A EUROPEAN JOURNAL OF CHEMICAL BIOLOGY

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**To be cited as:** *ChemBioChem* 10.1002/cbic.201700154

**Link to VoR:** http://dx.doi.org/10.1002/cbic.201700154

A Journal of

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# Design and synthesis of galactosylated bifurcated ligands with nanomolar affinity for lectin LecA from *Pseudomonas aeruginosa*

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**Abstract:** Lectin LecA of Pseudomonas aeruginosa is established as a virulent factor. Glycoclusters targeting LecA able to compete with human glycoconjugates present on epithelial cells are promising candidates to treat P. aeruginosa infection. A family of 32 glycodendrimers of generation 0 and 1 displaying bifurcated bis-galactosides has been designed to interact with LecA. The influence of both the central multivalent core and the aglycon of these glycodendrimers on their affinity toward LecA has been evaluated using a microarray technology both qualitatively for a rapid screening of the binding properties but also quantitatively (Kd) leading to high affinity LecA ligands with Kd values in the low nanomolar range (Kd = 22 nM for the best one).

## Introduction

*Pseudomonas aeruginosa* (PA) is an opportunistic Gram-negative bacterium involved in nosocomial diseases. It strongly affects immunocompromised people and especially cystic fibrosis patients as it is often related to pulmonary infections.[1] PA has two soluble lectins LecA (or PA-IL) and LecB (or PA-IIL) that recognize D-galactose and L-fucose respectively. Both lectins are involved in biofilm formation.[2-6] LecA (PDB code 1OKO) has a cobblestone shape with the four carbohydrate recognition domains (CRDs) located at the corner of the rectangle (32 and 70 Å away on the width and length, respectively).[6, 7] Several studies report that galactoclusters can reach simultaneously two CRDs along the width of one tetramer leading to chelation.[8] LecB (PDB code 2VUD) has more or less a tetrahedral spatial distribution of the CRDs that lead to a more difficult chelation.[9] Carbohydrate-lectin interactions usually occur with low affinities with dissociation constants between mM to μM. To increase the binding, Nature uses multivalency leading to a cluster effect.[10] Along this line, many multivalent glycoclusters have been synthesized to improve the avidity of carbohydrate ligands to lectins[11-20] and especially against PA.[8] However, a high number of carbohydrates is not a guarantee for a higher affinity due to some steric hindrance. Furthermore, it has been shown that the topology of the glycocluster is often more important than the number of carbohydrates to gain a high affinity for LecA or LecB. Hence glycoclusters targeting LecA or LecB exhibiting only 3 carbohydrates can reach the nanomolar range avidity.[21] Furthermore, the simplest the multivalent ligand, the best for therapeutic applications. There are a few examples in the literature of bis-galactosides that display high affinity for LecA. Three bis-galactosides exhibiting a phenyl aglycon display $K_d$ values between 82 and 550 nM corresponding to an increase of potency per residue between 91 and 159 fold (Figure 1).[22-24] The most remarkable bis-galactosides with an increase of potency per residue between 272 to 3778 fold were those with a rigid glucose-triazole backbone reported by Pieters with a $K_d$ value of 28 nM (Figure 1).[25-28] The docking studies suggested that such bis-glycosides could chelate simultaneously two adjacent CRDs.

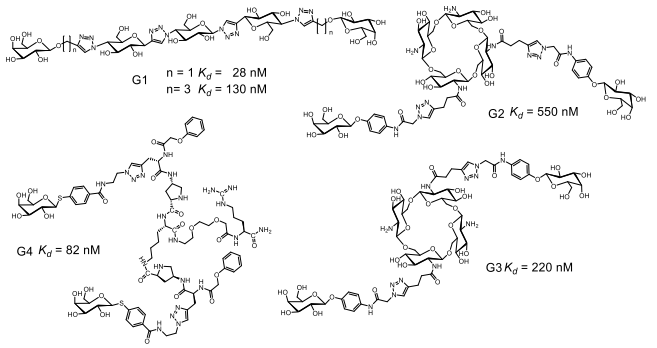


**Figure 1**. Schematic representation of some bis-galactosides against LecA with their $K_d$ values (Compounds G1,[25, 26] G2,[23], G3[23] and G4[24]).

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## Results and Discussion

We report herein the design, synthesis and binding studies toward LecA of bis-galactosylated glycodendrimers of generation 0 and 1 built on two different core scaffolds, one with a 1,3-propanediol aliphatic core (Figure 2**A**) and the other with a 1,3-dihydroxybenzene aromatic moiety (Figure 2**B**). Both structures have the same distance in terms of number of atoms between the two functional groups used for conjugation with galactosides. Since it has been shown that aromatic galactosides exhibit higher affinity for LecA,[2, 22, 29-32] we introduced seven different aromatic β-D-galactosides and an additional triethyleneglycol β-D-galactoside for comparison. These bifurcated bis-galactosides have been designed as a clamp to chelate two adjacent CRDs of LecA in line with the finding of Pieters [25, 26] (Figure 2).

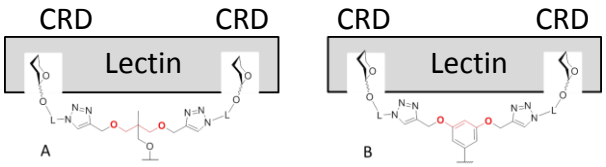



**Figure 2**. General structure of bis-galactosides with an aliphatic (A) or aromatic (B) core. The grey rectangle represents a part of the tetrameric lectin LecA with two CRDs.

The synthesis of the glycodendrimers was performed by copper catalyzed azide alkyne cycloaddition (CuAAC)[33, 34] using azido-functionalized glycosides with different aglycons and bis-propargylated tris(hydroxymethyl)ethane or 3,5-dihydroxybenzoic acid. In order to rapidly screen the affinity of these structures toward LecA, they were conjugated to an oligonucleotide for their subsequent immobilization on a DNA chip as previously reported.[35, 36] Both bis-propargylated scaffolds were prepared as phosphoramidite intermediates **1** and **2** (Figure 3) for their coupling with the oligonucleotide by phosphoramidite chemistry[37] on solid support. Finally the glycosides were introduced in solution by CuAAC using the corresponding azido-functionalized glycosides. The synthesis of **1** was previously reported[38] while phosphoramidite **2** was prepared from methyl 3,5-dihydroxybenzoate **3** that was propargylated to give **4**, then hydrolyzed with LiOH to reach the benzoic acid **5**.[39] Amidation with 6-amino-hexanol afforded **6** that was finally converted into **2** by reaction of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (Scheme 1).

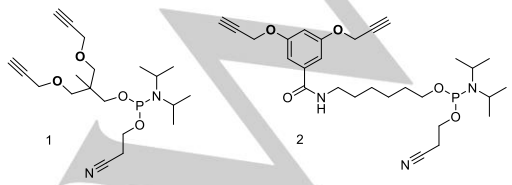
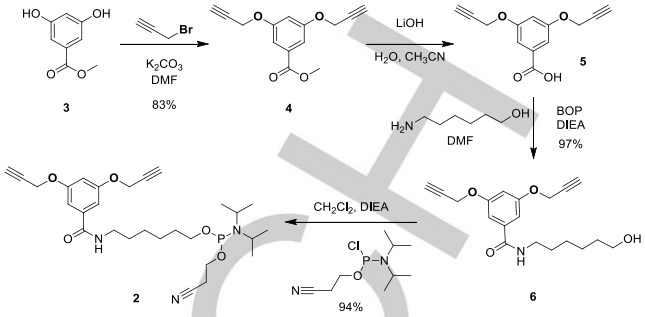



**Figure 3**. Structure of bis-propargyl phosphoramidites **1** and **2**.




**Scheme 1**. Synthesis of bis-propargyl phosphoramidite **2**.

The bis-galactosides were synthesized from eight galactosides exhibiting different aglycons (Figure 4). Amongst the galactosides, two were chosen to provide glycoclusters with low to medium affinities (**7a** and **7b**) based on our previously reported results,[32] four (**7c-f**) should display high affinities[30, 32] and two aglycons are new (**7g** and **7h**) as analogues of aromatic aglycons **7d** and **7e**.

