Combinatorial variation of branching length and multivalency in a large (390 625 member) glycopeptide dendrimer library: ligands for fucose-specific lectins†‡

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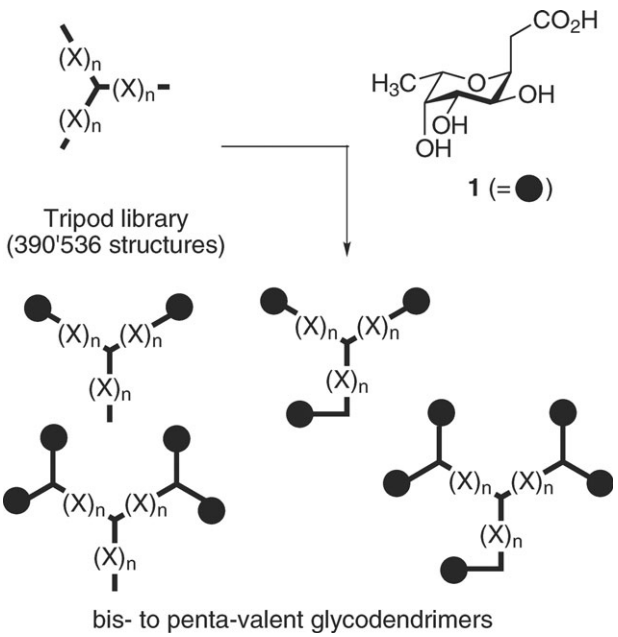
A new approach to dendrimer property tuning is demonstrated by combinatorial variation of dendrimer branch length and multivalency level, using a large (390 625-member) self-encoded glycopeptide dendrimer library. Ligands for the fucose lectins of *Ulex europaeus* were identified by screening a combinatorial library of dendrimers with variable arm length (0–4 amino acids) and multivalency (2–5 end groups). The ligands identified by this approach also bind to the fucose lectin LecB (PA-IIL) of *Pseudomonas aeruginosa*, and might provide a possible starting point to develop anti-infective agents against this antibiotic resistant pathogen which causes lethal respiratory tract infections in cystic fibrosis patients.

Introduction

The design of dendrimers with targeted physicochemical or biological properties has to date almost exclusively been based on variations of functional groups at the ends or the core of dendrimers with a preset branching structure.[1] Herein we demonstrate a new approach to dendrimer property tuning based on the combinatorial variation of dendrimer branch length and multivalency. Multivalency is known to enhance carbohydrate–protein interactions,[2] and has been studied with dendrimers,[3,4] however not in combinatorial manner. We report the identification of ligands for the fucose lectins of *Ulex europaeus*[5] by screening a combinatorial α-C-fucosyl-peptide dendrimer library with variable arm length and multivalency (Scheme 1). The ligands identified by this approach also bind to the fucose lectin LecB (PA-IIL) of *Pseudomonas aeruginosa*,[6] and might provide a possible starting point to develop anti-infective agents against this antibiotic resistant pathogen which causes lethal respiratory tract infections in cystic fibrosis patients.

We recently reported a new type of peptide dendrimer[7] whose branched structure consists of alternating proteinogenic α-amino acids with branching diamino acids.[8–10] The peptide dendrimer synthesis allowed the attachment of carbohydrate residues at the N-termini, and was used to deliver drugs selectively to cancer cells.[11] We also developed a combinatorial approach to these dendrimers in the context of enzyme models,[12,13] and used the method to screen a second-generation peptide dendrimer library of general structure (Fuc-α-CH_2CO-$X^8X^7X^6)_4(KX^5X^4X^3)_2KX^2X^1$ (X^{8-1} = variable amino acids, K = branching lysine), decorated with an α-C-fucosyl group at the four N-termini of the dendrimer.[14] Affinity-screening of this library against lectins provided a tetravalent dendritic ligand of structure (Fuc-α-CH_2CO-KPL)$_4$(KFKI)$_2$-KHI–NH_2, showing 115-fold stronger affinity to the lectins compared to fucose itself, corresponding to a 29-fold enhancement per fucose residue. The screening indicated a consensus



Scheme 1 Arm-length and valency variable peptide dendrimer combinatorial library for C-fucoside display; X = amino acids, n = 0–4. The central branching point is (*S*)-2,3-diaminopropanoic acid and the peripheral branching point is lysine.

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for a positively charged residue adjacent to the fucose, suggesting that binding was influenced by secondary interactions between the peptide portion of the dendrimers and the lectins. We were intrigued by the possibility that peptide dendrimers with arms of different lengths, or with a different level of multivalency, might also bind to the same lectins. In the present paper we address these two questions in a study demonstrating a new extension of our self-encoded combinatorial dendrimer library principle, which enlarges library sizes by one order of magnitude while increasing the structural diversity of the dendrimers.

