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FULL PAPER

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X-Ray Crystal Structure of a Second-Generation Peptide Dendrimer in Complex with *Pseudomonas aeruginosa* Lectin LecB

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This publication is dedicated to Prof. *Philippe Renaud* on the occasion of his 60th Birthday

Dendrimers are regularly branched molecular trees which are notoriously difficult to crystallize. Herein we report the crystal structure of a *C*-fucosylated second generation peptide dendrimer as complex with lectin LecB in which the only dendrimer-lectin contact is the LecB bound glycoside (PDB 6S5S). In contrast to a previously reported crystal structure of a first-generation peptide dendrimer as LecB complex in which the dendrimer formed trimers connected by intermolecular β-sheets (PDB 5D2A), the present structure features a globular monomeric state held together by intramolecular backbone hydrogen bonds and assembled into a non-covalent dimer stabilized by hydrophobic contacts between leucine side-chains and proline-phenylalanine CH-π stacking interactions. Molecular dynamics and circular dichroism studies suggest that this crystal structure resembles the structure of the peptide dendrimer in solution. Structures of a partially resolved dendrimer (PDB 6S5R) and of *C*-fucosylated disulfide bridged peptide dimers connecting different LecB tetramers are also reported (PDB 6S7G, PDB 6S5P).

Keywords: peptides, dendrimers, lectins, crystal structure, carbohydrates.

Introduction

The invention of dendrimers, or cascade molecules, by Vögtle and Tomalia 40 years ago generated great enthusiasm in the chemistry community as a simple strategy to assemble synthetic macromolecules and polymers resembling the globular shape of proteins.[1–6] The dendrimer concept, which consists in iteratively assembling dendrons into a regular molecular tree, was realized starting from a variety of building blocks such as benzyl alcohols,[7] glycerols,[8] phosphazenes,[9] poly(amidoamides),[10] polyphenylenes,[11] and amino acids.[12] The approach allowed designing microenvironment effects at the dendrimer core and multivalency effects at the dendrimer periphery, which proved useful in a variety of settings, most notably in catalysis and biomedical applications.[13–16] However, dendrimers turned out to be quite difficult to characterize structurally due to their symmetry and partly due to synthetic imperfections whenever they were prepared by polymer chemistry approaches. For instance, only a handful of dendrimers have yielded to X-ray crystallography, in all cases for relatively small dendrimers consisting of structurally rigid aromatic dendrons.[17,18]

Herein, we report the crystal structure of a second-generation peptide dendrimer (**SBD8**, PDB 6S5S). This structure provides the first direct structural insight into a broad class of peptide dendrimers developed in our group over the past 15 years as enzyme models,[13] drug and nucleic acid delivery agents,[19–22] biofilm inhibitors and antimicrobial agents,[23,24] but for which structural models to date were built indirectly by molecular dynamics (MD) studies supported by DOSY NMR for size estimation and circular dichroism (CD) for secondary structure content.[25–30]

The X-ray crystal structure presented herein features a fucosylated dendrimer in complex with the

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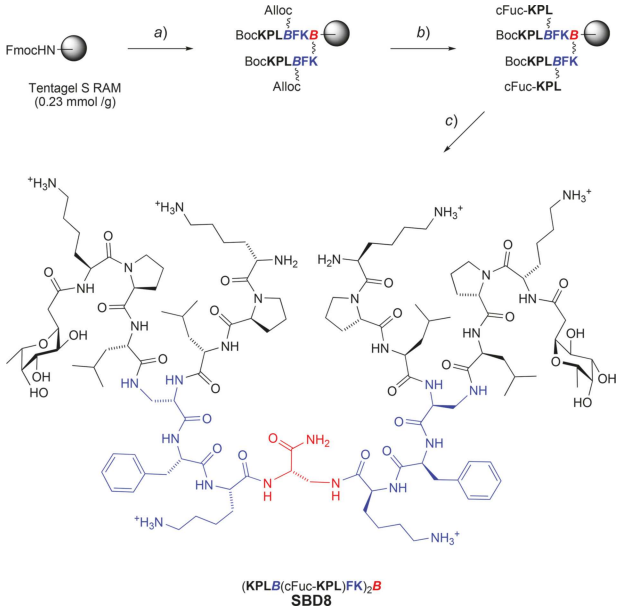
fucose specific *Pseudomonas aeruginosa* lectin LecB.[31] LecB mediated crystallography is very useful to elucidate the structure of molecules otherwise difficult to crystallize such as oligonucleotides,[32] cyclic,[33] bicyclic,[34,35] and linear peptides.[36] We focused on this approach because we had previously collected promising crystallographic data with dendrimer lectin complexes. In one case we had obtained diffracting crystals of a third generation galactosylated peptide dendrimer in complex with the galactose specific *P. aeruginosa* lectin LecA, however only the aromatic galactoside anchor was visible in the structure, while the dendrimer itself was not visible (PDB 5D21).[37] In a second case, we had obtained a crystal structure of a first generation glycopeptide dendrimer in complex with LecB, in which peptide branches were engaged in a trimeric network of intermolecular β-sheets, while the dendrimer itself was dimerized by disulfide bridge formation (PDB 5D2A).[38] Although none of these structures provided relevant information for the monomeric state known to be the prevalent form in solution for most of our dendrimers, they clearly suggested that lectin crystallography should be further pursued to obtain a peptide dendrimer structure.

Results and Discussion

Design and Synthesis

In view of crystallizing dendrimer complexes with the fucose specific lectin LecB, we synthesized fucosylated analogs of two series of dendrimers for which previous data suggested the presence of secondary structures in the peptide branches. In the first series, we focused on analogs of **G3KL** and **TNS18**, which are antimicrobial peptide dendrimers (AMDPs) active against multidrug resistant Gram-negative bacteria and for which CD and MD studies point to either α-helical (**G3KL**) or β-sheet (**TNS18**) secondary structures within the dendrimer.[27,28,30] In the second case, we prepared analogs of **FD2**,[39] a biofilm inhibitory glycopeptide dendrimer,[40] as a follow-up on previously obtained X-ray crystal structures of lectin complexes with glycosylated analogs of its terminal tripeptide branch and with a related first generation glycodendrimer.[38,41]

To favor crystallization, we limited ourselves to second generation structures small enough to fit within the crystal lattice of LecB. We prepared dendrimers using also D-amino acids because a previous project with LecB complexes of fucosylated analogs of α-helical linear antimicrobial peptides had only yielded diffracting crystals with D-enantiomeric sequences.[36] We attached a *C*-fucosyl group at the side chain amino group of diaminopropanoic acid or lysine placed either at the core, in the first or second generation branches, or on the N-terminus of the second generation, making use of orthogonal protecting groups at the branching point to obtain dendrimers with one or at most two fucosyl groups. In total, we prepared twelve dendrimers following these designs. All dendrimers were obtained as pure products after solid-phase peptide synthesis and purification by preparative reversed-phase HPLC in multimilligram quantities sufficient for characterization and various crystallization screens (*Table 1*). The synthesis of **SBD8**, which yielded to crystallization in the present study, is shown as a representative example (*Scheme 1*).