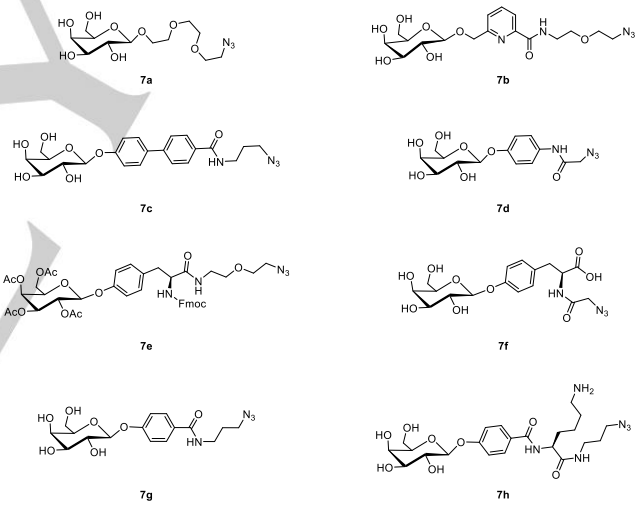
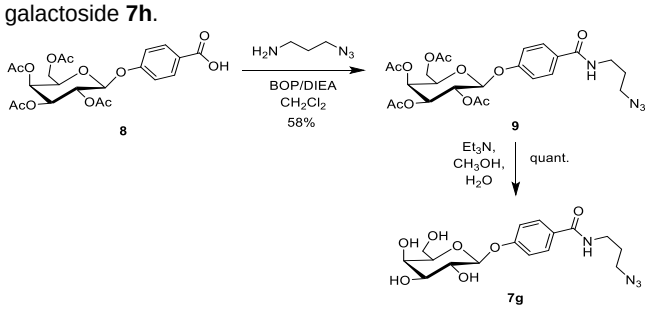


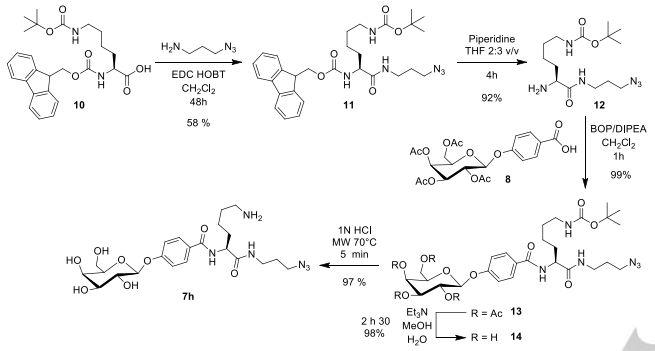

**Figure 4**. Structures of azido-functionalized β-D-galactosides used for the synthesis of bis-galactosides.

The synthesis of galactoside **7g** was performed from 4-carboxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **8**[22] that was conjugated to 3-azidopropylamine affording the fully protected derivative **9** (Scheme 2). Acetyl groups were removed by a solution of methanol, triethylamine, water affording pure **7g** after evaporation. Galactoside **7h** was prepared in few steps as described in Scheme 3. First, 3-azidopropylamine was conjugated to Fmoc-Lys(Boc)-OH affording **11**. The Fmoc group was removed leading to amine **12** and then condensation with acid **8** afforded the fully protected derivative **13**. Acetyl groups were removed by a solution of methanol, triethylamine and water then the Boc group was removed by treatment with a 1N HCl

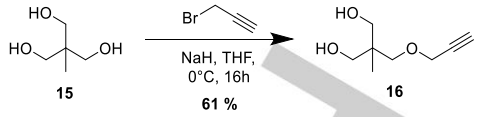
under microwaves assistance affording the fully deprotected galactoside **7h**.




**Scheme 2**. Synthesis of the phenylcarboxamido-propylazide galactoside **7g**.



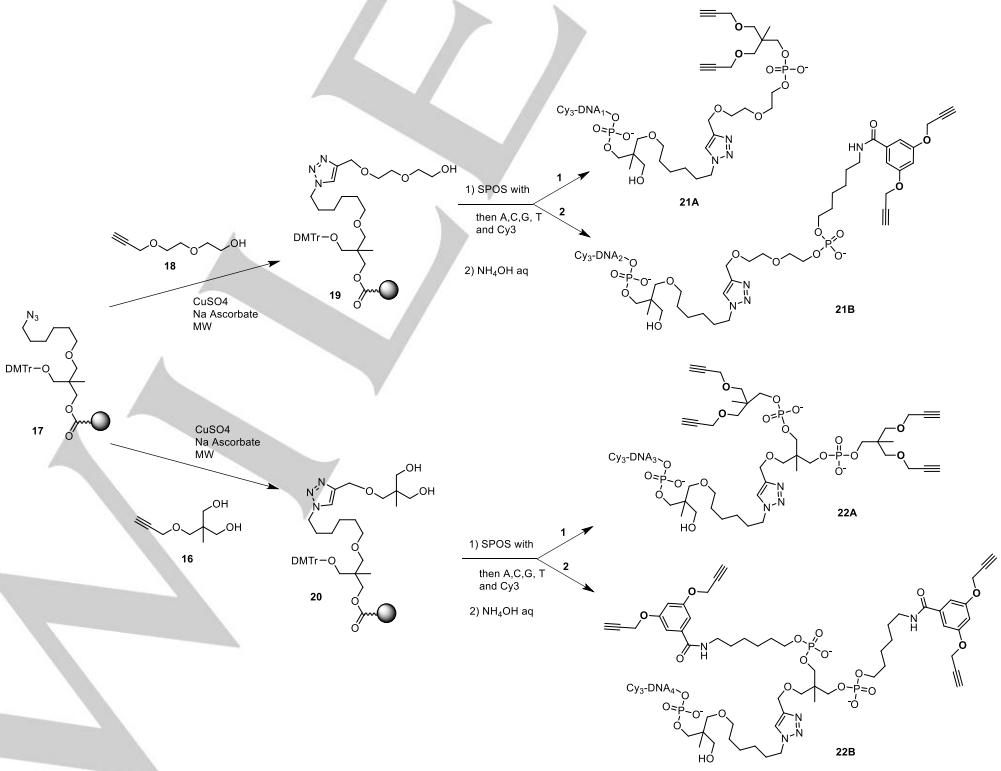

**Scheme 3**. Synthesis of the phenylcarboxamido-lysine-propylazide galactoside **7h**.



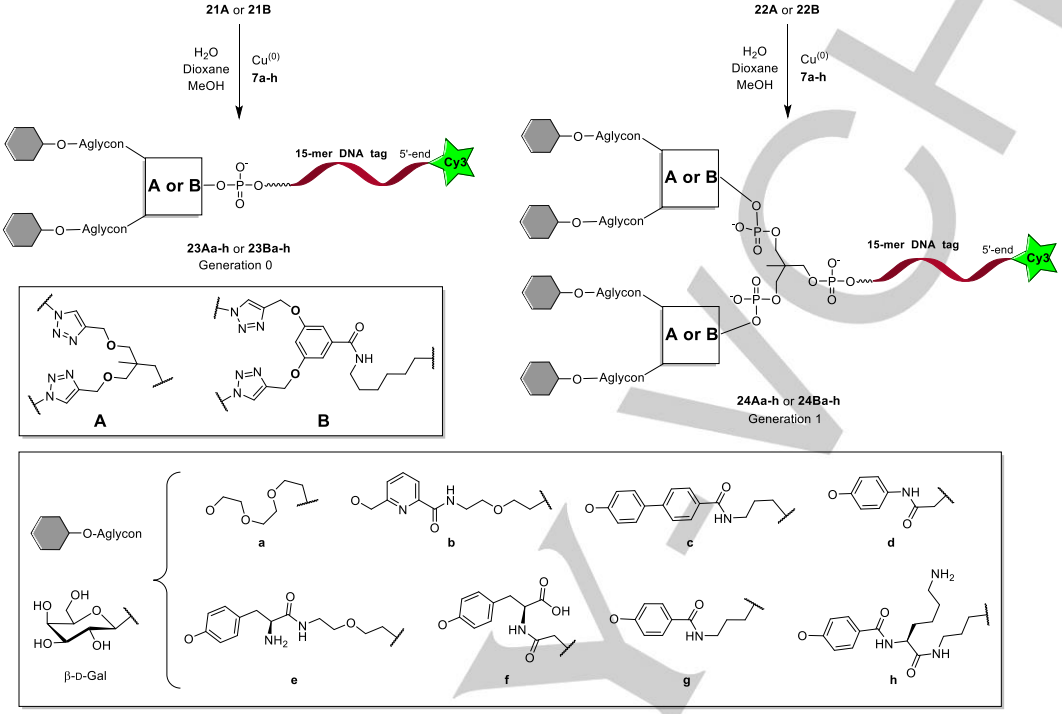

**Scheme 4**. Synthesis of propargyl tris(hydroxymethyl)ethane **16**

The glycodendrimers were designed using a propargyl diethyleneglycol **18** for the generation 0 series and the propargylated diol **16** for the generation 1. To this end the tris(hydroxymethyl)ethane **15** was monoalkylated with propargyl bromide affording the propargylated diol **16** (Scheme 4).

