

Discovery of Two Classes of Potent Glycomimetic Inhibitors of *Pseudomonas aeruginosa* LecB with Distinct Binding Modes

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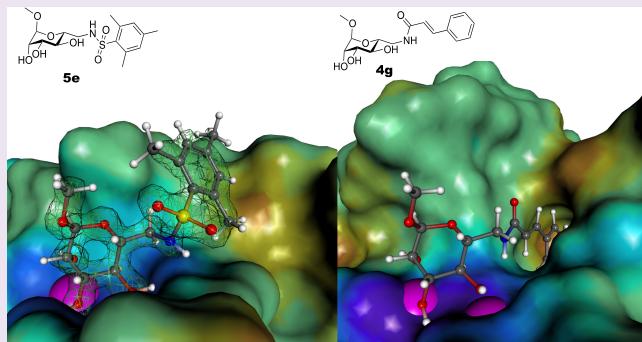
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S Supporting Information

ABSTRACT: The treatment of infections due to the opportunistic pathogen *Pseudomonas aeruginosa* is often difficult, as a consequence of bacterial biofilm formation. Such a protective environment shields the bacterium from host defense and antibiotic treatment and secures its survival. One crucial factor for maintenance of the biofilm architecture is the carbohydrate-binding lectin LecB. Here, we report the identification of potent mannose-based LecB inhibitors from a screening of four series of mannosides in a novel competitive binding assay for LecB. Cinnamide and sulfonamide derivatives are inhibitors of bacterial adhesion with up to a 20-fold increase in affinity to LecB compared to the natural ligand methyl mannoside. Because many lectins of the host require terminal saccharides (e.g., fucosides), such capped structures as reported here may offer a beneficial selectivity profile for the pathogenic lectin. Both classes of compounds show distinct binding modes at the protein, offering the advantage of a simultaneous development of two new lead structures as anti-pseudomonal drugs with an anti-virulence mode of action.



Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium accounting for a large number of nosocomial infections.^{1,2} Additionally, it frequently colonizes airways of cystic fibrosis patients, ultimately leading to lung failure.³ As a result of the bacterium's high resistance toward antibiotics and its ability to form biofilms, infections can turn chronic with a high mortality rate among patients.^{4,5} In a biofilm, bacteria are embedded in the extracellular matrix consisting of extracellular DNA, polysaccharides, and proteins.⁶ *P. aeruginosa* produces two soluble lectins under quorum-sensing control: LecA (or PA-IL) and LecB (or PA-IIL).^{7,8} Deletion of either *lecA* or *lecB* genes in *P. aeruginosa* resulted in strains with weaker lung colonization and systemic spread in a murine model of lung infections.⁹ Since the two lectins are both virulence factors and necessary for biofilm formation, their inhibition is considered a promising approach for anti-pseudomonal treatment.^{10–17} These tetrameric carbohydrate-binding proteins recognize specific monosaccharide residues in a calcium-dependent manner: LecA is specific for D-galactose, whereas LecB binds L-fucosides and D-mannosides.⁷ Williams and Reymond showed that treatment of *P. aeruginosa* with multivalent carbohydrate-based inhibitors of these lectins resulted in a reduced biofilm formation *in vitro*.^{13,18,19} Furthermore, inhalation of an aqueous galactose- and fucose-containing aerosol resulted in a reduction of respiratory tract infections with *P. aeruginosa*.^{20,21}

LecB has an unusual high affinity for fucose residues, which has been explained by the crystal structure of the complex: two calcium ions in the binding site mediate the binding of one saccharide ligand to the protein.^{22,23} This particularly high affinity has prompted various groups to synthesize fucose-based inhibitors of this lectin (reviewed in Imberty *et al.*^{12,15}). The Lewis blood group antigen Lewis^a, a trisaccharide containing an α-fucoside, is the best known monovalent ligand of LecB ($K_d = 210$ nM).²⁴ The crystal structure of its complex revealed, in addition to the recognition of fucose, an additional hydrogen bond of the attached GlcNAc-6-OH with the receptor but no direct interaction of the third saccharide moiety. Consequently, synthetic inhibitors were simplified and based on the Fuc-α-1,4-GlcNAc disaccharide or on fucose alone. This did, however, not lead to an increase in affinity compared to the parent saccharide Lewis^a, and its saccharide character was maintained.^{15,25} In another example, fucosylamides displayed a 3-fold lower affinity compared with that of methyl α-L-fucoside, which has a K_d of 430 nM.^{26,27}

These examples and all other synthetic fucose-derived inhibitors of LecB described to date bear terminal α-fucosides,^{12,15}

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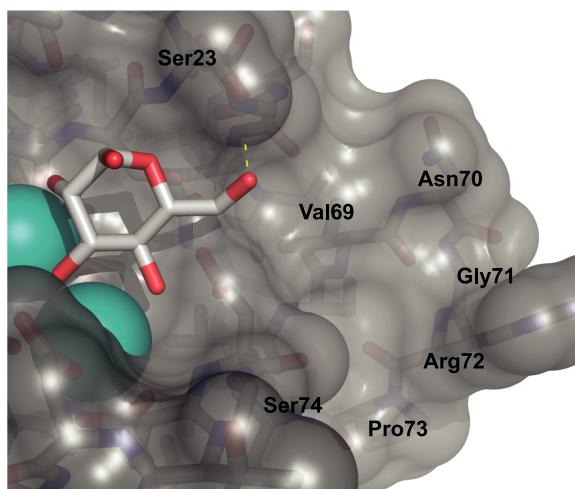


Figure 1. (Top) The crystal structure of LecB in complex with α -D-mannose reveals the hydrogen bond between O6 of the ligand and the Ser23 side chain (yellow line). The loop from Val69 to Ser74 confines a cleft adjacent to C6 of mannose (PDB code 1OUR²²). (Bottom) Methyl α -D-mannoside (**1**, $K_d = 71 \mu\text{M}$ ²⁷) served as a lead structure for the search for LecB inhibitors. Compound **1** was modified at its primary hydroxyl group to target the adjacent cleft using different linker chemistries. Libraries of triazoles **2**, amines **3**, amides **4**, and sulfonamides **5** were synthesized and analyzed for binding to LecB.

