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Synthesis of Novel DC-SIGN Ligands with an α-Fucosylamide Anchor

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The dendritic cell-specific intercellular adhesion molecule (ICAM) 3-grabbing nonintegrin (DC-SIGN) is a C-type lectin that appears to perform several different functions. Besides mediating adhesion between dendritic cells and T lymphocytes, DC-SIGN recognizes several pathogens some of which, including HIV, appear to exploit it to invade host organisms. The intriguing diversity of the roles attributed to DC-SIGN and their therapeutic implications have stimulated the search for new ligands that could be used as biological probes and possibly as lead compounds for drug development. The natural ligands of DC-SIGN consist of mannose oligosaccharides or fucose-containing Lewis-type determinants. Using the known 3D structure of the Lewis-x trisaccharide, we have identified some monovalent α-fucosylamides that bind to DC-SIGN with inhibitory constants 0.4–0.5 mM, as determined by SPR, and have characterized their interaction with the protein by STD NMR spectroscopy. This work establishes for the first time α-fucosylamides as functional mimics of chemically and enzymatically unstable α-fucosides and describes interesting candidates for the preparation of multivalent systems able to block the receptor DC-SIGN with high affinity and with potential biomedical applications.

Introduction

Dendritic cells (DCs) are antigen-presenting cells and currently understood as critical controllers of the immune response.[1] Immature DCs localized in peripheral mucosal tissues act as pathogen sentinels: specific receptors on DCs recognize and internalize pathogens, which are then degraded by lysosomal enzymes. The resulting fragments are presented by major histocompatibility complex (MHC) molecules at the DC surface and are used to activate naive T cells and eventually induce an effective immune response.

DC-SIGN is one of the dendritic cells' specific pathogen-uptake receptors. It was brought to the attention of the scientific community by the group of van Kooyk, who reported that HIV-1 targets DC-SIGN but escapes degradation in lytic compartments, and thus uses DCs as a Trojan horse to invade the host organism.[2] After this discovery, it was shown by several groups that many pathogens are recognized by DC-SIGN; this indicates that this lectin could participate in some way during the corresponding infection process.[3] Hence, this receptor is currently considered as an interesting new target for the design of anti-infective agents.[4–6] Furthermore, as the detailed molecular mechanisms by which this receptor operates are not known, effective modulators of DC-SIGN are needed to help clarify the different biological processes in which it can be involved.

In humans, DC-SIGN is expressed together with a closely related receptor, DC-SIGNR, which is found on a different subset of cells. Both receptors belong to the calcium-dependent C-type lectin family and recognize high-mannose N-linked oligosaccharides. DC-SIGN, but not DC-SIGNR, can also recognize branched fucosylated structures that bear terminal galactose residues, such as the Lewis antigens expressed at the surface of viruses and bacteria as glycoconjugates.[7–11]

Binding of fucose-containing oligosaccharides to DC-SIGN has been reported by several groups.[7,8,12,13] Fucose itself was reported to bind DC-SIGN with a dissociation constant of approximately 6 mM.[8] A glycan array study[10] performed with a Consortium for Functional Glycomics array of 130 glycan structures indicates that the presence of a terminal fucose residue is not a sufficient condition for DC-SIGN binding. However, 14 fucose-bearing glycans were found to bind selectively to DC-SIGN. All of these molecules contain a terminal fucose residue and have the structure of Lewis epitopes. It is well known that

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the Lewis trisaccharides assume a well-defined, highly conserved 3D structure, with the fucose ring stacked on top of the galactose residue.[14] The same feature was observed in the X-ray structure of the DC-SIGN–Lewis-x complex (PDB ID code: 1SL5).[10] In this complex, fucose coordinates the Ca^{2+} ion in its site and the galactose residue interacts weakly with a secondary binding site.

There are few reports describing noncarbohydrate inhibitors of lectins. Most examples are concerned with compounds designed to block selectins.[15–17] Glycomimetic inhibitors of the cholera toxin have been reported.[18–22] We have recently described a mannobioside mimic that binds to DC-SIGN and shows an effective antiviral activity in an infection model for the Ebola virus.[4a] The Kiessling group has also recently reported on the discovery of noncarbohydrate small-molecule inhibitors of DC-SIGN by high-throughput screening (HTS) of combinatorial libraries.[5] Herein, we present a novel fucose-based mimic designed to reproduce some salient features of the Lewis-x trisaccharide, and which appears to effectively interact with the DC-SIGN receptor.

α-Fucosides are both enzymatically and chemically labile.[23] C-Glycosides have often been used as mimics of monosaccharide units. However, the conformation of α-L-*C*-fucosides has been found to deviate significantly from the native $^1C_4$ chair.[24] α-Glicosylamides, although difficult to synthesize,[25–27] have the advantage of being chemically stable and essentially unknown in Nature,[28] they are therefore likely not to be recognized by hydrolytic enzymes. The only α-fucosylamide reported so far was found to adopt the $^1C_4$ chair conformation.[25b] In order to design a fucose-based DC-SIGN ligand, we selected to use an α-fucosylamide anchor and connected it to a galactose or galactose mimic,[29] and thus reproduced the basic 3D features of the Lewis-x trisaccharide **1**. As an additional requirement aimed to improve the metabolic stability of the construct and to simplify the synthesis, the linker and the sugar or sugar-like fragments were connected, avoiding glycosidic bonds. From the template **2**, the structure of the linker was chosen by performing a conformational search of the candidates (MacroModel's MC/EM, with the AMBER* force field[30]) and overlapping the resulting conformations within 3 kcal mol^{-1} to the Lewis-x trisaccharide. In this way, (1*S*,2*R*)-2-amino-cyclohexanecarboxylic acid was identified as an interesting candidate that appeared to promote stacking of the two sugars fragments. Furthermore, the use of a galactose mimic rather than galactose itself appeared to improve the structural similarity between **2** and **1** by reducing the H-bonding interactions between the sugar and linker that distorted the ligand away from the desired shape (Figure 1).

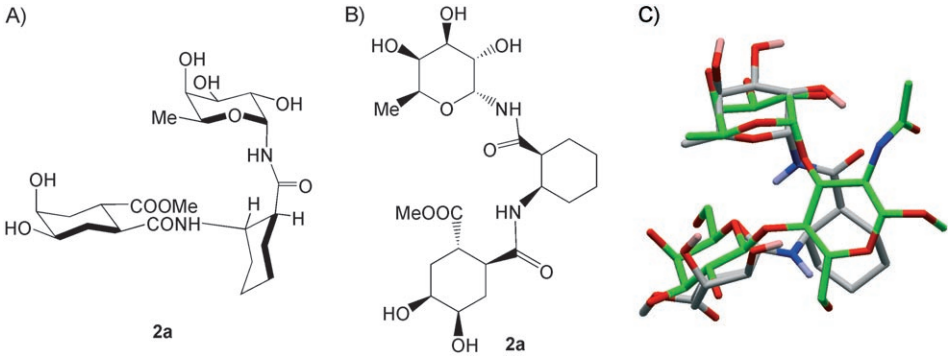
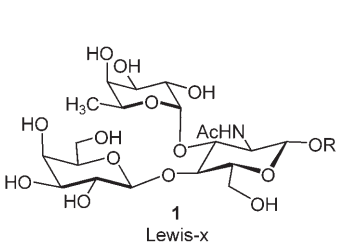
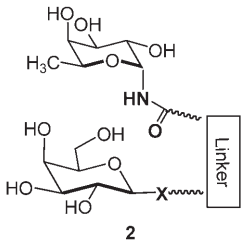


Figure 1. A) Structure of mimic **2a**, B) its predicted conformation, and C) superimposition with the Lewis-x trisaccharide (**2a** framework in gray and Lewis-x in green).

As a result of this design, compound **2a** (Figure 1) emerged as an interesting target. Compound **2a** is a diamide formed by an unnatural β-amino acid ((1*S*,2*R*)-2-amino-cyclohexanecarboxylic acid), and therefore it is expected to be stable to peptide hydrolases.[31] Computational analysis suggested that **2a** is dynamically rather flexible but it mainly adopts (by more than 60%) the conformation shown in Figure 1B and C (gray framework), which overlaps satisfactorily with the Lewis-x trisaccharide (Figure 1C, green framework). Herein, we report on the synthesis of **2a**, its NMR conformation, and on the initial binding studies with the DC-SIGN extracellular domain, which were performed by STD NMR spectroscopy and surface plasmon resonance (SPR).

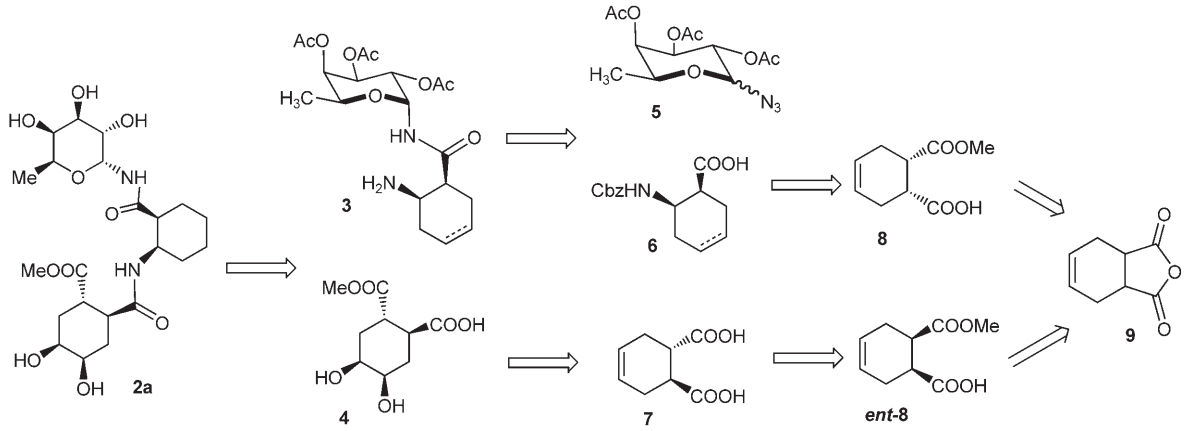




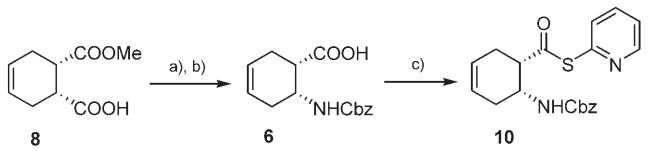
Results and Discussion

Synthesis of the target diamide 2a

Retrosynthetically, the diamide **2a** can be disconnected into the known dihydroxyacid **4**[32] and the α-fucosylamido-amine **3** (Scheme 1). As we have noted above, only a handful of processes are available for the synthesis of α-glycosylamides,[25–27] and the most efficient ones (see below) use a glycosylazide as the starting material. Based on this, the intermediate **3** can be envisaged as deriving from the *O*-acetyl-fucosylazide **5**[33] and the (1*S*,2*R*)-2-amino-cyclohexanecarboxylic acid **6** (Scheme 1). Like **4**, **6** is a known compound.[34] Both **6** and **4** share tetrahydrophtalic anhydride **9** as the common precursor via the intermediates shown in Scheme 1. Activation of acid **6** as a pyridyl thioester was achieved under Mukaiyama conditions[35] to afford **10** in 67% yield (Scheme 2).



