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Divergent and convergent synthesis of GalNAc-conjugated dendrimers using dual orthogonal ligations

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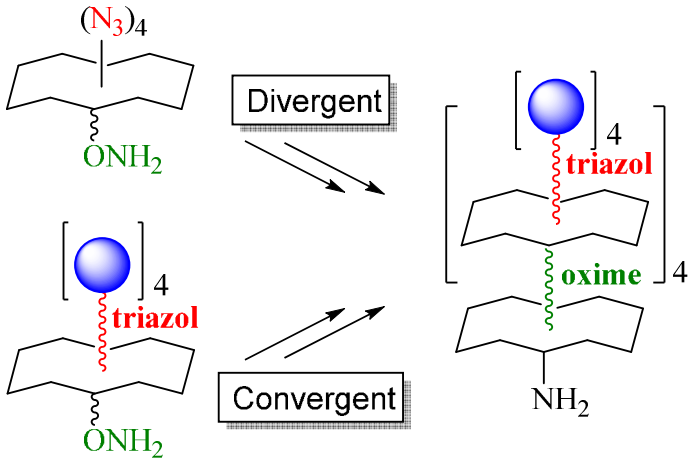
Abstract

The synthesis of glycodendrimers remains a challenging task. In this paper we propose a protocol based on both oxime ligation (OL) to combine cyclopeptide repeating units as the dendritic core and the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) to conjugate peripheral α and β propargylated GalNAc. By contrast with the oxime-based iterative protocol reported in our group, our current strategy can be used in both divergent and convergent routes with similar efficiency and the resulting hexadecavalent glycodendrimers can be easily characterized compared to oxime-linked analogues. A series of glyconjugates displaying four or sixteen copies of both α and β GalNAc have been prepared and their ability to inhibit the adhesion of the Soybean agglutinin (SBA) lectin to polymeric-GalNAc immobilized onto microtiter plates have been evaluated. As it was anticipated, the higher inhibitory effect (IC_{50} = 0.46 μM) was measured with the structure displaying αGalNAc with the higher valency (compound **13**), which demonstrates that the binding properties of these glycoconjugates are strongly dependent on the orientation and the distribution of the GalNAc units.

Graphical abstract



Introduction

The chemistry of multivalent glycosystems has made impressive progress over the past decade.[1] Various structures such as glycodendrimers,[2] glycocalixarenes,[3] or glycocyclodextrines[4] are now commonly used for biomedical applications[5,6] and represent relevant tools for studying carbohydrate-protein interactions.[7,8] In this broad research field, our group has developed glycoclusters and glycodendrimers which have been assembled at the surface of conformationally stable cyclopeptide scaffolds[9,10] using either identical or different sugar head groups - with valency of four,[11] sixteen[12] and sixty four[13] - and with other biomolecular entities in a well-defined spatial orientation.[14] Some of these structures have revealed sub-nanomolar affinities towards vegetal[15] and bacterial lectins[16] as well as potent immunoactivation effect against cancer cells in murine models.[17-19]

However, the construction of glycodendrimers remains a difficult and challenging task.[20,21] To this aim, we have developed a divergent strategy that allows the controlled assembly of cyclopeptide and carbohydrate building blocks in a repetitive fashion and with excellent yields. In this approach, properly functionalized cyclopeptides (*i.e.* with aminooxy and oxo-aldehyde functions) can be self-condensed using an iterative oxime ligation (OL) protocol to provide dendritic framework to be functionalized with aminooxy carbohydrates (Fig. 1A). While stable *in vivo*, the main drawback of the resulting compounds is the fragility of the peripheral oxime-linked carbohydrates during analysis by mass spectrometry which strongly hampers the complete structural characterization.[12,13] In addition, the utilization of the alternative convergent approach which is more reliable to construct glycodendrimers with lower risk of formation of partially glycosylated intermediates was found unsuccessful in our hands due to side reactions (Fig. 1B).[12] Herein, we report first a versatile synthetic strategy that can be used either in a divergent (Fig. 1C) or a convergent (Fig. 1D) protocol to build hexadecavalent glycocyclopeptide dendrimers. To demonstrate the feasibility of the proposed synthetic route, we have selected two orthogonal ligation methods[22] that are the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) strategy to attached sugar moieties while the

dendritic core was built using OL, similarly to the "onion peel" strategy previously described by the group of R. Roy.[23]

