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The influence of the aromatic aglycon of galactoclusters on the binding of LecA: a case study with *O*-phenyl, *S*-phenyl, *O*-benzyl, *S*-benzyl, *O*-biphenyl and *O*-naphthyl aglycons†

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A library of 24 new mannose-centered tetragalactoclusters with four different linkers (di- and triethyleneglycol with phosphodiester or phosphorothioate linkages) and six different aromatic aglycons (*O*-phenyl, *S*-phenyl, *O*-benzyl, *S*-benzyl, *O*-biphenyl and *O*-naphthyl) was synthesized. Their interactions with LecA were evaluated on a DNA Directed Immobilization (DDI) based glycocluster array allowing the determination of their IC_{50} against lactose and the evaluation of their dissociation constant (K_d). Finally, the docking simulations confirm the experimental results and demonstrated that the better affinity of *O*-biphenyl- and *O*-naphthyl-galactoside is due to a double interaction between the aromatic ring and the histidine 50 and proline 51 of LecA.

Introduction

Infection by bacteria or viruses is generally initiated through the selective recognition by pathogen lectins of the host carbohydrate motif leading to the adhesion of pathogens on epithelial surfaces. *Pseudomonas aeruginosa* (PA) is a ubiquitous Gram-negative, aerobic and clinically important opportunistic bacterium often related to pulmonary infections, it is responsible for 10–30% of hospital-acquired diseases.[1] It is threatening for immune-compromised patients and more specially for cystic fibrosis patients leading to the degradation of their respiratory tract.[2] Consequently, regarding the emergence of resistance of most pathogenic bacteria, especially PA, to antibiotics, the development of new antibacterial agents able to escape the mechanisms of resistance or of new modes of action had become imperative and is a major research challenge to treat or prevent infectious diseases. Thus, the design of multivalent ligands targeting bacterial lectins has arisen as a promising strategy to design anti-adhesive drugs.[3–5]

PA produces two soluble lectins LecA (or PA-IL) and LecB (or PA-IIL).[6] LecA which binds D-galactose is involved in the pathogenicity of PA.[7,8] LecA is a tetravalent lectin with a nearly rectangular shape with binding site distances of 71 Å on the long side, and 32 Å on the short side.[9,10]

The binding of LecA with monovalent galactosides spans the micromolar range and is influenced by the structure of the aglycon with an enhanced binding for aromatic β-D-galactopyranosides.[11,12] The phenyl β-D-galactopyranoside presents a 26-fold increase of affinity to LecA compared to methyl β-D-galactopyranoside.[10] Recently, it has been demonstrated that aromatic aglycons present a CH-π "T-shape" interaction with the His50 residue of the lectin.[13,14] Several galactosylated glycoclusters have been reported and their binding to LecA has been discussed.[3,4,13–28] Among them, only a few report the use of aromatic galactoside aglycons in multivalent galactosylated structures and all confirm this beneficial influence.[13,24,28]

We recently showed that a glycocluster built from a mannose core and exhibiting four or eight phenyl-galactoside moieties displayed high affinity for LecA with an increase of potency of 844 and 1361-fold respectively in comparison with a monotriethyleneglycol galactoside.[28] To evaluate the influence of the aromatic effect on the binding to LecA, we synthesized five new galactosides where the *O*-phenyl (Ph) moiety was replaced with *S*-phenyl, *O*-benzyl (Bn), *S*-benzyl, *O*-biphenyl (biPh) and *O*-naphthyl (Napht) motifs (Fig. 1).

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†Electronic supplementary information (ESI) available: HPLC profiles, NMR data and docking. See DOI: 10.1039/c4ob01599a

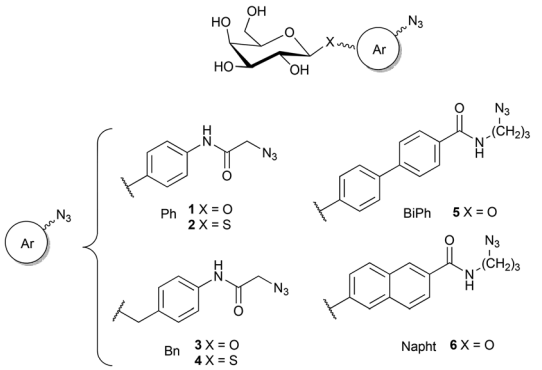


Fig. 1 Schematic representation of azide aromatic galactosides.

The flexibility and the length of the linker between the epitope and the scaffold also play a key role. Ideally, the topology of the carbohydrate residues should match on the atomic scale the binding sites of the lectin for a maximal interaction. However, since this is almost unachievable, some flexibility should be introduced through the linker and consequently, lead to an entropic cost of the binding. In this study, we evaluated two different linkers prepared from di- and triethyleneglycolmethylene triazole with phosphodiester or phosphorothioate linkages. Furthermore, thio-glycosides and phosphorothioate linkages are known to be resistant to glycosidases and phosphodiesterases respectively.

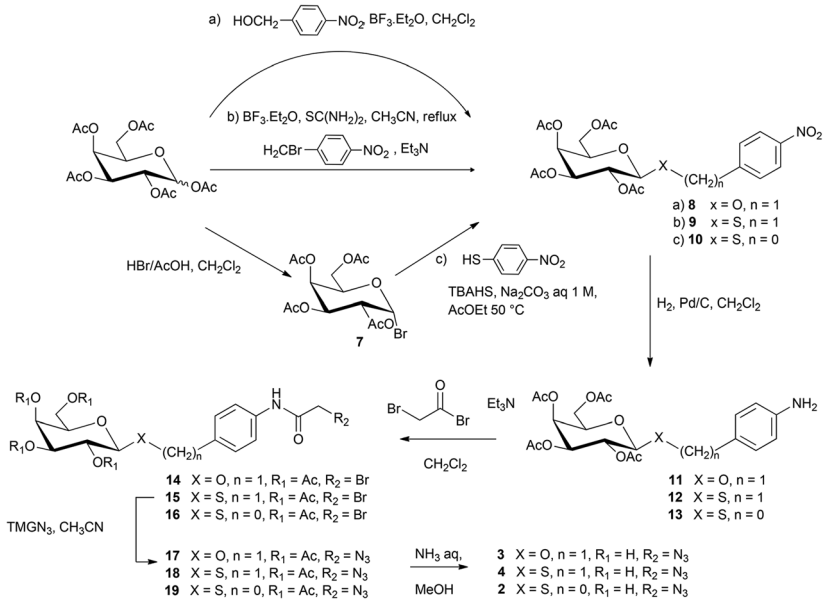
Herein we present the influence of the aromatic aglycon of mannose-centered tetragalactoside clusters on the affinity toward LecA. To this end, 24 glycoclusters conjugated to an oligonucleotide and exhibiting six different aromatic aglycon moieties on different scaffolds were synthesized and their binding to LecA was studied on a DNA-Directed Immobilized glycocluster array (DDI-glycoarray) allowing the determination of their IC_{50} value against lactose and the evaluation of their dissociation constant (K_d).[25,29]

Finally, the experimental results were compared with the docking simulation.

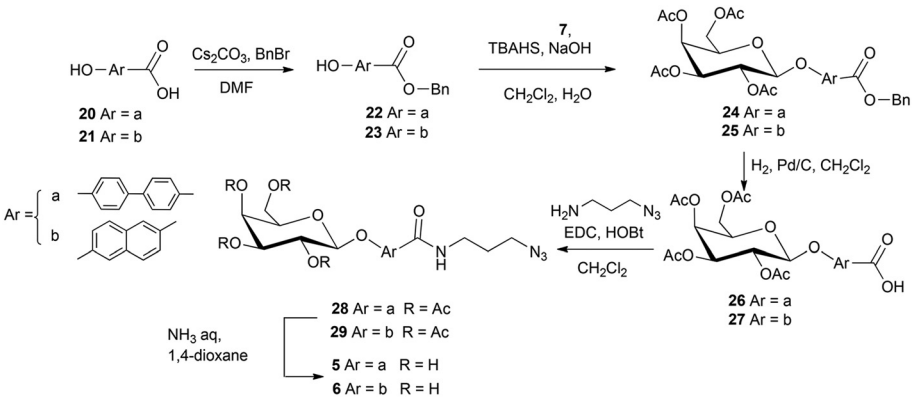
Results and discussion

For the synthesis of galactoclusters, we used a combination of nucleic acid chemistry on solid supports and copper catalysed azide alkyne cycloadditions (CuAAC) as previously reported.[28,30]

The *O*-phenyl-galactoside azide derivative **1** was synthesized according to the literature.[19] As shown in Scheme 1, the other aromatic galactoside azide derivatives **2–6** were synthesized in a limited number of steps. The *p*-nitro-benzyl-β-D-galactopyranoside derivatives **8** and **9**[31] were prepared by traditional Lewis acid mediated glycosidation starting from peracetylated galactopyranoside. The synthesis of *p*-nitrophenyl-β-D-thio-galactopyranosides **10** was achieved with a phase transfer catalysed (PTC) reaction[32] on the readily available 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **7**.[13] As expected, the phase transfer catalysed nucleophilic displacement of the bromide by the *p*-nitrothiophenol occurred with complete anomeric inversion to afford only the corresponding β-glycoside derivative. The anomeric configurations of all molecules have been proven by ^{1}H NMR and correlation experiments (COSY, HSQC) as all $JH_{1,2}$ coupling constants were in the range of 8–10 Hz, thus establishing the 1,2-*trans* relationship at the anomeric centers. The subsequent reduction of the nitro group of **8**, **9** and **10** was achieved by palladium on charcoal hydrogenation affording amino-aryl derivatives **11–13**, which were treated with bromoacetic bromide to obtain the corresponding bromoacetamide derivatives **14–16**. Azidation was performed with



Scheme 1 Synthesis of *S*-phenyl, *O*-benzyl, and *S*-benzyl azido galactosides **2–4**.



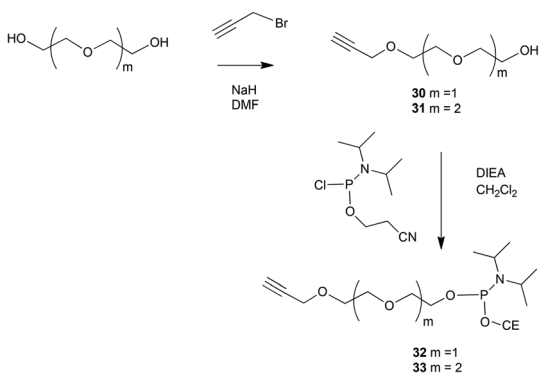
Scheme 2 Synthesis of biphenyl and naphthyl azido galactosides **5** and **6**.

tetramethylguanidinium azide affording **17–19**. The unprotected galactoside azido derivatives **2**, **3** and **4** were prepared by de-O-acetylation using aqueous ammonia in methanol.

The preparation of biphenyl **5** and naphthyl **6** galactoside azides is shown in Scheme 2. Commercially available 4′-hydroxy-4-biphenylcarboxylic acid **20** and 6-hydroxy-2-naphthoic acid **21** were benzylated with benzyl bromide to afford **22** and **23**, followed by glycosidation with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **7**. Chemoselective cleavage of the benzyl group to obtain **26** and **27** was performed with a palladium on charcoal hydrogenation. The azido group was then introduced by the coupling of acids **26** and **27** with 3-azidopropylamine using ethyl dimethylpropyl carbodiimine (EDC) and hydroxybenzotriazole (HOBt). A final de-O-acetylation (NH_4OH in MeOH or dioxane) led to the desired compounds **5** and **6**.