The synthesis of the oligonucleotide glycodendrimers started with the immobilization of propargyl diethyleneglycol 1**8** or propargyl tris(hydroxymethyl)ethane **16** on the azido-functionalized solid support **17** by means of CuAAC with $CuSO_4$ and sodium ascorbate under microwaves assistance[40] leading to **19** and **20** respectively (Scheme 5). Then, the phosphoramidites **1** or **2** were coupled on the free hydroxyls and the oligonucleotides ($DNA_{1-4}$) were elongated and labeled with a Cy3 fluorescent dye, using standard phosphoramidite cycle (solid phase oligonucleotide synthesis SPOS).[37] The cycle consists to remove the DMTr group, then couple the nucleoside phosphoramidite, cap the unreacted species and finally oxidize the phosphite linkage into phosphate one. A final ammonia treatment afforded the Cy3-oligonucleotides conjugated with one (**21A/B**) or two (**22A/B**) A or B motifs. Each construction was tagged with a different oligonucleotide sequence in order to performed multiplexed analysis of their binding with LecA. [41]



**Scheme 5.** Solid phase synthesis of Cy3-oligonucleotide conjugated with generation 0 (**21A, 21B**) or generation 1 (**22A, 22B**) dendrimer precursors. SPOS Solid Phase Oligonucleotides Synthesis: a) 3% $Cl_3CCO_2H$ in $CH_2Cl_2$; b) amidite, benzylthiotetrazole, $CH_3CN$; c) $Ac_2O$, *N*-methyl-imidazole, THF, pyridine; d) $I_2$, $H_2O$, THF, pyridine.




**Scheme 6.** Synthesis of glycodendrimers conjugated with Cy3-oligonucleotides.

After purification by $C_{18}$ reverse phase HPLC, the four scaffolds were conjugated by CuAAC in solution with the eight azido-functionalized β-D-galactosides **7a-h** (Figure 4). Thus, 32 oligonucleotide glycodendrimers exhibiting one (**23A/Ba-h**, generation 0) or two (**24A/Ba-h**, generation 1) A or B motifs were obtained (Scheme 6). Each construction was purified by HPLC and characterized by MALDI-TOF MS (Table S1).

The oligonucleotide glycodendrimers **23A/Ba-h** and **24A/Ba-h** labeled with the Cy3 dye were immobilized on the microarray by DNA Directed Immobilization (DDI). A tris-mannosylated glycocluster ($Man_3$) as a negative control, a mono-phenyl (Ph-Gal) galactoside and a tetra-phenyl galactoside [$(Ph\text{-}Gal)_4$] previously described[31] as positive controls were also immobilized (Figure 5). Their correct immobilization was readily verified by the Cy3 fluorescence signal which deviated by less than 11%. It has been demonstrated that immobilization of glycoconjugates by DDI leads to surface densities of $10^{11}$-$10^{12}$ molecules per $cm^2$ with a distance between the immobilized molecules over 30 nm.[42] Under such conditions, each lectin molecule interacts with only one glycoconjugate. Therefore, no surface cluster effect is expected. Alexa 647 labeled LecA was incubated on the resulting carbohydrate microarray and the Alexa fluorescence signal was recorded averaged over 32 spots to determine the binding of LecA to the immobilized glycodendrimers.

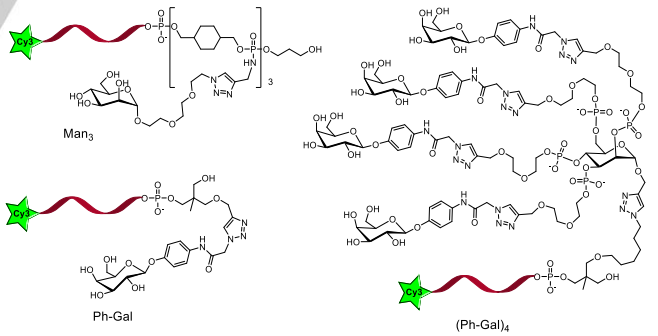



**Figure 5.** Structure of negative $(Man)_3$ and positive Ph-Gal and $(Ph\text{-}Gal)_4$ controls.

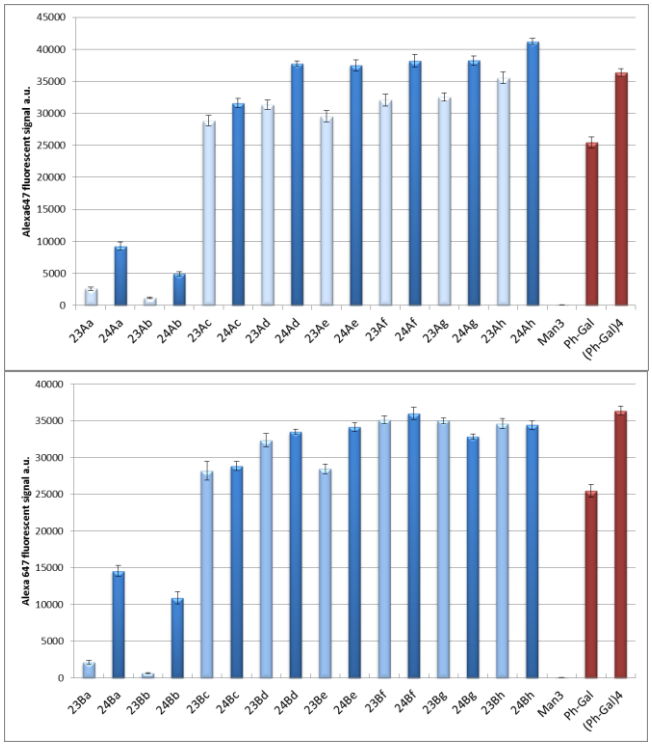



**Figure 6.** Screening of the binding properties towards LecA using the carbohydrate microarray through the Alexa 647 fluorescence signal of labeled LecA (arbitrary units a.u.). "Upper panel: **23-24Aa-h**; Lower panel: **23-24Ba-h**. Divalent compounds: light blue; Tetravalent compounds: blue. Controls: red". Lectin concentration of 0.12 µM.

The fluorescence signal of negative control $Man_3$ in the background (60 a.u.) confirmed that no unspecific adsorption of LecA occurred. For both motifs, excepted in the case of the methylenepyridyl aglycon (**b**), all aromatic aglycons lead to an increased affinity compared to the triethyleneglycol ($EG_3$) linker (**a**). This increased binding has been reported several times in the literature [13, 22, 29, 30, 32] and is the result of a face-to-edge or edge-to-edge $\pi$–$\pi$ stacking between the aromatic ring and the $His_{50}$ of the lectin. The fluorescence signal observed after binding of LecA to bis-galactosides with a $EG_3$ aglycon were low (**23Aa** 2569 a.u., **23Ba** 1672 a.u.), more surprisingly the fluorescent signals for bis-galactosides with a methylenepyridyl aglycon (**23Ab** and **23Bb**) were lower (<1000 a.u.). Increasing the valency from two (**23Ab** and **23Bb**) to four (**24Ab** and **24Bb**), improved significantly the binding by a factor of nearly 4 and 20, respectively. Interestingly, the lower the binding of the divalent clusters was, the higher was the gain due to increased valency from 2 to 4. The same trend was observed for glycocluster with $EG_3$ aglycon (**23Aa** vs **24Aa** and **23Ba** vs **24Ba**). These mono- and bis-digalactosides exhibited lower fluorescence signal than the mono-phenyl galactoside (Ph-Gal) (25 000 a.u.). This result confirmed the high affinity for LecA of glycodendrimers built with aromatic galactoside. All the other bis-galactosides with A and B motifs exhibited a high fluorescence signal above 28 000 a.u. showing a high affinity for LecA. However, aglycons biPh (**23A/Bc**) and $TyrNH_2$ (**23A/Be**) were slightly poorer (~ 28 500-29 000 a.u.) than the four other aglycons (> 30 000 a.u.). In general, a slightly higher fluorescence signal could be observed for the bis-galactosides built with a B motif.