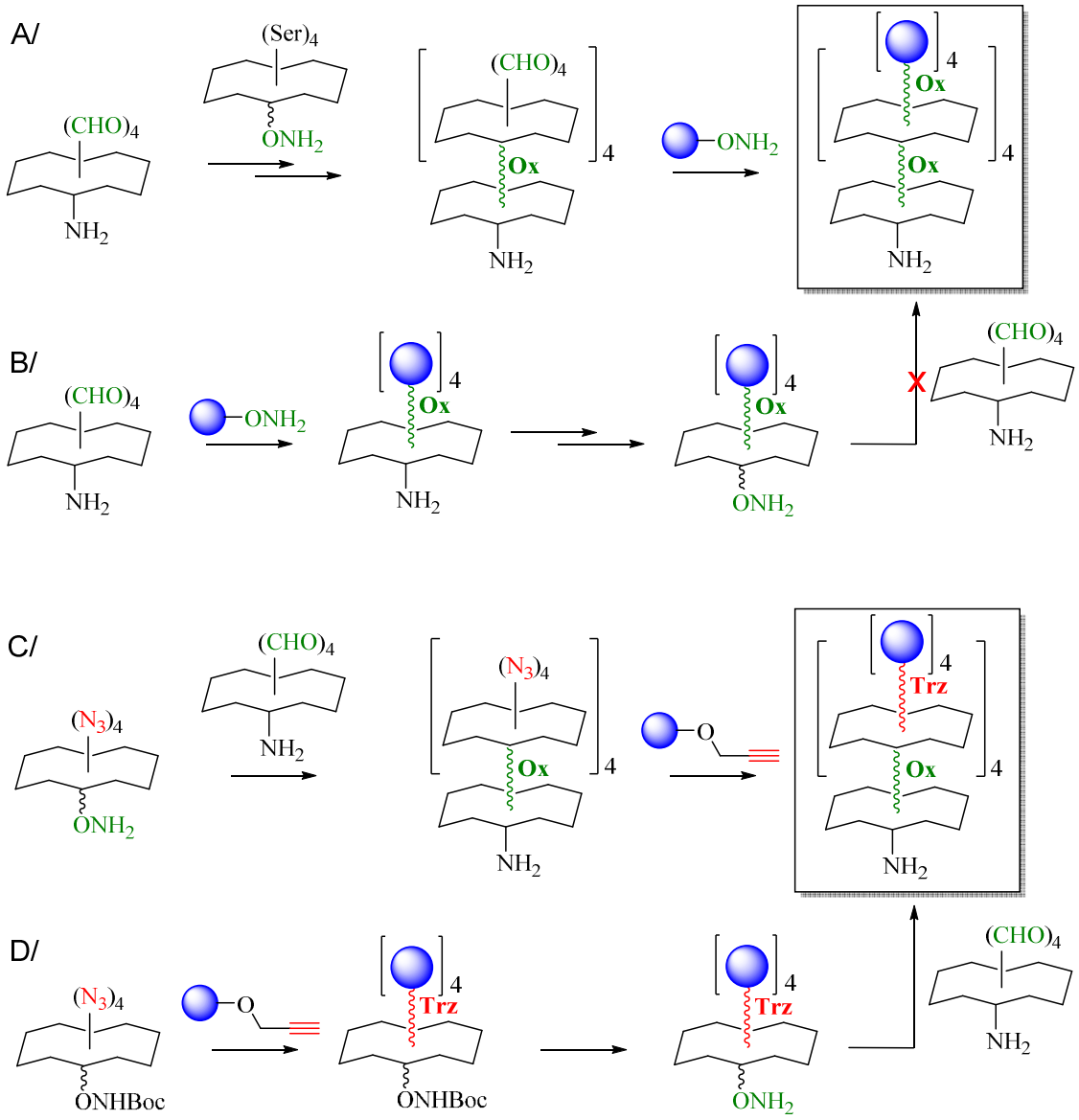


Fig. 1 General strategy for the divergent (A) and the convergent (B) assembly by OL; for the divergent (C) and the convergent (D) assembly by CuAAC/OL, similarly to the "onion peel" method. Trz: triazole; Ox: oxime ether.

Results and Discussion

We focused the present study on *N*-acetylgalactosamine (GalNAc) which represents key building block for the synthesis of antitumoral vaccines,[24,25] uridine diphosphate (UDP) mimics[26] or antifreeze glycoproteins (AFGP).[27] In addition, when GalNAc is presented in a suitable multivalent fashion, the resulting structures have shown interesting properties towards specific carbohydrate-binding proteins.[28-30] In the course of our activities in this field, we have designed here molecules displaying both α and β GalNAc that have been synthesized using propargylated compounds **1a** and **1b** (Fig. 2). It was indeed largely demonstrated that besides valency, the orientation of the sugar unit within the scaffold is a structural parameter that strongly impacts the binding affinity for these proteins.[1,16]

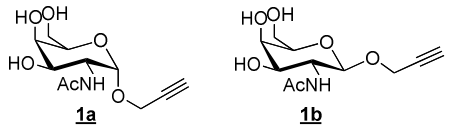


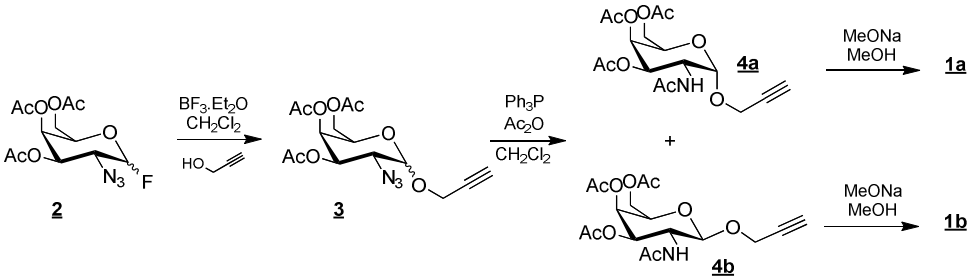
Fig. 2 Structure of compounds **1a** and **1b**.

Synthesis of prop-2-ynl 2-acetamido-2-deoxy-α-D-galactopyranoside

A few groups recently proposed different synthetic strategies for the preparation of *O*-propargyl GalNAc **1a** and **1b**. In 2009, Fairbanks and co-workers[26] described the preparation of the α anomer **1a** by a Fisher-type glycosylation. The unprotected GalNAc was treated with sulphuric acid in presence of silica and equimolar amount of propargyl alcohol. The α/β mixture (3:2) was obtained in 54% yield after recrystallization. In the same year, the Brimble group[31] described another protocol to obtain the acetylated compound **1a**, but later the authors confirmed that only the furanose form was synthesized.[32] To avoid this problem, the 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-galactose has been glycosylated with propargyl alcohol in the presence of $BF_3.OEt_2$ as promoter. After the reduction/acetylation of the azido group using Zn and Ac_2O/pyridine, both α and β anomers **1a** and **1b** have been separated by silica gel chromatography. In 2010, Sewald and co-workers[27] also described the synthesis of **1a** starting from the azidochloride precursor using a

Koenigs-Knorr reaction as the key step. The azido group was reduced and subsequently acetylated with AcSH/pyridine and the fully deprotected compound **1a** was obtained using Zemplén conditions after separation of α and β anomers.

As a synthetic alternative, we have used here the fluoride donor **2**[33] that was glycosylated with propargyl alcohol in CH_2Cl_2 using boron trifluoroetherate ($BF_3.OEt_2$) as promoter (Scheme 1). An inseparable mixture of α and β anomers **3** was obtained in 90% yield (α/β, 6:4). The azido group was next converted into -NHAc group using triphenylphosphine (PPh_3) in presence of acetic anhydride.[32]



Scheme 1. Synthesis of compounds **1a** and **1b**.

The separation of α and β anomers was performed by silica gel chromatography to obtain the alpha anomer **4a** in 45% (36% for the β anomer **4b**). Compound **4a** has been further crystallized as single crystals in a mixture of dichloromethane and pentane. Both the coupling constant measured by ^{1}H NMR ($J_{H1\text{-}H2}$ = 4.0 Hz) and the RX diffraction analysis (Fig. 3) have confirmed the stereochemistry of the anomeric carbon of **4a**, which is in good agreement with the literature data.[32] Both anomers were finally deacetylated using the Zemplén condition to afford the corresponding prop-2-ynl 2-acetamido-2-deoxy-α/β-D-galactopyranosides (**1a**-**b**) in 23% and 12% over yield, respectively.

