100.6 (C-7'), 79.3 (C-3'), 78.6 (C-12'), 76.2 (C-4'), 68.4 (C-5'), 66.6 (C-14), 63.1 (C-6'), 62.8 (C-9), 56.5 (C-2'), 52.9 (C-8), 48.0 (C-12), 31.2 (C-13), 29.5 (C-9'), 23.4 (C-11'), 19.8 (C-14'), 19.5 (C-8').

Compound 15 (85 % yield)
^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 8.11 (s, 2H, H-11), 7.27 (d, *J*=2.3 Hz, 2H, H-2, H-6), 6.96 (t, *J*=2.3 Hz, 1H, H-4), 5.22 (s, 4H, H-9), 4.57 – 4.47 (m, 4H, H-12), 4.44 (q, *J*=6.8 Hz, 2H, H-9'), 4.33 (d, *J*=8.1 Hz, 2H, H-1'), 3.90 (s, 3H, H-8), 3.89 – 3.80 (m, 4H, H-6, H-6'a, H-14a), 3.72 – 3.66 (m, 4H, H-6'b, H-2'), 3.51 (t, *J*=9.6 Hz, 2H, H-3'), 3.48 – 3.40 (m, 4H, H-4', H-14b), 3.27 (ddd, *J*=9.5, 5.7, 2.3 Hz, 2H, H-5'), 2.16 – 2.08 (m, 4H, H-13), 1.94 (s, 6H, H-8'), 1.37 (d, *J*=6.8 Hz, 6H, H-11').
^{13}C NMR (76 MHz, CD_3OD) δ (ppm): 176.4 (C-10'), 174.2 (C-7'), 168.0 (C-7), 160.9 (C-3, C-5), 144.4 (C-10), 133.4 (C-1), 125.9 (C-11), 109.7 (C-2,C-6), 108.1 (C-4), 103.4 (C-1'), 82.0 (C-3'), 77.9 (C-5', C-9'), 71.7 (C-4'), 66.4 (C-14), 62.7 (C-9), 62.6 (C-6'), 55.7 (C-2'), 52.8 (C-8), 48.1 (C-12), 31.3 (C-13), 22.0 (C-8'), 19.8 (C-11').
Anal. Calcd. for $C_{42}H_{60}N_8O_{20}$: C, 50.60; H, 6.07; N, 11.24. Found: C, 50.53; H, 6.06; N, 11.20.
HRMS: m/z calcd for $C_{42}H_{60}N_8O_{20}Na$ $[M+Na]^+$ 1019.3821, found 1019.3847.

Compound 18 (90 % yield)
^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 8.01 (s, 3H, H-9), 6.31 (s, 3H, H-2, H-4, H-6), 5.12 (s, 6H, H-7), 4.54 – 4.41 (m, 6H, H-10), 4.36 (d, *J*=8.3 Hz, 3H, H-1'), 4.28 (q, *J*=6.9 Hz, 3H, H-12'), 3.83 (dd, *J*=10.7, 5.4 Hz, 3H, H-6'), 3.76 (m, 6H, H-6'$_{ec}$, H-12a), 3.68 (dd, *J*=10.2, 8.3 Hz, 3H, H-2'), 3.64 (t, *J*=9.2 Hz, 3H, H-4'), 3.56 (dd, *J*=10.2, 8.9 Hz, 3H, H-3'), 3.43 – 3.36 (m, 3H, H-12b), 3.22 (ddd, *J*=9.9, 9.9, 5.4 Hz, 3H, H-5'), 2.10-1.9 (m, 6H, H-11), 2.01 (s, 9H, H-11'), 1.49 (s, 9H, H-8'), 1.34 (s, 9H, H-9'), 1.30 (d, *J*=7.0 Hz, 9H, H-14').
^{13}C NMR (126 MHz, CD_3OD) δ (ppm): 180.0 (C-13'), 174.6 (C-10'), 161.6 (C-1, C-3, C-5), 144.7 (C-8), 125.7 (C-9), 104.1 (C-1'), 100.6 (C-7'), 96.3 (C-2, C-4, C-6), 79.1 (C-3'), 78.6 (C-12'), 76.4 (C-4'), 68.5 (C-5'), 66.6 (C-12'), 63.2 (C-6'), 62.6 (C-7), 56.6 (C-2'), 48.0 (C-10), 31.2 (C-11), 29.5 (C-9'), 23.4 (C-11'), 19.8 (C-14'), 19.5 (C-8').

