Synthesis and binding properties of divalent and trivalent clusters of the Lewis a disaccharide moiety to *Pseudomonas aeruginosa* lectin PA-IIL

Karine Marotte,[a] Cathy Préville,[a] Charles Sabin,[b] Myriame Moumé-Pymbock,[a] Anne Imberty*[b] and René Roy*[a]

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The synthesis of oligomeric glycocomimetics has been performed for targeting the *Pseudomonas aeruginosa* PA-IIL lectin, which is of therapeutical interest for anti-adhesive treatment. The disaccharide α-L-Fuc*p*-(1→4)-β-D-GlcNAc, which is a high-affinity ligand of the lectin, has been coupled to dimeric and trimeric linkers with various lengths and geometries. A series of linear dimers displayed an efficient clustering effect and a very strong affinity, with a lower dissociation constant of 90 nM. The trimeric compound was less efficient in inhibition assays but displayed high affinity in solution. Titration microcalorimetry and molecular modeling allowed in-depth analysis and rationalization of the binding data. These glycoclusters could act by crosslinking the lectins present on the surface of bacteria and therefore interfere with host recognition or biofilm formation.

Introduction

Many pathogens exploit host cell-surface glycoconjugates as receptors for attachment, tissue colonization and/or invasion.[1] At the cell surface, the glycan structures offer a wide range of diversity for encoding information, a fact directly tied to the variability of possible isomeric configurations of monosaccharides, increased even further when taking into account the possible linkages between the sugar units.[2] As a counterpart of this variety of carbohydrate structures, pathogenic bacteria use a panel of carbohydrate-binding proteins such as toxins, soluble lectins or fimbrial adhesins.[3,4]

While plant and animal lectins display weak affinity for their carbohydrate ligands (millimolar range), a very different behavior is observed for bacterial lectins that interact with high affinity with glycoconjugates[5]. As a consequence, the design of glycomimetics that could interfere in host recognition and adhesion is an attractive antibacterial strategy.[6] In addition, glycoclusters and glycodendrimers can be constructed in order to increase the affinity of the ligands for the lectins by a "glycoside cluster effect".[7] Di-, tri- or multimeric glycoclusters, either in the form of polymeric or dendrimeric materials, have been used for a variety of biological applications.[7–9] Such multivalent analogs strongly enhanced the binding of glycoconjugates to microbial proteins with an avidity well above that expected from monomeric glycomimetics.[9]

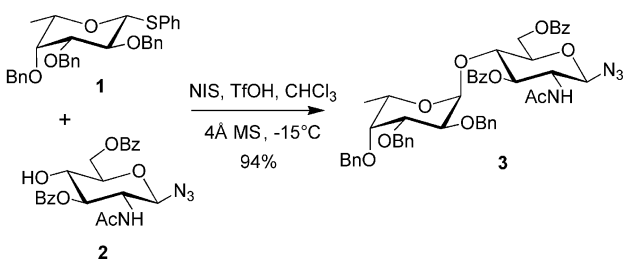
We focused our work on PA-IIL, a fucose-binding lectin from *Pseudomonas aeruginosa*, an opportunistic bacteria responsible for nosocomial infection that can cause in particular life-threatening damage to cystic fibrosis patients. The lectin has been widely characterized by both biochemical and structural approaches.[10–12] This soluble lectin is located on the outer membrane of the bacteria and it has been proposed to play a role in host recognition, adhesion and biofilm formation.[13] The isolated protein displays micromolar affinity for L-fucose (Fuc) and ten times higher affinity when this residue is attached to position 4 of *N*-D-acetylglucosamine (GlcNAc), as in the case of the Lewis a (Le[a]) antigen.[14] The lectin quaternary structure consists of a ball-shaped tetramer.[15] It is unlikely that multivalent glycoconjugates possessing short interglycosidic linkers can bind to different sites of the same tetrameric proteins, but it is expected that dimeric or trimeric ligands would be very efficient in the formation of often insoluble cross-linked complexes, resulting in higher avidity.