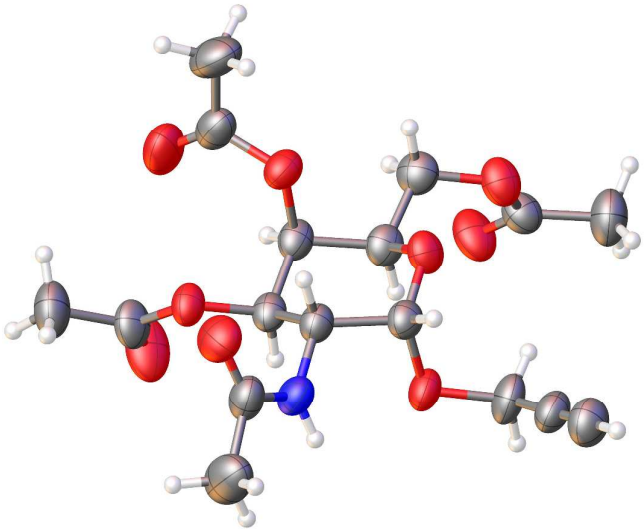
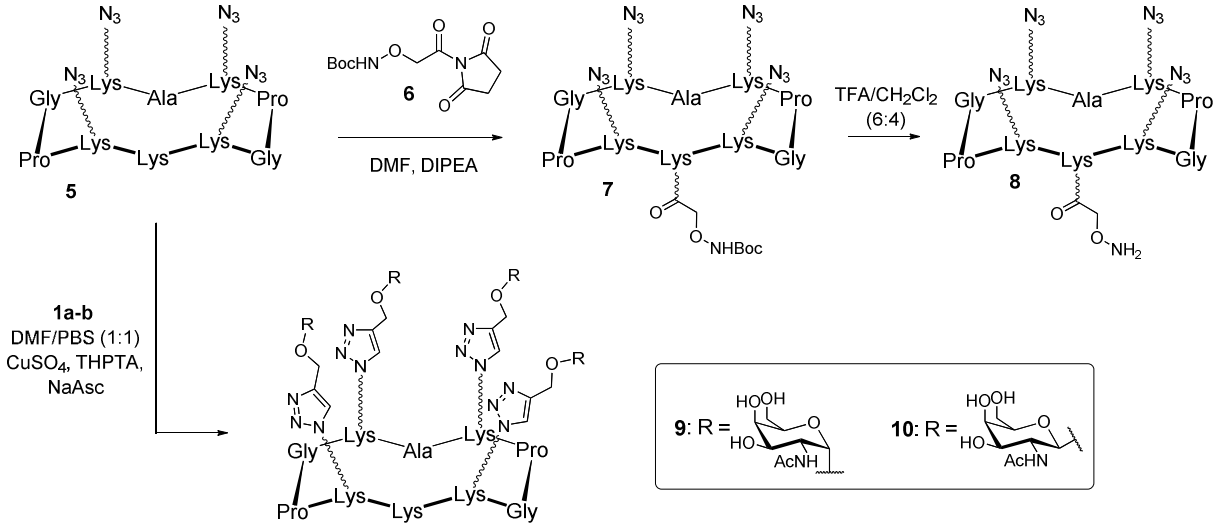


Fig. 3 X-ray structure of compound **4a** (Crystallization: CH_2Cl_2/pentane; Formula: $C_{17}H_{23}N_1O_9$; Unit Cell Parameters: a = 9.1230(18); b = 14.399(3); c = 15.260(3); $P2_12_12_1$). Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1423098. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

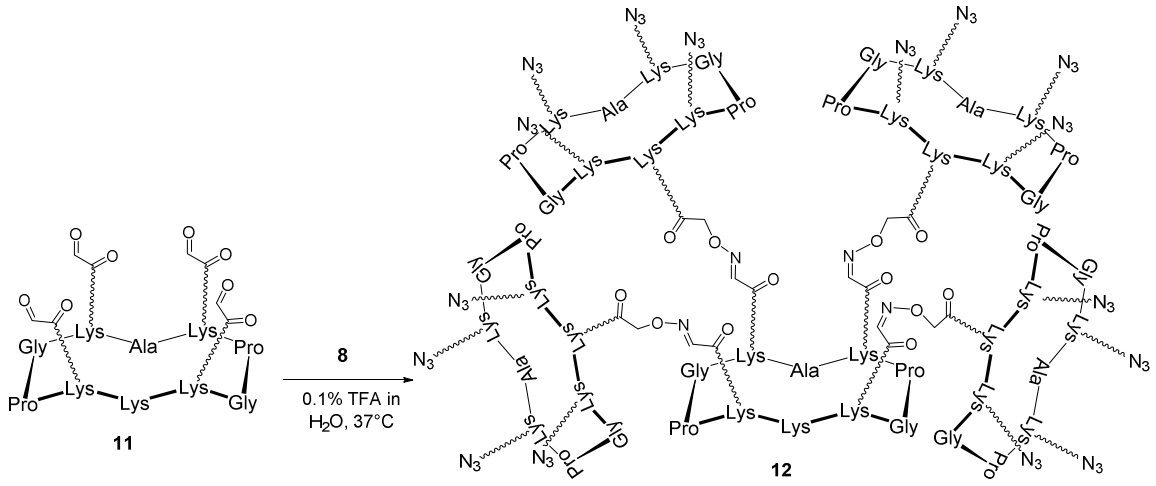
Synthesis of hexadecavalent glycodendrimers

We first followed a divergent route to prepare glycodendrimers following the onion peel method. To do this, we introduced an aminooxyacetyl linker using *N*-Boc-*O*-(carboxymethyl)hydroxylamine succinimide ester **6**[35] in presence of DIPEA to the scaffold **5** (Scheme 2). Further removal of the Boc protecting group gave the appropriate template **8** in 91% yield calculated over two steps.



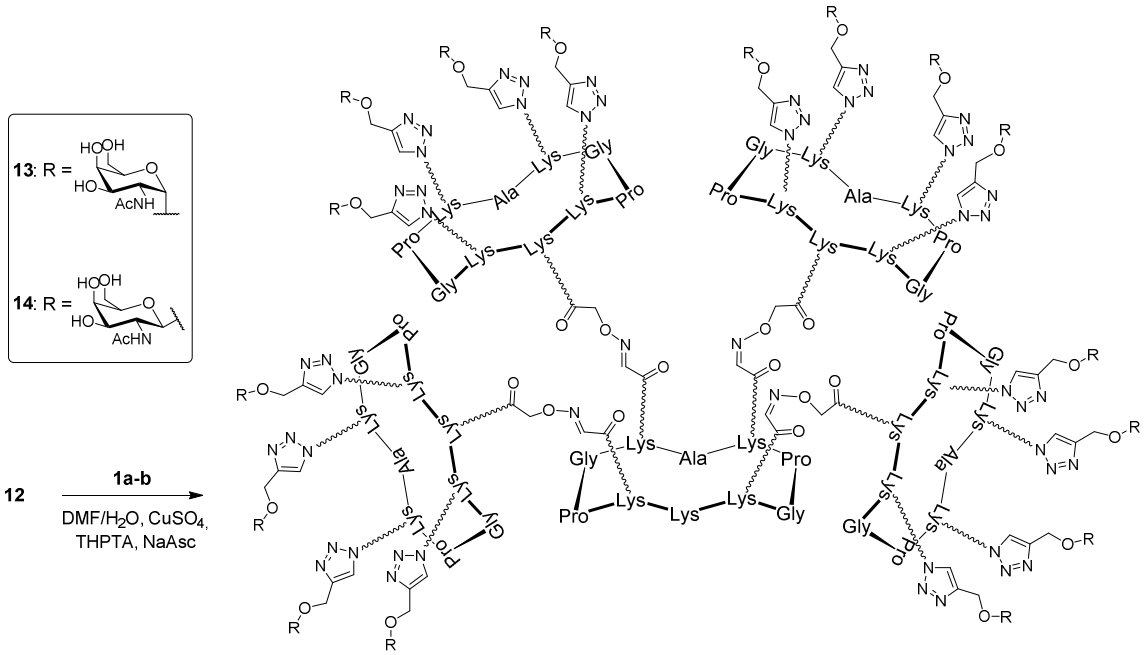
Scheme 2 Synthesis of compound **8** and glycoclusters **9-10**.