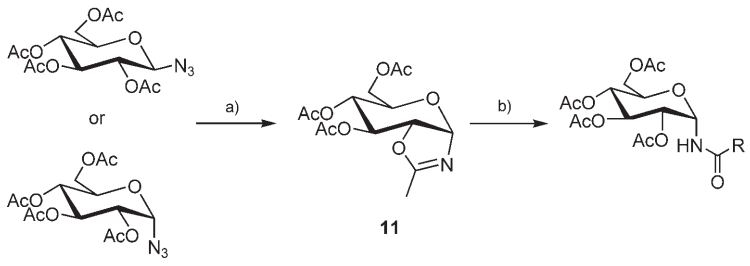
Scheme 1. Retrosynthetic analysis of **2a**.



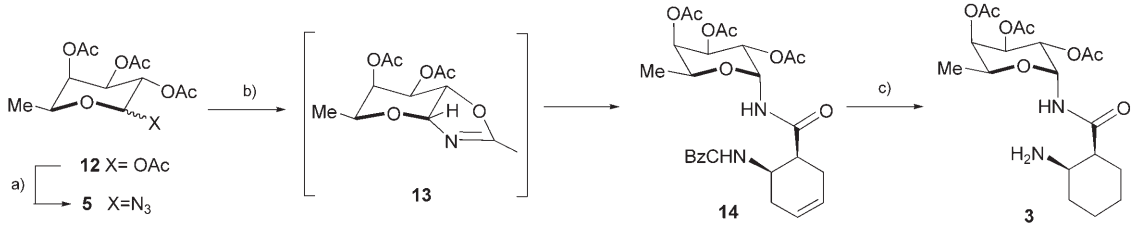
Scheme 2. Synthesis of the activated ester **10**: a) DPPA, Et_3N, then $PhCH_2OH$; b) LiOH, $MeOH/H_2O$; c) Ph_3P, dipyridyldisulfide.

The most demanding task in the retrosynthetic plan consists of the synthesis of the α-fucosylamide moiety **3**. The difficulties stem from the rapid α to β anomerization of 1-amino glycopyranosyl derivatives—a process so notoriously fast that a number of approaches have been investigated to circumvent it.[25–27] In a clever turn, the group of DeShong has taken advantage of the easy anomeric equilibration and has shown that treatment of α-or β-2,3,4,6-tetra-*O*-acetyl-glucopyranosyl-azide (Scheme 3) with Ph_3P in refluxing 1,2-dichloroethane under anhydrous conditions yields a single oxazoline **11**, formed by cyclization of the intermediate iminophosphorane, which can only occur in the α-anomer (Scheme 3). Acylation of the oxazoline with pyridylthiolesters occurs with retention of the configuration at the anomeric carbon to afford the α-glucosylamide with good selectivity (Scheme 3).[26]

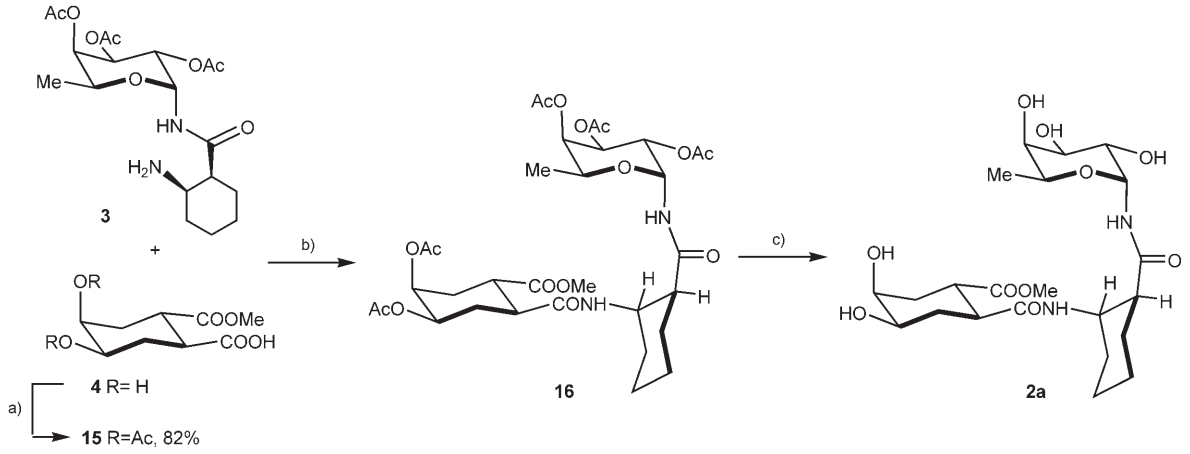
For its simplicity, the DeShong approach appears attractive for large-scale synthesis and we began to investigate its extension to the fucose series. The required tri-*O*-acetyl-fucosylazide **5** was prepared in 94% yield and in 9:1 (β/α) ratio from *O*-tetracetyl-fucose **12** by using trimethylsilyl azide and TMSOTf[33a] (Scheme 4). Application of the DeShong protocol revealed that the Ph_3P reduction of **5** is very slow in dichloroethane and many byproducts are formed. However, fucosyloxazoline **13** could be obtained quantitatively in 12 h in refluxing nitroethane (Scheme 4). The acylation reaction with **10** was best performed one-pot at 40 °C for 20 h to afford **14** in 67% overall yield, after chromatography. The anomeric configuration of **14** was unequivocally established on the basis of the coupling constant of the anomeric proton (H1 at 5.7 ppm) $J_{1-2} = 5.2$ Hz, which is typical of an α-glycosylamide.[25] Less than 10% of the β epimer was formed under this condition, as identified in the crude reaction mixtures by the presence of a second anomeric



Scheme 3. The DeShong synthesis of α-glucopyranosylamides:[26] a) Ph_3P, refluxing dichloroethane; b) RCOSPy, $CuCl_2$.



Scheme 4. Synthesis of α-fucosylamide **3**: a) TMSOTf 0.4 mol equiv, $TMSN_3$, DCM, room temperature, 94%; b) Ph_3P, $EtNO_2$, reflux 12 h, then **10**, 20 h, 40 °C; c) H_2, Pd-C.



Scheme 5. Synthesis of the target diamide **2a**: a) TBDMSCl, Et_3N, CH_2Cl_2, then AC_2O, DMAP, pyridine, dilute HCl work-up; b) HBTU, 69%; c) MeONa, MeOH.

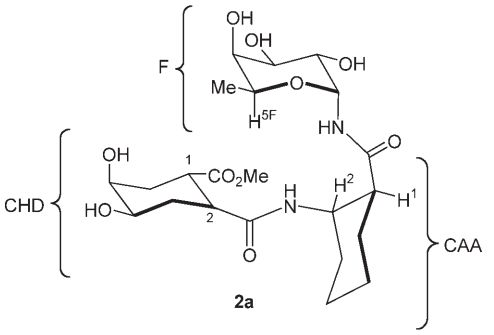
carbon at 81 ppm, coupled in the Hetcor spectrum to a proton at 5.2 ppm. The estimated α/β anomeric ratio was 11:1. Removal of the carbobenzyloxy protecting group by hydrogenolysis occurred with concomitant double bond reduction to yield **3** and set the stage for the final coupling reaction.

Direct activation of the dihydroxyacid **4** caused fast lactonization under a number of conditions. Lactone formation was also triggered under acetylation conditions (Ac_2O, pyridine). However, the diacetate **15** (Scheme 5) was obtained in good yields by a temporary protection of the carboxy group (*t*Bu-Me_2SiCl, Et_3N, CH_2Cl_2), followed by treatment with Ac_2O, and work-up with diluted HCl. Condensation of **15** with **3** was obtained by HBTU activation, and afforded **16** in 69% yield (Scheme 5). Removal of the protecting groups under standard[36] conditions gave the target **2a**.

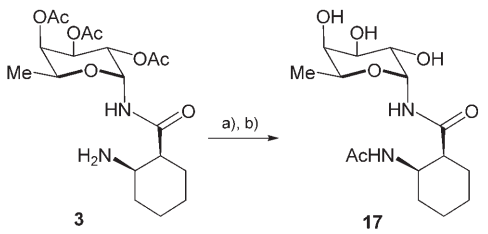
Given the limited contribution of the galactose fragment to the Lewis-x–DC-SIGN stabilizing interaction[10] and, in general, for comparison purpose, the unprotected fucosylamide **17** was also prepared by nitrogen acetylation of **3** followed by Zemplen's deprotection according to Scheme 6.