Results and discussion

Synthesis of dimeric and trimeric ligands

For the synthesis of both flexible and more rigid dimeric glycoclusters, oligoethylene glycols and methyl 3,5-bis(prop-2-ynyloxy)benzoate were used as linkers, respectively.[16] Different sizes of oligomeric ethylene glycol linkers were initially evaluated with the aim of optimizing the distance between two disaccharidic residues.

The key disaccharide **3**, bearing an azide aglycone, was prepared according to Scheme 1 following recently described optimized conditions.[17] The corresponding linkers were functionalized with

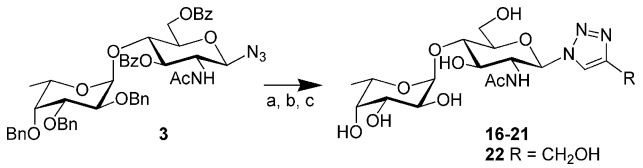


Scheme 1 Synthesis of the key α-L-Fuc*p*-(1→4)-β-D-GlcNAc azide derivative.

[a]*Département de Chimie et Biochimie, Université du Québec à Montréal, Case Postale 8888, Succ. Centre-Ville, Montréal, Québec, Canada H3C 3P8. E-mail: roy.rene@uqam.ca; Fax: +1 (514)987-4054; Tel: +1 (514)987-3000#2546*
[b]*CERMAV, BP53, 38041 Grenoble cedex 9, France. E-mail: Anne.Imberty@cermav.cnrs.fr; Fax: +33 476 547203; Tel: +33 476 037636*

alkyne end groups to be coupled to glycosyl azide **3** by a copper(I)-catalyzed 1,3-dipolar cycloaddition ("click chemistry").[18,19]

First, disaccharide **3** was coupled to dimeric linker **4**[16] using the original $CuSO_4$/sodium ascorbate conditions.[18] At room temperature (24 h), the yield of dimers **10** was only 48%, presumably because of the poor solubility of the reactants under the conditions employed (*t*-BuOH–H_2O). However, the yield could be raised to 83% when the reaction was run at 55 °C for 40 minutes. Hence, for the synthesis of the remaining dimers and trimers, the more organic-like conditions using soluble copper(I) salt were chosen (CuI, THF, DIPEA) (Scheme 2).[19]



Scheme 2 Synthesis of oligomeric ligands. *Reagents and conditions*: (a) for coupling conditions and linkers, see Table 1; (b) MeONa, MeOH; (c) H_2, $Pd(OH)_2$/C, MeOH

An excess of disaccharide **3** and one equivalent of the ethylene glycol linkers **5**,[20] **6**,[20] **7**,[21] or **8**[21] were dissolved in tetrahydrofuran and treated at room temperature with copper iodide (0.6 eq.) and DIPEA (2.0 eq.). Dimers **11–14**, as single 1,4-regioisomers, were obtained in very good yields (Table 1). The coupling reaction between 3.1 equivalents of **3** and the trifunctionalized linker **9**[22] in THF, catalysed by CuI and DIPEA, gave trimer **15** in excellent yield (85%).

The deprotection steps of the carbohydrate moieties occurred following a sequence of debenzoylation using a catalytic amount of sodium methanolate in methanol and debenzylation with hydrogen catalysed by palladium hydroxide on carbon. The final yields are reported in Table 1.

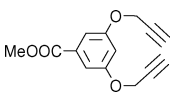
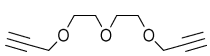
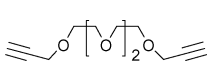
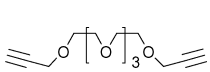
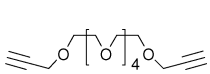
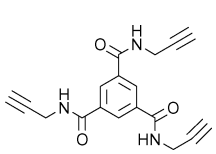
Interaction with PA-IIL

Dimeric and trimeric ligands were then evaluated for their relative capacity to inhibit the binding of biotinylated polymeric L-fucose to immobilized PA-IIL on the surface of microtitre plates, as previously described for the assays using milk oligosaccharides.[14] Two different strategies were used: the first one involved immobilized lectin following detection by biotinylated polyacrylamide–fucose and the second one with polyacrylamide–fucose immobilized in the wells and detection of binding by biotinylated PA-IIL. Since at the present time it is not clear if the lectin acts as a mobile entity in the extracellar medium or as one fixed on the bacterial cell surface, these two methodologies allowed both situations to be simulated.