which are also ubiquitous glycoconjugate epitopes in the host. Such inhibitors therefore possess the potential to interfere with other fucose-binding proteins of the host (e.g., DC-SIGN, MBL, the selectins, etc.), which have among others important roles in host defense and inflammatory response. The natural LecB ligand Lewis^a, for example, is also a potent ligand to DC-SIGN^{28,29} and is therefore unsuitable as a drug. In general, an inhibition with terminally unmodified saccharides may result in unwanted off-target side effects. In nature, carbohydrate-binding proteins generally recognize multivalently displayed epitopes and thereby circumvent the promiscuity of individual monosaccharides for a plethora of target receptors. Therefore, the design of small molecule inhibitors of carbohydrate-binding proteins with the potential for becoming a drug is a particular challenge.¹⁴ Consequently, besides the poor pharmacokinetic properties of structures such as Lewis^a (Supplementary Table S3), modifications of the natural saccharide ligands with the aim to improve affinity and specificity toward the target of interest are of crucial importance.

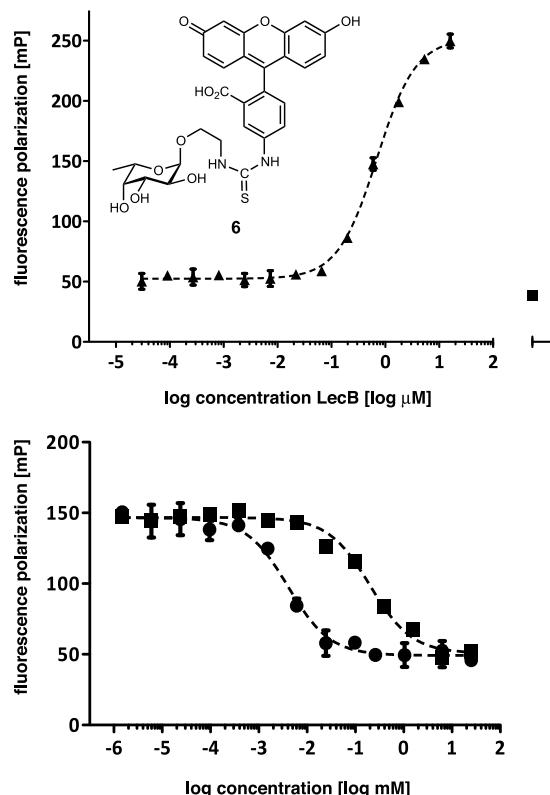


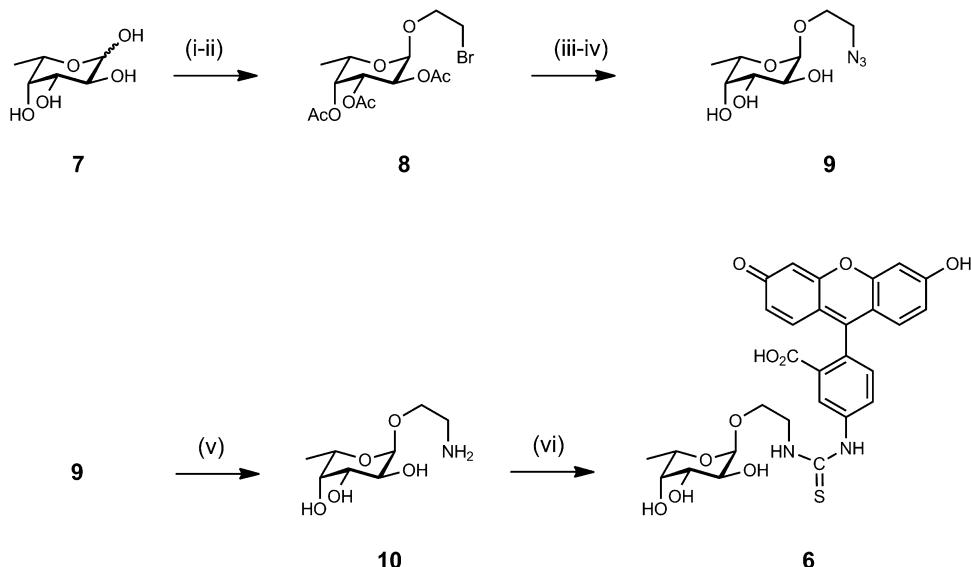
Figure 2. (Top) Titration of fluorescein-labeled fucose-based reporter ligand **6** with LecB and determination of the fluorescence polarization reveals a strong and specific binding ($EC_{50} = 697 \text{ nM}$, black triangles) that could be completely inhibited by the addition of excess fucose (1 mM, 16 μM LecB, black circle). Ligand **6** in the absence of LecB showed fluorescence polarization values at background level (black square). (bottom). Competitive inhibition of the binding of **6** to LecB with inhibitors allowed the determination of IC_{50} values for fucose ($IC_{50} = 2.74 \pm 1.44 \mu\text{M}$, black circles) and **1** ($IC_{50} = 157.8 \pm 52.8 \mu\text{M}$, black squares); one representative titration of triplicates is shown. IC_{50} values for fucose and **1** correspond to 21 and 17 independent experiments, respectively, and error bars show standard deviations.

LecB also binds mannosides through coordination of the two calcium ions to their secondary hydroxyl groups with the same relative orientation as in fucose. Interestingly, the additional 6-OH of mannose forms a hydrogen bond to Ser23.²² Mannose, however, lacks the equatorial methyl group of L-fucose, which served as an explanation for the increased affinity of fucose with respect to mannose derivatives (e.g., **1**, Figure 1; $K_d = 71 \mu\text{M}$) toward LecB.^{22,27} Adjacent to the primary 6-hydroxy group of **1**,²² a cleft on the protein surface that is confined by the loop of Val69 to Ser74 may be targeted by additional pharmacophores to address affinity, specificity, and drug-like properties (Figure 1).