The increase of the valency, from 2 to 4, is more effective for the binding to LecA for bis-galactosides with A than B motifs. For A motif, generation 1 tetravalent glycodendrimers **24** displayed a higher signal of fluorescence than the generation 0 divalent glycodendrimers **23**. For B motif, the change of fluorescence intensity was mostly negligible except for low affinity aglycons **a** and **b**, and also to a lower extent for the $TyrNH_2$ aglycon (**24Be**). As a result, glycodendrimers **24Ad-h** displayed higher binding to LecA than the control $(Ph\text{-}Gal)_4$ while all glycodendrimers with a B motif displayed lower fluorescence signals than the control, excepted **24Bf** ($TyrCO_2H$ aglycon).[32] We have recently showed that this $TyrCO_2H$ aglycon led to a mannose-centered tetragalactocluster of high affinity ($K_d$ = 19 nM).[32]. This difference of affinity could arise from the general structure and hence topology of the present glycodendrimers. For motif A, the distance between two triazole rings of two bis-galactosides is 19 atoms while for motif B it is 35 atoms due to the presence of the two hexylamide linkers. Hence glycodendrimers **24A** could be envisaged as tetraglycoclusters while glycodendrimers **24B** as two bifurcated bis-galactosides. The binding properties are mostly influenced by the aglycon while valency appears as a secondary parameter. The tetraphenyl galactoside on a mannose core $(Ph\text{-}Gal)_4$ displayed a fluorescence signal (36 400 a.u.) similar to that of the best digalactoside **23Ah** with LysPh aglycon. This data shows that the topology and the nature of the aglycon are two important parameters to consider for reaching high affinity.

In order to have a better insight of the influence of each aglycon and of the topology on the affinity, the values of dissociation constant ($K_d$) for the 12 glycodendrimers (**23A/Bc-h**) exhibiting high fluorescence signal (>28 000 a.u.) were measured and compared with the $K_d$ value of $(Ph\text{-}Gal)_4$ (Table 1).

**Table 1.** $K_d$ values of the best bis-galactosides.

| Glycodendrimer | | $K_d$ (nM) | Rank |
|---|---|---|---|
| **Valency 2** | **23Ac** | 62 ± 2 | 10 |
| | **23Ad** | 38 ± 1.5 | 5eq |
| | **23Ae** | 68 ± 1 | 11 |
| | **23Af** | 35 ± 1 | 4 |
| | **23Ag** | 38 ± 2.5 | 5eq |
| | **23Ah** | 32 ± 6 | 2 |
| | | | |
| **Valency 2** | **23Bc** | 75 ± 3.5 | 12eq |
| | **23Bd** | 56 ± 1.5 | 9 |
| | **23Be** | 75 ± 11.5 | 12eq |
| | **23Bf** | 40 ± 4.5 | 8 |
| | **23Bg** | 33 ± 1.5 | 3 |
| | **23Bh** | 22 ± 2 | 1 |
| | | | |
| **Valency 4** | **$(Ph\text{-}Gal)_4$** | 39[43] | 7 |

Dissociation constants ($K_d$) were measured using our previously reported protocol.[44] It is based on incubation of DDI immobilized glycoclusters with increasing concentrations of lectin. The resulting isotherms are linearized and the $K_d$ values calculated from the y-intercepts.[45] All measured $K_d$ values are ranked between 22 and and 75 nM. This data confirmed the high affinity of these glycodendrimers for LecA. $K_d$ values are in agreement with the fluorescence signals confirming that biPh (**c**) and $TyrNH_2$ (**e**) aglycons displayed lower affinities for LecA than the others (rank 10-12).

The bis-galactosides (**23A/Bh**) with the Lys-Ph aglycon displayed the lowest $K_d$ values regardless from the motif (A 32 nM or B 22 nM, rank 1-2). Glycoclusters built with Lys-Ph-galactoside have been reported previously.[22] In such a case, the increased binding was attributed to the interaction of the ammonium function from the lysine lateral chain with the $Asp_{47}$ residue of LecA. This improved binding was not observed for bis-galactosides **23Ag** and **23Bg** with $TyrNH_2$ aglycons ($K_d$ = 68 and 75 nM, respectively, rank 11-12). Therefore, for these glycoclusters, the amine function of the tyrosine did not seem to engage any additional interaction with the lectin. The amine is probably located too far from the $Asp_{47}$ residue. However, the bis-galactosides **23A/Bf** with $TyrCO_2H$ aglycon displayed a high affinity with $K_d$ values (**23Af** 35 nM and **23Bf** 40 nM; rank 4 and 8) that are nearly twice lower than the bis-galactosides $TyrNH_2$. Finally, bis-galactosides with phenyl aglycons (**d** and **g**) have similar $K_d$ values (**23Ad**,**g** and **23Bg** 33-38 nM, rank 3-5), excepted for **23Bd** (56 nM, rank 9) suggesting that inverting the amide function with respect to the aromatic ring and addition of two methylenes have a limited effect on the binding to LecA.

The two glycoclusters **23A/Bh** exhibiting the best affinities were further investigated by using molecular simulations and docking techniques. An empirical calculation of the potential energy of interaction $\Delta E$ of both bis-galactosides **23A/Bh** gave -216 and -260 kcal/mol respectively that is consistent with the $K_d$ values. For all galactoside, we observed the characteristic coordination of the C3 and C4 hydroxyls to the lectin-bound calcium ion and C6 hydroxyls of the galactose form a hydrogen bond with each $His_{50}$ residue of adjacent LecA monomer.[5, 22, 46, 47] For **23Ah**, one ammonium group of a lysine chain interacts through a hydrogen bond to Gln40 (monomer A) while the ammonium group of the other lysine chain gives a strong ionic interaction with Glu49 (monomer B). Both phenyl rings of the bis-galactoside form a σ-π interaction with both Pro38s (Figure 7).

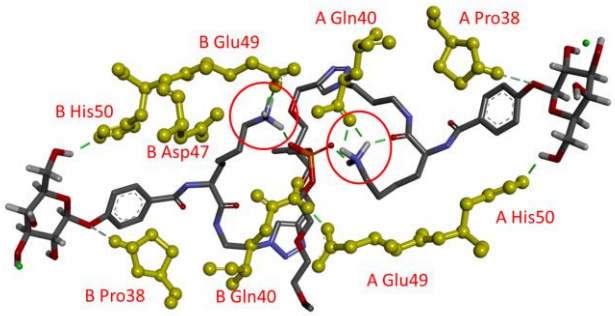



**Figure 7.** Bis-galactoside **23Ah** interacting with LecA. Red circles show the ammonium of the lysine chains. Sticks represent **23Ah** and ball and sticks in yellow represent some amino acids of monomers A and B of LecA.

For compound **23Bh**, one ammonium group of a lysine chain exhibits two strong ionic interactions with Asp47 and Glu49 of monomer B while the ammonium group of the other lysine chain interacts through a hydrogen bond donor to Gln40 with the monomer A (Figure 8). The phenyl ring in the monomer B has two π-π interactions with His50 and Tyr36 while the other phenyl ring has a σ-π interaction with Pro38 (monomer A). This double strong ionic interaction could explain why this bis-galactoside displayed the lowest $\Delta E$ and $K_d$ values.

