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Development of a competitive binding assay for the *Burkholderia cenocepacia* lectin BC2L-A and structure activity relationship of natural and synthetic inhibitors[1]

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Abstract

Burkholderia cenocepacia is an opportunistic Gram-negative pathogen and especially hazardous for cystic fibrosis patients. In analogy to its relative *Pseudomonas aeruginosa*, *B. cenocepacia* possess numerous lectins with roles in adhesion and biofilm formation. The LecB homolog BC2L-A is important for biofilm structure and morphology. Inhibitors of this D-mannose specific C-type lectin could be useful as tools in *B. cenocepacia* biofilm research and potentially as anti-biofilm compounds against chronic infections. Here, we report the development of a fluorescence polarization-based competitive binding assay and its application in an extensive structure-activity relationship study of inhibitors of BC2L-A. In contrast to its homolog LecB, BC2L-A is highly selective for D-mannose-based ligands with an absolute requirement of its hydroxyl group at C6. A strict diastereoselectivity was observed for (*6S*)-mannoheptose-derived ligands. Intriguingly, bioisosteric substitution or methylation of hydroxyl groups directly involved in the calcium-coordination resulted in loss of inhibition for the two homologous lectins BC2L-A and LecB.

Keywords

Lectin assay, glycomimetics, *Burkholderia cenocepacia*, BC2L-A, *Pseudomonas aeruginosa*, LecB

Introduction

Burkholderia cenocepacia is a Gram-negative bacterium and belongs to the *B. cepacia* complex (BCC). BCC currently contains at least 18 genetically different but phenotypically similar species, which could be isolated from different environments.[1] *B. cenocepacia* has been recognized as a problematic opportunistic pathogen, particularly to immunosuppressed patients and patients suffering from cystic fibrosis (CF). Compared to its relative *Pseudomonas aeruginosa*, mortality of patients infected with *B. cenocepacia* is increased and conditions like the cepacia syndrome often lead to pulmonary disfunction.[2,3] Treatment of such infections is difficult due to antibiotic resistance of *B. cenocepacia,*[4] which is increased by its ability to form biofilms. In these social colonies the bacteria are protected by a self-formed extracellular matrix and show up to fifteen-fold higher resistance against antibiotics than *B. cenocepacia* grown in planktonic culture.[5] Like for *P. aeruginosa*,[6] the inhibition of bacterial biofilm formation could be a promising approach to overcome antibiotic resistance.

Interestingly, *B. cenocepacia* was shown to form mixed species biofilms with CF isolates of *P. aeruginosa* and both pathogens often infect patients simultaneously.[7] In *P. aeruginosa* the lectins LecA and LecB are necessary for biofilm formation.[8,9] *lecB*-like genes were also identified in several other Gram-negative bacteria such as *Chromobacterium violaceum, Ralstonia solanacearum,* as well as in *B. cenocepacia*.[10-12] *B. cenocepacia* has three lectins homologous to *P. aeruginosa* LecB: BclA (BCAM0186), BclB (BCAM0184) or BclC (BCAM0185), also called BC2L-A, BC2L-B and BC2L-C. BC2L-A contains only a LecB domain, whereas BC2L-B and BC2L-C have additional N-terminal domains. This additional domain is without homology to known domains in BC2L-B, and in BC2L-C it contains a tumor necrosis factor-fold domain. The latter has been characterized and reported as super lectin where the additional TNF domain binds fucose in addition to the mannose-binding LecB domain.[13,14]

The expression of BC2L-A is positively regulated through activation of *Burkholderia* CepR, a

protein homologous to LuxR which is a member of the acyl homoserine lactone quorum sensing system.[15,16] Later, the *Burkholderia* lectin cluster *bclACB* was shown to be mainly regulated by the *Burkholderia* diffusable signal factor (BDSF) system with cis-2-dodecenoic acid as signal molecule and that maximal expression of the lectins is dependent on both signaling systems.[17] Surprisingly, deletion of the *bclACB* gene cluster in the CF isolate *B. cenocepacia* H111 had no effect on biofilm formation in a static microtitre plate biofilm assay.[18] However, under identical conditions the lectin cluster *bclACB* showed an effect on biofilm formation in absence of the major adhesin gene *bapA*. Expression of BapA was therefore suggested to compensate the effect of the lectins BclACB under static biofilm conditions. When analyzed under flow conditions, however, the absence of the lectins BclACB had a profound effect on the biofilm structure also in the presence of *bapA* and large hollow structures were formed. Based on complementation experiments with individual lectins and combinations, Eberl and coworkers suggested that all three lectins cooperate in the structural development of biofilms. Therefore, inhibitors targeting these lectins could serve as tools to study *Burkholderia* biofilm formation.

BC2L-A has been extensively structurally characterized.[10,19,20] In contrast to the tetramer forming LecB, BC2L-A forms a dimeric assembly of its 13.8 kDa monomers.[10] The carbohydrate specificity of BC2L-A was determined by glycan array analysis, which revealed a high specificity of this lectin for mannose-containing ligands. In contrast to the high affinity of LecB to L-fucose, BC2L-A shows a low affinity to L-fucose (IC_{50} = 2.3 mM). Methyl α-D-mannoside is a high affinity ligand with a K_d of 2.7 μM and co-operative effects upon binding were observed in microcalorimetry experiments. The crystal structure was solved and the carbohydrate specificity was explained on a structural basis: His112 stacks to lipophilic areas in mannosides but would clash with O-1 of fucose and its conjugates. Marchetti *et al.* later showed, that BC2L-A binds also to L-glycero-D-manno-heptose which is a major constituent of bacterial lipopolysaccharide.[20] The stereochemistry of the glycol side chain was important for binding and methyl α-L,D-mannoheptoside bound with a K_d of

54 μM, while its C6 epimer did not bind.

Here, we report the development of a competitive binding assay for BC2L-A based on fluorescence polarization and its application in a detailed structure-activity relationship study with more than 30 potential ligands based on the monosaccharides fucose and mannose.

Results and Discussion

Fluorescence polarization-based competitive binding assays have been developed for various lectins.[21-23] This assay format allows small scale volumes and convenient *in situ* quantification of the inhibitory potencies of given inhibitors. Because BC2L-A binds D-mannosides with high affinity and L-fucosides with low affinity, we designed the D-mannose-based fluorescent tracer **3** (Scheme 1) as assay probe. Its synthesis started from D-mannose (**1**) and an ethyl linker was introduced by borontrifluoride-catalyzed glycosylation of peracetylated mannose with 2-bromoethanol as published by Dahmen *et al.*.[24] After subsequent nucleophilic displacement of the bromide with NaN_3,[25] Zemplén deacetylation yielded intermediate azide, which was reduced to the fully unprotected amine **2**.[26] The fluorescent tracer **3** was then obtained in 79% yield after coupling with fluorescein isothiocyanate (FITC). Then, mannose-based **3** and fucose-based **4**[22] were titrated with BC2L-A and fluorescence polarization was determined (Figure 1A). Mannose derivative **3** showed strong binding to BC2L-A (K_d 3.1 ± 1.7 μM), whereas fucose derivative **4** only showed binding at protein concentrations greater than 100 μM. These results are consistent with the known dissociation constant of methyl α-D-mannoside and BC2L-A of K_d = 2.7 μM and the poor binding of L-fucose.[10] The assay was then evaluated by titration of BC2L-A (1.5 μM) in presence of the reporter ligand **3** (10 nM) with a dilution series of D-mannose (**1,** Figure 1B) and an IC_{50} value of 10.8 ± 0.7 μM was obtained. This data is in good agreement with published data for D-mannose (**1**, K_d 5.15 μM) obtained by isothermal microcalorimetry.[10]

