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Homo- and Heterovalent Neoglycoproteins as Ligands for Bacterial Lectins

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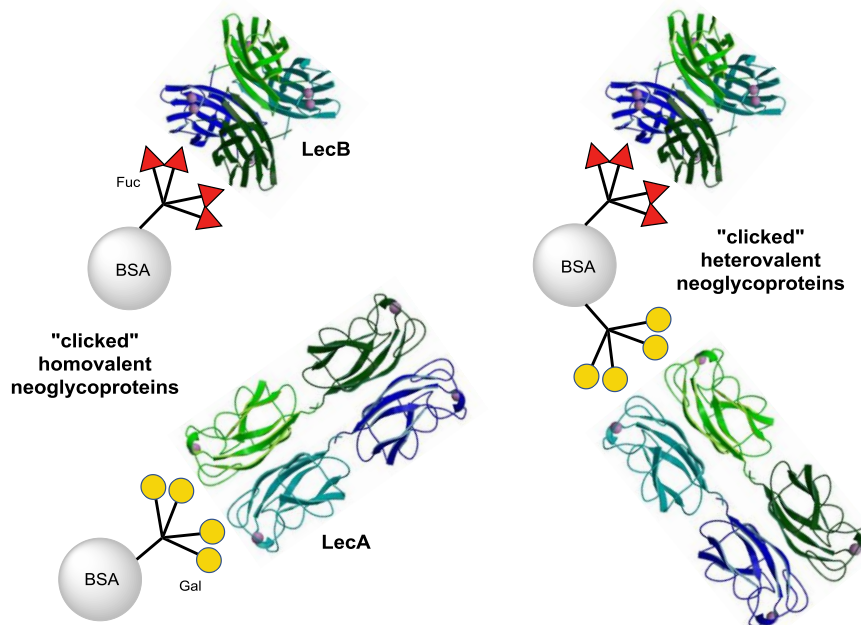
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Extending molecular diversity of multivalent systems: the utilization of click chemistry allowed the preparation of homo- and heterovalent neoglycoproteins from a carrier protein and glycodendrons. Binding assays with LecA and LecB lectins from *P. aeruginosa* highlighted the impact of the multivalent presentation of glycodendrons by the carrier protein and demonstrated that the heterovalent display of glycans allows the interaction with both lectins.



Abstract

Click chemistry gives access to unlimited set of multivalent glycoconjugates to explore carbohydrate-protein interactions and discover high affinity ligands. In this study, we have created supramolecular systems based on a carrier protein that was grafted by Cu(I)-catalyzed azide-alkyne cycloaddition with tetravalent glycodendrons presenting α Gal, β Gal and/or α Fuc. Binding studies of the homo- (**4a-c**) and heterovalent (**5**) neoglycoproteins (neoGPs) with the LecA and LecB lectins from *P. aeruginosa* has first confirmed the interest of the multivalent presentation of glycodendrons by the carrier protein (IC_{50} up to 2.8 nM). Moreover, these studies have shown that the heterovalent display of glycans (**5**) allows the interaction with both lectins (IC_{50} of 10 nM) despite the presence of unspecific moieties, and even with similar efficiency for LecB. These results demonstrate the potential of multivalent and multispecific neoGPs as a promising strategy to fight against resistant pathogens.

Introduction

In the past decades, glycosylated compounds based on multivalent synthetic scaffolds revealed essential tools for studying carbohydrate-protein (lectin) interactions and modulating biological events.^{[1],[2],[3]} To design high affinity molecules, computational methods and structural data of the target protein allow the choice of sugar units, valency and linking arm, however predicting the influence of the scaffold itself remains difficult. Besides being easy to synthesize, it should ideally be chosen to be both soluble and stable *in vivo*, to bring the sugar units at the closest proximity of the protein binding pockets and to have low flexibility to limit entropic cost of the interaction. If simple scaffolds fulfil these criteria, the development of chemical ligation has significantly extended the geometrical limits of multivalent systems to optimize their properties.^{[4],[5],[6]} By combining scaffolds having either identical or different characteristics, a diversity of glycosylated architectures are now accessible and some of them are currently investigated as pathogen inhibitors, immunomodulators, delivery or theranostic tools.^{[7],[8],[9],[10],[11]}

In the present study, we report the conjugation of glycodendrons to a carrier protein using a convergent approach based on the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC),^[12] thus providing a new series of neoglycoproteins (neoGP) with clusters of glycans distributed at different positions (Fig. 1). Given the dense and expanded expression of glycoconjugates at the surface of the cell membrane, we reasoned that the combination of synthetic glycodendrons within a protein carrier could easily provide a diversity of relevant neoglycoprotein-based reagents for studying and/or modulating carbohydrate-protein interactions. In particular, the utilization of glycans involved in bacterial infections might have the potential to inhibit the infection process and to provide alternative to antibiotic treatments against resistant pathogens.^[13] To this aim, we used Bovine Serum Albumin (BSA), a naturally unglycosylated protein as the carrier model.

BSA was first functionalized with alkyne linkers, then tetravalent α - and β -galactosylated (α Gal and β Gal) or α -fucosylated (α Fuc) dendrons functionalized with an azido group were conjugated to provide homovalent neoGPs. The resulting structures may thus bind selectively to the LecA and LecB lectins of *Pseudomonas aeruginosa*.^{[14],[15]} These two tetrameric calcium-dependent lectins are involved in the bacterial adhesion to the host cells, the biofilm formation and the lethal effect for immunocompromised patients, thus making them ideal biological targets for this study. In addition, recent studies have highlighted the importance of heterovalent structures as high affinity and selective ligands for carbohydrate-binding proteins, often leading to original properties.^{[16],[17],[18],[19],[20],[21],[22]} To this aim and to extend the interest of the present approach, another neoGP combining randomized grafting of the α Gal and α Fuc dendrons was prepared to provide a heterovalent structure. The binding ability of these homo- and heterovalent neoGPs was next evaluated with these two bacterial lectins.