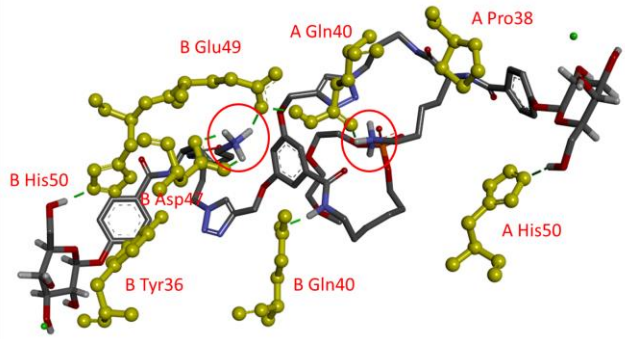



**Figure 8.** Bis-galactoside **23Bh** interacting with LecA. Red circles show the ammonium of the lysine chains. Sticks represent **23Bh** and ball and sticks in yellow represent some amino acids of monomers A and B of LecA.

## Conclusions

In the present contribution, 32 glycodendrimers exhibiting eight different aglycons were synthesized on two different central motifs (A corresponding to an aliphatic linker and B to an aromatic linker) and tagged with a Cy3-fluorescent oligonucleotide sequence. Thanks to their immobilization on a DNA microarray, their binding to LecA from *Pseudomonas aeruginosa* has been rapidly, qualitatively and quantitatively evaluated. The results showed that the effect of the central core on the affinity of the glycoclusters was limited. In contrast, the nature of the aglycon led to strong difference of affinity. The

galactoclusters with triethyleneglycol or methylenepyridyl aglycons bound poorly to LecA. In contrast, those with phenyl, biphenyl, naphthyl and phenyl-lysine aglycons showed high affinity with $K_d$ values between 22 to 75 nM. While the increase of valency from two to four has a noticeable beneficial effect on the binding of glycoclusters with A motif, it was mostly negligible for those with B motif. Our results are in good agreement with the literature and suggest that the primary parameter for enhanced binding is the structure of the aglycon. In particular, as already reported by several groups including ourselves, the aromatic aglycon is a major contributor to the binding through π–π and s-p interactions between the phenyl of the aglycon and the amino acids of lectin (His50, Tyr36and Pro38). In the present work, we showed that the introduction of an additional lysine (ammonium chain) allows additional ionic and hydrogen bound interactions with the amino acids of LecA leading to bis-galactosides **23A/Bh** with $K_d$ values of 32 and 22 nM respectively. Indeed, as investigated by molecular simulations and docking studies, additional electrostatic interactions further reinforced the affinity through strong ionic interactions between Asp47 and Glu49 and the ammonium of the lysine lateral chain and additional hydrogen bonds with Gln40.
The synthesis of both bis-galactosides **23A/Bh** without the DNA tag is in progress for subsequent evaluation of their anti-infectious activity against *Pseudomonas aeruginosa*.

## Experimental Section

General methods

All reagents for synthesis were commercial and used without further purification. Solvents were commercial and used without further purification. All moisture sensitive reactions were performed under an argon atmosphere. NMR solvents were purchased from Euriso-Top. NMR spectra were recorded at 293 K using a 200, 300, 400 or a 600 MHz spectrometer (Bruker). Shifts (δ) are referenced relative to deuterated solvent residual peaks and expressed in parts per million (ppm). Coupling constants are expressed in hertz (Hz). The following abbreviations are used to explain the observed multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), sbr (broad singlet). High-resolution (HR-ESI-QToF) mass spectra were recorded using a Q-Tof Micromass spectrometer. MALDI-TOF mass spectra were recorded on a Voyager mass spectrometer (Perspective Biosystems, Framingham, MA) equipped with a nitrogen laser. Thin layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F254 (Merck). TLC plates were inspected by UV light (□ = 254 nm) and developed by treatment with a mixture of 10% $H_2SO_4$ in EtOH/$H_2O$ (1:1 v/v), vanillin, $KMnO_4$ or phosphomolybdic acid followed by heating. Silica gel column chromatography was performed with silica gel Si 60 (40–63 mm). Reverse phase chromatography was performed with a $C_{18}$ reversed phase flash column.

Compounds **5**,[39] **7a**,[48] **7b**,[32] **7c**,[30] **7d**,[29] **7e**,[32] **7f**,[32] **7i**,[49] **8**,[22] were prepared according to the literature.

***N*-(6-Hydroxyhexyl)-3,5-bis(prop-2-ynoxy)benzamide 6:** 3,5-bis-(prop-2-ynoxy)benzoic acid **5**[39] (810 mg, 3.5 mmol) and 6-amino hexanol (450 mg, 3.85 mmol) were dissolved in anhydrous $CH_2Cl_2$ (10 mL) with dry DIEA (1.14 mL, 7.0 mmol) with 4Å molecular sieves under argon. After 30 min, benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (2.07 g, 4.2 mmol) was added and the mixture was stirred during 90 min. The solvent was removed *in vacuo*, and the crude product was dissolved in EtOAc. The organic layer was washed with satd $NaHCO_3$ (2 x 20 mL) and brine (2 x 20mL). The organic layer was dried ($Na_2SO_4$), and concentrated *in vacuo*. The crude product was purified on flash chromatography (20% EtOAc in cyclohexane) to afford the **6** (1.13 g, 97%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 2.3 Hz, 2H, Ar), 6.79 (t, *J* = 2.3 Hz, 1H, Ar), 4.77 (d, *J* = 2.4 Hz, 4H, $CH_2$CC), 3.55 (t, *J* = 6.6 Hz, 2H, $CH_2$OH), 3.36 (t, *J* = 7.2 Hz, 2H, $NHCH_2$), 2.97 (t, *J* = 2.4 Hz, 2H, CCH), 1.68-1.52 (m, 4H, $NHCH_2CH_2$ and $CH_2CH_2OH$), 1.47-1.39 (m, 4H, $EtCH_2CH_2EtOH$). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.5, 160.2, 137.9, 108.0, 106.2, 79.4, 77.1, 62.9, 57.0, 41.0, 33.6, 30.4, 27.9, 26.6. HR-ESI-QToF MS (positive mode): *m/z* calcd. for $C_{13}H_{11}O_4$ $[M+H]^+$ 330.1705, found 330.1705.

**6-(3,5-bis(Prop-2-ynoxy)benzamido)hexyl)-(2-cyanoethyl)-*N*-diisopropyl phosphoramidite 2:** *N*-(6-Hydroxyhexyl)-3,5-bis(prop-2-ynoxy)benzamide **6** (543 mg, 1.65 mmol) was added to a solution of DIEA (645 µL, 4.0 mmol), $CH_2Cl_2$ (5 mL) and acetonitrile (5 mL). The mixture was stirred for 30 min on 4Å molecular sieves under argon, then 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (239 mg, 2.0 mmol) was added and the mixture was stirred for 1 h. After addition of water (1 mL) and 15 min stirring, cyclohexane (20 mL) was added and the solvents weres removed *in vacuo*. The crude residue was purified on silica gel chromatography ($NEt_3$ 10% and EtOAc 40% in cyclohexane) leading to 2 (823 mg, 94%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.06 (t, *J* = 5.0 Hz, 1H, NH), 7.01 (d, *J* = 2.3 Hz, 2H, Ar), 6.71 (t, *J* = 2.3 Hz, 1H, Ar), 4.76 (d, *J* = 2.4 Hz, 4H, $CH_2$CC), 3.81-3.53 (m, 6H, 2x$CH_2$OP, N$CHMe_3$), 3.31 (q, *J* = 7.0 Hz, 2H, $NCH_2$), 2.84 (t, *J* = 2.4 Hz, 2H, CCH), 2.63 (t, *J* = 6.0 Hz, 2H, $CH_2CN$), 1.62-1.53 (m, 4H, $NHCH_2CH_2$ and $CH_2CH_2OH$), 1.43-1.35 (m, 4H, $EtCH_2CH_2EtOH$), 1.16 (m, 12H, 4x$CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 166.9, 159.5, 138.4, 118.3, 107.5, 105.7, 79.3, 77.1, 64.2 (d), 59.2 (d), 56.8, 43.7 (d), 40.5, 31.8 (d), 30.1, 27.3, 26.4, 24.9 (d), 21.1 (d). $^{31}$P NMR (162 MHz, $CDCl_3$) δ 147.0 (s, 1P). HR-ESI-QToF MS (positive mode): *m/z* calcd. for $C_{28}H_{41}N_3O_5P$ $[M+H]^+$ 530.2784, found 530.2784.