Di- and triethyleneglycolpropargyl phosphoramidites **32** and **33** were prepared in two steps starting from di- and triethyleneglycol which were first alkylated with propargyl bromide in the presence of sodium hydride and then converted into phosphoramidite using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in the presence of *N*,*N*-diisopropylethylamine (Scheme 3).

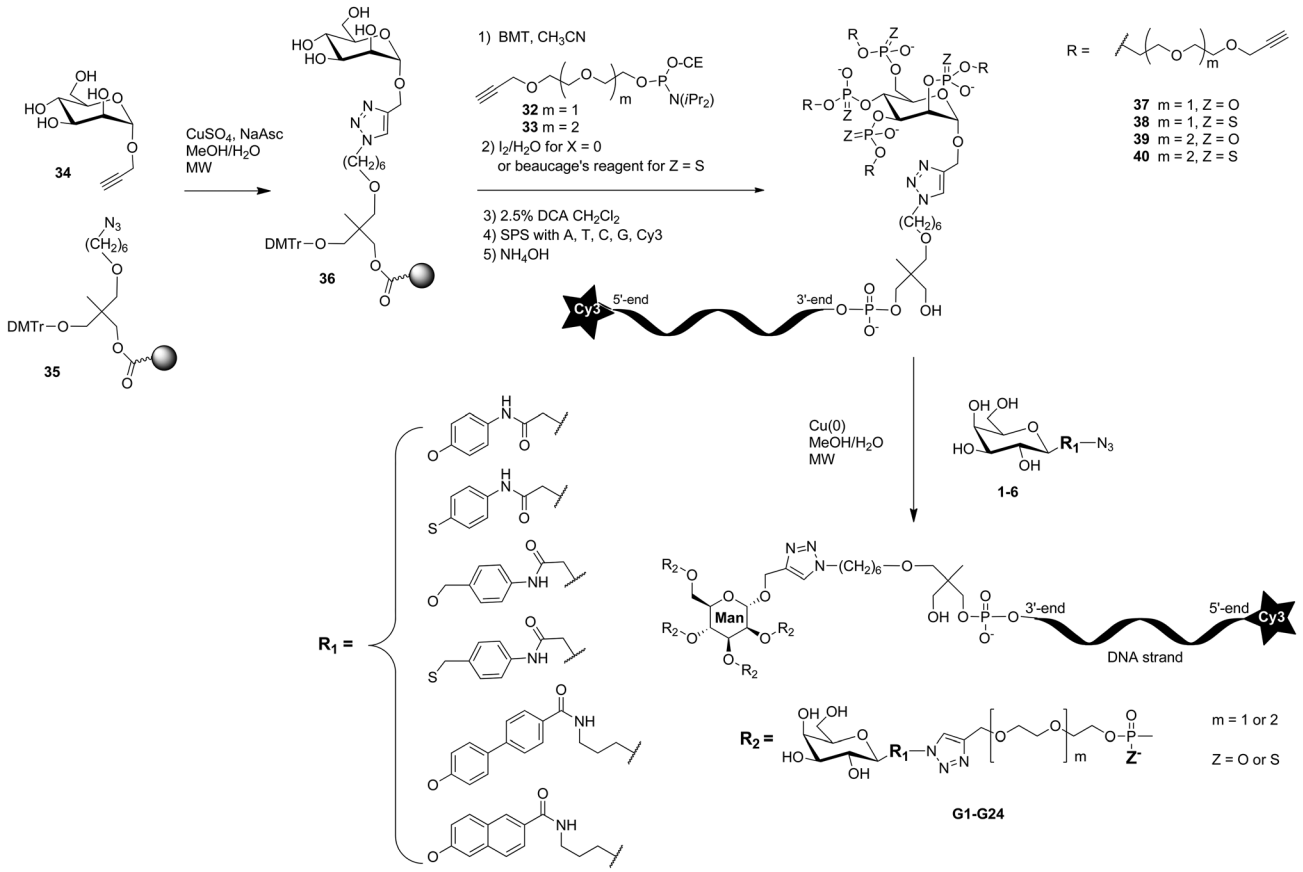
With all the building blocks in hand, the synthesis of glycoclusters was mainly performed on a solid support. First,



Scheme 3 Synthesis of propargyl di- and triethyleneglycol phosphoramidites **32** and **33**.

propargyl-α-D-mannopyranoside **34** was immobilized on an azide solid support **35** by CuAAC using $CuSO_4$ and sodium ascorbate with microwave assistance[30] (Scheme 4). Second, propargyl di- or tri-ethyleneglycol phosphoramidites **32** or **33** were introduced on each hydroxyl by activation with benzylmercaptotetrazole with a triple coupling to guarantee their full introduction using a DNA synthesizer. Oxidation of the phosphite linkages was carried out with iodide/water or 3*H*-1,2-benzodithiole-3-one-1,1,-dioxide (Beaucage reagent)[33] to form phosphotriester or thionophosphotriester linkages respectively. Third, elongation of the oligonucleotide sequence and labelling with Cy3 were performed by standard phosphoramidite chemistry. Seven different sequences were used allowing multiplexed studies on the DDI-glycoarray.[17,25] After ammonia treatment the resulting 5′-Cy3-DNA-3′-tetraalkynylated-mannosides **37–40** were analysed by C_{18} HPLC and characterized by MALDI-TOF mass spectrometry. Finally, the different aromatic galactosides **1–6** were conjugated by CuAAC starting from 100 nmol of crude leading, after HPLC purification, to 6 to 60 nmol of pure oligonucleotide mannose-centered tetragalactosides **G1–G24** according to the purity of the starting material. These glycoclusters exhibit two different linkers di- or tri-ethyleneglycolmethylene (EG_2M or EG_3M), phosphodiester or phosphorothioate linkages (PO or PS) and six different aromatic galactosides (*O*-Ph, *S*-Ph, *O*-Bn, *S*-Bn, *O*-Biph and *O*-Napht). Then, the Cy3-oligonucleotide galactoclusters **G1–G24** were immobilized on a DNA array matrix by DNA/DNA duplex formation, the so-called DNA Directed Immobilization (DDI).[29,34,35] Each molecule carried a Cy3 fluorescent tag for quality control of surface immobilization. The Cy3 fluorescence signal deviated by less than 32%. In our case, the galactocluster surface density is 3×10^{11} molecules cm^{-2}. In consequence, LecA can interact with only one immobilized galactocluster molecule at a time allowing the probing of the so-called cluster effect. The interaction of LecA with the immobilized galactocluster was probed using Alexa 647 labelled LecA.

We have already reported that this platform can be exploited for the evaluation of the IC_{50} value and dissociation constant (K_d).[25] In this study, the platform was a borosilicate



	EG_2M/PO m =1 Z =O	EG_2M/PS m =1 Z =S	EG_3M/PO m =2 Z =O	EG_3M/PS m =2 Z =S
O-Ph	**G1** Sq 1	**G2** Sq 4	**G3** Sq 4	**G4** Sq 1
S-Ph	**G5** Sq 2	**G6** Sq 5	**G7** Sq 6	**G8** Sq 1
O-Bn	**G9** Sq 3	**G10** Sq 4	**G11** Sq 2	**G12** Sq 6
S-Bn	**G13** Sq 5	**G14** Sq 5	**G15** Sq 2	**G16** Sq 6
Biph	**G17** Sq3	**G18** Sq 6	**G19** Sq 7	**G20** Sq 4
Napht	**G21** Sq 3	**G22** Sq 6	**G23** Sq 7	**G24** Sq 4

Scheme 4 Synthesis of mannose-centered galactoclusters **G1–G24**.

glass slide bearing 40 microwells allowing the lactose or lectin concentration to be varied in each well. Each microwell was divided into 64 spots as an 8 × 8 matrix. Furthermore, since the galactoclusters were tagged with seven different DNA sequences, four analyses were performed in the same microwell where each complementary sequence of the galactoclusters was grafted sixteen times. Thus the value of fluorescence was the average of 16 measurements.

The IC_{50} values were determined through competitive experiments using lactose as the inhibitor. Each microwell corresponded to one concentration of lactose from 0.01 nM to 0.3 M. In our case, the IC_{50Lac} is the concentration of lactose required to reduce by 50% the Alexa fluorescence signal observed in the absence of lactose. Hence, the higher is the IC_{50Lac}, the strongest is the affinity of LecA for the galactocluster.

The dissociation constant values (K_d) were determined by incubating the DDI immobilized glycoclusters with an increasing concentration of Alexa647-LecA from 0.1 nM to 2.0 μM. Each well corresponded to one concentration of LecA. The resulting isotherms were linearized allowing calculation of the K_d value at the y-intercept.[25]

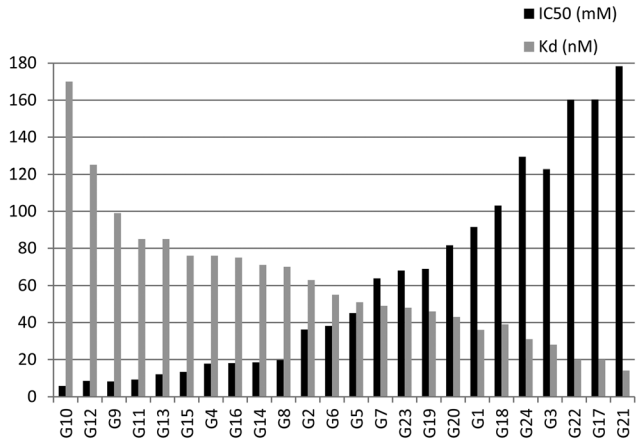


Fig. 2 IC_{50Lac} and K_d values obtained for 24 galactoclusters **G1–G24** interacting with the Alexa 647 labeled LecA. Galactoclusters are ranked from the smallest to the strongest interaction with LecA from the left to the right.

In Fig. 2, the IC_{50} and K_d values of the 24 galactoclusters **G1–G24** are presented.

According to Fig. 2, IC_{50Lac} and K_d values are in good agreement, so for the discussion we only refer to K_d values. All glycoclusters exhibited a high affinity for LecA with K_d values between 14 and 170 nM. At a first glance, the impact of the aromatic aglycon to LecA binding is benzyl < phenyl < biphenyl ≤ naphthyl.

For the benzyl-galactoside clusters, the *O*-benzyl ones (**G9–G12**) displayed a lower affinity than the *S*-benzyl ones (**G13–G16**) with K_d values between 85 and 170 nM for the former and K_d values between 71 and 85 nM for the latter. For the *O*-benzyl clusters, the introduction of the phosphorothioate linker (**G12** and **G10**) led to the lowest affinities (125 and 170 nM) and we can note a benefit due to the longest linker (EG_3M > EG_2M). For the *S*-phenyl, all the K_d values were close (71–85 nM), so the influence of the phosphodiester and the length of the linker are less visible.