NMR spectroscopy studies: conformation of 2a and interaction with DC-SIGN

Compound **2a** was analyzed by NMR spectroscopy in order to determine its conformation. Spectral overlap and low NOE signals did not allow definition of the relative orientation of the three cyclic fragments in **2a**. However, some key points could be addressed:



1) *The conformation of the* cis-*β-aminoacid (CAA ring)*: AMBER* calculations predicted a single chair conformation for this fragment that features the carboxy group in the equatorial position and the amino group in the axial position. Coupling constant analysis of **2a** in CD_3OD (600 MHz, 300 K) confirmed the modeling results. The H2 proton signal appears as a broad singlet at 4.27 ppm and H1 as a multiplet centered at 2.72 ppm. Irradiating at 4.27 ppm, the H1 signal is resolved into a doublet of doublets with one coupling constant of 10 Hz and one of 4.2 Hz ($J_{1,6ax}$ and $J_{1,6eq}$, respectively); this is consistent with an axial position for this proton and with the chair conformation of the CAA ring.
2) *The conformation of the cyclohexanediol (CHD ring)*: The conformational properties of this fragment have been discussed in detail previously.[18] Also, in the context of **2a**, this ring displays the single conformation, as revealed by the CHD-H_1 and CHD-H_2 proton signals, which appear at 3.04 and 2.66 ppm (600 MHz; CD_3OD), respectively, as doublets of triplets with coupling constants of 12 and 3.6 Hz.
3) *The conformation of the fucose ring (F)*: This was one point of major concern for the design of **2a**. The fucose ring, in fact, can undergo conformational equilibration between



Scheme 6. Synthesis of fucosylamide **17**: a) Ac_2O, pyridine; b) MeONa, MeOH.

the native $^1C_4$ chair and the isomeric $^4C_1$ chair. Bulky substituents in the axial position on the anomeric carbon tend to promote this equilibration. As we have noted above, α-L-*C*-fucosides do not adopt the native $^1C_4$ chair,[24] but recent results suggest that α-fucosylamide can do so.[25b] The large coupling constant value between the H2 and H3 protons of the pyranose ring (J_{2-3} = 10.8 Hz, 600 MHz; CD_3OD) in **2a** confirmed the *trans*-diaxial arrangement of these protons and supported the notion that α-fucosylamides are structural mimics of α-fucosides.

Saturation transfer difference (STD)[37] experiments allowed observation of the binding event between DC-SIGN and ligand **2a**. In STD experiments, irradiation of the protein is followed by transfer of magnetization to the ligand protons, which in turn causes a signal enhancement that can be best appreciated in the difference spectrum. STD experiments were carried out in the presence of the DC-SIGN extracellular (ECD) domain 40 μM in D_2O (d-Tris buffer, pD 8, 150 mM NaCl, 4 mM $CaCl_2$) at several ligand to protein ratios (from 12.5:1 to 500:1) and different saturation times (from 0.5 to 3 s). The experiments show clear signals that correspond to the fucose moiety Fuc-H1 (5.4 ppm), Fuc-H2 (3.9 ppm), Fuc-H3 (3.8 ppm), and Fuc-H4 and H5 (3.7 ppm; Figure 2). This confirms that indeed binding

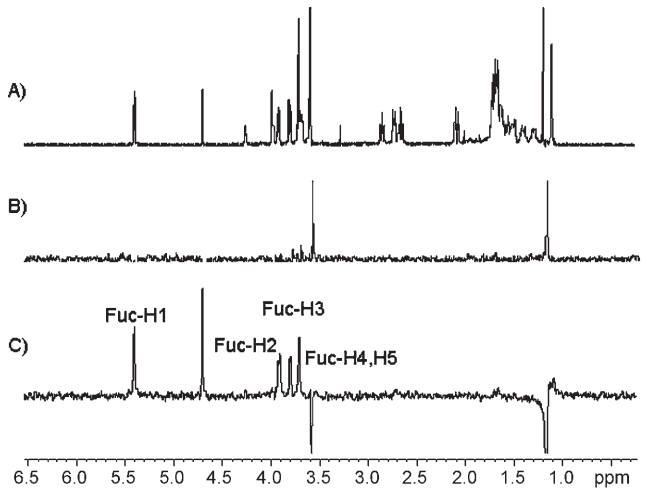


Figure 2. STD spectra of **2a** and DC-SIGN ECD: A) **2a** ^{1}H NMR spectrum (2 mM in buffer); B) **2a** STD irradiating frequency −300 Hz; C) **2a**: DC-SIGN ECD (250:1) irradiating frequency −300 Hz.

occurs, and indicates that the fucosylamide anchor is in close contact with the protein. Epitope mapping was performed by using relative STD values, as introduced by Mayer and Meyer[38] (Figure 3). Only the fucose protons showed saturation transfer; H1 and H2 appeared to be closer to the protein than H3, H4, and H5, which is in agreement with the expected binding mode of α-fucosides to DC-SIGN.[10]

The binding affinity was analyzed by performing STD experiments[38] at different ligand to protein ratios (from 12.5 to 500) at constant concentration of protein (40 μM in 200 μL d-Tris 25 mM, pD 8, $CaCl_2$ 4 mM, 150 mM NaCl), and by using a satu-

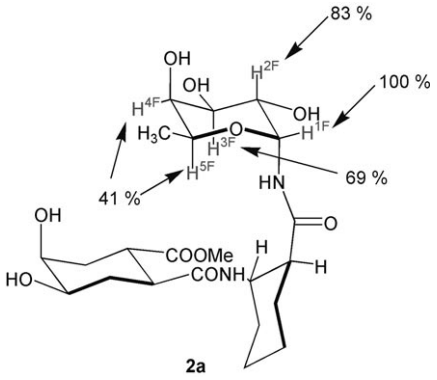


Figure 3. Relative values of STD amplification factors for **2a** (1 mM), 600 MHz, DC-SIGN ECD (40 μM) in 200 μL d-Tris (25 mM 25 pD 8), $CaCl_2$ (4 mM), NaCl (150 mM); 3 s saturation time.

ration time (T_{sat}) of 3 s. The plotting of the STD amplification factors against the concentration of added ligand for the proton with the largest STD amplification factor (Fuc-H1) allowed estimation of an EC_{50} value of 4 mM for the DC-SIGN ECD–**2a** complex (Figure 4). The same experiments were per-

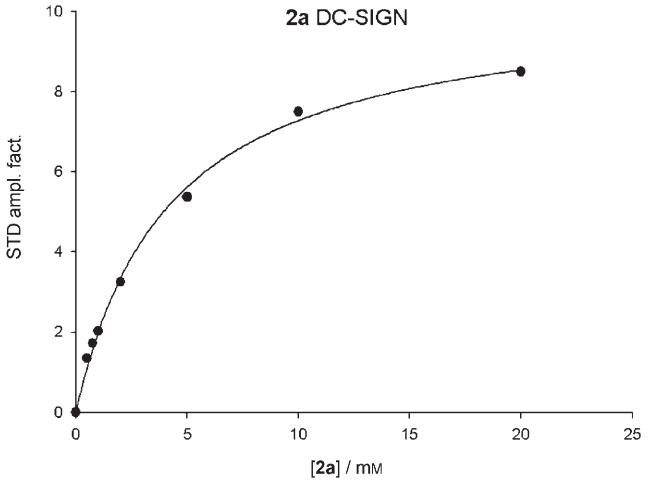


Figure 4. Observed STD amplification factors (STD ampl. fact.) of the Fuc-H1 resonance plotted against the concentration of the added ligand **2a** (600 MHz, DC-SIGN ECD 40 μM in 200 μL d-Tris 25 mM pD 8, $CaCl_2$ 4 mM, 150 mM NaCl; *T* sat 3 s); fitting was performed with Sigmaplot.

formed by using Lewis-x **1** (R = H, Carbosynth) as the substrate turned out to be rather noisy, but allowed estimation of an EC_{50} value of 7 mM (data not shown). Thus the NMR spectroscopy studies suggested that the affinity of artificial ligand **2a** is in the same range as that of the natural effector; but SPR studies were performed in order to obtain a better characterization of the inhibitory power of the artificial ligands.

SPR studies

Carbohydrate recognition domains (CRD) of C-type lectins have a weak affinity towards sugars. For example, DC-SIGN

CRD has a K_d of 13 mM for mannose.[7] In vivo, however, lectin oligomerization allows multivalent interactions and results in an avidity-based mechanism. In this study, we aim to compare DC-SIGN recognition properties for different sugars and their mimic derivatives, free in solution. Thus, even by using the whole ECD domain of DC-SIGN (tetrameric) such avidity based mechanism could not take place and for some of these compounds the weak level of affinity would not be measurable with SPR technology in direct interaction analysis mode. For these reasons, we performed a competition assay, which allows an affinity evaluation of all compounds relative to the others on the basis of an IC_{50} determination.

Mannosylated BSA was covalently attached to a CM4 dextran-functionalized gold SPR chip. Mannosylated BSA contains 15 glycosylation sites that display the manα1-3[manα1-6]man trisaccharide. DC-SIGN ECD exhibited good affinity for the chip. A series of measurements with increasing concentrations yielded a binding isotherm (Figure 5A). From this curve, the maximum of DC-SIGN ECD binding onto the generated mannosylated-BSA surface was evaluated. Thus, a concentration of 15 μM of DC-SIGN ECD was chosen for the competition assays. Inhibition studies were then performed by using DC-SIGN ECD, at the concentration defined above, injected alone or in the presence of an increasing amount of the ligands (Figures 5B–D).

To calibrate the competition-assay studies, we used mannose as a reference monosaccharide for C-type lectins and the Lewis-x trisaccharide as the natural version of the artificial ligand **2a** produced in this work. In order to evaluate the importance of the different units that compose our artificial ligand, we also tested L-fucose, **17**, and **2a**, which correspond to one, two, or all three units of the artificial ligand **2a**, respectively. The unnatural enantiomer D-fucose was used as negative control. The results are shown in Figure 5. In the presence of the various ligands used the binding response decreased with increasing amount of ligand; this indicates ECD binding inhibition with the mannosylated-BSA surface (as exemplified in Figures 4B and C for mannose and compound **2a**, respectively). The efficiency of inhibition as a function of the compound concentration is directly related to the ligand affinity towards DC-SIGN ECD. From Figure 4D, the IC_{50} value for each ligand could be determined. The reference compounds mannose, L-fucose, and Lewis-x were found to inhibit DC-SIGN binding to the mannosylated-BSA surface with an IC_{50} value of 1.8 mM, 1.2 mM, and 0.8 mM, respectively. The values measured for mannose and L-fucose are consistent with literature data,[7] whereas the affinity of Lewis-x has not been measured before. Compounds **17** and **2a** showed IC_{50} values of 0.5 and 0.35 mM, respectively. The activity of the ligands increased only slightly with the number of units. Satisfactorily, the full Lewis-x mimic **2a** and notably the α-fucosylamide **17**, which comprises only two units, were found to be a better inhibitor for DC-SIGN than the natural Lewis-x. Comparison between **2a** and **17** suggests that the cyclohexanediol unit in **2a** does not contribute significantly to the binding affinity. On the contrary, the transformation of fucose in the α-fucosylamide **17** has a positive effect on the DC-SIGN affinity with respect to fucose.