Our approach to dendrimer libraries is based on split-and-mix synthesis. Split-and-mix synthesis allows the simultaneous synthesis of a large number of compounds in very few steps.[15] The synthesis is carried out on a solid support consisting of millions of microscopic beads, and results in so-called one-bead-one-compound (OBOC) libraries in which each of the beads carries only one of the possible compounds. OBOC libraries are screened using an assay for the desired property, usually binding of a colored product, and the beads carrying an active compound are identified visually and separated. The key technical problem consists in identifying a compound on any polymer bead. This problem has been solved by different methods, such as: (1) analysis of the compound by mass spectrometry,[16] or in the case of peptides, by Edman sequencing;[15b] (2) reading out a code attached to each bead during the synthesis and allowing reconstitution of the synthesis sequence, for example GC-tags,[17] radiofrequency tags,[18] or MS-tags;[19] (3) deconvolution through resynthesis and activity testing of partial libraries, such as in positional scanning libraries.[15c]

While the above methods require additional synthetic steps for encoding or specific instrumentation, our peptide dendrimers can be decoded directly by quantitative amino acid analysis, which is a standard service analysis offered by bioanalytical laboratories, without using any encoding operations. An unequivocal dendrimer sequence assignment from amino acid composition data is possible if each amino acid is used only twice in successive branches during the synthesis. In this manner each amino acid appears in relative amounts of zero (not present), one (present in lower branch position), two (present in higher branch position), or three (present in both positions). Our original realization of this encoding principle involved a peptide dendrimer combinatorial library with the general structure $(X^8X^7)_8(DapX^6X^5)_4(DapX^4X^3)_2DapX^2X^1$, where X^i denotes a proteinogenic amino acid and Dap = 2,3-(*S*)-diaminopropanoic acid, the branching unit.[12] Four different amino acids were used in each of eight variable amino acid positions, resulting in 4^8 = 65 536 different dendrimers and the same number of unique amino acid composition profiles.

X^2	X^1
A	A
T	T
-	-

library member	composition A	T
A_2A	3	0
A_2T	2	1
A_2-	2	0
T_2A	1	2
T_2T	0	3
T_2-	0	2
-.A	1	0
-.T	0	1
-.-	0	0

Fig. 1 Illustration of encoding principle with a nine-member arm-length variable library; X = A (alanine) or T (threonine). The branching point is a diamino acid. Each library member has a unique amino acid composition profile.

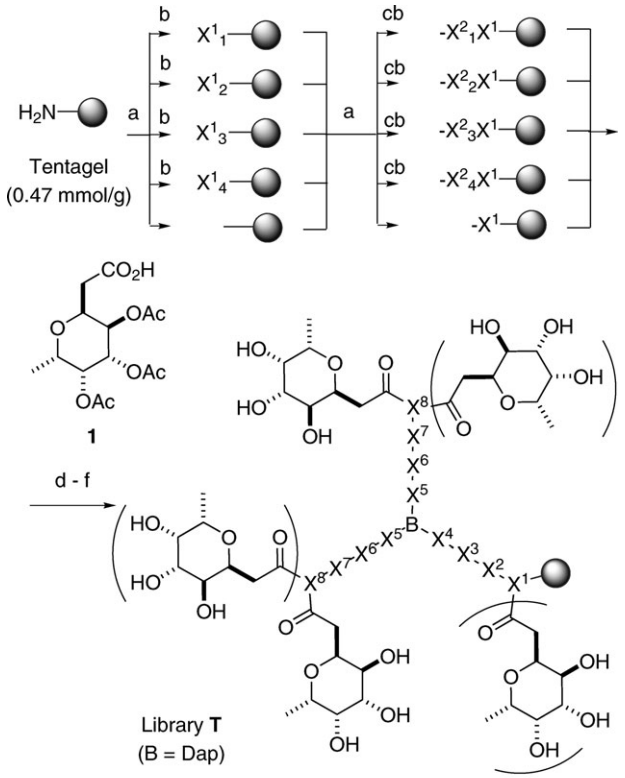
Results and discussion

Peptide dendrimer library design and synthesis

The information given by the amino acid analysis of library beads as discussed above is redundant. Indeed the HPLC-analysis indicates both the presence of one particular amino acid and the absence of the other ones at any variable position. We reasoned that this redundancy could be exploited to encode combinatorial libraries with length-variable arms by including amino acid deletions. This new, extended encoding principle for dendrimer libraries is illustrated for a first-generation dendrimer library with two variable positions and two amino acid building blocks, which results in 3^2 = 9 members including the arm-length variations (Fig. 1). Including such deletions in the split-and-mix synthesis of an eight-variable position dendrimer sequence with four variable amino acids per position provides a combinatorial library of 5^8 = 390 625 different dendrimers. Remarkably, this combinatorial synthesis with deletions thus delivers six times more sequences than without deletions (4^8 = 65 536) with exactly the same number of synthetic operations (4 × 8 coupling for variable positions + branching and capping).

This principle was implemented in a simple, first-generation dendrimer library containing a 4-amino acid stem followed by a single branching diamino acid and a pair of 4-amino acids branches (Scheme 2). If one ignores the orientation of the peptide chains, these first-generation dendrimers can be viewed topologically as tripods in which three peptide arms extend from the diamino acid branching point, which forms a trivalent node. This tripodal view would be fully realized if the three branches ends would be functionalizable simultaneously. This was realized by using a lysine with an allyloxycarbonyl (Aloc) side-chain protection as one of the variable amino acids at the first synthesis position X^1 corresponding to the tip of the first tripodal arm. The side-chain amino group of this lysine could be deprotected selectively at the end of the synthesis and made available for epitope attachment at the same time as the N-termini at position X^8. Furthermore, we used a bis-Fmoc protected lysine as variable amino acid at position X^8, which resulted in a doubling of N-termini in sequences bearing this amino acid.