For the phenyl-galactoside clusters (**G1–G8**), the *O*-phenyl aglycon was mainly more favorable to the binding of LecA compared to the *S*-phenyl except for **G2**. With both phenyl aglycons, the phosphorothioate linkage was not favourable and a slight preference for the shorter linker (EG_2M) was observed. The phosphorothioate linkages induced a strong destabilization for *O*-benzyl clusters (**G4** and **G2**) with K_d values of 76 and 63 nM *versus* 36 and 28 nM for the phosphodiesters (**G8** and **G3**). While the four *S*-phenyl clusters (**G5–G8**) displayed close K_d values between 49 and 70 nM, the four *O*-phenyl clusters (**G1–G4**) explored a larger range of affinity with K_d values varying from 28 to 76 nM.

Biphenyl (**G17–G20**) and naphthyl (**G21–G24**) galactoclusters exhibited high affinity with a slight preference for naphthyl ones. The clusters with shorter linkers were overall better ligands for LecA. Furthermore, no clear conclusions could be drawn on the impact of the phosphorothioate linkage as opposite conclusions can be drawn depending on the linker. According to this study the three best galactoclusters were those with the EG_2M linker and phosphodiester linkages with naphthyl (**G21** K_d 14 nM) and biphenyl (**G17** K_d 20 nM) and the phosphorothioate analog with naphthyl (**G22** K_d 20 nM). Surprisingly both *O*-phenyl aglycons with phosphodiester linkages displayed high affinity with a preference for the EG_3M linker with a K_d value of 28 nM *versus* 36 nM for the EG_2M one (**G3** and **G1**, respectively).

Concerning the length between the galactose moiety and the mannose core for each cluster we can hypothesize that an optimum is required for a better binding with LecA. Since the length between the galactopyranose and the azide is longer for the biphenyl and naphthyl (13 and 15 atoms) than for phenyl and benzyl (9 and 10 atoms) it could be the explanation for why the EG_2M linker was preferred for the biphenyl and naphthyl and the EG_3M linker was better for phenyl and benzyl clusters. The total length was 25 and 27 atoms for naphthyl and biphenyl respectively with the EG2M linker and 24 atoms for phenyl with the EG3M linker.

These data confirmed that the aromatic ring must be connected directly on the *O*/*S* anomeric atom of the galactoside. Thus when a methylene is present like in the benzyl aglycon, we observed a lower affinity for LecA. Along this line, we previously showed that galactoclusters with *O*-methylene-triazole galactoside motifs also display a lower binding than with *O*-phenyl motifs and glycoclusters with thymidine-galactoside motifs, where the heterocycle is directly connected on the C1 of the galactose, were unable to bind LecA.[28]

In a recent publication, Reymond[14] studied the binding with LecA of 17 galactosides, 14 of which had an aromatic aglycon. ITC measurements found K_d values of 9.9 and 8.8 μM for the *S*-phenyl and *O*-phenyl galactoside respectively. These results are similar to the trends observed with our galactoclusters where galactoclusters with *O*- and *S*-phenyl displayed close affinities for LecA. Furthermore, they found a twice increase of affinity when comparing *O*-phenyl galactoside with *O*-naphthyl-β-D-galactoside (K_d = 8.8 and 4.2 μM respectively). Herein, we also found that the naphthyl aglycon in our galactoclusters was beneficial with respect to their binding to LecA.

The dockings of the following aromatic galactosides *O*-phenyl, *S*-phenyl, *O*-benzyl, *S*-benzyl, *O*-biphenyl and *O*-naphthyl in the carbohydrate recognition domain of LecA have been investigated. In their study using X-ray crystallography, Kadam *et al.* showed that the histidine residue (H50) specifically interacts with the aromatic ring of the galactoside aglycone.[14] This information is of primary importance in order to select poses in docking experiments. For four of the aglycones (*O*-biphenyl, *O*-naphthyl and *O*/*S*-phenyl), all docking solutions are correctly oriented with respect to H50. Since the amino-acid side chains (including H50) within the binding site of the lectin are considered as flexible, not only the "T-shape" ("edge to face") but also the "face to face" and the "parallel displaced" conformations were found. Experimental estimates indicate that these interactions are energetically attractive by *ca.* 1.5–2.5 kcal mol^{-1} in the solid state or at low temperatures in solution but generally disfavored in

solutions by entropic factors due to the restricted internal rotations.[36–39] According to values of ΔE(interaction), the four aglycones interacting with H50 are ranked as follows: *O*-naphthyl (−66.21 kcal mol^{-1}), *O*-biphenyl (−65.72 kcal mol^{-1}), *S*-phenyl (−60.98 kcal mol^{-1}) and *O*-phenyl (−55.80 kcal mol^{-1}). It must be noted that using the present force field, the value of ΔE(interaction) obtained for β galactose and LecA is −53.24 kcal mol^{-1}.

The docked structures of *O*-biphenyl (H50, face to face) and *O*-naphthyl (H50, edge to face) are given in Fig. 3 and *O*-phenyl (H50, edge to face) and *S*-phenyl (H50, edge to face) in Fig. S2.†

It should be noted that the proline residue P51 may interact with the π electron cloud of the aromatic moiety of the aglycone. This unique role of the proline amino-acid has been extensively studied.[40] Contributions of H50 and P51 to the potential energy of the interaction with phenyl, biphenyl and naphthyl rings have been evaluated and displayed in Fig. 4: *O*-biphenyl: ΔE(interaction) = −9.29 kcal mol^{-1} (approximate contributions: H50 = −5.81 kcal mol^{-1}, P51 = −3.80 kcal mol^{-1}); *O*-naphthyl: ΔE(interaction) = −7.75 kcal mol (H50 = −3.38 kcal mol^{-1}, P51 = −4.14 kcal mol^{-1}); *O*-phenyl: ΔE(interaction) = −2.41 kcal mol^{-1} (H50 = −1.88 kcal mol^{-1}, P51 = −0.41 kcal mol^{-1}).

Due to the presence of a methylene group, all docking solutions for *O*-benzyl and *S*-benzyl aglycones do not orient towards H50 even if their interaction energy is of the same order as the former ones (*S*-benzyl: −64.8 kcal mol^{-1}, *O*-benzyl: −63.6 kcal mol^{-1}). In this case, the aromatic ring interacts with the proline residue P38 (Fig. S3†). The present observations are in agreement with experimental and theoretical results by Kadam *et al.*[14]

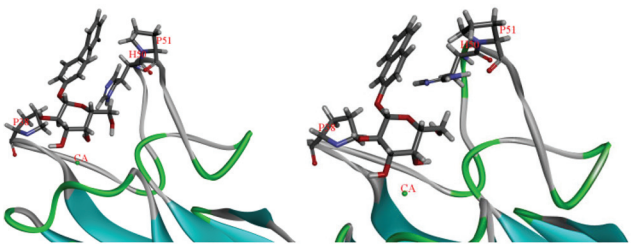


Fig. 3 Biphenyl and naphthyl-β-D-galactoside in the CRD of LecA.

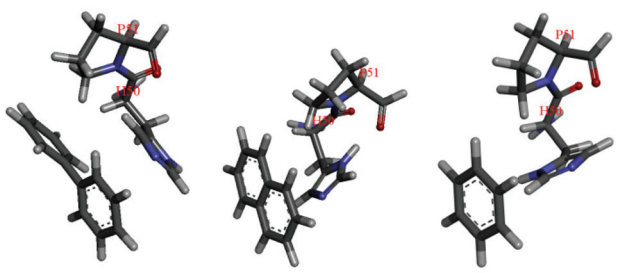


Fig. 4 Aromatic aglycon interaction with H50 and P51 of LecA. Left biphenyl: parallel displaced; middle naphthyl: edge to face and right phenyl: edge to face.

The **G21** galactocluster, exhibiting the lowest K_d value, was built in order to investigate some various docking arrangements with the tetrameric lectin LecA. The configurational space of **G21** was sampled using a Monte Carlo approach within the BOSS 4.9 software and the GB/SA methodology (20 million configuration attempts).[41,42] A conformational study was performed with **G21** alone.

The lowest energy conformation is shown below (Fig. 5). The corresponding potential energy (using the SPASIBA force field for comparison) is E = −63.7 kcal mol^{-1}. The very stable globular structure is maintained by a dense intramolecular hydrogen bond network. Among high energy conformers, an extended structure (E = 168.3 kcal mol^{-1}) is displayed in Fig. 5. Between these two forms a number of stable structures may be involved in the binding with LecA.

The galactocluster potential energy is used as a criterion for the complex stability. The most stable corresponds to one galactocluster (E = 31.4 kcal mol^{-1}) on one lectin with two interacting galactoses (Fig. 6).

Some possible arrangements were also found where one **G21** can bind two lectins according to a parallel arrangement (E = 53.7 kcal mol^{-1}, Fig. S4†) or to a linear arrangement E = 56.6 kcal mol^{-1}, Fig. S5†) or three galactoses of the same galactocluster interacting with three lectins in the Y (E = 79.8 kcal mol^{-1}) and T (E = 128.1 kcal mol^{-1}) arrangements (Fig. S6 and S7†).

The present docking simulations will be used in understanding future experiments by atomic force spectroscopy.

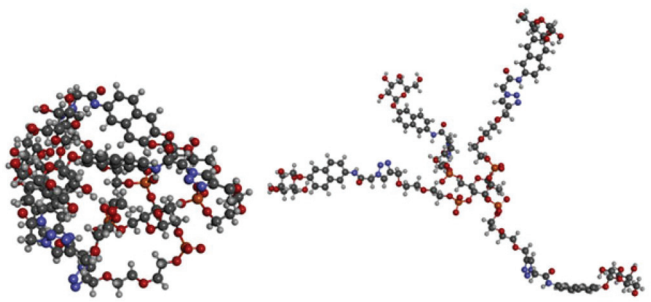


Fig. 5 Conformation of G21 at low energy (left) and high energy (right).

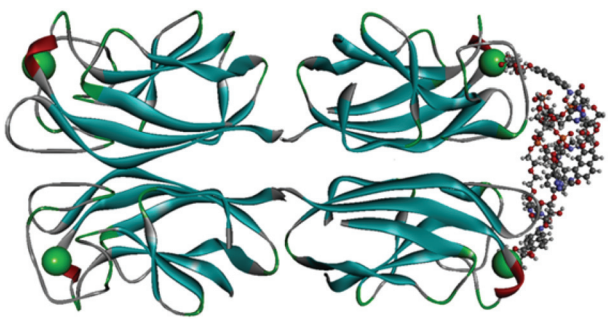


Fig. 6 Glycocluster **G21** bound to LecA. (Calcium ions are shown as green spheres).

Microarray experiments and docking simulation are in good agreement and underline the importance of the aromatic aglycon nature in the stabilization of the complex. Simulations also gave some insights into the energy involved during multiple contacts between one to three lectins and the galactocluster **G21**.