Scheme 1. Synthesis of peptide dendrimer **SBD8**. *a*) Standard Fmoc SPPS; *b*) i. $Pd(PPh_3)_4$ (0.25 equiv.), $PhSiH_3$ (25 equiv.), CH_2Cl_2, 2×45 min., ii. Standard Fmoc SPPS, iii. Peracetylated α-L-fucosyl-acetic acid/Oxyma/DIC in NMP/CH_2Cl_2, overnight; *c*) i. MeOH/H_2O/NH_3•25% aq. (8:1:1), 24 h, ii. CF_3CO_2H/$^iPr_3SiH$/H_2O (95:2.5:2.5), 3.5 h.

X-Ray Crystallography

We subjected each of the fucosylated dendrimers in combination with purified lectin LecB to a series of protein crystallization conditions (*Table S2*), typically 2×96 conditions from commercially available screens. In view of our previous data showing that similar

Table 1. Sequence and design of *C*-fucosylated peptide dendrimers for co-crystallization with LecB.

Name	Sequence[a]	Comments	Yield [mg][b]	MS calc/obs[c]
G3KL	$(KL)_8(KKL)_4(KKL)_2KKL$	Reference AMPD	52	4534.2/4535.0
TNS18	$(OF)_4(KDabL)_2KKLK(C_{10})$	Reference AMPD	121	2395.61/2395.61
SBD1	$(K)_2BKB((K)_2BB(cFuc))$	Small dendrimer with Dap branching to reduce flexibility	12.6	1189.76/1189.76
SBD2	$(KL)_4(BKL)_2BB(cFuc)$	**G3KL**-type with Dap branching	5.0	1996.36/1996.37
SBD3	$(KL)_4(kKL)_2kB(cFuc)L$	**G3KL**-type with D-Lys branching	6.4	2235.59/2235.59
SBD4	$(kl)_4(kkl)_2kB(cFuc)lll$	D-**G3KL**-type with leucines at G0 to induce α-helix folding	8.0	2461.75/2461.76
SBD5	$(kl)_2kklk((kl)_2kB(cFuc)l)llll$	as **SBD4** with fucose at G1 on one branch	10.0	2446.74/2446.75
SBD6	$(K)_2kKyK((K)_2kB(cFuc)y)lLlL$	Alternative D-L sequence to flatten the structure, Tyr to favor crystal contacts	11.6	2094.37/2094.37
SBD7	$(KW)_2KKK((KW)_2KB(cFuc))L$	Trp in G2 to favor aromatic stacking	6.7	2173.30/2173.31
FD2	$(cFuc\text{-}KPL)_4(KFKl)_2KHl$	Reference biofilm inhibitor	31	3534.16/3534.16
Het2G1-Cys	GalA-KPL*K*(cFuc-KPL)FC	crystallized G1 dendrimer	50	1542.82/1543.81
SBD8	$(KPLB(cFuc\text{-}KPL)FK)_2B$	Based on Het2G1-Cys dimer, branching as Dap to reduce flexibility, only fucose	28	2554.21/2554.57
SBD9	$(KPLK(cFuc\text{-}KPL)FK)_2K$	Same as **SBD8** with Lys branching	34	2680.70/2680.71
SBD10	$(KKLK(cFuc\text{-}KKL)KL)_2B$	Same as **SBD8** based on G3KL	19	2694.86/2694.86
SBD11	$(KPLK(cFuc\text{-}KPL)YC)_2$	Analog of **Het2G1**-Cys, Phe →Tyr, no galactose	10.2	2549.44/2549.43
SBD12	$(KPLB(cFuc\text{-}KPL)YC)_2$	Same as **SBD11** with Dap branching	8.4	2465.34/2465.35

[a] one-letter code for amino acids, branching residues in italics, brackets right of residue denote side-chain acylated section. *B* = diaminopropanoic acid, Dab = diaminobutanoic acid, cFuc = *C*-fucosylacetyl, GalA = 4-(β-galactosyloxy)benzoyl. Carboxy termini are carboxamide $CONH_2$, amino termini are free or fucosylated. [b] Yield of isolated product of lyophilized solid after SPPS and preparative RP-HPLC purification. [c] ESI-MS.

mono- and di-fucosylated peptide dendrimers bind LecB strongly ($K_D < 1\mu M$),[38] we expected all dendrimers to form lecB complexes under these conditions. Three samples (**SBD1**, **SBD7**, and **SBD12**) formed well-diffracting crystals but with insufficient electronic densities to resolve the dendrimers except for the anchoring fucosyl moiety or in one case up to three residues corresponding to the G1 branch (**SBD6**, *Figure 1*, *Table S3.1*). Another six samples (**SBD2**, **SBD3**, **SBD4**, **SBD5**, **SBD9**, and **SBD11**) did not crystallize over a period of 18 months yet gave good datasets when the dendrimers were soaked in Apo crystals of LecB, however again only the anchoring *C*-fucosyl group was visible. These data indicated that the dendrimers were present but remained conformationally disordered within the LecB crystal lattice, illustrating the inherent difficulty to crystallize dendrimers.

Nevertheless, to our delight one of the dendrimers, **SBD8**, provided diffracting crystals from which high-quality datasets could be collected (*Table S3.2*) with well resolved electron densities corresponding to the essentially complete peptide dendrimer, providing the first high resolution crystal structure of a second-generation peptide dendrimer (*Figure 2*).

In this structure, the dendrimer is bound to a single LecB monomer through one of its two N-terminal fucose moieties. Almost all atoms in the structure are visible (155 of 181 heavy atoms, 86% in **SBD8**) except for parts of two G2 branches, namely the second fucose residue pointing towards the solvent and the side chain of its adjacent lysine residue, and the N-terminal lysine in another G2 branch.