***N*-(3-Azidopropyl)-4-carboxamidophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 9:** To a solution of 4-carboxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **8**[22] (224 mg, 0.48 mmol) in dichloromethane (2 mL), 3-azidopropylamine (84 mg, 0.72 mmol) and DIEA (157 µL, 0.96 mmol) were added with a few beads of molecular sieves (4 Å). After 1 h stirring under argon, BOP (283 mg, 0.62 mmol) was added and the solution was stirred for 1 h at rt. After evaporation, the mixture was purified on silica gel (15 g) using cyclohexane/EtOAc (7:3 to 3:7 v/v) affording **9** (153.3 mg, 58%). $R_f$ = 0.43 (cyclohexane/EtOAc, 2:8, v/v). $[\alpha]_D$ = 0.0 (c = 1.0, MeOH). $^1$H NMR (400 MHz, $CD_3CN$) δ 7.82-7.64 (m, 2H, Ar), 7.12-6.99 (m, 2H, Ar), 5.40 (dd, *J* = 3.5, 1.1 Hz, 1H, H4), 5.32-5.27 (m, 2H, H1, H2), 5.24-5.12 (m, 1H, H3), 4.27-4.19 (m, 1H, H5), 4.15 and 4.09 (2dd, *J* = 11.3 and 5.4 Hz, 2H, H6), 3.41 and 3.39 (2t, *J* = 6.6 Hz, 4H, $CH_2CH_2CH_2N_3$), 2.11 (s, 1H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.93 (s, 3H, $COCH_3$), 1.80 (p, *J* = 6.6 Hz, 2H, $CH_2CH_2N_3$). $^{13}$C NMR (100 MHz, $CD_3CN$) δ 170.9, 170.8, 170. 5, 170.1, 166.9, 159.6, 130.1, 129.5, 116.7, 98.9, 71.8, 71.1, 69.1, 67.9, 62.0, 49.5, 37.4, 29.1, 20.5, 20.48, 20.4, 20.39. HR-ESI-QToF MS (positive mode): *m/z* calcd. for $C_{24}H_{31}N_4O_{11}$ $[M + H]^+$ 551.1989, found 551.1993.

***N*-(3-azidopropyl)-4-carboxamidophenyl β-D-galactopyranoside 7g**: Compound **9** was dissolved in a solution of MeOH/$H_2O$/$Et_3N$ (4:1:1 v/v/v) and stirred 3 h at rt. After evaporation, galactoside **7g** was obtained. HR-ESI-QToF MS (positive mode): *m/z* calcd. for $C_{16}H_{23}N_4O_7$ $[M+H]^+$ 383.1567, found 383.1565.

**FmocLys(Boc) (3-azidopropyl)amide 11**: To a solution of Fmoc-Lys(Boc)-OH (1.0 g, 2.13 mmol) in $CH_2Cl_2$ (20 mL), 3-azidopropylamine (5.5 mmol) followed by 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide EDC (1.2 g, 6.3 mmol) and HOBt (850 mg, 6.29 mmol) were added. The mixture was stirred at room temperature for 2 days. $CH_2Cl_2$ was added and the organic layer was washed with a 1M HCl (10 mL), a white precipitate was formed and removed by filtration. The organic layer was washed with water, dried ($Na_2SO_4$) and evaporated under vacuum. The residue was purified by silica gel flash chromatography ($CH_2Cl_2$/MeOH 95:5) to afford compound **11** (753 mg, 58%) as an amorphous white solid. $R_f$ = 0.33 ($CH_2Cl_2$/MeOH 95:5). $^1$H NMR (400 MHz, $CDCl_3$) δ ppm: 7.73 (d, *J* = 7.5 Hz, 2H, CHAr), 7.55 (d, *J* = 7.4 Hz, 2H, CHAr), 7.37 (t, *J* = 7.5 Hz, 2H, CHAr), 7.27 (t, *J* = 7.4 Hz, 2H, CHAr), 6.83 (bs, 1H, NH amide), 5.86 (bd, *J* = 5.6 Hz, 1H, NH urethane), 4.78 (s, 1H, NH urethane), 4.46-4.24 (m, 2H, C$H_2$Fmoc), 4.16 (m, 2H, αCHLys/CHFmoc), 3.37 – 3.19 (m, 4H, $CH_2CH_2CH_2N_3$), 3.08 (m, 2H, $\varepsilon CH_2$), 1.77-1.68 (m, 4H, $\beta CH_2$, $CH_2CH_2N_3$), 1.55-1.27 (m, 4H, $\gamma CH_2$, $\delta CH_2$, 1.42 (s, 9H, $3CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) δ ppm: 172.2, 156.6, 156.3, 127. 8, 127.1, 125.2, 120.0, 79.2, 67.1, 54.9, 49.1, 47.1, 39.9, 37.0, 32.1, 29.6, 28.7, 28.5, 22.6. HR-ESI-QToF MS (positive mode): *m*/*z* calcd. for $C_{29}H_{39}N_6O_5$ $[M+H]^+$ 551.2982, found 551.2985.

**Lys(Boc) (3-azidopropyl) amide 12**: A solution of FmocLys(Boc) (3-azidopropyl) amide **11** (639 mg, 1.16 mmol) in piperidine/THF (4:6, v/v) was stirred at rt for 4 h. The solvent was coevaporated with toluene (7 times) and $CHCl_3$ (3 times) to obtain a yellow oil purified by silica gel flash chromatography ($CH_2Cl_2$/MeOH 9:1) to afford Lys(Boc) (3-azidopropyl) amide (358 mg, 92%) as a clear oil. $R_f$ = 0.43 ($CH_2Cl_2$/MeOH 9:1). $^1$H NMR (400 MHz, $CDCl_3$) δ ppm: 7.51 (d, *J* = 4.3 Hz, 1H, NH), 4.59 (s, 1H, NH), 3.41-3.22 (m, 5H, $CH_2CH_2CH_2N_3$, αCH lys), 3.18-3.02 (m, 2H, $\varepsilon CH_2$), 1.90-1.64 (m, 5H, βCH, $\gamma CH_2$, $CH_2CH_2N_3$), 1.59-1.28 (m, 12H, δCH-7′-βCH, 3 x $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) δ ppm: 175.1, 156.1, 79.2, 55.0, 49.3, 40.1, 36.6, 34.5, 29.9, 28.9, 28.4, 22.9. HR-ESI-QToF MS (positive mode): *m*/*z* calcd. for $C_{14}H_{29}N_6O_3$ $[M+H]^+$ 329.2301, found 329.2300.

**4-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)-benzamide Lys(Boc) (3-azidopropyl) amide 13:** Lys(Boc) (3-azidopropyl)amide **4** (337 mg, 1 mmol) and 4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-benzoic acid **5** (472 mg, 1 mmol) were solubilised in $CH_2Cl_2$ (4.5 mL). The solution was stirred with molecular sieve 4Å for 30 min. BOP (505.6 mg, 1 mmol) and DIEA (340 µL, 2 mmol) were added and the mixture was stirred at rt for 1 h. The solvent was evaporated under vacuum and the residue was purified by silica gel flash chromatography (cyclohexane/EtOAc) to afford compound **13** (820 mg, 99%) as an amorphous white solid. $R_f$ = 0.41 ($CH_2Cl_2$/MeOH 95:5). $^1$H NMR (400 MHz, $CDCl_3$) δ ppm: 7.79 (d, *J* = 8.7 Hz, 2H, Ar), 7.06-6.99 (d, *J* = 8.7 Hz 2H, Ar), 6.90 (bs, 1H, NHCO), 5.55-5.43 (m, 2H, H2, H4), 5.22-5.04 (m, 2H, H1, H3), 4.59 (m, 1H, αCH Lys), 4.23-4.09 (m, 3H, H5, H6), 3.39-3.29 (m, 4H, $CH_2CH_2CH_2N_3$), 3.11 (m, 2H, $\varepsilon CH_2$), 2.18 (s, 3H, $CH_3CO$), 2.06 (s, 3H, $CH_3CO$), 2.06 (s, 3H, $CH_3CO$), 2.01 (s, 3H, $CH_3CO$), 1.99-1.88 (m, 1H, βCH), 1.84-1.74 (m, 3H, $CH_2CH_2N_3$, βCH), 1.59-1.48 (m, 2H, $\delta CH_2$), 1.47-1.35 (m, 11H, $\gamma CH_2$, 3x$CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) δ ppm: 171.0, 169.3, 169.2, 169.1, 158.5, 128.0, 127.3, 116.0, 98.0, 76.2, 70.2, 70.0, 67.5, 65.8, 60.4, 52.5, 48.1, 39.0, 36.1, 31.0 , 28.5, 27.6, 27.4 , 21.7, 19.7, 19.6, 19.5. HR-ESI-QToF MS (positive mode): *m*/*z* calcd. for $C_{35}H_{50}N_6O_{14}Na$ $[M+Na]^+$ 801.3283, found 801.3286.