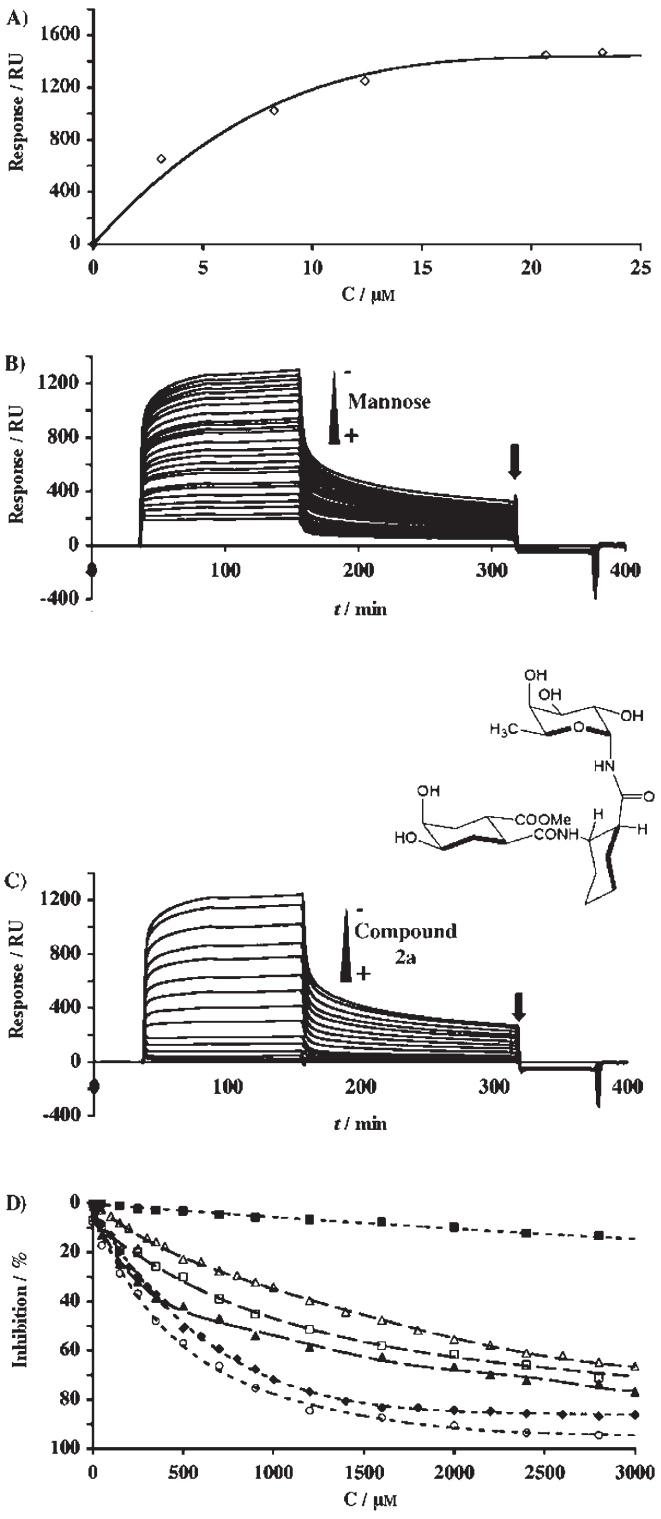


Figure 5. Inhibition of the DC-SIGN ECD–mannosylated-BSA surface. A) Binding isotherm of DC-SIGN ECD onto the mannosylated-BSA surface. DC-SIGN ECD (15 μM) was incubated for 1 h with B) mannose at 36 increasing concentrations from 0 to 5000 μM, and C) compound **2a** at 17 increasing concentrations from 0 to 4000 μM, and coinjected onto the mannosylated-BSA functionalized surface (1200 RU immobilized). Black arrow represents EDTA injection for surface regeneration between each injection cycle. D) Comparison of the inhibitory power of the ligands from B) and C) and of other competition assays realized for Lewis-x, fucose, and **17** towards the DC-SIGN ECD–mannosylated-BSA interaction; ■: D-fucose, △: D-mannose, □: L-fucose, ▲: Lewis-x, ◆: **17**, and ○: **2a**.

Conclusions

In this work we have demonstrated for the first time that α-fucosylamides are effective functional mimics of α-fucosides, and have established a simple protocol for their synthesis based on the DeShong's methodology.[26] The biological relevance of α-fucosides can hardly be overestimated, and yet these glycosides are notorious for their chemical and enzymatic instability; this makes their synthesis difficult and their use for antagonism or modulation of lectin activity in biological settings limited. The chemical stability of α-fucosylamides is likely to be accompanied by an improved stability to hydrolytic enzymes, which is going to be the subject of further investigations. This, together with the NMR spectroscopy data, which show that α-fucosylamides retain the characteristic $^1C_4$ chair of α-L-fucosides, establish this class of compounds as the molecule of choice to act as mimics of these unstable glycosides.

We have also described the first fucose-based unnatural ligands of DC-SIGN. Both **2a** and **17** inhibit DC-SIGN binding to mannosylated BSA with a potency that is similar to—and in fact slightly better than—the natural ligand Lewis-x and only one order of magnitude lower than the best small-molecule inhibitors identified so far by a HTS campaign of about 30000 compounds.[5] The fact that the full Lewis mimic **2a** (IC_{50} 0.35 mM) is not much better than **17** (IC_{50} 0.5 mM) suggests that there is still room for improvement of the fragment we have chosen to replace the galactose residue of Lewis-x. Further modeling work is in progress to optimize this element, based on the possible interactions with the DC-SIGN binding site, as mapped by STD experiments. On the other hand, the simple fucosylamide **17**, which shows a twofold affinity increase over fucose in our SPR assay and which is already equipped with a convenient handle for further functionalization, can be considered as an excellent candidate to prepare multivalent systems able to block DC-SIGN with high affinity. The advantage presented by this type of ligand in terms of chemical and enzymatic stability makes them very attractive for the development of new anti-infective drugs.

Experimental Section

Expression of ECD of DC-SIGN in *E. coli* and purification process: Plasmid pET30b (Novagen) containing cDNA that encoded the extracellular domain (ECD; corresponding to amino acids 66–404) of DC-SIGN was used for overproduction, as described previously.[39] Protein produced in inclusion bodies was refolded as described.[7] Purification of functional DC-SIGN protein was achieved by affinity chromatography on a mannan–agarose column (Sigma) equilibrated in buffer A (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 4 mM $CaCl_2$) and was eluted in the same buffer without $CaCl_2$ but supplemented with EDTA (10 mM; buffer B). This step was followed by a superose six size-exclusion chromatography equilibrated in buffer A.

SPR analysis: All experiments were performed by using a BIAcore 3000 with functionalized CM4 chips and the corresponding reagents from BIAcore. Two flow cells were activated as previously described.[39] Flow cell one was then blocked with 1 M ethanolamine (50 μL) and served as a control surface. The second one was treated with BSA–manα1-3[manα1-6]man (BSA–Mannotriose, Dextra; 60 μg mL^{-1}) in acetate buffer (10 mM, pH 4). Remaining activated groups were blocked with ethanolamine (1 M, 50 μL). The final density immobilized on the surface of the second flow cell was 1200 RU. The BSA–mannotriose used to functionalize the CM4 chips harbors 15 glycosylation sites according to the manufacturer. The affinity of the various sugars and mimics was then estimated by a DC-SIGN ECD binding inhibition assay. The ECD of DC-SIGN was injected onto the BSA–mannotriose surface at a constant concentration, either alone or in the presence of an increasing concentration of the sugar derivatives. Injections were performed at 20 μL min^{-1} by using buffer A, supplemented with P20 surfactant (0.005%) as running buffer.

STD NMR spectroscopy: Experiments were recorded with an Avance Bruker instrument that operated at 600 MHz, 278 K. Samples without lectin used as negative control, were prepared by dissolving **2a** and **17** in NaCl (150 mM), d-Tris (25 mM, pH 8.1), $CaCl_2$ (4 mM) in D_2O after three cycles of deuterium exchange. The samples in presence of DC-SIGN ECD were prepared by using 40 μM of lectin, which was assumed to have a monomeric state in the same D_2O buffer, by using 0.5, 0.75, 1.0, 2.0, 5.0, and 10.0 mM of the ligand. STD experiments were performed at 278 K by using watergate solvent suppression at 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0 s saturation times with a train of Gaussian shaped pulses of 49 ms and 100–60 Hz power spaced by 1.0 ms delays.[38] On-resonance irradiation was performed at 0.9 ppm, appropriate blank experiments were also performed to assure the absence of direct irradiation on the ligand. On-resonance and reference spectra were recorded interleaved and STD was quantified by manual fitting by superimposition of both spectra by using manufacturer software.

Synthesis

General methods: NMR spectra were recorded at 300 K on Bruker Avance 400 and Avance 600 spectrometers with TMS as the internal standard. Chemical shifts are reported in parts per million (ppm). Spin multiplicities are indicated by standard notation. The atoms of **2a**, **16**, and **17** were numbered as follows: n for the atoms of the fucose ring, n′ for the atoms of the β-aminoacid ring (CAA), n″ for the atoms of the cyclohexanediol ring (CHD). Optical rotation $[\alpha]_D$ was measured in a 1 dm length cell by using sodium D-line wavelength (589 nm) on a Perkin–Elmer 241 polarimeter. LC-MS analyses were performed with reversed-phase HPLC (Agilent 1100 HPLC with diode array, column Atlantis dc19×100 mm, 5 μm) with ESI mass ionization (iontrap MS detector Bruker Esquire 3000+). HRMS spectra were obtained with an Apex II ICR FTMS instrument (ESI ionization). Thin-layer chromatography (TLC) was performed on 0.25 mm Merck F254 silica-coated glass plates, and compounds were identified in one or more of the following manners: UV (254 nm) and molybdic reagent/sulfuric acid/ninhydrin/potassium permanganate charring. Flash chromatography was carried out with Macherey–Nagel silica gel 60 (230–400 mesh). Semipreparative HPLC was performed with Auto Purification System Waters, column Atlantis dC18 ($\Phi = 1.9$ mm, $h = 100$ mm, 5μ), detection at 220 nm. The solvents used were dried before use by standard procedures under nitrogen atmosphere. All reactions were performed under nitrogen atmosphere unless noted otherwise. The synthesis of **8**, *ent*-**8**, **7**, and **4** has been reported.[32]

