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# Discovery of potent 1,1-diarylthiogalactoside glycomimetic inhibitors of *Pseudomonas aeruginosa* LecA with antibiofilm properties

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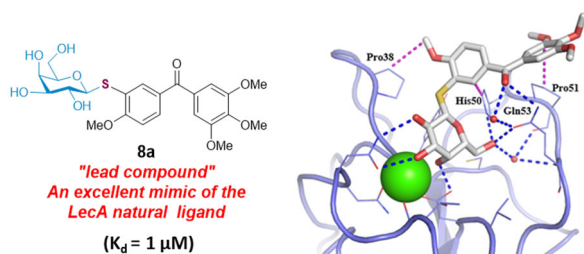
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## Abstract

In this work,  $\beta$ -thiogalactoside mimetics bearing 1,1-diarylmethylene or benzophenone aglycons have been prepared and assayed for their affinity towards LecA, a lectin and virulence factor from *Pseudomonas aeruginosa* involved in bacterial adhesion and biofilm formation. The hit compound presents higher efficiency than previously described monovalent inhibitors and the crystal structure confirmed the occurrence of additional contacts between the aglycone and the protein surface. The highest affinity (160 nM) was obtained for a divalent ligand containing two galactosides. The monovalent high affinity compound ( $K_d = 1 \mu\text{M}$ ) obtained through structure-activity relationship (SAR) showed efficient antibiofilm activity with no associated bactericidal activity.

**Keywords:** thiogalactoside, *Pseudomonas aeruginosa*, antibiofilm activity, structure-activity relationship.

## Graphical Abstract



- ✓ High affinity for LecA (*Pseudomonas aeruginosa*)
- ✓ Dose-dependent inhibition of biofilm formation
- ✓ No associated bactericidal activity
- ✓ Creation of an additional hydrogen bond with Gln53

## Highlights

- A series of new thiogalactosides were synthesized.
- Four ligands showed a strong affinity for a lectin from *Pseudomonas aeruginosa*
- The monovalent ligand **8a** displayed high affinity toward LecA ( $K_d = 1 \mu\text{M}$ ) and strong antibiofilm activity.

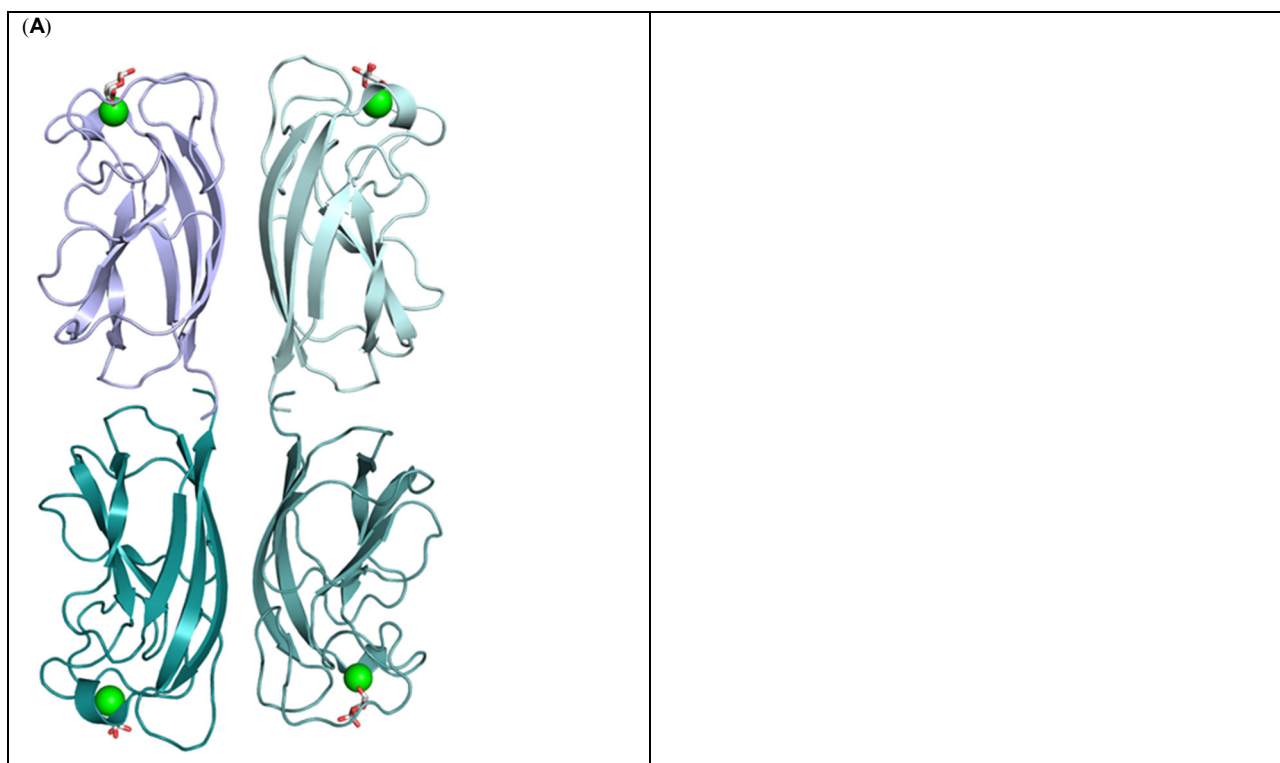
## 1. Introduction

*Pseudomonas aeruginosa* is a Gram-negative opportunistic human pathogen causing severe infections of the respiratory tract particularly in immune-suppressed patients. In addition, *P. aeruginosa* induces chronic infections in the lungs of patients with cystic fibrosis, damaging the lung epithelium by strains presenting high intrinsic or acquired resistance to antibiotics as loss of susceptibility when under biofilms and resulting in morbidity and mortality for young adults[1] *P. aeruginosa* adhesion to epithelial cells involves several proteins able to bind to the glycans at the surface of human tissues and, including two soluble ones: the galactose specific lectin LecA (PA-IL) and the fucose specific lectin LecB (PA-III).[2, 3] Experimental studies on mice and humans demonstrated that monosaccharides and more specifically galactose prevent lung colonization by *P. aeruginosa*. [4-6] Therefore, LecA appears as a target of interest and the development of high affinity glycomimetics as an attractive strategy to treat such infections.[7] Furthermore, LecA is also involved in biofilm formation which makes it a relevant target in the context of antibiotic resistance of *P. aeruginosa*. [7] Galactose-derivatives targeting this protein were demonstrated to disrupt biofilm formation and restore antibiotic sensitivity.[8-10]

LecA is a homotetramer with four galactose binding sites, each containing a calcium ion coordinated by both the carbohydrate and the surrounding amino acids (Figure 1A).[11] Because of the spatial organization of the four galactose binding sites, the lectin binds with high avidity to natural glycolipids on cell surfaces. [12] This avidity can be exploited for the design of multivalent glycoconjugates that reach high affinity for the lectin.[13] Although the multivalent galactosides showed a stronger affinity (nanomolar range) against LecA than monovalent analogues, their complex structures and high molecular weight result in lack of druglikeness and poor pharmacokinetic properties that are main drawbacks for reaching clinical trials. Divalent compounds able to cross-link two galactose binding sites on the surface of LecA, were demonstrated to present sufficient avidity to reach efficiency in the nanomolar range (20-80 nM) (Figure 1B).[14, 15] However, small monovalent ligands such as galactosides bearing an aromatic aglycon are considered to be more drug-like molecules with favorable bioavailability to reach the targeted infected tissues. As highlighted in the Figure 1B, a variety of monovalent aryl- $\beta$ -O-galactosides were reported to be more active ligands against LecA than galactose. Libraries were synthesized and tested with net gain in affinity for phenyl (Kd 8.8  $\mu$ M) and nitrophenyl (PNP- $\beta$ -Gal; Kd 14  $\mu$ M).[16, 17] The increase in affinity is rationalized by the occurrence of a hydrophobic pocket adjacent to the galactose binding site on LecA surface but also of an unusual T-stacking interaction between the ring of the aglycone and the one of His50.[17] The most affine monovalent ligand to date is a phenyl-galactoside extended by the tripeptide Lys-Pro-Leu (GalAG0) from Raymond group (Kd 3  $\mu$ M).[18] In parallel, Roy *et al.* reported a novel series of aryl 1-thioglycosides, which are more resistant to the metabolic hydrolysis under biological conditions, as a promising and innovative series of glycomimetics with high affinity for LecA.[19] Naphtyl thioglycoside was identified as a good candidate with a Kd of 6  $\mu$ M. In addition, the X-ray crystal analysis of the LecA/thionaphtyl- $\beta$ -Gal complex revealed that the naphtyl ring plays a pivotal role in the binding pocket by adapting two different conformations in sites A/D and B/C of the tetrameric LecA protein. This difference in the naphthyl group orientation was attributed to changes in both  $\Phi$  and  $\Psi$  torsion angles at the S-glycosidic linkage. Then, the aromatic ring was much closer to the protein surface in protomer B than in protomer A. These advanced achievements created an increased interest to discover new small glycomimetic thioglycosides.

In order to emulate this approach, we sought to explore a new chemical space around the hydrophobic ring with the purpose to identify unique structures with better affinity and antibiofilm activities. In this work, we report the synthesis of a novel series of monovalent and divalent aryl thioglycosides as LecA inhibitors (Figure 2). Their (*i*) affinity towards

LecA, (ii) biofilm inhibition against *P. aeruginosa* as well as (iii) cytotoxicity against MRC-5 cell line were also presented. Notably, the glycomimetics developed here were potent inhibitors of biofilm formation in stark contrast to the ineffective native carbohydrates.



**Figure 1.** (A) Tetrameric structure of LecA (PDB 1OKO) with protein represented as ribbon, calcium as a sphere and galactose ligand as sticks. (B) Selected examples of multivalent and mono-galactoside ligands of LecA.

