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Glycopeptide Dendrimers with High Affinity for the Fucose-Binding Lectin LecB from *Pseudomonas aeruginosa*

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The fucose-specific lectin LecB is implicated in tissue binding and biofilm formation by the opportunistic pathogen *Pseudomonas aeruginosa*, which causes severe respiratory tract infections mainly in immunocompromised patients or cancer patients undergoing chemotherapy. With a view to developing multivalent LecB inhibitors as novel antibacterial agents, a combinatorial library containing 15625 tetravalent *C*-fucosyl peptide dendrimers with the basic structure (CFuc-$X^6X^5X^4)_4$(Lys$X^3X^2X^1)_2$LysIleHisNH$_2$ (CFuc = α-L-fucosyl acetic acid, X^{1-6} = amino acids, Lys = lysine branching) was screened for lectin binding using on-bead binding assays. Ten tetravalent and three octavalent dendrimers derived from the identified sequences were prepared by solid-phase peptide synthesis (SPPS), cleaved from the resin, and purified by preparative HPLC. Relative affinities of these soluble ligands to LecB were determined by an enzyme-linked lectin assay (ELLA). Strong binding was observed for tetravalent and octavalent ligands, with up to 440-fold enhancement in potency over fucose for the octavalent cationic dendrimer **2G3** (CFuc-Lys-Pro)$_8$(*Lys*LeuPhe)$_4$(*Lys*LysIle)$_2$*Lys*HisIleNH$_2$). Mono- and divalent controls showed affinities similar to fucose, highlighting the importance of multivalency for binding. Docking studies showed that the *C*-fucosyl group of the dendrimers can adopt the same binding mode as fucose itself, with the peptide arms protruding from the binding pocket and establishing specific contacts with the lectin.

Introduction

Dendrimers are regularly branched, tree-like synthetic macromolecules under investigations for a variety of applications in technology and medicine.[1] Dendrimers can be assembled from organic building blocks that are either synthetic, such as polypropylene imine,[2] poly(amidoamide),[3] 3,5-dihydroxybenzyl ether,[4] phosphazenes,[5] or amino acids.[6] The dendritic topology enables the multivalent display of various groups at the branch termini, an effect which is particularly useful to obtain high affinity ligands for lectins[7] by dendritic carbohydrate display,[8,9,10] based on the well-known cluster effect.[11,12] Dendrimers with multivalent display of carbohydrates could potentially be therapeutically useful, for example, as immunomodulators and angiogenesis inhibitors in the case of multivalent glucosamine dendrimers,[13] or as inhibitors of bacterial adhesion.[14]

In the course of our investigation on peptide dendrimers as synthetic enzyme models and drug delivery agents,[15] we recently reported the synthesis of multivalent glycopeptide dendrimers displaying *C*-fucosyl groups as ligands for fucose-specific lectins.[16] The ligands were identified by functional selection from a combinatorial peptide dendrimer library on solid support using an enzyme-coupled lectin binding assay (ELLA) similar to those reported for oligosaccharides[17] and glycopeptides,[18] but directed towards the fucose-specific plant lectin UEA-I from *Ulex europeaus*, a case which had not been investigated previously. Variation of the number of fucose residues from two to five in a related combinatorial library showed that tetravalency provided the best ligands for this lectin.[19] The best glycopeptide dendrimer ligand identified for UEA-I, the tetravalent dendrimer **FD2**, also bound tightly to the bacterial lectin LecB from *Pseudomonas aeruginosa*. Similar reports on multivalent fucosylated dendrimers and polymers were reported shortly after, however, with lower potencies compared to our peptide dendrimers.[20,21] The fucose-specific lectin LecB, together with the galactose-specific lectin LecA, plays a role in specific recognition of host cells and attachment to target cells,[22] and seem to be virulence factors that might be potential drug targets.[23,24,25,26] A biological study involving biofilms showed that **FD2** also potently inhibits biofilm formation by *P. aeruginosa*, confirming that binding is also effective in vivo and suggesting that our peptide dendrimer ligands might be useful as novel antibacterial agents against this opportunistic human pathogen.[27] *P. aeruginosa* causes chronic airway infections that can be lethal in cystic fibrosis patients but has acquired resistance to classical antibiotics.

Herein we report a detailed investigation of *C*-fucosyl peptide dendrimers for binding to the fucose-specific lectin LecB,