Experimental section

Synthesis

1-(4-Nitro-benzyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [8]. Under a nitrogen atmosphere, at 0 °C boron trifluoride diethyl etherate (1.5 mL, 12 mmol) was added dropwise to a solution of pentaacetate β-D-galactoside (1.561 g, 4 mmol) and *p*-nitrobenzyl alcohol (1.225 g, 8 mmol) in 20 mL of CH_2Cl_2. After a few minutes, the mixture was heated to reflux and was kept stirring for 7 h. The reaction was then quenched with water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was collected, dried with Na_2SO_4, and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (0 to 15% AcOEt in cyclohexane) to give **8** as a white solid (1.148 g, 59%). R_f = 0.36 (AcOEt–cyclohexane, 1 : 1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.21 (d, $^3J_{9-10}$ = 8.9 Hz, 2H, H-10, H-12), 7.47 (d, $^3J_{9-10}$ = 8.9 Hz, 2H, H-9, H-13), 5.42 (dd, $^3J_{3-4}$ = 3.4 and $^3J_{4-5}$ = 0.8 Hz, 1H, H-4), 5.32 (dd, $^3J_{2-3}$ = 10.5 and $^3J_{1-2}$ = 7.9 Hz, 1H, H-2), 5.04 (dd, $^3J_{2-3}$ = 10.5 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 5.02–4.72 (2 × d, $^2J_{7-7'}$ = 13.2 Hz, 2H, H-7), 4.60 (d, $^3J_{1-2}$ = 7.9 Hz, 1H, H-1), 4.21 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6}$ = 6.5 Hz, 1H, H-6), 4.15 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6'}$ = 6.5 Hz, 1H, H-6′), 3.94 (dt, $^3J_{4-5}$ = 0.8 and $^3J_{5-6}$ = 6.5 Hz, 1H, H-5), 2.17 (s, 3H, CH_3CO), 2.06 (s, 6H, 2 × CH_3CO), 1.99 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.3, 170.2, 169.5 (4 × CO-Ac), 147.7 (C-11), 144.6 (C-8), 127.7 (C-9), 123.8 (C-10), 100.8 (C-1), 71.1 (C-5), 70.9 (C-3), 69.6 (C-7), 68.9 (C-2), 67.1 (C-4), 61.4 (C-6), 20.9, 20.8, 20.8, 20.7(4 × CH_3-Ac). HRMS (ESI/Q-TOF): calculated for $C_{21}H_{25}NO_{12}Na$ $[M + Na]^+$ 506.1274, found 506.1282. $[\alpha]_D^{20} = -19.1°$ (*c* 0.9, MeOH).

General procedure for the hydrogenolysis (Method A). Compound **8**, **9**[31,43] or **10**[32,44] was dissolved in distilled CH_2Cl_2 (35 mL mmol^{-1}) to which was added 10% palladium on charcoal (10% w/w). Hydrogen gas was bubbled into the reaction mixture until starting materials disappeared as judged by TLC. The reaction mixture was filtered over a celite pad and washed with CH_2Cl_2. The crude **11** and **12** were purified by silica gel flash column chromatography to afford the desired products while crude **13** was directly used for the next reaction affording **16** (see below).

General procedure for the synthesis of 4-bromoacetamidoaryl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (Method B). A solution of **11** or **12** (1 eq.) in distilled CH_2Cl_2 (50 mL mmol^{-1}) was flushed with argon, cooled to 0 °C, and Et_3N (1.4 eq.) was added. Bromoacetyl bromide (1.3 eq.) was added dropwise and the mixture was stirred for 1 h at 0 °C. The mixture was allowed to warm up at rt for 1 h. The crude mixture in CH_2Cl_2 was washed with 1 N HCl (2 × 25 mL), water (2 × 25 mL) and brine (25 mL). After drying (Na_2SO_4), concentration and total removal of CH_2Cl_2 under vacuum, the residue was purified by silica gel column chromatography to afford the desired products **14–15**.

General procedure for the synthesis of 4-azidooacetamidoaryl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (Method C). A solution of **14**, **15** or **16** (1 eq.) and tetramethylguanidinium azide ($TMGN_3$) (3 eq.) in anhydrous CH_3CN (25 mL mmol^{-1}) was stirred at 80 °C for 15 minutes with microwave assistance. After concentration under vacuum, the residues were purified by silica gel column chromatography to afford the desired products.

General procedure for deacetylation of carbohydrates (Method D). The acetylated glycosides (4-(azidoacetamide) phenyl-β-D-galactoside,[19] **17–19** or **28–29**) were suspended in MeOH or 1,4-dioxane and 30% ammonia solution was added (1 : 1, v/v). The mixture was stirred at room temperature for 6 hours to 1 day. The solvent was evaporated under vacuum to afford the desired products **1–6**, which were dissolved in methanol to give a 100 mM stock solution used for the CuAAc reactions.

General procedure for glycosidation (Method E). To a solution of **7** (1 eq.), **22** or **23** (2 eq.), and tetrabutylammonium-hydrogen sulfate (1 eq.) in CH_2Cl_2 at 0 °C a 1 M aq. solution of NaOH was added. The biphasic mixture was stirred at rt for 36 h, then diluted with CH_2Cl_2, washed with 1 M NaOH (2 × 30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography to afford the desired products.

General procedure for azidation of biarylgalactopyranosides (Method F). **26** or **27** (1 eq.) was dissolved in anhydrous DMF, followed by the addition of 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDC) (1.6 eq.) and hydroxybenzotriazole (1.1 eq.). 3-Azidopropylamine (2 eq.) was added and the reaction was stirred at room temperature for 12 h. The reaction was concentrated, quenched with water and extracted with CH_2Cl_2. The organic layer was dried with Na_2SO_4, concentrated and purified by silica gel column chromatography to afford the desired product.

1-(4-Amino-benzyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [11]. Obtained as a white solid (412 mg, 45%) following Method A: **8** (968 mg, 2.00 mmol), Pd/C 10% (96.8 mg), in distilled CH_2Cl_2 (30 mL). The mixture was worked up, the aqueous layer was extracted with CH_2Cl_2 and the crude product was purified on silica gel (0 to 5% MeOH in CH_2Cl_2) to afford the pure product. R_f = 0.43 (AcOEt–cyclohexane, 6 : 4, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 7.02 (d, $^3J_{9-10}$ = 8.1 Hz, 2H, H-10, H-12), 6.60 (d, $^3J_{9-10}$ = 8.1, 2H, H-9, H-13), 5.32 (d, $^3J_{3-4}$ = 3.3 Hz, 1H, H-4), 5.18 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 8.0 Hz, 1H, H-2), 4.91 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.3, 1H, H-3), 4.71–4.46 (2 × d, $^2J_{7-7'}$ = 11.9, 2H, H-7), 4.42 (d, $^3J_{1-2}$ = 8.0, 2H, H-1), 4.15 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6}$ = 6.5 Hz, 1H, H-6), 4.10 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6'}$ = 6.5 Hz, 1H, H-6′), 3.81 (t, $^3J_{5-6,5-6'}$ = 6.5, 1H, H-5), 2.09 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.91 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.4, 170.2, 169.5 (4 CO Ac), 146.6

(C-11), 129.8 (C-8), 126.3 (C-9), 115.0 (C-10), 99.2 (C-1), 71.1 (C-5), 70.8 (C-3), 70.7 (C-7), 69.0 (C-2), 67.3 (C-4), 61.5 (C-6), 20.8, 20.8, 20.7, 20.6 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{21}H_{28}NO_{10}$ $[M + H]^+$ 454.1713, found 454.1718. $[\alpha]_D^{20}$ = −25.0° (*c* 0.4, MeOH).

4-Amino-benzyl-1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [12]. Obtained as colourless oil (314 mg, 79%) following Method A: **9** (425 mg, 0.851 mmol), Pd/C 10% (42.5 mg), in distilled CH_2Cl_2 (15 mL). The mixture was worked up, the aqueous layer was extracted with CH_2Cl_2 and the crude product was purified on silica gel (0 to 5% MeOH in CH_2Cl_2) to afford **12**. R_f = 0.26 (AcOEt–cyclohexane, 6 : 4, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 7.08 (d, $^3J_{9-10}$ = 8.1 Hz, 2H, H-9, H-13), 6.63 (d, $^3J_{9-10}$ = 8.1 Hz, 2H, H-10, H-12), 5.40 (d, $^3J_{3-4}$ = 3.3 Hz, 1H, H-4), 5.26 (t, $^3J_{1-2,\ 2-3}$ = 9.9 Hz, 1H, H2), 4.96 (dd, $^3J_{2-3}$ = 9.9 and $^3J_{3-4}$ = 3.3 Hz, 1H, H-3), 4.27 (d, $^3J_{1-2}$ = 9.9 Hz, 1H, H-1), 4.17 (dd, $^2J_{6-6'}$ = 11.3 and $^3J_{5-6}$ = 6.6 Hz, 1H, H-6), 4.11 (dd, $^2J_{6-6'}$ = 11.3 and $^3J_{5-6'}$ = 6.6 Hz, 1H, H-6′), 3.86 (d, $^2J_{7-7'}$ = 12.9 Hz, 1H, H-7), 3.81 (t, $^3J_{5-6,\ 5-6'}$ = 6.6 Hz, 1H, H-5), 3.75 (d, $^2J_{7-7'}$ = 12.9 Hz, 1H, H-7), 2.14 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.4, 170.2, 169.7 (4 CO Ac), 145.8 (C-11), 130.3 (C-9), 126.5 (C-8), 115.3 (C-10), 82.5 (C-1), 74.5 (C-5), 72.0 (C-3), 67.5 (C-4), 67.3 (C-2), 61.8 (C-6), 33.7 (7), 20.9, 20.8, 20.8, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{21}H_{28}NO_9S$ $[M + H]^+$ 470.1485, found 470.1489. $[\alpha]_D^{20}$ = −73.8° (*c* 1.0, MeOH).

4-Bromoacetamidobenzyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [14]. Obtained as pale yellow oil (238 mg, 79%) following Method B: **11** (239 mg, 0.527 mmol), Et_3N (0.103 mL, 0.738 mmol), bromoacetyl bromide (0.059 mL, 0.685 mmol) in distilled CH_2Cl_2 (30 mL). The mixture was worked up, and the crude product was purified on silica gel (0 to 60% AcOEt in cyclohexane) to afford **14**. R_f = 0.31 (AcOEt–cyclohexane, 6 : 4, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.23 (s, 1H, H-14), 7.51 (d, $^3J_{9-10}$ = 8.4 Hz, 2H, H-10, H-12), 7.26 (d, $^3J_{9-10}$ = 8.4 Hz, 2H, H-9, H-13), 5.37 (dd, $^3J_{3-4}$ = 3.4 and $^3J_{4-5}$ = 0.9 Hz, 1H, H-4), 5.25 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 7.9 Hz, 1H, H-2), 4.97 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 4.85–4.59 (2 × d, $^2J_{7-7'}$ = 12.2 Hz, 2H, H-7), 4.50 (d, $^3J_{1-2}$ = 7.9 Hz, 1H, H-1), 4.19 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6}$ = 6.5 Hz, 1H, H-6), 4.13 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6'}$ = 6.5 Hz, 1H, H-6′), 3.99 (s, 2H, H-16), 3.88 (dt, $^3J_{4-5}$ = 0.9 and $^3J_{5-6,\ 5-6'}$ = 6.5 Hz, 1H, H-5), 2.13 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.6, 170.4, 170.3, 169.6 (4 × CO-Ac), 163.8 (C-15), 137.0 (C-11), 133.7 (C-8), 128.7 (C-9), 120.2 (C-10), 100.0 (C-1), 71.1 (C-3), 71.0 (C-5), 70.4 (C-7), 69.0 (C-2), 67.3 (C-4), 61.5 (C-6), 29.6 (C-16), 20.9, 20.8, 20.8, 20.7 (4 × CH_3-Ac). HRMS (ESI/Q-TOF): calculated for $C_{23}H_{29}BrNO_{11}$ $[M + H]^+$ 574.0924, found 574.0933. $[\alpha]_D^{20}$ = −13.1° (*c* 2.6, MeOH).

