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Synthesis and Characterization of Linker-Armed Fucose-Based Glycomimetics

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Glycomimetic molecules can be used to antagonize the action of carbohydrate-binding proteins (lectins) involved in biological processes of high relevance for human and plant disease. In this paper we describe the derivatization with appropriate linkers of a previously described glycomimetic containing an α-fucosyl amide anchor that is known to act as antagonist of the dendritic cell lectin DC-SIGN. Key steps of the functionalization were the stereoselective epoxidation of an intermediate β-amino-cyclohexene-carboxylic acid derivative, followed by regioselective opening with 2-chloroethanol. Introduction of the linker does not alter the DC-SIGN binding properties of the molecule, as shown by Surface Plasmon Resonance and NMR studies. Whereas the fucose-based anchor allows the mimic to interact efficiently with fucose-binding lectins, the linker could also be exploited for the synthesis of glycodendrimers, as well as for the study of ligand interactions with commercially available lectins by array technology. In particular, a tetravalent fucosylated dendrimer was obtained that displayed a low-micromolar range of activity against DC-SIGN. Additionally, screening of the ligand against lectins with common fucose specificity in an array format allowed the lectin from the bacterium *Ralstonia solanacearum* (RSL) to be identified as a potential target protein and suggested that even simple glycomimetic structures can attain a significant amount of selectivity among lectins with analogous specificity.

Introduction

As one of the major constituents of the biomass, carbohydrates display a broad range of functions in biological systems. Beside their most prominent role as a source and storage of energy, they are also involved in cellular communication, proliferation, adhesion, and apoptosis.[1] Moreover, numerous human and plant diseases are promoted by the interaction of carbohydrate-binding proteins (lectins) with carbohydrate structures expressed on cell surfaces.[2] Antagonism of lectin binding can lead to the control of many important biological processes. Such control can be achieved with small molecules mimicking the 3D structure of oligosaccharide epitopes.[3] In 2008 the Bernardi group introduced a mimic of the blood group antigen Lewis X [Fucα-1,4-(Galβ-1,3-)GlcNAc, Figure 1].[4] The fucose moiety of this branched trisaccharide binds to the Ca^{2+} present in the binding site of DC-SIGN,[5] a C-type lectin of dendritic cells that participates in infection processes of pathogens such as HIV or Ebola.[6] Blocking DC-SIGN affords a way of contrasting the initial phases of the infection and of preventing sexual transmission of AIDS.[7] With the aim of blocking that protein, compound **1** (Figure 1) was synthesized by employing fucose as an anchor and introducing 2-amino-cyclohexanecarboxylic acid as a central building block. The galactose fragment of Lewis X, on the other hand, was substituted by a mimic that helps the molecular shape to be consistent with the overall conformation of the trisaccharide. The IC_{50} value for mimic **1** was determined by means of surface plasmon resonance (SPR) and showed a slightly higher affinity for DC-SIGN when compared with Lewis X. Based on docking studies that suggested the interaction of the galactose mimic in **1** with lipophilic areas of the protein, a library of fucosyl amides with the general structure **2** was generated.[8] Apart from the exchange of the galactose mimic with predominantly aromatic systems, the central building block was also varied by exploiting all possible configurations of 2-amino-cyclohexanecarboxylic acid and by introducing the acyclic, commercially available β-alanine. A set of 40 compounds was pre-

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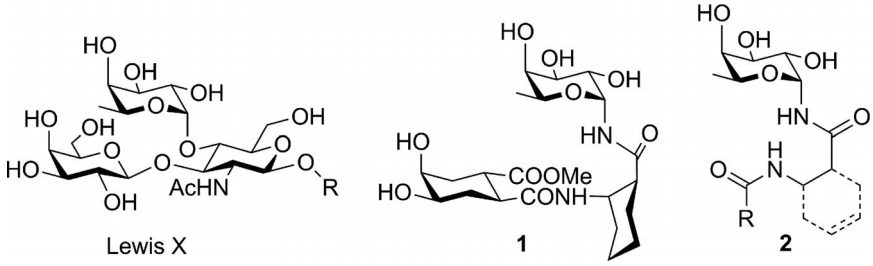


Figure 1. Structure of Lewis X, its mimic **1** and core **2** of a library of molecules.

pared and tested to establish their affinity for the target protein DC-SIGN, but no major improvement over Lewis X was achieved. Compound **3** (Figure 2), with an IC_{50} of 470 μM, was identified as the most potent inhibitor, and the synthetically more accessible compound **4** was found to be as active as Lewis X (IC_{50} 800 μM).

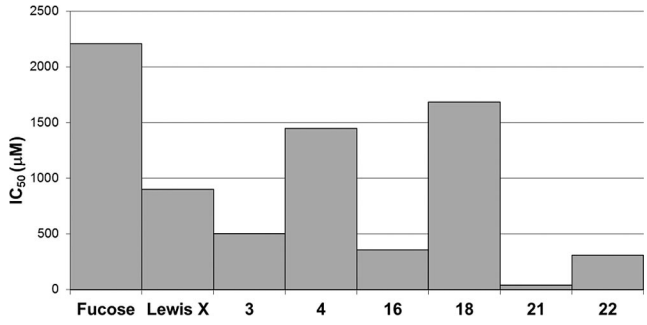
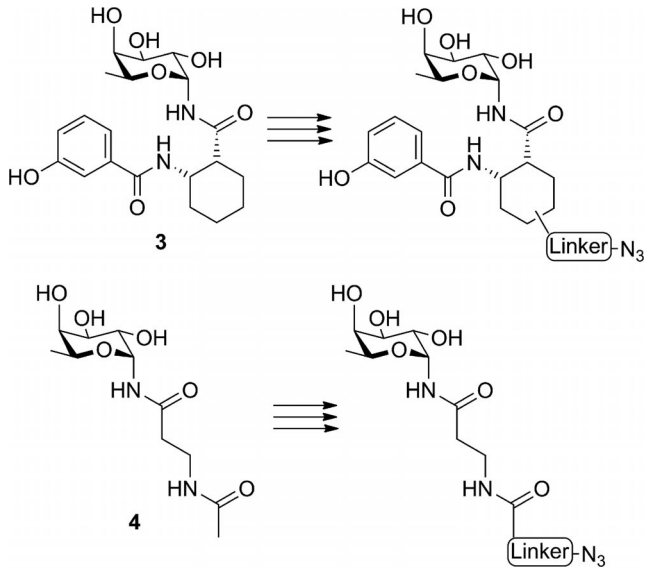


Figure 2. IC_{50} values (μM) obtained by SPR competition assay for fucose-based ligands inhibiting DC-SIGN binding to Man-BSA surface.

A general approach that can be used to compensate for weak protein-carbohydrate interaction is the synthesis of multivalent ligands that present several copies of the monomeric epitope.[9] Many of the available methods for a multivalent presentation require the presence of appropriate functional groups at the monomeric unit,[10] which allows the active ligand to be tethered to a polyvalent scaffold. Herein, we report on the functionalization of **3** and **4** to afford linker-armed molecules that could be used for the synthesis of glycodendrimers, as well as for the study of ligand interactions with commercially available lectins by glycan array technology (Scheme 1).

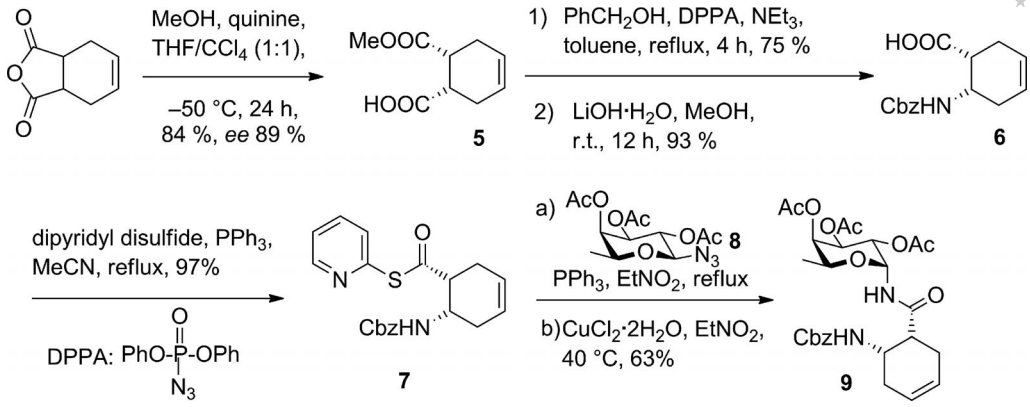


Scheme 1. Functionalization of monomeric ligands as a basis for multivalent presentation.

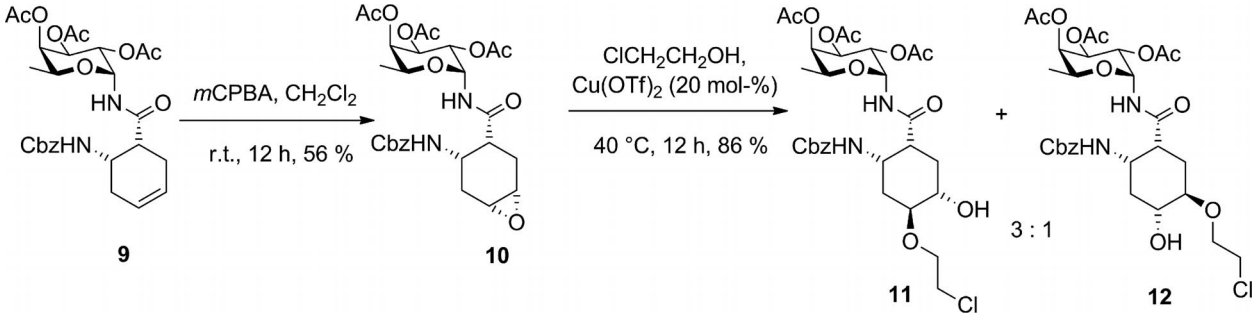
Results and Discussion

As previously described,[8] the central building block of ligand **3** originates from the desymmetrization of tetrahydrophthalic anhydride by quinine-promoted methanolysis using quinine as a chiral additive (Scheme 2). In the following step, carboxylic acid **5** was transformed into the *N*-Cbz-protected β-amino acid **6** by the Curtius rearrangement in the presence of benzyl alcohol, followed by hydrolysis of the methyl ester. Coupling of **6** to tri-*O*-acetyl-fucosyl azide **8** by using DeShong's[11] procedure led to the formation of α-fucosyl amide **9**.

