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Neoglycopeptide Dendrimer Libraries as a Source of Lectin Binding Ligands

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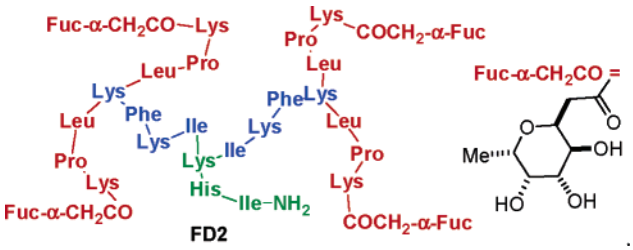
ABSTRACT



A 15 625-membered peptide dendrimer combinatorial library was acylated with an α-*C*-fucosyl residue at its four N-termini and screened for binding to fucose-specific lectins. Dendrimer FD2 (Fuc-α-CH_2CO-Lys-Pro-Leu)$_4$(Lys-Phe-Lys-Ile)$_2$Lys-His-Ile-NH_2 was identified as a potent ligand against *Ulex europaeus* lectin UEA-I (IC_{50} = 11 μM) and *Pseudomonas aeruginosa* lectin PA-IIL (IC_{50} = 0.14 μM).

One goal of organic chemistry is to create synthetic compounds useful for biomedical applications. While a very broad diversity of small molecules is available,[1] efforts toward synthetic macromolecules have mostly focused on resynthesis of naturally occurring linear or cyclic oligomers, in particular, peptides and analogues thereof.[2] More synthetic diversity is available from branched or dendritic macromolecules;[3] however, most of these macromolecules are polymeric,[4] and their synthesis has not been realized in a versatile combinatorial manner. We recently reported a general method for the synthesis and decoding of peptide dendrimer[5] combinatorial libraries[6] and demonstrated their use for the discovery of synthetic enzyme models and for drug delivery.[7] Herein, we report the synthesis of neoglycopeptide dendrimer combinatorial libraries and their use for the discovery of lectin ligands. The method is exemplified by the discovery of a potent ligand for the fucose-specific lectins UEA-I from the plant *Ulex europaeus*[8] and PA-IIL, a microbial lectin involved in biofilm formation in the opportunistic pathogen *Pseudomonas aeruginosa* responsible for lethal infections in cystic fibrosis patients.[9]

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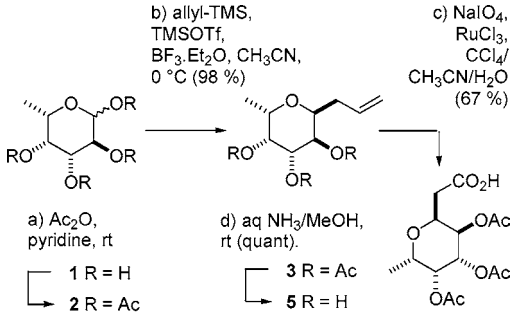
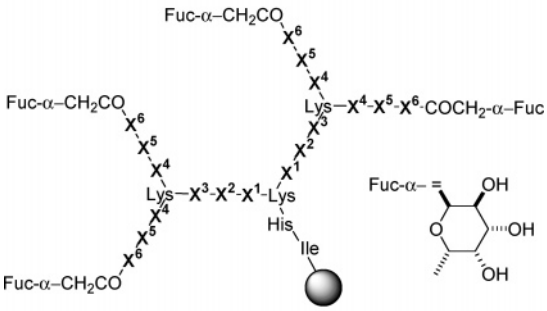
Our approach toward peptide dendrimers is based on solid-phase peptide synthesis alternating α-amino acids with branching diamino acids at every second, third, or fourth position to form dendrimers of up to 37 amino acids (ca. 4 kDa) in only 11 coupling steps, obtained in pure form by preparative HPLC purification.[7] Solid-supported combinatorial libraries of such peptide dendrimers can be prepared by split-and-mix synthesis.[10] To test the potential of our peptide dendrimer libraries to deliver protein ligands, we selected the fucose-specific lectins UEA-I from *Ulex europaeus*[8] and PA-IIL from *Pseudomonas aeruginosa*[9] as targets. These lectins bind to fucose with micromolar affinities, such that gaining 1 or 2 orders of magnitude in binding might provide nanomolar ligands. Lectin ligands are known on the basis of the multivalent display of glycosides (mostly mannose and galactose)[11] in dendrimers,[12] oligosaccharides,[13] and glycopeptides.[14] However, no synthetic multivalent ligands have been previously described for fucose-specific lectins.[15,16]