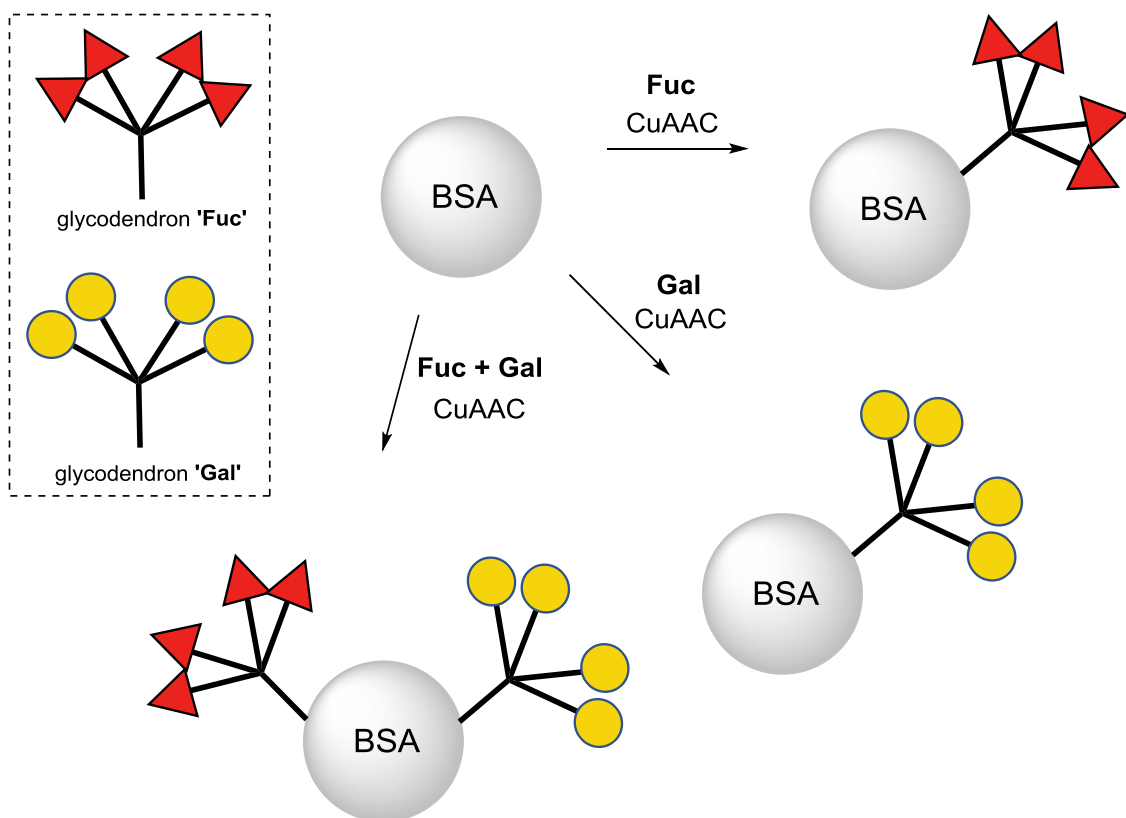
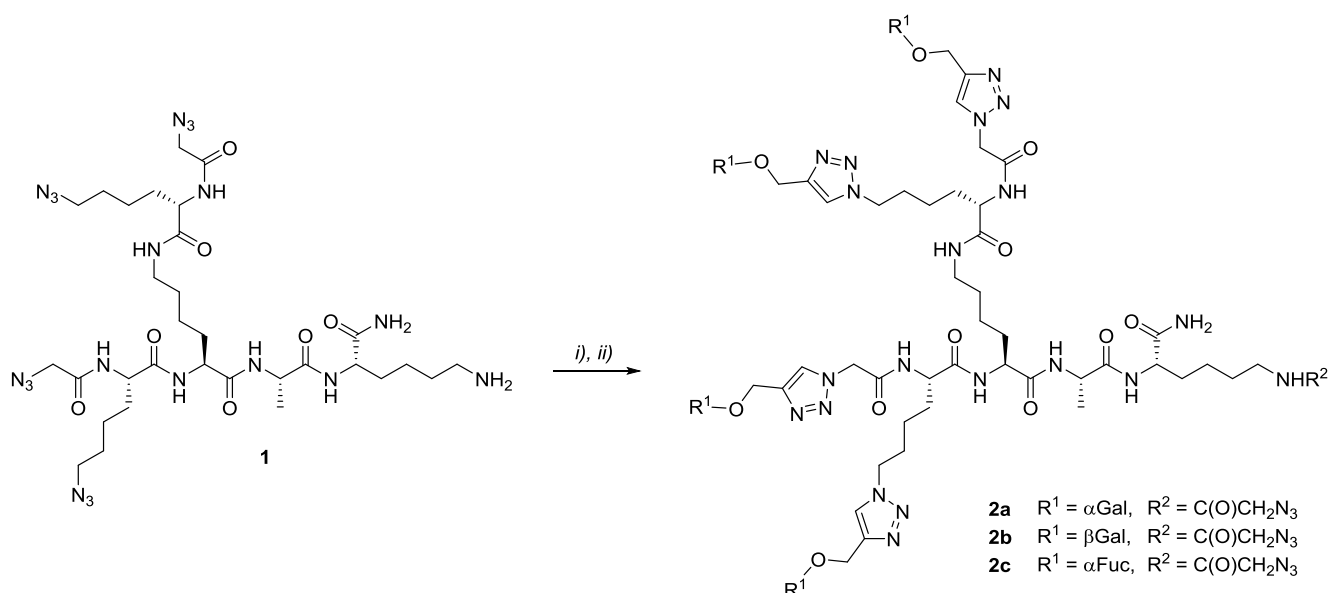


Figure 1. Assembly of homo- and heterovalent neoGPs from BSA and glycodendrons 'Fuc' and 'Gal' by CuAAC.