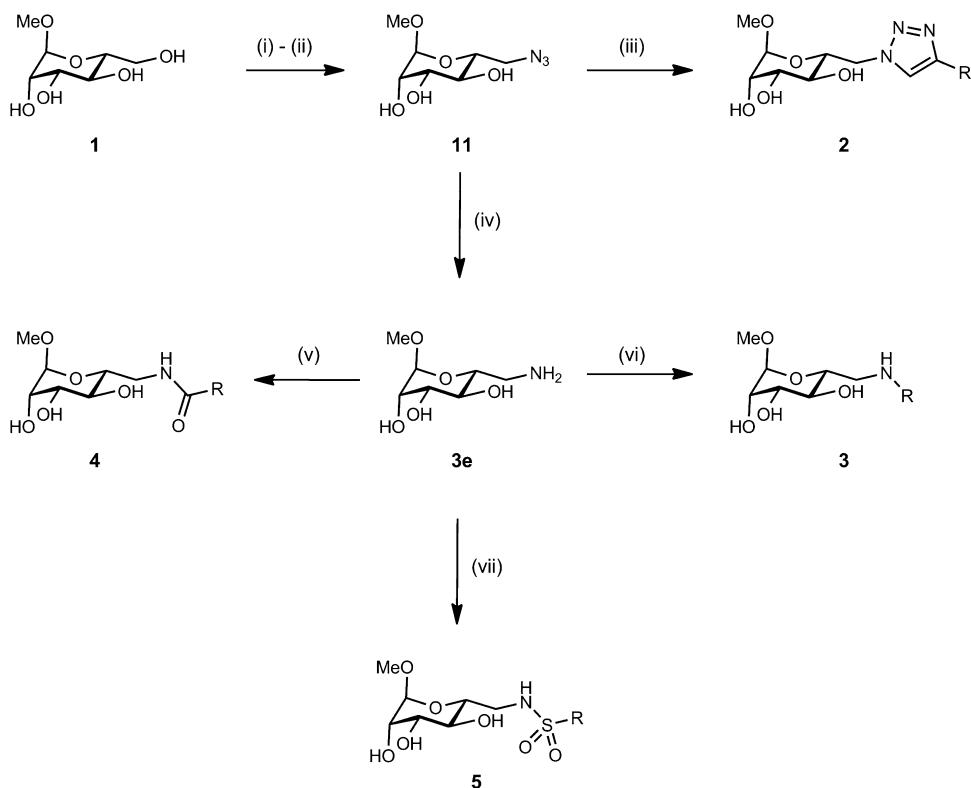
Here, we report on novel terminally modified mannosides as ligands of LecB and inhibitors of the adhesion of *P. aeruginosa* cells with a 20-fold increase in affinity compared to that of **1**. These small molecules may lead to more selective ligands for LecB over other lectins of the host through the terminal capping of mannose.

RESULTS AND DISCUSSION

To target the additional cleft on the protein surface, **1** was modified with a nitrogen at position 6, and a set of substituted triazoles **2**, amines **3**, amides **4**, and sulfonamides **5** were designed (Figure 1). The different linking functional groups were chosen on the

Scheme 1. Synthesis of Fucose-Based Fluorescein-Labeled Reporter Ligand 6^a

^aReagents and conditions: (i) La(OTf)₃, 2-bromoethanol, 7.5 h, 70°C; (ii) Ac₂O, pyridine, 12 h, rt, 46% (2 steps, α/β 4:1); (iii) NaN₃, DMF, 3.25 h, 70°C, 70%; (iv) NaOMe, MeOH, 12 h, rt; (v) H₂, Pd/C, EtOH, 20 h, rt, 66% (2 steps); (vi) FITC, NaHCO₃, DMF, 6.5 h, rt, 96%.

Scheme 2. Synthesis of Mannose-Derived Triazoles 2, Amines 3, Amides 4, and Sulfonamides 5^a

^aReagents and conditions: (i) TsCl, pyridine, rt, 24 h; (ii) NaN₃, DMF, 70°C, 4 h; (iii) alkyne, CuSO₄, Na ascorbate, rt, 2–48 h; (iv) H₂, Pd/C, EtOH, rt, 12 h; (v) acyl chlorides or carboxylic acids/EDC-HCl, TEA, DMF; (vi) RCHO, NaBH₄, 12 h; (vii) sulfonyl chlorides, TEA, DMF.

basis of facile access through selective reactions and to study their influence on possible hydrogen bonding with Ser23 or salt bridge formation with Asp96.

For a rapid biochemical evaluation of the LecB antagonists, we established a competitive binding assay based on fluorescence polarization. Such assays were shown useful for inhibitor

screening of other lectins, e.g., galectins³⁰ and FimH.³¹ A fluorescein-labeled, fucose-based reporter ligand 6 was synthesized (Scheme 1) starting from a lanthanum triflate catalyzed fucosylation of 2-bromoethanol following the procedure of Dasgupta *et al.*³² in an α/β anomeric ratio of 4:1. Acetylation of 2-bromoethyl fucoside, nucleophilic replacement of the bromide by sodium

Table 1. Mannose-Derived Libraries of Triazoles 2, Amines 3, Amides 4, and Sulfonamides 5 as LecB Inhibitors^a

triazoles				amines			
	R	IC ₅₀ [μM]	SD [μM]		R	IC ₅₀ [μM]	SD [μM]
2a	CH ₂ OH	578.1	76.5	3a	CH ₂ Ph	226.8	85.0
2b	CH ₂ CH ₂ Ph	264.1	113.6	3b	CH ₂ (4-NO ₂)Ph	116.9	65.2
2c	Ph	372.6	90.1	3c	CH ₂ (4-CN)Ph	114.9	19.4
2d	3-MePh	279.4	120.2	3d	CH ₂ (4-Cl)Ph	211.6	88.5
2e	4-MePh	293.8		3e	H	353.5	29.4
2f	4- ^t BuPh	ns					
2g	4-FPh	240.0	149.9				
amides				sulfonamides			
	R	IC ₅₀ [μM]	SD [μM]		R	IC ₅₀ [μM]	SD [μM]
4a	Me	281.3	123.8	5a	Ph	15.9	3.3
4b	Ph	110.2	28.1	5b	4-MePh	49.7	36.9
4c	4-NO ₂ Ph	75.8	15.8	5c	4-BrPh	20.9	4.6
4d	3,5-diNO ₂ Ph	121.0	39.2	5d	4-NO ₂ Ph	36.1	16.0
4e	CH ₂ Ph	73.8	13.2	5e	2,4,6-Me ₃ -Ph	3.4	0.4
4f	CHPh ₂	161.2	9.8	5f	2,4,6-iPr ₃ -Ph	ns	
4g	(E)-CHCHPh	37.4	5.0	5g	(E)-CHCHPh	86.9	11.1