The implementation of these two differently protected lysine building blocks at position X^1 and X^8 resulted in a library containing dendrimers with two (no lysine in X^1/X^8, 64% of the library), three (lysine in X^1 only, 16%), four (lysine in X^8 only, 16%), or five (lysine in X^1 and X^8, 4%) functionalization sites. 15 different amino acids were distributed into the



X^8	X^7	X^6	X^5	X^4	X^3	X^2	X^1
Lys*	Asp	Asn	Ser	Ser	Thr	Phe	Lys**
Phe	Tyr	His	Ala	Asn	Gly	Glu	Val
Gly	Glu	Ile	Leu	Arg	Tyr	His	Ala
Arg	Val	Thr	Pro	Pro	Ile	Leu	Asp
-	-	-	-	-	-	-	-

* BisFmoc protected Lysine,
** Aloc protected Lysine

Scheme 2 Design and synthesis of peptide dendrimer combinatorial library **T**. The solid support is tentagel and the branching unit B is L-2,3-diaminopropanoic acid (**Dap**). Using four Fmoc protected amino acids and 1 deletion at the variable position X^a (a = 1–8) gives 5^8 = 390 625 members. *Conditions*: (a) suspend the whole resin batch in DMF–CH_2Cl_2 (2 : 1, v/v), mix *via* nitrogen bubbling for 15 min, and split the batch in five equal portions 1–5 by volume; (b) in each portion X_b^a (b = 1–4): 3.0 eq. Fmoc-Xab-OH, 3.0 eq. PyBOP, 6.0 eq. DIEA, CH_2Cl_2–NMP (12 : 1, v/v), 1.5 h; (c) DMF–piperidine (42 : 1, v/v), 2 × 10 min. Steps (a)–(c) are repeated nine times, the fifth coupling being with only **Dap** and performed to the fifth portion as well. (d) Aloc/Fmoc removal: 25 eq. $PhSiH_3$, 0.2 eq. $Pd(PPh_3)_4$, CH_2Cl_2, 2 × 20 min followed by washing with 10 mL CH_2Cl_2, dioxane–H_2O (9 : 1), DMF and CH_2Cl_2, then DMF–piperidine (42 : 1, v/v), 2 × 10 min; (e) 5 eq. **1**, 5.5 eq. DIC, 5 eq. HOBt, CH_2Cl_2–NMP (12 : 1, v/v), 24 h; (f) TFA–TIS–H_2O (952 : 2.52 : 2.5) ,4 h, then MeOH–NH_3–H_2O (82 : 12 : 1, v/v), 24 h.

remaining variable positions X^1–X^8. The positioning was chosen such as to ensure a distribution of charged, aromatic, hydrophobic and small and polar amino acid throughout the sequence. The effect of including deletions in the library synthesis resulted in a lower mass distribution compared to the full length sequences due to the large number of shorter sequences included (Fig. 2).

The library was synthesized on non-cleavable tentagel beads with 0.47 mmol g^{-1} using Fmoc chemistry (Scheme 2). In each

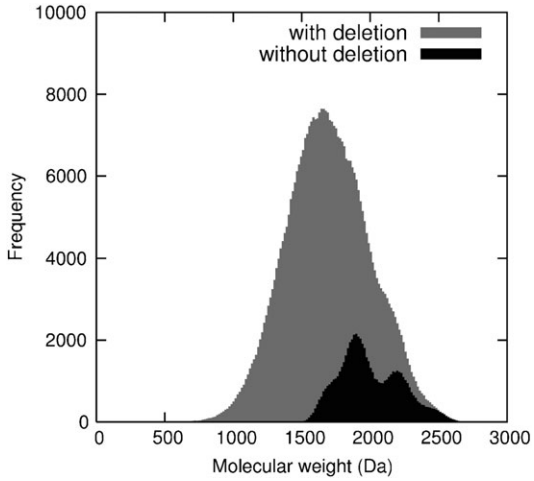


Fig. 2 Molecular weight distribution profile of the peptide dendrimer library **T** with or without amino acid deletions. The bin size for the frequency plot is 25 mass units. Note that library synthesis with or without deletions requires the same number of operations. The deletion library comprises six times more sequences (390 625) than the library without deletions (65 536 sequences).

step the resin was split into five equal portions. Four of these portions were deprotected with piperidine and coupled to the corresponding amino acids. The five portions were then recombined and split again for the next coupling. After coupling of the last amino acids at position X^8, all portions were combined and treated with $Pd(PPh_3)_4/PhSiH_3$ in dichloromethane to remove the Aloc protecting group at the lysine residue in position X^1. The N-terminal Fmoc groups were then removed by treatment with piperidine and the peracetylated derivative of the α-C-fucosyl carboxylic acid **1**,[20] was coupled. A fucose-free control library was also prepared by N-acetylation at the N-termini. Finally, the amino acid side-chain protecting groups were removed by acidic treatment, and the acetyl protecting groups of the C-fucosyl residue by ammonolysis.

On-bead lectin binding assay

The peptide dendrimer library was assayed for binding to biotinylated *Ulex europeaus* lectin (UEA-I), which is specific for α-L-fucose. Lectin binding was revealed with an alkaline phosphatase conjugated monoclonal anti-biotin antibody and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) as phosphatase substrate. This procedure was adapted from similar on-bead assays reported for screening oligosaccharide and glycopeptide libraries against various carbohydrate-binding proteins.[21] The non-fucosylated, N-acetylated control library gave less than 1% of stained beads, indicating that the assay was not subject to non-specific binding interactions. On the other hand, more than 90% of the beads in the fucosylated library had a strong dark purple coloration in this assay. Addition of L-fucose to the assay medium led to a gradual reduction in the number of stained beads, with only 2% dark purple beads in the presence of 3 M L-fucose. Furthermore, there was no bead coloration in the assay when the lectin was omitted, confirming that the assay