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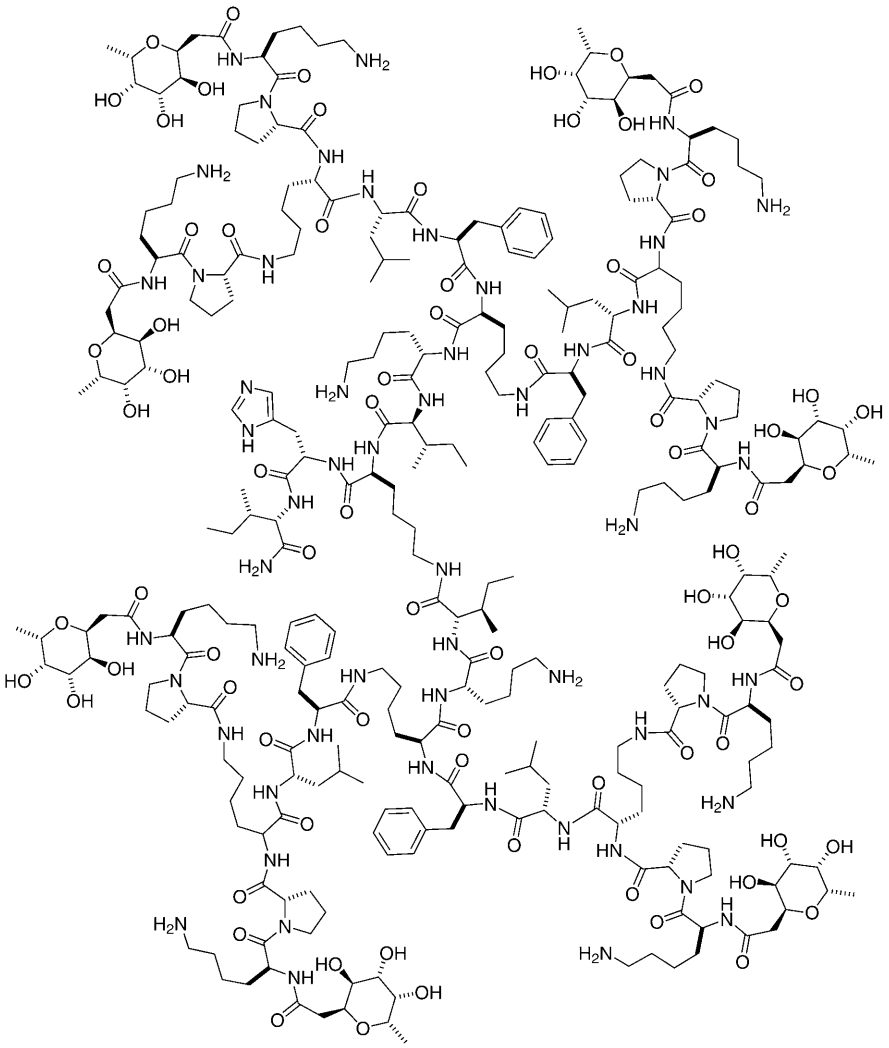


2G3

and the identification of potent dendritic inhibitors of that target by variation of the dendritic topology. A variety of peptide sequences appear to be compatible with strong lectin binding allowing the fine-tuning of the dendrimer solubility and pharmacological profiles. The best ligand identified in this study is the octavalent dendrimer **2G3** (IC_{50} = 0.025 mM, determined in ELLA). On the other hand, simple mono-, di- and trivalent *C*-fucosides do not show significant affinity enhancement to the lectin compared to fucose itself, implying a critical role of the peptide dendrimer structure in providing strong binding. A molecular modeling study of C-fucosylated ligands docked into the fucose binding pocket of the reported LecB crystal structure[23] provides a more detailed insight into the nature of the glycopeptide dendrimer–lectin interaction.

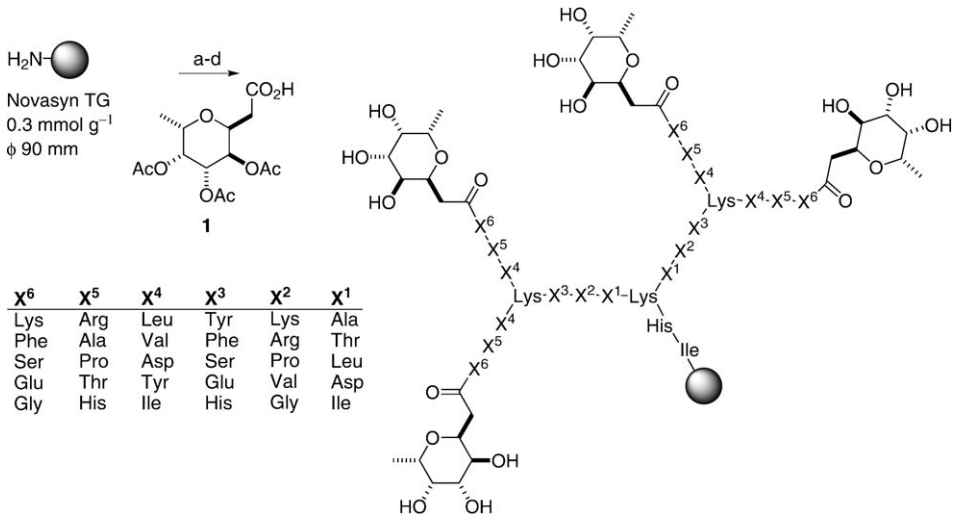
Results and Discussion

Library synthesis

The tetravalent glycopeptide dendrimer library was prepared by solid-phase peptide synthesis (SPSS) on noncleavable tentagel beads using Fmoc synthesis as described previously.[15] In this library, five different amino acids were used at each of the six variable positions, providing a total of 15625 different dendrimer sequences. The amino acids were selected to display cationic, anionic, hydrophobic and aromatic residues along the sequence. The synthesis was terminated by acylation with the acetyl protected *C*-fucosyl building block **1**, followed by acidic removal of the side-chain protecting groups and deacetylation (Scheme 1).

On-bead screening with rhodamine B labeled LecB

P. aeruginosa lectin LecB was expressed in *E. coli* and purified as previously described.[28] LecB was labeled with rhodamine B by reaction with the isothiocyanate in aqueous phosphate buffer saline (PBS) at pH 8.5, followed by purification on a size-exclu-



X^6	X^5	X^4	X^3	X^2	X^1
Lys	Arg	Leu	Tyr	Lys	Ala
Phe	Ala	Val	Phe	Arg	Thr
Ser	Pro	Asp	Ser	Pro	Leu
Glu	Thr	Tyr	Glu	Val	Asp
Gly	His	Ile	His	Gly	Ile

Scheme 1. Synthesis of the *C*-fucosyl peptide dendrimer library: a) split-and-mix SPPS; b) compound **1**, HCTU, DIEA, NMP (4×); c) TFA/TIS/H_2O (95:2.5:2.5), 4 h; d) MeOH/NH_3/H_2O (*v*/*v* 8:1:1).

sion column. Under optimized conditions, the glycopeptide dendrimer library screening was carried out by incubation of 50 mg samples of the library (~50000 beads) first with 1% bovine serum albumin (BSA) in phosphate buffer, and secondly with the rhodamine-labeled lectin in the presence of L-fucose (0.45 M) as a competitor. The beads were then washed with a fucose-containing solution to release the weaker binding ligands. While a general nonspecific staining was observed at the bead center, including for the N-acetylated control library, a specific ring of intense fluorescence was observed in the fucosylated library and interpreted as indicative of specific binding to the lectin (figure S1, Supporting Information).