Compound 19 (95 % yield)
^{1}H NMR (300 MHz, CD_3OD) □□(ppm): 8.29 (d, *J*=6.7 Hz, 3H, H-9), 6.33 (s, 3H, H-2, H-4, H-6), 5.18 (s, 6H, H-7), 4.59 – 4.47 (m, 9H, H-10, H-9'), 4.38 (d, *J*=8.1 Hz, 3H, H-1'), 3.87 – 3.75 (m, 6H, H-6'a, H-12a), 3.68 – 3.60 (m, 6H, H-6'b, H-2), 3.51 – 3.34 (m, 9H, H-12b, H-3, H-4), 3.25 – 3.20 (m, 3H, H-5'), 2.16 – 2.06 (m, 6H, H-11), 2.00 (d, *J*=7.3 Hz, 9H, H-8'), 1.31 (d, *J*=6.8 Hz, 9H, H-11').
^{13}C NMR (76 MHz, CD_3OD) δ (ppm): 175.7 (C-10'), 174.3 (C-7'), 161.2 (C-1, C-3, C-5), 143.6 (C-8), 126.7 (C-9), 103.2 (C-2, C-4, C-6), 102.38 (C-1'), 83.3 (C-3'), 77.7 (C-5'), 77.1 (C-9'), 72.2 (C-4'), 66.6 (C-12), 62.3 (C-6'), 61.8 (C-7), 56.3 (C-2'), 49.5 (C-10), 31.0 (C-11), 23.1 (C-8'), 19.4 (C-11').
Anal. Calcd. for $C_{57}H_{84}N_{12}O_{27}$: C, 50.00; H, 6.18; N, 12.27. Found: C, 49.84; H, 6.16; N, 12.25.
HRMS: m/z calcd for $C_{57}H_{84}N_{12}O_{27}Na$ $[M+Na]^+$ 1391.5466, found 1391.5453.

Compound 22 (75 %)
^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.94 (s, 4H, H-25), 7.13 (d, *J*=2.4 Hz, 2H; H-2, H-6), 6.75 (t, *J*=2.4 Hz, 1H, H-4), 6.66 (d, *J*=2.3 Hz, 4H; H-11, H-15, H-18, H-22), 6.56 (t, *J*=2.4 Hz, 2H; H-13, H-20), 5.08 (s, 8H; H-23), 4.95 (s, 4H; H-9, H-16), 4.46 – 4.3 (m, 8H, H-26), 4.34 (d, *J*=8.1 Hz, 4H, H-1'), 4.22 (q, *J*=7.3 Hz, 4H, H-12'), 3.82 (s, 3H, H-8), 3.80 – 3.76 (m, 4H, H-6'ax), 3.76 – 3.66 (m, 12H, H-2', H-6'ec, H-28a), 3.65 – 3.56 (m, 4H, H-4'), 3.55 – 3.44 (m, 4H, H-3'), 3.38 (dt, *J*=10.7, 5.5 Hz, 4H, H-28b), 3.18 (ddd, *J*=9.7, 9.7, 5.4 Hz, 4H, H-5'), 2.09 – 2.01 (m, 8H, H-27), 1.98 (s, 12H, H-11'), 1.44 (s, 12H, H-8'), 1.31 (s, 12H, H-9'), 1.29 (d, *J*=7.3 Hz, 12H, H-14').
^{13}C NMR (76 MHz, CD_3OD) δ (ppm): 180.5 (C-13'), 174.5 (C-10'), 167.9 (C-7), 160.9 (C-3, C-5), 160.9 (C-12, C-14, C-19, C-21), 144.6 (C-24), 140.8 (C-10, C-17), 133.1 (C-1), 125.6 (C-25), 109.5 (C-2, C-6), 107.9 (C-4), 107.6 (C-11, C-15, C-18, C-22), 104.0 (C-1'), 102.6 (C-13, C-20), 100.5 (C-7'), 79.5 (C-3'), 79.3 (C-12'), 75.9 (C-4'), 70.8 (C-9, C-16), 68.5 (C-5), 66.7 (C-28), 63.1 (C-6'), 62.6 (C-23), 56.5 (C-2'), 52.9 (C-8), 48.0 (C-26), 31.2 (C-27), 29.5 (C-9'), 23.4 (C-11'), 20.0 (C-14'), 19.5 (C-8').