4-Bromoacetamidobenzyl-1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [15]. Obtained as a yellow oil (367 mg, 93%) following Method B: **12** (314 mg, 0.669 mmol), Et_3N (0.130 mL, 0.937 mmol), bromoacetyl bromide (0.075 mL, 0.869 mmol) in distilled CH_2Cl_2 (10 mL). The mixture was worked up, and the crude product was purified on silica gel (0 to 40% AcOEt in cyclohexane) to afford the desired product. R_f = 0.28 (AcOEt–cyclohexane, 1 : 1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.23 (s, 1H, H-14), 7.47 (d, $^3J_{9-10}$ = 8.5 Hz, 2H, H-10, H-12), 7.26 (d, $^3J_{9-10}$ = 8.5 Hz, 2H, H-9, H-13), 5.37 (dd, $^3J_{3-4}$ = 3.3 and 0.8 Hz, 1H, H-4), 5.23 (t, $^3J_{1-2,\ 2-3}$ = 10.0 Hz, 1H, H-2), 4.94 (dd, $^3J_{2-3}$ = 10.0 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 4.25 (d, $^3J_{1-2}$ = 10.0 Hz, 1H, H-1), 4.12 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6}$ = 6.7 Hz, 1H, H-6), 4.05 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6'}$ = 6.4 Hz, 1H, H-6′), 3.98 (s, 2H, H-16), 3.90, 3.81 (2 × d, $^2J_{7-7'}$ = 13.0 Hz, each 1H, H-7), 3.78 (m, 1H, H-5), 2.12 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.6, 170.4, 170.2, 169.8 (4 CO Ac), 163.74 (C-15), 136.4 (C-11), 133.9 (C-8), 130.0 (C-9), 120.3 (C-10), 82.6 (C-1), 74.6 (C-5), 72.0 (C-3), 67.5 (C-4), 67.3 (C-2), 61.7 (C-6), 33.4 (C-7), 29.6 (C-16), 20.9, 20.8, 20.8, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{23}H_{29}NO_{10}BrS$ $[M + H]^+$ 590.0696, found 590.0688. $[\alpha]_D^{20}$ = −56.7° (*c* 2.0, MeOH).

4-Bromoacetamidophenyl-1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [16]. A solution of **10** (497 mg, 1.02 mmol) in anhydrous CH_2Cl_2 (20 mL) was degassed and then Pd/C 10% (49.7 mg) was added. The solution was subjected to a hydrogen atmosphere and stirred at rt for 3 days. After total disappearance of the starting material, a mixture of **13** was flushed with argon, cooled to 0 °C, and Et_3N (0.043 mL, 0.308 mmol) was added. Bromoacetyl bromide (0.025 mL, 0.286 mmol) was added dropwise and the mixture was stirred for 1 h at 0 °C. The mixture was allowed to warm up at rt for 1 h, filtered through a plug of celite and washed with CH_2Cl_2. The crude mixture in CH_2Cl_2 was washed with 1 N HCl (2 × 25 mL), water (2 × 25 mL) and brine (25 mL). After drying (Na_2SO_4), concentration and total removal of CH_2Cl_2 with vacuum, the residue was purified by silica gel column chromatography (0 to 30% AcOEt in cyclohexane) to give the product as a yellow oil (505.6 mg, 86% total). R_f = 0.33 (AcOEt–cyclohexane, 6 : 4, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.15 (s, 1H, H-13), 7.52 (m, 2H, H-9, H-11), 7.51 (m, 2H, H-8, H-12), 5.40 (dd, $^3J_{3-4}$ = 3.3 and $^3J_{4-5}$ = 0.8 Hz, 1H, H-4), 5.19 (t, $^3J_{1-2,\ 2-3}$ = 9.9 Hz, 1H, H-2), 5.04 (dd, $^3J_{2-3}$ = 9.9 and $^3J_{3-4}$ = 3.3 Hz, 1H, H-3), 4.65 (d, $^3J_{1-2}$ = 9.9 Hz, 1H, H-1), 4.17 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6}$ = 6.9 Hz, 1H, H-6), 4.11 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6'}$ = 6.9 Hz, 1H, H-6′), 4.01 (s, 2H, H-15), 3.91 (dt, $^3J_{4-5}$ = 0.8 and $^3J_{5-6,\ 5-6'}$ = 6.9 Hz 1H, H-5), 2.11 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.3, 170.2, 169.5 (4 CO Ac), 163.5 (C-14), 137.4 (C-10), 134.3 (C-8), 128.2 (C-7), 120.3 (C-9), 86.7 (C-1), 74.7 (C-5), 72.1 (C-3), 67.4 (C-4), 61.7 (C-6), 29.5 (C-15), 21.0, 20.8, 20.8, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{22}H_{26}NO_{10}NaSBr$ $[M + Na]^+$ 598.0358, found 598.0360. $[\alpha]_D^{20}$ = −13.0° (*c* 2.2, MeOH).

4-Azidoacetamidobenzyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [17]. Obtained as a white solid (148 mg, 94%) following Method C: **14** (168 mg, 0.292 mmol), $TMGN_3$ (138.6 mg, 0.876 mmol) in anhydrous CH_3CN (4 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 40% AcOEt in cyclohexane) to afford the desired product. R_f = 0.28 (AcOEt–cyclohexane, 1 : 1, v/v). ^{1}H NMR (600 MHz,

$CDCl_3$) δ ppm: 8.05 (s, 1H, NH), 7.50 (d, $^3J_{9-10}$ = 8.8 Hz, 2H, H-10, H-12), 7.24 (d, $^3J_{9-10}$ = 8.8 Hz, 2H, H-9, H-13), 5.35 (d, $^3J_{3-4}$ = 3.4 Hz, 1H, H-4), 5.23 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 7.9 Hz, 1H, H-2), 4.95 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 4.83 (d, $^2J_{7-7'}$ = 12.2 Hz, 1H, H-7), 4.57 (d, $^2J_{7-7'}$ = 12.2 Hz, 1H, H-7′), 4.48 (d, $^3J_{1-2}$ = 7.9 Hz, 1H, H-1), 4.17 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6}$ = 6.4 Hz, 1H, H-6), 4.13 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6'}$ = 6.4 Hz, 1H, H-6′), 4.10 (s, 2H, H-16), 3.85 (t, $^3J_{5-6,\ 5-6'}$ = 6.4 Hz, 1H, H-5), 2.12 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.4, 170.3, 170.1, 169.4 (4 CO Ac), 164.6 (C-15), 136.6 (C-11), 133.4 (C-8), 128.6 (C-9), 120.0 (C-10), 99.8 (C-1), 70.9 (C-3), 70.8 (C-5), 70.2 (C-7), 68.9 (C-2), 67.1 (C-4), 61.3 (C-6), 53.0 (C-16), 20.8, 20.7, 20.7, 20.6 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{23}H_{29}N_4O_{11}$ $[M + H]^+$ found 537.1840. $[\alpha]_D$ = −18.0° (*c* 1.0, MeOH).

4-Azidoacetamidobenzyl-β-D-galactopyranoside [3]. HRMS (ESI/Q-TOF): calculated for $C_{15}H_{21}N_4O_7$ $[M + H]^+$ 369.1410, found 369.1411.

4-Azidoacetamidobenzyl-1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [18]. Obtained as light brown crystals (99 mg 94%) following Method C: starting from **15** (112 mg, 0.190 mmol) and $TMGN_3$ (90.2 mg, 0.570 mmol) in anhydrous CH_3CN (5 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 40% AcOEt in cyclohexane) to afford the desired product R_f = 0.26 (AcOEt–cyclohexane, 1 : 1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.20 (s, 1H, NH), 7.50 (d, $^3J_{9-10}$ = 8.4 Hz, 2H, H-10, H-12), 7.27 (d, $^3J_{9-10}$ = 8.4 Hz, 2H, H-9, H-13), 5.39 (d, $^3J_{3-4}$ = 3.3 Hz, 1H, H-4), 5.25 (t, $^3J_{1-2,\ 2-3}$ = 10.0 Hz, 1H, H2), 4.96 (dd, $^3J_{2-3}$ = 10.0 and $^3J_{3-4}$ = 3.3 Hz, 1H, H-3), 4.28 (d, $^3J_{1-2}$ = 10.0 Hz, 1H, H-1), 4.13 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6}$ = 6.7 Hz, 1H, H-6), 4.10 (s, 2H, H-16), 4.07 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6'}$ = 6.7 Hz, 1H, H-6′), 3.92, 3.81 (2 × d, $^2J_{7-7'}$ = 13.0 Hz, each 1H, H-7), 3.80 (d, $^3J_{5-6,\ 5-6'}$ = 6.7 Hz, 1H, H-5), 2.14 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.4, 170.3, 170.1, 169.7 (4CO Ac), 164.9 (C-15), 136.1 (C-11), 133.6 (C-8), 129.8 (C-9), 120.2 (C-10), 82.4 (C-1), 74.4 (C-5), 71.2 (C-3), 67.4 (C-4), 67.09 (C-2), 61.6 (C-6), 52.90 (C-16), 33.3 (C-7), 20.8, 20.7, 20.7, 20.6 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{23}H_{29}N_4O_{10}S$ $[M + H]^+$ 553.1604, found 553.1621. $[\alpha]_D^{20}$ = −53.4° (*c* 1.0, MeOH).

4-Azidoacetamidobenzyl-1-thio-β-D-galactopyranoside [4]. HRMS (ESI+): calculated for $C_{15}H_{21}N_4O_6S$ $[M + H]^+$ 385.1182, found 385.1185.

4-Azidoacetamidophenyl-1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside [19]. Obtained as colourless oil (56 mg 55%) following Method C: **16** (109 mg, 0.189 mmol), $TMGN_3$ (89.7 mg, 0.567 mmol) in anhydrous CH_3CN (4 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 40% AcOEt in cyclohexane) to afford the desired product R_f = 0.28 (AcOEt–cyclohexane, 6 : 4, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.08 (s, 1H, NH), 7.51 (m, 2H, H-9, H-11), 7.49 (m, 2H, H-8, H-12), 5.39 (dd, $^3J_{3-4}$ = 3.3 and $^3J_{4-5}$ = 0.9 Hz, 1H, H-4), 5.18 (t, $^3J_{1-2,\ 2-3}$ = 9.9 Hz, 1H, H-2), 5.03 (dd, $^3J_{2-3}$ = 9.9 and $^3J_{3-4}$ = 3.3 Hz, 1H, H-3), 4.64 (d, $^3J_{1-2}$ = 9.9 Hz, 1H, H-1), 4.16 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6}$ = 6.9 Hz, 1H, H-6), 4.13 (s, 2H, H-15), 4.09 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6'}$ = 6.9 Hz, 1H, H-6′), 3.90 (dt, $^3J_{4-5}$ = 0.8 and $^3J_{5-6,5-6'}$ = 6.9 Hz, 1H, H-5), 2.10 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.3, 170.1, 169.5 (4 CO Ac), 164.7 (C-14), 137.3 (C-10), 134.2 (C-8), 128.0 (C-7), 120.4 (C-9), 86.7 (C-1), 74.6 (C-5), 72.1 (C-3), 67.4 (C-4), 61.7 (C-6), 53.08 (C-15), 20.9, 20.8, 20.7, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{22}H_{27}N_4O_{10}S$ $[M + H]^+$ 539.1448, found 539.1450. $[\alpha]_D^{20}$ = −12.3° (*c* 1.3, MeOH).