## 2. Results and discussion

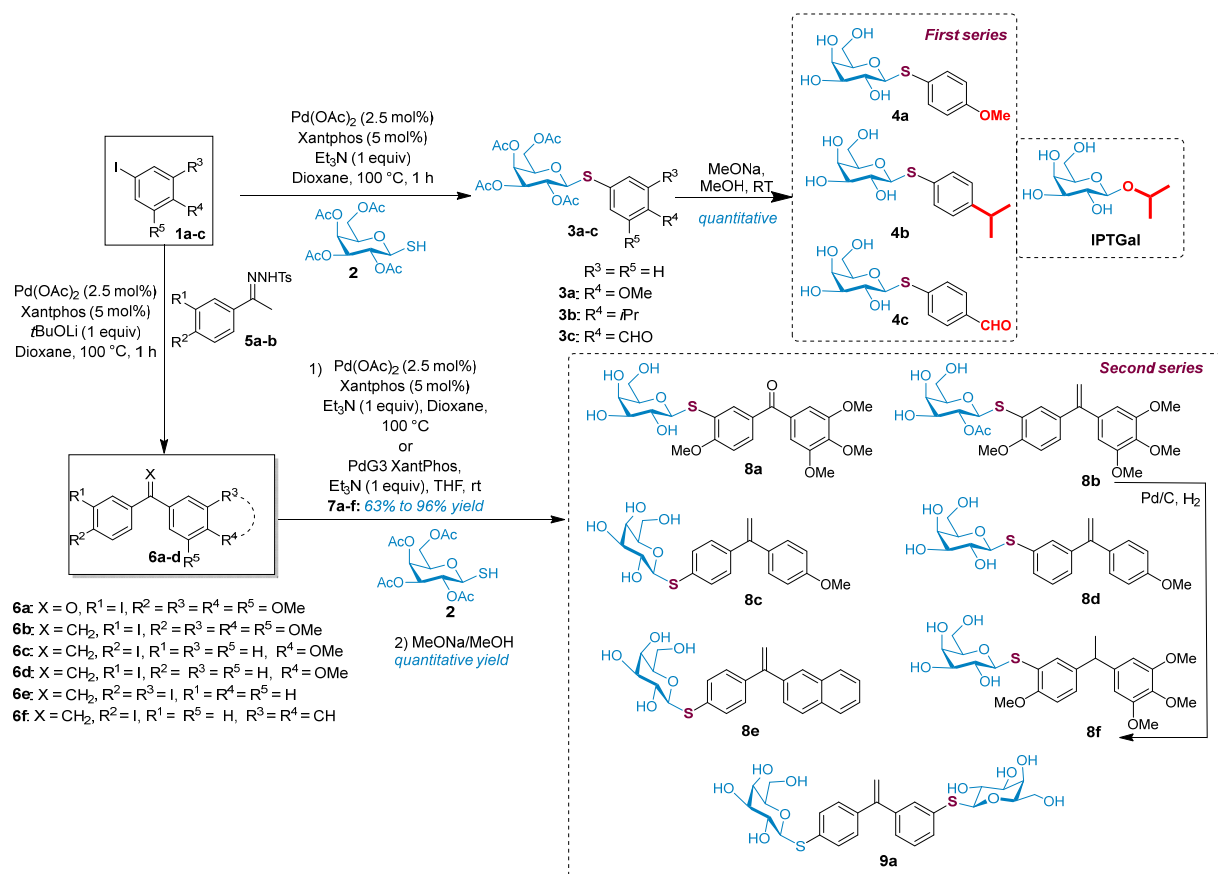
### 2.1. Chemistry

We recently reported an efficient Pd-catalyzed method allowing the introduction of glycosyl thiols to various iodo(hetero)aryls[20] and alkenyl/alkynyl halides.[21] This method is based on the use of Pd(OAc)<sub>2</sub> as the catalyst, combined with XantPhos as the ligand and Et<sub>3</sub>N as the base in THF or dioxane at 100 °C temperature. Moreover, a variant of this method implying the use of the palladium G3-Xanthphosbiphenyl precatalyst under mild reaction conditions (rt and aqueous solvent) was developed to functionalize challenging substrates with thiosugars[22] including nucleic acids[23] and unprotected long peptides.[24] With these conditions on hand, we first designed a short series of three phenyl β-*S*-galactopyranoside **4a-c** in the aim to evaluate the substitution of the aromatic ring on the affinity toward LecA. Thus, analogues bearing methoxyl, isopropyl and benzaldehyde groups at the *para* position of the phenyl ring were prepared in good yields through a sequence of Pd-catalyzed coupling of the iodinated starting materials **1a-c** with the tetra-*O*-acetylated 1-thio-β-D-galactopyranose **2** followed by the Zemplen deprotection of the acetate groups (Scheme 1). Interestingly, compound **4b** which may be considered as a direct aromatic glycomimetic of isopropyl β-D-1-thiogalactopyranoside (IPTG) will be a good indicator of the effect of introducing the *S*-aromatic linkage of IPTG toward the affinity for the lectin (Scheme 1).

Then, in another part of this study, we focused our synthesis on the 1,1 biaryl thiogalactosides **8a-e** and **9a** (Scheme 1). We speculated that the presence of two phenyl nucleus linked by Csp<sup>2</sup> carbone bridge could provide strong π-π stacking interactions with residues of the binding pocket and will allow us to understand the role played by hydrophobic aglycones in binding to LecA.

The synthesis of the second series of our thiogalactosides starts from the preparation of the iodinated diphenyl substrates **6a-f** through a Pd-catalyzed Barluenga-Valdes cross-coupling between aryl iodides **1a-c** and the corresponding tosyl hydrazones **5a-b**.<sup>[25]</sup> Then, the coupling of the tetra-*O*-acetylated 1-thio- $\beta$ -D-galactopyranose **2** with biaryls **6a-f** furnished the desired acetylated galactoside intermediates **7a-f** in yields ranging from 63% to 96%. Simple deprotection of the acetate groups by action of sodium methoxide in methanol led to the desired compounds **8a-e** and **9a** in a quantitative yield (Scheme 1).

Finally, to examine the flexibility *versus* rigidity of ethylene bridge of **8b**, the reduction of this later was performed by hydrogenation with Pd/C. Accordingly, the compound **8f** was obtained quantitatively as a mixture of the two diastereoisomers (Scheme 1).



**Scheme 1.** Synthetic route toward thiogalactoside analogues **3a-c**, **8a-f** and **9a**.

## 2.2. Interactions with LecA target

### 2.2.1. Determination of affinity towards LecA and thermodynamic parameters

The affinity of generated thiogalactosides has been assessed in solution by isothermal titration calorimetry (ITC), a bioanalytical technique for the study of ligand–receptor interactions that generates thermodynamic information on the ligand binding process. Typical thermograms of LecA interacting with the four best ligands are given in Figure 2 and all others are available in Supplementary Information. Titration of LecA by monovalent ligands resulted in exothermic peaks and saturation of 50% of protein occurred with a stoichiometry close to  $n = 1$ , indicating the expected binding interaction. As expected, the divalent compound **9a** displays a stoichiometry ligand/protein close to 0.5, indicating that each of the galactose residues is bound to a LecA protomer which is confirmed by aggregation observed in ITC tube.

Table 1 reports the affinity values for all compounds. For monovalent galactosides, the values vary between 1 and 12  $\mu\text{M}$  with higher affinity obtained for compounds **4b**, **8a** and **8c** with  $K_d$  lower than 3  $\mu\text{M}$ . This is 30-fold better than the

affinity observed for galactose and also stronger than the best affinity reported previously for best thio-galactoside ligands for LecA displayed in Figure 1.[19] Interestingly, the affinity of the isopropyl-phenyl-*S*-galactoside **4b** ( $K_d = 2.45 \mu\text{M}$ ) is 10-time higher than the corresponding non-aromatic IPTG ( $K_d = 32 \mu\text{M}$ ).[16] This result demonstrates clearly the gain obtained with *S*-aryl galactoside ligands compared to *S*-alkyl analogues. Importantly, the series bearing biaryl aglycones (compounds **8a-f**) showed a promising activity particularly with the benzophenone monovalent galactoside **8a** with a  $K_d$  of  $1 \mu\text{M}$ . To our knowledge, this is the strongest reported affinity for a monovalent galactoside towards LecA. The structure-activity relationship study (SAR) showed that replacing the carbonyl bridge in **8a** by a double bond (compound **8b**) lead to a lower affinity toward the lectin with a  $K_d$  of  $6.9 \mu\text{M}$ . In addition, reducing the double bond into a methyl group (compound **8e**) decreased the affinity ( $K_d = 9 \mu\text{M}$ ). This result suggests a role for the oxygen atom of the carbonyl bridge and indicates that an optimal positioning of the two aromatics is crucial for the binding to LecA.

It is worth noticing that since benzophenone scaffolds are often used as photoactivatable probes,[26-29] our hit compound **8a** could potentially display photoactivatable properties. It could therefore be envisaged that the irradiation of **8a** at 330-365 nm would generate radicals able to react with the protein target by photo-crosslinking. However, during our characterization, particularly during -crystallization experiments and subsequent analysis of crystal structure, we did not observe any photo-crosslinking arising from the alkylation of the benzophenone scaffold to the lectin protein.

The analysis of the thermodynamic contribution demonstrated that the interaction is driven by enthalpy of binding, which is opposed by an entropy barrier, as often observed in protein/glycocompound interactions.[30] The compounds that reach the stronger enthalpy, **4b**, **8a** and **8c**, are the ones with higher affinity, indicating that creation of additional contacts around the galactose binding sites is the appropriate strategy to obtain efficient ligands. Compound **8a** singles out by its stronger enthalpy and also by a heat integration curve that did not fit perfectly with one site model. Together with baseline noise around saturation of 50% of protein, usually associated with aggregation, this indicates additional interactions, probably with neighboring tetramers in solution (Figure 2).

The divalent galactoside **9a** displayed an excellent affinity toward LecA with a  $K_d = 160 \text{ nM}$  (Figure 2). In addition, the stoichiometry value of 0.44 indicates that the two thiosugar moieties interact with LecA. Considering the short size of the linker and the precipitation observed during titration, compound **9a** does not bind to two adjacent galactose binding sites on one LecA tetramer but rather cross-link two tetramers.

Table 1 . Microcalorimetry data and thermodynamics contribution for higher affinity compounds binding to LecA. The experiments were realized in duplicate at 298 K unless otherwise stated

ligand	$K_D$ ( $\mu\text{M}$ )	n	$-\Delta G$ (kJ/mol)	$-\Delta H$ (kJ/mol)	$T\Delta S$ (kJ/mol)
<b>4a</b>	11.9 <sup>a</sup>	1 <sup>b</sup>	28.1	42.3	-14.2
<b>4b</b>	2.45 ± 0.4	1 <sup>b</sup>	32.0	49.9 ± 0.0	-17.9
<b>4c</b>	9.2 <sup>a</sup>	1 <sup>b</sup>	28.7	41.2	-12.5
<b>8a</b>	1.02 ± 0.6	1 <sup>b</sup>	34.2	54.5 ± 2.7	-20.3
<b>8b</b>	6.9 ± 0.1	0.98 ± 0.03	29.4	36.0 ± 0.7	-6.6
<b>8c</b>	3.0 ± 0.6	1 <sup>b</sup>	31.6	46.6 ± 0.1	-15.0
<b>8d</b>	8.1 ± 0.2	0.97 ± 0.06	29.1	46.4 ± 0.9	-17.3
<b>8e</b>	9.0 ± 0.2	1 <sup>a</sup>	28.8	35.2 ± 1.0	-6.4
<b>9a</b>	0.16 ± 0.03	0.44 ± 0.04	38.7	90.2 ± 1.1	-51.5

<sup>a</sup> Only one experiment; <sup>b</sup> ligand concentration adjusted to reach stoichiometry of 1 (within 10% variation);

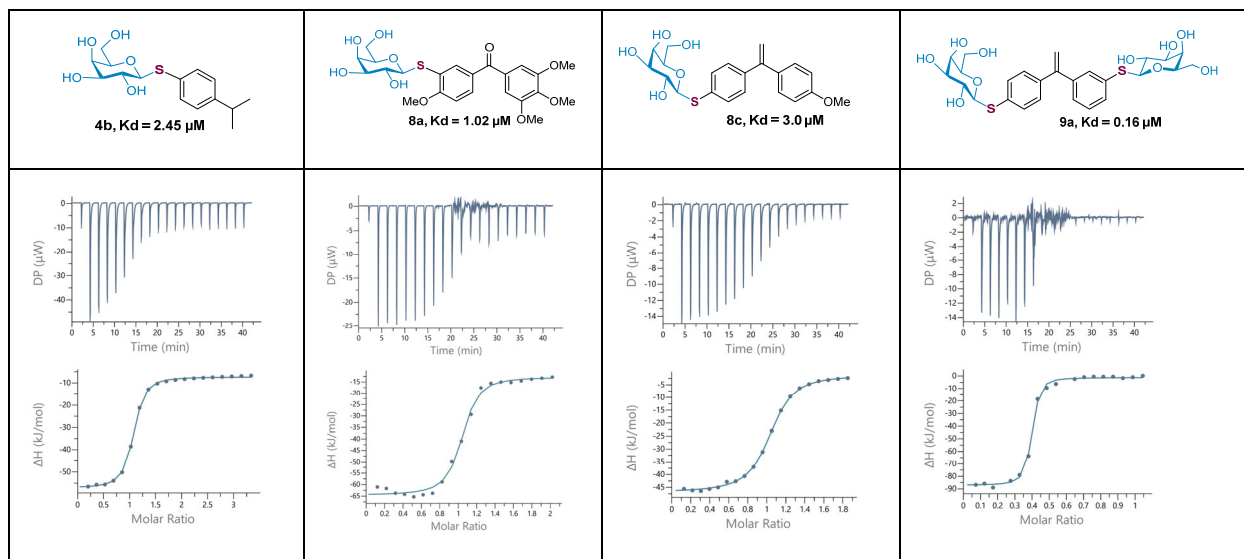


Figure 2: ITC titration of LecA with compound **4b**, **8a**, **8c** and **9a**. The plot in the lower panel shows the total heat released as a function of total ligand concentration for the titration shown in the upper panel. The solid line represents the best least-square fit to experimental data using a one site model.