The asymmetric unit contains one LecB monomer (*Figure 2,a*, chain A) and its bound fucosylated dendrimer. The dendrimer structure is stabilized by six intramolecular hydrogen bonds spread between the first and second generation as well as by a single proline-phenylalanine CH-π stacking contact between a phenylalanine in one of the G1 branches and a proline residue in one of the four G2 branches (*Figure 2,b*). This type of

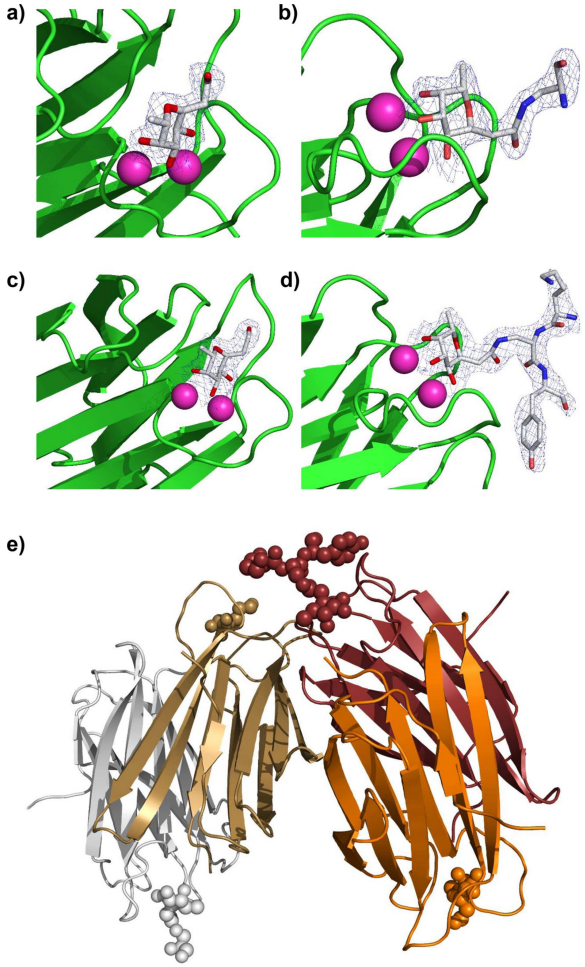


Figure 1. Details of the X-ray structure of the **SBD6**·LecB complex with incomplete dendrimer (PDB 6S5R). a)–d) Asymmetric peptide entities with corresponding electron density map as blue mesh, with Ca^{2+} atoms shown as magenta spheres and the bound LecB monomer as green cartoon. e) Full asymmetric unit representation. Peptides are shown as spheres and bound lectin monomers are displayed as cartoon of the same color.

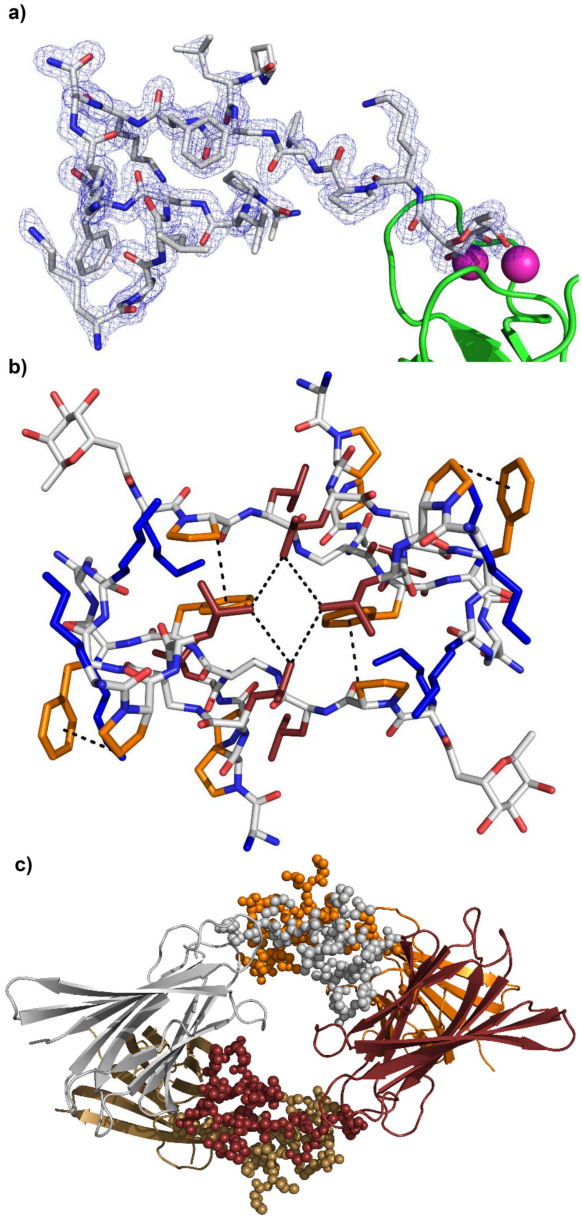


Figure 2. X-ray crystallography of peptide dendrimer **SBD8**. a) Stick representation of **SBD8** and electron density map as blue mesh, with Ca^{2+} atoms as magenta spheres and bound LecB monomer as green cartoon. b) Stick model of **SBD8** homodimer. Brown: Leucine, Orange: Phenylalanine and Proline, Blue: Lysine. CH-π and hydrophobic contacts represented with black dashes. c) View of the overall X-ray structure. Peptides are shown as spheres and bound lectin monomers are displayed as cartoon of the same color.

contact is often observed between adjacent residues within β-turns where they stabilize the turn structure, but also between distant residues within hydrophobic regions of proteins.[42–44] Within the crystal lattice the dendrimer assembles with itself to form a non-covalent homodimer stabilized by intermolecular hydrophobic interactions comprising four leucines (two per dendrimer) arranged in square, as well as one phenylalanine and one proline residue interacting in a proline-phenylalanine CH-π stacking contact at a distance of 3.5 to 4.0 Å from each other similar to the intramolecular case described above (*Figure 2,b, 2,c*).

Molecular Modeling

Remarkably, the only contact point between dendrimer and lectin in the **SBD8**·LecB complex was the fucose anchoring group, suggesting that the structure

of the dendrimer in the crystal might resemble its structure in solution. To test this hypothesis, we performed molecular dynamics (MD) simulations for 1000 ns in pure water using GROMACS,[45] starting either from a dendrimer molecule as monomer, or from the non-covalent homodimer, using the coordinates observed in the crystal structure. According to an acid-base titration showing an apparent pK_a value of approximately 6.5 for the amino termini and above 9 for lysine side chains (*Figure S3*), we set the protonation state of amino termini as free amines and all lysine side chains as ammoniums. In both the monomer and the homodimer, the overall dendrimer structure changed relatively little over the course of the simulation (*Figure S4*), but several new backbone hydrogen bonds were formed.

Clustering of the last 100 ns of the trajectories gave a small number of clusters (*Table S4*) and a relatively well-defined average structure in both cases. Compared to the starting X-ray structure, MD with the dendrimer monomer resulted in the formation of additional intramolecular backbone H-bonds, while the overall dendrimer geometry was preserved (*Figure 3,a*). The same situation occurred for MD of the non-covalent homodimer, which remained well assembled over the course of the trajectory, while additional intermolecular backbone H-bonds were formed which further stabilized the dimer (*Figure 3,b*, *Figure S5*).