Scheme 1. *Synthesis of fluorescently labeled mannose tracer* ***3***. *Reagents and conditions: (a)* Ac_2O, *pyridine, r.t.; (b)* BF_3•OEt_2, *2-bromoethanol; (c)* NaN_3, *DMF, 70 °C; (d) NaOMe, MeOH, r.t.; (e)* H_2, *Pd/C, EtOH, r.t.; (f) FITC,* $NaHCO_3$, *DMF, r.t.*

Figure 1. *(A) Titration of mannose-based reporter ligand* ***3*** *and fucose-based reporter ligand* ***4*** *with BC2L-A and determination of the fluorescence polarization. Binding of* ***3*** *(*K_d *= 3.1 ± 1.7 μM) compared to the low affinity fucose-based ligand* ***4*** *indicate the carbohydrate specificity of BC2L-A. (B) Competitive inhibition of the binding of* ***3*** *to BC2L-A with* D*-mannose (****1****,* IC_{50} *= 10.8 ± 0.7 μM). One representative titration of independent triplicates (three plates) is shown here and error bars represent standard deviation of triplicates on one plate, standard deviations given for* K_d *and* IC_{50} *represent data from three independent experiments (three plates of triplicates each).*

***Table 1**: Biochemical evaluation of selected* L*-fucose and* D*-mannose derivatives for BC2L-A binding. IC_{50} values were determined and are averages of at least three independent experiments, standard deviations are given. n.i.: no inhibition observed up to 666 μM.*

After establishing the assay, we tested various derivatives of L-fucose and D-mannose for inhibition of BC2L-A (Table 1). The common structural motif contained the 6-membered tetrahydropyran ring structure and all three calcium-coordinating secondary hydroxy groups were present. In a first set of compounds (**1**, **5**-**12**), fucose, mannose and derivatives thereof were tested. This series was previously generated to explain monosaccharide selectivity of the structurally related lectin LecB and dissect individual functional group contributions to the binding affinity.[27] For BC2L-A, only a weak affinity of L-fucose (**5**, IC_{50} 498 μM) was detected and no inhibition by the methyl fucosides α-**6** or β-**7** was observed up to 666 μM. Removal of the glycosidic linkage of fucose in **8** or introduction of a C-methyl substituent in **9** also resulted in inactive compounds. The observation that fucose-containing conjugates are poor BC2L-A inhibitors was further supported by the lack of inhibition of BC2L-A by various blood group antigens, *e.g.*, Lewis-type structures Le^x, Le^y, Le^a, Le^b or antigens of the ABO-system blood group A-, B- and H-antigens (data not shown). In contrast, mannose-derived inhibitors **1**, **10**-**12** were potent inhibitors with IC_{50} values ranging from 2.9 - 13 μM. These potent binders all contain the 6-OH group of D-mannose but possess variations at the anomeric center of mannose as C-glycoside **10**, 1-deoxy mannose **11**, free mannose **1** or the methyl glycoside **12**. A hydroxy group in position 6 of mannose was required for efficient binding to BC2L-A as observed by the comparison of inactive 6-deoxy-**9** and potent inhibition of 6-hydroxy-**10**. This data also reinforces the conclusion made by Marchetti *et al.*, that a lack of an STD effect of D-rhamnose (*i.e.*, 6-deoxy D-mannose) with BC2L-A by NMR spectroscopy resulted from the loss in affinity of such 6-deoxygenated mannose-derivatives.[20] Variation of the aglycon in mannosides **12**-**15** had only a minor effect (7.0 - 14.5 μM), and methyl glycoside α-**12** was as potent as the bulky β-**15** (IC_{50} = 7.0 μM for **12** and 7.4 μM for **15**).

Deoxygenation in position-6 of mannosides resulted in complete loss in binding. Therefore, we isosterically replaced this hydroxy group with other small substituents which could establish attractive interactions with amino acids of the protein, *i.e.* halogens[28,29] in **16-18** or an amino group in **19**. Halogenated mannosides **16-18** were synthesized by selective activation of the 6-hydroxy group in methyl mannoside **12** as tosylate[22] and its nucleophilic substitution with the corresponding halogenide salts in DMF at elevated temperatures (Scheme 2). Remarkably, none of the halogenated mannosides nor the amine substituted derivative were able to inhibit the lectin (Table 1), confirming that the presence of a free 6-OH group is essential for binding.

***Scheme 2**. Synthesis of 6-deoxy-6-halo mannosides **16**, **17** and **18**. Reagents and conditions: (a) TsCl, pyridine, DMF, 0 °C - r.t.; (b) $(CH_3)_4NCl$ for **16**, KBr for **17** or KI for **18**, DMF, 65 °C.*

Marchetti *et al.* previously reported the binding of BC2L-A to L, D-mannoheptose with a K_d of 54 μM for its α-methyl glycoside.[20] This heptose is a constituent of bacterial surface lipopolysaccharide (LPS) and may play a role in BC2L-A mediated bacterial adhesion. In a previous study, we synthesized a set of mannoheptose derivatives as inhibitors for the homologous lectin *P. aeruginosa* LecB.[30] These compounds differ in their stereochemical configuration at position 6 and bear amido- and sulfonamido-substituents in position 7. All compounds were tested for their inhibition of BC2L-A. In agreement with the selectivity reported by Marchetti *et al*., we observed a preferential binding of BC2L-A to the (*6S*)-diastereomers **20**-**22** in the amide series with IC_{50} values of 116, 104 and 64.0 μM, respectively, whereas the corresponding (*6R*)-diastereomers D,D-mannosides **23** and **24** did not show any inhibition. The (*6S*)-configurated sulfonamides **25** and **26** both inhibited BC2L-A function and were the most potent inhibitors in the mannoheptose series with IC_{50}s of 14-19 μM. This inhibitory potency is superior to the unsubstituted heptoside previously reported by Marchetti *et al.*. The mannohexose analogs bearing these amide and sulfonamide substituents in position 6 were previously reported as potent inhibitors of LecB.[22,31] However, since these compounds lack a free hydroxy group in position 6, the observed lack of inhibition of BC2L-A (data not shown) was consistent with the observations for the relative behavior of *e.g.*, compound **9** and **10**.