Compound 23 (80 % yield).
^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 8.05 (s, 4H, H-25), 7.21 (d, *J*=2.4 Hz, 2H; H-2, H-6), 6.87 – 6.75 (m, 1H, H-4), 6.77 – 6.69 (m, 4H, H-11, H-15, H-18, H-22), 6.64 (s, 2H; H-13, H-20), 5.16 (s, 8H, H-23), 5.04 (s, 4H, H-9, H-16), 4.60 (q, *J*=6.8 Hz, 4H, H-9'), 4.56 – 4.44 (m, 8H, H-26), 4.41 (d, *J*=8.3 Hz, 4H, H-1'), 3.92 – 3.82 (m, 8H, H-6'a, H-28a), 3.76 – 3.66 (m, 8H, H-6'b, H-2'), 3.54 – 3.43 (m, 12H, H-3', H-4', H-28b), 3.31 – 3.24 (m, 4H, H-5'), 2.19 – 2.08 (m, 8H, H-27), 2.03 (s, 12H, H-8'), 1.39 (d, *J*=6.8 Hz, 12H, H-11').
^{13}C NMR (76 MHz, CD_3OD) δ (ppm): 175.8 (C-10'), 173.8 (C-7'), 168.0 (C-7), 161.0 (C-3, C-5), 161.0 (C-12, C-14, C-19, C-21), 144.7 (C-10, C-17), 125.8 (C-25), 109.6 (C-2, C-6), 108.0 (C-4), 107.6 (C-11, C-15, C-18, C-22), 102.8 (C-13, C-20), 102.6 (C-1'), 83.1 (C.3'), 77.8 (C-5'), 77.0 (C-9'), 72.3 (C-4'), 70.9 (C-9, C-16), 66.5 (C-28), 62.5 (C-23, C-6'), 56.2 (C-8), 52.9 (C-8), 48.1 (C-26), 31.3 (C-27), 23.4 (C-8'), 19.5 (C-11').
Anal. Calcd. for $C_{90}H_{124}N_{16}O_{40}$: C, 52.22; H, 6.04; N, 10.83. Found: C, 52.03; H, 6.02; N, 10.79.
HRMS: m/z calcd for $C_{90}H_{124}N_{16}O_{40}Na$ $[M+Na]^+$ 2091.8058, found 2091.8032.

Compound 16. (85%).
^{1}H-RMN (500 MHz, $CDCl_3$) δ (ppm): 7.77 (s br, 2H, H-11), 7.25 (t, *J*=2.2 Hz, 2H, H-2 and H-6), 6.81 (t, *J*=2.2 Hz, 1H, H-4), 5.26 (t, *J*=9.6 Hz, 2H, H-3'), 5.24 (t, *J*=9.6 Hz, 2H, H-4'), 5.18 (s br, 4H, H-9), 4.99 (dd, *J*=9.6, 7.7 Hz, 2H, H-2'), 4.54 (d, *J*=7.7 Hz, 2H, H-1'), 4.51-4.46 (m, 2H, H-12a), 4.43-4.37 (m, 2H, H-12b), 4.01 (d, *J*=9.6 Hz, 2H, H-5'), 3.87 (s, 3H, H-8), 3.82-3.78 (m, 2H, H-14a), 3.67 (s, 6H, H-7'), 3.52-3.48 (m, 2H, H-14b), 2.18-2.11 (m, 4H, H-13), 2.04 (s, 6H, OAc), 1.99 (s, 12H, OAc).
^{13}C-RMN (125 MHz, $CDCl_3$) δ (ppm): 170.1 (CO), 169.5 (CO), 169.5 (CO), 167.4 (C-6'), 166.7 (C-7), 159.5 (C-3 and C-5), 143.4 (C-10), 132.2 (C-1), 124.1 (C-11), 108.7 (C-2 and C-6), 107.3 (C-4), 100.6 (C-1'), 72.4 (C-5'), 72.0 (C-3'), 71.3 (C-2'), 69.5 (C-4'), 65.8 (C-14), 62.2 (C-9), 53.0 (C-7'), 52.4 (C-8), 46.6 (C-12), 30.2 (C-13), 20.8 (AcO), 20.7 (AcO), 20.6 (AcO).
Anal. Calcd. for $C_{46}H_{58}N_6O_{24}$: C, 51.21; H, 5.42; N, 7.79. Found: C, 51.10; H, 5.40; N, 7.77.