A 15 625-membered library of second-generation dendrimers was prepared on tentagel beads (0.5 g, 0.30 mmol/g, bead diameter of 90 μm) and functionalized at the N-terminus by the *C*-fucosyl-acetic acid building block **4**, obtained in three steps from L-fucose via the known *C*-allyl fucoside **3** (Scheme 1).[17] Amino acids were distributed in six variable positions to allow positive, negative, hydrophobic, small, and polar or aromatic residues throughout the sequence (Scheme 2). Each amino acid was used twice at two positions in successive branches to allow decoding by amino acid analysis as described previously.[6] After attachment of the *C*-fucoside **4**, side chain protecting groups were removed ($CF_3CO_2H/(i\text{Pr})_3SiH/H_2O$ 95:2.5:2.5), followed by

Scheme 1. *C*-Fucoside Synthesis



Scheme 2. Neoglycopeptide Dendrimer Combinatorial Library (5^6 = 15 625 Sequences)[a]



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

[a] The solid support is tentagel. In the N-acetylated control library, an acetyl group replaces the *C*-fucoside, but the lysine side chains are free.

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extensive deacetylation (MeOH/NH_4/H_2O 8:1:1, 25 °C, 24 h). The quality of the library was checked by amino acid analysis of randomly selected beads, providing the expected statistical distribution of amino acids at the variable positions.

The library was assayed for binding to biotinylated UEA-I lectin and stained with an alkaline-phosphatase-conjugated antibiotin antibody and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) as a chromogenic substrate as described for a related on-bead assay for wheat-germ hemaglutinin.[14c] While the N-acetylated control library gave less than 1% of stained beads in the assay, over 90% of the beads in the fucosylated library were darkly stained. Addition of 3 M fucose during the lectin binding step reduced staining to less than 2% of the beads. Darkly stained beads were manually picked, and their sequence was determined by amino acid analysis. A strong consensus for positively charged lysines at the terminal positions X^6 and X^2 was observed (Table 1). Four hits were resynthesized on Rink-amide resin and purified by preparative HPLC.

Table 1. Hit Sequences from the Dendrimer Combinatorial Library[a]

hit	X^6	X^5	X^4	X^3	X^2	X^1
FD1	Lys	His	Val	His	Gly	Ala
FD2	Lys	Pro	Leu	Phe	Lys	Ile
FD3	Lys	His	Leu	Glu	Lys	Ile
FD4	Lys	Arg	Asp	Ser	Arg	Ala
FD5	Lys	Thr	Ile	Ser	Arg	Leu
FD6	Lys	Thr	Leu	His	Lys	Ala
FD7	Lys	Ala	Ile	His	Lys	Thr
FD8	Lys	Thr	Val	Ser	Arg	Ile

[a] Beads were soaked for 3 h at 25 °C with 5 μg/mL of biotinylated UEA-I lectin in PBST buffer (pH 7.2) with 3 M L-fucose and stained.

Lectin binding was characterized using an enzyme-linked lectin assay (ELLA) as described.[18] This assay measures competition between the ligand and a biotinylated polymeric fucose reagent for binding to the lectin. The ELLA test identified hit **FD2** as the most potent ligand with an IC_{50} = 11 μM and a relative potency of 115 in reference to L-fucose (Table 2, Figure 1). This corresponds to a 6-fold affinity enhancement on a per fucose basis relative to α-*C*-allyl fucoside **5**. The other three dendrimers showed relative potencies in the range 20−40, which is similar on a per residue basis to the α-*C*-allyl fucoside **5**.

The structure−activity relationship in **FD2** was investigated by testing the terminal arm **2G0** and the outer branch **2G1** (Figure 1), as well as two higher valency analogues obtained by distributing the **FD2** sequence on a third-generation dendrimer backbone using either lysine (**2G3**) or (*S*)-2,3-diaminopropionic acid (**2G3′**) as the branching unit (Table 2). A gradual increase in relative potency to fucose was observed in the series **2G0** (r.p. = 4) → **2G1** (r.p. = 11) → **FD2** (r.p. = 29). While this increase might reflect multivalency, it must be weighed against the fact that the other tetravalent dendrimers **FD1**, **FD3**, and **FD4** show almost no affinity increase per fucose unit compared to monovalent α-fucosides. Similarly, the higher valency analogues **2G3** and **2G3′** were again less potent than **FD2** on a per fucose basis. The affinity of **FD2** toward UEA-I is thus clearly dependent on its particular structure and amino acid sequence. The **FD2** structure is probably particularly

Table 2. IC_{50} Values with UEA-I Lectin as Measured by ELLA

no.	IC_{50} (μM)	r.p.[a]	n[b]	r.p./n[c]
1	1265 ± 5	1	1	1
5	261 ± 13	5	1	5
FD1	67 ± 4	19	4	5
FD2	11 ± 0.35	115	4	29
FD3	72 ± 9	18	4	4
FD4	31.5 ± 9	40	4	10
2G0	290 ± 40	4	1	4
2G1	56 ± 6	23	2	11
2G3[d]	9 ± 0.14	141	8	18
2G3′[e]	30 ± 3	42	8	5

[a] r.p. = relative potency to fucose = IC_{50} (fucose)/IC_{50} (ligand). [b] n = number of fucosyl units per dendrimer. [c] Relative potency per fucose residue. [d] Structure of **2G3**: (Fuc-α-CH_2CO-Lys-Pro)$_8$(*Lys*-Leu-Phe)$_4$ (*Lys*-Lys-Ile)$_2$ *Lys*-His-Ile NH_2 (branching lysines in italics). [e] Structure of **2G3′**: (Fuc-α-CH_2CO-Lys-Pro)$_8$(*Dap*-Leu-Phe)$_4$ (*Dap*-Lys-Ile)$_2$ *Dap*-His-Ile NH_2 (Dap = branching (*S*)-2,3-diaminopropionic acid).