Results and discussion

Synthesis of neoGPs

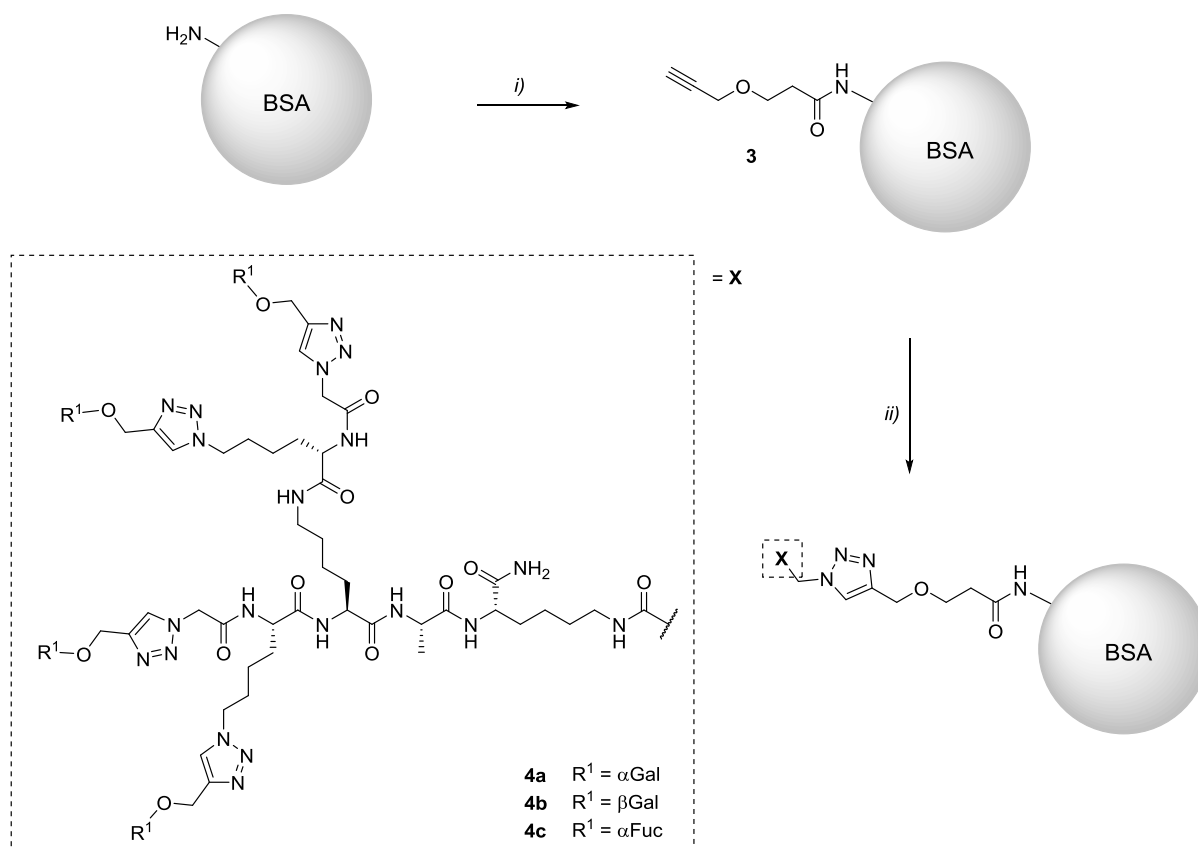
We previously reported several series of fully synthetic multivalent structures decorated with β Gal or α Fuc.^{[23],[24]} Binding studies revealed that their affinity with LecA and LecB was closely dependent on either the valency or the ligand geometry. For example, only fucosylated structures of higher valency (*i.e.* 16 copies of α Fuc) were found as excellent ligands for LecB with a significant impact of the sugar display on the affinity.^[23] By contrast, tetravalent galactosylated structures have shown nanomolar affinity for LecA, while the increase of valency to sixteen β Gal had only a limited effect on the binding.^[24] Here we first synthesized tetravalent dendrons displaying both glycans to be further conjugated to BSA as the carrier protein. A polylysine structure functionalized with azido groups (**1**) was synthesized by solid-phase peptide synthesis using the Fmoc/tBu strategy (Scheme 1).



Scheme 1. Synthesis of the polylysine-based glycodendrons **2a-c**. Reagents and conditions: i) Propargyl glycoside, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, THPTA, sodium ascorbate, DMF/PBS (pH 7.5, 1:1), r.t., 2h; ii) *N*-hydroxy-succinimidyl ester of azidoacetic acid, DIPEA, DMF, r.t., 2h.

Propargylated αFuc and βGal ^{[24], [25]} were next conjugated by CuAAC under standard condition using copper sulfate, tris(3-hydroxypropyl-triazolylmethyl)amine (THPTA) and sodium ascorbate in a mixture of DMF and PBS (pH 7.4). After 2 hours, semi-preparative RP-HPLC purification provided tetravalent compounds that were subsequently functionalized on the free side chain of the C-terminal lysine with azido *N*-hydroxy-succinimidyl ester of azidoacetic acid (Fig. S1-9).

The neoGPs were synthesized using a similar protocol. BSA was functionalized at the lysine side chain with a commercial propargyl linker by amide coupling (Scheme 2).



Scheme 2. Synthesis of homovalent neoGPs **4a-c**. Reagents and conditions: i) 3-(2-Propyn-1-yloxy)propanoic acid 2,5-dioxo-1-pyrrolidinyl ester, PBS (pH 8.0), r.t., 5h; ii) **2a-c**, CuSO₄·5H₂O, TBTA, sodium ascorbate, PBS (pH 7.5), r.t., 24h.