The double bond of the cyclohexene scaffold was elaborated at this level because functionalization of synthetic precursors was detrimental to the DeShong reaction. As described by Reina et al. for a model system,[12] epoxidation of the double bond followed by nucleophilic addition of chloroethanol is a viable strategy for the introduction of a linker on β-amino-cyclohexene-carboxylic acid derivatives. For the formation of the epoxide, *m*-chloroperoxybenzoic acid (*m*CPBA) was used as the oxygen source. The reaction of **9** was stereoselective, due to the directing property of the carbamate group;[13] a single isomer was formed in moderate yield and its structure assigned as the *cis* isomer **10** (Scheme 3). In fact, the product configuration could not be confirmed on the basis of its spectroscopic data at this stage, but was determined after epoxide opening (see below). Nucleophilic opening of the epoxide was performed with 2-chloroethanol, using $Cu(OTf)_2$ as catalyst, and afforded a 3:1 mixture of regioisomers **11** and **12** (Scheme 3). The major compound was assigned as the product of C4 opening **11** based on the chemical shifts in the ^{13}C NMR spectrum. The C5-peak (δ = 67.4 ppm) appears more upfield than the C4-peak (δ = 78.8 ppm), which is typical of an alcohol in comparison with an ether. In the case of product **12**, which is derived from C5 opening, the opposite



Scheme 2. Synthesis of the central building block **6** and coupling to fucosyl azide **8** to afford **9**.

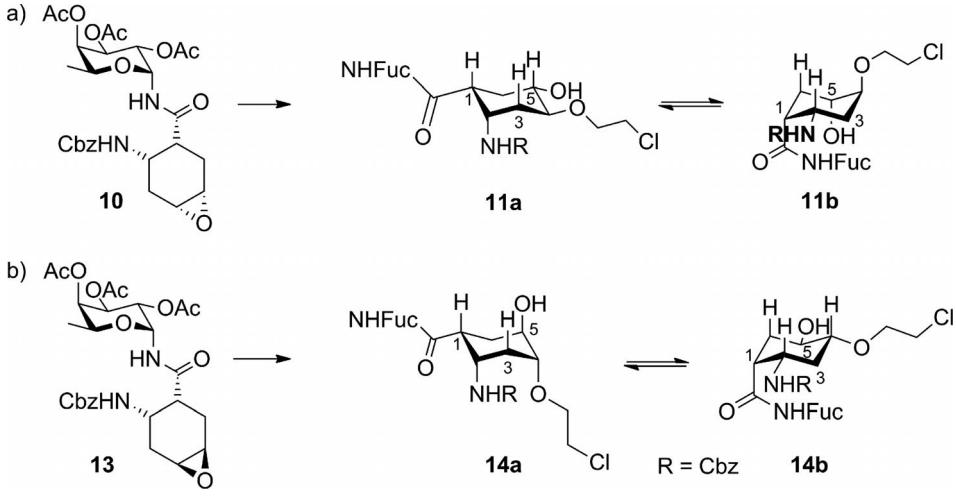


Scheme 3. Introduction of a linker by epoxidation of **9** and nucleophilic addition to **10**.

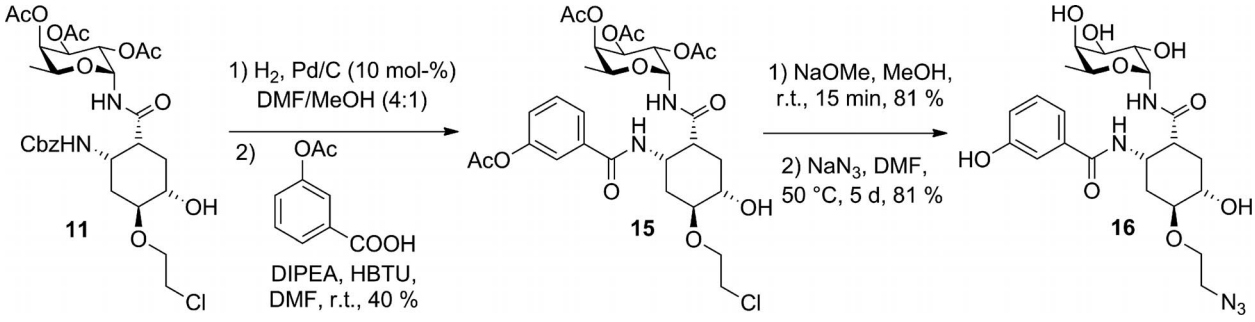
pattern could be observed in the ^{13}C NMR spectrum (δ_{C4} = 69.2 ppm and δ_{C5} = 78.0 ppm).

After chromatographic separation, the stereochemistry of the epoxidation step was confirmed by NMR analysis of **11** (Scheme 4). The NOESY spectrum of **11** ($CDCl_3$, 400 MHz) showed two cross-peaks for proton H5 of the cyclohexane ring with protons H1 and H3. These must arise from conformer **11a**, for which the three protons are in a 1,3-diaxial relationship and would not have been observed if the starting material had been the *trans* epoxide **13**, as illustrated in Scheme 4b. Analysis of the minor epoxide opening product **12** was also consistent with C5 opening of the *cis* epoxide **10** (see the Supporting Information Figures SI-1 and SI-2).

The CbzN-protecting group of the major product **11** was then removed (H_2/Pd, Scheme 5) and the resulting amine was coupled with 3-acetoxybenzoic acid by using *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as the coupling agent to afford **15**, which was fully deprotected with Zemplen's procedure. In the final



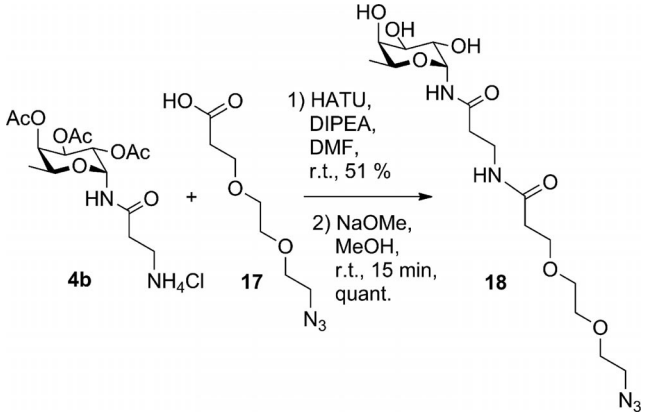
Scheme 4. Possible conformations of the cyclohexane ring after nucleophilic C4-opening of (a) *cis* epoxide **10** and (b) the hypothetical *trans* epoxide **13**.



Scheme 5. Coupling of 3-acetoxybenzoic acid, deprotection, and substitution of the chloride by an azide affords functionalized ligand **16**.

step of the synthesis, the chloride at the linker was replaced by an azide to afford **16** as a functionalized version of **3**.

For comparison (see below), an azido-functionalized version of **4** was prepared by reaction of **4b** with the PEG-like linker **17** (see the Supporting Information), followed by deprotection to afford **18** (Scheme 6).



Scheme 6. Functionalisation of **4b** to afford **18**.

Binding of **16** to DC-SIGN was tested by employing an SPR competition assay, according to a previously described protocol.[8] The assay allows the relative affinity of ligands to be compared on the basis of their ability to inhibit DC-SIGN binding to mannosylated bovine serum albumin (Man-BSA) immobilized on a carboxymethyl dextran-functionalized gold SPR sensor chip (CM4). Inhibition studies were performed by using the extracellular domain (ECD) of DC-SIGN (20 μM) injected either alone or in the presence of increasing concentrations of the ligands. The 50% inhibition concentration (IC_{50}) of **16** was determined and compared to that of unfunctionalized ligand **3**, the β-alanyl fucosyl azide **4**, its linker-armed variant **18**, Lewis X, and Fucose (Figure 2; sensorgrams and inhibition curves are collected in the Supporting Information, Figure SI–3).

The results show that functionalization of the scaffold does not reduce the activity of **3** (Figure 2). Both ligands **3** and **16** were found to be more active than Lewis X and **4** and very similar to one another. Functionalization of **4** to yield **18** also did not modify its activity significantly.

These results appear to imply that functionalization of the cyclohexane ring does not perturb the conformation of the scaffold. Indeed, coupling constant analysis of **16** in D_2O solution showed that, as in compound **3**,[8] the carboxyl group of the β-amino amide is equatorial on the cyclohexane ring, whereas the amino functionality is axial (Figure 3, a). In the ^1H NMR spectrum, H1 appears as a dt with $^3J_{1,6ax}$ = 12.4 Hz and $^3J_{1,6eq}$ = 5.4 Hz. The lack of cross peaks in the NOESY spectrum between the protons of fucose and the aromatic ring, along with a conformational analysis by means of OPLSA force field, suggested that the structure of **16** can be described by the extended conformation shown in Figure 3b.

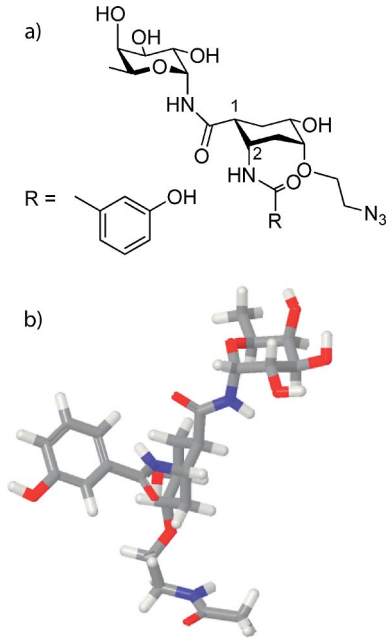


Figure 3. Preferred conformation of **16** in water solution (a) as determined by NMR coupling constant analysis, and (b) calculated by conformational analysis (OPLSA force field).