^aIC₅₀ values were determined with a competitive fluorescence polarization assay. A minimum of 3 independent experiments were performed, and standard deviations are given. ns = not soluble at 10 mM in aqueous buffer with 10% DMSO. SD = standard deviation.

azide, and deacetylation under Zemplén conditions gave **9** in good yield. After reduction, 2-aminoethyl fucoside (**10**) was subsequently treated with fluorescein-5-isothiocyanate (FITC) and base to yield the reporter ligand **6** in 96% yield. Upon incubation of **6** with a dilution series of LecB, a dose-dependent increase of fluorescence polarization was observed as a result of the binding to LecB (Figure 2, top). The observed binding of **6** to LecB (EC₅₀ = 697 nM) is in good agreement with the known dissociation constant of methyl α-L-fucoside.²⁷ The carbohydrate specificity of the binding of **6** was demonstrated by addition of excess fucose, resulting in complete displacement of **6** from the protein. For the screening of LecB ligands, constant concentrations of **6** and LecB were titrated with serial dilutions of inhibitors. The assay was evaluated using fucose and **1** as ligands with known K_d values (Figure 2, bottom): IC₅₀ values of 2.74 ± 1.44 μM (N = 21) for fucose and 157.8 ± 52.8 μM (N = 17) for **1** are in good agreement with the corresponding dissociation constant of fucose (K_d = 2.9 μM²⁴) and **1** (K_d = 71 μM²⁷).

The common precursor for the designed LecB ligands, methyl 6-azido 6-deoxy α-D-mannopyranoside (**11**), was obtained following the published protocol of Ferguson and co-workers,³³ omitting the acetylation/deacetylation sequence (Scheme 2). A library of substituted triazoles **2** was obtained through copper-catalyzed Huisgen 1,3-dipolar cycloaddition^{34,35} with alkynes. For the sulfonamide (**5**), amide (**4**), and amine (**3**) derived LecB ligands, the azido group in **11** was reduced by hydrogenation. The resulting primary amine **3e** was then reacted with either sulfonyl chlorides, acyl chlorides, or carbodiimide-activated carboxylic acids to give the corresponding 6-sulfonamido 6-deoxy mannosides **5** or 6-amido 6-deoxy mannosides **4**, respectively. For the amine derived library **3**, aldehydes were reductively aminated using amine **3e** and sodium borohydride.

We then analyzed triazoles **2**, amines **3**, amides **4**, and sulfonamides **5** in the competitive binding assay (Table 1). All

6-modified mannosides showed binding to LecB. However, a striking difference in potency was detected between the different linker groups. 1,2,3-Triazoles as in **2** are motifs resulting from the so-called ‘click chemistry’ cycloaddition, which proved to be a versatile method of diversification.³⁶ The use of triazoles as linkers in **2** led, however, to a strong reduction in binding affinity compared to **1** (IC₅₀ = 157.8 μM) with IC₅₀ values ranging from 240 to 578 μM (Table 1). Amines **3** were designed to form a putative salt bridge with Asp96 of LecB: Adam *et al.*¹⁰ analyzed a mutant form of LecB, where Ser22 was changed to Ala22 (S22A mutant), in complex with **1** by X-ray crystallography. In this mutant, the primary hydroxyl group of **1** no longer forms a hydrogen bond with Ser23 but establishes a hydrogen bond with Asp96. However, the existence of such a salt bridge between the protonated amines with Asp96 is unlikely according to the high IC₅₀ values for **3a–e** (115–353 μM, Table 1). We also studied the protonated form of **3e** in complex with LecB by molecular dynamics simulation (Supplementary Figures S1, S2). Consistent with the experimental data, the amino group in **3e** does not establish an interaction with Asp96 but forms a stable hydrogen bond to Ser23 as observed for **1** (Supplementary Figure S1). Even an enforced interaction of the protonated amino group in **3e** with Asp96 in the start conformation of the simulation resulted in a conformational change to establish the hydrogen bond with Ser23 after a few picoseconds (Supplementary Figure S2). This may explain the weaker affinities observed for amines **3a–e** compared to **1**.

Surprisingly, amides **4** were generally more potent inhibitors of LecB with IC₅₀ values from 37 to 161 μM (Table 1). Within the amide series **4**, only acetamide **4a** (IC₅₀ = 281 μM) was a weaker ligand than **1**. When lipophilic substituents were added (**4b–g**), an increase in binding affinity to LecB was observed and (E)-cinnamide **4g** (IC₅₀ = 37 μM) was identified as the best ligand among this series. **4g** was subsequently studied in LecB-mediated hemagglutination of sheep erythrocytes¹⁸ as well as agglutination of baker’s yeast.³⁷ In both assays, **4g** showed

a stronger activity in inhibiting agglutination than **1** (Supplementary Figure S9). Microcalorimetric titrations of LecB with **4g** revealed a K_d of $18.5 \mu\text{M}$ (Table 2, Supplementary Figure S10),

Table 2. Comparison of Binding Thermodynamics of **4g and **5e** with LecB Compared to **1**²⁷ by Isothermal Calorimetry^a**

$K_a [\text{M}^{-1}]$	$K_d [\mu\text{M}]$	$\Delta H [\text{kcal/mol}]$	$-T\Delta S [\text{kcal/mol}]$	N
4g	53 950	18.5	-4.3	-2.3
5e	302 000	3.3	-7.9	0.4
1	14 200	71.0	-4.3	-1.4