### 2.2.2. Co-crystal structures of LecA with compounds **8a** and **8c**

Co-rystallization experiments were conducted with **8a**, **8c** and **9a** ligand with the protein either preincubated with the ligand or deposited on the coverslip with dried ligand on it (dry-soaked method). Co-crystals were obtained for LecA with compounds **8a** and **8c** only, diffracting to 1.75 and 1.53 Å, respectively. The content of unit cell was different with one lectin tetramer and four ligands for the complex with **8a** and one lectin dimer with only one ligand for the complex with **8c** (Figure 3 A and B). This difference resulted from the ligand-induced packing. For **8a**, stacking contacts are observed between the aryl rings of two ligands in facing tetramer as frequently observed for LecA in complex with aromatic galacto compounds, while for **8c**, the distal aryl moiety contacts the binding site of neighbouring LecA, preventing the binding site to be occupied. Such contacts occur only during crystallization and are generally not relevant for analysing the affinity of LecA in solution, although some signs of aggregation were observed during titration of LecA by **8a**.

Analysis of contacts established by compound **8c** in the binding site reveals the classical contacts for galactose, including calcium coordination by oxygen O3 and O4, direct hydrogen bonds with the protein and water mediated hydrogen bonds for O6 (Figure 3B). The aglycone folds on the protein surface, creating hydrophobic contacts with CH2 of His50 and Pro51 with the proximal and distal benzyl groups, respectively. The rings of His50 and of the ligand are almost perpendicular, corresponding to an unusual type of hydrophobic interaction, described as T-shape and previously observed with other aromatic aglycones for LecA ligands.[17] The extensive hydrophobic interactions between the aglycone of **8c** and LecA surface provides the structural basis of its excellent affinity ( $K_d = 3 \mu\text{M}$ ).

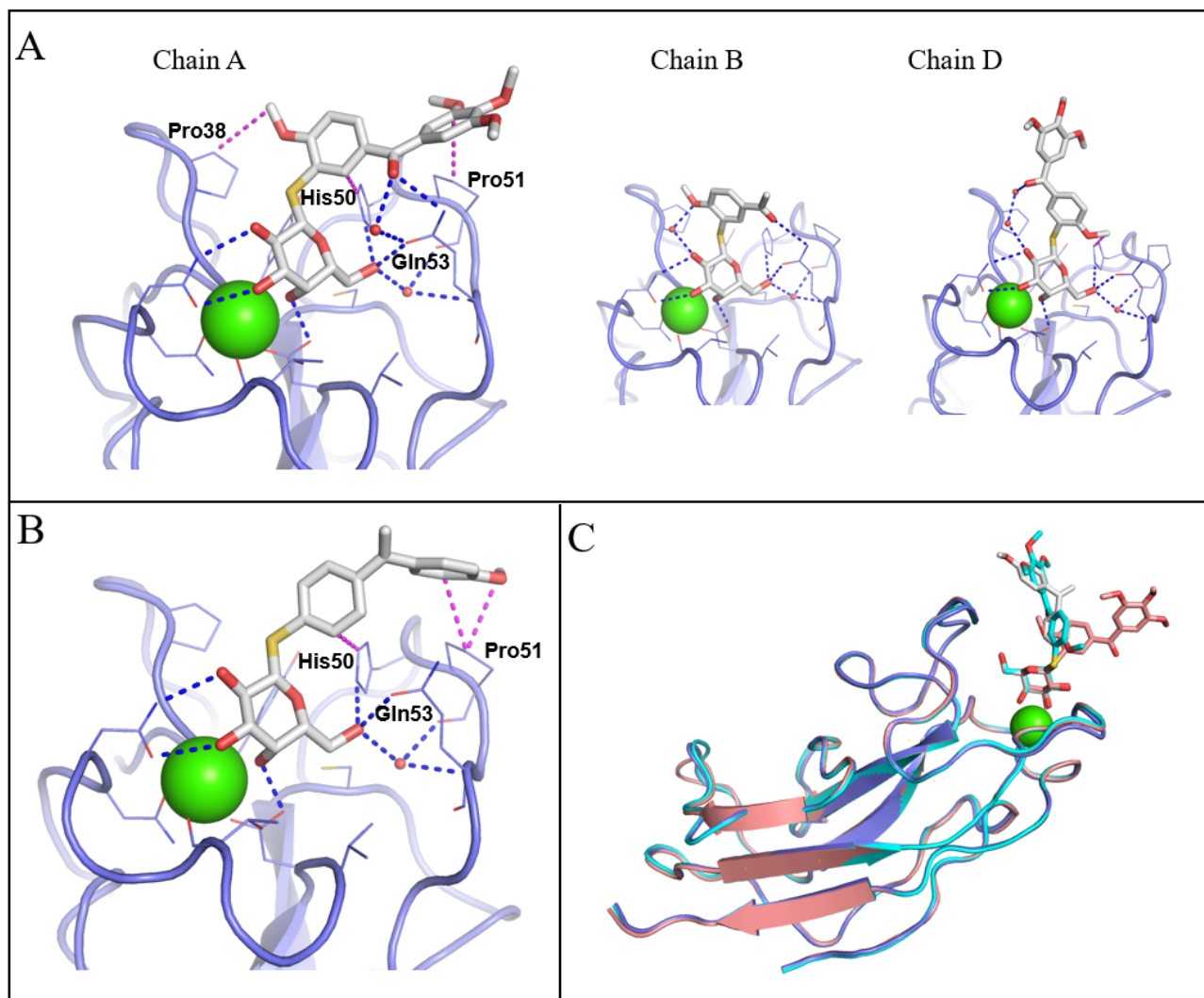


Figure 3: Crystal structures of LecA complexed with compounds **8a** and **8c**. The calcium cation is presented as a green sphere, the galactoside as sticks, water molecules as red spheres, hydrogen bonds and hydrophobic contacts as blue and magenta dotted lines, respectively. (A) Compound **8a** in three different conformations observed in the binding site of chain A, B and D. Binding site of chain C is similar to chain A and is not represented. (B) Compound **8c** in binding site A. (C) Superimposition of **8a** (two conformations) and **8c** in LecA protomer A.

The compound **8a**, which displays the highest affinity for LecA ( $K_d = 1 \mu\text{M}$ ) compared to the other monovalent ligands, adopts different conformations for its aglycone in the four binding sites, while the galactose always maintains the classical binding mode described above. In protomers A and C, the conformations are very similar, with the two aryl rings adopting an overall shape similar to the one observed for compound **8c** thus, creating same hydrophobic contacts with His50 and Pro51 (Figure 3A). In addition, the methoxy group on the proximal benzyl creates hydrophobic contact with Pro38 on the other wall of the binding site. Interestingly, the C=O group between the two benzyl rings establishes a direct hydrogen bond with the side chain of Gln53, that is already interacting with the O6 hydroxyl of the galactose residue. A water bridge is also observed between C=O and this side chain. These additional hydrogen bonds are in agreement with the more favourable enthalpy of binding measured for compound **8a**. The value of  $\Delta H$  is 50% higher than for homolog **8b** that differs only by C=C replacing C=O. The strong enthalpy is partially compensated by increase of entropy barrier but still results in higher affinity. Compound **8a** adopts a different conformation in the binding site of chain D and is partially



disordered in chain B, resulting in electron density visible only for proximal aryl group (SI, Figure S5 A and C). The resulting extended conformation of the aglycone moiety in chain D displays only limited contact with the protein surface, with methoxy group of first benzyl contacting the CH2 of His50. This illustrates the flexibility of the thio-linkage that allows for different conformations, here, probably induced by the crystal packing.

Since the origin for high affinity of compounds **8a** is related to the creation of a hydrogen bond with Gln53, we searched for similar contacts in LecA complexes available in Unilectin3D, the database of crystal structures of lectins.[31] Synthetic compounds from Winssinger and Titz groups do present a C=O bond linker on the benzyl ring but in *para* position compared to galactose that is therefore too far from Gln53 for creating a hydrogen bond.[14, 32] On the opposite, the natural ligand, isoGb3 trisaccharide ( $\alpha$ Gal1-3 $\beta$ Gal1-4Glc) demonstrates two hydrogen bonds between the  $\beta$ Gal residue and Gln53.[12] Furthermore, compound **8a** adopts a shape very similar to this trisaccharide in the binding site of LecA (Figure 4). The target of LecA in human is proposed to be Gb3, a sphingolipid present on human tissues, and presenting the  $\alpha$ Gal1-4 $\beta$ Gal1-4Glc epitope. No crystal structure is available but a modelling study also proposed that O2 and O3 of the second galactose interact with Gln53 side chain through hydrogen bonds.[12] Compound **8a** appears therefore as an excellent mimic of the natural glycoconjugates targeted by *P. aeruginosa* on the surface of lung epithelia.[33]

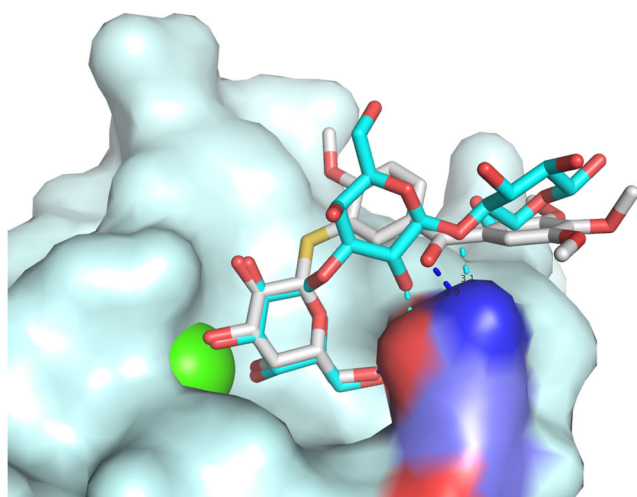


Figure 4: Superimposition of crystal structures of LecA complexed with compound **8a** (chain A – this work) and trisaccharide  $\alpha$ Gal1-3 $\beta$ Gal1-4Glc from PDB 2VXJ (stick colored in cyan).