4-Azidoacetamidophenyl-1-thio-β-D-galactopyranoside [2]. HRMS (ESI/Q-TOF): calculated for $C_{14}H_{19}N_4O_6S$ $[M + H]^+$ 371.1025, found 371.1031.

Benzyl 4′-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-biphenyl-4-carboxylate 24. Obtained as a white solid (2.189 g, 99%) following Method E: **7** (1.439 g, 3.5 mmol), benzyl 4′-hydroxy-biphenyl-4-carboxylate **22**[45] (2.464 g, 7.05 mmol), tetrabutylammoniumhydrogensulfate (1.188 g, 3.5 mmol) in distilled CH_2Cl_2 (15 mL), a 1 M aq. solution of NaOH (5 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 30% AcOEt in cyclohexane) to afford the desired product R_f = 0.39 (AcOEt–cyclohexane, 1 : 1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.13 (d, $^3J_{12-13}$ = 8.5 Hz, 2H, H-13, H-15), 7.60 (d, $^3J_{12-13}$ = 8.5 Hz, 2H, H-12, H-16), 7.56 (d, $^3J_{8-9}$ = 8.8 Hz, 2H, H-9, H-17), 7.46 (d, $^3J_{22-23}$ = 7.2 Hz, 2H, H-22, H-26), 7.40 (t, $^3J_{22-23,\ 23-24}$ = 7.2 Hz, 2H, H-23, H-25), 7.34 (t, $^3J_{23-24}$ = 7.2 Hz, 1H, H-24), 7.10 (d, $^3J_{8-9}$ = 8.8 Hz, 2H, H-8, H-18), 5.52 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 8.0 Hz, 1H, H-2), 5.48 (dd, $^3J_{3-4}$ = 3.4 and $^3J_{4-5}$ = 0.8 Hz, 1H, H-4), 5.39 (s, 2H, H-20), 5.14 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 5.11 (d, $^3J_{1-2}$ = 8.0 Hz, 1H, H-1), 4.25 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6}$ = 7.0 Hz, 1H, H-6), 4.18 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6'}$ = 6.4 Hz, 1H, H-6′), 4.09 (ddd, $^3J_{5-6}$ = 7.0 and $^3J_{5-6'}$ = 6.4 and $^3J_{4-5}$ = 0.8 Hz, 1H, H-5), 2.19 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.4, 170.3, 170.2, 169.5 (4 CO Ac), 166.4 (C-19), 157.2 (C-7), 145.1 (C-11), 136.3 (C-21), 135.3 (C-10), 130.4 (C-13), 128.9 (C-14), 128.7 (C-23), 128.6 (C-9), 128.4 (C-24), 128.3 (C-22), 126.9 (C-12), 117.5 (C-8), 99.7 (C-1), 71.3 (C-5), 71.0 (C-3), 68.8 (C-2), 67.0 (C-4), 66.8 (C-20), 61.5 (C-6), 20.9, 20.8, 20.7, 20.6 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{34}H_{34}O_{12}Na$ $[M + Na]^+$ 657.1948, found 657.1948. $[\alpha]_D^{20}$ = +6.0° (*c* 1.2, 1,4-dioxane).

4′-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)-biphenyl-4-carboxylic acid [26]. Obtained as a white solid (691 mg, 37%) following Method A: **24** (2.189 g, 3.45 mmol), Pd/C 10% (219 mg), in distilled CH_2Cl_2 (30 mL). The mixture was worked up, the aqueous layer was extracted with CH_2Cl_2 and the crude product was purified on silica gel (0 to 50% AcOEt in cyclohexane) to afford the desired product. R_f = 0.44 (MeOH–CH_2Cl_2, 1 : 9, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ δ ppm: 8.17 (d, $^3J_{12-13}$ = 8.4 Hz, 2H, H-13, H-15), 7.65 (d, $^3J_{12-13}$ = 8.4 Hz, 2H, H-12, H-16), 7.58 (d, $^3J_{8-9}$ = 8.7 Hz, 2H, H-9, H-17), 7.11 (d, $^3J_{8-9}$ = 8.7 Hz, 2H, H-8, H-18), 5.53 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 7.9 Hz, 1H, H-2), 5.48 (d, $^3J_{3-4}$ = 3.4 Hz, 1H, H-4), 5.15 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 5.12 (d, $^3J_{1-2}$ = 7.9 Hz, 1H,

H-1), 4.26 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6}$ = 7.0 Hz, 1H, H-6), 4.19 (dd, $^2J_{6-6'}$ = 11.4 and $^3J_{5-6'}$ = 6.4 Hz, 1H, H-6′), 4.11 (m, 1H, H-5), 2.20 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 171.3 (C-19), 170.5, 170.4, 170.3, 169.5 (4 CO Ac), 157.3 (C-7), 145.8 (C-11), 135.2 (C-10), 131.0 (C-13), 128.7 (C-9), 127.9 (C-14), 127.0 (C-12), 117.5 (C-8), 99.7 (C-1), 71.3 (C-5), 71.0 (C-3), 68.8 (C-2), 67.0 (C-4), 61.5 (C-6), 20.9, 20.8, 20.7, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{27}H_{28}O_{12}Na$ $[M + Na]^+$ 567.1478, found 567.1489. $[\alpha]_D$ = +6.6° (*c* 1.1, 1,4-dioxane).

4′-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)-biphenyl-4-carboxylic acid 3-azido-propyl-amide [28]. Obtained as a white solid (47 mg, 74%) following Method F: **26** (131 mg, 0.102 mmol), 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (25.3 mg, 0.163 mmol), hydroxybenzotriazole (15.1 mg, 0.112 mmol), 3-azidopropylamine (20.4 mg, 0.204 mmol) in anhydrous DMF (5 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 50% AcOEt in cyclohexane) to afford the desired product. R_f = 0.34 (MeOH–CH_2Cl_2, 2 : 98, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 7.81 (d, $^3J_{12-13}$ = 8.2 Hz, 2H, H-13, H-15), 7.58 (d, $^3J_{12-13}$ = 8.2 Hz, 2H, H-12, H-16), 7.52 (d, $^3J_{8-9}$ = 8.6 Hz, 2H, H-9, H-17), 7.06 (d, $^3J_{8-9}$ = 8.6 Hz, 2H, H-8, H-18), 6.46 (t, $^3J_{NH-21}$ = 5.7 Hz, 1H, H-20), 5.49 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 8.0 Hz, 1H, H-2), 5.45 (d, $^3J_{3-4}$ = 3.4 Hz, 1H, H-4), 5.11 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 5.08 (d, $^3J_{1-2}$ = 8.0, 1H, H-1), 4.22 (dd, $^2J_{6-6'}$ = 11.3 and $^3J_{5-6}$ = 6.7 Hz, 1H, H-6), 4.15 (dd, $^2J_{6-6'}$ = 11.3 and $^3J_{5-6'}$ = 6.7 Hz, 1H, H-6′), 4.08 (t, $^3J_{5-6,\ 5-6'}$ = 6.7 Hz, 1H, H-5), 3.56 (q, $^3J_{NH-21,\ 21-22}$ = 6.4 Hz, 2H, H-21), 3.44 (t, $^3J_{22-23}$ = 6.4 Hz, 2H, H-23), 2.17 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.91 (p, $^3J_{21-22,\ 22-23}$ = 6.4 Hz, 2H, H-22). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.3, 170.2, 169.5 (4 CO Ac), 167.4 (C-19), 157.1 (C-7), 143.6 (C-11), 135.3 (C-10), 133.0 (C-14), 128.5 (C-9), 127.6 (C-13), 127.1 (C-12), 117.4 (C-8), 99.7 (C-1), 71.2 (C-5), 70.9 (C-3), 68.8 (C-2), 67.0 (C-4), 61.5 (C-6), 49.8 (C-23), 38.0 (C-21), 28.9 (C-22), 20.9, 20.8, 20.8, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{30}H_{35}N_4O_{11}$ $[M + H]^+$ 627.2302, found 627.2304. $[\alpha]_D^{20}$ = +3.8° (*c* 3.2, 1,4-dioxane).

Benzyl 4′-(β-D-galactopyranosyloxy)-biphenyl-4-carboxylic acid 3-azido-propyl-amide [5]. HRMS (ESI/Q-TOF): calculated for $C_{22}H_{27}N_4O_7$ $[M + H]^+$ 459.1880, found 459.1884.

Benzyl-6-hydroxy-2-naphthoate [23]. To a solution of 6-hydroxy-2-naphthoic acid (1.882 g, 10 mmol), in aqueous methanol 90% (20 mL), Cs_2CO_3 (1.629 g, 5 mmol) was added. The solution was stirred at room temperature for 30 min. The solvent was evaporated at reduced pressure and then co-evaporated with toluene (2 × 10 mL). The resulting cesium salt was suspended in anhydrous DMF (10 mL), cooled to 0 °C and benzyl bromide (1.19 mL, 10 mmol) was added. After 1 h stirring, the solution was allowed to warm up to room temperature and stirring was continued for a further 10 h before the solvent was removed under reduced pressure. The residue was taken up into water (2 × 20 mL) and then extracted with AcOEt (200 mL) and the combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (0 to 30% AcOEt in cyclohexane) to give the product as a white solid (2.095 g, 75%). R_f = 0.47 (cyclohexane–AcOEt, 1 : 1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.57 (d, $^4J_{1-3}$ = 1.7 Hz, 1H, H-1), 8.05 (dd, $^3J_{3-4}$ = 8.6 and $^4J_{1-3}$ = 1.7 Hz, 1H, H-3), 7.85 (d, $^3J_{7-8}$ = 8.8 Hz, 1H, H-8), 7.69 (d, $^3J_{3-4}$ = 8.6 Hz, 1H, H-4), 7.50 (d, $^3J_{14-15}$ = 7.3 Hz, 1H, H-14), 7.42 (t, $^3J_{14-15,\ 15-16}$ = 7.3 Hz, 1H, H-15), 7.36 (t, $^3J_{15-16}$ = 7.3 Hz, 1H, H-16), 7.18 (d, $^4J_{5-7}$ = 2.4 Hz, 1H, H-5), 7.16 (dd, $^3J_{7-8}$ = 8.8 and $^4J_{5-7}$ = 2.4 Hz, 1H, H-7), 5.63 (s, 1H, OH), 5.43 (s, 2H, H-12). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 167.1 (C-11), 155.9 (C-6), 137.4 (C-10), 136.3 (C-13), 131.7 (C-8), 131.4 (C-1), 128.8 (C-15), 128.4 (C-16), 128.4 (C-14), 128.0 (C-10), 126.7 (C-4), 126.2 (C-3), 125.2 (C-9), 118.9 (C-7), 109.7 (C-5), 67.1 (C-12). HRMS (ESI/Q-TOF): calculated for $C_{18}H_{15}O_3$ $[M + H]^+$ 279.1021; found 279.1024.