Beads retaining an intense fluorescent ring after extensive washing with fucose were considered as positive hits and their sequence determined by amino acid analysis (Table 1). The hits obtained with the rhodamine B-labeled LecB contained predominantly cationic sequences similar to those identified with UEA-I.[15] Six out of nine sequences carried multiple positively charged residues (Lys at X^6 or Arg at X^5). Although, one sequence was neutral and one was anionic, suggesting that charged side chains might not be necessary for optimal LecB binding.

Table 1. Fluorescent (positive) hit sequences from glycopeptide dendrimer combinatorial library with *C*-fucosides screened with LecB.[a]

No.	X^6	X^5	X^4	X^3	X^2	X^1
PA1	Lys	His	Asp	Phe	Pro	Asp
PA2	Lys	His	Val	His	Pro	Leu
PA3	Lys	Pro	Leu	Glu	Pro	Thr
PA4	Lys	Arg	Leu	Ser	Gly	Ala
PA5	Gly	Arg	Val	Glu	Gly	Leu
PA6	Gly	Arg	Asp	Tyr	Arg	Asp
PA7	Gly	Ala	Ile	His	Arg	Ile
PA8	Gly	Thr	Val	His	Pro	Thr
PA9	Glu	His	Tyr	Tyr	Gly	Asp

[a] Screening conditions: Beads were incubated for 1 h at RT with 1 mL of rhodamine B-labeled lectin LecB in PBST (6 μg mL^{-1}, pH 6.8). After removal of lectin, L-(−)-fucose in PBST (1 mL, 0.45 M) was added and the beads were left to soaked for 1 h at 25 °C. (See figure S1 in the Supporting Information for pictures of the fluorescent beads)

Ligand selection and synthesis

A series of fucosylated dendritic ligands and controls were selected for affinity measurements to LecB in solution. In order to complement our existing series of fucosylated glycopeptide dendrimers, we selected sequences **PA5**, **PA8** and **PA9**, which were clearly different from the previous sequences identified against UEA-I. In addition, a 3rd generation analogue of the anionic dendrimer **FD10** was prepared to provide the octavalent ligand **10G3**, following the design used previously to derive the cationic octavalent dendrimer **2G3** from **FD2**. Synthesis of the 3rd generation analogue of the neutral dendrimer **PA8** was not successful; despite repeated attempts, only sequences with a maximum of seven fucosyl groups were formed due to incomplete couplings in the final steps. Nevertheless, dendrimer **PA8** could be modified by moving the 1st and 2nd branching point as in the 3rd generation sequence but omitting the 3rd branching, thus providing the 2nd generation dendrimer **PA8b** with a different spacer length between the fucosyl groups (Figure 1). All dendrimers were synthesized on

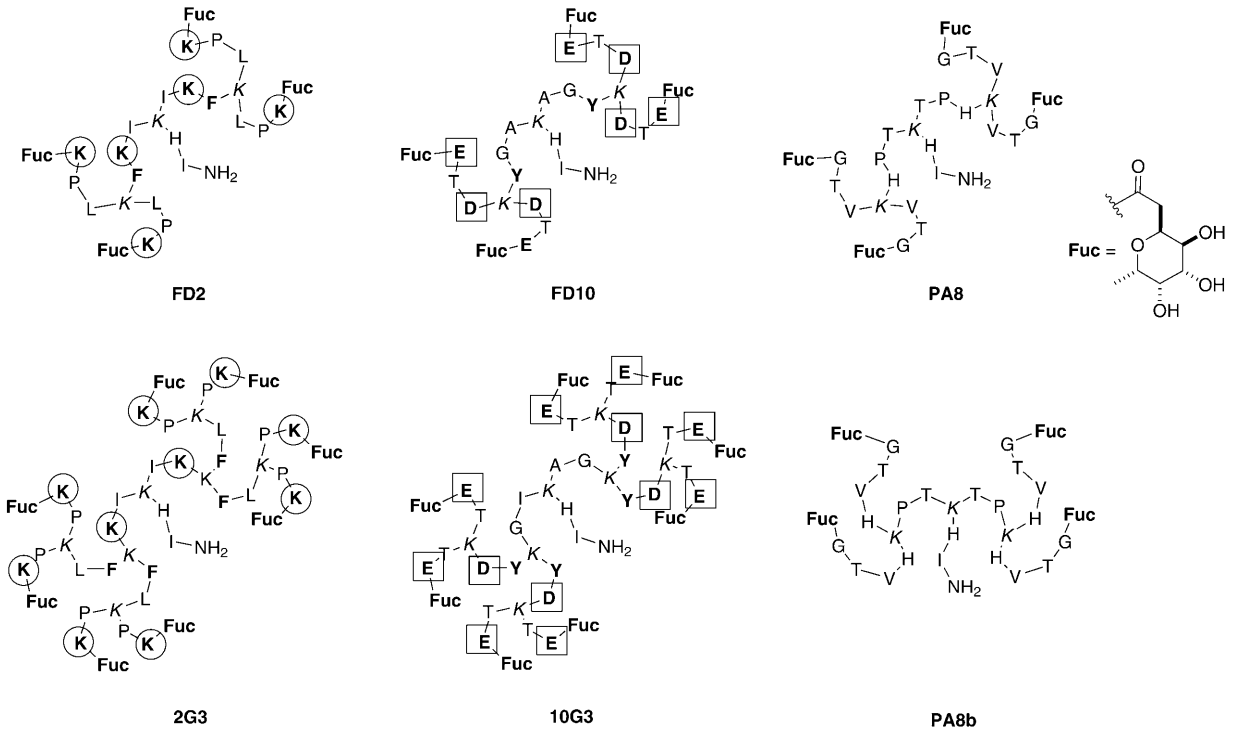
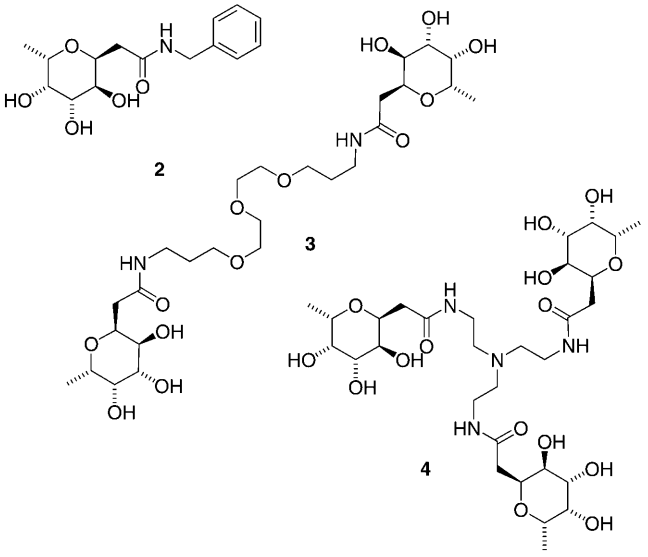