TR-NOESY spectra in the presence of DC-SIGN extracellular domain indicate that the conformation found in the free state is also the conformation that interacts with the protein. To obtain the ligand binding epitope, we performed STD NMR experiments at different saturation times by using the initial slope approach (STD_0) (Figure 4).[14] As expected, the fucose ring was the main ligand recognition element, coordinating the calcium atom as found for other

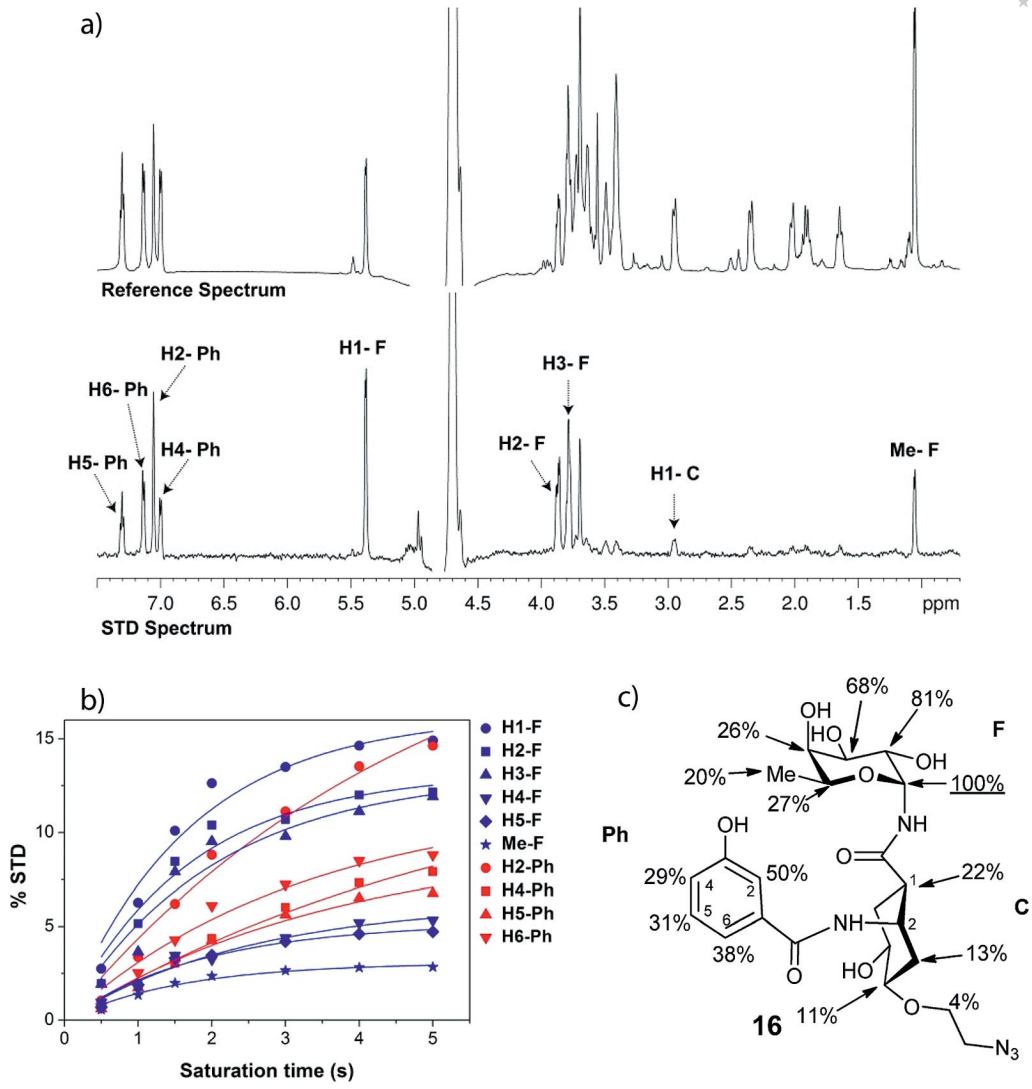


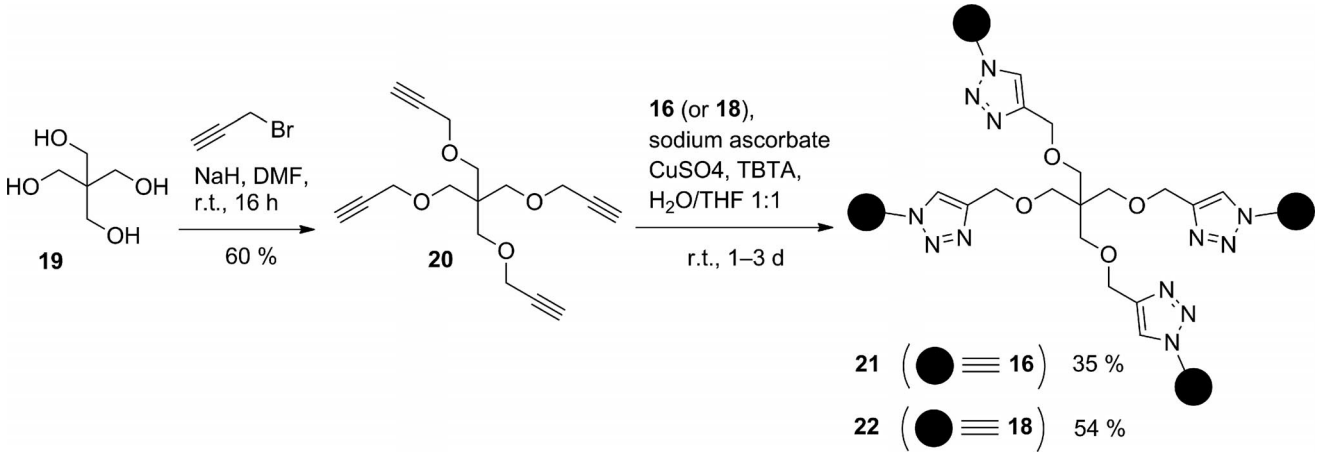
Figure 4. (a) Reference ^{1}H NMR spectrum (above) and STD spectrum (below) of **16** (1.6 mM) in the presence of DC-SIGN ECD (19 μM). Some key proton signals are labeled. (b) STD growth curves of the same sample (STD vs. the saturation time). The symbols represent experimental data, whereas lines show the mathematical fit, in blue for protons of fucose and in red for the aromatic ring. (c) Relative STD values (ligand epitope mapping) for **16** (same sample). The ratio of the intensities I_{STD}/I_0 was normalized by using the most intense STD value of the anomeric proton H1 of fucose residue (100%) as a reference.

fucose-based ligands of DC-SIGN.[4,8,15] Compared with previously examined ligands that contain the same elements as **3** but use a stereoisomeric β-amino acid scaffold,[15] the intensities of the STD signals for the aromatic ring of **16** are higher, which implies a closer proximity of this fragment to the protein. On the other hand, the cyclohexane scaffold displayed reduced contacts (Figure 4).

The presence of a functional linker in **16** and **18** allows polyvalent presentations of the ligands to be generated. We focused our attention on the tetravalent dendrimer **20** (Scheme 7), which is based on tetraerythritol **19**.[16] Compounds **16** and **18** were connected through 1,3-cycloaddition to **20** by using the Sharpless protocol.[17,18] Due to a challenging chromatographic purification, the tetravalent dendrimers **21** and **22** were obtained in low to moderate yields.

The relative affinity of **21** and **22** for DC-SIGN was examined in the SPR competition assay (Figure 2). The tetravalent presentation of **18**, compound **22**, resulted in an improved activity by a factor of five over the monomeric structure (β = 1.3). For the tetravalent presentation of **16**, compound **21**, an improvement of one order of magnitude was achieved (β = 2). Most likely, the amplification observed occur through statistical rebinding[19] because, with a distance of 35 to 38 Å in the DC-SIGN tetramer, the dendrimer is too small to bridge two binding sites.[20] Compound **21** was the most effective DC-SIGN blocker in the tested series, with an IC_{50} value of 39 μM.

Functionalization of the monovalent ligands could also be exploited for solid-phase support in an array format. Glycan arrays have been extensively used as a tool for rapid analysis of the interaction of different carbohydrates with a



Scheme 7. Synthesis of dendrimers **21** and **22** obtained through 1,3-cycloaddition.

wide variety of biological targets, including lectins.[21] This approach can be used to begin examining the question of selectivity for glycomimetic lectin ligands, which has very rarely been addressed.[8,22] Thus, in a preliminary experiment, the interactions of **16** and **18** with several commercially available fucose-binding lectins were tested by means of glass-supported arrays. The functionalized Lewis X derivative **23** and the mimics **24** and **25**, which were obtained after reduction of azides **16** and **18** by H_2/Lindlar-Pd (Figure 5), were immobilized on *N*-hydroxysuccinimide (NHS) activated glass slides. Additionally, fucosyl amide **26**, equipped with just a short β-alanine linker, was conjugated to the surface, together with the negative controls mannose and glucose. All compounds were spotted on the slide by using a printing robot. The interaction of the glycomimetics with FITC-labeled fucose-binding lectins was tested at different protein concentrations [Figure 6a (0.001 mg/mL of protein) and Figure 6b (0.01 mg/mL of protein)]. After incubation with plant lectin from *Ulex europaeus*[23] as well as bacterial lectins LecB (PA-IIL),[24] RSL,[25] and BC2LC-Nt,[26] intense binding-signals for the interaction of **24** and **25** with RSL were detected at the lowest tested protein concentration of 0.001 mg/mL (Figure 6, a). Isothermal calorimetry and SPR experiments have shown that RSL binds to α-Me-fucoside with a dissociation constant (K_d) of 0.7 μM and to Lewis X with an affinity 15 times lower.[25] Our array data suggest that *N*-fucosyl amides also bind strongly to RSL. The observation is consistent with the X-ray structure of the RSL/*O*-Me-fucoside complex, which shows that the anomeric oxygen of the sugar is in a relatively open area and is not involved in interactions with the protein.[25] Thus, O/NH replacement should not be detrimental for the formation of the complex. In our experiment, the response of both **24** and **25** was already maximal at 0.001 mg/mL concentration of RSL (Figure 6, a) and no increase in fluorescence was observed upon increasing the protein concentration by one order of magnitude (0.01 mg/mL, Figure 6, b), likely because saturation of the binding sites was already reached. Compound **26** was slightly less active, which suggests that the length of the linker may influence the accessibility of the surface-bound epitope to the lectin. Lewis X was identified as the weakest binder of RSL among those tested.