This compound was next conjugated to the cyclopeptide-containing aldehyde **11**[36] by oxime ligation in water containing trifluoroacetic acid (0.1% TFA in H_2O) at 37°C (Scheme 3). After 4 h, analytical HPLC indicated the quantitative conversion of **11** to **12**. The crude mixture finally was purified by semi-preparative HPLC to remove the excess of **8** and provided scaffold **12** with an excellent yield (85%) and purity.



Scheme 3 Synthesis of compound **12**.

The efficiency of the CuAAC reaction is closely dependent on the experimental conditions.[37] In a previous study, we observed that tetravalent glycoclusters can be obtained in good yields using a catalytic amount of copper micropowder in a mixture of isopropanol and sodium acetate buffer.[38] Because copper-mediated generation of oxygen species can lead to the formation of aggregates in these conditions, we have decided to follow another procedure reported recently.[39] We have first tested this procedure for the preparation of more simple compounds, *i.e.* tetravalent glycoclusters **9**-**10** (Scheme 2). Compounds **1a** and **1b** have been reacted with **5** in the presence of $CuSO_4$, THPTA (3(tris(3-hydroxypropyltriazolylmethyl)amine) and sodium ascorbate in a mixture of DMF and phosphate buffer. Complete reaction has been observed in 2 h for both compounds and the pure glycoclusters **9** and **10** have been isolated after purification by preparative HPLC in 84% and 83% respectively. Due to the efficiency of this protocol, we thus decided to follow the same procedure from **12** (Scheme 4).



Scheme 4 Divergent route to synthesize glycodendrimers **13**-**14**.

Despite steric hindrance generated during the molecular assembly of these glycodendrimers, the HPLC profile of the crude mixture indicated once again the formation of a single compound in both cases and no trace of partially glycosylated structures (Figure 3). After HPLC purification, compounds **13** and **14** have been recovered in 70% and 69% yields, respectively.

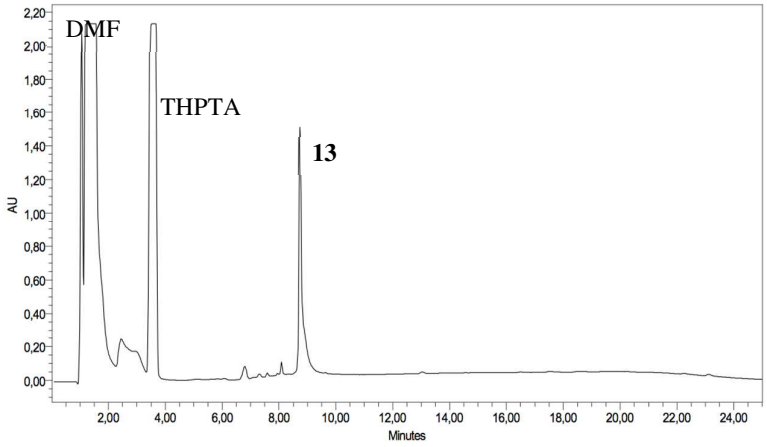
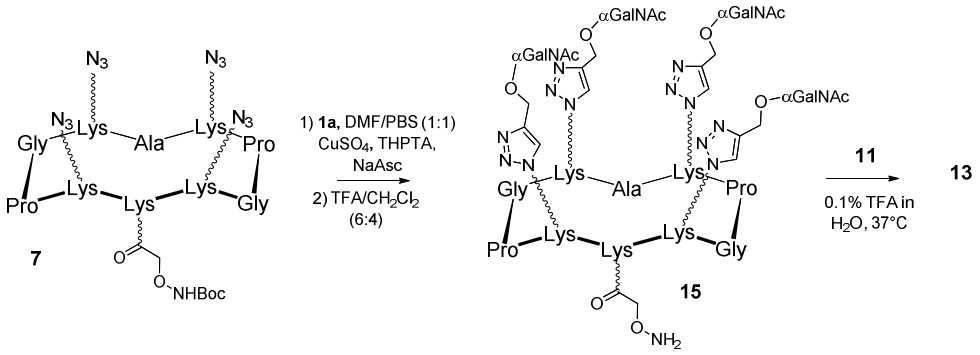


Fig. 3 RP-HPLC profile of crude mixture (λ = 214 nm) for compound **13**. Analysis was carried out at 1.0 mL/min using a linear A–B gradient (buffer A: 0.09% CF_3CO_2H in water; buffer B: 0.09% CF_3CO_2H in 90% acetonitrile) in 20 min.

These compounds have been first characterized by NMR spectroscopy. ^{1}H NMR has shown characteristic signal for the triazole protons at 8.04 - 7.75 ppm, the oxime protons at 7.77 - 7.74 ppm and the anomeric protons at 4.96 - 4.97 ppm (α anomer) and 4.93 - 4.90 ppm (β anomer) with the expected integration values (*i.e.* 16, 4 and 16, respectively). By contrast with the previous synthetic approach based on OL, mass spectrometry has provided the expected spectra without requiring specific sample preparation.[12] Moreover no peak corresponding to fragmentation during the analysis has been observed, thus confirming the monodispersity of the glycodendrimers.

We next evaluated whether the convergent approach can be used to prepare the same series of glycodendrimers. For this purpose, the compound **7** was first conjugated with the propargylated

compound **1a** under the conditions described previously (Scheme 5). After semi-preparative HPLC, the aminooxy group was deprotected with TFA and the resulting tetravalent structure **15** was coupled to **11** by OL. As it was expected, the glycodendrimer **13** was finally obtained in 90% yield after purification, thus confirming that the convergent approach can be followed successfully by using both oxime and CuAAC conjugations.



Scheme 5 Synthesis of glycodendrimer **13**.