Compound 17. (97%).
^{1}H-RMN (500 MHz, CD_3OD) δ (ppm): 8.20 (s br, 2H, H-11), 7.28 (t, *J*=2.2 Hz, 2H, H-2 an H-6), 6.96 (t, *J*=2.2 Hz, 1H, H-4), 5.24 (s br, 4H, H-9), 4.64 - 4.56 (m, 4H, H-12), 4.31 (d, *J*=7.7 Hz, 2H, H-1'), 3.91 (s, 3H, H-8), 3.87 (d, *J*=9.6 Hz, 2H, H-5'), 3.84-3.79 (m, 2H, H-14a), 3.55 (t, *J*=9.6 Hz, 2H, H-4'), 3.56-3.51 (m, 2H, H-14b), 3.42 (t, *J*=9.6 Hz, 2H, H-3'), 3.28 (dd, *J*=9.6, 7.7 Hz, 2H, H-2'), 2.24-2.16 (m, 4H, H-13).
^{13}C-RMN (125 MHz, CD_3OD) δ (ppm): 170.1 (C-6'), 166.7 (C-7), 142.9 (C-10), 159.5 (C-3 and C-5), 132.1 (C-1), 125.2 (C-11), 108.5 (C-2 and C-6), 106.9 (C-4), 103.2 (C-1'), 75.9 (C-3'), 75.3 (C-5'), 73.4 (C-2'), 71.9 (C-4'), 65.5 (C-14), 61.3 (C-9), 51.6 (C-8), 46.9 (C-12), 29.9 (C-13).
Anal. Calcd. for $C_{32}H_{42}N_6O_{18}$: C, 48.12; H, 5.30; N, 10.52. Found: C, 48.05; H, 5.28; N, 10.50.
HRMS: m/z calcd for $C_{32}H_{42}N_6O_{18}Na$ $[M+Na]^+$ 821.2453, found 821.2466.

Compound 20. (75%).

^{1}H-RMN (500 MHz, $CDCl_3$) δ (ppm): 7.76 (s br, 4H, H-25), 7.25 (d, *J*=2.3 Hz, 2H, H-2 and H-6), 6.77 (t, *J*=2.3 Hz, 1H, H-4), 6.69 (d, *J*=2.3 Hz, 4H, H-11, H-15, H-18 and H-22), 6.61 (t, *J*=2.3 Hz, 2H, H-13 and H-20), 5.25 (t, *J*=9.6 Hz, 4H, H-3'), 5.19 (t, *J*=9.6 Hz, 4H, H-4'), 5.17 (s br, 8H, H-23), 5.02 (dd, *J*=9.6, 7.7 Hz, 4H, H-2'), 5.01 (s br, 4H, H-9 and H-16), 4.54 (d, *J*=7.7 Hz, 4H, H-1'), 4.46-4.41 (m, 4H, H-26 a), 4.39-4.32 (m, 4H, H-26b), 4.02 (d, *J*=9.6 Hz, 4H, H-5'), 3.90 (s, 3H, H-8), 3.86-3.81 (m, 4H, H-28a), 3.68 (s, 12H, H-7'), 3.54-3.48 (m, 4H, H-28b), 2.22-2.12 (m, 8H, H-27), 2.06 (s, 12H, AcO), 2.01 (s, 12H, AcO), 2.00 (s, 12H, AcO).
^{13}C-RMN (125 MHz, $CDCl_3$) δ (ppm): 170.1 (CO), 169.6 (CO), 169.5 (CO), 167.3 (C-6'), 166.8 (C-7), 159.8 (C-12, C-14, C-19 and C-21), 159.8 (C-5 and C-3), 143.8 (C-24), 139.2 (C-10 and C-17), 132.2 (C-1), 124.0 (C-25), 108.6 (C-2 and C-6), 107.3 (C-4), 106.7 (C-11, C-15, C-18 and C-22), 101.7 (C-13 and C-20), 100.6 (C-1'), 72.4 (C-5'), 72.1 (C-3'), 71.3 (C-2'), 70.1 (C-9 and C-16), 69.5 (C-4'), 65.9 (C-28), 62.1 (C-23), 53.0 (C-7'), 52.4 (C-8), 46.7 (C-26), 30.2 (C-27), 20.8 (AcO), 20.7 (AcO), 20.6 (AcO).