Table 1 Sequences of positive and negative hits from libraries **T**[a]

Hit	X^8	X^7	X^6	X^5	X^4	X^3	X^2	X^1
T1+	Arg	—	—	Leu	Arg	Ile	Phe	Val
T2+	Arg	—	—	Leu	Pro	—	Leu	Ala
T3+	Arg		—	Pro	Ser	Gly	Phe	Ala
T4+	Arg	—	—	Leu	Ser	Gly	Phe	Ala
T5+	Arg	—	—	—	Arg	Ile	Phe	Val
T6+	Arg	—	—	Ala	Ser	Ile	Phe	Ala
T7−	Lys	Glu	—	Ala	Pro	Thr	Phe	Lys
T8−	Phe	Glu	—	Pro	Pro	—	Phe	Val
T9−	Phe	—	—	Ala	Pro	Thr	—	Ala
T10−	Gly	Glu		Ala	Pro	—	Phe	Val
T11−	Arg	Glu	—	Ser	Ser	Thr	Phe	Val
T12−	Phe	Glu		Ser	—	Tyr	Phe	Val

[a] Structures in Scheme 2. Beads were soaked for 3 h at 25 °C with 5 μg mL^{-1} biotinylated UEA-I lectin in PBST buffer pH 7.2 with (positive hits, stained) or without (negative hits, unstained) 3 M L-fucose, and stained as described.

measured lectin-dendrimer interactions rather than binding of the anti-biotin antibody to the beads.

The beads that were darkly stained even with 3 M competing L-fucose were picked and sequenced as positive hits. Beads that remained uncolored in the absence of L-fucose were analyzed as negative hits (Table 1). The positive hits showed a strong consensus for Arg at X^8, while glutamate, aspartate and hydrophobic amino acids prevailed in negative hits, a similar consensus to that observed in a previous tetravalent peptide dendrimer library for fucose display.[14] Strikingly, there was no significant selection for high-valency dendrimers (lysine at position X^8/X^1), with most hits having only a divalent display of fucose. The absence of high-multivalency hits might be caused by a strong selection for a positive charge (Arg) at X^8, which excludes the higher multivalence with lysine at that position due to the library design.

The positive hits **T1+** **T2+** and **T3+** and the negative hit **T7−** were synthesized on cleavable Rink amide resin and purified by preparative HPLC to give pure glycodendrimers. We also prepared an analogue of **T1+** by adding an additional bis-Fmoc lysine residue at the N-terminus to form the tetravalent ligand **K-T1+** to investigate multivalency. Dendrimers **T1+** and **T7−** were also resynthesized on the original non-cleavable TG resin to confirm the on-bead lectin assay. Indeed, all resin beads carrying the positive hit sequence **T1+** were darkly stained upon affinity testing, while the resin carrying the negative hit sequence **T7−** remained colorless under the same assay conditions (Fig. 3). The structure of **K-T1+** and **T7−** are shown in Fig. 4.

Lectin–dendrimer interactions by ELLA

The binding properties of the dendrimers towards UEA-I were investigated using a microtiter-plate enzyme-linked lectin assay. Direct inhibition of lectin binding to fucose-coated surfaces was unsuccessful either due to poor binding (coating with a bovine serum albumin conjugate of the α-C-fucoside) or because competitive inhibition with L-fucose was not observable (coating with *n*-octyl α-L-fucoside as described by Wong *et al.*[22]). We therefore used the published procedure involving competitive binding between the ligand and a biotinylated polymeric fucose for binding to a lectin-coated surface.[23] This assay gave IC_{50} = 1.2 mM for free L-fucose. The dendrimers gave lower IC_{50}, with a specific affinity increase of up to 20-fold potency increase per fucose residue (Table 2, Fig. 5). The strongest binding was observed with the tetravalent dendrimer **K-T1+** which combined multivalency with the presence of a positive charge in proximity to the carbohydrate. The negative hit **T7−** also showed significant binding with the lectin in the ELLA test, in contrast to the reproducible negative result in the on-bead assay (Fig. 3). Large differences between on-beads screening results and assays in solution have been reported previously in solid-supported oligosaccharides,[21] and might be caused by the very high ligand concentration present at the

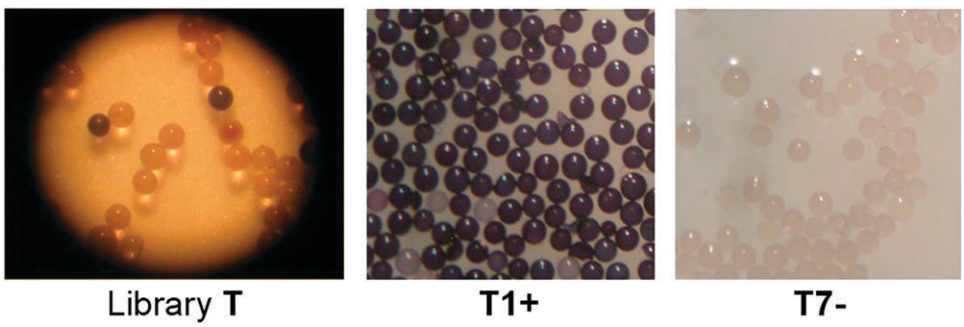


Fig. 3 On-bead lectin assay with UEA-I with tentagel beads from the library or with single sequences. See Experimental section for assay details.