After 5h in PBS, the crude mixture was purified by size-exclusion chromatography on Sephadex G25 and MALDI-TOF analysis of BSA-alkyne **3** indicated that among the 60 lysines present in BSA, approximately 33 were functionalized with an alkyne group (Fig. S10). Finally, glycodendrons **2a-c** (25 equiv. over the 33 reactive groups present in **3**) were conjugated to **3** by CuAAC, with tris(benzyltriazolylmethyl)amine (TBTA), L-sodium ascorbate and CuSO₄ in PBS similarly to previous reports.^{[26],[27],[28]} After 24h at room temperature, the resulting homovalent neoGPs **4a-c** were purified on Sephadex G25. The purity was confirmed by SDS-PAGE and MALDI-TOF experiments enabled to estimate that between 12 to 19 tetravalent glycodendrons were coupled to BSA, which corresponds to 49-75 glycans per BSA and molecular weights from 92 to 105 kDa for homovalent

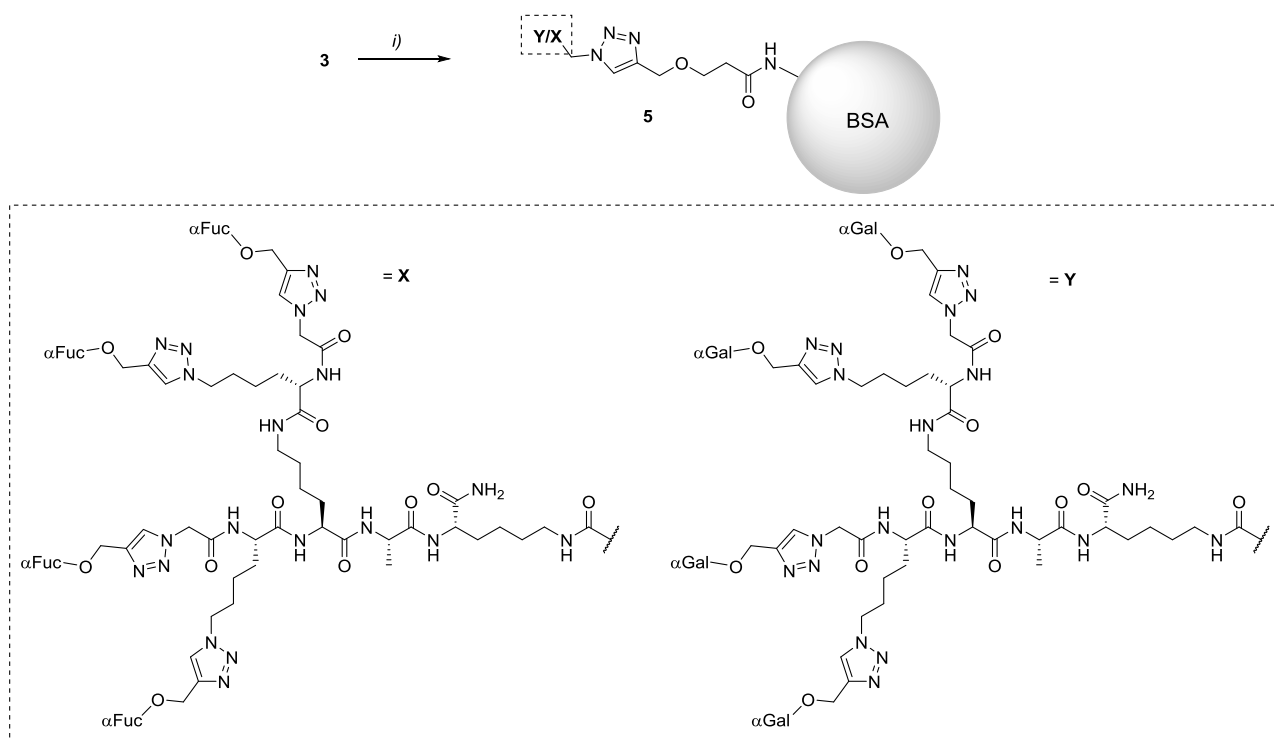
neoGPs **4a-c** (Table 1, Fig.S11 and S14). Of note, when the coupling reaction was performed with an excess of the fucosylated glycodendron **2c** over BSA-alkyne **3** (*i.e.* 50 equiv.), the quantity of α Fuc only increased to 73 (versus 53 using 25 equiv.), thus suggesting that the remaining alkyne groups are less accessible in the protein to be fully functionalized under these experimental conditions (Fig. S12).

Table 1. MALDI-TOF analysis of the functionalization of BSA-alkyne **3** with glycodendrons **2a-c** by CuAAC.

Compound	MW calc. [Da]	MW found [Da]	nDendron/BSA	nGlycan/BSA
neoGP 4a (α Gal, 25 eq.)	114578 ^[a]	103371	19 ^[d]	75 ^[e]
neoGP 4b (β Gal, 25 eq.)	114578 ^[a]	91894	12 ^[d]	49 ^[e]
neoGP 4c (α Fuc, 25 eq.)	112978 ^[a]	93322	13 ^[d]	53 ^[e]
neoGP 4c (α Fuc, 50 eq.)	126664 ^[b]	101321	18 ^[d]	73 ^[e]
neoGP 5 (α Fuc/ α Gal, 50/50) ^[f]	113778 ^[c]	103303	19 ^[d]	76 ^[e]

The value presented in the table are rounded values; ^[a] MW of neoGPs **4a-c** was calculated on the basis of the number of eq. of glycodendrons **2a-c**: MW calc. = MW of BSA-alkyne **3** + 25 x MW of **2a-c**; ^[b] MW of neoGP**4c** was calculated on the basis of the number of eq. of glycodendron **2c**, considering that BSA-alkyne **3** displays a maximum of 33 alkyne groups: MW calc. = MW of BSA-alkyne **3** + 33 x MW of **2c**; ^[c] MW of neoGP**5** was calculated on the basis of the number of eq. of mixed glycodendrons **2a-c**: MW calc. = MW of BSA-alkyne **3** + 25 x (50% MW of **2a** + 50% MW of **2c**); ^[d] nDendron/BSA = (MW found for **4a-c** – MW of BSA-alkyne **3**) / MW of **2a-c**; ^[e] n Glycan/BSA = n Dendron / BSA x 4; ^[f] The ratio of 50% of α Gal and 50% of α Fuc residues was arbitrarily set to determine the number of dendrons and glycans per BSA and nGlycan/BSA.