Compound 21. (90%).
^{1}H-RMN (500 MHz, CD_3OD) δ (ppm): 8.29 (s br, 4H, H-25), 7.17 (d, *J*=2.3 Hz, 2H, H-2 and H-6), 6.77 (t, *J*=2.3 Hz, 1H, H-4), 6.72 (t, *J*=2.3 Hz, 4H, H-11, H-15, H-18 and H-22), 6.65 (t, *J*=2.3 Hz, 2H, H-13 and H-20), 5.25 - 5.14 (m, 8H, H-23), 5.02 (s br, 4H, H-9 and H-16), 4.63 - 4.56 (m, 8H, H-26), 4.25 (d, *J*=7.7 Hz, 4H, H-1'), 3.85 (s, 3H, H-8), 3.82 (d, *J*=9.6 Hz, 4H, H-5'), 3.79 - 3.74 (m, 4H, H-28a), 3.53 - 3.46 (m, 4H, H-28b), 3.50 (t, *J*=9.6 Hz, 4H, H-4'), 3.38 (t, *J*=9.6 Hz, 4H, H-3'), 3.23 (dd, *J*=9.6, 7.7 Hz, 4H, H-2'), 2.21 - 2.13 (m, 8H, H-27)
^{13}C-RMN (125 MHz, CD_3OD) δ (ppm): 171.4 (C-6'), 168.1 (C-7), 161.0 (C-5 and C-3), 160.8 (C-12, C-14, C-19 and C-21), 141.1 (C-24), 139.7 (C-10 and C-17), 131.8 (C-1), 125.2 (C-25), 109.6 (C-2 and C-6), 108.1 (C-4), 108.0 (C-11, C-15, C-18 and C-22), 104.5 (CH, C-1'), 102.9 (C-13 and C-20), 77.2 (C-3'), 76.6 (C-5'), 74.7 (C-2'), 73.2 (C-4'), 70.9 (C-9 and C-16), 66.9 (C-28), 62.0 (C-23), 53.0 (C-8), 48.7 (C-26), 30.1 (C-27).
Anal. Calcd. for $C_{42}H_{57}N_9O_{24}$: C, 47.06; H, 5.36; N, 11.76. Found: C, 46.90; H, 5.34; N, 11.73.
HRMS: m/z calcd for $C_{42}H_{57}N_9O_{24}Na$ $[M+Na]^+$ 1094.3414, found 1094.3426.

Compound 24. (85%).
^{1}H-RMN (500 MHz, $CDCl_3$) δ (ppm): 7.77 (s br, 3H, H-9), 6.27 (s, 3H, H-2, H-4 and H-6), 5.25 (t, *J*=9.6 Hz, 3H, H-3'), 5.18 (t, *J*=9.6 Hz, 3H, H-4'), 5.12 (s br, 6H, H-7), 5.01 (dd, *J*=9.6, 7.7 Hz, 3H, H-2'), 4.55 (d, *J*=7.7 Hz, 3H, H-1'), 4.52-4.46 (m, 3H, H-10a), 4.44-4.38 (m, 3H, H-10b), 4.02 (d, *J*=9.6 Hz, 3H, H-5'), 3.85-3.79 (m, 3H, H-12a), 3.75 (s, 9H, H-7'), 3.55-3.49 (m, 3H, H-12b), 2.21-2.11 (m, 6H, H-11), 2.06 (s, 9H, AcO), 2.01 (s, 18H, AcO).
^{13}C-RMN (125 MHz, $CDCl_3$) δ (ppm): 170.1 (CO), 169.6 (CO), 169.5 (CO), 167.4 (C-6'), 160.2 (C-1, C-3 and C-5), 143.7 (C-8), 124.1 (C-9), 100.6 (C-1'), 95.2 (C-2, C-4 and C-6), 72.4 (C-5'), 72.0 (C-3'), 71.3 (C-2'), 69.5 (C-4'), 65.9 (C-12), 61.9 (C-7), 53.0 (C-7'), 46.7 (C-10), 30.2 (C-11), 20.8 (AcO), 20.7 (AcO), 20.6 (AcO).