Benzyl-6-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-2-naphthoate [25]. Obtained as a white solid (1.239 mg, 77%) following Method E: **7** (1.082 g, 2.63 mmol), **23** (1.464 g, 5.26 mmol), tetrabutylammoniumhydrogensulfate (0.823 g, 2.63 mmol) in distilled CH_2Cl_2 (15 mL), a 1 M aq. solution of NaOH (5 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 30% AcOEt in cyclohexane) to afford the desired product R_f = 0.38 (AcOEt–cyclohexane, 1 : 1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.59 (d, $^4J_{10-12}$ = 1.6 Hz, 1H, H-12), 8.09 (dd, $^3J_{9-10}$ = 8.7 and $^4J_{10-12}$ = 1.6 Hz, 1H, H-10), 7.89 (d, $^3J_{13-14}$ = 8.8 Hz, 1H, H-13), 7.76 (d, $^3J_{9-10}$ = 8.7 Hz, 1H, H-9), 7.49 (d, $^3J_{20-21}$ = 7.2 Hz, 2H, H-20, H-24), 7.41 (t, $^3J_{20-21,\ 21-22}$ = 7.2 Hz, 2H, H-21, H-23), 7.37 (d, $^4J_{8-14}$ = 2.4, 1H, H-8), 7.36 (m, 1H, H-22), 7.24 (dd, $^3J_{13-14}$ = 8.8 and $^4J_{8-14}$ = 2.4 Hz, 1H, H-14), 5.56 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 7.9, 1H, H-2), 5.50 (dd, $^3J_{3-4}$ = 3.4 and $^3J_{4-5}$ = 0.8 Hz, 1H, H-4), 5.42 (s, 2H, H-18), 5.24 (d, $^3J_{1-2}$ = 7.9 Hz, 1H, H-1), 5.17 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 4.27 (dd, $^2J_{6-6'}$ = 11.1 and $^2J_{5-6}$ = 6.8 Hz, 2H, H-6), 4.18–4.15 (m, 1H, H-5), 2.20 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.4, 170.3, 169.6 (4 CO Ac), 166.70 (C-17), 156.7 (C-7), 136.9 (C-15), 136.3 (C-19), 131.5 (C-13), 131.2 (C-12), 129.3 (C-16), 128.9 (C-21), 128.50 (C-20), 127.5 (C-9), 126.6 (C-10), 126.5 (C-11), 119.8 (C-14), 111.2 (C-8), 99.5 (C-1), 71.5 (C-5), 71.0 (C-3), 68.9 (C-2), 67.1 (C-4), 67.1 (C-18), 61.8 (C-6), 20.9, 20.9, 20.9, 20.8 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{32}H_{32}O_{12}Na$ $[M + Na]^+$ 631.1791, found 631.1788. $[\alpha]_D^{20}$ = −11.2° (*c* 1.1, MeOH).

6-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)-2-naphthoic acid [27]. Obtained as a white solid (806 mg, 76%) following Method A: **25** (1.239 g, 2.04 mmol), Pd/C 10% (124 mg), in distilled CH_2Cl_2 (15 mL). The mixture was worked up, the aqueous layer was extracted with CH_2Cl_2 and the crude product was purified on silica gel (0 to 50% AcOEt in cyclohexane) to afford the desired product. R_f = 0.44 (MeOH–CH_2Cl_2, 6 : 94, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.66 (d, $^4J_{10-12}$ = 1.6 Hz, 1H, H-12), 8.11 (dd, $^3J_{9-10}$ = 8.6 and $^4J_{10-12}$ = 1.6 Hz, 1H, H-10), 7.93 (d, $^3J_{13-14}$ = 9.0 Hz, 1H, H-13), 7.80 (d, $^3J_{9-10}$ = 8.6 Hz, 1H, H-9), 7.39 (d, $^4J_{8-14}$ = 2.4 Hz, 1H, H-8), 7.27 (dd, $^3J_{13-14}$ = 9.0 and $^4J_{8-14}$ = 2.4 Hz, 1H, H-14), 5.57 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 7.9 Hz, 1H, H-2), 5.51 (d, $^3J_{3-4}$ = 3.5 Hz, 1H,

H-4), 5.26 (d, $^3J_{1-2}$ = 7.8 Hz, 1H, H-1), 5.18 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.5 Hz, 1H, H-3), 4.30–4.19 (m, 2H, H-6), 4.19–4.17 (m, 1H, H-5), 2.20 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 171.6 (C-17), 170.6, 170.4, 170.3, 169.6 (4 CO Ac), 157.0 (C-7), 137.3 (C-15), 132.14 (C-12), 131.7 (C-13), 129.3 (C-16), 127.6 (C-9), 126.6 (C-10), 125.6 (C-11), 119.9 (C-14), 111.2 (C-8), 99.5 (C-1), 71.6 (C-5), 71.1 (C-3), 68.9 (C-2), 67.2 (C-4), 61.8 (C-6), 21.0, 20.9, 20.9, 20.8 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{25}H_{25}O_{12}$ $[M - H]^-$ 517.1346, found 517.1344. $[\alpha]_D^{20} = -6.4°$ (c 1.1, MeOH).

6-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)-2-naphthoic acid 3-azido-propyl-amide [29]. Obtained as a white solid (182 mg, 79%) following Method F: **27** (200 mg, 0.386 mmol), 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (96 mg, 0.618 mmol), hydroxybenzotriazole (57.4 mg, 0.425 mmol), 3-azidopropylamine (77.3 mg, 0.772 mmol) in anhydrous DMF (5 mL). The mixture was worked up and the crude product was purified on silica gel (0 to 50% AcOEt in cyclohexane) to afford the desired product. R_f = 0.32 (MeOH–CH_2Cl_2, 2 : 98, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.22 (d, $^4J_{10-12}$ = 1.5 Hz, H-12), 7.83 (d, $^3J_{13-14}$ = 9.0 Hz, 1H, H-13), 7.80 (dd, $^3J_{9-10}$ = 8.5 and $^4J_{10-12}$ = 1.5 Hz, 1H, H-10), 7.76 (d, $^3J_{9-10}$ = 8.5 Hz, 1H, H-9), 7.33 (d, $^4J_{8-14}$ = 2.4 Hz, 1H, H-8), 7.22 (dd, $^3J_{13-14}$ = 9.0 and $^4J_{8-14}$ = 2.4 Hz, 1H, H-14), 6.52 (t, J = 5.7 Hz, 1H, NH), 5.53 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{1-2}$ = 7.9 Hz, 1H, H-2), 5.47 (dd, $^3J_{3-4}$ = 3.4 and $^3J_{4-5}$ = 0.8 Hz, 1H, H-4), 5.19 (d, $^3J_{1-2}$ = 7.9 Hz, 1H, H-1), 5.13 (dd, $^3J_{2-3}$ = 10.4 and $^3J_{3-4}$ = 3.4 Hz, 1H, H-3), 4.24 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6}$ = 7.1 Hz, 1H, H-6), 4.16 (dd, $^2J_{6-6'}$ = 11.2 and $^3J_{5-6'}$ = 6.0 Hz, 1H, H-6′), 4.14 (ddd, $^3J_{5-6}$ = 7.1 Hz and $^3J_{5-6'}$ = 6.0 Hz and $^3J_{4-5}$ = 0.8 Hz, 1H, H-5), 3.59 (quad, $^3J_{NH\text{-}19,19-20}$ = 6.1 Hz, 2H, H-19), 3.46 (t, $^3J_{20-21}$ = 6.1 Hz, 2H, H-21), 2.17 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.93 (p, $^3J_{19-20,\ 20-21}$ = 6.1 Hz, 2H, H-20). ^{13}C NMR (151 MHz, $CDCl_3$) δ ppm: 170.5, 170.4, 170.3, 169.5 (5 CO ester), 167.6 (C-17), 156.1 (C-7), 135.9 (C-15), 130.9 (C-13), 130.7 (C-11), 129.4 (C-16), 127.7 (C-9), 127.4 (C-12), 124.5 (C-10), 119.9 (C-14), 111.1 (C-8), 99.5 (C-1), 71.4 (C-5), 71.0 (C-3), 68.8 (C-2), 67.0 (C-4), 61.6 (C-6), 49.8 (C-21), 38.1 (C-19), 29.0 (C-20), 20.9, 20.8, 20.8, 20.7 (4 CH_3CO). HRMS (ESI/Q-TOF): calculated for $C_{28}H_{33}N_4O_{11}$ $[M + H]^+$ 601.2146, found 601.2150. $[\alpha]_D^{20} = -7.0°$ (c 1.1, MeOH).

6-(β-D-Galactopyranosyloxy)-2-naphthoic acid 3-azido-propyl-amide [6]. HRMS (ESI/Q-TOF): calculated for $C_{20}H_{25}N_4O_7$ $[M + H]^+$ 433.1723, found 433.1722.

***O*-2-Cyanoethyl-O′-(3,6,9-trioxadodecan-11-ynyl)-N,N-diisopropyl-phosphoramidite [33].** To a solution of 3,6,9-trioxadodecan-11-yn-1-ol **31** (376 mg, 2 mmol) in dry dichloromethane (20 mL) in the presence of a 4 Å molecular sieve and under argon, diisopropylethylamine (520 μL, 3 mmol) was added and then *O*-(2-cyanoethyl)-*N*,*N*-diisopropyl-chlorophosphoramidite (480 μL, 2 mmol) was added dropwise. After 2 h stirring at room temperature, 1 mL of water was added. After 10 min, the solution was diluted with dichloromethane (40 mL) and then washed with a saturated aqueous $NaHCO_3$ (75 mL). The organic layer was extracted with dichloromethane (2 × 100 mL), dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude was chromatographed on silica gel with 0 to 50% of ethyl acetate in cyclohexane containing 4% Et_3N, affording **33** as a colourless syrup 563 mg, 73%. TLC: R_f = 0.55 cyclohexane–AcOEt–Et_3N 5 : 4 : 1, v/v/v. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.14 (dd, 12H, J = 6.8 Hz, isopropyl), 2.36 (t, 1H, J = 2.4 Hz, –CCH), 2.59 (t, 2H, J = 6.5 Hz, $–CH_2–CN$), 3.5–3.81 (m, 16H, –CH–, $–O–CH_2–CH_2–O–$, $–O–CH_2–P$), 4.14 (d, 2H, J = 2.5 Hz, $HCC–CH_2$). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 18.17, 18.26, 22.4, 22.4, 22.5, 22.6, 40.9, 41, 56.3, 56.5, 60.4, 60.6, 67, 68.3, 68.5, 68.6, 69, 69.2, 72.4, 77.5, 115.6. ^{31}P-NMR ($CDCl_3$, 121 MHz): δ 148.67 ppm. HRMS (ESI/Q-TOF): calculated for $C_{18}H_{36}N_2O_6P$ $[M + H_2O + H]^+$ 407.2311 found 407.2270.

Synthesis of mannose-centered aromatic galactoside oligonucleotide conjugates

Immobilization on azide solid support **35** of propargyl mannoside by Cu(I)-catalyzed alkyne azide 1,3-dipolar cycloaddition. An aqueous solution of propargyl α-mannopyranoside **34**[46] (100 mM, 175 μL), freshly prepared aqueous solutions of $CuSO_4$ (100 mM, 14 μL) and sodium ascorbate (500 mM, 14 μL), water (147 μL) and MeOH (350 μL) were added to 3.5 μmol of azide solid support **35**.[47] The resulting mixture in a sealed tube was heated at 60 °C for 45 min using a microwave synthesizer (monowave 300, Anton Paar). The temperature was monitored with an internal infrared probe. The solution was removed, and CPG beads were washed with H_2O (3 × 2 mL), MeOH (3 × 2 mL) and CH_3CN (3 × 2 mL), and dried affording the solid-supported mannoside **36**.