**4-(β-D-Galactopyranosyloxy)-benzamide Lys(Boc) (3-azidopropyl) amide 14:** Compound **6** was solubilized in MeOH/$H_2O$/$NEt_3$ (8 mL, 5:1:2, v/v/v) and the mixture was stirred at rt for 2.5 h. The solvent was evaporated to afford compound **14** (695 mg, quantitative) as an amorphous white solid. $R_f$ = 0.13 ($CH_2Cl_2$/MeOH 9:1). $^1$H NMR (400 MHz, $CD_3OD$) δ ppm: 7.91-7.77 (d, *J* = 8.8 Hz, 2H, Ar), 7.23-7.07 (d, *J* = 8.8 Hz, 2H, Ar), 4.95 (d, *J* = 7.7 Hz, 1H, H1), 4.46 (dd, *J* = 8.8, 5.7 Hz, 1H, αCH Lys), 3.92 (d, *J* = 3.4 Hz, 1H, H4), 3.85-3.69 (m, 4H, H2, H5, H6), 3.60 (dd, *J* = 9.7, 3.4 Hz, 1H, H3), 3.36 (t, *J* = 6.7 Hz, 2H, $CH_2N_3$), 3.30-3.27 (m, 2H, $CH_2EtN_3$), 3.04 (t, *J* = 6.7 Hz, 2H, $\varepsilon CH_2$), 1.90-1.74 (m, 4H, $\beta CH_2$, $CH_2CH_2N_3$ ), 1.38-1.56 (m, 4H, $\gamma CH_2$, $\delta CH_2$), 1.41 (s, 9H, 3 x $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) δ ppm: 174.8, 169.8, 162.0, 130.3, 128.8, 117.3, 102.4, 77.1, 74.8, 72.2, 70.2, 62.4, 55.6, 50.1, 41.0, 37.8, 32.8, 30.6, 29.7, 28.8, 24.4. HR-ESI-QToF MS (positive mode): *m*/*z* calcd. for $C_{27}H_{43}N_6O_{10}$ $[M+H]^+$ 611.3041, found 611.3045.

**4-(β-D-Galactopyranosyloxy)-benzamide Lys (3-azidopropyl) amide 7h:** Compound **14** (636 mg, 1 mmol) was solubilized in HCl 1N (10 mL). The mixture was stirred at 70°C for 5 min under microwave irradiation. Polyvinyl pyridine (PVP 2% cross-linked, 60 mesh, 8 meq/g) was added to neutralize the reaction. The mixture was filtered and the PVP washed with MeOH. The solvent was evaporated under vacuum to afford compound **8** (518 mg, 97%) as an amorphous white solid. $[\alpha]_D^{20}$ = -2.0 (c = 1.0 $H_2O$). $^1$H NMR (400 MHz, $D_2O$) δ ppm: 7.89-7.79 (m, 2H, Ar), 7.28-7.18 (m, 2H, Ar), 5.17 (d, *J* = 7.5 Hz, 1H, H1), 4.42 (dd, *J* = 8.7, 6.0 Hz, 1H, αCH Lys), 4.02 (dd, *J* = 3.2, 0.8 Hz, 1H, H4), 3.97-3.74 (m, 5H, H2, H3, H5, H6), 3.37 (t, *J* = 6.6 Hz, 2H, $CH_2N_3$), 3.35-3.28 (m, 2H, $NHCH_2EtN_3$), 3.01 (t, *J* = 7.7 Hz, 2H, $\varepsilon CH_2$), 1.98-1.65 (m, 6H, $\beta CH_2$, $CH_2CH_2N_3$, $\delta CH_2$), 1.50 (m, 2H, $\gamma CH_2$). $^{13}$C NMR (100 MHz, $D_2O$) δ ppm: 174.2, 170.2, 159.7, 129.4, 127.3, 116.2, 115.4, 100.1, 75.5, 72.5, 70.4, 68.4, 60.7, 54.6, 48.7, 39.2, 36.7, 30.4, 27.6, 26.3, 22.3. HR-ESI-QToF MS (positive mode): *m*/*z* calcd. for $C_{23}H_{35}N_6O_8$ $[M+H]^+$ 511.2516, found 511.2522.

**2-Methyl-2-[(prop-2-ynoxy)methyl]propane-1,3-diol 16**. Sodium hydride (400 mg, 10 mmol) was added to a solution of 1,1,1-tris(hydroxymethyl)ethane (1.2 g, 10 mmol) in dry THF (30 mL) under argon atmosphere. Then, a solution of propargyl bromide (0.52 mL, 5 mmol) in dry THF (10 mL) was added dropwise at 0°C. The mixture was stirred overnight and water was added to quench the reaction. After 15 min, the crude mixture was coevaporated with toluene, and directly purified by silica flash chromatography (EtOAc 50% to 100% in cyclohexane) leading to compound **16** (480 mg, 61%). $^1$H NMR (400 Mz, $CDCl_3$) 4.16 (d, *J* = 2.4 Hz, 2H, $CH_2C{\equiv}CH$), 3.62-3.54 (m, 6H, $CH_2$), 2.45 (t, *J* = 2.4 Hz, 1H, ≡CH), 0.85 (s, 3H, $CH_3$). In agreement with the literature data.[39]

**Immobilization of alkynes 16 and 18 on azido-functionalized solid support 17 by CuAAC:** An aqueous solution of propargyl diethylene glycol **18** or compound **16** (0.2 M, 50 µL, 10 µmol), freshly prepared aqueous solutions of $CuSO_4$ (0.04 M, 25 µL, 1 µmol) and sodium ascorbate (0.1 M, 50 µL, 5 µmol), TEAAc buffer (2 M, 25 µL), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 0.1 M, 20 µL, 2 µmol) and MeOH (80 µL) were added to 1 µmol of azide solid support 1**7**. The resulting mixture was heated at 65°C under microwave for 30 min at 250 rpm. The solution was removed and CPG beads were washed with $H_2O$ (5 mL), MeOH (5 mL) and $CH_2Cl_2$ (5 mL) then dried affording **19** and **20**.

**Glyco-oligonucleotide synthesis:** The oligonucleotide synthesis on solid support was performed on a DNA synthesizer Applied Biosystems (381A or 394 DNA synthesizer). Reactions under microwave activation were performed on an Anton Paar Monowave 300 system. Solutions of Cap A, Cap B and iodide were purchased from Link Technologies as well as the commercial solid supports. Solutions of TCA and $CH_3CN$ for DNA synthesis were purchased from Biosolve. Cy3-amidite was purchased from GE Healthcare. All glycooligonucleotides were purified and analyzed by $C_{18}$ reversed-phase HPLC (Macherey-Nagel, Nucleodur 4.6×75 mm, 3 mM) on a Dionex Ultimate 3000 system with a Reodyn injector and a detector UV DAD 3000. Oligonucleotides were dosed by

UV-Vis spectrophotometry at 550 nm on a Varian Cary 300 Bio UV-Vis spectrophotometer.

**General procedure for introduction of phosphoramidites 1 and 2 on solid-supported scaffolds 19 and 20:** Solid-supported scaffolds **19** and **20** (1 µmol) were treated, on a DNA synthesizer, with phosphoramidites **1** and **2** using only coupling and oxidation steps. For the coupling step, benzylmercaptotetrazole was used as an activator (0.3 M in anhydrous $CH_3CN$) and phosphoramidites 1 and 2 (0.2 M in anhydrous $CH_3CN$) were introduced once on 10 and twice on 11 with a 30 s coupling time. Oxidation was performed with 0.1 M commercial solution of iodide for 15 s.