Compound 25 (95%).
^{1}H-RMN (500 MHz, CD_3OD) δ (ppm): 8.27 (s br, 3H, H-9), 6.36 (s, 3H, H-2, H-4 and H-6), 5.19 (s br, 6H, H-7), 4.65 - 4.60 (m, 6H, H-10), 4.30 (d, *J*=7.7 Hz, 3H, H-1'), 3.87 (d, *J*=9.6 Hz, 3H, H-5'), 3.84 - 3.80 (m, 3H, H-12a), 3.57 - 3.53 (m, 3H, H-12b), 3.55 (t, *J*=9.6 Hz, 3H, H-4'), 3.42 (t, *J*=9.6 Hz, 3H, H-3'), 3.28 (dd, *J*=9.6, 7.7 Hz, 3H, H-2'), 2.28 - 2.17 (m, 6H, H-11).
^{13}C-RMN (125 MHz, CD_3OD) δ (ppm): 171.4(C-6'), 161.4 (C-1, C-3 and C-5), 140.3 (C-8), 125.2 (C-9), 104.2 (C-1'), 94.8 (C-2, C-4 and C-6), 77.0 (C-3'), 76.4 (C-5'), 74.4 (C-2'), 72.9 (C-4'), 66.6 (C-12), 61.9 (C-7), 48.3 (C-10), 30.8 (C-11).
Anal. Calcd. for $C_{70}H_{88}N_{12}O_{36}$: C, 50.24; H, 5.30; N, 10.04. Found: C, 50.09; H, 5.27; N, 10.01.
HRMS: m/z calcd for $C_{70}H_{88}N_{12}O_{36}Na$ $[M+Na]^+$ 1695.5322, found 1695.5307

SPR experiments

SPR experiments were performed at 258C with Biacore 3000 (GE Healthcare). PBST (10 mm phosphate, pH 7.40, 150 mm NaCl, and 0.005% v/v surfactant P20) was used as running buffer for CM4 experiments. A solution with Dengue Virus serotype 2 protein (DENV-2) was adjusted to 15 μg/mL in 10 mm citrate pH 4.00 buffer and the protein was immobilized in flow cell 1 of a CM4 sensor chip by following the amine coupling method according to the manufacturer's instructions. Prior to injection over the sensor chip, DENV2 was mixed with a 4-fold molar excess of the heparin, for 30 min at 4°C, to protect the glycosaminoglycan-binding domains of the envelope protein. Immobilization response was 1800 RU and then the sensor surface washed with 1 M NaCl to remove the bound heparin. Sensor chip flow cell 2 was activated, blocked and used as a reference surface. Blank samples and concentration series were injected on CM4 chip at a flow rate of 5 μL/min for 180 s and dissociation was registered for 180 s. Chip CM4 concentration series were 15-500 μm of the glycodendrimers (compound **14**: 50-500 μm; **18**: 125-225 μm; **22**: 15-175 μm; **12**: 50-500 μm: **16**: 50-500 μm; **20**: 150-400 μm). Data processing and analysis were carried out with BiaEvaluation v.4.1.1 (GE Healthcare). All signals were blank subtracted, reference corrected, and globally adjusted to an adequate kinetic model to obtain binding parameters. Goodness of fit was indicated by χ^2, and values of less than 10 indicated good fit.

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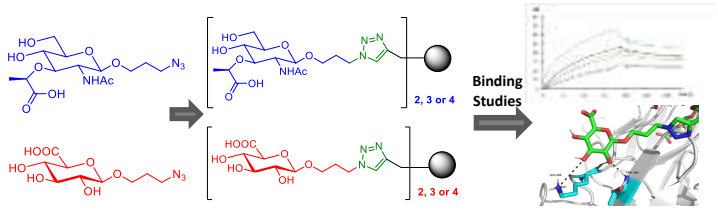

Glycodendrimers displaying muramic and glucuronic acids were efficiently synthesized. This multivalent systems showed high binding affinities to Dengue virus evelope protein.

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