Biological evaluation

Several groups have studied the recognition of GalNAc by specific lectins. For example, Bertozzi *et al.*[29] developed microarrays in which glycans are presented on linear polymer backbones mimicking the spatial arrangements of native mucins. By modulating the molecular composition and surface density of these mucin mimetics, they have shown how parameters such as GalNAc valency and interligand spacing affect their recognition by several GalNAc-specific lectins. In addition, if other studies have demonstrated the influence of glycan density on lectin binding, the recognition mechanism is still not fully addressed.[28] Dam and co-workers[40] reported isothermal titration microcalorimetry (ITC) and hemagglutination inhibition measurements for the binding of the Soybean agglutinin (SBA) lectin to modified forms of porcine submaxillary mucin (PSM), which possesses GalNAc residues. This SBA lectin is a tetramer, in which the GalNAc binding domains are located at the apexes of quadrangle spaced by 5 and 7 nm. Its high affinity (K_d = 0.2 nM) for a modified form of PSM (≈ 2300 GalNAc residues) indicates that increasing the numbers of GalNAc

epitopes leads to higher affinities. Further investigations suggested that a lectin "bind and jump" from carbohydrate to carbohydrate epitope along the mucin peptide backbone before complete dissociation from the mucin.[41,42] In our study, we have selected this lectin to evaluate how parameters such as GalNAc valency and anomeric configuration of GalNAc can affect the recognition process. To do this, we have performed competitive enzyme-linked lectin assays (ELLA) using GalNAc-polymer coated to microtiter plates with GalNAc as the monovalent reference, tetravalent glycoclusters **9** and **10** and the hexadecavalent glycodendrimers **13** and **14** (Fig. 4). IC_{50} values which correspond to the concentration of glycoconjugates required to prevent 50% of the binding are reported in Table 1.

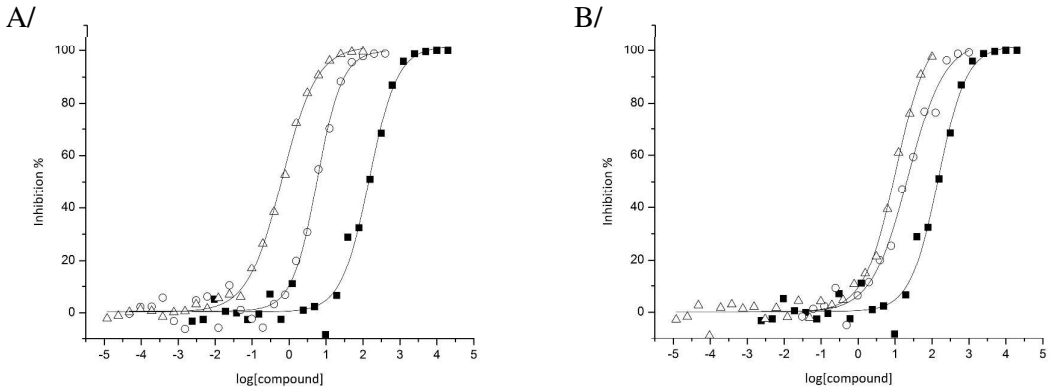


Fig. 4 Inhibition curves for the binding of SBA-HRP to GalNAc-polymer by (A) GalNAc monomer (■), **9** (○) or **13** (△); (B) GalNAc monomer (■), **10** (○) or **14** (△).

Table 1. Inhibition of the adhesion of SBA lectin to a GalNAc-coated plates determined by ELLA.

Compound	n[a]	IC_{50} (μM)[b]	rp [c]	rp/n[d]

GalNAc	1	154.8±26	1	1
9	4	4.9±0.6	31.6	7.9
10	4	27.3±2	5.6	1.4
13	16	0.46±0.1	330.8	20.6
14	16	8.6±0.3	17.9	1.1

[a] Number of sugars in the glycoconjugate; [b] Average of three independent experiments; [c] Relative potency "rp" = IC_{50}(monosaccharide)/IC_{50}(glycoconjugate); [d] Relative potency/sugar "rp/*n*" = relative potency/n.

The results showed that all glycoclusters and glycodendrimers, in both anomer series are able to prevent SBA lectin adhesion at micromolar concentrations. In the beta series, an improvement of the lectin binding was obtained when the valency increases, since tetravalent **10** and hexadecavalent **14** derivatives exhibited IC_{50} of 27.3 µM and 8.6 µM, respectively, while an IC_{50} of 154.8 µM was measured for GalNAc. Although, when reported to the number of sugars (rp/*n*), the value only reaches 1, which denotes that inhibition is only due to a simple concentration effect instead of multivalency. More interestingly, stronger inhibition effects were obtained in the alpha series. Indeed, if significant improvement was obtained with tetravalent glycocluster **9** (IC_{50} = 4.9 µM, rp = 31), the higher inhibitory effect was measured with the hexadecavalent structure **13** since an IC_{50} of 0.46 µM corresponding to a 330-fold improvement compared to the GalNAc monomer. When reported to the number of GalNAc, the inhibition enhancement achieved 20, suggesting that the glycodendrimer **13** display alpha GalNAc residues in more favourable orientation to ensure multivalent interaction with the SBA binding sites. While moderate effects have been observed, all these results demonstrate that the binding properties of these glycoconjugates are strongly dependent on structural parameters that are the orientation and the distribution of the GalNAc units.

Conclusions

We have reported an "onion peel" strategy based on OL to combine cyclopeptide repeating units as the dendritic core and CuAAC to conjugate peripheral α and β propargylated GalNAc. By contrast with our previous oxime-based protocol, this strategy offers the advantage to allow both divergent and convergent routes with similar efficiency. In addition, the characterization by mass spectrometry of the resulting glycodendrimers is significantly easier than for the oxime-linked analogues. The capacity of the resulting glycodendrimers **13** and **14** to prevent the binding of the SBA lectin to polymeric-GalNAc has been studied by ELLA. As it was anticipated, the structure displaying αGalNAc (**13**) inhibit the interaction with higher efficacity (IC_{50} = 0.46 μM, 330-fold improvement compared to GalNAc), which suggests that the binding properties of these glycoconjugates are strongly dependent on the orientation and the distribution of the GalNAc units. Due to both facility and versatility, this strategy will be used in our laboratory to synthesize large glycodendrimers[13] as inhibitors against bacterial lectins.