General procedure for introduction of alkynyl phosphoramidites on mannose hydroxyls

Solid-supported mannoside **36** (1 μmol scale) was treated with alkynyl phosphoramidites **32**[28] or **33**, on a DNA synthesizer (ABI 394) according to phosphoramidite chemistry. Only coupling and oxidation steps were performed. For the coupling step, benzylmercaptotetrazole (BMT) was used as an activator (0.3 M in anhydrous CH_3CN) and phosphoramidite **32** or **33** (0.2 M in anhydrous CH_3CN) was introduced three times (3 × 40 μmol) with a 180 s coupling time (3 × 180 s). Oxidation was performed with a commercial solution of iodide (0.1 M I_2, THF–pyridine–water 90 : 5 : 5) for 15 s to form phosphotriesters or with 3*H*-1,2-benzodithiole-3-one-1,1,-dioxide with Beaucage reagent, 0.05 M in dry acetonitrile[33] for 60 s to form thionophosphotriesters.

General procedure for elongation of DNA sequences and labelling with Cy3

The DNA sequences were synthesized on the solid-supported tetraalkynyl scaffolds on the 1 μmol-scale on a DNA synthesizer (ABI 394) by standard phosphoramidite chemistry. For the coupling step, BMT was used as an activator (0.3 M in anhydrous CH_3CN), commercially available nucleosides phosphoramidites (0.075 M in anhydrous CH_3CN) were introduced with a 20 s coupling time and Cy3 amidite (0.067 M in anhydrous

CH_3CN) with a 180 s coupling time. The capping step was performed with acetic anhydride using a commercial solution (Cap A: Ac_2O–pyridine–THF, 10 : 10 : 80 and Cap B: 10% *N*-methylimidazole in THF) for 15 s. Oxidation was performed for 15 s using 0.1 M I2, THF–pyridine–water 90 : 5 : 5. Detritylation was performed with 2.5% dichloroacetic acid (DCA) in CH_2Cl_2 for 35 s.

General procedure for deprotection of solid-supported oligonucleotides

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 room temperature and warmed to 55 °C for 2 h. For each compound, the supernatant was withdrawn and evaporated to dryness. The residue was dissolved in water for subsequent analysis and characterization.

General procedure for CuAAC reaction

Procedure for introduction of azido-functionalized D-galactoside derivatives **1–6**: to a solution of 5′-fluorescent-3′-alkyne oligonucleotide (100 nmol in 100 μL of H_2O) were added azido-functionalized galactosides **1–6** (3 equiv. per alkyne function, 100 mM in MeOH), ~0.1 mg of Cu(0) nanopowder, triethylammonium acetate buffer 0.1 M, pH 7.7 (25 μL), water and MeOH to obtain a final volume of 250 μL (water–MeOH, 1 : 1, v/v). The tube containing the resulting preparation was sealed and placed in a microwave synthesizer Monowave 300 from Anton Paar at 60 °C for 60 min.

Work-up of CuAAC reactions and HPLC purification

EDTA (400 μL, 0.1 M) was added to the mixture and after centrifugation, the supernatants were withdrawn to eliminate Cu(0) and were desalted by size-exclusion chromatography on Sephadex G25 (NAP10). After evaporation, the 5′-fluorescent 3′-(glycomimetic) oligonucleotides were dissolved in water and purified by C_{18} reversed-phase preparative HPLC using a linear gradient from 8% to 32% of acetonitrile in TEAAc buffer pH 7 over 20 min. Residues were dissolved in water for subsequent analyses.

Fabrication of the DDI-microarray

Microarray design. Microarrays were made on microstructured borosilicate glass slides (Nexterion glass D, Schott). In brief, microwells were fabricated on the slides by the photolithography and wet etching process according to the protocol described by Mazurczyk *et al.*[48,49] The resulting slides bear 40 square wells (3 mm width, 60 ± 1 μm depth, with 4.5 mm spacing between each wells).

Silanization of microarrays. The microstructured slides were functionalized according to the protocol developed by Dugas *et al.*[50,51] After a piranha treatment, the slides were heated under dry nitrogen at 150 °C for 2 h. Next, the slides were incubated for 2 h at room temperature in a solution of *tert*-butyl-11-(dimethylamino)silylundecanoate in dry pentane. After pentane evaporation, slides were heated at 150 °C overnight. Functionalized slides were obtained after washing in THF and rinsing in water (10 minutes, under ultrasound). To convert the ester function in the corresponding acid, slides were incubated in formic acid for 7 h at room temperature. The slides were washed subsequently with dichloromethane and water, and the acid functions were activated for amine coupling using *N*-hydroxysuccinimide (0.1 M) and di(isopropyl)carbodiimide (0.1 M) in dry THF overnight at room temperature. Finally, slides were washed with THF and dichloromethane.

Immobilization of amino-modified oligonucleotides. Seven amino-modified oligonucleotides (18 to 25-mers) were purchased from Eurogentec. Spotting of 0.3 nL of the various oligonucleotides at 25 μM in PBS10X (pH 8.5) on the microarray was done using a spotting robot, Scienion sciFLEXARRAYER s3 system piezo electric. The substitution reaction between the amino-modified oligonucleotides and the NHS-activated microarray surface was performed for 3 h under a water saturated atmosphere and overnight at room temperature to allow a gentle drying of the spots. Slides were washed for 30 min at 70 °C in SDS 0.1% and rinsed in water.

Slides were treated for 2 h with bovine serum albumin (BSA) at 4% in PBS1X (pH 7.4) to prevent nonspecific adsorption during the following steps of galactocluster immobilization on the surface and lectin recognition. To remove excess blocking solution, slides were washed for 3 × 3 min in PBS-Tween20 (0.05%) followed by 3 × 3 min PBS1X and rinsed with water before being dried by centrifugation.

Immobilization of galactoclusters

Each galactocluster bears a Cy3 fluorophore and an oligomeric DNA sequence (called Sq, see Table 1) complementary to one sequence immobilized on the glass slide. Galactoclusters were diluted in PBS1X (pH 7.4) and mixed to give a final solution containing 1 μM of each galactocluster. In each microwell of the slide, 1.5 μL of the resulting solution was incubated for 3 h at 37 °C under a water saturated atmosphere. Then, slides were washed with SSC2X SDS 0.1% at 51 °C for 1 min, SSC2X for 5 min and briefly rinsed with water before drying by centrifugation.

Slides were scanned at 532 nm (excitation) to detect the Cy3 fluorophore using the Axon microarray scanner, GenePix 4100A software package.

The fluorescence signal of each galactocluster was determined as the average of the mean fluorescence signal of 16 spots and allowed the control of galactocluster immobilization

Table 1 Sequence (Sq) of the oligonucleotides conjugated to the mannose-centered galactocluster used for DDI

Sq name	5′-sequence-3′
Sq 1	CTG CCT CTG GGC TCA
Sq 2	CCG CGT TGG ATT AGC
Sq 3	GCT TGG TGC CTC CAC
Sq 4	TGC CAC CTC GCT TGG
Sq 5	GCT CTC CAC TGC TGG
Sq 6	TGG CAC GCT CTC TGC
Sq 7	GAA ACC AAG TCC ACA

on the microarray. The fluorescence signal deviated by less than 32% between each molecule.

LecA (PA-IL) labeling. LecA lectin was labeled with the Alexa Fluor® 647 Microscale Protein Labelling Kit (A30009) from Invitrogen. The labeling process was previously detailed by Goudot *et al.*[25]

IC_{50} value determination. The labelled lectin was diluted at 0.12 μM with BSA 2%, 1 μg mL^{-1} $CaCl_2$ in PBS1X (pH 7.4). Lactose was added to the solution at different final concentrations from 0.01 nM to 0.3 M to provide an IC_{50} of 20 discrete points. 1.5 μL of each solution was deposited in one well. The fluorescence signal at 635 nm due to the complex galactoclusters/alexa647-LecA was detected and an average of the mean fluorescence signal of 16 spots per galactocluster was calculated. IC_{50} values were determined thanks to the BioDataFit 1.02 Program. A Sigmoidal fit (Log EC_{50}) was chosen:

$$FI = FI_{min} + (FI_{max} - FI_{min})/[1 + 10(\log\,[LecA] - \log[IC_{50}])]$$

FI is the fluorescence signal at 635 nm observed for a given galactocluster at a given lactose concentration. FI_{min} and FI_{max} are respectively the minimum and maximum Alexa647 fluorescence signals observed for galactoclusters. [LecA] is the Alexa647 labelled lectin concentration.

K_d determination. Solutions with different final concentrations of Alexa 647 labelled lectin ranking from 0.1 nM to 2.0 μM, BSA 2% and 1 μg mL^{-1} $CaCl_2$ were prepared in PBS1X (pH 7.4). 1.5 μL of each solution was deposited in one well of the glycoarray with increasing concentration of LecA per well. The fluorescence signal at 635 nm due to the galactoconjugate/Alexa647-LecA complex was detected and an average of the mean fluorescence signal of 16 spots per galactocluster was calculated. K_d values were determined from the intercept with the *y*-axis of eqn (1) for each galactocluster using a linear regression:

$$[LecA]/FI = 1/FI_{max} \times [LecA] + K_d/FI_{max} \quad (1)$$

FI is the fluorescence signal at 635 nm observed for a given galactocluster at a given lectin concentration.

Fluorescence scanning. Scanning was performed using an Axon 4100A fluorescent scanner. The genepix software was used for data mining.

In silico molecular docking

The lectin structure file was retrieved from the RCSB Protein Data Bank website (PDB code 4LJH). Docking experiments were performed with the GOLD software (The Cambridge Crystallographic Data Centre, Cambridge, UK). The six heavy atoms of the ligand galactose ring found in the structure file were used as a scaffold in the active site. The rest of the ligand was considered as flexible during the docking process. Side chains of the following residues in the active site vicinity, Y36, H50, C62, Y98, D100, V101, T104, Y105, N107 and N108, were also defined as flexible during the docking procedure. For each ligand 10 poses that are energetically reasonable were kept while searching for the correct binding mode of the ligand. The decision to keep a trial pose is based on a computed ligand–receptor interaction energy (score) of that pose. The ChemPLP fitness scoring function is used to rank poses. The ChemPLP scoring function is the default in Gold version 5.2 used here. Additionally an empirical potential energy of interaction ΔE for the ranked complexes is evaluated using the simple expression (2):

$$\Delta E(\text{interaction}) = E(\text{complex}) - (E(\text{protein}) + E(\text{ligand})) \quad (2)$$

For that purpose the Spectroscopic Empirical Potential Energy function SPASIBA and the corresponding parameters were used.[52,53] Molecular graphics and analysis were performed using the Discovery Studio Visualizer 4.0 software (Accelrys, San Siego, CA, USA).

Conclusions

Among the present 24 glycoclusters exhibiting aromatic aglycons, those with a naphthyl or a biphenyl aglycon displayed the strongest affinity towards LecA with a K_d value, determined using a DDI-glycoarray, of 14 and 20 nM for **G21** and **G17** respectively. It was shown that the high affinity was due to the interaction of the aglycon motif with H50 and P51 of LecA. The glycocluster **G21** will be synthesized on the mg scale for further studies including AFM and inhibition of *Pseudomonas aeruginosa*.

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