**Table 2.** Statistics on Data Collection and Refinement

Structure	<i>LecA-8c</i>			<i>LecA-8a</i>		
<b>Data collection</b>						
Space Group	P2 <sub>1</sub> 2 <sub>1</sub> 2			P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>		
Unit cell a, b, c (Å)	48.12 57.09 83.40			42.26 61.93 180.01		
Beamline	PX1 SOLEIL			PX1 SOLEIL		
Wavelength (Å)	0.97918			0.97856		
Resolution limits (Å)	41.70-1.53 (1.56-1.53)			45.00-1.75 (1.78-1.75)		
Reflections: measured	326966 (16132)			305761 (16876)		
unique	35361 (1733)			48775 (2627)		
Completeness (%)	99.8 (99.4)			100 (99.9)		
R <sub>merge</sub> (% , within I <sup>+</sup> /I <sup>-</sup> )	4.2 (26.4)			6.7 (29.1)		
R <sub>meas</sub> (% , within I <sup>+</sup> /I <sup>-</sup> )	4.7 (29.7)			8.0 (34.7)		
R <sub>pim</sub> (% , within I <sup>+</sup> /I <sup>-</sup> )	2.1 (13.4)			4.3 (18.6)		
Mean I / σI	26.5 (7.3)			16.1 (5.3)		
Multiplicity	9.2 (9.3)			6.3 (6.4)		
CC (1/2)	99.9 (98.7)			99.8 (97.6)		
<b>Refined Statistics</b>						
Resolution (Å)	41.70-1.53			45.00-1.75		
No. reflections / free reflections	33577 / 1749			46191 / 2506		
R-factor / R <sub>free</sub> (%)	16.5 / 20.6			16.9 / 21.3		
R.m.s.d bond lengths (Å)	0.0155			0.0157		
R.m.s.d bond angles (deg)	1.89			1.77		
R.m.s.d chiral (Å <sup>3</sup> )	0.0965			0.100		
No. of atoms/ Bfac (Å <sup>2</sup> )	A	B	A	B	C	D
-Protein	942 / 16.5	914 / 20.9	914 / 16.0	907 / 13.8	918 / 9.1	916 / 10.9
-Calcium	1 / 14.8	1 / 24.6	1 / 15.1	1 / 11.3	1 / 7.6	1 / 9.1
-Sugar	28 / 21.6		34 / 18.9	23 / 24.0	34 / 15.5	34 / 22.0
-Waters	211 / 29.5	192 / 32.2	201 / 25.7	178 / 24.7	257 / 21.8	225 / 23.3
Ramachandran	100 / 97.3 / 0			100 / 97.0 / 0		
Allowed / Favored /Outliers (%)						
PDB Code	7Z62			7Z63		

Values in parentheses correspond to high resolution shell

† 5 % of the reflections were set aside for a R<sub>free</sub> test before initiating any refinement

### 2.3 In vitro cytotoxicity and antibiofilm activity

#### 2.3.a. Antibiofilm activity

Microbial biofilms are becoming increasingly difficult to treat in the medical setting due to their intrinsic resistance to antibiotics. In order to study the potential antibiofilm activity of the most active ligands **8a** and **9a**, their effect on the inhibition of the biofilm formation was evaluated in a *P. aeruginosa* assay (Figure 5). Solution with 3 % methanol was considered for control. At first, the effect of monovalent galactoside **8a** was investigated with concentrations of 0.08 to 5 mM. Compound **8a** showed a dose-response effect on the bacterial biofilm formation with 100 % of inhibition at 5 mM (log reduction > 4) (Figure 5).

The antibiofilm activity with the divalent glycomimetic **9a** was also examined. The concentration of 5 mM was fixed first to compare the effect of divalent **9a** with the monovalent **8a** which showed a strong effect (100 % biofilm inhibition) at this concentration. Surprisingly, at 5 mM, **9a** showed a slightly lower reduction of biofilm formation with approximately 90% inhibition (1 log reduction compared to the 3 % methanol control) (Figure 5). This result is not in agreement with the high affinity observed with **9a** ( $K_d = 0.16 \mu\text{M}$ ) against LecA and at this stage no any obvious correlation between the affinity toward the lectin LecA and the antibiofilm activity could be established. A possible explanation of this result would be a potential aggregating effect displayed by the compound **9a**. Several scenari can be envisage for lack of antibiofilm effect of cross-linking compounds, such as the formation of microbial microcolonies that would be favourable for the bacteria.

Of note, since the biofilm activity of **9a** is less than **8a** at 5 mM, the study of its effect at lesser concentrations are not warranted.

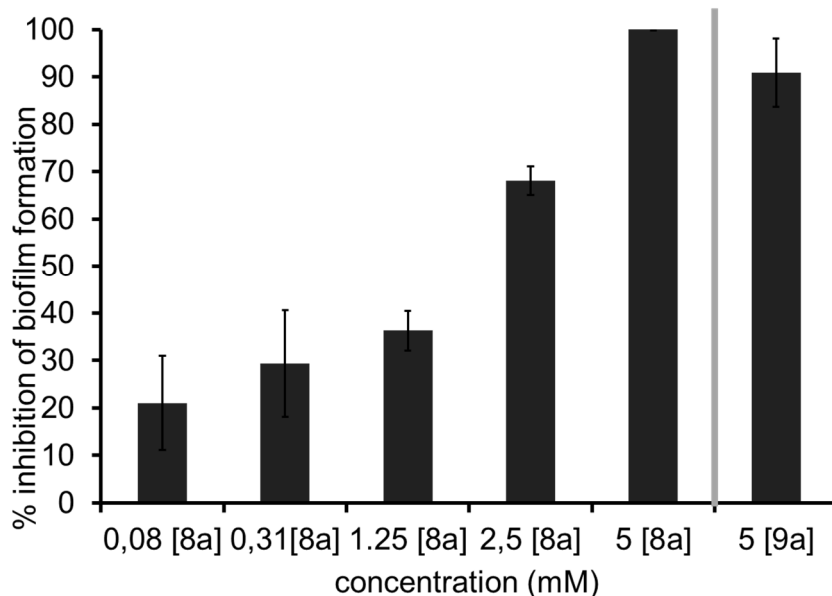


Figure 5: Antibiofilm activity of compounds **8a** and **9a**

### 2.3. b. *In vitro* cytotoxicity

The toxicity of ligands **8a** and **9a** against MRC-5 cell line (Human Fetal Lung Fibroblast Cells) was evaluated after a 24 h of contact (Figure 6). In such conditions, no significant loss of cell viability was observed for the higher methanol concentration (3%) and **8a** and **9a** compounds for concentrations less than or equal to 1.25 mM or 2.5 mM respectively. At the higher concentration tested (5 mM) the cell viability versus control was 7.1% and 5.2% for respectively compounds **8a** and **9a**. Then diluted 2 fold (2.5 mM) cell viability increased to 41.1% for **8a** and 65.1% for **9a** to be above 50% for both compounds at the concentration of 1.25mM (61.5% and 91.4% respectively). These results indicate that these two hit compounds display a cytotoxic effect at concentration higher than 1.25 mM. It can be noted that at non-toxic concentration of 1.25 mM (Figure 5), the best compound identified **8a** displays approximately 40% of biofilm inhibition.

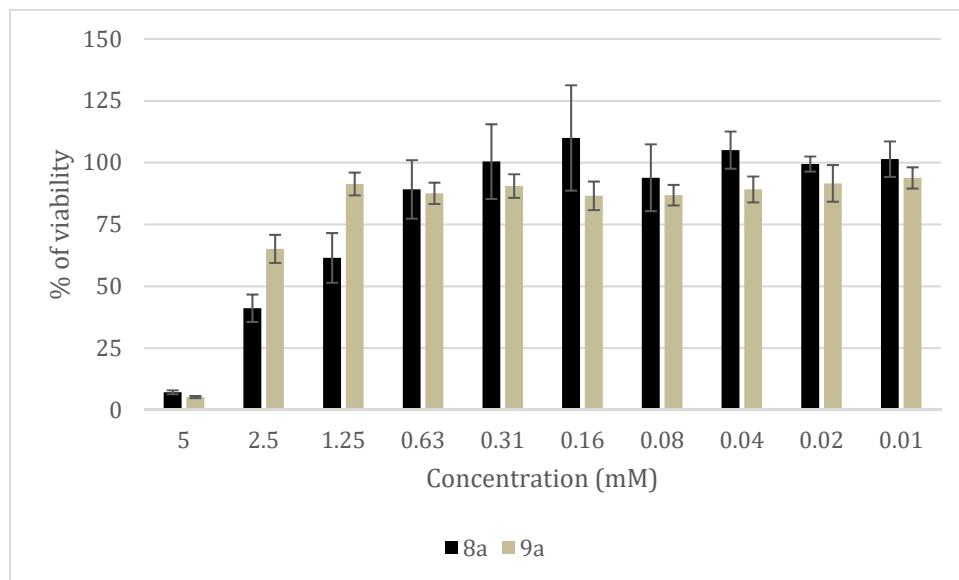


Figure 6: % of viability of MRC-5 cells after 24h of contact with compounds **8a** and **9a** (mean  $\pm$  SD).

Finally, to exclude any unwanted antibiotic effect on biofilm formation, the MICs (the concentration at which no bacterial growth was visible after 24 h of incubation at 37°C) and MBCs (minimal bactericidal concentration; the concentration at which the number of CFU was reduced by 99.9%) of compounds **8a** and **9a** against *P. aeruginosa* PAO1 were recorded as previously described.[34] The MICs and MBCs were higher than 5 mM (the highest concentration tested) for both compounds which demonstrated clearly the absence of classical antibacterial effect (on planktonic cells) for the tested compounds.

## Conclusion

In the context of the global threat of antimicrobial resistance, the development of strategies to support or complement antibiotics is crucial.[35] Strategies for developing compounds that would fight infection without or with limited risk inducing resistance include anti-adhesion and anti-biofilm therapy. Glycomimetics that are able to compete with attachment site on tissues and/or biofilm formation have been used to target pathogens.[10, 36-37] Different designs have been proposed, from large multivalent glycodendrimers[38] to non-carbohydrate glycomimetics[39], but the simpler monosaccharide derivatives, as the ones described here, present the advantage of small size, fine specificity through the monosaccharide moiety and reasonable pharmacokinetic and physicochemical properties. Furthermore, the use of thio-glycoside linkage insures the metabolic stability.

In summary, we synthesized a series of thio-galactosides compounds directed towards LecA from *P. aeruginosa* with the aim of increasing the chemical diversity around the aglycone phenyl group, with substituents in meta and ortho positions that were not explored previously. The affinities of a variety of mono-aryl and bi-aryl derivatives were analyzed by ITC as well as a divalent compound. This SAR investigation demonstrated that the bi-aryl derivative with a carbonyl linker on position *meta* and methoxy on position *ortho* results in the affinity value that was not previously reached for monovalent ligand of LecA. The crystal structure of the complex rationalized this observation by confirming the establishment of new ligand-protein contacts. It also uncovered the strong similarity with the contact established with natural oligosaccharides in LecA binding site, demonstrating that this hit compound is an excellent mimetic of the natural ligand. This hit compound demonstrated dose-dependent inhibition of biofilm formation, with no antibacterial effect on

planktonic cells at the efficient dose, and no toxicity on human cells. It is therefore a very promising hit-compound for therapeutical development, for example as adjuvant to antibiotic actions.

