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CH−π "T-Shape" Interaction with Histidine Explains Binding of Aromatic Galactosides to *Pseudomonas aeruginosa* Lectin LecA

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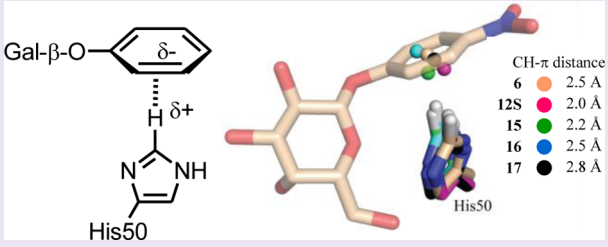
Supporting Information

ABSTRACT: The galactose specific lectin LecA mediates biofilm formation in the opportunistic pathogen *P. aeruginosa*. The interaction between LecA and aromatic β-galactoside biofilm inhibitors involves an intermolecular CH−π T-shape interaction between C(ε1)−H of residue His50 in LecA and the aromatic ring of the galactoside aglycone. The generality of this interaction was tested in a diverse family of β-galactosides. LecA binding to aromatic β-galactosides (K_D ∼ 8 μM) was consistently stronger than to aliphatic β-galactosides (K_D ∼ 36 μM). The CH−π interaction was observed in the X-ray crystal structures of six different LecA complexes, with shorter than the van der Waals distances indicating productive binding. Related XH/cation/π−π interactions involving other residues were identified in complexes of aromatic glycosides with a variety of carbohydrate binding proteins such as concanavalin A. Exploiting such interactions might be generally useful in drug design against these targets.



The CH−π interaction[1] is a weak noncovalent interaction (∼1 kcal/mol)[2] in which an aliphatic or aromatic CH bond interacts with the π-face of an aromatic system, similar to the interaction between H-bond donors (OH and NH) and benzene rings. XH−π interactions are collectively known as "unconventional hydrogen bonds" because the aromatic ring acts as a hydrogen acceptor. While electrostatic forces dominate in the case of OH−π and NH−π interactions, CH−π interactions reflect mostly dispersion interactions.[2−5] CH−π interactions influence the structure, function, and properties of various molecular assemblies,[6,7] for example, they stabilize proteins,[4] protein−protein,[8] protein−nucleic acid,[9] and protein−carbohydrate[10] interactions, and have been employed in the design of constrained peptides[11] and peptidomimetics,[12] and for small molecule recognition.[13]

In the context of developing glycopeptide dendrimers as multivalent lectin ligands and *Pseudomonas aeruginosa* biofilm inhibitors,[14,15] we recently observed an unusual intermolecular CH−π interaction, engaging the C(ε1)−H of His50 of LecA with the aromatic glycosidic group of the glycotripeptide GalAG0 (Gal-β-OC_6H_4CO-Lys-Pro-Leu-NH_2) in a "T-shape" edge-to-face interaction (I, Figure 1).[16] This CH−π T-shape interaction also occurred in the LecA complex with 4-nitrophenyl β-galactoside (NPG) but was missing in the related aliphatic thioglycoside GalBG0 (Gal-β-SCH_2CH_2CO-Lys-Pro-Leu-NH_2), which was also a 4-fold weaker binder, thus providing a structural basis for understanding the previously noted strong binding of aromatic galactosides to LecA.[17−19]

Updating on previous analyses,[20] we found similar HisC-(ε1)H−π T-shape interactions in only 152 (0.52%) of 29 585 histidine−aromatic side chain contacts and 7 (0.4%) of 1749 histidine−aromatic ligand contacts in 33 091 pdb entries with resolution ≤2.0 Å, assessing to the rarity of the interaction (Table S1, Supporting Information). HisCH−π prevailed 3.5:1 over HisNH−π contacts in this analysis, suggesting a more stable arrangement in condensed phase contrasting with the more stable NH−π interaction reported for gas-phase computations of imidazole−benzene dimers.[21] In the case of LecA−galactoside interactions, a conserved hydrogen bond to C(6)−OH of galactose engages the N(ε2) of His50 and restricts the movement of the imidazole allowing only the C(ε1)−H mediated T-shape interaction to take place.