^aFour and two independent titrations were performed for **4g** and **5e**, and average values are given. Compound aggregation was not observed for all ITC titrations and was further verified by UV spectroscopy (Supplementary Figure S19).

which is in good agreement with its IC_{50} value of $37.4 \mu\text{M}$ obtained from the competitive binding assay, as well as the increase in potency observed in both agglutination assays. Encouraged by the results for the terminally amide capped mannoside **4g**, we investigated sulfonamides **5** as linking groups and common

replacements³⁸ for amides with distinct differences to carboxyamides in conformational and hydrogen bonding properties. A set of aryl sulfonamides was synthesized and tested in the competitive binding assay (**5a–f**, Table 1). All members of this subset were equal or stronger ligands than cinnamide **4g**, with **5e** being the most potent inhibitor ($\text{IC}_{50} = 3.4 \mu\text{M}$, $K_d = 3.3 \mu\text{M}$) of all identified 6-modified mannosides corresponding to a 20-fold increase in binding affinity compared to **1** ($K_d = 71 \mu\text{M}$) as determined by isothermal microcalorimetry (Table 2).

To get insight into the binding modes of the potent members of both amides and sulfonamides, we performed co-crystallization of both ligands (**4g**, **5e**) with LecB. Crystals diffracting to 1.41\AA resolution were obtained for the complex of **5e**. The asymmetric unit contains one tetramer of LecB, with very similar orientation of the ligand **5e** in each binding site (Figure 3). The mannoside residue of **5e** coordinates to the two protein-bound calcium ions, in an identical orientation as mannose²² is recognized by LecB. In addition, the sulfonamide nitrogen forms a hydrogen bond with Asp96 and the phenyl ring has lipophilic contacts with the protein surface. This hydrogen bond likely accounts for the enthalpy-driven binding of **5e** (Table 2).

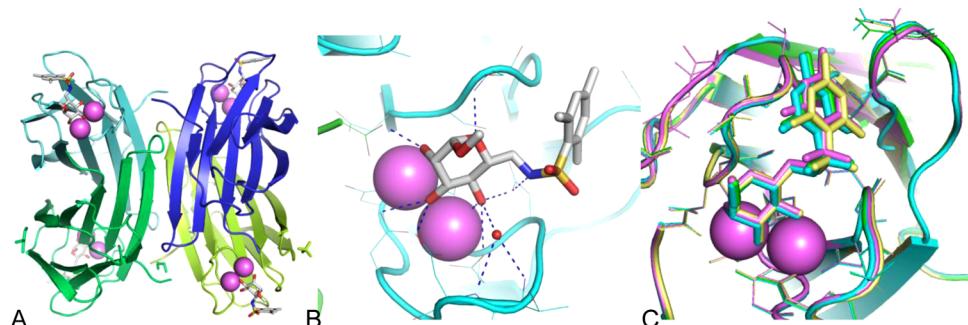


Figure 3. (A) General structure of the LecB tetramer complexed with sulfonamide **5e**. (B) Binding site of chain A with representation of hydrogen bond network between protein amino acids, ligand, and conserved water molecules. (C) Superimposition of the four binding sites.

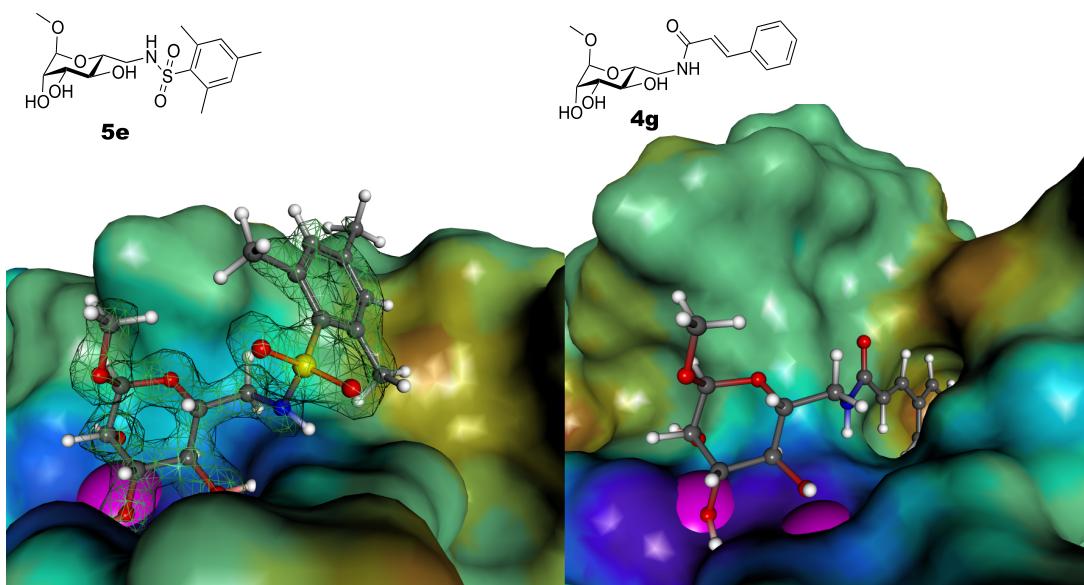


Figure 4. (Left) The crystal structure of sulfonamide **5e** in complex with LecB shows an extended binding of the lipophilic substituent to the surface of the protein; the sulfonamide nitrogen forms a hydrogen bond with the carboxylate of Asp96. (Right) One representative pose of one 40-ns molecular dynamics simulation of **4g** with LecB indicates an opening of the β -sandwich lectin fold to accommodate the lipophilic cinnamide moiety. The saccharide moiety of **4g** binds in the same orientation as in sulfonamide **5e** in the crystal structure; calcium ions are shown in pink, and the surface is colored by lipophilicity ranging from blue (hydrophilic) to orange (lipophilic).