## 4. Experimental

### 4.1. Materials

Solvents and reagents are obtained from commercial suppliers and were used without further purification. Analytical TLC was performed using Merck silica gel F254 (230-400 mesh) plates and analyzed by UV light or by staining upon heating with vanillin solution. For silica gel chromatography, the flash chromatography technique was used, with Merck silica gel 60 (230-400 mesh) and p.a. grade solvents unless otherwise noted. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded in either  $\text{CDCl}_3$ , MeOD, or DMSO-*d*<sub>6</sub> on Bruker Avance 300 or 400 spectrometers. The chemical shifts of  $^1\text{H}$  and  $^{13}\text{C}$  are reported in ppm relative to the solvent residual peaks. IR spectra were measured on a Perkin Elmer spectrophotometer. High resolution mass spectra (HR-MS) were recorded on a MicroMass LCT Premier Spectrometer. Thiosugars were synthesized as according the following protocols.[40-41] The Xantphos palladium precatalyst third generation was synthesized according to literature protocol.[42] **6** and Acetophenone **7** was prepared adopting reported methods spectral data are in agreement with the published ones.[43]

### 4.2. Synthetic Chemistry

#### 4.2.1. Synthesis of the intermediates **6a-f**

##### General procedure for Palladium-Catalyzed coupling of N-Tosylhydrazones with Iodoarenes

A round-bottom flask with a condenser under an argon atmosphere was charged with *N*-tosylhydrazone (1 equiv),  $\text{Pd}(\text{OAc})_2$  (4 mol %), SPhos (8 mol %), diiodoarene (1.0 equiv, 1 mmol) and LiO-*t*-Bu (2.4 equiv). Then 7 mL of Dioxane was added via syringe at room temperature. The flask was put into a preheated oil bath and stirred at reflux for 1 h. The resulting suspension was cooled to room temperature and filtered through celite eluting with ethyl acetate. The filtrate was concentrated and purification of the residue by silica gel column chromatography gave the desired products.

##### 1-iodo-3-(1-(4-methoxyphenyl)vinyl)benzene **6d**

Yellow oil;  $R_f = 0.81$  (Cyclohexane/EtOAc 8/2); 52% after column chromatography (Cyclohexane); IR (neat): 3392, 1730, 1608, 1510, 1483, 1283, 1232, 1084  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.72 (t,  $J = 1.7$  Hz, 1H), 7.67 (dd,  $J = 7.8$ , 1.1 Hz, 1H), 7.34 – 7.28 (m, 1H), 7.29 – 7.22 (m, 2H), 7.08 (t,  $J = 7.8$  Hz, 1H), 6.93 – 6.86 (m, 2H), 5.43 (d,  $J = 1.0$  Hz, 1H), 5.35 (d,  $J = 1.0$  Hz, 1H), 3.85 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  159.7(C), 148.3(C), 144.3(C), 137.3(CH), 136.7(CH), 133.4(C), 130.0(CH), 129.4(2CH), 127.8(CH), 114.0( $\text{CH}_2$ ), 113.8(2CH), 94.3(C), 55.5( $\text{CH}_3$ ). HR-MS(APCI): $m/z$  calcd for  $\text{C}_{15}\text{H}_{14}\text{IO}$  [ $\text{M}+\text{H}$ ] $^+$  337.0084; found 337.0086

##### 1-iodo-4-(1-(4-methoxyphenyl)vinyl)benzene **6c**

White solid;  $R_f = 0.81$  (Cyclohexane/EtOAc 8/2); 37% after column chromatography (Cyclohexane); IR (neat): 2953, 2833, 1753, 1667, 1511, 1483, 1292, 1252, 1180, 1029  $\text{cm}^{-1}$ . m.p.=129.2-130.2 $^\circ\text{C}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.66 (d,  $J = 8.5$  Hz, 2H), 7.25 (d,  $J = 9.0$  Hz, 2H), 7.09 (d,  $J = 8.5$  Hz, 2H), 6.87 (d,  $J = 8.9$  Hz, 2H), 5.37 (dd,  $J = 16.5$ , 1.1 Hz, 2H), 3.83 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  159.6(C), 148.7(C), 141.5(C), 137.4(2CH), 133.5(C), 130.3(2CH), 129.5(2CH), 113.8(2CH), 113.6( $\text{CH}_2$ ), 93.4(C), 55.5( $\text{CH}_3$ ). HR-MS(APCI): $m/z$  calcd for  $\text{C}_{15}\text{H}_{14}\text{IO}$  [ $\text{M}+\text{H}$ ] $^+$  337.0084; found 337.0084

##### 1-iodo-3-(1-(4-iodophenyl)vinyl)benzene **6e**

White solid;  $R_f = 0.62$  (Cyclohexane); 45% after column chromatography (Cyclohexane); IR (neat): 3287, 2952, 2925, 1649, 1558, 1414, 1079, 1015  $\text{cm}^{-1}$ . m.p.=64.5-66.1 $^\circ\text{C}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.72 – 7.61 (m, 4H), 7.31 – 7.19 (m, 1H), 7.12 – 6.96 (m, 3H), 5.47 (d,  $J = 0.6$  Hz, 1H), 5.44 (d,  $J = 0.6$  Hz, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  148.0(C), 143.3(C), 140.4(C), 137.6(2 $\text{CH}_3$ ), 137.2( $\text{CH}_3$ ), 137.1( $\text{CH}_3$ ), 130.1(3 $\text{CH}_3$ ), 127.6( $\text{CH}_3$ ), 115.9( $\text{CH}_2$ ), 94.5(C), 93.9(C). HR-MS(APCI): $m/z$  calcd for  $\text{C}_{14}\text{H}_{11}\text{I}_2\text{O}$  [ $\text{M}+\text{H}$ ] $^+$  432.8945; found 432.8941

##### 2-(1-(4-iodophenyl)vinyl)naphthalene **6f**

White solid;  $R_f = 0.47$  (Cyclohexane); 51% after column chromatography (Cyclohexane); IR (neat): 3056, 2924, 2853, 1752, 1655, 1557, 1581, 1432, 1368, 1221, 1135, 1111, 1079, 1047, 1006  $\text{cm}^{-1}$ . m.p.=94.8-95.9 $^\circ\text{C}$ ;  $^1\text{H}$  NMR (300 MHz, Chloroform-*d*)  $\delta$  7.86 – 7.78 (m, 3H), 7.75 (dd,  $J = 1.8$ , 0.6 Hz, 1H), 7.72 – 7.65 (m, 2H), 7.52 – 7.43 (m, 3H), 7.19 – 7.06 (m, 2H), 5.60 (d,  $J = 1.0$  Hz, 1H), 5.54 (d,  $J = 1.0$  Hz, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  149.3(C), 141.2(C), 138.4(C), 137.5(2CH), 133.4(C), 133.2(C), 130.4(2CH), 128.3(CH), 128.0(CH), 127.8(CH), 127.4(CH), 126.4(C), 126.3(2C), 115.4(C), 93.6(C). HR-MS(APCI): $m/z$  calcd for  $\text{C}_{18}\text{H}_{14}\text{I}$  [ $\text{M}+\text{H}$ ] $^+$  357.0140; found 357.0147.

#### 4.2.2. Synthesis of the intermediates **7a-g**

##### General procedure A for Pd-Catalyzed coupling of thiogalactose with iodoarenes.

A flame-dried resealable Schlenk tube was charged with  $\text{Pd}(\text{OAc})_2$  (3 mol%), Xantphos (1.5 mol%), thiogalactose **2** (0.375mmol), aryl halide **6a-f** (0.25 mmol), and  $\text{Et}_3\text{N}$  (0.25 mmol). The Schlenk tube was capped with a rubber septum,

evacuated and backfilled with argon; then, dioxane (1.5 mL) was added through the septum. The septum was replaced with a teflon screwcap. The Schlenk tube was sealed, and the mixture was stirred at 100 °C for 1 h. The resulting suspension was cooled to room temperature and filtered through celite eluting with ethyl acetate. The filtrate was concentrated and purification of the residue by silica gel column chromatography gave the desired products.

### General procedure B for Pd-G3 XantPhos Catalyzed coupling of thiogalactose with iodoarenes at room temperature.

A round bottom flask is charged with Xantphos PdG<sub>3</sub> precatalyst (1 mol%), thiogalactose 2 (1 equiv.), halide compound (1 equiv.). After Argon flushing, THF (0.25M) is added. Upon stirring the reaction mixture, NEt<sub>3</sub> (1 equiv.) is added to the medium. The reaction mixture was stirred at room temperature under Argon. After completion and solvent evaporation, the residue is purified by silica gel column chromatography, unless otherwise noted, to give the desired product.

*(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-([2-methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl]thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 7a* [21]

**Compound 7a** was prepared from the iodophenstatine in 97% yield by using the general method A.

White-yellow solid; R<sub>f</sub> = 0.32 (cyclohexane/ AcOEt, 9:1mp 108–1108C; [a]<sub>D</sub> 24 : +61.0 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.01 (d, *J* = 2.1 Hz, 1H), 7.71 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.98 (s, 2H), 6.91 (d, *J* = 8.6 Hz, 1H), 5.45–5.36 (m, 1H), 5.27 (t, *J* = 10.0 Hz, 1H), 5.06 (dd, *J* = 9.9, 3.4 Hz, 1H), 4.88 (d, *J* = 10.0 Hz, 1H), 3.99–4.02 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.85 (s, 6H), 2.10 (s, 3H), 2.05 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 193.91 (C=O), 170.43 (C=O), 170.29 (C=O), 170.06 (C=O), 169.57 (C=O), 161.13 (C), 152.91 (C), 141.89 (C), 134.03 (CH), 132.88 (C), 131.86 (CH), 130.74 (C), 122.10 (C), 109.88 (CH), 107.52 (2CH), 84.69 (CH), 74.49 (CH), 71.96 (CH), 67.18 (CH), 61.40 (CH<sub>2</sub>), 61.02 (CH), 56.39 (3CH<sub>3</sub>), 56.23 (CH<sub>3</sub>), 20.87 (CH<sub>3</sub>), 20.69 (CH<sub>3</sub>), 20.63 (2 CH<sub>3</sub>); IR (neat): ν = 3439, 3323, 3270, 3204, 3131, 2358, 2159, 2027, 1942, 1751, 1647, 1582, 1502, 1413, 1368, 1225 cm<sup>-1</sup>; HR-MS (ESI): *m/z* = 687.1708, calcd. for C<sub>31</sub>H<sub>36</sub>NaO<sub>14</sub>S: 687.1718.