None of the structures displayed any extended secondary structures. Consistent with the X-ray and MD data, circular dichroism (CD) measured in water and analyzed using Dichroweb[46] showed that the dendrimer predominantly contained random coil conformations and approximately one third of residues in a β-sheet conformation (*Figure 3,c*, *Figure S1*, *Table S1*). The propensity of the dendrimer to form random coil/β-sheet conformations was further highlighted by the observation that the CD spectrum only changed minimally upon addition of trifluoroethanol (TFE), which normally behaves as a strong inducer of α-helices.[47]

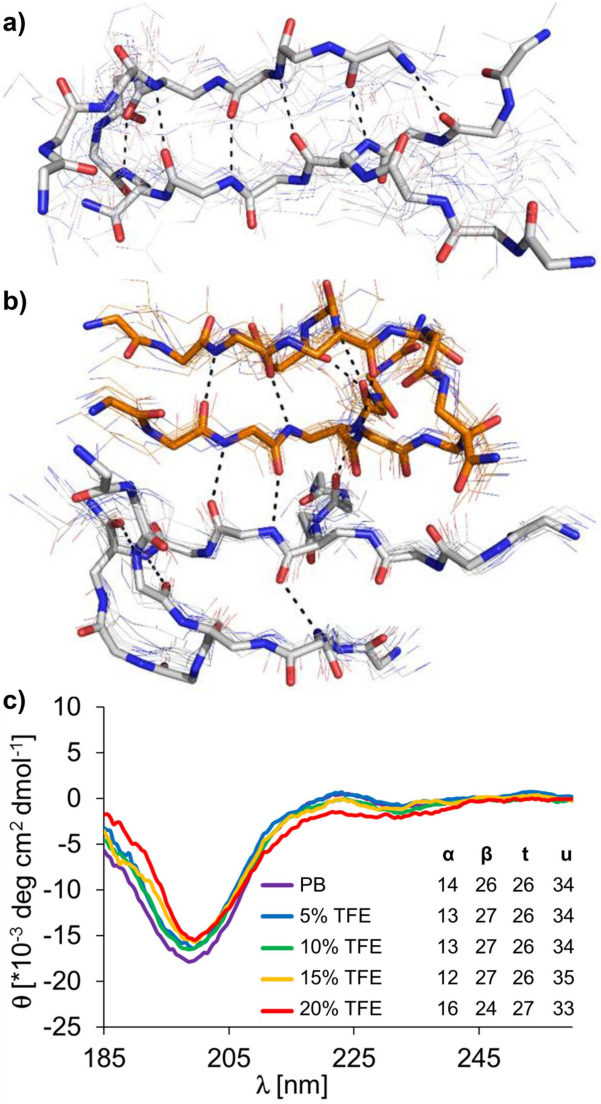


Figure 3. MD and CD data of dendrimer **SBD8**. a) Average backbone structure across the last 100 ns of a 1000 ns MD trajectory of the **SBD8** monomer in water starting from the X-ray coordinates. b) Average backbone structure for an MD run with the **SBD8** homodimer starting from the X-ray coordinates. Carbon atoms are color-coded to differentiate the two monomers. c) CD spectra of **SBD8** (0.10 mg/mL) in 10 mM aqueous phosphate buffer pH 7.2 with addition of TFE and percentages of secondary structures as calculated from the CD trace using Dichroweb. α = alpha, β = beta, t = turn, u = unordered.

Antimicrobial Activity

Since our dendrimers were analogs of antimicrobial peptide dendrimers, we tested their possible activity against a small panel of bacteria using the cyclic peptide polymyxin B (PMB) as positive control (*Table 2*). Indeed, two of the peptide dendrimers, **SBD4** and **SBD5**, showed significant activity against several Gram-negative bacteria, with potencies similar to their parents **G3KL**/**TNS18**,[48–50] while three further analogs **SB3**, **SB6**, and **SB7** showed marginal activity. We believe that these compounds act by membrane disruption similarly to **G3KL** and **TNS18**. Although biofilm inhibition has been reported with monovalent fucose analogs,[51] we did not test biofilm inhibition here because previous studies showed that biofilm inhibition requires four fucosyl groups per peptide dendrimer.[40]

Table 2. Antibacterial activity of peptide dendrimers.[a]

	P. aeruginosa PAO1	*A. baumannii* 19606	*K. pneumoniae*	*E. coli* (W3110)	*S. epidermiditis*	MRSA
SBD3	32	>64	>64	32	32	>64
SBD4	2	4	32	4	4	>64
SBD5	8	4	16	16	8	>64
SBD6	32	>64	>64	>64	32	>64
SBD7	8	>64	>64	32	16	32
PMB	0.25	0.25	1	0.25	4	>16

[a] MIC (minimum inhibitory concentration) in μg/mL were measured by serial two-fold dilutions in 96 well plates in Mueller–Hinton (MH) broth after incubation overnight at 37 °C. MIC Values were measured in independent duplicates with at least two experiments giving the same value. PMB is polymyxin B used as positive control. MRSA = methicillin resistant *Staphylococcus aureus*. There was no activity (MIC >64 μg/mL) on any of the six strains tested with **SBD1-3** and **SBD8-12**.

Difucosylated Peptides Bridging Two LecB Monomers

The relatively low success rate of crystallization of dendrimer LecB complexes probably reflects the intrinsic structural flexibility of peptide dendrimers, as illustrated by the fact that several dendrimers gave diffracting crystals in which only the anchoring fucose residue was visible. Nevertheless, we were intrigued by the fact that dendrimers bearing two fucose residues either did not crystallize, or as with **SBD8**, crystallized with only a single bound fucose residue. Compared to the related galactose specific lectin LecA, where pairs of binding sites on the tetramer point in the same direction and can be chelated by compounds presenting two carbohydrates,[52–54] the situation is intrinsically more difficult in the LecB tetramer because its four fucose binding sites point in different directions and cannot be easily bridged by the same ligand. This structural difference might explain the strong binding enhancement effects observed with multivalent galactosides for LecA versus the very weak multivalency effects observed with LecB.[55] However, bridging between two fucose binding sites on two different LecB tetramer should be possible within the crystal lattice.

Inspecting several crystal structures of LecB complexes with fucosylated linear antimicrobial peptides[36] showed that two fucose binding sites on different tetramers were separated by a distance of approximately 30 Å within the crystals, a distance which should be covered by a peptide of 8 to 12 residues. To test the feasibility of bridging two such sites with a divalent ligand, we investigated LecB complexes with seven different linear peptides of four to six residues acylated with a *C*-fucosyl group at their N-terminus and dimerized through a disulfide bond involving cysteine at the C-terminus. As residues we used alanines and optionally lysines to enhance aqueous solubility and aromatic residues and proline to favor structure forming stacking interactions (*Table 3*).

Table 3. *C*-Fucosylated peptide dimers.