***Table 2**: Biochemical evaluation of mannoheptose derivatives for BC2L-A binding. IC_{50} values were determined using competitive binding assay and are averages of at least three independent experiments, standard deviations are given. n.i.: no inhibition observed up to 666 μM.*

[a]

The crystal structure of methyl α-L,D-mannoheptoside in complex with BC2L-A was solved by Marchetti *et al*..[20] In this structure, the 6-OH of the glycol side chain establishes a hydrogen bonding network with the side chain of Asp110 and the main chain nitrogen of Glu31, an interaction pattern similar to 6-OH of mannose[10] in complex with BC2L-A. Both, (*6S*)-**22** and (*6R*)-**24** were docked into the ligand-free form of the mannoheptose-BC2L-A crystal structure previously published by Marchetti *et al*. (Figure 2). The docking pose of mannoheptose amide (*6S*)-**22** corresponds to the crystal structure of methyl α-L,D-mannoheptoside and the 6-OH group forms the previously mentioned hydrogen bonding interactions since both heptoses have the same stereochemistry at position 6. The diastereomeric analog (*6R*)-**24** could also be docked with its pyranose ring in the same position as the one observed for methyl α-L,D-mannoheptoside, but the OH group at C6 cannot take the same position since it would generate steric hindrance between the rest of the side chain and the protein surface. Thus, the side chain is rotated in its docking pose and the 6-OH group in this (*6R*)-diastereomer cannot be optimally accommodated in the binding site. In (*6S*)-**22**, the cinnamide substituent extends along the cleft and interacts with BC2L-A, whereas in (*6R*)-**24**, the same substituent is oriented towards the solvent as a result of the altered stereochemistry at C6. Both properties, the hydrogen-bonding network of 6-OH as well as the van der Waals interaction of the amide substituents as in (*6S*)-**22** could explain the strong selectivity of BC2L-A for (*6S*)-heptose derivatives over their (*6R*)-diastereomers.

Figure 2. *Molecular docking of cinnamide modified mannoheptose C-6 (S) isomer* ***22*** *(A) and C-6 (R) isomer* ***24*** *(B) with BC2L-A. The two C-6 diastereomers show same orientation of the carbohydrate ring but differ in orientation of 6-OH and the cinnamide moiety. Carbohydrate recognition domain of BC2L-A is presented as cartoon with transparent surface. Ligands and amino acids forming interaction with 6-OH are depicted as sticks colored by elements (N: blue, O: red, C: grey). Two* Ca^{2+}*-ions in the binding site are shown as green spheres.*

We were further interested in the importance of the ring hydroxy groups for binding, which are directly coordinating to the two Ca^{2+} ions in BC2L-A or in the related *P. aeruginosa* LecB. Is it possible to replace or to modify one or more of these hydroxy groups and retain or improve affinity to LecB-like proteins? In 2004, Plenio described attractive Ca^{2+}-F interaction in organofluorine compounds.[32] In addition, due to similar size, fluorine became an established bioisoster for hydrogens and hydroxyl groups in medicinal chemistry.[33] The introduction of fluorine can have profound effects on a diverse set of properties of drugs, *e.g.*, changes in acidity, lipophilicity or metabolic stability. On the other hand, some lectins are known to preferentially bind to O-methylated carbohydrates, for example *Laccaria bicolor* tectonin[34] or *Bos taurus* galectin-1.[35] Both lectins, however, do not belong to the C-type lectin family. Galectin-1 was also probed with fluorine-substituted galactose derivatives and binding was completely lost for the 4- and the 6-fluorine analog, whereas a 3-fluoro substitution was well tolerated by galectin-1.[35] Fluorinated saccharides as ligands for other carbohydrate binding proteins have been described, *e.g.* glycogen phosphorylase,[36] *Toxoplasma* adhesin TgMIC1,[37] calnexin and calreticulin,[38,39] as well as NMR-active probes for cyanovirin.[40] However, none of these carbohydrate-binding proteins are C-type lectins. One example of C-type lectins binding fluorinated glycan ligands has been reported by Hoechst AG for fluorinated sialyl Lewis^x and E-selectin.[41] It is important to note, that in this study fluorine was introduced at the galactose moiety of the tetrasaccharide, which is not involved in direct coordination to the protein-bound calcium ion.

Scheme 3. *Synthesis of 1,2-dideoxy-2-fluoro L-fucose (**30**) and allyl 4-O-methyl α-mannopyranoside (**33**). Reagents and conditions: (a) benzaldehyde dimethyl acetal, camphorsulfonic acid, DMF, r.t.; (b) XtalFluor-E, Et_3N•3HF, CH_2Cl_2, r.t., 2 h; (c) Pd/C, H_2, EtOH, r.t.; (d) AllOH, Amberlite/H^+, 70 °C; (e) Acetone/H_2O=40:1, pTsOH, 40 °C; (f) Triphenylmethyl chloride, pyridine, 50 °C; (g) NaH, MeI, DMF, 0 °C-r.t.; (h) Acetone, conc. HCl, 50 °C.*

Table 3: *Biochemical evaluation of derivatives substituted in one of the three Ca^{2+}-chelating hydroxyl groups for binding to BC2L-A and its homolog from P. aeruginosa LecB. n.i.: no inhibition observed up to 666 µM.*

Two commercially available fluorinated mannose derivatives (**34** and **35**, Table 3) were used to study the effect of the substitution of 2-OH and 3-OH with a fluorine atom. A fluorine derivative corresponding to the substitution of mannose 4-OH was not commercially accessible. Therefore, we synthesized 1,2-dideoxy-2-fluoro-L-fucose (**30**) bearing the fluorine at position C-2 of fucose which corresponds to position C-4 in mannose (Scheme 3). An inversion of the free hydroxy group, its activation as leaving group and fluorination *via* S_N2-type substitution are the key steps in the synthesis of such fluorinated carbohydrates. However, all attempts to invert the equatorial hydroxyl group at position 2 in selectively protected **27** into its axial epimer **28** failed (Scheme 3), although various conditions were tested. After activation of the hydroxyl group as mesylate, tosylate or triflate leaving groups, the subsequent inversion using various nucleophiles (NaOH, KOH, BzOH or cesium benzoate) was unsuccessful. An attempt to invert the stereochemistry under Mitsunobu conditions was also unsuccessful. Finally, Lattrel-Dax conditions using nitrite ions as nucleophile reagent was tested on the triflate due to previous success in epimerisation of carbohydrate triflates.[42] Also under these conditions access to **28** remained unsuccessful. In contrast, a direct fluorination of the free hydroxy group in **27** using the reagent Deoxo-Fluor yielded the fluorinated compound **29** with the desired stereochemistry, however, this was only successful in a small scale test reaction. Finally, **29** could be smoothly obtained in good yields using *N*,*N*-diethylamino-*S*,*S*-difluorosulfinium tetrafluoroborate (XtalFluor-E), a new generation fluorination reagent[43] with enhanced stability and selectivity. The stereochemistry of **29** and hydrogenolytically deprotected **30** was confirmed using ^{1}H-NMR (for **29**, **30**) and ^{1}H,^{1}H-NOESY NMR (for **29**) spectroscopy. The proton NMR spectrum and observed NOE cross peaks (see Supporting Information for spectra) between the benzylidene aliphatic proton and H2 in **29** unambiguously confirmed the presence of only one diastereomer, *i.e., exo*-**29**. The question whether exclusively the *exo*-isomer of **27** reacted to *exo*-**29** or the *endo*/*exo*-mixture of **27** anomerized during the acidic reaction conditions was not

further analyzed.