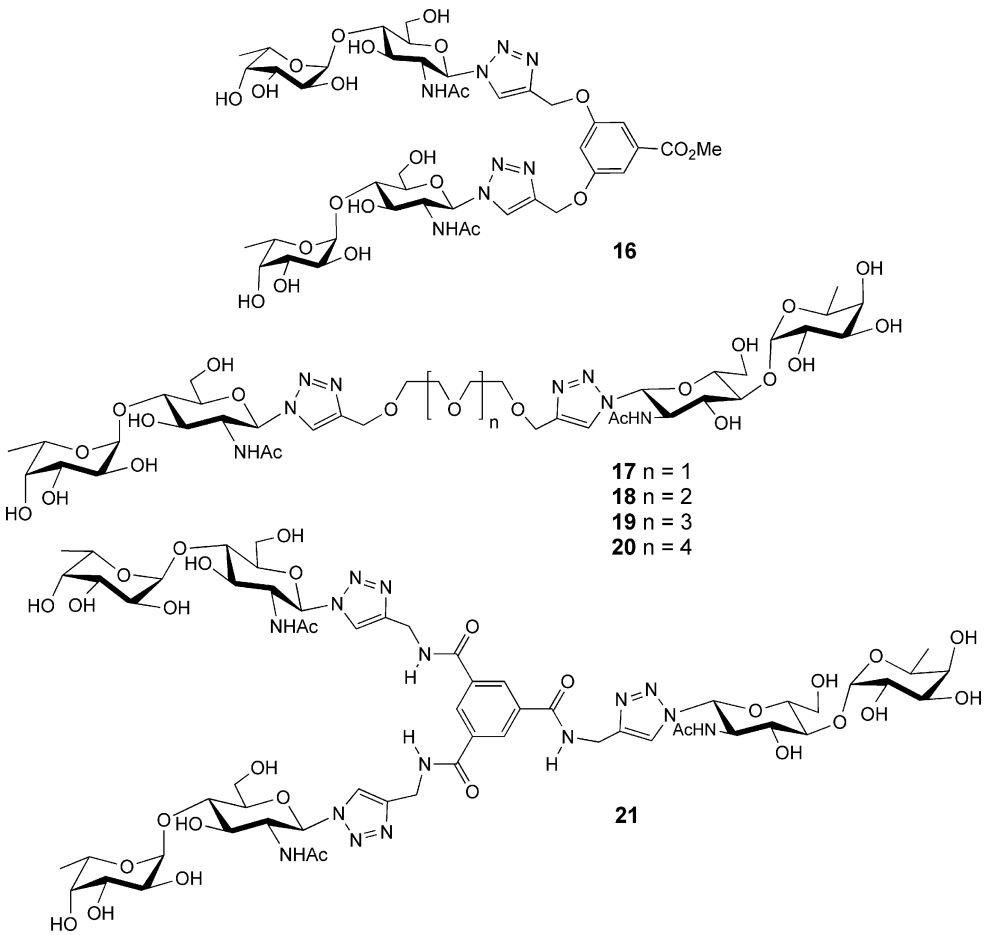
All dimeric glycoclusters displayed competition power two or three times better than for the entire Lewis a trisaccharide. The differences due to both coating methods used were generally negligible. For the linear molecules, the IC_{50} did not seem to depend on the length of the linkers, but rather on its geometry in space, since **16**, having a more rigid methyl 3,5-dihydroxybenzoate core, was the most potent inhibitor in the ELLA tests, with an IC_{50} value of 0.24 μM. The flexibility of the clusters also seemed to play an important role, since the trimeric construct **21**, with rather rigid branches, did not perform significantly better than the Lewis a trisaccharide in the ELLA assays.