Name	Sequence [a]	Dist.[b] [Å]	Yield [mg][c]	MS calc/obs[d]
SBL1	$(cFuc\text{-}KAKAC)_2$	30	17.2	1410.72/ 1410.72
SBL2	$(cFuc\text{-}KAAKAC)_2$	35	7.4	1552.79/ 1552.79
SBL3	$(cFuc\text{-}KAAC)_2$	25	10.3	1155.53/ 1155.54
SBL4	$(cFuc\text{-}KAKLYC)_2$	35	18.1	1820.94/ 1820.94
SBL5	$(cFuc\text{-}KPLKFC)_2$	35	20.8	1841.98/ 1841.98
SBL6	$(cFuc\text{-}LKFC)_2$	25	18.8	1390.69/ 1390.69
SBL7	$(cFuc\text{-}KLKFC)_2$	30	14.0	1646.88/ 1646.88

[a] One-letter code for amino acids, cFuc = *C*-fucosylacetic acid. [b] Distance between *C*-fucosyl groups predicted for a stretched conformation. [c] Yield of isolated product from SPPS, oxidative dimerization, and HPLC purification. [d] ESI-MS.

From the seven peptides, five either did not crystallize as LecB complexes or gave structures where only the fucose residue was visible, suggesting that bridging had not occurred properly. However, the two simplest peptides **SBL1** (PDB 6S7G, *Table S3.3*) and **SBL2** (PDB 6S5P, *Table S3.4*), which contained only alanine and lysine residues besides the anchoring fucosyl group and the C-terminal cysteine, provided well-resolved LecB complexed structures showing a peptide dimer bridging two different fucose binding

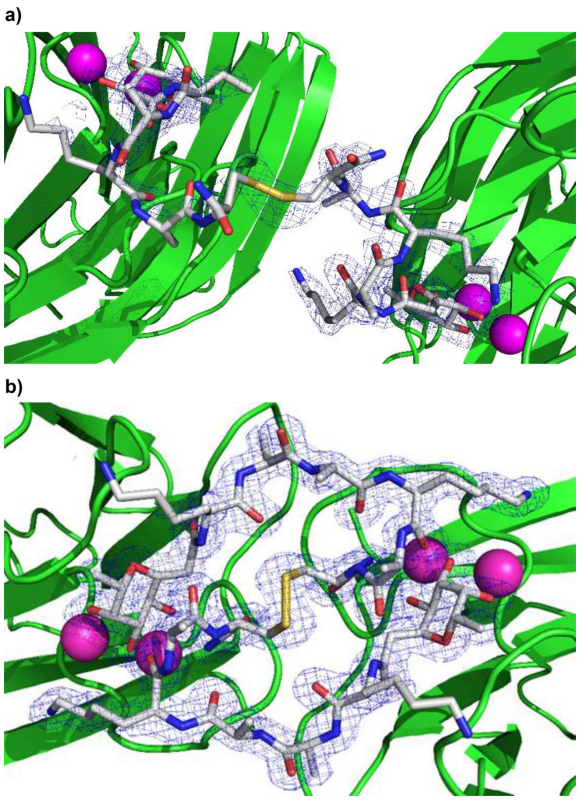


Figure 4. Details of the X-ray structure of the (a) **SBL1**·LecB (PDB 6S7G) and (b) **SBL2**·LecB (PDB 6S5P) complexes. Peptides as stick models with corresponding electron density map as blue mesh, Ca^{2+} atoms shown as magenta spheres and the bound LecB monomer as green cartoon.

sites on different lectin tetramers (*Figure 4*). In these two structures, the asymmetric unit contained four non-equivalent LecB monomers (chains A to D) each bound to a different **SBL1** or **SBL2** peptide (*Figure S2.1* and *Figure S2.2,a* to *S2.2,d*, chains E to H). In the case of **SBL1**, two of the four bound peptides were well-resolved and formed a heterodimer between chain F and G (*Figure 4,a*). On the other hand, **SBL2** formed a symmetrical homodimer of chain H (*Figure 4,b*), while in the three other chains only the fucosyl group was visible, indicating structural flexibility without bridging (*Figure S2.2*). The dimers both adopt an S-shape chain bridging two fucose binding sites separated by only 20 and 16 Å in the lattice respectively, suggesting that an even shorter peptide might be suitable to bridge different LecB tetramers.

Discussion

Taken together, the MD and CD studies above suggest that the structure of **SBD8** in aqueous solution is relatively close to that of its LecB complex and features a monomeric state stabilized by intramolecular backbone H-bonds and proline-phenylalanine CH-π stacking contacts, with possible formation of a non-covalent homodimer enabled by hydrophobic contacts. In most of our synthetic studies, peptide dendrimers are generally well behaved and monomeric in solution, suggesting that **SBD8** is more representative of our peptide dendrimers than the previously reported G1 dendrimer **Het2G1**-Cys as a LecB complex, where an intermolecular β-sheet between different dendrimers led to the formation of a supramolecular trimeric state.

Although the overall shape of **SBD8** or its dimer is approximately globular, it must be noted that only 32% of the residues in **SBD8** are engaged in an intramolecular backbone H-bond, which is much less than in a folded peptide (*e.g.* **SB4**, a 13-residue α-helical AMP)[36] or protein (*e.g.* insulin or lectin B) where a larger fraction of residues engage in secondary structures and are backbone H-bonded (*Table 4*, *Figure 5*).[56,57] This low percentage of backbone H-bonded residues in the dendrimer is in good agreement with our previously reported modeling study with a third generation catalytic peptide dendrimer **RMG3** which had the compactness of a 'molten globule' equivalent to that of a partially folded protein.[25]

Table 4. Analysis of backbone H-bonds.

	H-bonds[a]	# residues	Ratio [%]
Peptide dendrimers			
SBD8	6	19	32
Het2G1-Cys	4*	9*	44*
G3KL	10	37	27
TNS18	6	18	33
RMG3	12	38	32
Linear peptides and proteins			
SB4	8	13	61
Insulin	35	51	69
LecB	64	115	56

[a] Backbone H-bond count. Data for **G3KL**, **TNS18**, and **RMG3** from MD models, all other data from X-ray crystallography. Values are for intramolecular H-bonds except for Het2G1-Cys, marked *, which are intermolecular backbone H-bonds with a symmetrical dendrimer in the crystal lattice.