Figure 1. Structure of glycopeptide dendrimers: tetravalent hits **FD2**, **FD10**, **PA8**; octavalent analogues **2G3** and **10G3** and the modified hit **PA8b**. Amino acids are given as one-letter codes. Branching lysine residue in italics (*K*). Cationic residues are in circles, anionic residues are in squares.

cleavable Rink amide support, cleaved from the resin and purified as soluble ligands by preparative HPLC.

We also prepared three ligands derived from the *C*-fucosyl building block **1** in the form of the monovalent amide **2**, the divalent bisamide ligand **3**, and the trivalent trisamide ligand **4** by acylation of benzylamide, trioxaundecanediamine, and tris-(aminoethyl)amine, respectively, to test the role of the multivalent display of our *C*-fucosylamide ligand independent of the peptide backbone. The earlier generation analogues of **FD2** featuring the monovalent tripeptide **2G0** and the divalent 1st generation dendrimer **2G1** corresponding to the outer branches of **FD2** were also included in the study as additional lower valency controls.



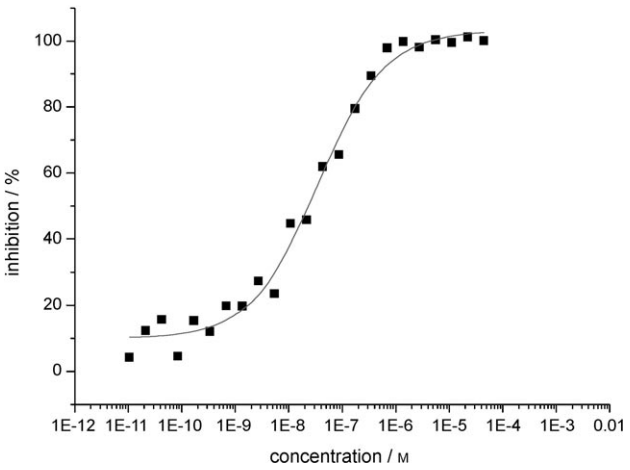


Figure 2. Percentage inhibition curve of dendrimer **2G3** from ELLA results with LecB. See Experimental Section for assay details.

LecB affinity of C-fucosylated dendrimers and ligands

Lectin binding by the soluble dendrimers and ligands was investigated with an ELLA. This assay measures competition between the ligand and a biotinylated polymeric fucose reagent for binding to the lectin coated on the plate surface.[29] Binding of the biotinylated polymeric fucose is revealed using a horseradish peroxidase conjugate of streptavidin and ABTS as chromogenic substrate. The IC_{50} values are expressed as ligand concentration reducing the color signal by 50% (Figure 2). L-(−)-Fucose was used as the reference throughout the study ($IC_{50} = 11 \pm 1.5$ μM). The results of the ELLA measurements are summarized in Table 2.

The LecB ELLA data showed that the monovalent *C*-fucosides had potencies comparable to free fucose with, at most, a

Table 2. ELLA data[a] for LecB binding (see Figure 2).