The array data also show that amides **24**–**26** bind to LecB (Figure 6), although a strong response was detected only at high protein concentration (0.01 mg/mL). Lewis X

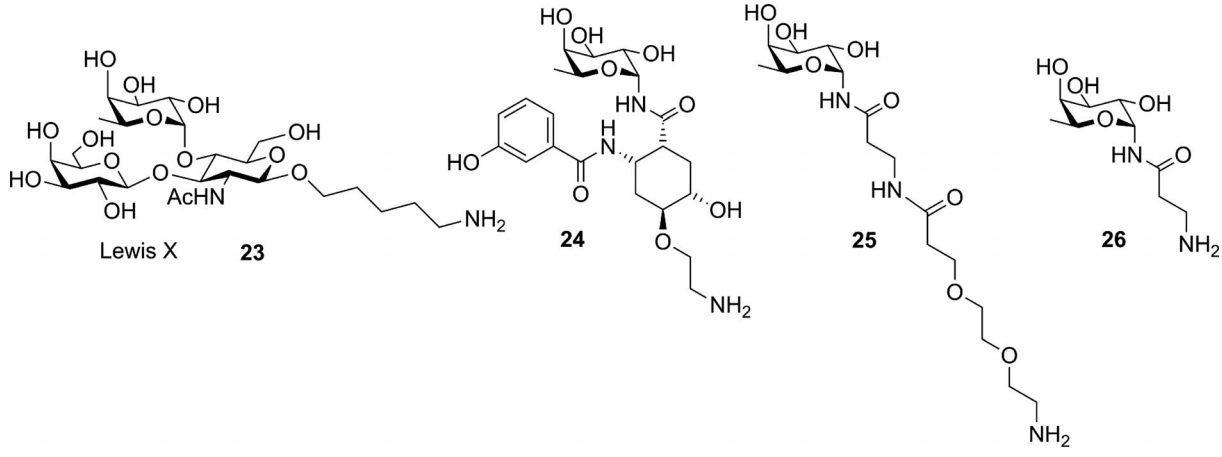


Figure 5. Compounds tested on the array.

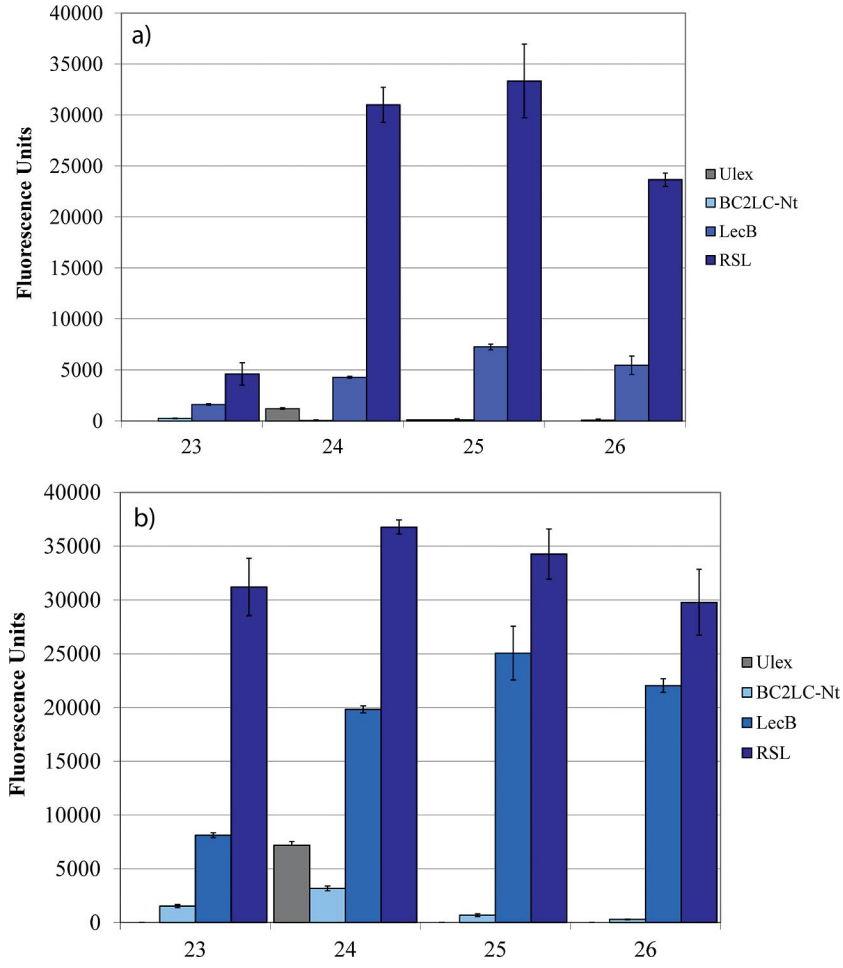


Figure 6. Array interaction studies. Fluorescence intensities for ligand-lectin interactions at (a) 0.001 mg/mL and (b) 0.01 mg/mL protein concentration. Each ligand was printed as a solution at the same concentration (0.5 M) in three replicates. The black bars represent the standard deviations obtained from the average of three values. Selectivity RSL >> LecB >> BC2LC-Nt > Ulex.

was also the weakest binder. This is consistent with previous ITC experiments that established the affinity of α-Me-fucoside for Lec-B to be about twofold higher than that of **26** and other α-fucosylamides.[27] On the other hand, Lewis X was reported to bind to Lec-B one order of magnitude less effectively than fucose itself.[28] Analysis of the Lec-B fucose binding site shows that the NH group at the anomeric position of α-fucosylamides perturbs a crystallographically observed water molecule, which is highly conserved in all structures of Lec-B complexes and participates in a H-bonding network that involves the protein and the ligands. Thus, compared with simple *O*-fucosides, *N*-fucosylamides are weaker ligands of Lec-B.

Ulex europaeus I agglutinin is a lectin that is specific for the H blood group antigen (αFuc1–2Galβ1–4GlcNAc), which is recognized mainly through its α-L-fucose residue.[23] BC2L-C has a strict specificity for fucosylated oligosaccharides but it was noted that short fragments such as fucose itself and disaccharides (αFuc1–2Gal, αFuc1–3GlcNAc, αFuc1–4GlcNAc) as well as the Lewis X trisaccharide are not efficient ligands.[26a] The tested products showed no affinity for *Ulex europaeus* or BCIILC-Nt. At 0.01 mg/mL protein concentration only weak binding of **24** to *Ulex* was registered.

Conclusions

In this work we have described the functionalization of previously reported functional Lewis X mimics, which are synthetically more accessible and biologically more effective than the natural ligand of DC-SIGN. Functionalization of these monovalent ligands allowed polyvalent constructs to be generated, which increased the statistical binding to DC-SIGN and therefore resulted in higher activity. The tetravalent construct **21** allowed a low-micromolar range of activity to be reached, which makes this approach useful for use in cellular tests.

Addition of a functionalized tether to the ligands could also be exploited to support the molecules on solid phase in an array format. By using these arrays as analytical tools for screening the mimics with commercially available lectins with common fucose specificity, we have identified the lectin from the bacterium *Ralstonia solanacearum* (RSL) as

a potential target protein of α-fucosylamide ligands. This bacterium is responsible for lethal wilt of more than 200 plants and thereby causes substantial economic damage to farmers in tropical regions. LecB (PA-IIL) is highly homologous to RSL and has similar affinity for fucose.[29] Thus, this experiment suggests that even simple glycomimetic structures can attain a significant amount of selectivity among lectins with analogous specificity. Work is in progress in our group to exploit array analysis of a larger group of glycomimetic structures towards the identification of lectin-selective glycomimetic entities.

Experimental Section

Glycan Arrays

General Considerations: FITC-labeled LecB, RSL, and BCIILC-Nt were purchased from Elicityl, OligoTech, France. FITC-labeled *Ulex europaeus* was purchased from Sigma–Aldrich, Germany. DC-SIGN ECD protein (residues 66–404) was overexpressed and purified as described previously.[20]

Surface Plasmon Resonance: SPR experiments were performed with a Biacore 3000 at 25 °C using a CM4 chip. During functionalization steps, HBS-P running buffer was used at 5 μL/min flow rate. Flow cells (Fc) 1 and 2 were activated with a 0.2 M EDC/0.05 M NHS mixture (50 μL); after this step Fc2 was functionalized with mannosylated bovine serum albumin (Man α1–3[Manα1–6]Man BSA (Man-BSA), Dextra Laboratories) by injecting 60 μg/mL of protein prepared in 10 mM sodium acetate pH 4, and finally the remaining activated groups of both flow cells were blocked with 1 M ethanolamine (30 μL). After blocking, both flow cells were treated with 10 mM HCl (10 μL) to remove unspecific bound protein and 50 mM EDTA (20 μL) to expose the surface to the regeneration protocol. After these steps, 1600 RU of Man-BSA was immobilized on Fc2.