To test whether the CH−π T-shape interaction observed in GalAG0 and NPG might generally explain the preferential binding of aromatic over aliphatic galactosides by lectin LecA, we set out to examine the complexation of various galactosides by LecA. Herein, we show that LecA binds aromatic β-galactosides consistently stronger (12 examples, K_D ∼ 8 μM) than aliphatic β-galactosides (4 examples, K_D ∼36 μM), as determined by isothermal titration calorimetry (ITC). The CH−π interaction is directly observed in the X-ray crystal structures of six LecA complexes of structurally diverse aromatic β-galactosides, with CH−π distances shorter than

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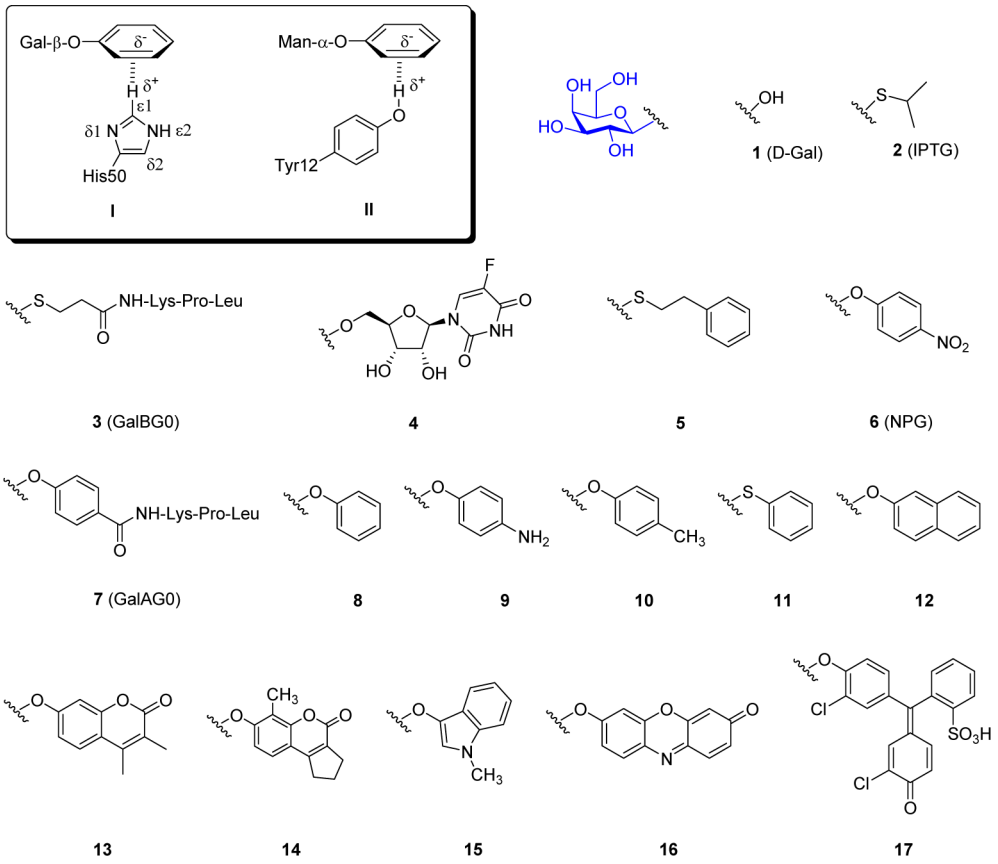

Figure 1. CH−π T-shape and OH−π interactions in lectin aryl glycoside interactions. Model of aromatic glycosides interaction with LecA (I) and concanavalin A (II) and structures of O and S linked galactosides **1**−**17** used in the study.