In addition, with respect to the role of LecA and LecB in the infectious and lethal processes mediated by *P. aeruginosa*, heteroclusters combining β Gal and α Fuc could simultaneously bind these two lectins to strengthen inhibitory effect as demonstrated in previous studies based on heterovalent peptide glycodendrimers,^[29] pillar[5]arene-containing [2]rotaxane^[30] or hexadecaivalent cyclopeptide-based glycodendrimers.^[24] To this aim, we performed the CuAAC reaction between BSA-alkyne **3** and a mixture of **2a** and **2c** to afford the heterovalent neoGP **5** with randomized distribution of glycodendrons exposed by BSA (Scheme 3, Table 1, Fig. S13 and Fig. S15).



Scheme 3. Synthesis of heterovalent neoGP **5** with randomized distribution of glycodendrons. Reagents and conditions: i) **2a** (12.5 eq.) and **2c** (12.5 eq.), CuSO₄·5H₂O, TBTA, sodium ascorbate, PBS (pH 7.5), r.t., 24h.

Direct binding assays

The binding ability of the homovalent neoGPs **4a-c** was studied with LecA and LecB using the GLYcoPROFILE[®] technology (GLYcoDiag, France).^{[26],[27],[28],[31]} NeoGPs **4a-c** were first labelled with biotin using the protocol described previously then were incubated in microtitration plates coated with lectins. Streptavidin labelled with 5-(4,6-dichlorotriazinyl)-aminofluorescein (DTAF) was finally incubated in each well and the interaction was directly detected by reading the fluorescence intensity (FI) provided by the neoGP bound to the lectin. While no interaction was observed with the unspecific Wheat Germ Agglutinin (WGA), this experiment indicated that the galactosylated **4a-b** and fucosylated **4c** neoGPs were able to bind LecA and LecB, showing a FI proportional to the neoGP concentration (Fig. S16-18). In addition, at lower concentration, we observed higher FI for **4a** than for **4b** which could be attributed to the higher valency in **4a** rather than a clear preference of LecA for the alpha anomer of galactose. Altogether, these results confirm the benefit of the multivalent presentation of glycans.

Finally, we evaluated the interaction of the heterovalent neoGP **5**, which statistically displays identical content of α Gal and α Fuc units, with LecA and LecB (See Fig. S19-20). As expected, the fluorescent signal observed with LecA is higher when BSA only displays α Gal due to the higher valency (75 in **4a** vs 38 in **5**). Conversely, when the grafting ratio of α Fuc increases (**4c**) compared to **5**, the fluorescent signal observed with LecB is of higher intensity. More interestingly, while containing half less specific glycan for a given lectin, the heterovalent neoGP **5** shows slightly lower binding ability with LecA and LecB than the parent homovalent neoGPs **4a-c**. This result suggests that the heterovalent display of glycans in BSA allows the simultaneous interaction with both lectins without significant loss of specificity and efficiency.

Inhibition assays

To confirm these observations, we next performed inhibition assays with LecA and LecB using biotinylated neoGPs as tracers (respectively neoGP **4a** for LecA and neoGP **4c** for LecB).^{[31],[26],[27],[28]} The neoGPs **4 a-c** and **5**, the two commercial neoglycoproteins NeoGa and NeoF functionalized with monovalent α Gal or α Fuc (grafting ratio of 13 and 14 units, respectively), and the corresponding monosaccharides have been tested as inhibitors (Table 2).

Table 2. Inhibition of the interaction of biotinylated neoGPs to LecA and LecB-coated plates with homo- and heterovalent neoGPs **4a-c** and **5**.

Compound	n ^[a]	IC ₅₀ [μ M]	rp ^[b]	rp/n ^[c]
Gal (LecA)	1	13.2 \pm 1.2	1	1
NeoGa ^[d] (LecA)	13	0.247 \pm 0.025	53	4
neoGP 4a (LecA)	75	0.0069 \pm 0.0009	1913	26
neoGP 4b (LecA)	49	0.0028 \pm 0.0007	4714	96
neoGP 5 (LecA)	76 (38 α Gal)	0.010 \pm 0.0012	1320	34
Fuc (LecB)	1	42.0 \pm 2.9	1	1
NeoF ^[e] (LecB)	14	0.208 \pm 0.017	201	14
neoGP 4c (LecB)	53	0.010 \pm 0.0013	4200	79
neoGP 5 (LecB)	76 (38 α Fuc)	0.010 \pm 0.0014	4200	110

[a] Number of sugar units; [b] Relative potency “rp” = IC₅₀(monosaccharide) / IC₅₀(neoGP); [c] Relative potency/sugar “rp/n”; [d] Neoglycoprotein displaying α Gal commercialized by GLYcoDiag, France; [e] Neoglycoprotein displaying α Fuc commercialized by GLYcoDiag, France.

In this assay, lectin is immobilized in microplate wells and simultaneously incubated with a solution containing the biotinylated tracer and various concentration of the inhibitors.