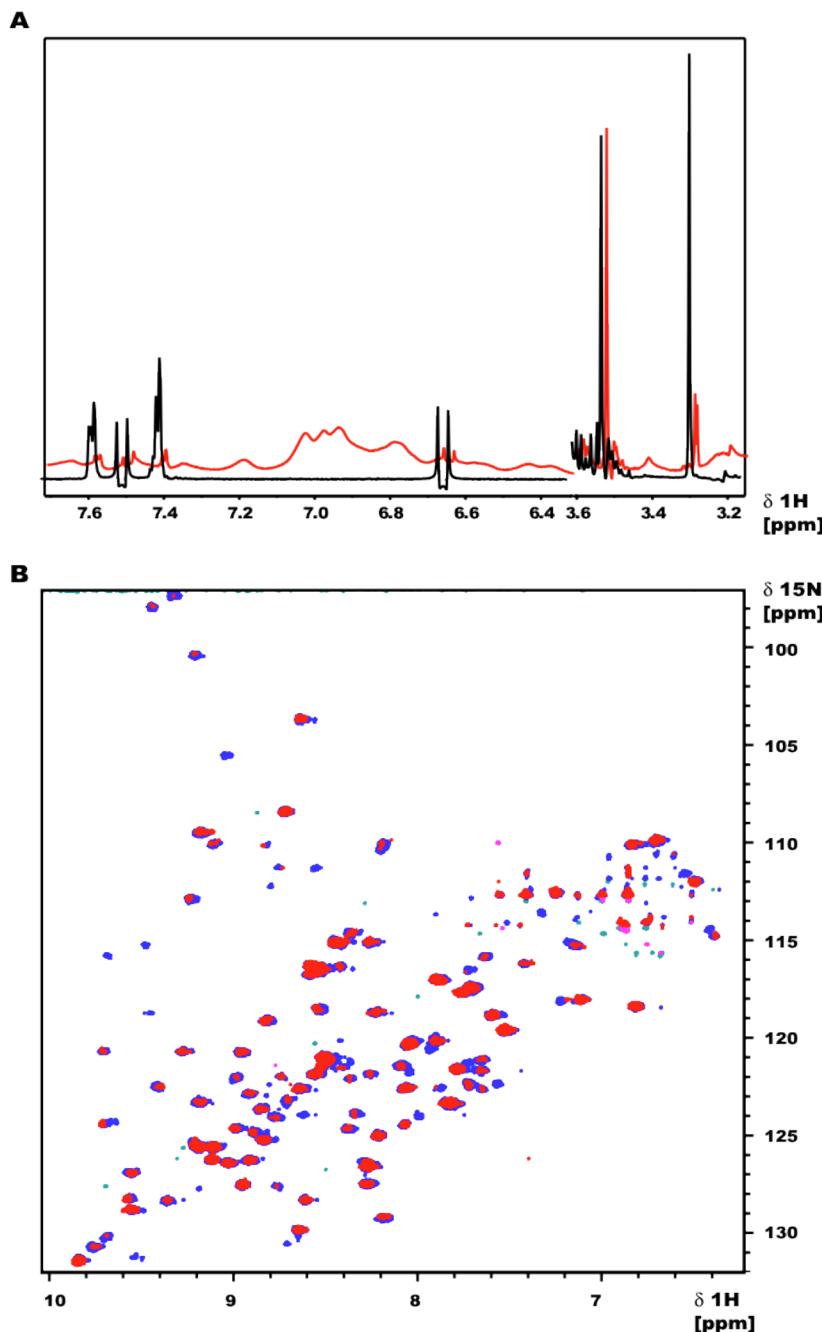


Figure 5. (A) Binding of **4g** to LecB was detected by ¹H NMR. The spectra were referenced to equal intensity on the ¹³C satellite peak of Tris at 3.53 ppm. A strong reduction of signal intensity of **4g** (in buffer, black trace) in the presence of 2 equiv of LecB (red trace) indicates binding to the protein. The region containing the water and main Tris signal do not contain indicative signals for **4g** (3.6 to 6.4 ppm) and was removed from the spectrum for clarity reasons. (B) ¹⁵N-labeled LecB was analyzed by ¹H, ¹⁵N-TROSY-HSQC NMR experiments in the absence (blue) and in presence of 0.5 equiv **4g** (red). Reduction in intensities of numerous peaks were observed, indicative for a large area of interaction and/or significant structural changes upon ligand binding that occur on an intermediate time scale. The signals below 102 ppm are folded.

Since crystals obtained for cinnamide **4g** in complex with LecB did not diffract, we performed extensive molecular dynamics (MD) simulations: starting structures were generated by aligning the saccharide moiety of **4g** onto **1** in the crystal structure²² and the cinnamido substituent manually oriented in 12 different poses, and 40-ns MD simulations were performed for each of these starting poses. All simulations resulted in only two distinctly different poses (Supplementary Figure S5). In the first one, the saccharide part of **4g** is coordinated to both Ca^{2+} ions and the cinnamido moiety opens a cleft and binds

inside the formerly closed β -sandwich (Figure 4, right). In a superposition with the crystal structure, the lowest energy structure of **4g** bound to LecB indicated nearly unchanged β -sheets of the β -sandwich fold despite the intercalating ligand, whereas slight changes in the loops were observed (Supplementary Figure S6). This model is consistent with the large entropic contribution to binding of **4g** to LecB (Table 2). In contrast, the other pose of the simulation positioned the cinnamido moiety into the solvent and thus contradicts the thermodynamic fingerprint of **4g** in comparison to **1** and can therefore be ruled out.