*2,3,4-Tri-O-acetyl-α-L-fucopyranosylazide (**5**)*:[33] $TMSN_3$ (2.138 mL, 16.25 mmol; 2 equiv) and TMSiOTf (0.600 mL, 3.25 mmol; 0.4 equiv) were added to a solution of **12** (2.700 g, 8.13 mmol; 1 equiv) in CH_2Cl_2. The reaction mixture was stirred, overnight, at room temperature. Et_3N was added (0.5 mL) and the solvent evaporated under vacuum. The residue was dissolved in EtOAc,

and the organic phase was washed with HCl (1 M), water (to neutral pH), and brine. The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to obtain crude product. The crude product was recrystallized from isopropyl ether to obtain 1.79 g of pure β-fucosylazide as a white crystalline solid (70%); m.p. = 125–128 °C; $[\alpha]_D^{20} = +24.7$ (*c* 1.98, EtOH). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.28$ (d, $J_{6\text{-}5} = 6.4$ Hz, 3H, *H*6), 2.21–2.10–2.01 (3s, 3×3H, 3*Me-CO*), 3.9 (qd, $J_{5\text{-}4} = 0.8$ Hz, $J_{5\text{-}Me} = 6.4$ Hz, 1H, *H*5), 4.60 (d, 1H, *H*1, $J_{1\text{-}2} = 8.6$ Hz), 5.05 (dd, 1H, *H*3, $J_{3\text{-}2} = 10.4$ Hz, $J_{3\text{-}4} = 3.4$ Hz), 5.16 (dd, 1H, *H*2, $J_{2\text{-}1} = 8.6$ Hz, $J_{2\text{-}3} = 10.4$ Hz), 5.29 (dd, 1H, *H*4, $J_{4\text{-}3} = 3.4$ Hz, $J_{4\text{-}5} = 0.8$ Hz). ^{13}C NMR (100 MHz, CD_3OD): $\delta = 16.0$ (*C*6), 20.67–20.60–20.54 (*Me-CO*), 68.2 (*C*2), 69.9 (*C*4), 71.1 (*C*3), 71.5 (*C*5), 88.2 (*C*1), 170.5–170.0–169.4 (*C*=O).

(1S,6R)-6-(Benzyloxycarbonylamino)cyclohex-3-enecarboxylic acid (***6***):[34] DPPA (1.74 mL, 8.06 mmol; 1 equiv) and benzyl alcohol (1.67 mL, 16.1 mmol; 2 equiv) were added to a stirred solution of **8** (1.500 g, 8.08 mmol; 1 equiv) in 30 mL of toluene Et_3N (1.12 mL, 8.06 mmol; 1 equiv). The solution was heated under reflux for 4 h, cooled to room temperature and diluted with EtOAc. The organic phase was washed with HCl (5%), sat. solution of $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum. The residue was purified with flash chromatography (gradient elution: toluene/AcOEt (90:10)–toluene/AcOEt (93:7)) to yield 0.466 g (1.77 mmol) of the Curtius rearrangement product as a white foam (64%). $[\alpha]_D^{20}$: +2.18 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.39$–2.24 (m,2H, *H*3), 2.53–2.39 (m, 2H, *H*6), 2.85 (brs, 1H, *H*1), 3.72 (s, 3H, *Me*-O-), 4.28 (d, 1H, *H*2, $J_{2\text{-}HN} = 6$ Hz), 5.81 (s, 2H, -*CH*2-Ph), 5.42 (d, 1H, *H*N, $J_{HN\text{-}2} = 6$ Hz), 5.65–5.62 (m, 1H, *H*4), 5.71–5.67 (m, 1H, *H*5), 7.38–7.37 (m, 5H, *H*Ph). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 25.5$ (*C*6), 30.6 (*C*3), 42.0 (*C*1), 46.8 (*C*2), 51.8 (*C*11), 66.6 (*C*9), 124.7 (*C*4), 124.9 (*C*5), 128.5–127.1–128.1 (*C*Ph), 136.5 (*C*10), 155.8 (*C*8), 173.7 (*C*7); HPLC-MS: calcd for $[C_{16}H_{19}NO_4Na]^+$: 312.2; found: 311.7.

LiOH monohydrate (0.245 g, 5.80 mmol; 2.5 equiv) was added to a stirred solution of the Curtius rearrangement product (0.696 g, 2.33 mmol; 1 equiv) in MeOH/H_2O (4:1; 15 mL) at 0 °C. The solution was warmed to room temperature and stirred until no starting product was detected by TLC (hexane/AcOEt (8:2) + 1% AcOH). The reaction mixture was concentrated under vacuum to approximately one third of the initial volume, the pH was adjusted to 9 with $NaHCO_3$, and the solution was washed with Et_2O. The water phase was acidified with HCl (6 M) to pH 2, and extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to obtain 0.616 g (2.24 mmol) of pure acid **6** as a white crystalline solid (96%). $[\alpha]_D^{20}$: +12.4 (*c* 2.04, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.39$–2.22 (m, 2H, *H*3), 2.58–2.43 (m, 2H, *H*6), 2.91 (brs, 1H, *H*1), 4.30 (brs, 1H, *H*2), 5.15 (dd, 2H, -*CH*2-Ph, $J_{9\text{-}9'} = 18$ Hz), 5.45 (d, 1H, *H*N, $J_{HN\text{-}2} = 9.6$ Hz), 5.64 (d, 1H, *H*4, $J_{4\text{-}5} = 10$ Hz), 5.70 (d, 1H, *H*5, $J_{5\text{-}4} = 10$ Hz), 7.38–7.36 (m, 5H, *H*Ph). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 25.9$ (*C*6), 30.3 (*C*3), 42.0 (*C*1), 46.7 (*C*2), 66.8 (*C*9), 124.8 (*C*4), 124.9 (*C*5), 128.5–128.2–128.1 (*C*Ph), 136.3 (*C*10), 156.0 (*C*8), 178.7 (*C*7); HRMS (ESI): calcd for $[C_{15}H_{17}NO_4Na]^+$: 298.1057; found: 298.1049.

(1S,6R)-S-Pyridin-2-yl 6-(benzyloxycarbonylamino)cyclohex-3-enecarbothioate (***10***): PPh_3 (0.390 g, 1.50 mmol; 1.2 equiv) and 2,2′-dithiodipyridine (0.330 g, 1.50 mmol; 1.2 equiv) were added to a solution of **6** (0.342 g, 1.24 mmol; 1 equiv) in CH_3CN (12 mL). The solution was heated under reflux for 2 h, cooled to room temperature, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 7:3) to yield 0.331 g (0.898 mmol) of pure product as a yellowish oil (72%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.45$–2.25 (m, 2H, *H*3), 2.68–2.49 (m, 2H, *H*6), 3.25–3.21 (m, 1H, *H*1), 4.42–4.39 (m, 1H, *H*2), 5.11 (s, 2H, -*CH*2-Ph), 5.29 (d, 1H, *H*N, $J_{HN\text{-}2} = 8.4$ Hz), 5.70–5.67 (m, 1H, *H*4), 5.76–5.72 (m, 1H, *H*5), 7.37–7.28 (m, 6H, *H*Ph + *H*12), 7.65 (d, 1H, *H*14, $J_{14\text{-}13} = 7.6$ Hz), 7.73 (td, 1H, *H*13, $J_{13\text{-}12} = J_{13\text{-}14} = 7.6$ Hz, $J_{13\text{-}11} = 2$ Hz), 8.64 (ddd, $J_{11\text{-}12} = 4.8$ Hz, $J_{11\text{-}13} = 1.6$ Hz, $J_{11\text{-}14} = 0.8$ Hz, 1H, *H*11).

N-*((1S,6R)-6-(Benzyloxycarbonylamino)cyclohex-3-enecarboxyl)-2,3,4-tri*-O-*acetyl-α-*L*-fucopyranosylamine* (***14***): Grounded activated molecular sieves (4 Å) were added to a solution of fucosyl azide **5** (0.113 g, 0.358 mmol; 1 equiv) in dry $EtNO_2$ (5 mL). PPh_3 (0.103 g, 0.394 mmol; 1.1 equiv) was dissolved in $EtNO_2$ (5 mL) and was added; the mixture was refluxed for 18 h. The reaction was monitored by TLC ($CHCl_3$/AcOEt, 1:1) and the disappearance of the starting material and appearance of the oxazoline **13** was observed. The reaction mixture was used directly in the next step without isolation. In a separate vessel, the pyridyl thioester **10** (0.171 g, 0.465 mmol; 1.3 equiv) and $CuCl_2 \cdot H_2O$ (0.079 g, 0.465 mmol; 1.3 equiv) were dissolved in $EtNO_2$ (1 mL) and added to the solution of **13**. The reaction mixture was heated to 40 °C and monitored by TLC ($CHCl_3$/AcOEt, 1:1). After 20 h the mixture was filtered through a celite pad, and celite washed abundantly with EtOAc. The filtrate was washed with an aqueous solution of NH_3/NH_4Cl (pH 9), then with water to neutral pH. The organic phase was dried over Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 45:55) to obtain 0.126 g (0.231 mmol) of pure product as a white crystalline solid (64%). $[\alpha]_D^{20}$: −43.91 (*c* 0.94, $CHCl_3$).). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.06$ (d, 3H, *Me*Fuc, $J_{MeFuc\text{-}5} = 6.4$ Hz), 1.93 (s, 3H, *Me*Ac), 1.99 (s, 3H, *Me*Ac), 2.08 (s, 3H, *Me*Ac), 2.16 (m, 1H, *H*3′ax), 2.33 (d, 1H, *H*6′ax), 2.51 (d, 1H, *H*3′eq), 2.61 (d, 1H, *H*6′eq), 2.77 (m, 1H, *H*1′), 3.85 (brd, 1H, *H*5, $J_{5\text{-}MeF} = 6.4$ Hz), 4.32 (brs, 1H, *H*2′), 5.13 (s, 2H, C*H*2Ph), 5.22 (d, 1H, *H*4), 5.33 (brd,1H, *H*3, $J_{2\text{-}3} = 11.2$ Hz), 5.39 (dd, 1H, *H*2, $J_{2\text{-}1} = 5.2$ Hz, $J_{2\text{-}3} = 11.2$ Hz), 5.44 (d, 1H, *CONH*, $J_{NH\text{-}2'} = 8.8$ Hz), 5.69 (brd, 1H, *H*4′, $J_{4'\text{-}5'} = 10$ Hz), 5.79 (brd, 1H, *H*5′, $J_{5'\text{-}4'} = 10$ Hz), 5.73 (dd, 1H, *H*1, $J_{1\text{-}NHFuc} = 7.6$ Hz, $J_{1\text{-}2} = 5.2$ Hz), 7.31 (m, 1H, *CONH*Fuc), 7.37–7.31 (m, 5H, *H*Ph). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.1$ (*C*H_{3Fuc}), 21.1–20.6–20.5 (*C*H_{3Ac}), 25.4 (*C*6′), 31.4 (*C*3′), 43.4 (*C*1′), 46.1 (*C*2′), 65.5 (*C*5), 65.7 (*C*5), 66.0 (*C*2), 66.4 (*C*H_2Ph), 67.9 (*C*3), 70.4 (*C*4), 74.5 (*C*1), 124.8 (*C*5′), 125.4 (*C*4′), 128.6–128.1 (*C*Ph), 136.1 (*C*Ph), 167.0, 169.0, 170.0, 170.6, 173.0 (5× *C*O); HPLC-MS: calcd for $[C_{27}H_{34}N_2O_{10}Na]^+$: 569.2; found: 569.2; HRMS (ESI): calcd for $[C_{27}H_{34}N_2O_{10}Na]^+$: 569.21057; found: 569.21087.