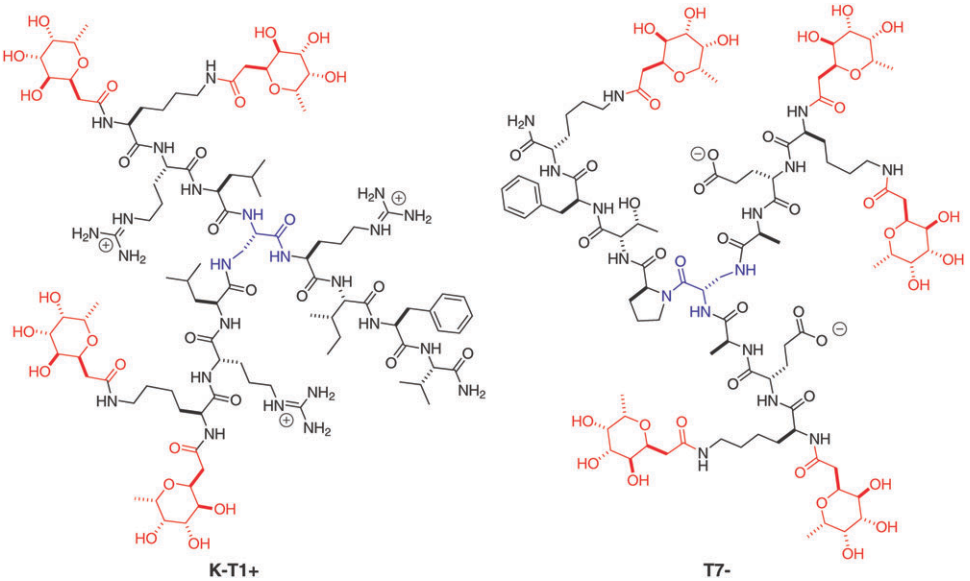


Fig. 4 Structures of dendrimers **K-T1+** and **T7−**.

bead surface, which might allow binding interactions not possible using dilute ligands in solutions.

The dendrimers were also tested for binding to LecB (PA-IIL). This fucose lectin mediates tissue attachment and biofilm formation by *Pseudomonas aeruginosa*.[6] L-Fucose is reported to bind PA-IIL with K_D = 0.34 μM by ITC, and gave an IC_{50} of 17 μM in the competition ELLA used here. In the same assay dendrimer **T1+** had an IC_{50} of 5 μM, significantly more potent than fucose, and dendrimer **K-T1+**, with an IC_{50} of 0.6 μM, was 30-fold stronger than fucose. These data showed that the effects obtained with UEA-I lectin are partly transferable to this other lectin, as expected from multivalency.

The dendrimers studied here provide up to 90-fold increase in potency relative to fucose in the case of **K-T1+** against UEA-I, an effect which is comparable to the tetravalent peptide dendrimer ligand (Fuc-α-CH_2CO-KPL)$_4$(*K*FKI)$_2$-*K*HI-NH_2 identified previously.[14] While these dendrimer carried multiple positive charges, peptide dendrimers with two positive charges or less (**T2+**, **T3+** and **T7−**) were less potent on a per fucose basis. The on-bead assay in both studies indicated a strong consensus for multiple positive charges in the dendrimers. These observations suggest that the presence of multiple positive charges is favorable for binding, probably by electrostatic interactions with the negatively charged residues that are found near the fucose binding site in both lectins.[5,6] Electrostatic effects might also explain the fact that **T7−** with two negative charges from glutamate residues is a relatively poor ligand on a per fucose basis despite of being pentavalent. Electrostatic effects mediated by charged residues might be strongly enhanced on the solid support, and could explain the very strong difference between **K-T1+** and **T7−** in the on-bead assay (Fig. 3).

The weaker binding of the pentavalent ligand **T7−** compared to the divalent **T1+** or its tetravalent analog **K-T1+** show that multivalency is not the governing factor for lectin binding in our peptide dendrimers. On the other hand, while in our previous study the monovalent ligand Fuc-α-CH_2CO-KPL-NH_2 corresponding to the outer branch of the active dendrimer bound the lectins with the same affinity as α-methyl-L-fucoside, engineering of the divalent **T1+** to its tetravalent analog **K-T1+** was effective in enhancing its affinity to the lectins and produced the best ligand in the series, supporting the concept of multivalency as a design feature. Although the affinity enhancement effects observed

Table 2 The IC_{50} values measured by ELLA test

Hits	Structure[a]	Valency	UEA-I IC_{50}/μM	R.p.[b]	R.p. per fucose	PA-IIL IC_{50}/μM	R.p.[b]	R.p. per fucose
	L-Fucose	1	1265 ± 5	1	1	17 ± 0.62	1	1
	Allyl-(α(L)-C-Fuc	1	261 ± 13	5	5			
	Me-α(L)-Fuc	1	269 ± 23	5	5			
T1+	(RL)$_2$*Dap*RIFV	2	84 ± 24	15	8	5 ± 0.45	3.5	1.7
T2+	(RL)$_2$*Dap*PLA	2	150 ± 15	8	4			
T3+	(RP)$_2$*Dap*SGFA	2	310 ± 32	4	2			
K-T1+	(KRL)$_2$*Dap*RIFV	4	14 ± 1.9	90	23	0.59 ± 0.059	29	7.2
T7−	(KEA)$_2$*Dap*PTFK	5	54 ± 6.6	23	5			

[a] *Dap* = Dap branching, UEA-I = *Ulex europeaus lectin* PA-IIL = LecB = *Pseudomonas aeruginosa lectin*. See Experimental section for assay details. [b] R.p. = Relative potency = IC_{50}(fucose)/IC_{50}(ligand).