For the selective methylation of calcium-coordinating hydroxy groups, we used the previously reported[34] 3-O-methyl mannoside **36** (Table 3) and designed the synthesis of its 4-methoxy analogon **33**. Fischer glycosylation of D-mannose (**1**) in allylic alcohol, isopropylidene masking of the cis-diol OH-2/3 and subsequent tritylation of the primary hydroxyl group yielded the selectively protected mannoside **31** in 23% over 3 steps (Scheme 3). Methylation of **31** using methyl iodide and subsequent simultaneous deprotection of the trityl and isopropylidene protecting groups yielded 4-O-methyl mannoside **33** in good yield.

All fluorinated (**30**, **34**, **35**) and methylated (**33**, **36**) potential ligands were evaluated in the corresponding competitive binding assays for both C-type lectins, *B. cenocepacia* BC2L-A and its homolog LecB from *P. aeruginosa.* Surprisingly, no competitive binding was observed for any of these derivatives up to concentrations of more than 650 μM, a concentration more than 10-100-fold above the affinities of the corresponding derivatives with unmodified hydroxy groups. Based on our previous observations, 2-fluoro fucose **30** was not expected to bind to BC2L-A due to the lack of the primary hydroxy group (see Table 1 for the parent compound **8**). However, **8** is a potent inhibitor of LecB[27] and no inhibition of LecB function with its fluoro-analog **30** was observed even at concentrations of more than 400-fold higher than the reported IC_{50} of **8**. These observations indicate the crucial importance of free hydroxy groups in all calcium-coordinating positions for binding to LecB and its homolog BC2L-A.

The fluorinated mannose analogs **34** and **35** are hemiacetals and their conformation is not fixed as glycoside in a pyranose ring. Introduction of a strongly electronegative fluorine substituent in positions 2 or 3 could therefore influence the reactivity of the adjacent aldehyde and hydroxyl groups. The influence of these substitutions on the equilibrium of the various cyclic forms in

aqueous solution was therefore analyzed by NMR spectroscopy and J-coupling analysis (Table 4). When comparing the proton NMR spectra of D-mannose (**1**) and its fluorinated analogs **34** and **35** under identical conditions, in all three cases both pyranose forms were observed with comparable α/β-ratios. In addition, comparable ^{3}J-coupling constants between H2 and H3 (2.2 - 3.5 Hz) and between H3 and H4 (9.4 - 9.8 Hz) indicate comparable conformations for D-mannose (**1**) and its fluorinated analogs **34** and **35** in aqueous solution. Thus, an influence of the fluorine substituents on the conformational equilibrium of mannose could not account for the loss in lectin binding activity.

***Table 4**: Conformational analysis of D-mannose (**1**), 2-deoxy-2-fluoro-D-mannose (**35**) and 3-deoxy-3-fluoro-D-mannose (**34**) by ^{1}H-NMR in D_2O. The depicted ^{1}H-NMR spectra show the anomeric protons and quantification of anomeric ratios. Anomeric ratios (α:β) of the pyranose ring forms were comparable in **1**, **34**, **35**. ^{3}J-coupling analysis of between H2/H3 and H3/H4 indicate comparable coupling constants, and thus comparable conformations in all three compounds analyzed.*

Summary

In summary, we have developed a fluorescence polarization-based competitive binding assay to quantify inhibitory potencies of different inhibitors of BC2L-A, a potential target for anti-infectives against infections with *B. cenocepacia*. With the help of this assay, we extensively studied the structure-activity relationship of its glycan ligands based on thirty natural carbohydrates and synthetically derived inhibitors. The previously proposed role of the 6-OH group of D-mannosides for the binding to BC2L-A was found to be fundamental for activity, based on a set of approximately 10 compounds including this hydroxyl group, potential bioisosters thereof or with compounds lacking this hydroxyl group. In addition, we tested a set of diastereomeric mannoheptose derivatives, quantified the diastereoselectivity of BC2L-A and proposed the binding mode of the potent derivative which could assist the design of novel heptose-based inhibitors. The

diastereoselectivity of BC2L-A is very tight compared to the previously reported data for its homolog LecB.[30] This stringent selectivity is likely to originate from its high requirement for optimal coordination of the hydroxyl group at mannose C6, whereas for LecB[27] this hydroxyl group does not contribute to the overall binding affinity. Surprisingly, bioisosteric substitution or methylation of hydroxyl groups directly involved in the calcium-coordination resulted in complete loss of inhibition for the two homologous lectins BC2L-A and LecB. Thus, the assay developed here and the detailed information gained from this study will guide future development of lectin-directed inhibitors and anti-virulence drugs against *B. cenocepacia* and *P. aeruginosa*.

Experimental

Chemical Synthesis

Thin layer chromatography (TLC) was performed using silica gel 60 coated aluminum sheets containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) using UV light (254 nm) and by charring either in anisaldehyde solution (1% v/v 4-methoxybenzaldehyde, 2% v/v concentrated H_2SO_4 in EtOH), in aqueous $KMnO_4$ solution or in a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4) with heating. Medium pressure liquid chromatography (MPLC) was performed on a Teledyne Isco Combiflash Rf200 system using pre-packed silica gel 60 columns from Teledyne Isco, SiliCycle or Macherey-Nagel. Commercial chemicals and solvents were used without further purification. Deuterated solvents were purchased from Eurisotop (Saarbrücken, Germany). Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker Avance III 400 or 500 UltraShield spectrometer at 400/500 MHz (^{1}H) or 101/126 MHz (^{13}C), respectively. Chemical shifts are given in ppm and were calibrated on residual solvent peaks as internal standard.[44] Multiplicities were specified as s (singlet), d (doublet), t (triplet) or m (multiplet). The signals were assigned with the help of ^{1}H,^{1}H - COSY, DEPT-135-edited ^{1}H,^{13}C -HSQC, ^{1}H,^{13}C–HMBC and ^{1}H,^{1}H-NOESY experiments. Mass spectra were obtained on a Bruker amaZon SL spectrometer, high resolution mass spectra on a Bruker micrOTOF II ESI spectrometer and the data were analyzed using DataAnalysis from Bruker.

L-fucose (**5**), D-mannose (**1**), umbelliferyl α-D-mannoside (**14**) and umbelliferyl β-D-mannoside (**15**) were from Dextra Laboratories (Reading, UK), methyl α-L-fucoside (**6**), methyl β-L-fucoside (**7**) and fluoromannoses **34** and **35** from Carbosynth Ltd. (UK). Methyl α-D-mannoside (**12**) was purchased from Sigma Aldrich (Germany). Fluorescein isothiocyanate isomer I was from Serva Biochemicals (Heidelberg, Germany).