Compound	*n*[b]	Sequence	IC_{50} [μM]	rel. pot.[c]	rel. pot. /fucose[d]
L-fucose	1	NA	11 ± 1.5[e]	1.0	1.0
2G0	1	CFuc-LysProLeuNH_2	5.94 ± 1.24[e]	1.9	1.9
2	1	NA	5.65 ± 1.55	1.9	1.9
3	2	NA	5.35 ± 0.05	2.1	1.0
2G1	2	(CFuc-LysProLeu)$_2$*Lys*PheLysIleNH$_2$	2.7 ± 0.56[e]	4.1	2.0
4	3	NA	3.53 ± 0.12	3.1	1.0
FD1	4	(CFuc-LysHisVal)$_4$(*Lys*HisGlyAla)$_2$*Lys*HisIleNH$_2$	0.43 ± 0.11[e]	25.6	6.4
FD2	4	(CFuc-LysProLeu)$_4$(*Lys*PheLysIle)$_2$*Lys*HisIleNH$_2$	0.14 ± 0.035[f]	78.6	19.6
FD3	4	(CFuc-LysHisLeu)$_4$(*Lys*GluLysIle)$_2$*Lys*HisIleNH$_2$	0.19 ± 0.05[e]	57.9	14.5
FD4	4	(CFuc-LysArgAsp)$_4$(*Lys*SerArgAla)$_2$*Lys*HisIleNH$_2$	0.54 ± 0.14[e]	20.4	5.1
FD9	4	(CFuc-SerProAsp)$_4$(*Lys*GluValAsp)$_2$*Lys*HisIleNH$_2$	0.63 ± 0.15	17.5	4.4
FD10	4	(CFuc-GluThrAsp)$_4$(*Lys*TyrGlyAla)$_2$*Lys*HisIleNH$_2$	0.25 ± 0.08	44.0	11.0
PA5	4	(CFuc-GlyArgVal)$_4$(*Lys*GluGlyLeu)$_2$*Lys*HisIleNH$_2$	0.74 ± 0.035	14.9	3.7
PA8	4	(CFuc-GlyThrVal)$_4$(*Lys*HisProThr)$_2$*Lys*HisIleNH$_2$	0.21 ± 0.02[e]	52.4	13.1
PA9	4	(CFuc-GluHisTyr)$_4$(*Lys*TyrGlyAsp)$_2$*Lys*HisIleNH$_2$	0.26 ± 0.04[e]	42.3	10.6
PA8b	4	(CFuc-GlyThrValHis)$_4$(*Lys*ProThr)$_2$*Lys*HisIleNH$_2$	2.4 ± 0.36	4.6	1.1
2G3	8	(CFuc-LysPro)$_8$(*Lys*LeuPhe)$_4$(*Lys*LysIle)$_2$*Lys*HisIleNH$_2$	0.025 ± 0.005	440.0	55.0
2G3′	8	(CFuc-LysPro)$_8$(*Dap*LeuPhe)$_4$(*Dap*LysIle)$_2$*Dap*HisIleNH$_2$	0.94 ± 0.14	11.7	1.5
10G3	8	(CFuc-GluThr)$_8$(*Lys*AspTy)$_4$(*Lys*GlyAla)$_2$*Lys*HisIleNH$_2$	0.12 ± 0.03	91.7	11.5

[a] Assays were carried out in triplicate. [b] Number of fucosyl groups in the ligand. [c] Relative potency = IC_{50}(fucose)/IC_{50}(ligand). [d] Relative potency/fucose = relative potency/*n*. [e] Data from reference [26]. [f] Data from reference [15].

twofold increase in potency for the tripeptide **2G0** corresponding to the outer branch of **FD2** or the simple benzyl amide **2**. Similarly, divalent ligands **3** and **2G1** and the symmetrical trivalent *C*-fucosylamide **4** showed negligible enhancement of potency relative to fucose in the assay. By contrast, the nine tetravalent dendrimers selected from library screening were 15- and 80-fold more potent than fucose, corresponding to a 4- to 20-fold enhancement of potency per fucose residue. The best tetravalent ligand was the cationic dendrimer **FD2** originally selected against the plant lectin UEA-I. However, neutral (**PA8**) and anionic (**FD10**, **PA9**) ligands bound almost equally well, implying that the charge was not a primary determinant of affinity for LecB. The tetravalent dendrimer **PA8b**, an analogue of **PA8** where the branching point was moved one position up the sequence, had a much lower potency, suggesting a strong influence of the overall dendrimer structure on affinity.

The octavalent dendrimer **2G3** showed a particularly strong enhancement of potency relative to its tetravalent analogue **FD2**, and displayed the strongest potency of all ligands tested. This was quite surprising considering that the sequence redesign of **FD2**→**2G3** only had minor effects on the affinity for UEA-I.[15] Dendrimer **2G3′**, an analogue of **2G3** with diaminopropanoic acid branching points instead of Lys, and **10G3**, the 3rd generation analogue of **FD10**, both showed only relatively small increase in potency against LecB, further highlighting the unique nature of the dendrimer **2G3** and LecB interaction.

Molecular modeling

The availability of the LecB–fucose co-crystal structure allowed structure-based modeling of the possible binding mode of the peptide dendrimer with the lectin.[24] A docking study was carried out using GLIDE.[30] L-Fucose, the *C*-fucosylamide **2**, and dendrimers **FD10, PA8, PA5, FD2, FD9** were investigated. Due to multiple degrees of freedom that limit flexible docking programs, small-molecule models of the *C*-fucosyl dipeptide end of the dendrimers ($CFucX^8X^7NHCH_3$) were used for modeling. GLIDE correctly positioned the reference crystallographic ligand α-L-fucose in its crystallographic position, confirming the suitability of this program for investigating binding to LecB. Various *C*-fucosyl ligands were placed in similar positions to fucose, with the fucosyl residue engaging in the same set of interactions with the protein as free fucose (figure S3, Table 3). The *C*-fucosylamide **2** showed one additional hydrogen bond whereas the various dipeptides contacted the protein with one additional hydrogen bond and two additional hydrophobic contacts compared with fucose.

The absence of tight contacts between the dipeptides and the lectin is consistent with a LecB–**2G0** co-crystal structure in which the peptide portion is largely disordered.[26] The small number of interactions between the peptide portion of the ligands and the lectin are consistent with the observation that monovalent ligands bind comparably to fucose itself, and suggests that the increased binding upon multivalent display reflects a multivalency effect. The dendrimers are too small to bridge two distinct fucose binding sites within the same lectin tetramer. It is conceivable that additional protein–dendrimer contacts, outside those of the fucose binding site, are taking place between the fucosyl tripeptide arms not directly bound to the fucose binding site and the protein. Such interactions could lead to different affinities for LecB with different amino acid sequences in the dendrimers investigated. Another possibility is that a particular peptidic structure displays the fucose with the right orientation for binding to the lectin. Further studies are now under way to better understand the nature of the peptide dendrimer–lectin interactions.