It was previously demonstrated that the whole Lewis a trisaccharide is not necessary to obtain high affinity and that several α-L-Fuc*p*-(1→4)-β-D-GlcNAc derivatives displayed similar IC_{50} values.[17] A series of triazole derivatives have been recently synthesized and assayed, and we could check that the presence of the triazole ring does not hamper the interaction with the bacterial

Table 1 Dimer and trimer synthesis

Azide	Multivalent linker		Coupling conditions	Product	Yield (%)	Deprotected compound[a]	Yield (%)[b]
3		**4**	$CuSO_4$, ascorbic acid, *t*BuOH–H_2O (1 : 1), 55 °C, 40 min	**10**	83	**16**	96
3		**5**	CuI, DIPEA, THF, rt, overnight	**11**	Quant.	**17**	95
3		**6**	CuI, DIPEA, THF, rt, overnight	**12**	Quant.	**18**	55
3		**7**	CuI, DIPEA, THF, rt, overnight	**13**	81	**19**	79
3		**8**	CuI, DIPEA, THF, rt, overnight	**14**	Quant.	**20**	56
3		**9**	CuI, DIPEA, THF, rt, overnight	**15**	85	**21**	65

[a] For structural formulae, see Scheme 3. [b] Yields are for two steps: debenzoylation and debenzylation.



Scheme 3 Glycoclusters tested for the interaction with PA-IIL.

lectin.[17] The inhibition potency of the monovalent compound **22** is displayed in Fig. 1 for comparison.

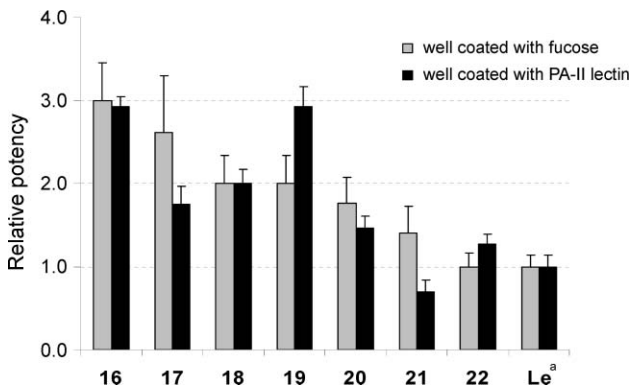


Fig. 1 Inhibition potency (relative to Lewis a trisaccharide with IC_{50} of 0.65 μM) of several compounds towards PA-IIL–fucose interaction. (Data for compound **22** are taken from ref. 17).

Binding affinity with PA-IIL

In all binding assays, the addition of dimeric or trimeric glycoclusters to PA-IIL resulted in precipitation of the protein. Consequently, all of the tested multivalent ligands have the proper geometry to cross-link the PA-IIL protein tetramer, resulting in an insoluble three-dimensional network. The microcalorimetry assays were therefore performed at low concentration of protein, and only limited precipitation was observed. Fig. 2 displays a typical ITC curve obtained when titrating PA-IIL with a dimeric ligand, with a very sharp decrease in the amplitude of the exothermic peaks, correlated with the high affinity of binding.

ITC experiments have been performed on selected compounds, *i.e.* trimeric ligand **21**, dimeric ligand **16** and dimeric compound **20** (which has the longest linker in the series **17**–**20**). As displayed in Table 2, the two divalent compounds **16** and **20** displayed stoichiometries of 0.6 to 0.7, indicating that the disaccharides at both extremities bound efficiently in solution. The longest linear dimer **20** had the highest affinity for PA-IIL, with a dissociation constant of 90 nM.