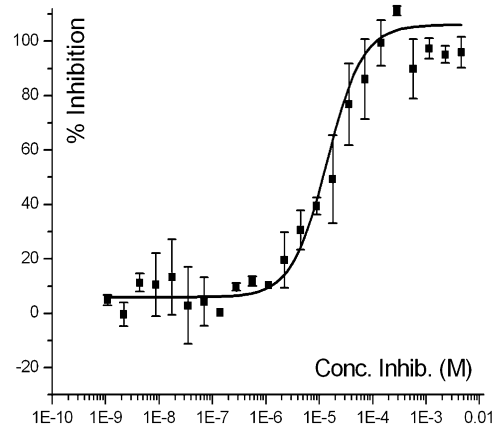


Fig. 5 Curve for the inhibition of binding of biotinylated polymeric fucose with **K-T1+** to UEA-I lectin, obtained from three independent ELLA tests. See experimental section for assay details.

are relatively modest, one must bear in mind that no other binding studies with multivalent ligands have been reported to date in the literature towards the fucose-specific lectins UEA-I and PA-IIL. One may therefore speculate that these lectins might not respond well to multivalency effects, perhaps due to the fact that they bind to their parent monovalent carbohydrate ligands with much higher affinities than previously studied lectins such as concanavalin A. Alternatively, the high degree of functionalization of our peptide dendrimers might favour secondary effects for protein–ligand interactions over multivalency effects, in contrast to more classical dendrimers with less functionalized arms.

While our methodology also addressed arm-length variations, there was no striking selection for very long or very short arms during screening of the library with the on-bead lectin binding assay, suggesting that this parameter is not critical in this assay within the series investigated (0–4 amino acids). The presence of at least one deletion in most sequences is probably a consequence of their frequent occurrence in the combinatorial library. An extended combinatorial experiment tailored to the active sequences reported here might be required to fully address the question of arm-length variations.

Conclusion

The experiment above demonstrates the first dendrimer combinatorial library with variable arm length and multivalency and its use to discover ligands for fucose-specific lectins UEA-I and PA-IIL by displaying an of α-L-C-fucosyl residue at the N-termini. The preparation of a variable arm-length combinatorial dendrimer library represents an important extension of the self-encoded dendrimer library principle, which allows us to prepare much larger combinatorial libraries using the exact same number of synthetic operations, and relying as before on the simple amino acid analysis for sequence determination. Such topologically variable libraries should be generally useful to clarify the role of multivalency and secondary interactions in dendritic ligands. In the present study, the combinatorial survey strengthens our previous finding that a cationic tetra-fucosylated peptide dendrimer ligand provides a favourable structure for lectin binding compared to lower valency ligands. Nevertheless, harnessing the full potential of these combinatorial experiments will require to overcome the difficulties encountered due to the differences between activities on-bead and in solution. Further investigations and optimization experiments are in progress along these lines, with the goal of improving the binding affinity towards PA-IIL in view of the therapeutic potential of these dendrimers as antibacterial agents.

Experimental

Materials and reagents

Peptide syntheses were performed manually in a glass reactor or plastic syringes (5 or 10 ml). All reagents were either purchased from Aldrich or Fluka Chemica (Switzerland) or synthesized following literature procedures. Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), Benzotriazol-1-yloxytri(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), hydroxybenzotriazole hydrate (HOBt), *N*,*N*′-Diisopropyl carbodiimide (DIC), amino acids and their derivatives were purchased from Senn Chemicals or Novabiochem (Switzerland). Amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-Asp(O*t*-Bu)-OH, Fmoc-Asn-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Boc)-OH, Fmoc-Leu-OH, Fmoc-Phe-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Thr(*t*-Bu)-OH, Fmoc-Ile-OH, Fmoc-Gly-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(*t*-Bu)-OH, Fmoc-Pro-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH. FmocDapFmoc-OH was purchased from BACHEM. NovaSyn® TGR (loading: 0.18–0.29 mmol g^{-1}), and Rink amide NovaGel (loading: 0.63 mmol g^{-1}), was purchased from Novabiochem (Switzerland). TentagGel HL NH_2 (loading: 0.47 mmol g^{-1}) was purchased from RAPP Polymere (Germany). All solvents used were analytical grade. Analytical RP-HPLC was performed in a Waters (996 Photo diode array detector) chromatography system using a chromolith performance RP-C18, 4.6 × 100 mm, flow rate 3 mL min^{-1} column. Compounds were detected by UV absorption at 214 nm. Preparative RP-HPLC was performed with HPLC-grade acetonitrile and MilliQ deionized water in a Waters Prepak cartridge 500 g (RP-C18 20 mm, 300 Å pore size) installed on a Waters Prep LC4000 system from Millipore (flow rate 100 mL min^{-1}, gradient 1 or 1.25% min^{-1} CH_3CN). MS spectra were provided by the Service of Mass Spectrometry of the Department of Chemistry and Biochemistry, University of Bern.