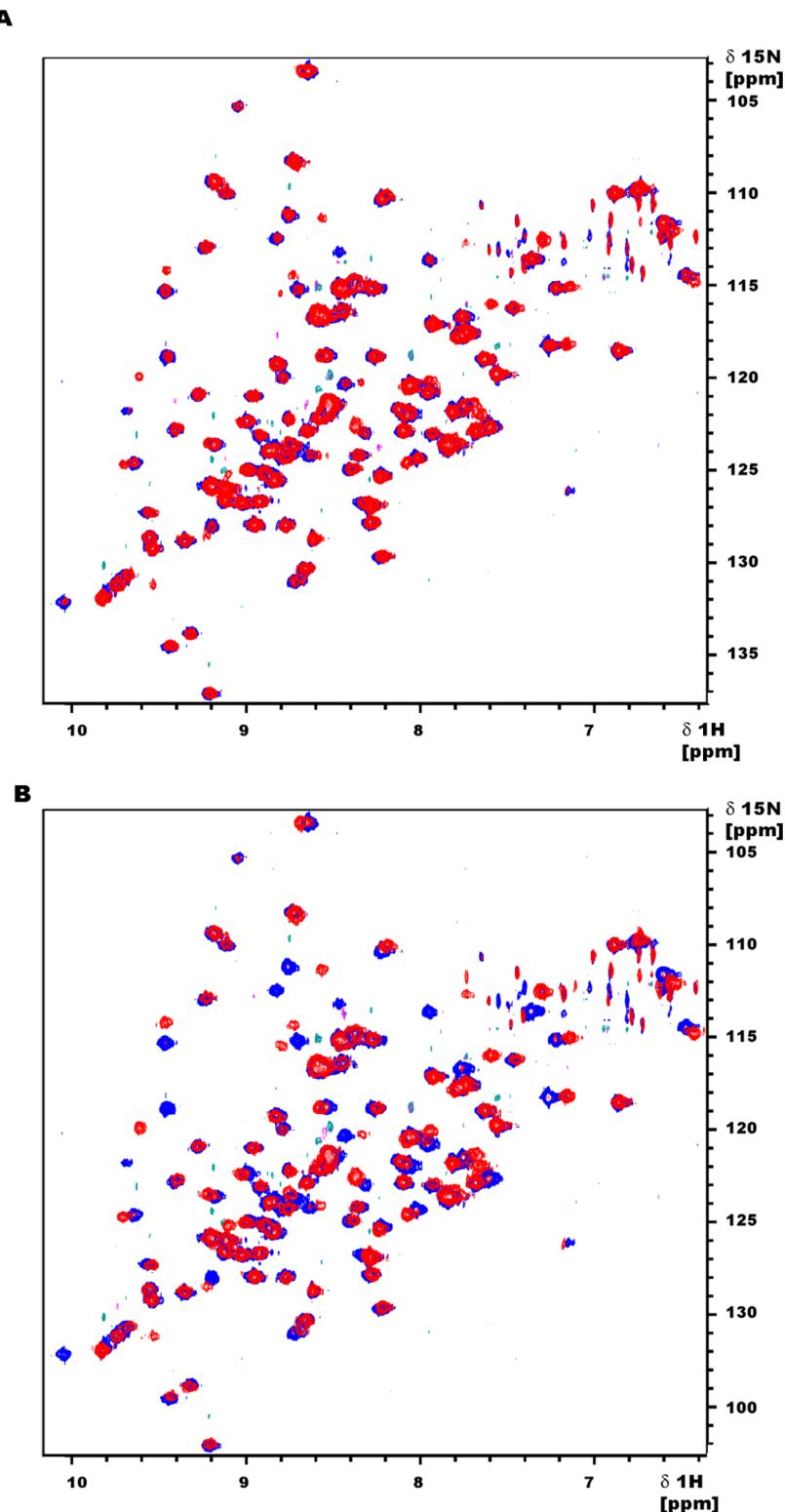


Figure 6. Binding of **5e** to LecB was analyzed by $^1\text{H},^{15}\text{N}$ -TROSY-HSQC NMR experiments in the absence (blue) and presence of **5e** (red). (A) Loss of signal intensity of defined signals of ^{15}N -labeled LecB was observed upon addition of **5e** to 2 equiv of LecB, and new signals appeared. (B) For Sulfonamide **5e**, the 1:1 complex with LecB could be analyzed, and chemical shift perturbation was observed for ca. 15 signals. In contrast to intermediate exchange of **4g**, ligand binding of **5e** to LecB occurs on a slow time scale, as deduced from the appearance of distinct sets of signals.

To gain further insight into the molecular recognition of ligands by LecB, we monitored the interaction of **1** and **4g** by NMR spectroscopy (Figure 5, Supplementary Figure S12). Interestingly, $^1\text{H},^{15}\text{N}$ -TROSY-HSQC experiments of

^{15}N -labeled LecB with **1** did not show significant chemical shift perturbation but led to strong line broadening of distinct amide signals. Nevertheless, binding of **1** was clearly evident also in our NMR experiments. At ligand-to-protein ratios of less

than or equal to 1:1 the resonances of **1** showed extensive line broadening leading to vanishing signal intensities. At higher excess of **1** line widths characteristic of the free sugar were observed. This indicates that even this monosaccharide ligand is in slow exchange on the NMR time scale and that the resonances of the bound state are broadened beyond detection. In contrast, cinnamide **4g** causes massive changes of the ^1H , ^{15}N correlation of the ^{15}N -labeled LecB. Addition of **4g** showed extensive global line broadening of protein resonances and vanishing of LecB as a whole (Figure 5). This indicates that the interaction of **4g** with its binding site on LecB causes chemical shift changes not only locally at the binding site but also extending to the whole lectin. Apparently, the binding is taking place on an intermediate time scale leading to extensive exchange broadening. This behavior is consistent with the proposed binding mode of **4g**, where the cinnamide moiety penetrates into the β -sandwich causing a slight but global rearrangement of the whole lectin domain (Figure 4, Supplementary Figure S6). To further compare the binding between LecB and **4g** and **5e**, respectively, we analyzed sulfonamide **5e** in complex with LecB by ^1H , ^{15}N -TROSY-HSQC NMR spectroscopy. In contrast to the global effect due to binding of the cinnamide **4g** (Figure 5B), line broadening of distinct signals of LecB was observed upon binding of **5e** (Figure 6A). The appearance of signals in addition to the signals of the unligated protein indicates a slow exchange between bound and unbound state on the NMR time scale (Figure 6A). For the fully ligated protein (Figure 6B), *ca.* 15 signals differ in chemical shift and/or signal intensity. This number is in good agreement with the approximate area of the binding site of LecB for **5e** as determined by X-ray crystallography.