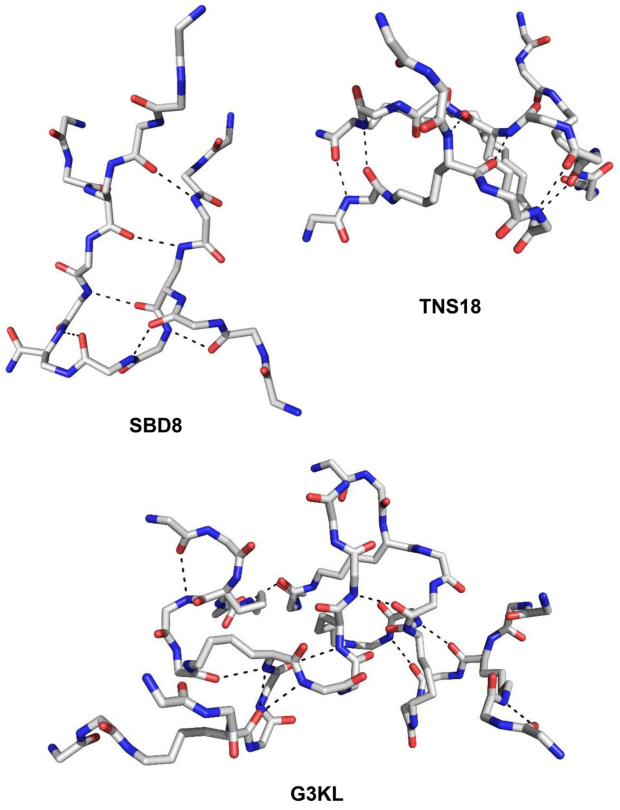


Figure 5. Backbone structure representation of peptide dendrimers **SBD8** (X-ray structure), **TNS18** and **G3KL** (MD models) with backbone H-bonds highlighted as dashed black lines.

Conclusions

In summary, the structure of the **SBD8**·LecB complex represents the first X-ray crystal structure of a second-generation peptide dendrimer. In contrast to our previous reported structure of a first-generation dendrimer **Het2G1**-Cys, also as LecB complex, in which the dendrimer formed a trimeric assembly mediated by an intermolecular β-sheet, **SBD8** only contains intramolecular H-bonds and forms an approximately globular structure assembled as a non-covalent homodimer through hydrophobic and proline-phenylalanine π-stacking interactions within the crystal. MD and CD studies suggest that the structure observed in the crystal represents a good approximation of the structure in solution, and probably resembles the structure of most of our peptide dendrimers which generally are monomeric in solution and do not aggregate. Remarkably, only one of the two fucosyl groups in **SBD8** was bound to a LecB binding site in the crystal structure. Nevertheless, two crystal structures of LecB complexes of disulfide bridged *C*-fucosylated peptide dimers showed that such double coordination is indeed possible in a LecB crystal.

Experimental Section

Experimental procedures for the synthesis and characterization of all peptides and peptide dendrimers, X-ray data collection tables, detailed procedures for CD spectroscopy, acid-base titration, molecular dynamics and microbiology are described in the *Supporting Information*.

Acknowledgements

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Author Contribution Statement

S. B. synthesized peptides, performed X-ray crystallography, MIC assays, MD, measured CD, interpreted data, and wrote the paper. S. J. designed and supervised MD studies, interpreted data and wrote the paper. A. S. supervised X-ray crystallography. T. D. and J.-L. R. designed and supervised the study, interpreted data, and wrote the paper.

References

[1] E. Buhleier, W. Wehner, F. Vögtle, '"Cascade"- and "Non-skid-Chain-like" Syntheses of Molecular Cavity Topologies', *Synthesis* **1978**, 155–158.
[2] D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith, 'A New Class of Polymers: Starburst–Dendritic Macromolecules', *Polym. J.* **1985**, *17*, 117–132.
[3] G. R. Newkome, Z. Yao, G. R. Baker, V. K. Gupta, 'Micelles. Part 1. Cascade molecules: a new approach to micelles. A [27]-arborol', *J. Org. Chem.* **1985**, *50*, 2003–2004.
[4] A. W. Bosman, H. M. Janssen, E. W. Meijer, 'About Dendrimers: Structure, Physical Properties, and Applications', *Chem. Rev.* **1999**, *99*, 1665–1688.
[5] J. M. J. Fréchet, 'Dendrimers and supramolecular chemistry', *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4782–4787.
[6] D. K. Smith, F. Diederich, 'Functional Dendrimers: Unique Biological Mimics', *Chem. Eur. J.* **1998**, *4*, 1353–1361.
[7] C. J. Hawker, J. M. J. Frechet, 'Preparation of polymers with controlled molecular architecture. A new convergent approach to dendritic macromolecules', *J. Am. Chem. Soc.* **1990**, *112*, 7638–7647.