For inhibition studies, DC-SIGN ECD (20 μM) mixed with increasing concentrations of inhibiting compounds were prepared in a running buffer composed of 25 mM Tris (pH 8), 150 mM NaCl, 4 mM $CaCl_2$, 0.005% P20 surfactant, and each sample (13 μL) was injected onto the surfaces at a 5 μL/min flow rate. Concentrations of inhibiting compounds ranged from 0.67 to 4367 μM, except for L-fucose (1.33–8733 μM) and dendrimers (0.27–1747 μM). The bound lectin was washed off by a 1 min injection of 50 mM EDTA (pH 8). DC-SIGN ECD equilibrium binding responses (R_{eq}) for each sample were obtained from the reference surface corrected sensorgrams 150 s after the start of the injection.

The DC-SIGN ECD binding responses were extracted from the sensorgrams, converted into percent residual activity values (y) with respect to lectin alone binding, and plotted against the corresponding compound concentration. The four-parameter logistic model [Equation (1)] was fitted to the plots, and the IC_{50} values were calculated by using the values of fitted parameters (R_{hi}, R_{lo}, A_1, and A_2) and Equation (2).

$$y = R_{hi} - \frac{R_{hi} - R_{lo}}{1 + \left(\frac{Conc}{A_1}\right)^{A_2}} \quad (1)$$

$$IC_{50} = A_1 \cdot \left(\frac{R_{hi} - R_{lo}}{R_{hi} - 50} - 1\right)^{\frac{1}{A_2}} \quad (2)$$

Fabrication of Glycan Arrays: The carbohydrate samples were dissolved in disodium hydrogen phosphate buffer (50 mM, pH 8.5) at three different concentrations (0.25, 0.50, and 1.00 mM; data shown in Figure 6 were obtained for a ligand concentration of 0.5 mM). Each solution (0.4 nL) was printed in three replicates by means of a piezoelectric spotting device (S3, Scienion, Berlin, Germany) onto NHS-coated CodeLink slides (SurModics). The slides were stored for 16 h in a moisture chamber. Before further treatment, the slides were washed with water, the unreacted NHS-groups were quenched with 100 mM ethanolamine in disodium hydrogen phosphate buffer (50 mM Na_2HPO_4, pH 9) for 1 h at 50 °C. After removal of the quencher, the slides were washed three times with water. In the final preparation step, the slides were treated with 1% BSA in HBS buffer (pH 7.4) for 1 h at room temp. and washed three times with HBS buffer (10 mM HEPES, 150 mM NaCl, pH 7.4).

The prepared microarrays were incubated with FITC-labeled lectins (Ulex, RSL, LecB, BCIILC-Nt) at different concentrations (0.001 mg/mL, 0.01 mg/mL) in lectin-binding buffer (10mM HEPES, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4) with 1% BSA for 40 min at room temp. During that time the slides were kept in the dark. After incubation, the slides were washed three times with HBS buffer, dried by centrifugation and read out by a fluorescence micro array scanner (Genepix 4300A, Molecular Devices). For the evaluation and interpretation of the results, the average of three values, obtained for the same sugar concentration, were considered as the resulting signal intensity.

Synthesis

General: All reactions were performed in an N_2 atmosphere using anhydrous solvents. Deuterated solvents as well as anhydrous dimethylformamide (DMF), dichloromethane (CH_2Cl_2), methanol and *N*,*N*-diisopropylethylamine (DIPEA) were purchased from Sigma–Aldrich. THF was dried by the application of standard procedures. ^{1}H NMR, ^{13}C NMR and HSQC spectra were recorded with a Bruker Avance 400 at 298 K using $CDCl_3$ (δ = 7.24 ppm), CD_3OD (δ = 3.31 ppm), or D_2O (δ = 4.80 ppm) as solvent and internal standard. Multiplicity of signals has been described as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), dt (doublet of triplet), t (triplet), td (triplet of doublets), q (quartet), m (multiplet), and br (broad). Chemical shifts (δ) are reported in ppm scale, and coupling constants (J) are stated in Hz. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization) or an Autospec Fission Instrument (FAB ionization). An Apex (FT-ICR) instrument from the Centro Interdipartimentale Grandi Apparecchiature (Università degli Studi di Milano) was used. HRMS (FT-ICR, ESI) were obtained with an Apex II instrument. MALDI-TOF mass spectra were recorded with an Ultra Flex III MALDI TOF-TOF instrument (Bruker Daltonics). Optical rotation values were measured with a Perkin–Elmer 241 polarimeter at 589 nm (sodium lamp). Silica gel 60M (mesh 230–400) from Macherey–Nagel was used for purification by flash-chromatography. For thin-layer chromatography (TLC), silica gel coated aluminum foils were used (Sigma–Aldrich). The synthesis of linker **17** and amines **4b**, and **24**–**26** as well as ^{1}H and ^{13}C NMR spectra of the new compounds are collected in the Supporting Information. Below we report the procedures and characterization of compounds **10**–**16** and the dendrimers **21** and **22**. Compounds **3**, **4**, **9**,[8] and **20**[16] as well as **23**[30] were previously described.

***N*-[(1*R*,2*S*,4*R*,5*S*)-2-(Benzyloxycarbonylamino)-4,5-epoxycyclohexane]-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine (10):** Compound **9** (550 mg, 1.00 mmol, 1.0 mol equiv.) and *m*-CPBA (275 mg, 1.59 mmol, 1.6 mol equiv.) were dissolved in CH_2Cl_2 (7.3 mL) and

the reaction mixture was stirred for 4 h at room temperature. The mixture was diluted in CH_2Cl_2 and extracted with a satd. aq $NaHCO_3$. The organic phase was dried with Na_2SO_4 and the solvent was evaporated. The product was purified by flash-chromatography ($CHCl_3$/MeOH, 98:2; R_f = 0.33). Product **10** was obtained as a white foam, yield 288 mg (51%); $[\alpha]_D$ = –50.8 (c = 0.50, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15 (d, $J_{F5,F6}$ = 6.4 Hz, 3 H, H_{F6}), 1.93 (s, 3 H, CH_3COO), 1.95 (s, 3 H, CH_3COO), 2.05–2.12 (m, 1 H, H_{Cy6ax}), 2.15 (s, 3 H, CH_3COO), 2.16–2.25 (m, 2 H, H_{Cy3}), 2.40–2.51 (m, 1 H, H_{Cy1}), 2.51–2.61 (m, 1 H, H_{Cy6eq}), 3.20–3.31 (m, 1 H, H_{Cy4}), 3.31–3.39 (m, 1 H, H_{Cy5}), 4.09 (q, $J_{F5,F6}$ = 6.3 Hz, 1 H, H_{F5}), 4.20 (m, 1 H, H_{Cy2}), 5.04 (d, J = 12.3 Hz, 1 H, Cbz), 5.17 (d, J = 12.3 Hz, 1 H, Cbz), 5.33 (m, 1 H, H_{F4}), 5.34 (dd, $J_{F1,F2}$ = 5.6, $J_{F2,F3}$ = 11.0 Hz, 1 H, H_{F2}), 5.43 (dd, $J_{F3,F4}$ = 3.1, $J_{F2,F3}$ = 11.0 Hz, 1 H, H_{F3}), 5.94 (dd, $J_{F1,F2}$ = 5.6, $J_{F1,NH}$ = 8.4 Hz, 1 H, H_{F1}), 6.03 (d, $J_{NH,Cy2}$ = 9.3 Hz, 1 H, NH-Cy2), 7.28–7.36 (m, 5 H, Cbz), 8.06 (d, $J_{NH,F1}$ = 8.3 Hz, 1 H, NH-F1) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.5 (C_{F6}), 20.8 (CH_3COO), 20.89 (CH_3COO), 24.2 (C_{Cy6}), 31.0 (C_{Cy3}), 43.3 (C_{Cy1}), 45.2 (C_{Cy2}), 51.8 (C_{Cy5}), 52.5 (C_{Cy4}), 66.3 (C_{F5}), 66.5 (C_{F2}), 67.7 (Cbz), 68.3 (C_{F3}), 70.9 (C_{F4}), 74.63 (C_{F1}), 128.3 (Cbz), 128.5 (Cbz), 128.7 (Cbz), 136.3 (C_{Ar}), 157.47 (-CO-), 169.80 (-CO-), 170.16 (-CO-), 170.90 (-CO-), 172.54 (-CO-) ppm. ESI-MS: m/z (%) = 585 (100) $[M + Na]^+$.

Synthesis of 11 and 12 by Ring Opening of Epoxide 10: $Cu(OTf)_2$ (37 mg, 0.10 mmol) was added to a solution of epoxide **10** (280 mg, 0.51 mmol) in 2-chloroethanol (1.7 mL) and the mixture was stirred for 16 h at 40 °C. After evaporation of the solvent, the crude material was dissolved in EtOAc and extracted with NH_4Cl/NH_3 solution (1:1). The organic phase was dried with Na_2SO_4 and the solvent was evaporated. Purification by flash-chromatography on SiO_2 (Et_2O) afforded **12** (62 mg, 19%). Further column chromatography ($CHCl_3$/MeOH, 9:1) gave **11** (220 mg, 67%).