Table 1. Data for Binding to *P. aeruginosa* Lectin LecA

	HAI assay[a]	isothermal titration calorimetry (ITC)[b]				
ligands	MIC (mM)	N	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)	ΔG (kcal/mol)	K_D (μM)
1 (D-gal)[c]	17	1.1 ± 0.1	−7.9 ± 0.4	2.3	−5.5	87.5 ± 3.5
2 (IPTG)[c]	8	1.1 ± 0.1	−8.9 ± 0.5	2.8	−6.1	32.4 ± 2.7
3 (GalBG0)[c]	2.5	1.2 ± 0.1	−7.3 ± 1.0	1.5	−5.9	51.5 ± 6.7
4	2.1	1.1 ± 0.1	−8.4 ± 1.4	2.5	−5.9	44.8 ± 2.7
5	ND	0.9 ± 0.1	−11.4 ± 1.0	4.9	−6.6	15.6 ± 2.4
6 (NPG)[c]	4.2	0.9 ± 0	−10.0 ± 0.1	3.4	−6.6	14.1 ± 0.2
7 (GalAG0)[c]	0.1	1.0 ± 0.1	−10.8 ± 0.6	3.4	−7.4	4.2 ± 0.9
8	2.1	0.9 ± 0	−11.7 ± 0.3	4.8	−6.9	8.8 ± 0.4
9	1.0	1.1 ± 0	−10.7 ± 0.3	3.8	−6.9	8.4 ± 0.4
10	2.1	1.0 ± 0	−11.4 ± 0.3	4.4	−7.0	7.4 ± 0.3
11	2.1	1.0 ± 0	−11.9 ± 0.4	5.1	−6.8	9.9 ± 0.4
12	0.7	1.2 ± 0	−9.0 ± 0.1	1.6	−7.3	4.2 ± 0.2
13	ND	1.2 ± 0	−8.9 ± 0.1	1.6	−7.3	4.7 ± 0.2
14	ND	0.9 ± 0.1	−8.8 ± 0.7	1.6	−7.2	5.4 ± 1.0
15	6.7	0.8 ± 0.1	−12.6 ± 1.2	6.1	−6.4	19.4 ± 1.5
16	ND	1.0 ± 0	−10.3 ± 0.4	3.4	−6.9	9.1 ± 0.6
17	2.1	1.1 ± 0	−10.6 ± 0.1	3.5	−7.1	6.3 ± 0.2

[a]MIC = minimal inhibitory concentration for the hemagglutination inhibition assay (HAI). Conditions: 2-fold serial dilutions of the tested compounds were incubated with the LecA lectin for 30 min at 4 °C; after that time, rabbit erythrocytes (5% solution in PBS) were added and further incubated for another hour at RT. The MIC corresponds to the highest dilution causing a complete inhibition of hemagglutination. ND = HAI assay data could not be obtained for these ligands due to limited solubility. [b]Thermodynamic parameters and dissociation constant K_D reported from ITC measurements in 0.1 M Tris-base, pH 7.5, 25 mM $CaCl_2$, 25 °C. Stoichiometry N = number of occupied lectin galactose binding site per ligand. [c]ITC and HAI assay data for these compounds from ref 16.

the van der Waals distance, indicating productive binding. Analyzing the published structures of aromatic glycosides in complex with various carbohydrate binding proteins reveals related interactions, for example, a previously unidentified