[8] E. Burakowska, R. Haag, 'Dendritic Polyglycerol Core-Double-Shell Architectures: Synthesis and Transport Properties', *Macromolecules* **2009**, *42*, 5545–5550.
[9] J.-P. Majoral, A.-M. Caminade, V. Maraval, 'The specific contribution of phosphorus in dendrimer chemistry', *Chem. Commun.* **2002**, 2929–2942.
[10] V. R. Araújo, D. S. Santos, E. I. Ferreira, J. Giarolla, 'New Advances in General Biomedical Applications of PAMAM Dendrimers', *Molecules* **2018**, *23*, 2849.
[11] B. A. G. Hammer, K. Müllen, 'Expanding the limits of synthetic macromolecular chemistry through Polyphenylene Dendrimers', *J. Nanopart. Res.* **2018**, *20*, 262.
[12] L. Crespo, G. Sanclimens, M. Pons, E. Giralt, M. Royo, F. Albericio, 'Peptide and Amide Bond-Containing Dendrimers', *Chem. Rev.* **2005**, *105*, 1663–1682.
[13] J. Kofoed, J.-L. Reymond, 'Dendrimers as artificial enzymes', *Curr. Opin. Chem. Biol.* **2005**, *9*, 656–664.
[14] C. C. Lee, J. A. MacKay, J. M. J. Fréchet, F. C. Szoka, 'Designing dendrimers for biological applications', *Nat. Biotechnol.* **2005**, *23*, 1517–1526.
[15] D. Astruc, E. Boisselier, C. Ornelas, 'Dendrimers Designed for Functions: From Physical, Photophysical, and Supramolecular Properties to Applications in Sensing, Catalysis, Molecular Electronics, Photonics, and Nanomedicine', *Chem. Rev.* **2010**, *110*, 1857–1959.
[16] B. N. S. Thota, L. H. Urner, R. Haag, 'Supramolecular Architectures of Dendritic Amphiphiles in Water', *Chem. Rev.* **2016**, *116*, 2079–2102.
[17] R. E. Bauer, V. Enkelmann, U. M. Wiesler, A. J. Berresheim, K. Müllen, 'Single-Crystal Structures of Polyphenylene Dendrimers', *Chem. Eur. J.* **2002**, *8*, 3858–3864.
[18] E. Badetti, V. Lloveras, K. Wurst, R. M. Sebastián, A.-M. Caminade, J.-P. Majoral, J. Veciana, J. Vidal-Gancedo, 'Synthesis and Structural Characterization of a Dendrimer Model Compound Based on a Cyclotriphosphazene Core with TEMPO Radicals as Substituents', *Org. Lett.* **2013**, *15*, 3490–3493.
[19] T. Darbre, J.-L. Reymond, 'Peptide Dendrimers as Artificial Enzymes, Receptors, and Drug-Delivery Agents', *Acc. Chem. Res.* **2006**, *39*, 925–934.
[20] A. Kwok, G. A. Eggimann, J.-L. Reymond, T. Darbre, F. Hollfelder, 'Peptide Dendrimer/Lipid Hybrid Systems are Efficient DNA Transfection Reagents: Structure-Activity Relationships Highlight the Role of Charge Distribution Across Dendrimer Generations', *ACS Nano* **2013**, *7*, 4668–4682.
[21] G. A. Eggimann, E. Blattes, S. Buschor, R. Biswas, S. M. Kammer, T. Darbre, J.-L. Reymond, 'Designed cell penetrating peptide dendrimers efficiently internalize cargo into cells', *Chem. Commun.* **2014**, *50*, 7254–7257.
[22] M. Heitz, S. Javor, T. Darbre, J.-L. Reymond, 'Stereoselective pH Responsive Peptide Dendrimers for siRNA Transfection', *Bioconjugate Chem.* **2019**, DOI: 10.1021/acs.bioconjchem.9b00403.
[23] J.-L. Reymond, M. Bergmann, T. Darbre, 'Glycopeptide dendrimers as *Pseudomonas aeruginosa* biofilm inhibitors', *Chem. Soc. Rev.* **2013**, *42*, 4814–4822.
[24] J.-L. Reymond, T. Darbre, 'Peptide and glycopeptide dendrimer apple trees as enzyme models and for biomedical applications', *Org. Biomol. Chem.* **2012**, *10*, 1483–1492.
[25] S. Javor, J.-L. Reymond, 'Molecular Dynamics and Docking Studies of Single Site Esterase Peptide Dendrimers', *J. Org. Chem.* **2009**, *74*, 3665–3674.
[26] N. A. Uhlich, A. Natalello, R. U. Kadam, S. M. Doglia, J.-L. Reymond, T. Darbre, 'Structure and Binding of Peptide-Dendrimer Ligands to Vitamin B12', *ChemBioChem* **2010**, *11*, 358–365.
[27] M. Stach, T. N. Siriwardena, T. Köhler, C. van Delden, T. Darbre, J.-L. Reymond, 'Combining Topology and Sequence Design for the Discovery of Potent Antimicrobial Peptide Dendrimers against Multidrug-Resistant *Pseudomonas aeruginosa*', *Angew. Chem. Int. Ed.* **2014**, *53*, 12827–12831.
[28] T. N. Siriwardena, M. Stach, R. He, B.-H. Gan, S. Javor, M. Heitz, L. Ma, X. Cai, P. Chen, D. Wei, H. Li, J. Ma, T. Köhler, C. van Delden, T. Darbre, J.-L. Reymond, 'Lipidated Peptide Dendrimers Killing Multidrug-Resistant Bacteria', *J. Am. Chem. Soc.* **2018**, *140*, 423–432.
[29] T. N. Siriwardena, A. Capecchi, B.-H. Gan, X. Jin, R. He, D. Wei, L. Ma, T. Köhler, C. van Delden, S. Javor, J.-L. Reymond, 'Optimizing Antimicrobial Peptide Dendrimers in Chemical Space', *Angew. Chem. Int. Ed.* **2018**, *57*, 8483–8487.
[30] T. N. Siriwardena, A. Lüscher, T. Köhler, C. van Delden, S. Javor, J.-L. Reymond, 'Antimicrobial Peptide Dendrimer Chimera', *Helv. Chim. Acta* **2019**, *102*, e1900034.
[31] E. Mitchell, C. Houles, D. Sudakevitz, M. Wimmerova, C. Gautier, S. Pérez, A. M. Wu, N. Gilboa-Garber, A. Imberty, 'Structural basis for oligosaccharide-mediated adhesion of *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients', *Nat. Struct. Biol.* **2002**, *9*, 918–921.
[32] P. Roethlisberger, A. Istrate, M. J. Marcaida Lopez, R. Visini, A. Stocker, J.-L. Reymond, C. J. Leumann, 'X-ray structure of a lectin-bound DNA duplex containing an unnatural phenanthrenyl pair', *Chem. Commun.* **2016**, *52*, 4749–4752.
[33] R. He, I. Di Bonaventura, R. Visini, B.-H. Gan, Y. Fu, D. Probst, A. Lüscher, T. Köhler, C. van Delden, A. Stocker, W. Hong, T. Darbre, J.-L. Reymond, 'Design, crystal structure and atomic force microscopy study of thioether ligated D,L-cyclic antimicrobial peptides against multidrug resistant *Pseudomonas aeruginosa*', *Chem. Sci.* **2017**, *8*, 7464–7475.
[34] I. Di Bonaventura, X. Jin, R. Visini, D. Probst, S. Javor, B.-H. Gan, G. Michaud, A. Natalello, S. M. Doglia, T. Köhler, C. v. Delden, A. Stocker, T. Darbre, J.-L. Reymond, 'Chemical space guided discovery of antimicrobial bridged bicyclic peptides against *Pseudomonas aeruginosa* and its biofilms', *Chem. Sci.* **2017**, *8*, 6784–6798.
[35] I. Di Bonaventura, S. Baeriswyl, A. Capecchi, B.-H. Gan, X. Jin, T. N. Siriwardena, R. He, T. Köhler, A. Pompilio, G. Di Bonaventura, C. van Delden, S. Javor, J.-L. Reymond, 'An antimicrobial bicyclic peptide from chemical space against multidrug resistant Gram-negative bacteria', *Chem. Commun.* **2018**, *54*, 5130–5133.
[36] S. Baeriswyl, B.-H. Gan, T. N. Siriwardena, R. Visini, M. Robadey, S. Javor, A. Stocker, T. Darbre, J.-L. Reymond, 'X-ray Crystal Structures of Short Antimicrobial Peptides as *Pseudomonas aeruginosa* Lectin B Complexes', *ACS Chem. Biol.* **2019**, *14*, 758–766.
[37] M. Bergmann, G. Michaud, R. Visini, X. Jin, E. Gillon, A. Stocker, A. Imberty, T. Darbre, J.-L. Reymond, 'Multivalency effects on *Pseudomonas aeruginosa* biofilm inhibition and

dispersal by glycopeptide dendrimers targeting lectin LecA', *Org. Biomol. Chem.* **2016**, *14*, 138–148.

[38] G. Michaud, R. Visini, M. Bergmann, G. Salerno, R. Bosco, E. Gillon, B. Richichi, C. Nativi, A. Imberty, A. Stocker, T. Darbre, J.-L. Reymond, 'Overcoming antibiotic resistance in *Pseudomonas aeruginosa* biofilms using glycopeptide dendrimers', *Chem. Sci.* **2015**, *7*, 166–182.