To assess the potential of **4g** and **5e** as possible lead structures for the search of inhibitors of *P. aeruginosa* host cell binding and biofilm formation, we finally developed a bacterial adhesion assay. In analogy to a plasmid-based system developed for fimbrial adhesion of *E. coli* bacteria by Lindhorst *et al.*,³⁹ we generated genetically CFP-tagged wild type *P. aeruginosa* and analyzed the adhesion to fucose-coated microtiter plates (Figure 7). The expression of both lectins, LecA and LecB, is

induced in the stationary phase.⁴⁰ *P. aeruginosa* cultures grown for 48 h showed LecB-dependent and fucose-specific binding to the surface, and this adhesion could be inhibited by addition of LecB-specific ligands, *i.e.*, fucose and **1**. The controls glucose or methyl α -D-glucoside showed only a negligible effect, which was also observed by Lindhorst *et al.*⁴¹ for *E. coli* adhesion. All inhibitors of LecB showed only a partial reduction of bacterial adhesion. The fact that LecA has a low affinity for fucose⁴² is likely to account for the observed residual binding to the coated fucose, which increases its affinity due to its polyvalent nature. The binding of a *P. aeruginosa* lecA knockout strain to a surface coated with ligands of LecB could be completely inhibited with inhibitors of LecB, supporting this hypothesis.⁴³ For a therapeutic application, this partial redundancy needs to be addressed by development and co-application of a suitable LecA inhibitor. Here, both, cinnamide **4g** and sulfonamide **5e** proved to be even slightly more potent than fucose for the inhibition of LecB-mediated adhesion of *P. aeruginosa* and thus will serve as lead structures for further development.

Conclusion. In summary, we developed a new competitive binding assay for LecB that allows *in situ* detection of the binding. Because it is run in a 384-well format, this assay is amenable to high-throughput screening of small molecule libraries. Current enzyme linked lectin assays (ELLA) for LecB^{19,25} are based on the ELISA protocol, offering the advantage of evaluation of inhibitors over a broad affinity range. However, this format requires numerous handling steps and suffers from low reproducibility between plates,⁴⁴ as a result of the low affinity nature of carbohydrate–protein interactions as compared to antibody–antigen binding in ELISA. Our new assay allowed the screening of four novel series of ligands for LecB inhibition. Using the crystal structure of D-mannose with LecB, we synthesized various 6-modified mannoses targeting a cleft on the protein surface. Mannose-derived amides were identified as potent inhibitors with cinnamide **4g** as the best identified inhibitor among this series. In addition, a set of sulfonamide-derived ligands were screened, and **5e** was identified as a mannose-derived, terminally modified, high affinity inhibitor of LecB ($K_d = 3.3 \mu\text{M}$). The crystal structure of sulfonamide **5e** in complex with LecB revealed an additional hydrogen bond of the sulfonamide nitrogen with Asp96 and a binding of the aromatic ring to the surface of LecB. Because we could not obtain diffracting crystals of LecB with **4g**, we characterized the binding in detail using a combination of biochemical assays, biomolecular NMR spectroscopy, molecular dynamics simulation, and bacterial adhesion. Our results suggest that the cinnamide moiety in **4g** opens a hydrophobic cavity within the β -sandwich of LecB and binds distinctly differently than all previously crystallized ligands of LecB, including **5e**. Noteworthy, **5g**, the sulfonamide homologue of **4g** carrying the same substituent, is a 2-fold weaker ligand. This supports the two different binding modes, which are likely to result from the conformational difference of carboxamides (planar, *trans*) and sulfonamides (staggered, *gauche*). Finally, it was shown for the first time that such glycomimetic mannose-derived inhibitors are able to inhibit the adhesion of whole *P. aeruginosa* bacteria. In contrast to the natural ligand Lewis^a, which is unsuitable as potential drug (see earlier), both **4g** and **5e** comply with Lipinski's rule of five and its extensions for oral availability (Supplementary Table S3). Because many lectins in the host recognize terminal fucosides, inhibitors based on this epitope are likely to interfere with important receptors involved in inflammation and immunity. We have identified and optimized

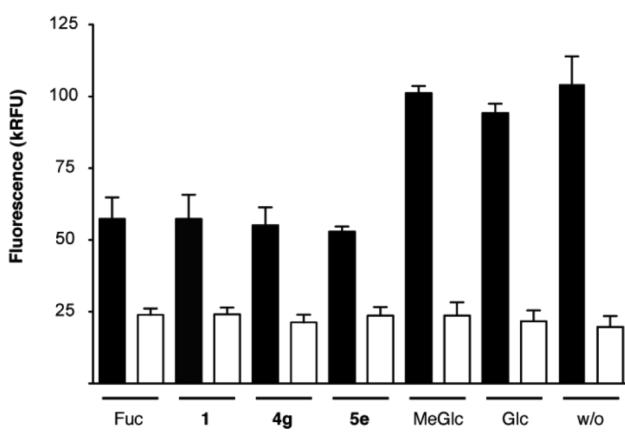


Figure 7. Cinnamide **4g** and sulfonamide **5e** can inhibit the adhesion of *P. aeruginosa* to a fucose-coated surface (black bars). The inhibition of bacterial adhesion was tested in the presence of fucose (Fuc), **1**, **4g**, **5e**, methyl α -D-glucoside (MeGlc), and D-glucose (Glc) all at 200 μM or in the absence of ligands (w/o). Furthermore, control experiments with a glucose-coated surface were conducted (white bars). Three independent experiments were performed. One representative graph is shown, and error bars represent the standard deviation of triplicates of one experiment.

terminally capped mannoses as inhibitors of LecB. Such capping modifications are expected to yield selective inhibitors for the pathogenic lectin over lectins of the host. Studies addressing the selectivity and *in vivo* efficacy of the reported compounds are ongoing. Knowledge gained from this work will further improve LecB inhibitors, which may lead to a novel class of anti-virulence drugs. Due to such a mode of action, a strongly reduced potential of developing resistant strains can be anticipated,⁴⁵ and follow-up compounds may constitute a suitable treatment of nosocomial infections and respiratory tract infections of cystic fibrosis patients.

■ ASSOCIATED CONTENT

Supporting Information

Details of molecular modeling studies and all experimental procedures, as well as ¹H NMR and ¹³C NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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