Experimental section

General procedures

All chemical reagents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. All protected amino acids and Fmoc-Gly-Sasrin®resin was obtained from Advanced ChemTech Europe (Brussels, Belgium), BachemBiochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). For peptides and glycopeptides, analytical RP-HPLC was performed on Waters system equipped with a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. Analysis was carried out at 1.0 mL/min (EC 125/3 nucleosil 300-5 C_{18}) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF_3CO_2H in water; buffer B: 0.09% CF_3CO_2H in 90% acetonitrile). Preparative HPLC was performed on Gilson GX 281 equipped with a fraction collector or on Waters equipment consisting of a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. Purifications were carried out at 22.0 mL/min (VP 250/21 nucleosil 100-7

C_{18}) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient. For carbohydrate, progress of reactions was monitored by thin layer chromatography using silica gel 60 F254 precoated plates (Merck). Spots were visualised by UV light and by charring with 10% H_2SO_4 in EtOH for protected derivatives or 1% ninhydrine in EtOH for hydroxylamine derivatives. Silica gel 60 (0.063-0.2 mm or 70-230 mesh, Merck) was used for column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on BrukerAvance 400 MHz or BrukerAvance III 500 MHz spectrometers and chemical shifts (δ) were reported in parts per million (ppm). Spectra were referenced to the residual proton solvent peaks relative to the signal of $CDCl_3$ (δ 7.27 and 77.23 ppm for ^{1}H and ^{13}C) and D_2O (4.79 ppm for ^{1}H), assignments were done by GCOSY and GHMQC experiments. The anomeric configuration was established from $J_{1,2}$ coupling constant. Standard abbreviations s, d, t, dd, br s, m refer to singlet, doublet, triplet, doublet of doublet, broad singlet, multiplet. ESI mass spectra of peptides and glycopeptides were measured on an Esquire 3000 spectrometer from Bruker or on an Acquity UPLC/MS system from Waters equipped with a SQ Detector 2. MALDI-TOF were performed on a AutoFlex I Bruker after sample pre-treatment in an OligoR3 microcolumn (Applied Biosystems, USA) using 2,5-dihydroxybenzoic acid matrix. HRMS analyses were performed on a Waters Xevo® G2-S QTof.

3-*O*-(2'-Deoxy-2'-azido-3',4',6'-tri-*O*-acetyl-*α*/*β*-D-galactopyranosyl)propyne 3

To a stirred solution of glycosyl fluoride **2**[33] (533 mg, 1.6 mmol) in CH_2Cl_2 (10 mL) at 0°C propargyl alcohol (369 µL, 6.4 mmol) and $BF_3.Et_2O$ (471 µL, 3.2mmol) were slowly added. After 2h the reaction mixture was diluted with water (25 mL) and extracted with CH_2Cl_2 (3x 25 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (2x25 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The resulting residue was purified by flash column chromatography (Et_2O:cyclohexane, 1:1) to afford **3** (531 mg, 90%) as a mixture of anomers (α/β, 3/2).

3-*O*-(2'-Deoxy-2'-acetamido-3',4',6'-tri-*O*-acetyl-*α*/*β*-D-galactopyranosyl) propyne 4a-4b

Triphenylphosphine (472 mg, 1.8 mmol) and acetic anhydride (440 µL, 4.31 mmol) were added to a solution of **3** (531 mg, 1.44 mmol) in CH_2Cl_2 (5 mL). After 16 h stirring at room temperature, the reaction mixture was diluted with water (25 mL) and extracted with CH_2Cl_2 (4x 25 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (2x 25 mL), dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The resulting residue was purified by flash chromatography (AcOEt:CH_2Cl_2, 4:1) to afford **4a** (248 mg, 45%) and **4b** (200 mg, 36%), both as a white solid.

For **4a:** R_f = 0.39 (AcOEt:CH_2Cl_2, 4:1); $[\alpha]_D^{25}$ = -21 (c, 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.60 (1H, d, *J* = 9.8 Hz, N*H*Ac), 5.38 (1H, app dd, *J* = 1.2, 4.0 Hz, H-4), 5.17 (1H, dd, *J* = 12.0, 4.0 Hz, H-3), 5.06 (1H, d, *J* = 4.0 Hz, H-1), 4.62 (1H, ddd, *J* = 12.0, 9.8, 4.0 Hz, H-2), 4.26 (2H, dd, *J* = 5.4, 2.4 Hz, OC*H*$_2$-), 4.19 (1H, m, H-5), 4.10 (2H, dd, *J* = 6.4, 1.8 Hz, H-6$_{a,b}$), 2.47 (1H, t, *J* = 2.4Hz, -C≡C*H*), 2.16 (3H, s, OAc), 2.04 (3H, s, OAc), 1.99 (3H, s, OAc), 1.97 (3H, s, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 171.02-170.21 (C=O), 96.82 (C-1), 78.40 (C-3'), 75.5 (C≡*C*H), 68.37 (C-3), 67.40(C-4)**,** 67.44(C-5), 61.85 (C-6), 55.41 (O*C*H$_2$), 47.7 (C-2), 23.41 (CH_3), 20.94 (CH_3), 20.83 (CH_3); HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{17}H_{23}NO_9Na$ $[M+Na]^+$: 408.1270, found: 408.1264 (error = -1.5 ppm).

For **4b:** R_f = 0.36 (AcOEt:CH_2Cl_2, 4:1); $[\alpha]_D^{25}$ = +83 (c, 1.0 in $CHCl_3$); ^{1}H NMR:($CDCl_3$, 400 MHz) δ = 5.58 (1H, d, *J* = 8.8 Hz, N*H*Ac), 5.36 (1H, br d, *J* = 3.4 Hz, H-4), 5.32 (1H, dd, *J* = 11.0, 3.4 Hz, H-3), 4.88 (1H, d, *J* = 8.0 Hz, H-1), 4.38 (2H, br d, *J* = 2.4 Hz, -C*H*$_2$), 4.14 (2H, m, H-6$_{a,b}$), 4.03 (1H, td, *J* = 11.0, 8.0 Hz, H-2), 3.94 (1H, t, *J* = 6.6 Hz, H-4), 2.46 (1H, t, *J* = 2.4 Hz, -C≡C*H*), 2.14 (3H, s, OAc), 2.04 (3H, s, OAc), 1.99 (3H, s, OAc), 1.96 (3H, s, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ170.63-170.39 (C=O), 98.75 (C$_\beta$-1), 78.71 (C-3), 75.39 (-C≡*C*H), 70.94 (-*C*≡CH), 70.04(C-4)**,** 66.83(C-5), 61.54 (C-6), 56.03 (O*C*H$_2$), 52.29 (C-2), 23.61 (CH_3), 20.85 (CH_3), 20.83 (CH_3); HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{17}H_{23}NO_9Na$ $[M+Na]^+$: 408.1270, found: 408.1280 (error = +2.4 ppm), *m/z*: calcd for $C_{17}H_{24}NO_9$ $[M+H]^+$: 386.1451, found: 386.1458 (error = +1.8 ppm).