*(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 7b*

**Compound 7b** was prepared from the diiodide **6b** in 80% yield by using the general method A.

White solid; R<sub>f</sub> = 0.22 (Cyclohexane/EtOAc 75/25); 63% after column chromatography (Cyclohexane / EtOAc 75/25); IR (neat): 2970, 2900, 1753, 1647, 1604, 1512, 1368, 1268, 1222, 1084, 1057 cm<sup>-1</sup>. m.p. = 69.6–71.4 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.56 (d, *J* = 2.2 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 1H), 6.52 (s, 2H), 5.40 (d, *J* = 3.7 Hz, 2H), 5.33 (s, 1H), 5.27 (t, *J* = 10.0 Hz, 1H), 5.06 (dd, *J* = 9.9, 3.4 Hz, 1H), 4.83 (d, *J* = 10.0 Hz, 1H), 4.06 (dd, *J* = 6.7, 3.1 Hz, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.80 (s, 6H), 2.10 (s, 3H), 2.05 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 170.5(C), 170.4(C), 170.2(C), 169.6(C), 164.5(C), 158.1(C), 153.1(C), 149.2(C), 138.1(C), 137.2(C), 134.4(C), 132.9(CH), 129.5(CH), 120.7(C), 113.1(CH<sub>2</sub>), 110.6(CH), 105.8(2CH), 85.4(CH), 74.5(CH), 67.5(CH), 67.3(CH), 61.4(CH<sub>2</sub>), 61.0(CH), 56.3(3CH<sub>3</sub>), 56.1(CH<sub>3</sub>), 20.9(CH<sub>3</sub>), 20.8(3CH<sub>3</sub>). HR-MS(ESI):*m/z* calcd for C<sub>32</sub>H<sub>38</sub>O<sub>13</sub>NaS [M+Na]<sup>+</sup> 685.1931; found 685.1934

*(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((1-(4-methoxyphenyl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 7c*

**Compound 7c** was prepared from the diiodide **6c** in 94% yield by using the general method B. White solid; R<sub>f</sub> = 0.14 (Cyclohexane/EtOAc 8/2); after column chromatography (Cyclohexane / EtOAc 8/2); IR (neat): 2921, 2849, 1747, 1650, 1603, 1511, 1367, 1210, 1083, 1057 cm<sup>-1</sup>. m.p. = 51.1–53.0 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.46 (d, *J* = 8.4 Hz, 2H), 7.33–7.20 (m, 4H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.43 (dd, *J* = 3.3, 0.8 Hz, 1H), 5.40 (d, *J* = 1.1 Hz, 1H), 5.36 (d, *J* = 1.1 Hz, 1H), 5.27 (t, *J* = 10.0 Hz, 1H), 5.06 (dd, *J* = 9.9, 3.4 Hz, 1H), 4.74 (d, *J* = 10.0 Hz, 1H), 4.15 (d, *J* = 13.4 Hz, 2H), 3.96 (t, *J* = 6.6 Hz, 1H), 3.82 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 170.5(C), 170.3(C), 170.2(C), 169.6(C), 159.6(C), 148.8(C), 141.9(C), 133.6(C), 132.2(2CH), 132.0(C), 129.5(2CH), 128.9(2CH), 113.7(2CH), 113.6(C), 86.8(CH), 74.6(CH), 72.1(CH), 67.4(CH), 67.3(CH), 61.7(CH<sub>2</sub>), 55.4(CH<sub>3</sub>), 21.0(CH<sub>3</sub>), 20.8(2CH<sub>3</sub>), 20.7(CH<sub>3</sub>). HR-MS(ESI):*m/z* calcd for C<sub>29</sub>H<sub>32</sub>O<sub>10</sub>NaS [M+Na]<sup>+</sup> 595.1614; found 595.1608.

*(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((3-(1-(4-methoxyphenyl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 7d*

**Compound 7d** was prepared from the diiodide **6d** in 80% yield by using the general method A. Yellow solid; R<sub>f</sub> = 0.27 (Cyclohexane/EtOAc 7/3); 75% after column chromatography (Cyclohexane / EtOAc 8/2); IR (neat): 2936, 1744, 1603, 1511, 1368, 1212, 1177, 1083, 1056 cm<sup>-1</sup>. m.p. = 48.3–50.2 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.49 (s, 1H), 7.48–7.41 (m, 1H), 7.34–7.21 (m, 4H), 6.92–6.83 (m, 2H), 5.42 (d, *J* = 1.2 Hz, 2H), 5.38 (d, *J* = 1.1 Hz, 1H), 5.25 (t, *J* = 10.0 Hz, 1H), 5.05 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.73 (d, *J* = 10.0 Hz, 1H), 4.08 (t, *J* = 6.4 Hz, 2H), 3.90 (t, *J* = 6.2 Hz, 1H), 3.82 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 170.5(C), 170.3(C), 170.2(C), 169.6(C), 159.6(C), 148.9(C), 142.8(C), 133.6(C), 132.7(C), 132.0(CH), 131.4(CH), 129.5(2CH), 128.8(CH), 128.2(CH), 113.8(CH<sub>2</sub>), 86.9(CH), 74.5(CH), 72.1(CH), 67.3(CH), 67.3(CH), 61.6(CH), 55.4(CH<sub>3</sub>), 21.0(CH<sub>3</sub>), 20.8(2CH<sub>3</sub>), 20.7(CH<sub>3</sub>). HR-MS(ESI):*m/z* calcd for C<sub>29</sub>H<sub>32</sub>O<sub>10</sub>NaS [M+Na]<sup>+</sup> 595.1614; found 595.1609

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-((3-(1-(4-(((2*R*,3*S*,4*R*,5*R*,6*S*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl)thio)phenyl)vinyl)phenyl)thio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate **7e**

**Compound 7e** was prepared from the diiodide **6e** in 96% yield by using the general method B: Light yellow solid;  $R_f = 0.27$  (Cyclohexane/EtOAc 5/5); 96% after column chromatography (Cyclohexane / EtOAc 5/5); IR (neat): 2928, 1747, 1637, 1428, 1387, 1368, 1211, 1154, 1083, 1057  $\text{cm}^{-1}$ . m.p.=80.0-81.9°C;  $^1\text{H}$  NMR (300 MHz, Acetone)  $\delta$  7.59 – 7.51 (m, 3H), 7.50 (t,  $J = 1.7$  Hz, 1H), 7.42 – 7.31 (m, 3H), 7.26 (ddd,  $J = 7.7, 1.8, 1.2$  Hz, 1H), 5.56 (dd,  $J = 4.8, 1.0$  Hz, 2H), 5.43 (ddd,  $J = 6.8, 2.5, 1.1$  Hz, 2H), 5.26 – 5.08 (m, 6H), 4.36 – 4.24 (m, 2H), 4.14 (dd,  $J = 6.3, 1.7$  Hz, 2H), 4.10 – 4.07 (m, 1H), 4.05 – 4.00 (m, 1H), 2.11 (d,  $J = 7.3$  Hz, 6H), 2.03 (d,  $J = 2.6$  Hz, 6H), 1.99 – 1.95 (m, 6H), 1.91 (d,  $J = 3.2$  Hz, 6H).  $^{13}\text{C}$  NMR (75 MHz, Acetone)  $\delta$  170.7(2C), 170.5(2C), 170.2(2C), 169.9(2C), 149.6(C), 142.8(C), 141.0(C), 134.4(C), 134.3(C), 131.8(2CH), 131.6(2CH), 129.7(CH), 129.4(2CH), 128.2(CH), 115.8(CH<sub>2</sub>), 86.2(CH), 86.1(CH), 75.2(CH), 75.1(CH), 72.5(2CH), 68.5(CH), 68.5(CH), 68.0(CH), 67.9(CH), 62.6(CH<sub>2</sub>), 62.5(CH<sub>2</sub>), 20.7(2CH<sub>3</sub>), 20.7(2CH<sub>3</sub>), 20.6(2CH<sub>3</sub>), 20.5(2CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>42</sub>H<sub>48</sub>O<sub>18</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup> 927.2180; found 927.2177

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-((4-(1-(naphthalen-2-yl)vinyl)phenyl)thio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate **7f**

**Compound 7f** was prepared from the diiodide **6f** in 80% yield by using the general method A. White solid;  $R_f = 0.16$  (Cyclohexane/EtOAc 7/3); 80% after column chromatography (Cyclohexane/EtOAc 7/3); IR (neat): 3059, 1748, 1652, 1591, 1367, 1211, 1153, 1083, 1057, 1041, 1015  $\text{cm}^{-1}$ . m.p.=62.6-64.1°C;  $^1\text{H}$  NMR (300 MHz, Chloroform-*d*)  $\delta$  7.86 – 7.75 (m, 4H), 7.53 – 7.43 (m, 5H), 7.37 – 7.31 (m, 2H), 5.61 (d,  $J = 1.1$  Hz, 1H), 5.56 (d,  $J = 1.1$  Hz, 1H), 5.44 (dd,  $J = 3.4, 1.1$  Hz, 1H), 5.29 (t,  $J = 9.9$  Hz, 1H), 5.08 (dd,  $J = 10.0, 3.4$  Hz, 1H), 4.77 (d,  $J = 10.0$  Hz, 1H), 4.24 – 4.09 (m, 2H), 4.01 – 3.92 (m, 1H), 2.14 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  170.5(C), 170.3(C), 170.2(C), 169.6(C), 149.4(C), 141.5(C), 138.5(C), 133.4(C), 133.1(C), 132.3(C), 132.2(2CH), 129.0(2CH), 128.3(CH), 128.0(CH), 127.8(CH), 127.4(CH), 126.4(CH), 126.4(CH), 126.3(CH), 115.5(CH<sub>2</sub>), 86.8(CH), 74.6(CH), 72.1(CH), 67.4(CH), 67.4(CH), 61.8(CH<sub>2</sub>), 21.0(CH<sub>3</sub>), 20.8(2CH<sub>3</sub>), 20.7(CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>32</sub>H<sub>32</sub>O<sub>9</sub>SNa [M+Na]<sup>+</sup> 615.1665; found 615.1677.

(2*R*,3*S*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-((2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)ethyl)phenyl)thio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate **7g**

**Compound 7g** (reduction of the double bond of **7b**) was prepared from the galactoside **7b** in a quantitative yield: A round bottom flask was charged with Pd/C (10 weight%) and the acetylated thiogalactoside **7a** (1 equiv. 1.5 mmol). MeOH (3mL) was then added. The reaction mixture was stirred at room temperature under hydrogen atmosphere (1atm) for 96h. After completion and solvent evaporation, the residue is purified by silica gel column chromatography to give the desired product. Light brown solid;  $R_f = 0.14$  (Cyclohexane/EtOAc 7/3); Quant.; IR (neat): 3391, 2961, 1752, 1590, 1511, 1419, 1369, 1220, 1128, 1084, 1058  $\text{cm}^{-1}$ . m.p.=66.7-67.9°C;  $^1\text{H}$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.40 (dd,  $J = 4.6, 2.3$  Hz, 1H), 7.11 (d,  $J = 8.2$  Hz, 1H), 6.81 (d,  $J = 8.5$  Hz, 1H), 6.41 (s, 2H), 5.43 – 5.39 (m, 1H), 5.26 (td,  $J = 9.9, 7.0$  Hz, 1H), 5.08 (dd,  $J = 3.4, 1.4$  Hz, 1H), 4.81 (dd,  $J = 10.0, 1.6$  Hz, 1H), 4.09 (d,  $J = 6.7$  Hz, 2H), 4.06 – 3.96 (m, 1H), 3.92 – 3.85 (m, 1H), 3.84 (s, 3H), 3.83 – 3.78 (m, 9H), 2.10 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.60 (d,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  170.4(C), 170.3(C), 170.2(C), 169.6(C), 157.0(C), 153.3(2C), 142.2(C), 138.9(C), 133.2(CH), 132.2(C), 128.8(CH), 120.2(C), 111.2(CH), 104.9(2CH), 85.5(CH), 74.5(CH), 72.2(CH), 67.6(CH), 67.3(CH), 61.4(CH<sub>2</sub>), 60.9(CH<sub>3</sub>), 56.3(2CH<sub>3</sub>), 56.0(CH<sub>3</sub>), 44.3(CH), 22.3(CH<sub>3</sub>), 20.9(CH<sub>3</sub>), 20.7(3CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>32</sub>H<sub>40</sub>O<sub>13</sub>NaS [M+Na]<sup>+</sup> 687.2087; found 687.2080

#### General procedure for the synthesis of unprotected glycosides **8a-f** and **9a**

To the corresponding acetylated glycoside under argon was added a solution of sodium methoxide [sodium in MeOH (0.25M)]. The reaction mixture was stirred at room temperature during 10 min approx. After control by TLC, the reaction mixture was acidified with Dowex-50®, previously rinsed with MeOH, the resin was taken off by filtration and the solvent evaporated to afford the corresponding unprotected glycoside.