After a final incubation with streptavidin-DTAF, the intensity of fluorescence enables to determine the inhibition potency of the tested compounds (Fig. S21-24). With galactosylated derivatives and LecA, we observed a modest IC₅₀ value (247 nM) for the monovalent NeoGa, which promotes a 53-fold improvement compared to galactose (13 μM). Additionally, both homovalent galactosylated neoGPs **4a-b** revealed IC₅₀ in the nanomolar range with LecA. The best neoGP **4b** displaying βGal even reaches a relative potency (rp) of 4700 over free galactose, *i.e.* almost 100-fold improvement when reported to the number of sugar units (rp/n). While containing 12 β-galactosylated dendrons (*i.e.* 49 βGal), this compound indeed shows a rp/n value 3.6 times better than **4a** having higher valency (*i.e.* 75 αGal). Unlike what was suggested by the direct binding assays, this result seems to indicate that βGal units allow stronger inhibitory effect than αGal. However, when alpha galactosylated ligands are compared, the heterovalent neoGP **5**, which contains 38 copies of αGal, was found slightly less efficient than **4a** (75 αGal) while showing comparable rp/n (34 for **5** vs. 26 for **4a**). This highlights that the inhibition potency is closely related to the number of αGal and more importantly, that the presence of fucose units does not interfere with the inhibition process.

With LecB, the neoF with 14 sugar units showed a 200-fold better inhibition over fucose (rp/n = 14), whereas neoGP **4c** displaying 53 glycans showed a remarkable improvement factor of 4200 times over fucose to inhibit the interaction, which corresponds to a rp/n of 79. Additionally, with the heterovalent neoGP **5** displaying only 38 αFuc (combined with 38 αGal units), the rp value reached similar efficiency albeit with lower rp/n. This unexpected result suggests that the presence of αGal in proximity of αFuc in **5** could induce a favourable orientation of the fucose units to inhibit the interaction of the tracer with LecB. This observation demonstrates the importance of spatial presentation of glycans rather than the valency for inhibition as already observed in other studies.^[3]

Conclusions

In this paper, we report the synthesis of multivalent neoglycoproteins by click chemistry. The functionalization of lysine residues of BSA with alkyne linkers provided approximately 33 anchoring groups to the protein that were subsequently conjugated with tetravalent glycodendrons presenting α Gal, β Gal and/or α Fuc by CuAAC. Homo- and heterovalent neoGPs (**4a-c** and **5**) functionalized with up to 19 randomly distributed dendrons were thus prepared and their binding ability were studied with the LecA and LecB lectins from *Pseudomonas aeruginosa* using the GLYcoPROFILE[®] technology. In a direct assay, we first showed the potential of the homovalent neoGPs **4a-c** for recognition with the specific lectin and we observed that the presence of randomized fucosylated and galactosylated glycodendrons in BSA (**5**) allows the interaction with both LecA and LecB without any loss of efficiency. Moreover, inhibition assays have highlighted that the presence of β Gal (**4b**) ensures a stronger inhibition for LecA, whereas α Gal (**4a**) at higher valency is required to promote similar effect. Unexpectedly, the inhibitory potency of the heterovalent neoGP **5** with LecB proved similar to the fucosylated neoGP (**4c**), thus suggesting that a more favorable orientation of the fucose units in **5**. Overall the simplicity of the synthetic strategy and the promising biological potency of carrier protein grafted with glycodendrimers opens the route towards the development of new therapeutic agents against resistant pathogens. In particular, due to their structural feature, such neoGP could efficiently mimic the cell surface and prevent the infection of host cells from pathogens. A large diversity of neoGPs is currently developed to explore this perspective.

Experimental section

General methods

All chemical reagents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. Commercial neoglycoproteins NeoGa and NeoF (*i.e.* BSA functionalized with α Gal and α Fuc, respectively), LecA and LecB 96-well plates (LEctPROFILE plates) were obtained from GLYcoDiag (France). All protected amino acids and Fmoc-Gly-Sasrin[®] resin was obtained from Advanced ChemTech Europe (Brussels, Belgium), Bachem Biochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). The EZ-link sulfo-NHS-LC-biotin was purchased from ThermoFisher Scientific (Ref: 21335) and the number of conjugated biotin quantified by the HABA/avidine method (ThermoFisher Scientific_UserGuide: HABA). For glycopeptides, analytical RP-HPLC was performed on a Waters alliance 2695 separation module, equipped with a Waters 2489 UV/visible detector. Analyses were carried out at 1.23 mL min⁻¹ (Waters XBridge Shield RP18 3.5 μ M, C₁₈, 100 \times 4.6 mm) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF₃CO₂H in water; buffer B: 0.09% CF₃CO₂H in 90% acetonitrile). Preparative HPLC was performed on Waters equipment consisting of a Waters 2545 controller and a Waters 2487 dual absorbance detector. Purifications were carried out at 22 mL min⁻¹ (VP 250 \times 21 mm nucleosil 100-7 C₁₈) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient. ¹H NMR spectra were recorded on Bruker Avance 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 D₂O (4.79 ppm). All mass spectrometry characterizations were performed at Mass Spectrometry facility, PCN-ICMG, Grenoble. ESI high resolution mass spectra of glycopeptides were measured on a LTQ Orbitrap XL spectrometer from Thermo Scientific. For the neoGPs, Sephadex-G25 was obtained from GE Healthcare. Chemicals and solvents were used as received from commercial sources without further purification. Fluorescence was recorded with a Fluostar Optima spectrometer (BMG labtech,

Offenburg, Germany). The MALDI-TOF analysis were carried out with an UltrafleXtreme from Bruker.