3-*O*-(2'-Deoxy-2'-acetamido-α-D-galactopyranosyl) propyne 1a

NaOMe (63 µL, 0.06 mmol) was added to a solution of **4a** (222 mg, 0.58 mmol) in MeOH (10 mL) at room temperature. After 4 h, the reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered and concentrated *in vacuo* to afford **1a** (143 mg, 96%) as a white solid. R_f = 0.39 (CH_2Cl_2:MeOH, 4:1); $[\alpha]_D^{25}$ = +230 (c, 1.0 in MeOH); ^{1}H NMR (D_2O, 400 MHz) δ = 5.12 (1H, d, *J* = 4.0 Hz, H-1), 4.41-4.36 (2H, m, H-2, H-3), 4.27 (1H, dd, *J* = 12.0, 3.8 Hz, H-4), 4.06-4.03 (2H, m, H-5, H-6_a), 3.95 (1H, dd, *J* = 12.0, 4.0 Hz, H-6_b), 3.82-3.80 (2H, m, -OCH_2), 2.94 (1 H, br s, -C≡C*H*), 2.10 (3H, s, NH*Ac*); ^{13}C NMR (D_2O, 100 MHz) δ = 174.7 (C=O), 96.1 (C-1), 71.42 (C-4), 68.51 (C-5), 67.64 (C-3), 61.17 (C-6), 55.15 (OCH_2), 49.6 (-*C*≡CH), 21.9 (CH_3); HRMS (ESI^+-TOF) *m/z*: calcd for $C_{11}H_{17}NO_6Na$ $[M+Na]^+$: 282.0954, found: 282.0962 (error = +2.8 ppm).

3-*O*-(2'-Deoxy-2'-acetamido-β-D-galactopyranosyl) propyne 1b

β Anomer was obtained following the conditions described for **1a** (49 mg, 95%). R_f = 0.35 (CH_2Cl_2:MeOH, 4:1); $[\alpha]_D^{25}$ = -38(c, 1.0 in H_2O); ^{1}H NMR (D_2O, 400 MHz,) δ = 4.71 (1H, d, *J* = 8.0 Hz, H-1), 4.38 (2H, d, *J* = 2.2 Hz, OCH_2-), 3.90 (1H, t, *J* = 3.4 Hz, H-3), 3.86 (1H, dd, *J* = 8.6, 3.4 Hz, H-2), 3.73 (3H, m, H-6, H-5), 3.65 (1H, dd, *J* = 7.6, 3.4 Hz, H-4), 2.96 (1H, t, *J* =4.0 Hz, -C≡C*H*), 2.10 (3H,s, NHO*Ac*); ^{13}C NMR (D_2O, 100 MHz,) δ = 175.02 (C=O), 99.73 (C-1), 79.01 (C-2), 75.78 (-C≡*C*H), 75.29 (C-3), 70.58 (C-4), 67.49 (C-5), 60.98 (C-6), 56.65 (O*C*H_2), 51.98 (-*C*≡CH), 22.4 (CH_3); HRMS (ESI^+-TOF) *m/z*: calcd for $C_{11}H_{17}NO_6Na$ $[M+Na]^+$: 282.0954, found: 282.0956 (error = +0.7 ppm).

General procedure for solid-phase peptide synthesis

Assembly of protected linear peptide was performed manually or automatically (Syro II, Biotage) by employing solid-phase peptide synthesis (SPPS) protocol using the Fmoc/tBu strategy and the Fmoc-Gly-SasrinTM resin (loading of 0.7 mmol/g). Coupling reactions were performed using,

relative to the resin loading, 1.5-2 eq. of *N*-Fmoc-protected amino acid *in situ* activated with PyBOP (1.5-2 eq.) and DIPEA (3-4 eq.) in DMF (10 mL/g resin) for 30 min. Coupling reaction was checked by TNBS test using a solution of 1% trinitrobenzenesulfonic acid in DMF. *N*-Fmoc protecting groups were removed by treatment with piperidine/DMF (1:4, 10 mL/g resin) for 10 min. The process was repeated three times and the resin was further washed five times with DMF (10 mL/g resin) for 1 min. The peptide was released from the resin using cleavage solution of TFA: CH_2Cl_2 (1:99) and linear protected peptide was obtained as a white solid powder after precipitation, triturating and washing with diethyl ether and was used without further purification.

General procedure for CuAAC ligation

A solution of $CuSO_4$ (1 eq./azide) and THPTA (5 eq./azide) in PBS buffer (pH 7.4, 100 mM) was added to a mixture of cyclopeptide and propargyl glycoside (1.5 eq./azide) in DMF at room temperature. To this reaction mixture was added a solution of sodium ascorbate (7 eq./azide) in PBS buffer (10 mM). All solutions were previously degassed under argon. The reaction was stirred at room temperature under argon, after 2h analytical HPLC indicated complete reaction coupling. Chelex^{TM} resin was added to remove excess copper and the reaction mixture was purified by RP-HPLC to afford pure compound as a white powder.

Compound 5

The linear peptide **A** (0.44 mmol) was synthesized following the general procedure for solid phase peptide synthesis, then dissolved in DMF (0.5 mM) and the pH was adjusted to 9 by addition of DIPEA. PyBOP (1.2 eq.) was added and the solution stirred at room temperature for 1h. Solvent was removed under reduced pressure and precipitation in diethyl ether afforded Boc-protected cyclic peptide **B** as white solid. After cleavage of Boc protecting group, using a solution of TFA/CH_2Cl_2 (10 mL, 3:2, v/v) for 1h, the crude reaction mixture was purified by RP-HPLC affording **5** (455 mg, 92 %) as a white powder after lyophilisation. RP-HPLC: R_t = 12.2 min (C_{18}, λ

= 214 nm 5-100% B in 20 min); HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{47}H_{77}N_{23}O_{10}Na$ $[M+Na]^+$: 1146.6121, found: 1146.6146 (error = +2.2 ppm).

Compound 8

To a solution of cyclopeptide **5** (11.7 mg, 10.4 µmol) and *N*-Boc-*O*-(carboxymethyl)hydroxylamine succinimide ester[35] (4.5 mg, 15.6 µmol) in DMF (4 mL), DIPEA was added and the pH adjusted at 8. After 2h stirring at room temperature analytical HPLC indicated formation of compound **7** and the reaction mixture was evaporated *in vacuo*. The crude product was then subjected to deprotection of Boc protecting group by using a solution of TFA/CH_2Cl_2 (20 mL, 3:2 v/v) at room temperature. The crude mixture was purified (acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) and lyophilized to give **8** (11.4 mg, 91%) as a white powder. R_t = 12.1 min (C_{18}, λ = 214 nm, gradient 5-100% B in 20 min); HRMS (ESI$^+$-TOF) *m/z*: calcd for $C_{49}H_{81}N_{24}O_{12}$ $[M+H]^+$: 1197.6466, found: 1197.6475 (error = +0.8 ppm), *m/z*: calcd for $C_{49}H_{80}N_{24}O_{12}Na$ $[M+Na]^+$: 1219.6285, found: 1219.6302 (error = +1.4 ppm).