[39] E. Kolomiets, E. M. Johansson, O. Renaudet, T. Darbre, J.-L. Reymond, 'Neoglycopeptide Dendrimer Libraries as a Source of Lectin Binding Ligands', *Org. Lett.* **2007**, *9*, 1465–1468.

[40] E. M. V. Johansson, S. A. Crusz, E. Kolomiets, L. Buts, R. U. Kadam, M. Cacciarini, K.-M. Bartels, S. P. Diggle, M. Cámara, P. Williams, R. Loris, C. Nativi, F. Rosenau, K.-E. Jaeger, T. Darbre, J.-L. Reymond, 'Inhibition and Dispersion of *Pseudomonas aeruginosa* Biofilms by Glycopeptide Dendrimers Targeting the Fucose-Specific Lectin LecB', *Chem. Biol.* **2008**, *15*, 1249–1257.

[41] R. U. Kadam, M. Bergmann, D. Garg, G. Gabrieli, A. Stocker, T. Darbre, J.-L. Reymond, 'Structure-Based Optimization of the Terminal Tripeptide in Glycopeptide Dendrimer Inhibitors of *Pseudomonas aeruginosa* Biofilms Targeting LecA', *Chem. Eur. J.* **2013**, *19*, 17054–17063.

[42] N. J. Zondlo, 'Aromatic-Proline Interactions: Electronically Tunable CH/π Interactions', *Acc. Chem. Res.* **2013**, *46*, 1039–1049.

[43] R. Bhattacharyya, P. Chakrabarti, 'Stereospecific Interactions of Proline Residues in Protein Structures and Complexes', *J. Mol. Biol.* **2003**, *331*, 925–940.

[44] L. Biedermannova, K. E. Riley, K. Berka, P. Hobza, J. Vondrasek, 'Another role of proline: stabilization interactions in proteins and protein complexes concerning proline and tryptophane', *Phys. Chem. Chem. Phys.* **2008**, *10*, 6350–6359.

[45] M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess, E. Lindahl, 'GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers', *SoftwareX* **2015**, *1–2*, 19–25.

[46] L. Whitmore, B. A. Wallace, 'DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data', *Nucleic Acids Res.* **2004**, *32*, W668–W673.

[47] A. I. Arunkumar, T. K. S. Kumar, C. Yu, 'Specificity of helix-induction by 2,2,2-trifluoroethanol in polypeptides', *Int. J. Biol. Macromol.* **1997**, *21*, 223–230.

[48] J. Pires, T. N. Siriwardena, M. Stach, R. Tinguely, S. Kasraian, F. Luzzaro, S. L. Leib, T. Darbre, J.-L. Reymond, A. Endimiani, '*In Vitro* Activity of the Novel Antimicrobial Peptide Dendrimer G3KL against Multidrug-Resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*', *Antimicrob. Agents Chemother.* **2015**, *59*, 7915–7918.

[49] A. Pompilio, C. Geminiani, P. Mantini, T. N. Siriwardena, I. Di Bonaventura, J.-L. Reymond, G. Di Bonaventura, 'Peptide dendrimers as "lead compounds" for the treatment of chronic lung infections by *Pseudomonas aeruginosa* in cystic fibrosis patients: in vitro and in vivo studies', *Infect. Drug Resist.* **2018**, *11*, 1767–1782.

[50] X. Han, Y. Liu, Y. Ma, M. Zhang, Z. He, T. N. Siriwardena, H. Xu, Y. Bai, X. Zhang, J.-L. Reymond, M. Qiao, 'Peptide dendrimers G3KL and TNS18 inhibit *Pseudomonas aeruginosa* biofilms', *Appl. Microbiol. Biotechnol.* **2019**, *103*, 5821–5830.

[51] R. Sommer, S. Wagner, K. Rox, A. Varrot, D. Hauck, E.-C. Wamhoff, J. Schreiber, T. Ryckmans, T. Brunner, C. Rademacher, R. W. Hartmann, M. Brönstrup, A. Imberty, A. Titz, 'Glycomimetic, Orally Bioavailable LecB Inhibitors Block Biofilm Formation of *Pseudomonas aeruginosa*', *J. Am. Chem. Soc.* **2018**, *140*, 2537–2545.

[52] F. Pertici, R. J. Pieters, 'Potent divalent inhibitors with rigid glucose click spacers for *Pseudomonas aeruginosa* lectin LecA', *Chem. Commun.* **2012**, *48*, 4008–4010.

[53] F. Pertici, N. J. de Mol, J. Kemmink, R. J. Pieters, 'Optimizing Divalent Inhibitors of *Pseudomonas aeruginosa* Lectin LecA by Using a Rigid Spacer', *Chem. Eur. J.* **2013**, *19*, 16923–16927.

[54] R. Visini, X. Jin, M. Bergmann, G. Michaud, F. Pertici, O. Fu, A. Pukin, T. R. Branson, D. M. E. Thies-Weesie, J. Kemmink, E. Gillon, A. Imberty, A. Stocker, T. Darbre, R. J. Pieters, J.-L. Reymond, 'Structural Insight into Multivalent Galactoside Binding to *Pseudomonas aeruginosa* Lectin LecA', *ACS Chem. Biol.* **2015**, *10*, 2455–2462.

[55] A. Bernardi, J. Jiménez-Barbero, A. Casnati, C. De Castro, T. Darbre, F. Fieschi, J. Finne, H. Funken, K.-E. Jaeger, M. Lahmann, T. K. Lindhorst, M. Marradi, P. Messner, A. Molinaro, P. V. Murphy, C. Nativi, S. Oscarson, S. Penadés, F. Peri, R. J. Pieters, O. Renaudet, J.-L. Reymond, B. Richichi, J. Rojo, F. Sansone, C. Schäffer, W. B. Turnbull, T. Velasco-Torrijos, S. Vidal, S. Vincent, T. Wennekes, H. Zuilhof, A. Imberty, 'Multivalent glycoconjugates as anti-pathogenic agents', *Chem. Soc. Rev.* **2013**, *42*, 4709–4727.

[56] C. N. Pace, H. Fu, K. L. Fryar, J. Landua, S. R. Trevino, D. Schell, R. L. Thurlkill, S. Imura, J. M. Scholtz, K. Gajiwala, J. Sevcik, L. Urbanikova, J. K. Myers, K. Takano, E. J. Hebert, B. A. Shirley, G. R. Grimsley, 'Contribution of hydrogen bonds to protein stability', *Prot. Sci.* **2014**, *23*, 652–661.

[57] D. F. Stickle, L. G. Presta, K. A. Dill, G. D. Rose, 'Hydrogen bonding in globular proteins', *J. Mol. Biol.* **1992**, *226*, 1143–1159.

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