***N*-(Fluorescein-5-yl)-*N*'-(α-D-mannopyranosyloxyethyl)-thiocarbamide (3)**. 2-Aminoethyl α-D-mannopyranoside (**2**) (53 mg, 0.24 mmol) was dissolved in DMF (3 mL) and FITC (93 mg, 0.24 mmol, 1.0 eq) was added. The reaction mixture was stirred at r.t. for 17 h, concentrated *in vacuo* and the residue was purified by MPLC (SiO_2; solvent A: CH_2Cl_2 with 1% HOAc, solvent B: EtOH with 2% HOAc; gradient of 0 - 57% B) to give the title compound as orange solid (115 mg, 0.19 mmol, 79%). ^{1}H NMR (500 MHz, MeOH-d_4) 8.17 (d, J = 1.8 Hz, 1H, ArH), 7.79 (dd, J = 8.3, 2.0 Hz, 1H, ArH), 7.17 (d, J = 8.3 Hz, 1H, ArH), 6.75 - 6.65 (m, 4H, ArH), 6.55 (dd, J = 8.7, 2.4 Hz, 2H, ArH), 4.83 (d, J = 1.7 Hz, 1H, H-1), 3.99-3.80 (m, 5H), 3.77-3.68 (m, 3H), 3.65-3.55 (m, 2H); ^{13}C NMR (126 MHz, MeOH-d_4) 183.1 (C=S), 171.3 (C=O), 154.4 (ArC), 142.4 (ArC), 131.6 (ArCH), 130.4 (ArCH), 129.5 (ArC), 125.9 (ArCH), 120.1 (ArCH), 114.0 (ArC), 111.7 (ArCH), 103.5 (ArCH), 101.8 (C-1), 74.8, 72.6, 72.1, 68.6 (C-2, -3, -4, -5), 67.0 (CH_2), 62.9 (CH_2), 45.4 (CH_2); HR-MS calcd. for $C_{29}H_{27}N_2O_{11}S^-$: calcd: 611.1341 found: 611.1320.

Methyl 6-chloro-6-deoxy-α-D-mannopyranoside (16). Methyl mannoside **12** was tosylated according to Wang *et al.,*[45] crude methyl 6-O-tosyl-α-D-mannopyranoside (200 mg, 0.57 mmol, 1 equiv.) was then stirred in DMF (5.7 mL) in presence of $(CH_3)_4NCl$ (314 mg, 2.87 mmol, 5 equiv.) at 65 °C for 2 d. The solvent was removed *in vacuo* and the residue was purified by MPLC (SiO_2; gradient of CH_2Cl_2 to CH_2Cl_2/MeOH = 10:1) to give **16** as colorless solid (32 mg, 26%, 2 steps). ^{1}H NMR (500 MHz, MeOH-d_4) δ 4.64 (d, *J* = 1.7 Hz, 1H, H-1), 3.92 – 3.88 (m, 1H, H-6a), 3.79 (dd, *J* = 3.3, 1.7 Hz, 1H, H-2), 3.69 – 3.61 (m, 3H, H-3, -4, -6b), 3.60 – 3.54 (m, 1H, H-5), 3.39 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, MeOH-d_4) δ 102.8 (C-1), 74.2 (C-3 or -4), 72.5 (C-3 or -4), 71.9 (C-2), 69.8 (C-5), 55.2 (OCH_3), 45.7 (C-6); ESI-MS calcd. $C_7H_{13}ClNaO_5^+$: 235.0; found: 234.8. **16** was first reported by Jennings and Jones[46] and the proton NMR data corresponded to the data disclosed in the literature.[47]

Methyl 6-bromo-6-deoxy-α-D-mannopyranoside (17). Methyl mannoside **12** was tosylated according to Wang *et al.,*[45] crude methyl 6-O-tosyl-α-D-mannopyranoside (300 mg, 0.86 mmol, 1

equiv.) was stirred in DMF (8.6 mL) in presence of KBr (512 mg, 4.31 mmol, 5 equiv.) at 65 °C for 3 d. The solvent was removed *in vacuo* and the residue was purified by MPLC (SiO_2; gradient of CH_2Cl_2 to CH_2Cl_2/MeOH = 10:1) to give **17** as colorless solid (60.7 mg, 31%, 2 steps). ^{1}H NMR (500 MHz, MeOH-d_4) δ 4.64 (d, J = 1.6 Hz, 1H, H-1), 3.81 – 3.77 (m, 2H, H-2, -6a), 3.66 – 3.60 (m, 2H, H-3, -4), 3.56 – 3.48 (m, 2H, H-5, -6b), 3.41 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, MeOH-d_4) δ 102.8 (C-1), 74.0 (C-3 or -4), 72.5 (C-3 or -4), 71.9 (C-2), 70.9 (C-5), 55.3 (OCH_3), 33.9 (C-6); ESI-MS calcd. $C_7H_{13}BrNaO_5^+$: 279.0; found: 278.8. **17** was first reported by Valentin[48] and the carbon NMR data corresponded to disclosed data in the literature.[49]

Methyl 6-deoxy-6-iodo-α-D-mannopyranoside (18). Methyl mannoside **12** was tosylated according to Wang *et al.,*[45] crude methyl 6-O-tosyl-α-D-mannopyranoside (300 mg, 0.86 mmol, 1 equiv.) was stirred in DMF (8.6 mL) in presence of KI (715 mg, 4.31 mmol, 5 equiv.) at 65 °C for 3 d. The solvent was removed *in vacuo* and the residue was purified by MPLC (SiO_2; gradient of CH_2Cl_2 to CH_2Cl_2/MeOH = 10:1) to give **18** as colorless solid (88.1 mg, 34%, 2 steps). ^{1}H NMR (500 MHz, MeOH-d_4) δ 4.62 (d, J = 1.7 Hz, 1H, H-1), 3.78 (dd, J = 3.4, 1.7 Hz, 1H, H-2), 3.68 – 3.60 (m, 2H, H-3, -6a), 3.47 – 3.41 (m, 5H, H-4, -5, OCH_3), 3.27 – 3.19 (m, 1H, H-6b). ^{13}C NMR (126 MHz, MeOH-d_4) δ 102.9 (C-1), 74.2 (C-5), 72.5 (C-4), 72.3 (C-3), 72.1 (C-2), 55.5 (OCH_3), 6.4 (C-6); ESI-MS calcd. $C_7H_{13}INaO_5^+$: 327.0; found: 326.8. **18** was first reported by Lehmann and Benson[50] and the NMR data corresponded to disclosed data in DMSO-d_6 in the literature.[51]

3,4-*O*-Benzylidene-1-deoxy-L-fucose (27). 1-deoxy-L-fucose[27] (**8**) (315 mg, 2.13 mmol) was dissolved in DMF (14 mL) and to the solution were added camphorsulfonic acid (50 mg, 0.21 mmol, 0.1 eq) and benzaldehyde dimethyl acetal (1 mL, 6.38 mmol, 3 eq). The mixture was stirred at r.t. for 19 h. Then, triethylamine (30 µL) was added, the volatiles were removed *in vacuo* and the residue was purified by MPLC (gradient petrol ether to petrol ether/EtOAc = 2:1) to give **27** as diastereomeric mixture (442 mg, 1.87 mmol, 88%) as colorless solids (ratio *S*/*R* = 1/1.5). ^{1}H NMR (400 MHz, MeOH-d_4) *S*-isomer: δ 7.59 – 7.29 (m, 5H, ArCH), 5.91 (s, 1H, PhC<u>H</u>(OR)$_2$), 4.15 (dd,