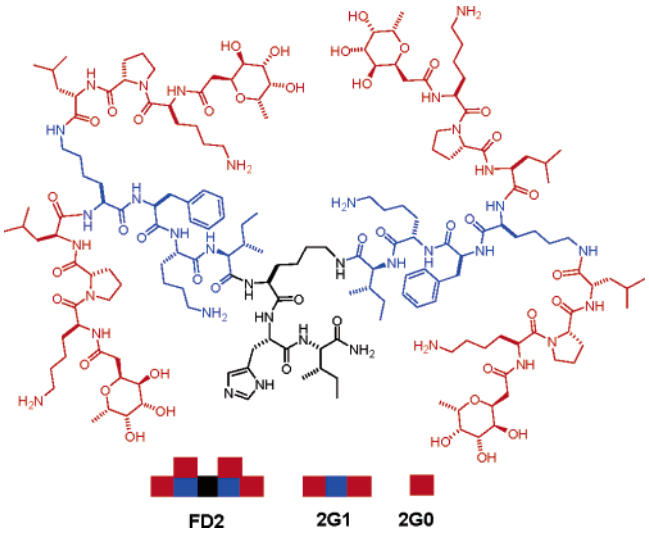

Figure 1. Structures of glycopeptide dendrimers **FD2** and lower-generation analogues **2G1** and **2G0** (both with $CONH_2$ at the C-terminus; see Supporting Information for details).

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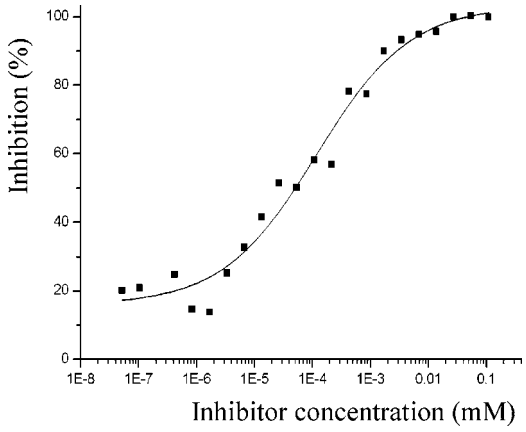

Figure 2. ELLA test with **FD2** and PA-IIL lectin for the inhibition of binding of biotinylated polymeric fucose. See Supporting Information for assay details. The assays were done in triplicate, providing an $IC_{50} = 0.14 \pm 0.035$ μM.

favorable for presenting fucosyl groups to the lectin and might also enable productive secondary interactions with the lectin. For example, electrostatic interactions between the 6-fold positively charged dendrimer and the negatively charged residues found near the fucose binding site in UEA-I[8] might enhance binding.

When tested against the fucose-specific *Pseudomonas aeruginosa* lectin PA-IIL, dendrimer **FD2** gave an inhibition concentration of $IC_{50} = 0.14 \pm 0.035$ μM, which corresponds to a relative potency of 80 relative to L-fucose, indicating that the effects observed with UEA-I lecting are partly transferable to this other lectin (Figure 2). L-Fucose, which gives an IC_{50} of 11 μM in the competition ELLA used here, binds to PA-IIL with $K_D = 2.9$ μM as reported for an isothermal calorimetry measurement.[19] As for UEA-I, negatively charged residues are found near the fucose binding site in PA-IIL.[9]

In summary, neoglycopeptide dendrimers represent a new class of synthetic macromolecules which can be readily prepared by standard peptide coupling chemistry. The experiments above show that ligands for fucose-binding lectins can be selected by affinity screening of combinatorial libraries of such dendritic ligands. A potent tetravalent *C*-fucosyl peptide dendrimer **FD2** was identified with 100-fold higher potency than fucose for binding to the lectins UEA-I and PA-IIL. This represents the first multivalent ligand to these lectins. Further optimization of **FD2** is in progress to increase binding affinity toward PA-IIL with regards to the possible therapeutic relevance of lectin ligands as inhibitors of biofilm formation in the *Pseudomonas aeruginosa* pathogen.[9c]

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Supporting Information Available: Experimental procedures for synthesis, details of inhibition tests, and characterization of dendrimers. This material is available free of charge via the Internet at http://pubs.acs.org.

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