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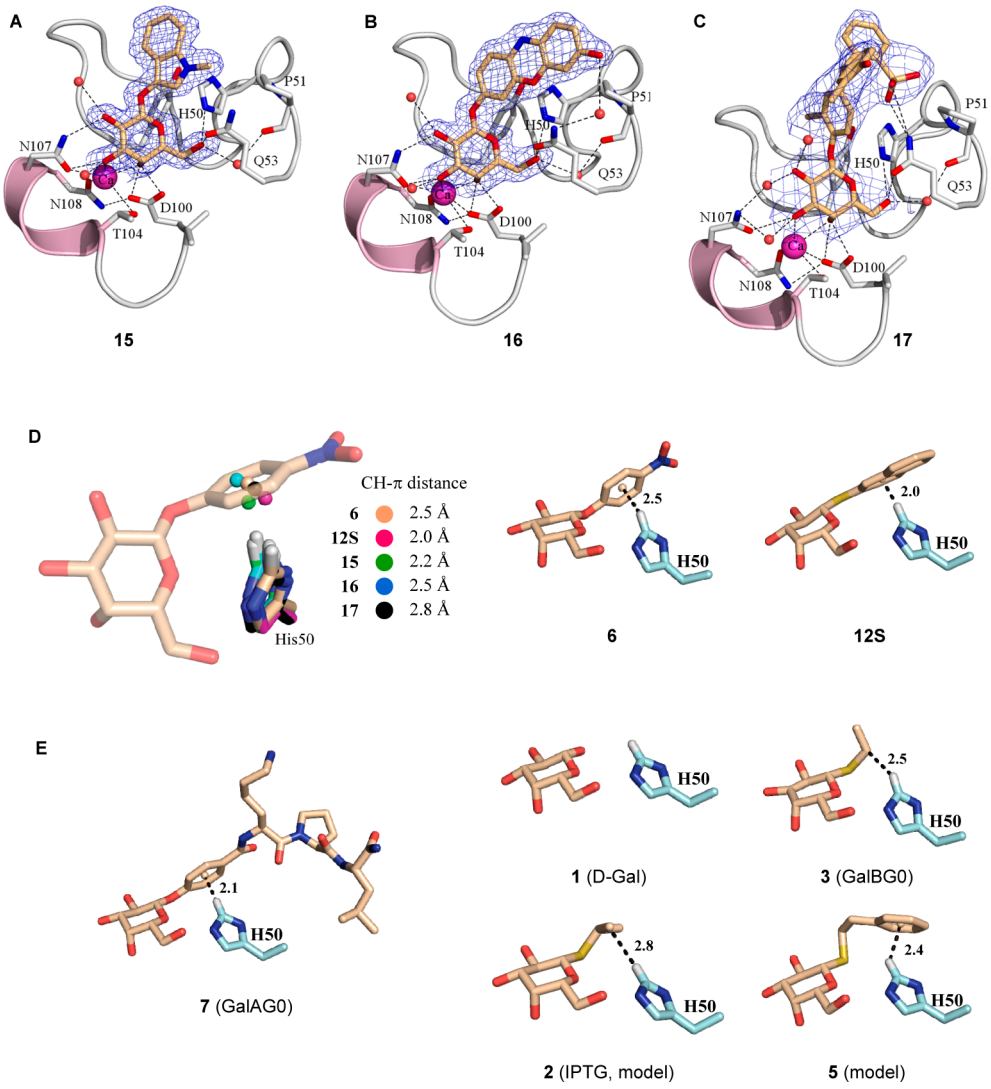

Figure 2. Structures and models of His^{50}C(ε1)H–π interactions in LecA-galactosides complexes. (a–c) Structures of cocrystallized ligands (in sticks) **15**, **16**, and **17** with LecA. The fit of the ligand models to the electron density map is depicted. H-bond interactions between the ligand and LecA are shown by dotted lines. (d) Comparison of CH–π T-shape interactions from an overlay of the structures of cocrystallized galactosides **6** (beige), **12S** (pink), **15** (green), **16** (cyan), and **17** (black). The centroids of the aromatic ring of the galactosides depicted in colored spheres and their distance from C(ε1)–H of His50 (LecA) is reported in Å. (e) Experimental structures of galactose binding in LecA complexes with **7** (3ZYB), **1** (1OKO), **3** (3ZYH), and docked models with IPTG (**2**) and phenylethyl-thio-β-galactoside (**5**). All galactosides are depicted in beige and His 50 from LecA in cyan colored sticks.