Synthesis of the split-and-mix combinatorial library T

The peptide-dendrimer library T was prepared from a 1 g resin batch of TentagGel HL NH_2 (loading: 0.47 mmol g^{-1}) divided equally in five reactors. In four reactors the resin was acylated with one of the four amino acids (3 eq.) in the presence of PyBOP (3 eq.) and *N*,*N*-diisopropylethylamine (DIEA) (5 eq.). Amino acids were acylated for 1.5 h, and Dap was coupled in all reactors for 2 h to all five parts. After each coupling, the five resin batches were mixed together and again split into five parts, equally introduced in the five reactors. These split-and-

mix steps were repeated after each amino acid coupling. After each coupling the resin was successively washed with *N*-methylpyrrolidone (NMP), MeOH, CH_2Cl_2 (3× with each solvent) and subsequently checked for free amino groups with the trinitrobenzene sulfonate (TNBS test). Proline coupling was checked with the chloranil test. If the test indicated the presence of free amino groups, the coupling was repeated. The Fmoc protecting groups were removed with a solution of 20% piperidine in DMF (2 × 10 min) and the solvent was removed by filtration. The final Fmoc protected resin was dried and stored at –4 °C. Immediately before screening, the Aloc protecting group was removed by treating the resin with $PhSiH_3$ (25 eq.) in 5 mL of anhydrous CH_2Cl_2 for 3 min under argon. After addition of $Pd(PPh_3)_4$ (0.2 eq.) the mixture was stirred for 20 min. The resin was washed with CH_2Cl_2 (10 mL), dioxane–H_2O (92 : 1, 10 mL), DMF (10 mL) and CH_2Cl_2 (2 × 10 mL). The Aloc deprotection procedure was repeated. The Fmoc protecting groups were removed and the library was capped with $\mathbf{1}^{20}$(5 eq.) in the presence of DIC (5 eq.) and HOBt (5 eq.) in CH_2Cl_2 /NMP (12 : 1, v/v) overnight. The carbohydrate was deprotected with a solution of MeOH–NH_3–H_2O (82 : 1 : 1, v/v) for 24 h. The side-chain protecting groups were removed with trifluoroacetic acid (TFA)–triisopropylsilane (TIS)–H_2O (95 : 2.5 : 2.5, v/v) for 4 h, resulting in a fucosylated-dendrimer library on beads.

On-bead immunosorbent lectin binding assay

About 50 mg of glyco-dendrimer on TG resin were 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). The mixture was shaken for 30 min and the beads were washed three times with 1 mL of PBST buffer containing 1% BSA and subsequently incubated for 3 h at room temperature with 2 mL of a solution of biotinylated lectin-UEA1 (5 μg mL^{-1} in PBST buffer). The beads were washed three times with 1 mL of TBST buffer (20 mM tri(hydroxymethyl)aminomethane hydrochloride (Tris · HCl), 500 mM NaCl, 0.05% Tween-20, pH 7.5), incubated for 1 h at 37 °C with 2 mL of an alkaline phosphatase-coupled monoclonal antibiotin antibody solution (1 : 10 000 in TBST buffer) and washed three times with 0.5 ml of TBST buffer with 0.5% BSA and three times with 1 mL alkaline phosphatase buffer (100 mM Tris · HCl), 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5). The beads were incubated for 10 min with 5 mL of a solution of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT). The staining was terminated by washing the beads twice with 1 mL of water and monitored under a stereo microscope. Darkly stained beads were manually selected and washed with 8 M urea containing 100 M acetic acid (2 × 10 mL) followed by water (10 × 1 mL), MeOH (10 × 1 mL), CH_2Cl_2 (10 × 1 mL), MeOH (10 × 1 mL), water (10 × 1 mL) in order to remove bound lectin as well as water-insoluble dye. The competition assay was carried out as above with 3 M free L(−)-fucose during the lectin binding step and 0.5 M free L(−)-fucose during the washing and incubation of the monoclonal anti-biotin antibody.

Sequence determination

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

Dendrimer synthesis

The resin NovaSyn® TGR (loading: 0.18–0.29 mmol g^{-1}) or Rink amide NovaGel (loading: 0.63 mmol g^{-1}) was acylated with each amino acid or diamino acid (3 eq.) in the presence of BOP or PyBOP (3 eq.) and DIEA (5 eq.) for 1.5 h for the zero generation and 3 h for 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. The potential remaining free amino groups were capped with acetic anhydride–CH_2Cl_2 for 10 min. The Fmoc and Aloc protecting groups were removed, the sequence was capped with **1** and the carbohydrate was deprotected as described above for library synthesis. Cleavage from the resin was carried out with TFA–TIS–H_2O (95 : 2.5 : 2.5, v/v) for 4 h. The peptide was precipitated with methyl *tert*-butyl ether then dissolved in water–acetonitrile. All dendrimers were purified by preparative HPLC.

T1+ (Fucose-Arg-Leu)$_2$*Dap*-Arg-Ile-Phe-ValNH$_2$. From 250 mg of NovaSyn® TGR resin (0.26 mmol g^{-1}) dendrimer **T1+** was obtained as a white foamy solid after preparative RP-HPLC purification (7.7 mg, 7.7%); analytical-HPLC: t_r = 5.9 min (λ = 214 nm, A : D = 70 : 30 to 50 : 50 in 8 min); MS (ES+): calc. for $C_{69}H_{120}N_{20}O_{19}$ $[M + H]^+$: 1533.91, found: 1533.80.

T2+ (Fucose-Arg-Leu)$_2$*Dap*-Pro-Leu-AlaNH$_2$. From 200 mg of NovaSyn® TGR resin (0.25 mmol g^{-1}) dendrimer **T2+** was obtained as a white foamy solid after preparative RP-HPLC purification (15.5 mg, 24.0%); analytical-HPLC: t_r = 5.5 min (λ = 214 nm, A : D = 80 : 20 to 40 : 60 in 12 min); MS (ES+): calc. for $C_{57}H_{102}N_{16}O_{18}$ $[M + H]^+$: 1299.76, found: 1299.80.