J = 6.1, 2.3 Hz, 1H, H-4), 4.08 (t, *J* = 6.5 Hz, 1H, H-3), 3.94 – 3.68 (m, 3H, H-2, -5, -1^{eq}), 3.23 – 3.10 (m, 1H, H-1^{ax}), 1.36 (d, *J* = 6.6 Hz, 3H, H-6); *R*-isomer: δ 7.59 – 7.29 (m, 5H, ArCH), 6.10 (s, 1H, PhC<u>H</u>(OR)$_2$), 4.24 (dd, *J* = 6.9, 5.2 Hz, 1H, H-3), 4.05 (dd, *J* = 5.4, 2.0 Hz, 1H, H-4), 3.94 – 3.68 (m, 3H, H-2, H-5, H-1^{eq}), 3.23 – 3.10 (m, 1H, H-1^{ax}), 1.32 (d, *J* = 6.6 Hz, 3H, H-6). ^{13}C NMR (101 MHz, MeOH-d_4) δ 140.9 (ArC), 139.4 (ArC), 130.2 (ArCH), 129.9 (ArCH), 129.3 (2C, ArCH), 129.2 (2C, ArCH), 127.8 (2C, ArCH), 127.3 (2C, ArCH), 105.3 (Ph<u>C</u>H(OR)$_2$, *S*-isomer), 104.2 (Ph<u>C</u>H(OR)$_2$, *R*-isomer), 81.8 (C-3, *R*-isomer), 80.0 (C-4, *S*-isomer), 80.0, 77.6 (C-4, *R*-isomer), 73.9, 73.2, 70.4, 69.6 (C-1, *R*-isomer), 69.5 (C-1, *S*-isomer), 67.2, 17.3 (C-6, *R*-isomer), 17.1 (C-6, *S*-isomer). HR-MS calcd. for $C_{13}H_{16}NaO_4^+$: 259.0941; found: 259.0933.

(*R*)-3,4-*O*-Benzylidene-1,2-dideoxy-2-fluoro-L-fucose (29). To a solution of selectively protected (*R*/*S*)-**27** (100 mg, 423 µmol) in CH_2Cl_2 (1.3 mL) was added XtalFluor-E (145 mg, 635 µmol, 1.5 eq) and Et_3N•3HF (173 µL, 0.85 mmol, 2 eq) at r.t. and the mixture was stirred for 4 h. The mixture was poured into saturated $NaHCO_3$ solution (0.5 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 2 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the residue was purified by MPLC (petrol ether to petrol ether/EtOAc = 6:1) to give only the (*R*)-3,4-*O*-benzylidene-1,2-dideoxy-2-fluoro-L-fucose (**29**) (65 mg, 272 µmol, 65%) as colorless solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.52 – 7.30 (m, 5H, ArCH), 6.15 (s, 1H, PhC<u>H</u>(OR)$_2$), 4.75 (dddd, *J* = 49.7, 9.6, 9.6, 6.2 Hz, 1H, H-2), 4.52 (dt, *J* = 18.9, 6.1 Hz, 1H, H-3), 4.14 (dt, *J* = 5.8, 1.7 Hz, 1H, H-4), 4.08 (ddd, *J* = 11.5, 7.2, 5.8 Hz, 1H, H-1^{eq}), 3.82 (qd, *J* = 6.6, 1.8 Hz, 1H, H-5), 3.42 (ddd, *J* = 11.6, 9.4, 6.6 Hz, 1H, H-1^{ax}), 1.32 (d, *J* = 6.6 Hz, 3H, H-6). ^{13}C NMR (101 MHz, MeOH-d_4) δ 140.5 (ArC), 130.1 (ArCH), 129.3 (2C, ArCH), 127.3 (2C, ArCH), 104.8 (Ph<u>C</u>H(OR)$_2$), 88.2 (d, J_{CF}= 177.3 Hz, C-2), 78.5 (d, J_{CF} = 23.8 Hz, C-3), 78.0 (d, J_{CF} = 7.0 Hz, C-4), 73.5 (C-5) , 66.6 (d, J_{CF} = 26.8 Hz, C-1), 17.0 (C-6). ^{19}F NMR (376 MHz, MeOH-d_4) δ -197.0. HR-MS calcd. for $C_{13}H_{15}FNaO_3^+$: 261.0897; found: 261.0778.

1,2-Dideoxy-2-fluoro-L-fucose (30). Protected 2-fluoro-fucose **29** (35 mg, 0.147 mmol) was stirred in MeOH (10 mL) under hydrogen atmosphere (1 atm) with 10% Pd–C (10 mol-%) at r.t. over night. The mixture was filtered through celite and the solvent was removed *in vacuo*. The residue was purified by MPLC (CH_2Cl_2 to CH_2Cl_2/MeOH = 8:1) to give 1,2-dideoxy-2-fluoro-L-fucose (**30**) (21 mg, 0.142 mmol, 97%) as colorless solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 4.60 (dddd, J = 51.3, 10.4, 9.0, 5.8 Hz, 1H, H-2), 4.01 (dd, J = 10.9, 5.7 Hz, 1H, H-1^{eq}), 3.72 – 3.60 (m, 2H, H-3, H-4), 3.55 (qd, J = 6.5, 1.2 Hz, 1H, H-5), 3.27 (td, J = 10.7, 4.2 Hz, H-1^{ax}), 1.22 (d, J = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, MeOH-d_4) δ 89.7 (d, J_{CF} = 176.1 Hz, C-2), 76.5 (C-5), 74.7 (d, J_{CF} = 16.6 Hz, C-3), 73.9 (d, J_{CF} = 9.4 Hz, C-4), 68.1 (d, J_{CF} = 28.2 Hz, C-1), 16.8 (C-6). ^{19}F NMR (376 MHz, MeOH-d_4) δ -208.4; ESI-MS calcd. $C_6H_{11}FNaO_3^+$: 173.1; found: 172.8.