OH–π interaction between the hydroxyl group of Tyr12 from concanavalin A and an aromatic α-mannoside (II, Figure 1). [22] These experiments establish that the CH–π T-shape interaction with His50 of LecA causes the stronger binding of aromatic galactosides to the lectin.

To test the possible generality of the CH–π T-shape interaction observed in the β-galactoside–LecA complexes NPG–LecA and GalAG0–LecA, additional complexation studies were performed with aromatic β-galactosides **8**–**17**, the aliphatic β-galactoside **4**, and phenethyl β-thiogalactoside (**5**) (Figure 1; Table S2, Supporting Information). Thermodynamic parameters were determined by ITC of LecA (20 μM) with the galactosides (1–2 mM) (Table 1; Figure S1, Supporting Information). The β-galactosides bound the lectin 2 to 20-fold stronger than free galactose (K_D = 88 μM), indicating a generally positive contribution of the aglycone to binding. However, the binding affinity was independent of the molecular weight (MW), showing that aglycones do not simply build additional productive contacts with LecA as their size increases (Figure S1c, Supporting Information). On the contrary, the nature of the aglycone played a major role in determining the binding strength. Thus, the aliphatic thiogalactosides **2** (IPTG), **3** (GalBG0) and the riboglycoside **4** were weak binders (K_D > 30 μM), while the aromatic galactosides ranging from the smallest ligand phenyl-β-galactoside **8** to the rather large galactotripeptide **7** (GalAG0) showed relatively strong binding (K_D < 10 μM). Aromatic galactosides had stronger binding enthalpies ($\Delta H \sim -11$ kcal/mol) than the aliphatic galactosides ($\Delta H \sim -8$ kcal/mol), compensated by more unfavorable binding entropies ($-T\Delta S \sim +4$ kcal/mol vs ~+2.5 kcal/mol), resulting in a net gain of 1–2 kcal/mol in the free energy of binding, consistent with literature values for CH–π interactions.[5,21]

Exceptions to this general trend included strong binding by the aliphatic thiogalactoside **5** (K_D ~16 μM) bearing a homobenzylic aromatic group, which showed binding en-

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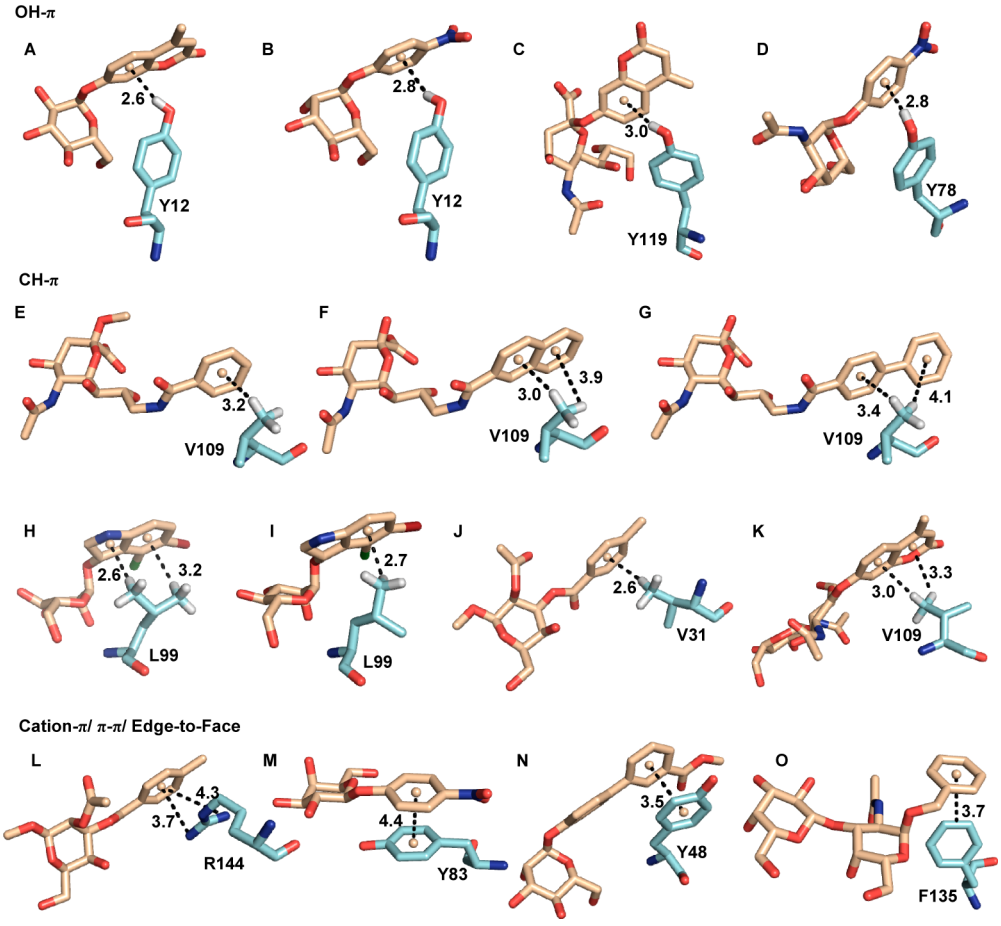