General procedure for the preparation of glycodendrons by CuAAC (2a-c)

Propargyl glycoside (4.4 eq.) and azide-functionalized scaffold **1** (1 eq.) were dissolved in 1 mL of a 1:1 mixture of DMF and PBS buffer (pH 7.5). A solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5 eq.) and THPTA (1 eq.) in PBS was added to a solution of sodium ascorbate (3 eq.) in PBS. This mixture was added to the solution containing the azide and alkyne which was degassed with argon and stirred at r.t. for 2 hours after which RP-HPLC showed completion of the reaction. Chelex[®] resin was then added to the reaction mixture which was stirred for 45 minutes. The resin was filtered off, rinsed with water and the filtrate was concentrated under reduced pressure. The crude product was used directly in the next step. The resulting tetravalent compound **2** (1 eq.) was dissolved in dry DMF (1 mL), DIPEA was added to reach pH ~ 9-10 (c.a. 20 μL) then *N*-hydroxy-succinimidyl ester of azidoacetic acid (1.5 eq.) was added. The reaction mixture was stirred at r.t. for 1 hour after which RP-HPLC showed completion of the reaction. The mixture was diluted with water (3 mL) and purified by semi-preparative RP-HPLC. Fractions containing the product were combined and lyophilized. Glycodendron **2a**: 82% yield; HRMS (ESI⁻) *m/z*: calc. for $\text{C}_{69}\text{H}_{109}\text{N}_{23}\text{O}_{32}$ $[\text{M}-2\text{H}]^{2-}$: 885.8810, found 885.8808; RP-HPLC: $R_t = 5.09$ min (C_{18} , $\lambda = 214$ nm, 0-30% B in 15 min). Glycodendron **2b**: 84% yield; HRMS (ESI⁺) *m/z*: calc. for $\text{C}_{69}\text{H}_{113}\text{N}_{23}\text{O}_{32}$ $[\text{M}+2\text{H}]^{2+}$: 887.8956, found 887.8971; RP-HPLC: $R_t = 6.65$ min (C_{18} , $\lambda = 214$ nm, 0-30% B in 15 min). Glycodendron **2c**: 74% yield; HRMS (ESI⁺) *m/z*: calc. for $\text{C}_{69}\text{H}_{113}\text{N}_{23}\text{O}_{28}$ $[\text{M}+2\text{H}]^{2+}$: 855.9058, found 855.9074; RP-HPLC: $R_t = 9.12$ min (C_{18} , $\lambda = 214$ nm, 0-30% B in 15 min).

Functionalization of BSA with an alkyne chain (3)

A solution of 3-(2-Propyn-1-yloxy)propanoic acid 2,5-dioxo-1-pyrrolidiny ester (2 mg, 60 eq.) was added in a solution of BSA (10 mg) in PBS and the reaction mixture was stirred for 5 h at room temperature. BSA-alkyne (**3**) was purified on a Sephadex G25 and its purity was controlled by SDS-PAGE (Fig. S14-15). 90% yield. The number of alkyne group per BSA was determined by MALDI-TOF (Fig. S10).

*Conjugation of BSA-alkyne **3** with glycodendrons **2a-c** by CuAAC*

TBTA (5 mg/mL), L-ascorbic acid (3 mg/mL), CuSO₄·5H₂O (3 mg/mL) and glycodendron **2a**, **2b** or **2c** (10 mg/mL, 25 eq. or 50 eq.) was added to a solution of BSA-alkyne **3** (2 mg/mL) in PBS. The solution was stirred 24 h at room temperature then the crude mixture was purified by size-exclusion chromatography on a Sephadex G25 gel. The purity of each neoglycoproteins was controlled by SDS-PAGE (Fig. S14-15) and the ratio of glycodendrimers by BSA was determined by MALDI-TOF (Fig. S11-13).

Biotinylated of NeoGPs

The labelling of NeoGPs was carried out by amide coupling with the EZ-link sulfo-NHS-LC-biotin (10 equivalents) in PBS at pH 7.2 for 30 min at room temperature. The excess of biotin reagent was removed by dialysis process. The number of biotin coupled on the neoglycoprotein is determined according to the standard HABA/avidine quantification method.

Direct binding assays

The assays were performed according GlycoDiag's protocol already described.^[21,22,23,24] For direct binding mode, each neoglycoproteins were preliminary labeled with biotin according to standard protocol already described.^[23] Briefly, the different compounds (range of concentrations) prepared in PBS supplemented with 1 mM CaCl₂ and 0.5 mM

MgCl₂ were deposited in each well of lectin (50 μL each) in triplicate and incubated for two hours at room temperature. After washing with PBS buffer, the streptavidin-DTAF conjugate was added (50 μL) and incubated for 30 min. The plate was then washed again with PBS. Finally, 100 μL of PBS were added for reading the plate using a fluorescence reader ($\lambda_{\text{ex}} = 485 \text{ nm}$, $\lambda_{\text{em}} = 530 \text{ nm}$). The intensity of the signal was directly correlated with the ability of the compound to be recognized by the lectin.

Inhibition assays

The interaction profiles of each compound were determined through an indirect method based on the inhibition by the compound of the interaction between a specific couple lectin-glycan (a neoglycoprotein labeled with biotin and used as a tracer). Briefly, a mix of tracer (fixed concentration) and the corresponding compounds (range of concentrations) prepared in PBS supplemented with 1 mM CaCl₂ and 0.5 mM MgCl₂ is deposited in each well (50 μL each) in triplicates and incubated two hours at room temperature. After washing with PBS buffer, the conjugate streptavidin-DTAF was added (50 μL) and incubated 30 min more. The plate was washed again with PBS. Finally, 100 μL of PBS was added for the readout of fluorescent plate performed with a fluorescence reader ($\lambda_{\text{ex}} = 485 \text{ nm}$, $\lambda_{\text{em}} = 530 \text{ nm}$). The signal intensity was inversely correlated with the capacity of the compound to be recognized by the lectin and expressed as inhibition percentage with comparison with the corresponding tracer alone.