Conclusions

The results of our investigation showed that multivalent peptide dendrimers with N-terminal *C*-fucosyl residues represent potent inhibitors of the fucose-specific lectin LecB, with increased potency up to 440-fold over fucose (55 per fucosyl group) for the octavalent cationic dendrimer **2G3**. The sequences were initially identified from a combinatorial library of tetravalent dendrimers on solid support using on-bead binding assays with fucose-specific lectins (UEA-I and LecB). While the assays identified predominantly cationic sequences related to **2G3**, anionic and neutral amino acids were also present. Affinity measurements with purified soluble dendrimers by LecB ELLA confirmed binding of the various tetravalent dendrimers identified in the initial screen. While mono- and divalent control ligands derived from the *C*-fucosyl moiety did not bind significantly stronger than fucose, octavalent analogues provided the best ligands, showing that multivalency is an important factor for binding. Docking studies show that the *C*-fucosyl

Table 3. Docking score and molecular interactions of fucose and *C*-fucosyl dipeptides with *P. aeruginosa* LecB.[a]

Compound	Glide Score [kcal mol^{-1}]	H bonds	Residues involved	HI[b]	Residues involved
L-fucose	−6.82	5	Ser 23, Asp 99, Asp 101, Gly 114, Asp 96	6	Thr 98, Gly 114, Thr 45, Ser 22, Ser 23, Gly 24
CFuc-NH-Bn (**2**)	−6.24	6	Ser 23, Asp 99, Asp 101, Gly 114, Asp 96, Asp 104	6	Thr 98, Gly 114, Thr 45, Ser 22, Ser 23, Gly 24
CFuc**GluThr**-$NHCH_3$ (**FD10**)	−5.57	+1	Ser23	NA	NA
CFuc**GlyThr**-$NHCH_3$ (**PA8**)	−5.32	NA	NA	+2	Val 69, Thr 25
CFuc**GlyArg**-$NHCH_3$ (**PA5**)	−4.53	+1	Asn 70	+2	Val 69, Thr 25
CFuc**LysPro**-$NHCH_3$ (**FD2**)	−4.90	+1	Asp 96	+2	Val 69, Thr 25
CFuc**SerPro**-$NHCH_3$ (**FD9**)	−5.29	+1	Asp 96	+2	Val 69, Thr 25

[a] L-Fucose or dendrimer *C*-fucosyl dipeptide branch termini were docked into the fucose binding pocket of LecB (1GZT.pdb) using GLIDE. All *C*-fucosyl dipeptide have the same contacts as the reference *C*-fucoside **2** for the fucosyl group itself, only additional contacts involving the dipeptide (in bold) are shown. [b] Number of hydrophobic interactions.

group of the dendrimers can adopt an identical binding mode to fucose itself, with the peptide arms protruding from the binding pocket and establishing specific contacts with the lectin. The absence of multiple additional interactions between monovalent ligands and lectin suggest that enhanced binding of the dendrimers compared to monovalent ligand is caused by multivalency. In addition, the architecture of the dendritic moiety and the nature of the amino acids present in the branches may modulate the presentation of the fucose residues for lectin binding.

In view of the versatility and reliability of SPPS to produce the peptide dendrimers as tunable multivalent fucosylated ligands, the dendrimers reported here seem particularly well suited to the development of polyvalent inhibitors of *P. aeruginosa* adhesion and biofilm. The aqueous solubility of the fucosyl peptide dendrimers is generally very good in contrast to other synthetic multivalent fucosyl dendrimers reported to date where aqueous solubility is often low. Multivalent glycosides such as our peptide dendrimers might eventually result in novel treatments against *P. aeruginosa*.[31] Further experiments along these lines are currently in progress.

Experimental Section

Synthetic procedures

Split and mix combinatorial library synthesis

The peptide dendrimer library was prepared from a 500 mg resin batch of NovaSyn® TG (0.30 mmol g^{-1}) divided equally in five reactors. Deprotection of the Fmoc group and coupling of each amino acid in each reactor were carried out as described for dendrimer synthesis (below). After each coupling, the five resin batches were then mixed together and split into five parts, equally introduced in the five reactors. These split and mix steps were repeated after each amino acid coupling. At the end of the synthesis, the Fmoc protected resin was dried and stored at $-4\,^{\circ}C$. Just before screening, the Fmoc protecting groups were removed, and the library was capped with a *C*-fucoside residue **1** (5 equiv) in the presence of DIEA (5 equiv) and HCTU (3 equiv) in NMP overnight. The side-chain protecting groups were then removed with TFA/TIS/H_2O (95:2.5:2.5) for 4 h. Finally the carbohydrate was deprotected with a solution of MeOH/NH_3/H_2O (*v*/*v* 8:1:1) for 24 h, resulting in a glycopeptide dendrimer library on beads.

N-Acetylated control library: Fmoc protecting groups were removed and the library was capped with Ac_2O/CH_2Cl_2 (1:1) for 30 min. The side-chain protecting groups were removed with TFA/TIS/H_2O (95:2.5:2.5) for 4 h to give a library with acetylated N termini and free ε-lysine side chains.