**General procedure for elongation of DNA sequences and labelling with Cy3:** The DNA sequences were synthesized on the solid-supported scaffolds (LCAA-CPG, 500 Å) at the 1 µmol scale on a DNA synthesizer (ABI 394) by phosphoramidite chemistry. For the coupling step, benzylmercaptotetrazole was used as an activator (0.3 M in anhydrous $CH_3CN$), and commercially available nucleoside phosphoramidites (0.1 M in anhydrous $CH_3CN$) were introduced with a 20 s coupling time and Cy3 amidite (0.06 M in anhydrous $CH_3CN$) with a 180 s coupling time. The capping step was performed with acetic anhydride using commercial solution (Cap A, $Ac_2O$/Pyridine/THF, 1:1:8, v/v/v; Cap B, 10% *N*-methylimidazole in THF) for 15 s. Each oxidation was performed with commercial solution of iodide (0.1 M in THF/pyridine/$H_2O$, 78:20:2, v/v/v) for 15 s. Detritylation was performed with 3% TCA in $CH_2Cl_2$ for 65 s.

**General procedure for déprotection:** The CPG beads bearing modified oligonucleotides were transferred to a 4 mL screw top vial and treated with 2 mL of concentrated aqueous ammonia for 15 h at 25°C. The supernatants were withdrawn and evaporated affording crude **21A-B** and **22A-B**.

**General procedures for CuAAC in solution:**

**Method A**: To a solution of 5'-fluorescent-3'-alkyne platform oligonucleotides **21A-B** and **22A-B** (1 mM in water) were added glycoside derivatives (20 mM, 12 eq.), THPTA (10 mM, 3 eq.), and 1 mg $Cu^0$. The resulting mixture was sonicated, stirred for 15 min at 65°C then 15 min at room temperature. The crude solutions were centrifuged and the supernatant were directly purified by $C_{18}$ reversed phase HPLC. The products were coevaporated with water and lyophilized.

**Method B**: To a solution of 5'-fluorescent-3'-alkyne platform oligonucleotides **21A-B** and **22A-B** (1 mM in water) were added glycoside derivatives (20 mM, 12 eq.), THPTA (10 mM, 3 eq.), TEAAc (2 M, 10 µL), water (20 µL) and 1 mg $Cu^0$. The resulting mixture was sonicated, stirred for 15 min at 65°C then 15 min at room temperature. The crude solutions were centrifuged and the supernatant were directly purified by $C_{18}$ reversed phase HPLC. The products were coevaporated with water and lyophilized.

**Method C**: To a solution of 5'-fluorescent-3'-alkyne platform oligonucleotides **21A-B** and **22A-B** (1 mM in water) were added glycoside derivatives (20 mM, 12 eq.), THPTA (10 mM, 3 eq.), TEAAc (2 M, 8 µL), MeOH (70 µL), water (30 µL) and 1 mg $Cu^0$. The resulting mixture was sonicated then stirred for 3h under microwave at 65°C. The crude solutions were centrifuged and the supernatant were directly applied on a Nap10. After lyophilization the residue was treated overnight with concentrated ammonia. After evaporation, the solution was extracted with ethyl acetate. Then the compound was purified by $C_{18}$ reversed phase HPLC. The products were coevaporated with water and lyophilized. The Table S1 reports the method used according to each glycocluster.

**Microarray studies**

**Microarray fabrication**: Borosilicate glass slides (Glass D, Schott) bearing 40 microwells fabricated by xurography [42] were chemically modified with tert-butyl (dimethylamino)silylundecanoate (TDSUM) to introduce carboxylic acids on the surface. After deprotection (glacial formic acid) and activation (DIC, NHS in THF, 0.1 M), amino-modified oligonucleotides (25 µM in PBS 10X, pH 8.5) were printed with a sciFLEXARRAYER s3 system piezoelectric leading to 32 repetitions of each sequence per well. The slides were washed at 70°C in SDS 0.1% for 30 min. To avoid non-specific adsorption, blocking was performed with a 4% solution of BSA in PBS 1X (pH 7.4) for 2 h and washed (3×3 minutes in PBS 1X-Tween20 0.5%, pH 7.4 then 3×3 min in PBS 1X then rinsed with water).

Each galactocluster was labelled with Cy3 and displayed a specific DNA sequence complementary to one of the sequence covalently immobilized in the microwell. Consequently, the galactoclusters were specifically addressed on the array by DNA/DNA duplex formation. Galactoclusters (in PBS 1X, pH 7.4) were mixed (final concentration 1 µM each) and 2 µL of the resulting mixture were poured into each microwell. Incubation was performed in a humidity saturated chamber at 37°C for 3 h. Next, the slides were washed successively with SSC 2X, SDS 0.1% at 51°C for 1 min, SSC 2X for 5 min at rt and rinsed with water. In order to assess the relative surface density of the clusters, slides were scanned at 532 nm (excitation of Cy3) by using the Axon microarray scanner. Image analysis was performed using the Genepix 4100 A software package. For each galactoclusters, the Cy3 mean fluorescence signal was averaged.

**Probing Galactocluster-LecA interaction on microarray**: The interaction of LecA (purchased from Elicityl, Crolles, France) with the galactoclusters was interrogated with Alexa647-LecA (Invitrogen microscale Alexa labeling kit). 2 µL of Alexa647-LecA (0.12 µM, 7.5 µM CaCl2, 2% BSA in PBS1X) were added in each well. Incubation was performed at 37°C for 3 h in a water saturated chamber. The slides were washed successively with PBS1X (pH 7.4)-Tween20 0.02% for 5 min, rinsed with water and scanned at 635 nm (excitation).

**Dissociation constant determination on microarray**:[42] LecA concentration was varied from 0.01 nM to 2 µM. Alexa647 fluorescence signal was recorded and from the resulting isotherm, the Kd values were determined using the following equation:

$$[LecA]/FI = 1/FI_{max}*[LecA] + Kd/FI_{max}$$

Where FI represents the fluorescence signal recorded at 635 nm for the given concentration of LecA and $FI_{max}$ is the maximum fluorescence signal obtained at 635 nm.

**In silico molecular docking:** The three dimensional structure of LecA was retrieved from the Protein Data Bank (www.rcsb.org) under the PDB code 2VXJ. For both glycoclusters (**23Ah** and **23Bh**) the building procedure of the lectin-ligand complex is depicted as follows: The galactoses endings are brought close to the crystallographic galactose ligands. The terminal sugars of the two glycocluster branches are then removed and the chemical bonds with galactose moieties of LecA are built. LecA and its two galactose groups are considered as aggregates and the complex is optimized. A classical Monte Carlo conformational searching procedure has been performed as described in the BOSS software.[50]

For the final retained structure, the constraints are removed and the whole complex is relaxed. In the minimization procedures, the spectroscopic empirical energy function SPASIBA and the corresponding parameters are used.[51-53] In the same way, an empirical potential energy of interaction $\Delta E$ for the lectin-ligand complexes using the simple expression $\Delta E_{interaction} = E_{complex} - E_{protein} - E_{ligand}$ was evaluated using the same force field.

## Acknowledgements

This project was supported by the Agence Nationale de la Recherche (Glycomime project ANR-12-BSV5-0020). The authors thank the University Claude Bernard Lyon 1, Ecole Centrale de Lyon, University of Lille 1, University of Montpellier and the CNRS for financial support. A.A. thanks the University of Montpellier for a research studentship. F.M. is from Inserm.

**Keywords:** glycoclusters • microarrays • multivalency • oligonucleotides • *Pseudomonas aeruginosa* • LecA

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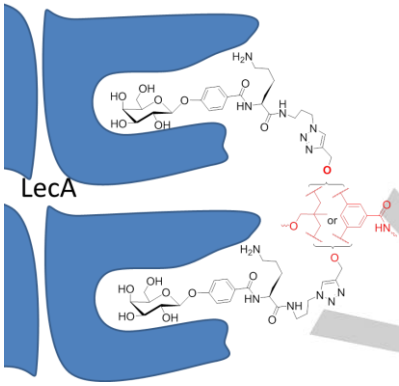
A series of 32 glycodendrimers of generation 0 and 1 targeting Lectin A (LecA) has been synthesized. Bis-galactosides exhibiting phenyl and lysine aglycons are able to tightly bind LecA with a low nanomolar $K_d$.( 22 to 32 nM)

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**Design and synthesis of galactosylated bifurcated ligands with nanomolar affinity for lectin LecA from *Pseudomonas aeruginosa***

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