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Keywords

bacterial lectin; click chemistry; glycodendrimer; multivalency; neoglycoprotein

References

- [1] O. Renaudet, R. Roy, *Chem. Soc. Rev.* **2013**, *42*, 4515–4517.
- [2] Y. Kim, J. Y. Hyun, I. Shin, *Chem. Soc. Rev.* **2021**, *50*, 10567–10593.
- [3] S. Cecioni, A. Imberty, S. Vidal, *Chem. Rev.* **2015**, *115*, 525–561.
- [4] E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2009**, *48*, 6974–6998.
- [5] K. Villadsen, M. C. Martos-Maldonado, K. J. Jensen, M. B. Thygesen, *ChemBioChem* **2017**, *18*, 574–612.
- [6] W. Tang, M. L. Becker, *Chem. Soc. Rev.* **2014**, *43*, 7013–7039.
- [7] A. Bernardi, J. Jiménez-Barbero, A. Casnati, C. De Castro, T. Darbre, F. Fieschi, J. Finne, H. Funken, K.-E. Jaeger, M. Lahmann, T. K. Lindhorst, M. Marradi, P. Messner, A. Molinaro, P. V. Murphy, C. Nativi, S. Oscarson, S. Penadés, F. Peri, R. J. Pieters, O. Renaudet, J.-L. Reymond, B. Richichi, J. Rojo, F. Sansone, C. Schäffer, W. B. Turnbull, T. Velasco-Torrijos, S. Vidal, S. Vincent, T. Wennekes, H. Zuilhof, A. Imberty, *Chem. Soc. Rev.* **2013**, *42*, 4709–4727.
- [8] L. Mousavifar, R. Roy, *Molecules* **2021**, *26*, 2428.
- [9] C. Pifferi, A. Ruiz-De-Angulo, D. Goyard, C. Tiertant, N. Sacristán, D. Barriales, N.

- Berthet, J. Anguita, O. Renaudet, A. Fernández-Tejada, *Chem. Sci.* **2020**, *11*, 4488–4498.
- [10] R. Sharma, K. Naresh, Y. M. Chabre, R. Rej, N. K. Saadeh, R. Roy, *Polym. Chem.* **2014**, *5*, 4321–4331.
- [11] B. Todaro, S. Achilli, B. Liet, E. Laigre, C. Tiertant, D. Goyard, N. Berthet, O. Renaudet, *Biomater. Sci.* **2021**, *9*, 4076–4085.
- [12] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
- [13] A. Imberty, A. Varrot, *Curr. Opin. Struct. Biol.* **2008**, *18*, 567–576.
- [14] A. Imberty, M. Wimmerová, E. P. Mitchell, N. Gilboa-Garber, *Microbes Infect.* **2004**, *6*, 221–228.
- [15] E. Mitchell, C. Houles, D. Sudakevitz, M. Wimmerova, C. Gautier, S. Pérez, A. M. Wu, N. Gilboa-Garber, A. Imberty, *Nat. Struct. Biol.* **2002**, *9*, 918–921.
- [16] J. L. Jiménez Blanco, C. Ortiz Mellet, J. M. García Fernández, *Chem. Soc. Rev.* **2013**, *42*, 4518–4531.
- [17] C. Müller, G. Despras, T. K. Lindhorst, *Chem. Soc. Rev.* **2016**, *45*, 3275–3302.
- [18] M. González-Cuesta, C. Ortiz Mellet, J. M. García Fernández, *Chem. Commun.* **2020**, *56*, 5207–5222.
- [19] M. I. García-Moreno, F. Ortega-Caballero, R. Rísquez-Cuadro, C. Ortiz Mellet, J. M. García Fernández, *Chem. Eur. J.* **2017**, *23*, 6295–6304.
- [20] R. S. Bagul, M. Hosseini, T. C. Shiao, N. K. Saadeh, R. Roy, *Polym. Chem.* **2017**, *8*, 5354–5366.
- [21] C. Ortiz Mellet, J.-F. Nierengarten, J. M. García Fernández, *J. Mater. Chem. B* **2017**, *5*, 6428–6436.
- [22] J. P. Ribeiro, S. Villringer, D. Goyard, L. Coche-Guerente, M. Höferlin, O. Renaudet, W. Römer, A. Imberty, *Chem. Sci.* **2018**, *9*, 7634–7641.

- [23] N. Berthet, B. Thomas, I. Bossu, E. Dufour, E. Gillon, J. Garcia, N. Spinelli, A. Imberty, P. Dumy, O. Renaudet, *Bioconjugate Chem.* **2013**, *24*, 1598–1611.
- [24] D. Goyard, B. Thomas, E. Gillon, A. Imberty, O. Renaudet, *Front. Chem.* **2019**, *7*, 666-.
- [25] E. Fernandez-Megia, J. Correa, I. Rodríguez-Meizoso, R. Riguera, *Macromolecules* **2006**, *39*, 2113–2120.
- [26] C. Assailly, C. Bridot, A. Saumonneau, P. Lottin, B. Roubinet, E. Krammer, F. François, F. Vena, L. Landemarre, D. Alvarez Dorta, D. Deniaud, C. Grandjean, C. Tellier, S. Pascual, V. Montembault, L. Fontaine, F. Daligault, J. Bouckaert, S. G. Gouin, *Chem. – A Eur. J.* **2021**, *27*, 3142–3150.
- [27] M. Cauwel, A. Sivignon, C. Bridot, M. C. Nongbe, D. Deniaud, B. Roubinet, L. Landemarre, F.-X. Felpin, J. Bouckaert, N. Barnich, S. G. Gouin, *Chem. Commun.* **2019**, *55*, 10158–10161.
- [28] Y. Brissonnet, C. Assailly, A. Saumonneau, J. Bouckaert, M. Maillason, C. Petitot, B. Roubinet, B. Didak, L. Landemarre, C. Bridot, R. Blossey, D. Deniaud, X. Yan, J. Bernard, C. Tellier, C. Grandjean, F. Daligault, S. G. Gouin, *Chem. Eur. J.* **2019**, *25*, 2358–2365.
- [29] G. Michaud, R. Visini, M. Bergmann, G. Salerno, R. Bosco, E. Gillon, B. Richichi, C. Nativi, A. Imberty, A. Stocker, T. Darbre, J.-L. Reymond, *Chem. Sci.* **2016**, *7*, 166–182.
- [30] S. P. Vincent, K. Buffet, I. Nierengarten, A. Imberty, J.-F. Nierengarten, *Chem. Eur. J.* **2016**, *22*, 88–92.
- [31] L. Landemarre, E. Duverger, **2013**, pp. 221–226.