Surprisingly, the order of affinities obtained in solution were almost opposite to those measured with PA-IIL attached to a plastic surface. The flexible dimer **20** behaved similarly in both situations, with affinities twice as high as that for Le^a. The inhibitory activity, when divided by the number of ligands on **20**, was therefore identical to the one obtained for Le^a. In contrast, **16**, which is a powerful inhibitor in the surface assay, had half the inhibitory power in solution, although the *n* value close to $\frac{1}{2}$ indicated its ability to cross-link PA-IIL. Trimer **21** behaved better

Table 2 Microcalorimetry data for the interaction of PA-IIL with Lewis a and multimeric compounds

	$K_a \times 10^{-4}/M^{-1}$ [a]	K_d/nM	n	$-\Delta G$/kJ mol^{-1}	$-\Delta H$/kJ mol^{-1}	$-T\Delta S$/kJ mol^{-1}
Le^a [b]	470	210	1.08	38.1	35.0	−3.1
16	576	170	0.62	38.6	73.9	35.3
20	1104	90	0.66	40.2	69.5	29.3
21	968	100	1.11	39.9	37.2	−2.7
22[c]	320	310	0.98	37.1	43.4	6.3

[a] Experimental data are averaged from three independent experiments and standard deviations are lower than 10%. [b] Data from ref. 14. [c] Data from ref. 17.

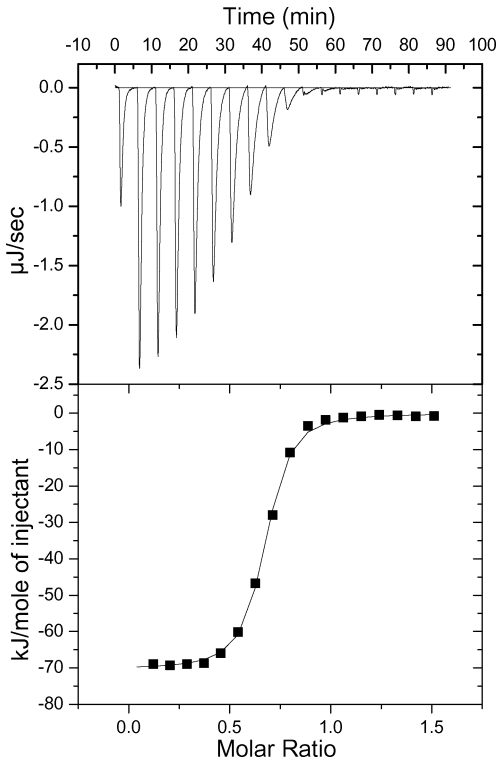


Fig. 2 ITC analysis of the interaction of PA-IIL (18 µM) with compound **20** (0.21 mM). Upper panel: data obtained from 18 injections (10 µL each) of **20** in the PA-IIL-containing cell. Lower panel: plot of the total heat released as a function of total ligand concentration. The solid line represents the best fit obtained with a one-site model.

in solution, with a high affinity, but its stoichiometry was close to 1, indicating that, when the ligand was not strongly concentrated, it had a tendency to bind to only one PA-IIL tetramer.

Analysis of the thermodynamical contribution (Table 2) demonstrated that both dimeric molecules paid a high entropic price for binding, compensated for by the very strong enthalpy of binding. Interestingly, trimer **21**, which also displayed high affinity, had a very different behavior in solution. Once one disaccharide extremity was bound, the remaining two were not readily accessible to other proteins.

The rigidity of **21** resulted in a slightly favorable entropy of binding. Nevertheless, precipitation of the protein solution was observed when the ligand concentration reached a two-fold excess, indicating that it was also capable of bridging the binding sites of several proteins.

Molecular modeling

In order to evaluate the potential cross-linking abilities of the various glycoclusters to form insoluble cross-linked lattices between different lectins, modeling experiments were conducted. The molecular modeling study was performed in two steps. All ligands were built using the disaccharide conformation observed in the crystal structure of PA-IIL complexed with a αFuc14GlcNAc derivative,[17] that corresponds closely to the main low energy conformation in solution.[23]

All linkers were generated in their most extended conformation, yielding fucose–fucose distances of 26 Å for **16**, and distances increasing from 30 Å to 41 Å for compounds **17**–**20**. For the trimeric compound **21**, the three arms could be arranged on the same side of the central ring, yielding a bowl-shaped molecule with distances of 22 Å between terminal fucose residues, or they could be arranged with one arm pointing to the other sides, resulting in a longer distance between two of the fucose residues (30 Å).