T3+ (Fucose-Arg-Pro)$_2$*Dap*-Ser-Gly-Phe-AlaNH$_2$. From 200 mg of Rink amide NovaGel (loading: 0.63 mmol g^{-1}) dendrimer **T3+** was obtained as a white foamy solid after preparative RP-HPLC purification (34 mg, 20%); analytical-HPLC: t_r = 5.7 min (λ = 214 nm, A : D = 90 : 10 to 60 : 40 in 12 min); MS (ES+): calc. for $C_{69}H_{120}N_{20}O_{19}$ $[M + H]^+$: 1347.68, found: 1348.1.

K-T1+ (Fucose)$_2$Lys-Arg-Leu)$_2$*Dap*-Arg-Ile-Phe-ValNH$_2$. From 200 mg of Rink amide NovaGel (loading: 0.63 mmol g^{-1}) dendrimer **K-T1+** was obtained as a white foamy solid after preparative RP-HPLC purification (20 mg, 7.3%); analytical-HPLC: t_r = 8.2 min (λ = 214 nm, A : D = 70 : 30 to 60 : 40 in 12 min); MS (ES+): calc. for $C_{97}H_{168}N_{24}O_{31}$ $[M + H]^+$: 2167.24, found: 2167.8.

T7− (Fucose)$_4$-Lys-Glu-Ala)$_2$*Dap*-Pro-Thr-Phe-(Fucose)-LysNH$_2$. From 300 mg of NovaSyn® TGR resin (0.25 mmol g^{-1}) dendrimer **T7−** was obtained as a white foamy solid after

preparative RP-HPLC purification (14.9 mg, 8.8%); analytical-HPLC: t_r = 7.9 min (λ = 214 nm, A : D = 90 : 10 to 65 : 35 in 12 min); MS (ES+): calc. for $C_{57}H_{102}N_{16}O_{18}$ $[M + H]^+$: 2174.31, found: 2174.1.

Expression and purification of LecB (PA-IIL) from *Pseudomonas aeruginosa*[6b]

A 345 bp DNA fragment containing the *lecB* gene and carrying a *Bam*HI or an *Nde*I site, respectively, was amplified by PCR using *P. aeruginosa* PAO1 DNA as the template. This fragment was digested with *Nde*I/*Bam*HI and cloned into pET22b resulting in plasmid pEC2 which was transformed into *E. coli* BL21(DE3). Expression cultures were grown at 30 °C in 1 liter of Luria–Bertani medium containing 0.4% (w/v) glucose in 5 liter Erlenmeyer flasks to an absorbance of 0.6 and then induced with 0.5 mM isopropyl-β-D-thiogalactoside. After 3 h of growth, the cells were harvested by centrifugation at 8000 g for 10 min and suspended in 20 ml of 100 mM Tris · HCl buffer, pH 8.0.

LecB was purified by affinity chromatography after lysis of the bacterial cells by three subsequent passages through a French press (SLM Aminco) at 20 000 psi and centrifugation at 10 000 g for 10 min. The following steps were carried out at 37 °C: the supernatant obtained after centrifugation was loaded onto a 5 ml mannose agarose column (Sigma-Aldrich) and the column washed with 100 ml of saline to remove unspecifically bound proteins. LecB was then eluted with 50 ml of 0.2 M mannose in 100 mM Tris · HCl, pH 8. The sample was concentrated by ultrafiltration using Vivaspin 6 devices (Virafines, cut-off 5 kDa) and washed with 100 mM Tris · HCl (pH 8) without mannose; purified LecB was stored at −20 °C.

ELLA (enzyme-linked lectin assay)

ELLAs were conducted using 96-well microtitre plates (Nunc Maxisorb) coated with *Ulex europaeus* UEA-I lectin (5 μg mL^{-1}) or *Pseudomonas aeruginosa* LecB (PA-IIL) lectin diluted in carbonate buffer, pH 9.6 (100 μL) for 1 h at 37 °C. After removal of lectin, the wells were blocked with 100 μL per well of 3% (w/v) BSA in PBS at 37 °C for 1 h. BSA solution was removed and each inhibitor was added in serial twofold dilutions (60 μL $well^{-1}$) in PBS to lectin-coated microplates and incubated at 37 °C for 1 h. Biotinylated polymeric fucose (Lectinity Holding, Inc.) (60 μL, 0.78 (UEA-I) or 5 (PA-IIL) μg mL^{-1}) was added to the solutions of inhibitors and the plates were incubated for another hour at 37 °C. The wells were washed three times with 150 μL $well^{-1}$ with T-PBS (PBS containing 0.05% Tween) and 100 μL of streptavidin peroxidase conjugate (dilution 1 : 5000 in PBS) was added and incubated for 1 h at 37 °C. The wells were washed three times with 150 μL $well^{-1}$ with T-PBS, once with DI water. A solution of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg per 4 mL) in citrate phosphate buffer (0.2 M, pH 4.0 with 0.015% (v/v) H_2O_2) 50 μL $well^{-1}$ was added. The reaction was stopped after 20 min by addition of 50 μL $well^{-1}$ 1 M H_2SO_4. Absorbance at 415 nm was recorded using a microtitre plate reader (spectra MAX 250). Every experiment was conducted in triplicate. The logarithm of the concentration of the dendrimer was plotted *vs.* the % of inhibition = $((A_{max} - A)/A_{max}) \times 100$. A_{max} corresponds to the mean value of the higher absorption limit reached in the dilution series. The sigmodial curve was fitted and the concentration at 50% of inhibition was determined as the IC_{50} value given in Table 2.

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