Figure 3. Non-covalent π interactions in aromatic glycosides–protein complexes. OH–π interactions: (A, B) conconavalin A (1CJP; 1VAM), (C) *Trypanosoma cruzi* trans-sialidase (1S0J), (D) *Maclura pomifera* agglutinin (3LM1). CH–π inreactions: (E–G) sialoadhesin (1OD9; 1OD7; 1ODA). (H) lectin from *Dioclea violacea* (3AX4), (I) pro-inflammatory lectin from the seeds of *Dioclea wilsonii* Standl (3SH3), (J) human galectin-1 (3T2T). (K) Lysozyme (1BB7). Cation–π interaction: (L) human galectin-3 (3T1L). π–π interaction: (M) galactoside acetyltransferase (1KRV), (N) FimH lectin (3MCY). Edge-to-face interaction: (O) *Amaranthus caudatus* agglutinin (1JLX).

thalpies and entropies typical of an aromatic galactoside presumably reflecting a CH–π interaction with the phenethyl group (see structural discussion below). Furthermore, unusually weak binding occurred with the aromatic galactoside **6** (K_D = 14 μM) due to a less favorable binding enthalpy (ΔH = −10.0 kcal/mol) and with the indoxyl galactoside **15** ($K_D \sim 19$ μM), for which a rather strong binding enthalpy (ΔH = −12.6 kcal/mol) was compensated by an unusually unfavorable binding entropy ($-T\Delta S$ = +6.1 kcal/mol) tentatively indicating a particularly small contribution of desolvation to binding. In both cases, the effects on binding enthalpies can be attributed to the nature of the aromatic group (see structural discussion below). Interestingly, the strong binding aromatic galactosides **12**–**14** ($K_D \sim 5$ μM) showed a weaker binding enthalpy ($\Delta H \sim -9$ kcal/mol) than the other aromatic galactosides compensated by a smaller entropy penalty ($-T\Delta S \sim +1.6$ kcal/mol). This effect was probably caused by their particularly hydrophobic aromatic aglycone leading to a stronger desolvation effect upon binding.