N-*(1S,2R)-2-(Aminocyclohexanecarboxyl)-2,3,4-tri*-O-*acetyl-α-*L*-fucopyranosylamine* (***3***): 10% wt. Pd/C (10%, Degussa type) was added to a solution of **14** (0.750 mg, 1.373 mmol; 1 equiv) in dry EtOH (1 mL). The reaction mixture was hydrogenated (1 bar) at room temperature until no trace of starting compound was detected by TLC ($CHCl_3$/MeOH, 92:2). The catalyst was filtered over a celite bed and the residual solvent evaporated under vacuum. The crude product was purified with flash chromatography ($CHCl_3$/MeOH, 9:1 + 2% Et_3N) to yield 0.398 g (0.960 mmol) of white solid (70%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.05$ (d, 3H, *Me*Fuc, $J_{MeFuc\text{-}5} = 6.4$ Hz), 1.34 (m, 1H, *H*4′ax), 1.50 (m, 1H, *H*4′eq), 1.40 (m, 1H, *H*3′ax), 1.56 (m, 1H, *H*3′eq), 1.57 (m, 1H, *H*6′ax), 1.84 (m, 1H, *H*6′eq), 1.93 (m, 1H, *H*5′ax), 2.04 (s, 6H, *Me*Ac), 2.10 (m, 1H, *H*5′eq), 2.19 (s, 3H, *Me*Ac), 2.43 (brs, 1H, *H*1′), 2.70 (brs, 2H, N*H*2), 3.24 (brs, 1H, *H*2′), 3.91 (q, 1H, *H*5, $J_{5\text{-}MeFuc} = 6.4$ Hz), 5.12 (d, 1H, *H*3, $J_{3\text{-}2} = 10.7$ Hz, $J_{3\text{-}4} = 3.2$ Hz), 5.20 (d, 1H, *H*4, $J_{4\text{-}3} = 3.2$ Hz), 5.40 (d, 1H, *H*2, $J_{2\text{-}1} = 4.8$ Hz, $J_{2\text{-}3} = 10.7$ Hz), 5.83 (d, 1H, *H*1, $J_{1\text{-}2} = 4.8$ Hz, $J_{1\text{-}N\ HFuc} = 8.4$ Hz), 10.22 (brs, 1H, CON*H*Fuc); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 16.1$ (*Me*Fuc), 20.05 (*C*5′), 21.01–20.64–20.57 (*C*H_{3Ac}), 21.3 (*C*3′), 23.6 (*C*4′), 25.8 (*C*6′), 32.2 (*C*2′), 32.7 (*C*1′), 65.7 (*C*5), 68.3 (*C*4), 66.2 (*C*2), 70.8

(*C*3), 74.0 (*C*1), 167–170 (CO_{Ac}), 173.0 ($CONH_{Fuc}$); HPLC-MS (ESI): calcd for $C_{19}H_{30}N_2O_8$: 414.45; found: 415.1 $[M+H]^+$; HRMS (ESI): calcd for $[C_{19}H_{31}N_2O_8]$: 415.20749; found: 415.20800; calcd for $[C_{19}H_{30}N_2O_8Na]^+$: 437.18944; found: 437.18994.

*(1S,2S,4S,5R)-4,5-Diacetoxy-2-(methoxycarbonyl) cyclohexanecarboxylic acid (**15**)*: Et_3N (0.191 mL, 1.374 mmol; 2.0 equiv) was added to an ice-cooled solution of **4** (0.150 g, 0.687 mmol; 1 equiv) in CH_2Cl_2 (7.5 mL). TBDMSCl (0.156 g, 1.031 mmol; 1.5 equiv) in CH_2Cl_2 (1 mL) was added and the reaction mixture stirred. After 20 h, quantitative transformation to TBDMS ester was observed by TLC ($CHCl_3$/MeOH, 9:1). In a separate vessel, DMAP (0.017 g, 0.137 mmol; 0.2 equiv) and pyridine (0.168 mL, 2.06 mmol; 3 equiv) were dissolved in CH_2Cl_2, cooled to 0 °C, and Ac_2O (0.196 mL 2.06 mmol; 3 equiv) was added over a period of 30 min. This solution was added to the ice-cooled solution of the TBDMS ester. The reaction mixture was stirred for another 18 h and monitored by TLC ($CHCl_3$/MeOH, 9:1). The solvent was evaporated under vacuum, the residue dissolved in EtOAc, and water phase was washed with HCl (0.5 M) and brine. The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (petroleter/AcOEt, 1:1 +1% AcOH) to obtain a colorless oil, which gave 0.156 g of white crystalline solid (0.515 mmol) after drying under vacuum (75%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.76$ (dt, 1H, *H*3ax, $J_{3ax\text{-}3eq} = 14.4$ Hz, $J_{3ax\text{-}2} = 12$ Hz, $J_{3ax\text{-}4} = 2.4$ Hz), 2.02 (s, 3H, *Me*Ac), 2.08 (m, 1H, *H*6ax), 2.13 (s, 6H, *Me*Ac), 2.20 (dt, 1H, *H*6eq, $J_{6eq\text{-}6ax} = 12.8$ Hz, $J_{6eq\text{-}1} = J_{6eq\text{-}5} = 4.4$ Hz), 2.30 (dt, 1H, *H*3eq, $J_{3eq\text{-}3ax} = 14.4$ Hz, $J_{3eq\text{-}2} = J_{3eq\text{-}4} = 4.4$ Hz), 2.92 (td, 1H, *H*1, $J_{1\text{-}2} = 11.04$ Hz, $J_{1\text{-}6ax} = 11.04$ Hz, $J_{1\text{-}6eq} = 4.4$ Hz), 3.16 (td, 1H, *H*2, $J_{2\text{-}1} = 11.04$ Hz, $J_{2\text{-}3ax} = 11.04$ Hz, $J_{2\text{-}3eq} = 3.9$ Hz), 3.71 (s, 3H, *Me*OOC), 4.89 (dt, 1H, *H*5, $J_{5\text{-}6eq} = 4.4$ Hz, $J_{5\text{-}6ax} = 11.4$ Hz, $J_{5\text{-}4} = 2.8$ Hz), 5.32 (br d, 1H, *H*4); HPLC-MS: calcd for $[C_{13}H_{18}O_8Na]^+$: 325.0; found: 325.0; HRMS (ESI): calcd for $[C_{13}H_{18}O_8Na]^+$: 325.08939; found: 325.08966; calcd for $[C_{13}H_{17}O_8Na_2]^+$: 347.07133; found: 347.07154.

N-*((1S,2R)-2-(1S,2S,4R,5S)-4,5-Diacetoxy-1-(methoxycarbonyl) cyclohexane-2-(carboxamido)cyclohexancarboxyl)-2,3,4-tri*-O-*acetyl-α-*L*-fucopyranosylamine (**16**)*: Et_3N (12 μL, 0.085 mmol; 3 equiv) and **15** (11 mg, 0.035 mmol; 1.25 equiv) in 0.25 mL of CH_2Cl_2 were added to a solution of **3** (2 mg, 0.028 mmol; 1 equiv) in CH_2Cl_2 (0.30 mL). Subsequently, HBTU (16 mg, 0.042 mmol; 1.5 equiv) was added and the reaction mixture stirred at room temperature. After 18 h, CH_2Cl_2 (10 mL) was added to the reaction mixture and the organic phase was washed with NaOH (0.5 M), $KHSO_4$ (1 M), water, and brine. The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (hexane/AcOEt, 8:2) to yield 0.010 g (0.0145 mmol) of white solid (78%). ^{1}H NMR (400 MHz, $CHCl_3$): $\delta =$ 1.15 (d, $J_{Me\text{-}5} = 6.8$ Hz, 3H, *Me*Fuc), 1.42–1.92 (inseparable m, 8H, C3′*H*2, C4′*H*2, C5′*H*2, C6′*H*2), 1.72 (m, 1H, *H*6″ax), 1.85 (m, 1H, *H*3″), 1.85 (m, 1H, *H*3″ax), 1.98 (s, 3H, *Me*Ac), 2.01(s, 6H, *Me*Ac), 2.06 (m, 1H, *H*3″eq), 2.07 (m, 1H, *H*4′eq), 2.12 (s, 3H, *Me*Ac), 2.17 (s, 3H, *Me*Ac), 2.24 (dt, $J_{6''eq\text{-}1''} = J_{6eq\text{-}5''} = 4.0$ Hz, $J_{6''eq\text{-}6''ax} = 14.4$, 1H, *H*6″eq), 2.58 (dt, 1H, *H*2″, $J_{2''\text{-}3''eq} = 3.6$ Hz, $J_{2''\text{-}3''ax} = J_{2''\text{-}1''} = 13$ Hz), 2.64 (m, 1H, *H*1′), 3.01 (dt, 1H, *H*1″, $J_{1''\text{-}6''eq} = 4.0$ Hz, $J_{1''\text{-}6''ax} = J_{1''\text{-}2''} = 13$ Hz), 3.67 (s, 3H, *Me*OOC), 4.00 (dd, 1H, *H*5, $J_{5\text{-}Me} = 6.4$ Hz), 4.25 (br s, 1H, *H*2′), 4.80 (dt, 1H, *H*4″, $J_{4''\text{-}3''ax} = 12.4$ Hz, $J_{4''\text{-}3''eq} = 7.1$ Hz, $J_{4''\text{-}5''} = 4.4$ Hz), 5.23 (d, 1H, *H*3, $J_{3\text{-}4} = 3.2$ Hz), 5.26 (s, 1H, *H*5″), 5.36 (d, 1H, *H*4, $J_{4\text{-}3} = 3.2$ Hz), 5.38 (d, 1H, *H*2, $J_{2\text{-}3} = 10.4$ Hz, $J_{2\text{-}1} = 5.4$ Hz), 5.86 (t, 1H, *H*1, $J_{1\text{-}NHFuc} = 7.6$ Hz, $J_{1\text{-}2} = 5.4$ Hz), 6.48 (d, 1H, CO*NH*′, $J_{CONH'\text{-}2'} =$ 8.8 Hz), 6.72 (d, 1H, CO*NH*Fuc, $J_{NHFuc\text{-}1} = 7.6$ Hz). ^{13}C NMR (100 MHz, $CHCl_3$): $\delta = 16.1$ (*Me*Fuc), 20.6, 20.7, 20.7, 20.9, 21.0, (*CH*$_3$Ac), 22.6 (*C*5′), 22.9 (*C*6′), 26.5 (*C*3′), 28.0 (*C*4′), 28.2 (*C*3″), 31.7 (*C*6″), 39.3 (*C*1″), 44.7 (*C*2″), 45.3 (*C*1′), 47.1 (*C*2′), 52.0 (*Me*OOC), 65.7 (*C*5), 66.2 (*C*2), 67.3 (*C*4), 67.9 (*C*3), 70.50 (*C*4″), 70.55 (*C*5″), 74.4 (*C*1), 169.3, 170.1, 170.1, 170.2, 170.6, 172.3, 173.9, 174.5 (8×*CO*); HPLC-MS: calcd for $[C_{32}H_{47}N_2O_{15}]^+$: 699.7; found: 699.6; HRMS (ESI): calcd for $[C_{32}H_{46}N_2O_{15}Na]^+$: 721.27904; found: 721.27927.