(4-methoxy-3-(((2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)thio)phenyl)(3,4,5-trimethoxyphenyl)methanone **8a**

**Compound 8a** was prepared from the galactoside **7a** in quantitative yield. White solid;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3408, 2942, 1751, 1652, 1581, 1414, 1326, 1256, 1229, 1127, 1056  $\text{cm}^{-1}$ . m.p.=89.9-91.7°C;  $^1\text{H}$  NMR (300 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  8.07 (d,  $J = 2.3$  Hz, 1H), 7.73 (dd,  $J = 8.5, 2.1$  Hz, 1H), 7.13 (d,  $J = 8.7$  Hz, 3H), 4.75 (d,  $J = 9.7$  Hz, 1H), 4.00 (s, 3H), 3.96 (d,  $J = 4.2$  Hz, 1H), 3.91 (d,  $J = 1.6$  Hz, 9H), 3.72 (t,  $J = 9.5$  Hz, 1H), 3.64 (t,  $J = 5.8$  Hz, 2H), 3.57 – 3.50 (m, 2H).  $^{13}\text{C}$  NMR (75 MHz, MeOD)  $\delta$  196.43(C), 161.94(C), 154.29(2C), 134.50(C), 133.16(CH), 131.82(2C), 131.57(CH), 125.86(C), 111.00(CH), 108.77(2CH), 87.99(CH), 80.34(CH), 76.36(CH), 71.20(CH), 70.19(CH), 62.13(CH<sub>2</sub>), 61.25(CH<sub>3</sub>), 56.83(2CH<sub>3</sub>), 56.76(CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>23</sub>H<sub>28</sub>O<sub>10</sub>SNa [M+Na]<sup>+</sup> 519.1301; found 519.1308.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-((2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenyl)thio)tetrahydro-2*H*-pyran-3,4,5-triyl **8b**



**Compound 8b** was prepared from the galactoside **7b** in quantitative yield.

White solid;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3338, 2989, 1649, 1636, 1536, 1508, 1452, 1415, 1242, 1128, 1056, 1015  $\text{cm}^{-1}$ . m.p.=185.1-186.0°C;  $^1\text{H NMR}$  (300 MHz, MeOD)  $\delta$  7.55 (d,  $J = 2.2$  Hz, 1H), 7.17 (dd,  $J = 8.5, 2.2$  Hz, 1H), 6.93 (d,  $J = 8.6$  Hz, 1H), 6.59 (s, 2H), 5.44 (d,  $J = 1.2$  Hz, 1H), 5.34 (d,  $J = 1.2$  Hz, 1H), 4.60 (d,  $J = 9.7$  Hz, 1H), 3.90 (d,  $J = 2.6$  Hz, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 3.79 (s, 6H), 3.69 – 3.55 (m, 3H), 3.50 – 3.41 (m, 2H).  $^{13}\text{C NMR}$  (75 MHz, MeOD)  $\delta$  171.8(C), 158.3(C), 154.2(C), 150.8(C), 149.3(C), 139.3(C), 135.8(C), 131.2(CH), 128.6(CH), 124.7(C), 113.5(CH<sub>2</sub>), 111.4(CH), 106.9(2CH), 88.4(CH), 80.3(CH), 76.3(CH), 71.1(CH), 70.2(CH), 62.2(CH<sub>2</sub>), 61.2(CH<sub>3</sub>), 56.6(2CH<sub>3</sub>), 56.5(CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>24</sub>H<sub>30</sub>O<sub>9</sub>NaS [M+Na]<sup>+</sup> 517.1508; found 517.1504

*(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((4-(1-(4-methoxyphenyl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triol 8c*

**Compound 8c** was prepared from the galactoside **7c** in quantitative yield.

Light yellow solid;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3366, 1650, 1637, 1511, 1466, 1242, 1179, 1089, 1030  $\text{cm}^{-1}$ . m.p.=96.6-98.5°C;  $^1\text{H NMR}$  (300 MHz, MeOD)  $\delta$  7.52 (d,  $J = 8.4$  Hz, 2H), 7.25 (d,  $J = 5.8$  Hz, 2H), 7.22 (d,  $J = 6.4$  Hz, 2H), 6.89 (d,  $J = 8.8$  Hz, 2H), 5.35 (dd,  $J = 7.2, 1.3$  Hz, 2H), 4.61 (d,  $J = 9.6$  Hz, 1H), 3.91 (dd,  $J = 3.4, 1.1$  Hz, 1H), 3.81 (s, 3H), 3.78 – 3.69 (m, 2H), 3.66 – 3.56 (m, 2H), 3.51 (dd,  $J = 9.2, 3.3$  Hz, 1H).  $^{13}\text{C NMR}$  (75 MHz, MeOD)  $\delta$  151.3(C), 150.7(C), 141.9(C), 135.6(C), 131.7(2CH), 130.4(2CH), 129.6(2CH), 126.3(C), 114.6(2CH), 113.2(CH<sub>2</sub>), 90.2(CH), 80.7(CH), 76.4(CH), 71.0(CH), 70.5(CH), 62.7(CH<sub>2</sub>), 55.7(CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>21</sub>H<sub>24</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 427.1191; found 427.1192

*(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((3-(1-(4-methoxyphenyl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triol 8d*

**Compound 8d** was prepared from the galactoside **7d** in quantitative yield.

Yellow solid;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3366, 2947, 1647, 1607, 1511, 1432, 1415, 1249, 1180, 1084, 1015  $\text{cm}^{-1}$ . m.p.=75.1-75.7°C;  $^1\text{H NMR}$  (300 MHz, MeOD)  $\delta$  7.63 – 7.46 (m, 2H), 7.37 – 7.17 (m, 4H), 6.99 – 6.89 (m, 2H), 5.42 (dd,  $J = 5.3, 1.2$  Hz, 2H), 4.62 (d,  $J = 9.6$  Hz, 1H), 3.95 (d,  $J = 2.4$  Hz, 1H), 3.85 (s, 3H), 3.72 (d,  $J = 6.1$  Hz, 2H), 3.65 (t,  $J = 9.4$  Hz, 1H), 3.57 (d,  $J = 6.6$  Hz, 1H), 3.53 (dd,  $J = 9.2, 3.3$  Hz, 1H).  $^{13}\text{C NMR}$  (75 MHz, MeOD)  $\delta$  159.6(C), 149.3(C), 142.5(C), 134.6(C), 133.5(C), 130.4(CH), 130.0(CH), 128.9(CH), 128.2(CH), 126.6(CH), 113.3(2CH), 112.2(CH<sub>2</sub>), 88.8(CH), 79.0(CH), 74.9(CH), 69.6(CH), 68.9(CH), 61.0(CH<sub>2</sub>), 54.3(CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>21</sub>H<sub>24</sub>O<sub>6</sub>NaS [M+Na]<sup>+</sup> 427.1191; found 427.1194

*(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)ethyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triol 8e*

**Compound 8e** was prepared from the galactoside **7g** in quantitative yield.

Light brown solid;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3385, 2966, 2938, 1647, 1591, 1490, 1462, 1421, 1332, 1245, 1127, 1063  $\text{cm}^{-1}$ . m.p.=61.3-63.2°C;  $^1\text{H NMR}$  (300 MHz, MeOD)  $\delta$  7.48 (dd,  $J = 8.6, 2.2$  Hz, 1H), 7.13 (dd,  $J = 8.5, 2.2$  Hz, 1H), 6.91 (dd,  $J = 8.5, 1.7$  Hz, 1H), 6.58 (s, 1H), 6.57 (s, 1H), 4.60 (d,  $J = 9.7$  Hz, 1H), 4.10 (q,  $J = 6.7$  Hz, 1H), 3.95 (d,  $J = 3.3$  Hz, 1H), 3.86 (s, 3H), 3.84 (m, 6H), 3.78 (s, 3H), 3.72 (dd,  $J = 5.4, 1.2$  Hz, 2H), 3.67 (dd,  $J = 9.4, 5.3$  Hz, 1H), 3.60 – 3.50 (m, 2H), 1.64 (d,  $J = 7.2$  Hz, 3H).  $^{13}\text{C NMR}$  (75 MHz, MeOD)  $\delta$  156.9(C), 154.3(C), 144.3(C), 140.6(C), 130.9(CH), 127.6(CH), 124.2(C), 122.2(C), 119.8(C), 111.8(CH), 106.1(2CH), 88.3(CH), 80.6(CH), 76.3(CH), 71.1(CH), 70.4(CH), 62.5(CH<sub>2</sub>), 61.0(CH<sub>3</sub>), 56.7(2CH<sub>3</sub>), 56.5(CH<sub>3</sub>), 45.5(CH), 22.4(CH<sub>3</sub>). HR-MS(ESI):m/z calcd for C<sub>24</sub>H<sub>32</sub>O<sub>9</sub>NaS [M+Na]<sup>+</sup> 519.1665; found 519.1669

*(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((4-(1-(naphthalen-2-yl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triol 8f*

**Compound 8f** was prepared from the galactoside **7f** in quantitative yield.

White solid;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3359, 1652, 1592, 1347, 1231, 1123, 1057, 1021  $\text{cm}^{-1}$ . m.p.=143.5-144.0°C;  $^1\text{H NMR}$  (300 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  7.93 – 7.80 (m, 3H), 7.80 – 7.75 (m, 1H), 7.65 – 7.56 (m, 2H), 7.49 (td,  $J = 8.3, 7.7, 2.6$  Hz, 3H), 7.33 (d,  $J = 8.4$  Hz, 2H), 5.61 (s, 1H), 5.58 (s, 1H), 4.68 (d,  $J = 9.6$  Hz, 1H), 3.95 (dd,  $J = 3.3, 1.1$  Hz, 1H), 3.87 – 3.73 (m, 2H), 3.69 (t,  $J = 9.5$  Hz, 1H), 3.63 (ddd,  $J = 6.7, 5.1, 1.2$  Hz, 1H), 3.56 (dd,  $J = 9.1, 3.3$  Hz, 1H).  $^{13}\text{C NMR}$  (75 MHz, MeOD)  $\delta$  151.1(C), 141.5(C), 139.9(C), 135.8(C), 134.7(C), 134.5(C), 131.8(2CH), 129.7(2CH), 129.2(CH), 128.8(CH), 128.6(CH), 128.2(CH), 127.3(CH), 127.2(2CH), 115.2(CH<sub>2</sub>), 90.1(CH), 80.7(CH), 76.4(CH), 71.0(CH), 70.5(CH), 62.7(CH<sub>2</sub>). HR-MS(ESI):m/z calcd for C<sub>24</sub>H<sub>24</sub>O<sub>5</sub>SNa [M+Na]<sup>+</sup> 447.1242; found 427.1237.