Binding affinities were also determined by hemagglutination assay measuring the inhibition of LecA induced agglutination of human erythrocytes in comparison to D-galactose as the reference (Table 1). All compounds were tested except for **5**, **13**, **14** and **16** due to their limited solubility in water. The results of HA followed the same trend as the ITC study with stronger binding of aromatic over aliphatic galactosides (Figure S2, Supporting Information). On the other hand, none of the galactosides showed any significant effect in a biofilm inhibition assay, in agreement with our previous finding that multivalency is essential to reduce biofilm formation via inhibition of LecA.[16]

To provide a direct observation linking the stronger binding of the aromatic galactosides to a CH–π T-shape interaction, all complexes were subjected to crystallization screening. Good quality crystals were obtained in the case of **15**, **16**, and **17**, leading to three new structures (Figure 2, panels a–c; Table S3, Supporting Information) complementing the already existing crystal structures of LecA complexes with D-galactose (**1**), NPG (**6**), GalAG0 (**7**) and GalBG0 (**3**). In all cases, the ligand occupied the galactose binding pocket with the galactose bound in the same orientation as free galactose, with coordination of Ca^{2+} with the C(4)–OH group of galactose. His50 formed an H-bond with the C(6)–OH group of galactose, and its C(ε1)–H engaged in a CH–π T-shape interaction with the aromatic aglycone of all the aromatic β-galactosides.

The CH–π bond distances, calculated from the histidine C(ε1)–H to the centroid of the aromatic aglycone, were between 2.2 and 2.8 Å (Figure 2, panel d), which is comparable to the relatively short distances reported for other CH–π interactions (2.53–2.75 Å).[23] The same binding geometry with a CH–π binding distance of 2.0 Å was also observed in the

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complex of LecA with 2-naphtyl-1-thio-β-D-galactopyranoside **12S** recently filed by Imberty et al. (PDB ID 4A6S). These CH−π distances were below the expected van der Waals distance of 2.9 Å, [25] indicating productive binding, with generally shorter CH−π distances in electron rich aromatic aglycones (**12S** and **15**) showing stronger binding enthalpies (**15**: ΔH = −12.6 kcal/mol), and longer CH−π distances in electron poor aromatic aglycones showing weaker binding enthalpies (**6**, **16**, and **17**, $\Delta H \sim -10$ kcal/mol), in agreement with the notion that CH−π T-shape interactions involve electron donation from an aromatic group to the electropositive H−C bond.[5,24]

By comparison, the structure of the LecA complexes with the thiogalactoside GalBG0 (**3**) and with free galactose showed essentially identical binding pattern for the galactosyl group. Residue His50 had the same position as in the other complexes and engaged in the conserved H-bond between its distal N(ε2) atom and the C(6)−OH of galactose. Although the CH−π T-shape interaction was absent, the C(ε1)−H was in van der Waals contact with the alkyl groups in GalBG0 (**3**) and in a docked complex with IPTG (**2**) (Figure 2, panel e). The tripeptide portion of GalBG0 (**3**) was disordered in the crystal structure of its LecA complex, while the same tripeptide was well resolved in the case of the stronger binding aromatic analog GalAG0 (**7**), which accounts for the smaller entropy loss upon binding of **3** ($-T\Delta S$ = 1.5 kcal/mol) compared to **7** ($-T\Delta S$ = 3.4 kcal/mol). Nevertheless, the binding enthalpy of **7** was comparable to that of other smaller aromatic galactosides, suggesting that the protein−ligand contacts at the level of the tripeptide did not contribute to the stronger binding enthalpy of **7** compared to **3** ($\Delta\Delta H$ = −3.5 kcal/mol).

The binding and structural data above can be interpreted in terms of the CH−π T-shape interaction of the aromatic aglycone with His50 providing the key productive interaction enhancing binding of the aromatic β-galactosides by approximately 4-fold over the aliphatic β-galactosides. The significantly stronger binding of phenethyl-β-thiogalactoside **5** (K_D = 16 μM) compared to the other nonaromatic galactosides might indicate a CH−π interaction with the phenyl ring despite of its more remote position in the ligand. Although a crystal structure could not be obtained in this case, a docking study indeed positioned the phenyl group of this ligand in the correct position for this CH−π interaction to take place but without the T-shape interaction (Figure 2, panel e).