***N*-[(1*R*,2*S*,4*S*,5*S*)-2-(Benzyloxycarbonylamino)-4-(2-chloroethoxy)-5-hydroxycyclohexane]-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine (11):** $[\alpha]_D$ = –41.5 (c = 0.43, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13 (d, $J_{F5,F6}$ = 6.4 Hz, 3 H, H_{F6}), 1.67–1.72 (m, 1 H, H_{Cy3}), 1.83–1.90 (m, 1 H, H_{Cy6}), 1.91 (s, 3 H, CH_3COO), 1.96 (s, 3 H, CH_3COO), 2.15 (s, 3 H, CH_3COO), 2.16 (m, 1 H, H_{Cy3}), 2.19 (m, 1 H, H_{Cy6}), 2.86–3.01 (m, 1 H, H_{Cy1}), 3.48–3.55 (m, 1 H, H_{Cy4}), 3.59 (t, $J_{CH2,CH2}$ = 5.5 Hz, 2 H, -CH_2-Cl), 3.64–3.69 (m, 1 H, -CH_2-O), 3.70–3.76 (m, 1 H, H_{Cy5}), 3.76–3.86 (m, 1 H, -CH_2-O), 3.93 (q, $J_{F5,F6}$ = 6.3 Hz, 1 H, H_{F5}), 4.12–4.20 (m, 1 H, H_{Cy2}), 4.97 (d, J = 12.2 Hz, 1 H, Cbz), 5.15 (d, J = 12.2 Hz, 1 H, Cbz), 6.03 (m, 1 H, NH-Cy), 5.20 (m, 1 H, H_{F4}), 5.23 (m, 1 H, H_{F3}), 5.36 (dd, $J_{F1,F2}$ = 5.3, $J_{F2,F3}$ = 10.9 Hz, 1 H, H_{F2}), 5.85 (dd, $J_{F1,F2}$ = 5.6, $J_{F1,NH}$ = 8.4 Hz, 1 H, H_{F1}), 7.21 (br. s, 1 H, NH-F), 7.27–7.36 (m, 5 H, Cbz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.4 (C_{F6}), 20.7 (CH_3COO), 20.9 (CH_3COO), 20.89 (CH_3COO), 28.4 (C_{Cy6}), 39.9 (C_{Cy3}), 43.6 (-CH_2-Cl), 44.6 (C_{Cy1}), 46.9 (C_{Cy2}), 66.2 (C_{F2}), 66.4 (C_{F5}), 67.3 (Cbz), 68.0 (C_{Cy5}), 68.1 (C_{F4}), 69.7 (-CH_2-O), 70.6 (C_{F3}), 74.9 (C_{F1}), 79.1 (C_{Cy4}), 128.4 (Cbz), 128.6 (Cbz), 128.8 (Cbz), 128.8 (Cbz), 136.2 (C_{Ar}), 152.7 (-CO-), 169.7 (-CO-), 170.5 (-CO-), 170.8 (-CO-) ppm. ESI-MS: m/z (%) = 665 (100) $[M + Na]^+$.

***N*-[(1*R*,2*S*,4*R*,5*R*)-2-(Benzyloxycarbonylamino)-4-hydroxy-5-(2-chloroethoxy)cyclohexane]-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine (12):** $[\alpha]_D$ = –65.2 (c = 0.75, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (d, $J_{F5,F6}$ = 6.4 Hz, 3 H, H_{F6}), 1.88 (m, 1 H, H_{Cy3}), 1.91 (s, 3 H, CH_3COO), 1.95 (s, 3 H, CH_3COO), 1.99–2.08 (m, 3 H, H_{Cy3} and H_{Cy6}), 2.15 (s, 3 H, CH_3COO), 2.90 (dt, $J_{ax/eq}$ = 5.3, $J_{ax/ax}$ = 10.9 Hz, 1 H, H_{Cy1}), 3.55–3.62 (m, 2 H, -CH_2-Cl), 3.62–3.71 (m, 2 H, -CH_2-O and H_{Cy5}), 3.80–3.92 (m, 2 H, H_{Cy4} and -CH_2-O), 4.01 (q, $J_{F5,F6}$ = 7.1 Hz, 1 H, H_{F5}), 4.11–4.24 (m, 1 H, H_{Cy2}), 4.99 (d, J_{ipsoH} = 12.2 Hz, 1 H, Cbz), 5.14 (d, J_{ipsoH} = 12.2 Hz, 1 H, Cbz), 5.28 (m, 1 H, H_{F4}), 5.32–5.41 (m, 2 H, H_{F2} and H_{F3}), 5.94 (dd, $J_{F1,F2}$ = 3.0, $J_{F1,NH}$ = 8.9 Hz, 1 H, H_{F1}), 6.03 (br. s, 1 H, NH-Cy), 7.28–7.37 (m, 6 H, NH-F1 and Cbz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.5 (C_{F6}), 20.8 (CH_3COO), 20.9 (CH_3COO), 33.3 (C_{Cy3}), 34.5 (C_{Cy6}), 42.8 (C_{Cy1}), 43.7 (-CH_2-Cl), 47.7 (C_{Cy2}), 66.3 (C_{F5}), 66.4 (C_{F2} or C_{F3}), 67.5 (CH_2-Cbz), 68.2 (C_{F2} or C_{F3}), 69.2 (C_{Cy4}), 69.6 (-CH_2-O), 70.8 (C_{F4}), 74.6 (C_{F1}), 78.0 (C_{Cy5}), 128.4 (Cbz), 128.5 (C_{Ar}), 128.8 (C_{Ar}), 136.4 (C_{Ar}), 157.2 (-CO-), 169.8 (-CO-), 169.8 (-CO-), 170.3 (-CO-), 170.9 (-CO-) ppm. ESI-MS: m/z (%) = 665 (100) $[M + Na]^+$.

***N*-[(1*R*,2*S*,4*S*,5*S*)-2-(3-Acetoxybenzamido)-4-(2-chloroethoxy)-5-hydroxycyclohexane]-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine (15):** Pd/C (10 wt.-%, 10%, Degussa type) was added to a solution of **11** in DMF/methanol (4:1, 0.05 M) and the reaction mixture was stirred under H_2 (1 bar) at room temperature until full conversion was observed by TLC. The catalyst was filtered through Celite and the solvent was evaporated to obtain the crude amine.

In the following step, 3-acetoxybenzoic acid (2.0 mol equiv.), HBTU (2.0 mol equiv.), and DIPEA (3.7 mol equiv.) were added to a solution of the crude amine in DMF (0.03 M). The reaction mixture was stirred for 15 h at room temperature, then the solvent was evaporated, the crude material was diluted in EtOAc and extracted with HCl (1 M), saturated Na_2CO_3 and water, and the organic phase was dried with Na_2SO_4. Product **15** was purified by flash-chromatography (*n*-hexane/ethyl acetate, 7:3; R_f = 0.21), yield 90 mg (40%). $[\alpha]_D$ = –33.4 (c = 0.75, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.07 (d, $J_{F5,F6}$ = 6.4 Hz, 3 H, H_{F6}), 1.67 (s, 3 H, CH_3COO), 1.73–1.77 (m, 1 H, H_{Cy3ax}), 1.91 (s, 3 H, CH_3COO), 1.92–1.97 (m, 1 H, H_{Cy6ax}), 2.09 (s, 3 H, CH_3COO), 2.15–2.24 (m, 1 H, H_{Cy6eq}), 2.25 (s, 3 H, CH_3COO), 2.27–2.35 (m, 1 H, H_{Cy3eq}), 2.86–2.94 (dd, $J_{eq/ax}$ = 5.2, $J_{ax/ax}$ = 11.2 Hz, 1 H, H_{Cy1}), 3.45–3.52 (m, 1 H, H_{Cy4}), 3.52–3.60 (m, 2 H, -CH_2-Cl), 3.60–3.66 (m, 1 H, -CH_2-O), 3.67–3.76 (m, 1 H, H_{Cy5}), 3.83–3.90 (m, 1 H, -CH_2-O), 3.95 (q, $J_{F5,F6}$ = 6.3 Hz, 1 H, H_{F5}), 4.40–4.49 (m, 1 H, H_{Cy2}), 5.14 (dd, $J_{F3,F4}$ = 3.3, $J_{F3,F2}$ = 10.4 Hz, 1 H, H_{F3}), 5.15–5.18 (m, 1 H, H_{F4}), 5.26 (dd, $J_{F1,F2}$ = 5.3, $J_{F2,F3}$ = 10.4 Hz, 1 H, H_{F2}), 5.77 (dd, $J_{F1,F2}$ = 5.4, $J_{F1,NH}$ = 7.8 Hz, 1 H, H_{F1}), 7.07 (d, $J_{NH,Cy2}$ = 6.2 Hz, 1 H, NH-Cy), 7.17 (ddd, J_{CH3ta} = 0.9, J_{CH3ta} = 2.9, J_{ortho} = 8.1 Hz, 1 H, H_{Ar4}), 7.37 (t, J_{ortho} = 7.9 Hz, 1 H, H_{Ar5}), 7.45 (t, J_{CH3ta} = 1.9 Hz, 1 H, H_{Ar2}), 7.55 (d, J_{ortho} = 7.9 Hz, 1 H, H_{Ar6}), 7.71 (br. d, $J_{NH,F1}$ = 6.2 Hz, 1 H, NH-F1) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 16.3 (C_{F6}), 20.4 (CH_3COO), 20.9 (CH_3COO), 20.9 (CH_3COO), 21.3 (CH_3COO), 29.2 (C_{Cy6}), 29.9 (C_{Cy3}), 43.8 (-CH_2-Cl), 43.9 (C_{Cy1}), 46.3 (C_{Cy2}), 66.1 (C_{F2}), 66.1 (C_{F5}), 68.3 (C_{F3}), 69.6 (C_{Cy5}), 70.7 (-CH_2-O), 75.1 (C_{F4}), 75.1 (C_{F1}), 79.1 (C_{Cy4}), 120.9 (C_{Ar2}), 124.5 (C_{Ar6}), 125.5 (C_{Ar4}), 130.0 (C_{Ar5}), 135.6 (C_{Ar1}), 151.2 (C_{Ar3}), 166.7 (-CO-), 169.4 (-CO-), 169.8 (-CO-), 170.7 (-CO-), 170.9 (-CO-), 175.1 (-CO-) ppm. ESI-MS: m/z (%) = 693 (100) $[M + Na]^+$.