N-*((1S,2R)-2-(1S,2S,4R,5S)-4,5-Dihydroxy-1-(methoxycarbonyl) cyclohexane-2-(carboxamido)cyclohexancarboxyl)-α-*L*-fucopyranosylamine (**2a**)*: NaOMe (31 μL, 1 M) was added to a solution of **16** (10 mg, 0.0143 mmol; 1 equiv) in dry MeOH (1.5 mL). The reaction mixture was stirred at room temperature for 2.5 h and the progress was followed by TLC (hexane/EtOAc, 2:8). Amberlite IRA 120$^+$ was added until pH ~7 and the beads were filtered off. The solvent was evaporated under vacuum to yield 6.6 mg (0.0135 mmol) of crude product as a white solid (94%). The product was purified with reversed-phase HPLC to yield 4.1 mg (8.4×10^{-3} mmol) of white solid (59%). Chromatographic conditions; column Atlantis dC18 (1.9×100 mm, 5 μm), 1 min of initial isocratic elution with a mixture of 0.1% formic acid and CH_3CN with 0.1% of formic acid (5:95, *v/v*) following elution with the gradient mobile phase: 5–50% mixture of 0.1% formic acid and CH_3CN with 0.1% of formic acid (5:95–50:50, *v/v*) in 7 min at a flow rate of 20.0 mL min^{-1} at 25 °C. ^{1}H NMR (600 MHz, CD_3OD): $\delta = 1.20$ (d, 1H, *H*MeFuc, $J_{MeFuc\text{-}5} = 6.3$ Hz), 1.37 (m, 1H, *H*4′ax), 1.49 (m, 1H, *H*5′ax), 1.55 (m, 1H, *H*3′ax), 1.60 (m, 1H, *H*6″ax), 1.62 (m, 1H, *H*5′eq), 1.66 (m, 1H, *H*6′ax), 1.74 (m, 1H, *H*4′eq), 1.80 (m, 1H, *H*3″ax), 1.84 (m, 1H, *H*3″eq), 1.89 (m, 1H, *H*6′eq), 1.92 (m, 1H, *H*3′eq), 2.14 (m, 1H, *H*6″eq), 2.66 (dt, 1H, *H*2″, $J_{2''\text{-}1''} = J_{2''\text{-}3''ax} = 12$ Hz, $J_{2''\text{-}3''eq} = 3.6$ Hz), 2.72 (m, 1H, *H*1′), 3.04 (dt, 1H, *H*1″, $J_{1''\text{-}2''} = J_{1''\text{-}6''ax} = 12$ Hz, $J_{1''\text{-}6''eq} = 3.6$ Hz), 3.60 (m, 1H, *H*4″), 3.64 (s, 3H, *Me*OOC), 3.65 (m, 1H, *H*4), 3.77 (d, 1H, *H*5, $J_{5\text{-}Me} = 6.3$ Hz), 3.80 (dd, 1H, *H*3, $J_{3\text{-}4} = 3.6$ Hz, $J_{3\text{-}2} = 10.8$ Hz), 3.94 (d,1H, *H*5″, $J_{5''\text{-}4''} =$ 6 Hz), 3.96 (dd, 1H, *H*2, $J_{2\text{-}1} = 6$ Hz, $J_{2\text{-}3} = 10.8$ Hz), 4.27 (br s, 1H, *H*2′), 5.48 (d, 1H, *H*1, $J_{1\text{-}2} = 6$ Hz). ^{1}H NMR (600 MHz, d-Tris buffer, pD 8, 150 mM NaCl, 4 mM $CaCl_2$): $\delta = 1.08$ (d, 1H, *H*MeFuc, $J_{MeFuc\text{-}5} =$ 7.1 Hz), 1.25 (m, 1H, *H*3″ax), 1.36 (m, 1H, *H*4′ax), 1.37 (m, 1H, *H*5′ax), 1.46 (m, 1H, *H*5′eq), 1.52 (m, 1H, *H*3′ax), 1.56 (m, 1H, *H*3″eq), 1.62 (m, 1H, *H*6″ax), 1.64 (m, 1H, *H*4′eq), 1.64 (m, 1H, *H*3′eq), 1.65 (m, 1H, *H*6′ax), 1.65 (m, 1H, *H*6′eq), 2.07 (td, 1H, *H*6″eq, $J_{6''eq\text{-}1''} = 14.4$ Hz, $J_{6''eq\text{-}5''} = 3.8$ Hz), 2.65 (td, 1H, *H*2″, $J_{2''\text{-}1''} = J_{2''\text{-}6''ax} = 11.6$ Hz, $J_{2''\text{-}6'eq'} = 4.5$ Hz), 2.72 (dt, 1H, *H*1′, $J_{1'\text{-}6'eq'} = J_{1'\text{-}2'} = 3.5$ Hz, $J_{1'\text{-}6'ax'} = 11.6$ Hz), 2.84 (ddd, 1H, *H*1″, $J_{1''\text{-}2''} = 11.7$ Hz, $J_{1''\text{-}6ax''} = 11.4$ Hz, $J_{1''\text{-}6''eq} = 3.8$ Hz), 3.59 (s, 3H, *Me*OOC), 3.64 (m, 1H, *H*4″), 3.67 (dd, 1H, *H*4, $J_{4\text{-}3} = 3.0$ Hz, $J_{4\text{-}5} = 5.3$ Hz,), 3.68 (m, 1H, H_5), 3.80 (dd, 1H, *H*3, $J_{3\text{-}2} = 11.2$ Hz, $J_{3\text{-}4} = 3.0$ Hz), 3.92 (dd,1H, *H*2, $J_{2\text{-}3} = 10.7$ Hz, $J_{2\text{-}1} =$ 5.6 Hz), 3.98 (m, 1H, *H*5″), 4.26 (d, 1H, *H*2′, $J_{2'\text{-}1'} = 3.4$ Hz), 5.40 (d, 1H, *H*1, $J_{1\text{-}2} = 6.0$ Hz), 7.76 (br s, 1H, CO*NH*′), 8.19 (br s, 1H, CO*NH*-Fuc); ^{13}C NMR (150 MHz, CD_3OD): $\delta = 16.1$ (*Me*Fuc), 19.9 (*C*5′), 22.7 (*C*6′), 23.0 (*C*4′), 30.0 (*C*3′), 30.5 (*C*5″), 32.7 (*C*6″), 38.6 (*C*1″), 44.3 (*C*2″), 45.9 (*C*1′), 47.3 (*C*2′), 52 (*Me*OOC), 65.8 (*C*2), 67.0 (*C*4, *C*5), 69.3 (*C*3), 67.7 (*C*5″), 70.2 (*C*4″), 76.8 (*C*1); HPLC-MS (ESI): calcd for $[C_{22}H_{36}N_2O_{10}Na]^+$: 488.2; found: 488.9; HRMS (ESI): calcd for $[C_{22}H_{36}N_2O_{10}Na]^+$: 511.22622; found: 511.22616.

N-*((1S,2R)-2-Acetamidocyclohexanecarboxyl)-α-*L*-fucopyranosylamine (**17**)*: Compound **3** (0.030 g, 0.072 mmol; 1 equiv) was dissolved in dry CH_2Cl_2 (2 mL); pyridine (7.1 μL, 0.087 mmol; 1.2 equiv) and Ac_2O (8.2 μL, 0.087 mmol; 1.2 equiv) were added, and the solution was stirred for 3 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with water (5 mL), and brine (5 mL), and the organic phase was dried over Na_2SO_4. The drying agent was filtered and the solvent was evaporated under vacuum. The residue was purified by flash chromatography (AcOEt). The resulting white solid was dissolved in dry MeOH (2.0 mL) and freshly prepared 1 M MeONa (129 μL) in dry MeOH was added to the solution. The reaction mixture was stirred at room temperature for 6 h (TLC hexane/

EtOAc, 2:8). Amberlite IRA 120$^+$ was added until pH ~ 7, and the beads were filtered off. The solvent was evaporated to yield 19.8 mg (0.0497 mmol) of crude product as a white solid (yield of two consecutive steps = 69 %). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.20$ (d, 1H, *H*MeFuc, $J_{MeFuc\text{-}5} = 6.4$ Hz), 1.35 (m, 1H, *H*5ax), 1.51 (m, 1H, *H*4ax), 1.56 (m, 2H, *H*4eq, *H*6ax), 1.65 (m, 1H, *H*6ax), 1.75 (m, 1H, *H*5eq), 1.86 (m, 1H, *H*6eq), 1.89 (m, 1H, *H*3eq), 1.95 (s, 3H, *Me*Ac), 2.74 (m, 1H, *H*1′), 3.32 (s, 3H, *Me*OOC), 3.63 (d,1H, *H*4, $J_{4\text{-}5} =$ 12.4 Hz), 3.73 (dd, 1H, *H*5, $J_{5\text{-}MeFuc} = 12.4$ Hz, $J_{5\text{-}4} = 6.0$ Hz), 3.78 (dd, 1H, *H*3, $J_{3\text{-}2} = 10.2$ Hz, $J_{3\text{-}4} = 3.2$ Hz), 3.95 (dd, 1H, *H*2, $J_{2\text{-}1} = 5.6$ Hz, $J_{2\text{-}3} = 10.2$ Hz), 4.34 (br s, 1H, *H*2′), 5.48 (d, 1H, *H*1, $J_{1\text{-}2} = 5.6$ Hz). ^{13}C NMR (100 MHz, CD_3OD): $\delta = 15.6$ (*Me*Fuc), 21.0 (*C*4′), 21.3 (*Me*Ac), 23.4 (*C*5′), 23.5 (*C*6′), 30.0 (*C*3′), 44.9 (*C*1′), 47.5 (*C*2′), 47.6 (*Me*OOC), 66.5 (*C*2), 66.8 (*C*5), 70.0 (*C*3), 71.9 (*C*4), 76.9 (*C*1), 171.2 (MeCONH), 176.0 (CONHFuc); HRMS (ESI): calcd for $[C_{15}H_{26}N_2O_6Na]^+$: 353.16831; found: 353.16795.