Dendrimer synthesis

Peptide syntheses were performed manually in a glass reactor or plastic syringes (5 or 10 mL). The resin NovaSyn® TGR (loading: 0.18–0.29 mmol g^{-1}) was acylated with each amino acid or diamino acid (3 equiv) in the presence of BOP or PyBOP (3 equiv) and DIEA (5 equiv) for 1.5 h, 3 h after the first generation. After each coupling the resin was successively washed with NMP, MeOH, and CH_2Cl_2 (3× with each solvent), then checked for free amino groups with the TNBS test. If the TNBS test indicated the presence of free amino groups, the coupling was repeated. After each coupling the potential remaining free amino groups were capped with Ac_2O/CH_2Cl_2 (1:1) for 10 min. The Fmoc protecting groups were removed with a solution of 20% piperidine in DMF (2× 10 min) and the solvent was removed by filtration. At the end of the sequence the resin was capped with *C*-fucosyl acid **1** (5 equiv) in the presence of DIC (5 equiv) and HOBt (5 equiv) or DIEA (5 equiv) and HCTU (3 equiv) in NMP overnight. The carbohydrate was deprotected with a solution of MeOH/NH_3/H_2O (*v*/*v* 8:1:1) for 24 h. The resin was dried and the cleavage was carried out with TFA/TIS/H_2O (95:2.5:2.5) for 4 h. The peptide was precipitated with methyl *tert*-butyl ether then dissolved in a H_2O/MeCN mixture. All dendrimers were purified by preparative HPLC with detection at $\lambda = 214$ nm. Eluent A: H_2O/TFA (0.1%); eluent B: MeCN/H_2O/TFA (3:2:0.1%). Yields and analytical data for all dendrimers and ligands are summarized in Table 4 and further detailed in the Supporting Information.

Labeling of LecB with rhodamine B isothiocyanate

The lectin LecB (0.5 mg) in phosphate-buffered saline (PBS, pH 8.3, 400 μL) was labeled with rhodamine B isothiocyanate (0.18 mg) in PBS (400 μL) giving a lectin/dye ratio of 1:4 (per lysine) after 21 h

Table 4. Synthetic yields of *C*-fucosyl peptide dendrimers.

Compound	*n*[a]	Sequence	Mass [mg][b]	Yield [%][b]	MW[c]	MS ES(+)[d]
2 G0	1	CFuc-LysProLeuNH_2	140	68	544.65	544.28
2 G1	2	(CFuc-LysProLeu)$_2$*Lys*PheLysIleNH$_2$	82.3	33	1586.95	1586.63
FD1	4	(CFuc-LysHisVal)$_4$(*Lys*HisGlyAla)$_2$*Lys*HisIleNH$_2$	18	6.7	33.91.85	3392.13
FD2	4	(CFuc-LysProLeu)$_4$(*Lys*PheLysIle)$_2$*Lys*HisIleNH$_2$	38.5	14	3536.34	3536.0
FD3	4	(CFuc-LysHisLeu)$_4$(*Lys*GluLysIle)$_2$*Lys*HisIleNH$_2$	12.4	4.4	3658.12	3657.88
FD4	4	(CFuc-LysArgAsp)$_4$(*Lys*SerArgAla)$_2$*Lys*HisIleNH$_2$	13.2	4.7	3629.96	3629.50
FD9	4	(CFuc-SerProAsp)$_4$(*Lys*GluValAsp)$_2$*Lys*HisIleNH$_2$	14	5.4	3288.34	3288.13
FD10	4	(CFuc-GluThrAsp)$_4$(*Lys*TyrGlyAla)$_2$*Lys*HisIleNH$_2$	8	5	3369.38	3368.0
PA5	4	(CFuc-GlyArgVal)$_4$(*Lys*GluGlyLeu)$_2$*Lys*HisIleNH$_2$	26	10	3252.68	3253.0
PA8	4	(CFuc-GlyThrVal)$_4$(*Lys*HisProThr)$_2$*Lys*HisIleNH$_2$	22	9	3104.4	3103.8
PA9	4	(CFuc-GluHisTyr)$_4$(*Lys*TyrGlyAsp)$_2$*Lys*HisIleNH$_2$	22	6	3791.6	3792.0
PA8 b	4	(CFuc-GlyThrValHis)$_4$(*Lys*ProThr)$_2$*Lys*HisIleNH$_2$	12	5	3378.7	3378.0
2G3	8	(CFuc-LysPro)$_8$(*Lys*LeuPhe)$_4$(*Lys*LysIle)$_2$*Lys*HisIleNH$_2$	11.7	2.5	5994.53	5994.25
2G3′	8	(CFuc-LysPro)$_8$(*Dap*LeuPhe)$_4$(*Dap*LysIle)$_2$*Dap*HisIleNH$_2$	10.4	2.3	5699.20	5699.88
10G3	8	(CFuc-GluThr)$_8$(*Lys*AspTy)$_4$(*Lys*GlyAla)$_2$ *Lys*HisIleNH$_2$	6	1.4	5880.59	5881.50[e]

[a] Number of fucosyl groups in the ligand. [b] Isolated by preparative HPLC. [c] Calculated molecular weight. [d] Mass obtained by MS ES(+). [e] MS ES(−).

at RT. The Labeled LecB was purified using a HiTrap desalting column (eluent = PBS, pH 7.2). The fractions were checked using SDS-PAGE 20% Standard and labeled lectin was observed at wavelength of 473 nm.