***N*-[(1*R*,2*S*,4*S*,5*S*)-2-(3-Hydroxybenzamido)-4-(2-azidoethoxy)-5-hydroxycyclohexane-carboxyl]-α-L-fucopyranosylamine (16):** A 0.01 M solution of protected sugar **15** in MeOH was treated with a 1M solution of NaOMe in MeOH (1 mol equiv.). The reaction mixture was stirred for 15 min at room temperature, then the pH was adjusted to pH 7 by addition of Amberlite IRA 120$^+$, which was then removed by filtration. The solvent was evaporated to afford the deacetylated product, which was purified by flash-chromatography (chloroform/methanol, 4:1; R_f = 0.31), yield 26 mg (81%). $[\alpha]_D$ = –42.8 (c = 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.19 (d, $J_{F5,F6}$ = 6.5 Hz, 3 H, H_{F6}), 1.66 (m, 1 H, H_{Cy3ax}), 1.96–2.10 (m, 2 H, H_{Cy6ax}), 2.46 (ddd, $J_{eq/ax}$ = 3.7, $J_{eq/eq}$ = 6.2, ^2J =

13.6 Hz, 1 H, H_{Cy3eq}), 2.93 (dt, $J_{eq/ax}$ = 4.7, $J_{ax/ax}$ = 9.2 Hz, 1 H, H_{Cy1}), 3.53 (m, 1 H, H_{Cy4}), 3.61–3.67 (m, 4 H, H_{Cy5}, -CH_2-Cl, H_{F4}), 3.73 (dd, $J_{F3,F4}$ = 3.4, $J_{F3,F2}$ = 10.2 Hz, 1 H, H_{F3}), 3.77–3.88 (m, 3 H, -CH_2-O and H_{F5}), 3.93 (dd, $J_{F1,F2}$ = 5.6, $J_{F2,F3}$ = 10.2 Hz, 1 H, H_{F2}), 4.58 (dt, $J_{eq/ax}$ = 3.9, $J_{ax/ax}$ = 7.5 Hz, 1 H, H_{Cy2}), 5.52 (d, $J_{F1,F2}$ = 5.6 Hz, 1 H, H_{F1}), 6.94 (dt, J_{CH3ta} = 2.6, J_{ortho} = 6.6 Hz, 1 H, H_{Ar4}), 7.22–7.28 (m, 3 H, $H_{Ar2,5,6}$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 17.0 (C_{F6}), 31.3 (C_{Cy6}), 32.9 (C_{Cy3}), 44.4 (-CH_2-Cl), 45.2 (C_{Cy1}), 48.3 (C_{Cy2}), 68.2 (C_{F2}), 69.0 (C_{F5}), 71.4 (-CH_2-O), 71.5 (C_{F4} or C_{Cy5}), 71.75 (C_{F3}), 73.2 (C_{F4} or C_{Cy5}), 78.7 (C_{F1}), 80.5 (C_{Cy4}), 115.5 (C_{Ar}), 119.6 (C_{Ar}), 119.8 (C_{Ar}), 130.8 (C_{Ar}), 137.5 ($C_{quat.}$, C_{Ar}), 158.9 ($C_{quat.}$, C_{Ar}), 170.6 (NH*C*O), 177.0 (NH*C*O) ppm. ESI-MS: *m*/*z* (%) = 525 (100) [M + Na]$^+$. The product isolated from the Zemplen deprotection (26 mg, 0.052 mmol) was dissolved in DMF (500 μL). After addition of NaN_3 (25 mg, 0.448 mmol), the reaction mixture was stirred for ten days at 50 °C, then the solvent was evaporated and product **16** was purified by flash-chromatography (chloroform/methanol, 9:1; R_f = 0.32), yield 26 mg (81%). $[\alpha]_D$ = –42.4 (*c* = 0.95, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 1.09 (d, $J_{F5,F6}$ = 6.5 Hz, 3 H, H_{F6}), 1.61–1.73 (m, 1 H, H_{Cy3ax}), 1.87–2.00 (m, 1 H, H_{Cy6ax}), 2.04 (m, 1 H, H_{Cy6eq}), 2.37 (m, 1 H, H_{Cy3eq}), 2.96 (dt, $^3J_{1,6ax}$ = 12.4, $^3J_{1,6eq}$ = 5.4 Hz, H_{Cy1}), 3.36–3.46 (m, 2 H, -CH_2-Cl), 3.46–3.55 (m, 1 H, H_{Cy4}), 3.77–3.85 (m, 2 H, H_{F5} and H_{F3}), 3.85–3.62 (m, 4 H, -CH_2-O, H_{Cy5} and H_{F4}), 3.90 (dd, $J_{F1,F2}$ = 5.6, $J_{F2,F3}$ = 10.5 Hz, 1 H, H_{F2}), 4.66 (q, ^3J = 3.5 Hz, 1 H, H_{Cy2}), 5.41 (d, $J_{F1,F2}$ = 5.6 Hz, 1 H, H_{F1}), 7.02 (ddd, J_{CH3ta} = 0.9, J_{CH3ta} = 2.6, J_{ortho} = 8.2 Hz, 1 H, H_{Ar4}), 7.06–7.11 (m, 1 H, H_{Ar2}), 7.16 (ddd, J_{CH3ta} = 0.9, J_{CH3ta} = 2.6, J_{ortho} = 8.2 Hz, 1 H, H_{Ar6}), 7.32 (t, J_{ortho} = 7.9 Hz, 1 H, H_{Ar5}) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 15.5 (C_{F6}), 29.2 (C_{Cy6}), 32.9 (C_{Cy3}), 43.7 (C_{Cy1}), 47.5 (C_{Cy2}), 50.7 (-CH_2-Cl), 65.9 (C_{F2}), 67.5 (C_{F5}), 68.2 (-CH_2-O), 69.4 (C_{F3}), 71.1 (C_{F4} or C_{Cy5}), 71.3 (C_{F4} or C_{Cy5}), 77.0 (C_{F1}), 78.7 (C_{Cy4}), 114.1 (C_{Ar2}), 119.11 (C_{Ar4} and C_{Ar6}), 130.10 (C_{Ar5}), 135.31 (C_{Ar1}), 155.8 (C_{Ar3}), 170.7 (NH*C*O), 175.6 (NH*C*O) ppm. ESI-MS: *m*/*z* = 508 (100) [M – 1 H]$^-$. HRMS (FT-ICR, ESI): *m*/*z* calcd. for $C_{22}H_{31}N_5O_9Na$ [M + Na]$^+$ 532.20140; found 532.20055.

***N*-{3-[2-(2-Azidoethoxy)ethoxy]propanecarboxyl}-α-L-fucopyranosylamine (18):** Compound **17** (17.0 mg, 0.0837 mmol) and HATU (33.0 mg, 0.0868 mmol) were dissolved in DMF (450 μL) and the solution was treated with DIPEA (40.0 μL, 0.229 mmol) and stirred for 15 min. After addition of a solution of **4b** (30 mg, 0.0768 mmol) in DMF (300 μL), the reaction was stirred at room temperature for 16 h. The solvent was evaporated and the product was purified by flash-chromatography on SiO_2 [MeOH/EtOAc, 0–3%; R_f = 0.36 (MeOH/$CHCl_3$, 3:97)], yield 20.0 mg (50%). $[\alpha]_D$ = –76.0 (*c* = 0.60, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.11 (d, $J_{F5,F6}$ = 6.4 Hz, 3 H, H_{F6}), 1.97 (s, 3 H, CH_3COO), 2.02 (s, 3 H, CH_3COO), 2.16 (s, 3 H, CH_3COO), 2.44 (t, ^3J = 6.1 Hz, 1 H, CH_2), 2.52 (t, ^3J = 6.8 Hz, 1 H, CH_2), 3.34–3.40 (m, 2 H, CH_2), 3.45 (td, ^3J = 3.7, ^3J = 6.7 Hz, 1 H, CH_2), 3.56–3.69 (m, 6 H, CH_2), 3.73 (d, ^3J = 6.1 Hz, 2 H, CH_2), 4.03 (q, $J_{F5,F6}$ = 6.3 Hz, 1 H, H_{F5}), 5.23 (dd, $J_{F1,F2}$ = 5.6, $J_{F2,F3}$ = 11.1 Hz, 1 H, H_{F2}), 5.29 (m, 1 H, H_{F4}), 5.48 (dd, $J_{F3,F4}$ = 3.5, $J_{F2,F3}$ = 11.2 Hz, 1 H, H_{F3}), 5.84 (d, $J_{F1,F2}$ = 5.6 Hz, 1 H, H_{F1}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.7 (C_{F6}), 20.6 (*C*H_3COO), 20.7 (*C*H_3COO), 20.7 (*C*H_3COO), 36.5 (CH_2), 36.8 (CH_2), 37.7 (CH_2), 51.9 (CH_2), 67.0 (C_{F5}), 67.8 (C_{F2}), 68.4 (CH_2), 69.5 (C_{F3}), 71.3 (CH_2), 71.6 (CH_2), 71.7 (CH_2), 72.3 (C_{F4}), 75.6 (C_{F1}), 171.6 (-CO), 171.7 (-CO-), 172.5 (-CO-), 174.5 (-CO-), 174.8 (-CO-) ppm. ESI-MS: *m*/*z* = 568 (100) [M + Na]$^+$.