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[1] J. Banchereau, R. M. Steinman, *Nature* **1998**, *392*, 245–252.
[2] T. B. Geijtenbeek, D. S. Kwon, R. Torensma, S. J. Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, Y. van Kooyk, *Cell* **2000**, *100*, 587–597.
[3] Y. van Kooyk, T. H. B. Geijtenbeek, *Nat. Rev. Immunol.* **2003**, *3*, 697–709.
[4] a) J. J. Reina, S. Sattin, D. Invernizzi, S. Mari, L. Martínez-Prats, G. Tabarani, F. Fieschi, R. Delgado, P. M. Nieto, J. Rojo, A. Bernardi, *ChemMedChem* **2007**, *2*, 1030–1036; b) J. J. Reina, O. S. Maldonado, G. Tabarani, F. Fieschi, J. Rojo, *Bioconjugate Chem.* **2007**, *18*, 963–969; c) G. Tabarani, J. J. Reina, C. Ebel, C. Vivès, H. Lortat-Jacob, J. Rojo, F. Fieschi, *FEBS Lett.* **2006**, *580*, 2402–2408.
[5] M. J. Borrok, L. L. Kiessling, *J. Am. Chem. Soc.* **2007**, *129*, 12780–12785.
[6] N. Frison, M. E. Taylor, E. Soilleux, M.-T. Bousser, R. Mayer, M. Monsigny, K. Drickamer, A.-C. Roche, *J. Biol. Chem.* **2003**, *278*, 23922–23929.
[7] B. J. Appelmelk, I. van Die, S. J. van Vliet, C. M J. E. Vandenbroucke-Grauls, T. B. H. Geijtenbeek, Y. van Kooyk, *J. Immunol.* **2003**, *170*, 1635–1639.
[8] D. A. Mitchell, A. J. Fadden, K. Drickamer, *J. Biol. Chem.* **2001**, *276*, 28939–28945.
[9] S. Pöhlmann, E. J. Soilleux, F. Baribaud, G. J. Leslie, L. S. Morris, J. Trowsdale, B. Lee, N. Cleman, R. W. Doms, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 2670–2675.
[10] Y. Guo, H. Feinberg, E. Conroy, D. A. Mitchell, R. Alvarez, O. Blixt, M. E. Taylor, W. I. Weis, K. Drickamer, *Nat. Struct. Mol. Biol.* **2004**, *11*, 591–598.
[11] E. Van Liempt, C. M. C. Bank, P. Mehta, J. J. García-Vallejo, Z. S. Kawar, R. Geyer, R. A. Alvarez, R. D. Cummings, Y. van Kooyk, I. van Die, *FEBS Lett.* **2006**, *580*, 6123–6131.
[12] S. Meyer, E. van Liempt, A. Imberty, Y. van Kooyk, H. Geyer, R. Geyer, I. van Die, *J. Biol. Chem.* **2005**, *280*, 37349–37359.
[13] M. A. Naarding, I. S. Ludwig, F. Groot, B. Berkhout, T. H. B. Geitenbeek, G. Pollakis, W. A. Paxton, *J. Clin. Invest.* **2005**, *115*, 3256–3264.
[14] For a review on structural studies on the conformation of Lewis oligosaccharides see: E. Yuriev, W. Farrugia, A. M. Scott, P. A. Ramsland, *Immunol. Cell Biol.* **2005**, *83*, 709–717.
[15] E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C. H. Wong, *Chem. Rev.* **1998**, *98*, 833–862.
[16] N. Kaila, K. Janz, S. DeBernardo, P. W. Bedard, R. T. Camphausen, S. Tam, D. H. H. Tsao, J. C. Keith, C. Nickerson-Nutter, A. Shilling, R. Young-Sciame, Q. Wang, *J. Med. Chem.* **2007**, *50*, 21–39.
[17] N. Kaila, K. Janz, A. Huang, A. Moretto, S. DeBernardo, P. W. Bedard, S. Tam, V. Clerin, J. C. Keith, Jr., D. H. H. Tsao, N. Sushkova, G. D. Shaw, R. T. Camphausen, R. G. Schaub, Q. Wang, *J. Med. Chem.* **2007**, *50*, 40–64.
[18] A. Bernardi, A. Checchia, P. Brocca, S. Sonnino, F. Zuccotto, *J. Am. Chem. Soc.* **1999**, *121*, 2032–2036.
[19] A. Bernardi, D. Potenza, A. M. Capelli, A. García-Herrero, F. J. Cañada, J. Jiménez-Barbero, *Chem. Eur. J.* **2002**, *8*, 4597–4612.
[20] A. Bernardi, D. Arosio, L. Manzoni, D. Monti, H. Posteri, D. Potenza, S. Mari, J. Jiménez-Barbero, *Org. Biomol. Chem.* **2003**, *1*, 785–792.
[21] A. Bernardi, D. Arosio, D. Potenza, I. Sanchez-Medina, S. Mari, F. J. Cañada, J. Jiménez-Barbero, *Chem. Eur. J.* **2004**, *10*, 4395–4406.
[22] A. Bernardi, C. Podlipnik, J, Jiménez-Barbero in *Protein-Carbohydrate Interactions in Infectious Diseases* (Ed.: C. A. Bewley), RSC, Cambridge, **2006**, pp. 73–91.
[23] a) H. Kunz, C. Unverzagt, *Angew. Chem.* **1988**, *100*, 1763–1765; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1697–1699; b) S. Peters, T. L. Lowary, O. Hindsgaul, M. Meldal, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1995**, 3017–3022; c) C. Unverzagt, H. Kunz, *Bioorg. Med. Chem.* **1994**, *2*, 1189–1201.
[24] A. Dondoni, N. Catozzi, A. Marra, *J. Org. Chem.* **2004**, *69*, 5023–5036.
[25] a) A. Bianchi, A. Bernardi, *Tetrahedron Lett.* **2004**, *45*, 2231–2234; b) A. Bianchi, A. Bernardi, *J. Org. Chem.* **2006**, *71*, 4565–4577.
[26] F. Damkaci, P. DeShong, *J. Am. Chem. Soc.* **2003**, *125*, 4408–4409.
[27] a) A. J. Ratcliffe, B. Fraser-Reid, *J. Chem. Soc. Perkin Trans. 1* **1989**, 1805–1810; b) A. J. Ratcliffe, P. Konradsson, B. Fraser-Reid, *Carbohydr. Res.* **1992**, *216*, 323–335.
[28] One example of α-linked glycopeptide has been described: S. Shibata, T. Takeda, Y. Natori, *J. Biol. Chem.* **1988**, *263*, 12483–12485.
[29] A. Bernardi, D. Arosio, L. Manzoni, F. Micheli, A. Pasquarello, P. Seneci, *J. Org. Chem.* **2001**, *66*, 6209–6216.
[30] a) http://www.schrodinger.com/ProductDescription.php?mID = 6&sID = 8&cID = 0; b) F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
[31] a) J. Frackenpohl, P. I. Arvidsson, J. V. Schreiber, D. Seebach, *ChemBioChem* **2001**, *2*, 445–455; b) H. N. Gopi, G. Ravindra, P. P. Pal, P. Pattanaik, H. Balaram, P. Balaram, *FEBS Lett.* **2003**, *535*, 175–178.
[32] a) A. Bernardi, D. Arosio, L. Manzoni F. Micheli, A. Pasquarello, P. Seneci, *J. Org. Chem.* **2001**, *66*, 6209–6216; b) A. Bernardi, D. Arosio, D. Dallavecchia, F. Micheli, *Tetrahedron: Asymmetry* **1999**, *10*, 3403–3407.
[33] a) M. Hayashi, H. Kawabata, *Recent Dev. Carbohydr. Res.* **2003**, *1*, 195–208; b) S. Cao, R. Roy, *Carbohydr. Lett.* **2006**, *2*, 27.
[34] I. Atodiresei, I. Schiffers, C. Bolm, *Chem. Rev.* **2007**, *107*, 5683–5712; and references therein.
[35] T. Mukaiyama, R. Matsueda, M. Suzuki, *Tetrahedron Lett.* **1970**, *11*, 1901–1904.
[36] G. Zemplen, *Ber. Dtsch. Chem. Ges.* **1927**, *60*, 1554–1564.
[37] a) M. Mayer, B. Meyer, *Angew. Chem.* **1999**, *111*, 1902–1906; *Angew. Chem. Int. Ed.* **1999**, *38*, 1784–1788; b) J. Klein, R. Meinecke, M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **1999**, *121*, 5336–5337; c) M. Vogtherr, T. Peters, *J. Am. Chem. Soc.* **2000**, *122*, 6093–6099.
[38] M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 6108–6117.
[39] F. Halary, A. Amara, H. Lortat-Jacob, M. Messerle, T. Delaunay, C. Houlès, F. Fieschi, F. Arenzana-Seisdedos, J. F. Moreau, J. Déchanet-Merville, *Immunity* **2002**, *17*, 653–664.

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