An analysis of other reported aromatic glycoside−protein complex structures revealed the presence of similar intermolecular XH/cation/π−π interactions (Table S4, Supporting Information). One example from each class is discussed here. An OH−π interaction was observed for the complex of conconavalin A with aryl mannosides (Figure 3, panels a and b).[25,26] This, together with additional hydrophobic contacts and an H-bond interaction, results in a 12-fold improvement in binding as compared to the unsubstituted mannose.[22] Similarly, CH−π interactions are observed in the complexes of sialoadhesin with sialic acid based siglec inhibitors,[27] where one or both CH groups from Val109 stack against the π face of the aromatic aglycone. These inhibitors present an interesting example of contribution of the CH−π interactions toward ligand binding (Figure 3, panels e−g). Formation of a single CH−π interaction (Figure 3, panel e) and hydrophobic contacts with two residues result in 2 fold affinity improvement for the benzyl containing ligand (Me-α-9-N-benzoyl-amino-9-deoxy-Nuc5Ac) over the nonaromatic ligand methyl-α-Neu5Ac. Naphthyl and biphenyl containing ligands (Me-α-9-N-(naphthyl-2-carbonyl)-amino-9-deoxy-Nuc5Ac and Me-α-9-N-(biphenyl-4-carbonyl)-amino-9-deoxy-Nuc5Ac respectively), which forms an additional CH−π interaction with the side chain of Val109 and van der Waals contacts with Ser45 and Asn95 (Figure 3, panels e and g), were respectively 11 and 13 times stronger binders than methyl-α-Neu5Ac. In another somewhat different system, formation of a cation−π interaction (Figure 3, panel l), which is significantly stronger than a CH−π interaction, together with an additional hydrogen bond, resulted in 20-fold improvement in affinity of the aromatic taloside inhibitor against human galectin-3 as compared to methyl β-D-talopyranoside.[28] A π−π interaction and an H-bond found in the complex of a biphenyl containing glycoside antagonist of FimH mediated bacterial adhesion, which can explain the 30 and 1000 fold stronger binding compared to phenyl-α-mannoside and methyl-α-mannoside, respectively.[29] In the case of *Amaranthus caudatus* agglutinin, formation of an edge-to-face contact along with additional van der Waals interactions is observed in the complex of α-benzyl T-disaccharide, which results in a 2-fold improvement in affinity over the α-methyl T-disaccharide (Figure 3, panel o). [30]

Overall, this study establishes the significance of the CH−π T-shape interaction between C(ε1)−H of His50 and the aromatic ring of the galactoside aglycone in ligand binding to lectin LecA from *P. aeruginosa*. Related XH/cation/π−π T-shape interactions involving other residues also occur in complexes of aromatic glycosides with a variety of carbohydrate binding proteins such as concanavalin A and contribute to complex stability. Exploiting such interactions might be generally useful in drug design against these targets.

METHODS

Procedures for ITC, hemagglutination assays, X-ray data collection and refinement statistics, and modeling and computational methods are described in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Compound vendors, methods, and data for ITC integrated titration curves, X-ray data collection, and refinement statistics. Details of computational methods used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

Coordinates and structure factors of refined LecA complexed with compound **15**, **16**, and **17** are available from the Protein Data Bank with accession codes 4LJH, 4LK7, and 4LK6, respectively.

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Author Contributions

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Author Contributions

R.U.K. designed the study, performed crystallography, computation, and hemagglutination, analyzed data, and wrote

the paper; D.G. designed the study, performed ITC, and wrote the paper; J.S. performed computational analysis of pdb; R.V. performed crystallography and hemagglutination; M.S. supervised ITC; A.S. supervised and performed crystallography; T.D. supervised the study and analyzed data; J.L.R. designed and supervised the study, analyzed data, and wrote the paper.

Notes

The authors declare no competing financial interest.

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