A 0.01 M solution of the coupling product in MeOH was treated with a 1M solution of NaOMe in MeOH (1 mol equiv.). The reaction mixture was stirred for 15 min at room temperature, then the pH was adjusted to pH 7 by addition of Amberlite IRA 120$^+$, which was removed by filtration. The solvent was evaporated to afford the deacetylated product **18**, yield quantitative. $[\alpha]_D$ = –39.5 (*c* = 0.24, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.18 (d, $J_{F5,F6}$ = 6.5 Hz, 3 H, H_{F6}), 2.44 (t, ^3J = 6.2 Hz, 2 H, CH_2), 2.51 (t, ^3J = 6.7 Hz, 2 H, CH_2), 3.36–3.40 (m, 2 H, CH_2), 3.46 (t, ^3J = 6.8 Hz, 2 H, CH_2), 3.58–3.70 (m, 7 H, H_{F4} and 3 × CH_2), 3.73 (t, ^3J = 6.2 Hz, 2 H, CH_2), 3.75–3.82 (m, 2 H, H_{F3} and H_{F5}), 3.96 (dd, $J_{F1,F2}$ = 5.6, $J_{F2,F3}$ = 10.3 Hz, 1 H, H_{F2}), 5.56 (d, $J_{F1,F2}$ = 5.6 Hz, 1 H, H_{F1}) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 16.9 (C_{F6}), 36.6 (CH_2), 36.7 (CH_2), 37.6 (CH_2), 51.8 (CH_2), 68.0 (C_{F2}), 68.3 (CH_2), 68.6 (C_{F3}), 71.1 (CH_2), 71.4 (CH_2), 71.4 (CH_2), 71.4 (C_{F5}), 73.2 (C_{F4}), 78.3 (C_{F1}), 174.0 (-*C*ONH-), 175.1 (-*C*ONH-) ppm. HRMS (FT-ICR, ESI): *m*/*z* calcd. for $C_{22}H_{33}N_3O_9Na$ [M + Na]$^+$ 442.19083; found 442.19061. ESI-MS: *m*/*z* (%) = 442 (100) [M + Na]$^+$.

Dendrimer 21: A solution of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)-methyl]amine (TBTA) (2.28 mg, 0.0043 mmol, 1.0 mol equiv.) in THF (325 μL), a solution of $CuSO_4$·5H_2O (10 mol-%) in degassed water (135 μL) and a solution of sodium ascorbate (0.376 mg, 0.4 mol equiv.) in degassed water (87 μL) were added to a solution of core **20** (1.24 mg, 0.0043 mmol, 1.0 mol equiv.) in freshly distilled THF (155 μL) under a N_2 atmosphere. Addition of **16** (6 mol equiv.) in water (260 μL) led to a water/THF (1:1 mixture) and the reaction was stirred for 3 d at room temperature (progress monitored by MALDI-MS analysis). Sodium ascorbate (0.1 mol equiv.) was added at 24 h intervals. After 3 d, full conversion was observed by MALDI mass spectrometry (DHB; CH_3CN/H_2O, 1:1). The product was purified by flash-chromatography on SiO_2 ($CHCl_3$/MeOH, 1:1) to give the product as a white solid, yield 4 mg (35%). ^{1}H NMR (400 MHz, D_2O): δ = 1.10 (d, $J_{F1,F2}$ = 6.5 Hz, 12 H, H_{F6}), 1.40–1.51 (m, 4 H, H_{Cy3ax}), 1.85–2.09 (m, 12 H, H_{Cy3eq} and H_{Cy6}), 2.91 (dt, $J_{eq/ax}$ = 4.1, $J_{ax/ax}$ = 11.9 Hz, 4 H, H_{Cy1}), 3.23 (s, 8 H, CH_2 core), 3.36–3.45 (m, 4 H, H_{Cy4}), 3.56–3.62 (m, 4 H, H_{Cy5}), 3.68–3.73 (m, 4 H, H_{F4}), 3.77–3.83 (m, 8 H, H_{F3} and H_{F5}), 3.92 (dd, $J_{F1,F2}$ = 5.6, $J_{HF2,HF3}$ = 10.5 Hz, H_{F2}), 3.99–4.02 (m, 8 H, CH_2-*N*-), 4.37 (s, 8 H, CH_2 core), 4.46–4.53 (m, 8 H, CH_2-O-), 4.53–4.54 (m, 4 H, H_{Cy2}), 5.42 (d, $J_{F1,F2}$ = 5.6 Hz, 4 H, H_{F1}), 6.99 (dd, J_{CH3ta} = 2.5, J_{ortho} = 8.1 Hz, 4 H, H_{Ar4}), 7.02–7.08 (m, 4 H, H_{Ar2}), 7.13 (d, J_{ortho} = 7.8 Hz, 4 H, H_{Ar6}), 7.27 (t, J_{ortho} = 7.9 Hz, 4 H, H_{Ar5}), 7.89 (s, 4 H, triazole) ppm. ^{13}C NMR (HSQC, 100 MHz, D_2O): δ = 15.3 (C_{F6}), 28.6 (C_{Cy3}), 32.4 (C_{Cy6}), 43.3 (C_{Cy1}), 47.3 (C_{Cy2}), 50.3 (CH_2-O-), 63.4 (CH_2 core), 65.6 (C_{F2}), 67.2 (C_{F3} or C_{F5}), 67.7 (CH_2 core), 67.9 (CH_2-*N*-), 69.1 (C_{F3} or C_{F5}), 70.6 (C_{Cy5}), 71.2 (C_{F4}), 76.7 (C_{F1}), 78.8 (C_{Cy4}), 114.0 (C_{Ar2}), 119.0 (C_{Ar4}), 119.2 (C_{Ar6}), 130.0 (C_{Ar5}) ppm. HRMS (FT-ICR, ESI): *m*/*z* calcd. for $C_{105}H_{144}N_{20}O_{40}Na_2$ (+2): 2370.96331 observed at 1185.48166, found 1185.48429.

Dendrimer 22: A solution of TBTA (2.9 mg, 0.0054 mmol, 1.0 mol equiv.) in THF (250 μL), a solution of $CuSO_4$·5H_2O (10 mol-%) in degassed water (170 μL) and a solution of sodium ascorbate (0.4 mg, 0.4 mol equiv.) in degassed water (100 μL) were added to a solution of the core **20** (1.6 mg, 0.0054 mmol, 1.0 equiv.) in freshly distilled THF (195 μL) under a N_2 atmosphere. Addition of **18** (10 mg, 0.024 mmol, 4.4 mol equiv.) in water (175 μL) led to a water/THF (1:1) mixture that was stirred under N_2 and monitored by ESI-MS. After ca. 2 h, additional sodium ascorbate (0.4 mg, 0.4 mol equiv. in 100 μL degassed water) was added. After 24 h, ESI-MS showed that the reaction had reached completion. The reaction mixture was then loaded on a Sephadex LH20 column and eluted with MeOH to give product **22** (6.6 mg, 62) as a white foamy solid. ^{1}H NMR (400 MHz, D_2O): δ = 1.15 (d, $J_{F5,F6}$ =

6.5 Hz, 12 H, H_{F6}), 2.46 (t, ^3J = 6.1 Hz, 8 H, CH_2-linker), 2.57 (t, ^3J = 6.7 Hz, 8 H, CH_2-β-Ala), 3.40 (s, 8 H, CH_2 core), 3.46 (t, ^3J = 6.5 Hz, 8 H, CH_2-β-Ala), 3.54–3.63 (m, 16 H, CH_2-linker), 3.67 (t, ^3J = 6.1 Hz, 8 H, CH_2-linker), 3.79–3.82 (m, 4 H, H_{F4}), 3.85 (dd, $J_{F3,F4}$ = 3.4, $J_{F2,F3}$ = 10.6 Hz, 4 H, H_{F3}), 3.85–3.92 (m, 4 H, H_{F5}), 3.92–3.98 (m, 8 H, CH_2-linker), 4.03 (dd, $J_{F1,F2}$ = 5.7, $J_{F2,F3}$ = 10.5 Hz, 4 H, H_{F2}), 4.52 (s, 8 H, CH_2 core), 4.58–4.63 (m, 8 H, CH_2-linker), 5.56 (d, $J_{F1,F2}$ = 5.7 Hz, 4 H, H_{F1}), 7.99 (s, 4 H, triazole) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 15.7 (C_{F6}), 35.0 (CH_2-β-Ala), 35.6 (CH_2-β-Ala), 35.9 (CH_2-linker), 44.6 ($C_{quat.}$ – core), 50.0 (CH_2-linker), 63.5 (CH_2, core), 66.0 (C_{F2}), 66.6 (CH_2-linker), 67.5 (C_{F5}), 68.1 (CH_2, core), 68.8 (CH_2-linker), 69.4 (CH_2-linker), 69.5 (C_{F3}), 69.6 (CH_2-linker), 71.4 (C_{F4}), 76.7 (C_{F1}), 125.3 (CH, triazole), 144.1 ($C_{quat.}$ – triazole), 173.8 ($C_{quat.}$, -C=O), 175.3 ($C_{quat.}$, -C=O) ppm. ESI-MS: *m*/*z* = 1987.9 $[M + Na]^+$. HRMS (FT-ICR, ESI): *m*/*z* calcd. for $C_{81}H_{136}N_{20}O_{36}$ $[M]^+$ 1964.94261; found 1964.94286 (after deconvolution).

STD Experiments: STD NMR experiments were performed at 25 °C with a Bruker Avance 600 instrument. Saturation was achieved by using a train of Gaussian shaped pulses of 49 ms (field strength of ca. 80 Hz), an inter-pulse delay of 1 ms[1] and 15 ms spin-lock pulse (field strength of 3.7 kHz) prior to acquisition. The on-resonance frequency was set to 0 ppm and the off-resonance frequency was 40 ppm. Appropriate blank experiments were performed to assure the lack of direct saturation of the ligand protons. Several saturation times were used to obtain the STD build-up curves. The binding epitope was characterized by analysis of initial slopes of the STD intensities: the experimental ($I_0 - I_{sat}/I_0$) curves were fitted to an exponential function described by the equation: STD (t_{sat}) = STD_{max} ($1 - e^{-ksat \cdot tsat}$), which allows the STD at zero saturation time (initial slopes) to be calculated from the product of resulting parameters STD_{max} and $k_{satd.}$

Supporting Information (see footnote on the first page of this article): Synthesis of linker **17**; synthesis of amines **4b** and **24**–**26**; ^1H and ^{13}C NMR spectra of new compounds; NOESY spectra and configuration assignment of **11** and **12**; primary data of SPR experiments (sensorgrams and inhibition curves).

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