Allyl 2,3-*O*-isopropylidene-6-*O*-trityl-α-D-mannopyranoside (31). Allyl α-D-mannopyranoside[34] (**13**) was converted to allyl 2,3-*O*-isopropylidene-α-D-mannopyranoside as described by Kochetkov *et al*.[52] ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.88 (dddd, J = 17.2, 10.4, 6.0, 5.2 Hz, 1H, C<u>H</u>CH$_2$-allyl), 5.24 (dq, J = 17.2, 1.7 Hz, 1H, CHC<u>H</u>$_2$-allyl), 5.16 – 5.08 (m, 2H, CHC<u>H</u>$_2$-allyl, OH-5), 4.92 (br s, 1H, H-1), 4.54 (t, J = 6.0 Hz, 1H, OH-6), 4.12 (ddt, J = 13.1, 5.2, 1.6 Hz, 1H, OC<u>H</u>$_2$-allyl), 4.02 (dd, J = 5.8, 0.9 Hz, 1H, H-2), 3.93 (ddt, J = 13.1, 6.0, 1.4 Hz, 1H, OC<u>H</u>$_2$-allyl), 3.88 (t, J = 6.2 Hz, 1H, H-3), 3.62 (ddd, J = 11.9, 6.0, 1.8 Hz, 1H, H-6a), 3.41 (dt, J = 11.8, 5.8 Hz, 1H, H-6b), 3.30 – 3.25 (m, 2H, H-4, H-5), 1.35 (s, 3H, OC<u>H</u>$_3$), 1.23 (s, 3H, OC(C<u>H</u>$_3$)$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 134.4 (<u>C</u>HCH$_2$-allyl), 117.1 (CH<u>C</u>H$_2$-allyl), 108.2 (O<u>C</u>(CH$_3$)$_2$), 95.5 (C-1), 78.5 (C-3), 75.2 (C-2), 71.3 (C-4/C-5), 68.2 (C-4/C-5), 66.8 (O<u>C</u>H$_2$-allyl), 60.6 (C-6), 28.0 (OC(<u>C</u>H$_3$)$_2$), 26.2 (OC(<u>C</u>H$_3$)$_2$). ESI-MS calcd. $C_{12}H_{20}NaO_6^+$: 283.1; found: 283.1. Allyl 2,3-*O*-isopropylidene-α-D-mannopyranoside was transformed to allyl 2,3-*O*-isopropylidene-6-*O*-trityl α-D-mannopyranoside (**31**) following the protocol from Gigg *et al*.[53] ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.45 – 7.36 (m, 6H, ArCH), 7.37 – 7.29 (m, 6H, ArCH), 7.29 – 7.21 (m, 3H, ArCH), 6.03 (dddd, J = 17.3, 10.4, 5.9,

5.2 Hz, 1H, C<u>H</u>CH$_2$-allyl), 5.33 (dq, *J* = 17.3, 1.7 Hz, 1H, CHC<u>H</u>$_2$-allyl), 5.27 – 5.21 (m, 1H, CHC<u>H</u>$_2$-allyl), 5.13 (d, *J* = 6.9 Hz, 1H, OH-4), 5.08 (s, 1H, H-1), 4.41 (ddt, *J* = 13.0, 5.3, 1.6 Hz, 1H, OC<u>H</u>$_2$-allyl), 4.15 (ddt, *J* = 12.9, 6.0, 1.4 Hz, 1H, OC<u>H</u>$_2$-allyl), 4.10 (dd, *J* = 5.7, 0.8 Hz, 1H, H-2), 3.93 (dd, *J* = 7.3, 5.8 Hz, 1H, H-3), 3.67 (ddd, *J* = 9.7, 7.6, 1.7 Hz, 1H, H-5), 3.35 – 3.28 (m, 1H, H-6a), 3.21 (dt, *J* = 10.5, 7.1 Hz, 1H, H-4), 3.03 (dd, *J* = 9.9, 7.6 Hz, 1H, H-6b), 1.38 (s, 3H, OC(C<u>H</u>$_3$)$_2$), 1.27 (s, 3H, OC(C<u>H</u>$_3$)$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 143.9 (3C, ArC), 134.4 (<u>C</u>HCH$_2$-allyl), 128.3 (6C, ArCH), 127.9 (6C, ArCH), 127.0 (3C, ArCH), 117.1 (CH<u>C</u>H$_2$-allyl), 108.3 (O<u>C</u>(CH$_3$)$_2$), 95.5 (C-1), 85.8 (C-Tr), 78.5 (C-3), 75.2 (C-2), 69.8 (C-5), 68.5 (C-4), 66.9 (O<u>C</u>H$_2$-allyl), 63.5 (C-6), 27.9 (OC(<u>C</u>H$_3$)$_2$), 26.2 (OC(<u>C</u>H$_3$)$_2$); ESI-MS calcd. $C_{31}H_{34}NaO_6^+$: 525.2; found: 525.2.

Allyl 2,3-*O*-isopropylidene-4-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside (32). Allyl 2,3-*O*-isopropylidene-6-*O*-trityl-α-D-mannopyranoside (**31**) (310 mg, 0.62 mmol, 1 equiv) was dissolved in dry DMF (1.5 mL) and NaH (60%, 74 mg, 1.85 mmol, 5 equiv) was added at 0 °C under nitrogen atmosphere. After 10 min, MeI (116 µL, 1.85 mmol, 5 equiv) was added dropwise and the reaction was stirred for 1 h at 0 °C. The reaction mixture was quenched with EtOH (1 mL), sat. $NaHCO_3$ solution (2 mL) was added and the reaction mixture was extracted with EtOAc (3x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The title compound **32** (262 mg, 0.51 mmol, 83%) was obtained after purification by MPLC (petrol ether/EtOAc = 9:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.45 – 7.38 (m, 6H, ArCH), 7.38 – 7.30 (m, 6H, ArCH), 7.29 – 7.23 (m, 3H, ArCH), 5.97 (dddd, *J* = 17.3, 10.4, 5.9, 5.2 Hz, 1H, C<u>H</u>CH$_2$-allyl), 5.30 (dq, *J* = 17.3, 1.7 Hz, 1H, CHC<u>H</u>$_2$-allyl), 5.24 – 5.17 (m, 1H, CHC<u>H</u>$_2$-allyl), 5.11 (s, 1H, H-1), 4.29 (ddt, *J* = 13.0, 5.2, 1.5 Hz, 1H, OC<u>H</u>$_2$-allyl), 4.13 (dd, *J* = 5.7, 0.8 Hz, 1H, H-2), 4.12 – 4.04 (m, 2H, OC<u>H</u>$_2$-allyl, H-3), 3.60 (ddd, *J* = 10.2, 5.7, 1.9 Hz, 1H, H-5), 3.29 (dd, *J* = 10.2, 6.9 Hz, 1H, H-4), 3.25 (dd, *J* = 9.9, 1.9 Hz, 1H, H-6a), 3.21 (s, 3H, OCH$_3$), 3.08 (dd, *J* = 9.9, 5.7 Hz, 1H, H-6b), 1.48 (s, 3H, OC(C<u>H</u>$_3$)$_2$), 1.30 (s, 3H, OC(C<u>H</u>$_3$)$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 143.7 (3C, ArC), 134.2 (<u>C</u>HCH$_2$-allyl), 128.2 (6C, ArCH), 127.8 (6C, ArCH), 127.0 (3C, ArCH), 117.2

(CH<u>C</u>H$_2$-allyl), 108.6 (O<u>C</u>(CH$_3$)$_2$), 95.5 (C-1), 85.7 (Ph$_3$<u>C</u>O-), 77.8 (C-3), 77.4 (C-4), 75.1 (C-2), 68.0 (C-5), 67.1 (O<u>C</u>H$_2$-allyl), 62.5 (C-6), 58.3 (O<u>C</u>H$_3$), 27.7 (OC(<u>C</u>H$_3$)$_2$), 26.1 (OC(<u>C</u>H$_3$)$_2$); ESI-MS calcd. $C_{32}H_{36}NaO_6^+$: 539.2; found: 539.2.