*(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((3-(1-(4-(((2R,3S,4R,5S,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)thio)phenyl)vinyl)phenyl)thio)tetrahydro-2H-pyran-3,4,5-triol 9a*

**Compound 9a** was prepared from the galactoside **7e** in quantitative yield.

Light yellow solid ;  $R_f = 0.00$  (Cyclohexane/EtOAc 6/4); IR (neat): 3366, 2952, 2840, 1750, 1651, 1419, 1370, 1222, 1059, 1015  $\text{cm}^{-1}$ . m.p.=116.1-118.0°C;  $^1\text{H NMR}$  (300 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  7.57 – 7.45 (m, 4H), 7.33 – 7.19 (m, 3H), 7.18 (d,  $J = 7.7$  Hz, 1H), 5.46 (s, 2H), 4.63 (d,  $J = 9.5$  Hz, 1H), 4.56 (d,  $J = 9.7$  Hz, 1H), 3.92 – 3.87 (m, 2H), 3.81 – 3.69 (m, 2H), 3.68 – 3.54 (m, 5H), 3.49 (td,  $J = 8.8, 3.1$  Hz, 3H).  $^{13}\text{C NMR}$  (75 MHz, DMSO)  $\delta$  148.0(C), 141.2(C), 138.1(C), 135.6(2C), 128.9(3CH), 128.8(2CH), 128.2(2CH), 125.9(CH), 115.1(CH<sub>2</sub>), 87.6(2CH), 79.2(CH), 79.1(CH), 74.7(2CH), 69.2(2CH), 68.4(CH), 68.3(CH), 60.6(CH<sub>2</sub>), 60.5(CH<sub>2</sub>). HR-MS(ESI):m/z calcd for C<sub>26</sub>H<sub>32</sub>O<sub>10</sub>NaS<sub>2</sub> [M+Na]<sup>+</sup> 591.1335; found 591.1342.

### 4.3. Isothermal Titration Calorimetry

LecA was expressed and purified as previously described.[44] All experiments were performed at 25 °C with an ITC200 isothermal titration calorimeter (Microcal-Malvern Panalytical, Orsay, France). The lyophilized LecA protein was dissolved in a buffer composed of 20 mM Tris HCl pH 7.5, 100 mM NaCl and 100 μM CaCl<sub>2</sub> with 5% DMSO final. All compounds were first dissolved in DMSO then in same buffer for a final concentration of 5% DMSO. The 200 μL sample cell containing LecA (concentrations ranging from 50 to 200 μM) was subjected to injections of ligand solution: 20 to 39 injections of 1 μL or 70 injections of 0.5 μL (0.5 to 2 mM, depending on the ligand) at intervals of 100, 120 or 200s while stirring at 850 rpm. Control experiments were performed by repeating the same protocol, but injecting the ligand into buffer solution. The supplied software Origin 7 or MicroCal PEAQ-ITC was used to fit the experimental data to a theoretical titration curve allowing the determination of affinity (i.e., association constant,  $K_a$ ), binding enthalpy ( $\Delta H$ ), and stoichiometry ( $n$ ). Values for free energy change ( $\Delta G$ ) and entropy contributions ( $T\Delta S$ ) were derived from the equation  $\Delta G = \Delta H - T\Delta S = -RT \ln K_a$  (with  $T = 298.15$  K and  $R = 8.314$  J.mol<sup>-1</sup>.K<sup>-1</sup>).

### 4.4. Crystallization and structure determination of LecA thiogalatosides complexes

The hanging drop vapor diffusion method was used with 2 μL drops containing 50/50 of protein and reservoir solution at 19°C. Crystals of the complex with compound **8c** were obtained by cocrystallisation where lyophilized LecA was dissolved in 20 mM Tris/HCl pH 8.0 and 100 mM NaCl to a concentration of 16.9 mg/mL and incubated for 3h at room temperature with 3.8 mM of compound (1 μL of 100 mM compound in 100% DMSO added to 25 μL of protein). Drops were made using the supernatant after centrifugation at 20000 g for 5 minutes since precipitation was observed with a crystallization solution containing 18% PEG3350, 150 mM potassium thiocyanate (KSCN) and 100 mM Tris/HCl pH 8.5. Rod like crystals were transferred in 30% PEG3350 for cryoprotection prior mounting in a cryoloop and flashed frozen in liquid nitrogen. For the complex with compound **8a**, the dry soak method was used where 0.2 μL of 25mM compound dissolved in 100% DMSO were deposited on the coverslip prior drying at room temperature for a night. Then, 1 μL of lyophilised LecA dissolved in 20 mM Hepes 7.5 10 μM CaCl<sub>2</sub> to 9.8 mg/mL was added and 1 μL of reservoir solution containing 18% PEG3350, 100 mM KSCN and 100 mM sodium acetate pH 4.5. Rod like crystals were obtained and a single rod was transferred in a solution where the PEG concentration was increased to 28% prior mounting and freezing. Data were collected at SOLEIL synchrotron, Saint Aubin, France on Proxima 1 beamline using an Eiger pixel detector (Dectris Ltd).

The data were processed using XDS[45] and XDSME.[46] All further computing was performed using the CCP4 suite.[47] The structures were solved by molecular replacement using as search model the monomer A coordinates of LecA-Gal complex PDB1OKO in PHASER 2.8 with a search for 2 and 4 copies for LecA-**8c** and LecA-**8a** complexes, respectively.[48] The model was improved using Buccaneer 1.5 [49] and ARP/wARP 8.0 [50] for LecA-**8c** and LecA-**8a** complexes, respectively. Restrained maximum likelihood refinement using REFMAC 5.8 [51] was iterated with manual rebuilding in Coot [52] to refine the structures. Five percent of the observations were set aside to perform cross-validation analysis. Hydrogen atoms were added in their riding positions and used for geometry and structure factor calculations. Ligand library were made using JLigand 2.6 (AceDRG mode) before modelling after inspection of the electron density maps and checked using Privateer.[53] Prior deposition in the Protein Data Bank, the models were validated using both the wwPDB Validation server (<http://wwpdb-validation.wwpdb.org>) and Molprobitry.[54] Coordinates and structure factors have been deposited under PDB accession codes 7Z62 and 7Z62 for LecA in complex with compound **8c** and **8a**, respectively. Data quality and refinement statistics are described in Table 2.

### 4.5 Antibiofilm activity [29, 55-56]

*P. aeruginosa* PAO1 (CIP 104116) was obtained from the Institute Pasteur collection (Paris, France). The strain was frozen and kept at -80°C. The inoculum used in each experiment came from a second subculture on Trypticase soy agar (BioMérieux, Craponne, France) that was incubated under aerobic conditions at 37 °C for 24 h. Bacterial suspensions were freshly prepared in sterile distilled water before each experiment. A low-nutritive medium, named minimum biofilm broth (MBB) was used for the biofilm formation and the evaluation of the antibiofilm activity of extracts to create stressful conditions and subsequently promote biofilm formation and growth of adherent cells rather than planktonic growth. The MBB 10X medium is composed of FeSO<sub>4</sub>, 7 H<sub>2</sub>O (0.005 g/L), Na<sub>2</sub>HPO<sub>4</sub> (12.5 g/L), KH<sub>2</sub>PO<sub>4</sub> (5.0 g/L), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (1.0 g/L), glucose (0.5 g/L) and MgSO<sub>4</sub>, 7 H<sub>2</sub>O (0.2 g/L)

Biofilms were developed in 24-well plates (Falcon, TC-treated, polystyrene). The bacterial suspension prepared in MBB (2X) was initially adjusted to 10<sup>8</sup> CFU/mL followed by a serial dilution to 10<sup>-6</sup> with the same medium. One milliliter of the 10<sup>-6</sup> dilution (equivalent to 10<sup>2</sup> CFU/mL) was introduced into each well. In order to test its effect on the biofilm, 1.0 mL of test compound solution prepared at different concentrations in sterile distilled water was added to each well (final concentrations 5, 2.5, 1.25, 0.31 and 0.08 mM). Wells containing 1.0 mL of SDW + 1.0 mL of un-inoculated MBB or 1.0 mL SDW + 1.0 mL inoculated MBB, were considered as sterility and biofilm growth controls, respectively. Plates were then incubated at 37°C. After 2, 4, 6, 20 and 24 h of incubation, the wells were rinsed twice with sterile distilled water and the medium (BBM ± tested product) was replaced. All assays were performed in triplicate.

After incubation, wells were rinsed twice with 2.0 mL of SDW, and then the attached cells were scraped (for 1 min) with a sterile spatula into 1 mL of SDW. The recovered suspension was diluted by serial dilution (from  $10^{-1}$  to  $10^{-6}$ ) and each dilution was inoculated by inclusion in TSA agar plates. After 48 h of incubation at 37 °C, the numbers of CFU were counted by considering only plates with 15 to 300 CFU. The adhered biomass was then calculated and subjected to logarithmic transformation by Formula (1). The logarithmic reduction and the percentage of biofilm inhibition with respect to the corresponding untreated control were also calculated using Formulas (2) and (3).

$$\log \text{ of adhered biomass (log CFU/mL)} = \log \frac{\text{number of colonies (CFU)}}{\text{Dilution factor} \times \text{inoculated volume}}$$

$$\log \text{ CFU/ml reduction} = \log \text{ CFU/ml for control} - \log \text{ CFU/ml for treated biofilm}$$

$$IP_{\text{CFU}} (\%) = \frac{\text{Adhered cells}_{\text{Control}} (\text{CFU/mL}) - \text{Adhered cells}_{\text{Sample}} (\text{CFU/mL})}{\text{Adhered cells}_{\text{Control}} (\text{CFU/mL})} \times 100$$

#### 4.6 MICs/MBS determination

Determination of the MICs and MBCs of evaluated compounds was performed in Mueller-Hinton broth and Mueller-Hinton agar, respectively, according to EUCAST/CA-SFM guidelines (2020). The range of tested concentrations was from 5,0 mM to 0,0098 mM. The highest used methanol concentration was also tested to confirm no inhibitory of growth nor bactericidal activity.

#### 4.7 Cytotoxicity on mammalian cells (MRC-5)

Evaluation of compounds cytotoxicity was performed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay based on published procedure[57] with some modifications. The potential cytotoxic activity of the ten most active compounds was assessed against MRC-5 cell lines (Human Fetal Lung Fibroblast Cells: ATCC® CCL-171) purchased from the ATCC® (Manassas, United-States) and cultivated in Modified Eagle Medium (MEM: (Dominique Dutsher, Plaisance-du-Touch, France) in flask. Solutions (2-fold serial dilutions) of selected compounds were prepared in RPMI medium.

96-wells microtiter plates were first filled with 100  $\mu$ L of a cell suspension prepared in RPMI medium ( $2 \times 10^4$  cells/100  $\mu$ L). After overnight incubation at 37 °C in a humidified 5-6.5% CO<sub>2</sub> incubator, 100  $\mu$ L of each product dilution were added to achieve final concentrations ranging from 5,0 mM to 0,0098 mM (final volume 200  $\mu$ L/well). Wells corresponding to the untreated control were supplemented with 100  $\mu$ L of fresh medium. Phenol was used as positive control for cytotoxicity. The microplate was then incubated for 24h at 37 °C in a humidified 5-6.5% CO<sub>2</sub> incubator. The supernatant was then discarded followed by a rinsing with 100  $\mu$ L of Dulbeccos's phosphate-buffered saline (D-PBS, Sigma, St. Quentin Fallavier, France). Then 100  $\mu$ L of a MTT solution prepared in D-PBS at a concentration of 0.5 mg/mL were added in all wells. After 60 min of incubation at 37 °C and in order to solubilize the formed formazan, indicator of cell viability, 100  $\mu$ L of DMSO were added. After agitation, the OD was measured at 570 nm and the viability percentage was calculated using the following formula. Assays were performed in duplicate with four technical replicates.

$$Viability (\%) = \frac{OD_{570nm} \text{ of treated cells}}{OD_{570nm} \text{ of control untreated cells}} \times 100$$

Cytotoxicity was defined for viability percentages below 50%.[33]

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/>

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