On-bead lectin binding assay

The glycodendrimer library on TG resin[15] (50 mg) was washed three times with 1 mL of PBST buffer (2 mM Na_2HPO_4, 10 mM NaH_2PO_4, 150 mM NaCl, 0.05% Tween-20, pH 6.8) and then suspended in 2 mL of PBST buffer containing 3% bovine serum albumin (BSA). After having been shaken for 30 min, the beads were washed three times with 1 mL of PBST buffer containing 1% BSA and subsequently incubated with 1 mL of a solution of rhodamine B labeled lectin LecB (6 $\mu g\,mL^{-1}$) for 1 h at RT. After removal of lectin solution, the beads were incubated with L-(−)-fucose (0.45 M) in PBST buffer for a further 1 h. The beads were washed with PBST buffer (×3, 1 mL) and water (×1, 1 mL) and monitored under a fluorescence microscope Axiovert 35. Fluorescent beads were manually selected and washed with 90% aq TFA (10×1 mL), water (10×), MeOH (2×), CH_2Cl_2 (5×), MeOH (2×), and water (5×) in order to remove bound lectin. The screening was repeated with L-(−)-fucose (3 M) solution and without fucose competition. Negative control was conducted using the acetylated library and no lectin binding was observed in this case.

Sequence determination

Single dendrimer-containing resin beads were hydrolyzed with aq HCl (6 M) at 110 °C for 22 h. The amino acids were derivatized with phenylisothiocyanate (PITC) and the phenylthiocarbamyl (PTC) derivatives analyzed on a reverse phase C18 Novapack column.

Enzyme-linked lectin assay (ELLA)

ELLAs were conducted using 96-well microtiter plates (Nunc Maxisorb) coated with LecB (5 $\mu g\,mL^{-1}$) diluted in carbonate buffer (pH 9.6, 100 μL) for 1 h at 37 °C. After lectin removal, the wells were blocked with BSA in PBS (3% *w/v*, 100 $\mu L\,well^{-1}$) at 37 °C for 1 h. BSA solution was removed and the test compound was added in twofold serial dilutions (54 $\mu L\,well^{-1}$) in PBS and incubated at 37 °C for 1 h. Biotinylated polymeric fucose (54 μL, 5 $\mu g\,mL^{-1}$, Lectinity Holding, Inc.) was added to the solutions of inhibitors and the plates were further incubated for 1 h at 37 °C. After washing with PBST (PBS+0.05% Tween, 3×150 $\mu L\,well^{-1}$), streptavidin–peroxidase conjugate (100 μL, dilution 1:5000 in PBS) was added and left for 1 h at 37 °C. The wells were then washed with PBST (3× 150 $\mu L\,well^{-1}$) and H_2O (×1), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 50 $\mu L\,well^{-1}$, 0.25 $mg\,mL^{-1}$) in citrate phosphate buffer (0.2 M, pH 4.0, 0.015% (*v/v*) H_2O_2) was added. The reaction was stopped after 20 min by adding H_2SO_4 (1 M, 50 $\mu L\,well^{-1}$). Absorbance was read at 415 nm using a microtiter plate reader (spectra MAX 250). Experiment were carried out in triplicate.

Determination of IC_{50} values

The logarithm of the concentration of the dendrimer was plotted against the percentage inhibition. The sigmodial curve was fitted and the concentration at 50% inhibition of binding of biotinylated polymeric fucose to the LecB-coated microtiter plate wells was determined. The percent of inhibition was calculated as follows: Inhibition (%) = $((A_{max}-A)/A_{max})\times 100$, where A_{max} corresponds to the mean value of the highest absorption limit reached in the dilution series and not to an external reference. Percent inhibition was plotted against log [inhibitor].

Molecular modeling of glycopeptide dendrimers

Structure construction and minimization

L-Fucose, the *C*-fucosylamide **2**, and dendrimers **FD10, PA8, PA5, FD2, FD9** were investigated. Due to high degrees of freedom that limit flexible docking programs, small-molecule models of the *C*-fucosyl dipeptide dendrimers termini ($CFucX^8X^7NHCH_3$) were used for modeling. The ligands were constructed using the co-crystallized fucose ligand (1GZT.pdb) as a template for the fucosyl group, while taking into account the required conformation of dendrimers, using the fragment dictionary of Maestro 8.0. Subsequently, the constructed dendrimers were geometry optimized by Macromodel (version 9.1, Schrödinger LLC) using the OPLS-AA (optimized potentials for liquid simulations-all atom) force field with the truncated Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-AA force field.

Protein structure preparation and refinement

The X-ray crystal structure of *P. aeruginosa* LecB in complex with fucose (1GZT.pdb) obtained from the Protein Data Bank (PDB) (http://www.rcsb.org) was used as the protein model. Water molecules of crystallization were removed from the complex, and the protein was optimized for docking using the protein preparation and refinement utility (Schrödinger LLC). Partial atomic charges were assigned according to the OPLS-AA force field.

Docking methodology and protocol

All docking calculations were performed using the Standard Precision (SP) mode of Glide (Schrödinger LLC). The accuracy of a docking procedure can be evaluated by determining how closely the lowest energy binding conformation predicted by the object scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, Standard Precision Glide docking procedure was validated by removing the fucose from and redocking it into the binding site of lectin LecB. A good agreement was observed between the localization of the inhibitor upon docking and the crystal structure. Similar hydrogen-bonding interactions were observed between fucose and Ser23, Asp99, Asp101, Gly114 and Asp96. The root mean square deviations between the predicted conformation and the observed X-ray crystallographic conformation of fucose equaled 0.56 Å. This value suggests the reliability of the docking in reproducing the experimentally observed binding mode for *P. aeruginosa* LecB, and also confirms that the parameter set for the Glide docking is reasonable to reproduce the X-ray crystal structure. The validated docking protocol was then used for docking dendrimers into the crystal structure of the protein. The fucose of the docked dendrimers presented a similar binding mode as crystallographic fucose. The scoring function, glide docking score (G_{score}), which is a modified and extended version of the empirically based Chemscore function, was used to evaluate the binding of the dendrimers.

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