Allyl 4-*O*-methyl-α-D-mannopyranoside (33). Allyl 2,3-*O*-isopropylidene-4-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside (**32**) (131 mg, 0.254 mmol) was stirred in acetone (1.6 mL) in presence of aqueous acetic acid (80%, 0.8 mL) for 21 h at r.t.. The reaction mixture was neutralized with NaOH, the volatiles were removed *in vacuo* and the residue was purified by MPLC (CH_2Cl_2/EtOH = 95:5) to give **33** as solid (16.2 mg, 0.07 mmol, 27%). ^{1}H NMR (500 MHz, MeOH-d_4) δ 5.92 (dddd, J = 17.2, 10.5, 5.9, 5.1 Hz, 1H, C<u>H</u>CH$_2$-allyl), 5.28 (dq, J = 17.3, 1.7 Hz, 1H, CHC<u>H</u>$_2$-allyl), 5.21 – 5.13 (m, 1H, CHC<u>H</u>$_2$-allyl), 4.77 (d, J = 1.3 Hz, 1H, H-1), 4.18 (ddt, J = 13.1, 5.1, 1.6 Hz, 1H, OC<u>H</u>$_2$-allyl), 3.99 (ddt, J = 13.1, 5.9, 1.4 Hz, 1H, OC<u>H</u>$_2$-allyl), 3.81 – 3.74 (m, 3H, H-2,-3,-6a), 3.69 (dd, J = 11.8, 5.1 Hz, 1H, H-6b), 3.54 (s, 3H, OC<u>H</u>$_3$), 3.48 (dddd, J = 9.9, 5.2, 2.3, 0.6 Hz, 1H, H-5), 3.41 – 3.32 (m, 1H, H-4); ^{13}C NMR (126 MHz, MeOH-d_4) δ 135.4 (<u>C</u>HCH$_2$-allyl), 117.3 (CH<u>C</u>H$_2$-allyl), 100.6 (C-1), 78.4 (C-4), 73.8 (C-5), 72.7 (C-2/3), 72.5 (C-2/3), 68.8 (O<u>C</u>H$_2$-allyl), 62.5 (C-6), 60.9 (O<u>C</u>H$_3$); ESI-MS calcd. $C_{10}H_{18}NaO_6^+$: 257.1; found: 256.9.

Expression of recombinant BC2L-A and LecB and Competitive binding assays

BC2L-A[10] and LecB[22] were produced according to the previously published protocols. The competitive binding assay for BC2L-A based on fluorescence polarization was performed in analogy to the published protocol[22] for LecB. For titration of **3** and **4** with BC2L-A, 10 µL of fluorescent reporter ligand *N*-(fluorescein-5-yl)-*N*'-(α-D-mannopyranosyloxyethyl)-thiocarbamide **3** (30 nM) or *N*-(fluorescein-5-yl)-*N*'-(α-L-fucopyranosyl-*O*-ethyl)-thiocarbamide **4** (30 nM) in TBS/Ca (20 mM Tris, 137 mM NaCl, 2.6 mM KCl at pH 7.4 supplemented with 100 µM $CaCl_2$) were mixed with 20 µL serial dilutions (507 µM to 0.247 µM, *i.e.*, a final concentration of 338 µM to 0.165 µM) of BC2L-A in TBS/Ca in triplicates. For compound inhibition assay, 20 µL of a stock solution of BC2L-A (2.25 µM) and fluorescent reporter ligand **3** (15 nM) in TBS/Ca were mixed

with 10 µL serial dilutions (2 mM to 25.6 nM) of testing compounds in TBS/Ca in triplicates in black 384-well microtiter plates (Greiner Bio-One, Germany, cat no 781900). In all experiments, the microtiter plates were centrifuged at 800 rpm for 1 min at 23 °C and incubated for 3 - 4 h at r.t. Fluorescence emission parallel and perpendicular to the excitation plane was measured on a PheraStar FS (BMG Labtech, Germany) plate reader with excitation filters at 485 nm and emission filters at 535 nm. The measured intensities were reduced by buffer values and fluorescence polarization was calculated. The data were analyzed using BMG Labtech MARS software and/or with Graphpad Prism and fitted according to the four parameter variable slope model. A minimum of three independent measurements of triplicates each was performed for every ligand. To assure reliability of the read signal and exclude any influence of the test compounds on the total intensity of the fluorescence of the tracer molecule **3**, total fluorescence intensities of each well are monitored. Concentrations of test compounds yielding deviations in fluorescence intensity of > 20% of tracer **3** in absence of test compound are generally not taken for determination of IC_{50} values. Here, none of the tested compounds showed any influence on the total fluorescence intensity of tracer **3**, yielding reliable fluorescence polarization data.

Measurements with LecB were performed according to previously reported protocol using the fucose based fluorescent reporter ligand **4**.[22]

Molecular Docking

The docking study was performed using PLANTS v1.1.[54] The calculation of charge and energy minimization of the protein and tested compounds was done using Molecular Operating Environment (MOE), 2014.09 (Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015). Thereafter, the standard docking procedure (PLANTS Manual, available at http://www.tcd.uni-konstanz.de/plants_download/download/manual1.1.pdf) was validated by removing the mannoheptose ligand and redocking it inside the active site of BC2L-A (PDB code: 4AOC). The

docking site was limited inside a 13.3 Å radius sphere centered in the mass center (coordination: X = -27.73, Y = 53.64 and Z = -12.64) of the crystallized ligand. Glu31, Asp110, Gly111 and His112 were set as flexible residues in the input file. After docking the original mannoheptose ligand for validation of the process, a good agreement was observed between the localization of the docked ligand and in the crystal structure (rmsd = 2.0 Å). This validated docking protocol was then used for docking the derivatives **22** and **24** into the crystal structure of the protein.

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Supplementary data

Supplementary data (^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR traces of all synthesized compounds and NOESY spectra for compounds **27** and **29**).

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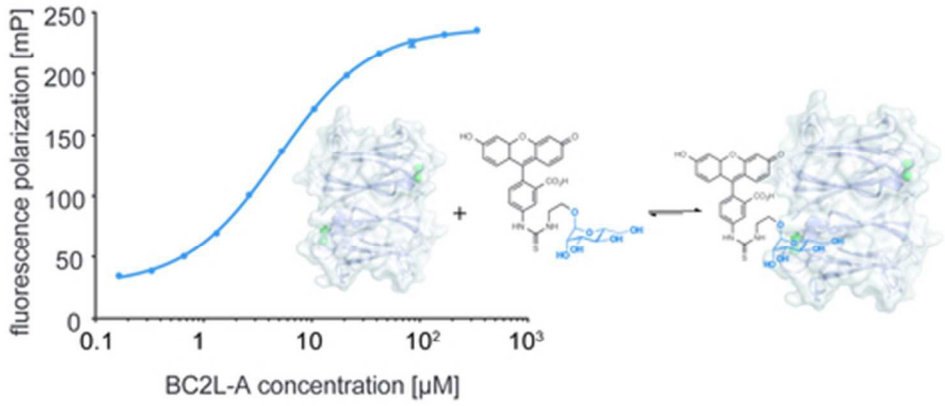

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