Compound 12

A solution of **8** (27.1 mg, 22.7 µmol) and **11**[36] (4.7 mg, 3.8 µmol) in a mixture water/acetonitrile (50:50) with 0.1% TFA (10 mL) was incubated at 37°C for 45 min. The crude reaction mixture was purified by semi-preparative HPLC (acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) and lyophilized to give **12** (19.2mg, 85%) as a flocculent white powder. RP-HPLC R_t = 17.0 min; (C_{18}, λ = 214 nm 5-100% B in 20 min); MALDI-TOF *m/z*: calcd for $C_{251}H_{398}N_{111}O_{62}$ $[M+H]^+$: 5962.6, found: 5962.7

Compound 9

A mixture of $CuSO_4$ (8.9 mg, 0.03 mmol) and THPTA (77.3 mg, 0.18 mmol) in PBS buffer (0.5 mL, pH 7.4, 10 mM) was added to a solution of **5** (10 mg, 8.9 µmol) and **1α** (13.8 mg, 53.2 µmol)

in DMF (1 mL) at room temperature under argon atmosphere. To this reaction mixture was added a solution of sodium ascorbate (49.4 mg, 0.25 mmol) in PBS buffer (0.5 mL, pH 7.4, 10 mM) and the reaction was stirred for 2 h. The crude reaction mixture was purified by semi-preparative HPLC (acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) to afford **9** (16.2 mg, 84%) as a white powder after lyophilisation. RP-HPLC R_t = 11.5 min (C_{18}, λ = 214 nm, gradient 5-100% B in 20 min); HRMS (ESI^+-TOF) *m/z*: calcd for $C_{91}H_{146}N_{27}O_{34}$ $[M+H]^+$: 2161.0525, found : 2161.0474 (error = -2.3 ppm).

Compound 10

The synthesis was performed using **5** (10 mg, 8.8 µmol) and **1b** (13.8 mg, 53.2 µmol) by following the procedure described for **9**. Compound **10** (15.8, 83%) was obtained as a white powder after purification (semi-preparative HPLC, acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) and lyophilisation. RP-HPLC R_t = 11.0 min (C_{18}, λ = 214 nm, gradient 5-100% B in 20 min); HRMS (ESI^+-TOF) *m/z*: calcd for $C_{91}H_{146}N_{27}O_{34}$ $[M+H]^+$: 2161.0525, found : 2161.0471 (error = -2.5 ppm).

Compound 13

The synthesis was performed using **12** (3.5 mg, 0.59 µmol) and **1a** (3.6 mg, 13.9 µmol) by following the procedure described for **9**. Compound **13** (4.2 mg, 70%) was obtained as white powder after purification (semi-preparative HPLC, acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) and lyophilisation. RP-HPLC R_t = 11.9 min (C_{18}, λ = 214 nm, gradient 5-100% B in 20 min); MALDI-TOF *m/z*: $C_{427}H_{670}N_{127}O_{158}$ $[M+H]^+$: 10110.6, found: 10106.1; ESI^+-MS *m/z*: calcd for $C_{427}H_{670}N_{127}O_{158}$ $[M+H]^+$: 10110.6, found: 10111.2

Compound 14

The synthesis was performed using **12** (4.5 mg, 0.75 µmol) and **1b** (4.8 mg, 18.5 µmol) by following the procedure described for **9**. Compound **14** (5.2 mg, 69%) was obtained as white powder after purification (semi-preparative HPLC, acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) and lyophilisation. RP-HPLC R_t = 11.5 min (C_{18}, λ = 214 nm, gradient 5-100% B in 20 min); MALDI-TOF *m/z*: calcd for $C_{427}H_{670}N_{127}O_{158}$ $[M+H]^+$: 10110.6 , found : 10106.1; ESI^+-MS *m/z*: calcd for $C_{427}H_{670}N_{127}O_{158}$ $[M+H]^+$: 10110.6, found: 10110.6

Compound 15

Compound **15** (13.2 mg, 77% over two steps) was obtained using **7** (10 mg, 7.7 µmol) and **1a** (12 mg, 46.3 µmol) by following the procedure described for **9**. The resulting Boc-protected glycosylated scaffold (15.6 mg, 6.7 µmol) RP-HPLC R_t = 11.1 min (C_{18}, λ = 214 nm, gradient 5-100% B in 20 min); HRMS (ESI^+-TOF) *m/z*: calcd for $C_{98}H_{156}N_{28}O_{38}Na$ $[M+Na]^+$: 2356.1033; found 2356. 0977 (error = -2.4 ppm) was treated with 60% TFA in CH_2Cl_2 (10 mL) at room temperature for 1 h. Solvents were removed under reduced pressure and the crude reaction mixture was purified by semi-preparative HPLC (acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) to afford the expected product as a white powder after lyophilisation. RP-HPLC R_t = 10.4 min; (C_{18}, λ = 214 nm 5-100% B in 20 min); HRMS (ESI^+-TOF) *m/z*: calcd for $C_{93}H_{148}N_{280}O_{36}Na$ $[M+Na]^+$; 2256.0509; Found 2256.0588 (error = +3.5 ppm).

Compound 13 (Convergent pathway)

A solution of **15** (10.7 mg, 4.8 µmol) and **11** (1.0 mg, 0.8 µmol) in 0.1% aq. TFA (10 mL) was incubated at 37°C for 45 min. The crude reaction mixture was purified by semi-preparative HPLC (acetonitrile/water with 0.1% TFA with gradient 5-60% B in 25 min) and lyophilized to afford **13** (7.3 mg, 90%) as a white powder.

ELLA (enzyme-linked lectin assay) experiments

ELLA experiments were conducted using 96-well microtiter Nunc-Immuno plates (Maxi-Sorp) coated with 100 µL of polymeric sugar (PAA-α-N-acetyl-D-galactosamine, 5 $\mu g.mL^{-1}$; Lectinity Holding, Inc., Moscow) diluted in carbonate buffer, pH 9.6 for 1 h at 37°C. Excess of PAA-sugar was removed, then wells were blocked with BSA in PBS (3% w/v, 100 $\mu L.well^{-1}$) at 37°C for 1 h. The Soybean agglutinin (SBA) lectin conjugated HRP (0.1 $\mu g.mL^{-1}$) was mixed with various concentrations of inhibitors for 1 h at 37°C. Then the mixture was added to the PAA-sugar-coated microwells and incubated for 1 h at 37°C. The wells were washed with T-PBS (3x100 $\mu L.well^{-1}$) then the colour was developed using 100 µL per well of 0.05 M phosphate/citrate buffer containing *O*-phenylenediamine dihydrochloride (OPD, 0.4 $mg.mL^{-1}$) and urea hydrogen peroxide (0.4 $mg.mL^{-1}$, Sigma-Aldrich). The reaction was stopped after 10 min by the addition of 50 µL of 30% H_2SO_4. The absorbance was read at 490 nm using a microtiter plate reader (SPECTRAmax, model PLUS384, Molecular Devices). Percentage inhibition was calculated as follows: inhibition $(\%)=((A_{max}-A)/A_{max})*100$, where A_{max} is the absorbance of the SBA lectin without inhibitor and A is the absorbance of the SBA lectin with inhibitor. Percent of inhibition was plotted against log [inhibitor].

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