The complexes with PA-IIL were built by fitting the disaccharide at each of the extremities in the position that it occupies in coordination with two calcium ions, in one binding site of a PA-IIL tetramer. The models could be built with no steric conflict (Fig. 3), except for trimer **21** when the three arms were oriented on the same face of the ring. The model displayed in Fig. 3C corresponds therefore to the other conformation of **21** with two fucose residues far apart.

Discussion and conclusion

Dimeric and trimeric linkers were coupled to disaccharide **3** and then deprotected in good yields. This resulted in the synthesis of five dimeric clusters and one trimeric cluster bearing the αFuc14GlcNAc epitopes with various geometries. There have been a limited number of fucosylated dendrimer syntheses reported in the literature recently,[24–26] but the present work describes the first synthesis of glycoclusters bearing the αFuc14GlcNAc disaccharide.

Molecular modeling was performed in order to rationalize the experimental binding data obtained in the presence of the bacterial lectin PA-IIL. Indeed, the series **17**–**20** allowed for easy and independent binding of two tetramers. Even the shorter linker resulted in the terminal fucosides being 30 Å apart (Fig. 3B), explaining the observed stoichiometry and cross-linking properties. The enthalpy is doubled when compared to monomeric Lewis a, since both disaccharides can bind efficiently. As described above, the entropy term does not follow in direct proportion to the valency.[27] In the

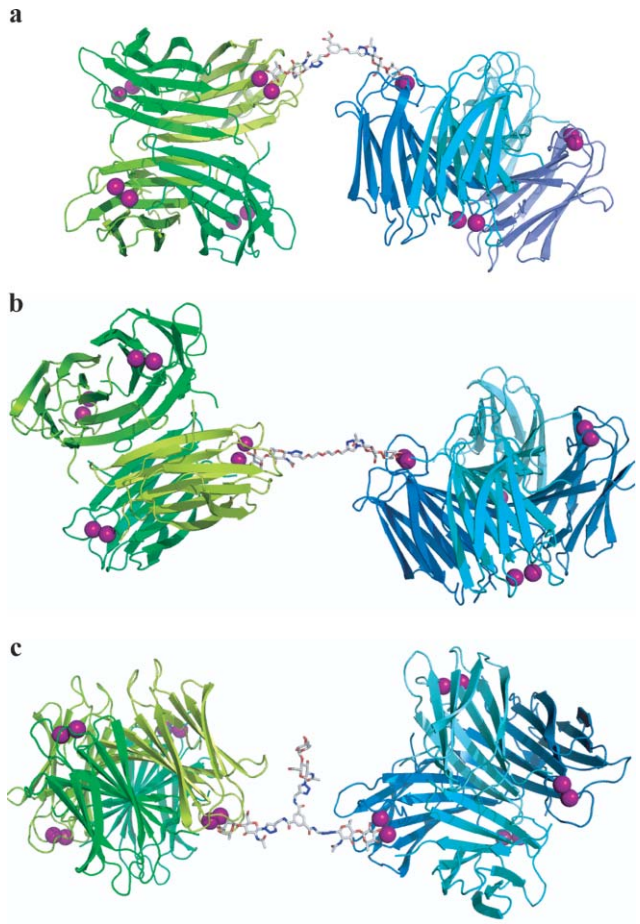


Fig. 3 Models of compounds (a) **16**, (b) **17** and (c) **21** interacting with two tetramers of PA-IIL. Polypeptide chains are represented by ribbons, calcium ions by purple spheres and synthetic ligands by sticks. Hydrogen atoms are not displayed for sake of clarity.

present case, a large entropic cost arises when two Lewis a moieties are joined by a linker.

The more rigid dimeric **16**, having a shorter inter-fucosidic distance of 26 Å, had the same overall behavior to that of the slightly more elongated dimer **17** possessing an inter-fucosidic distance of 30 Å. However, the entropy cost for binding is higher, resulting in a lower affinity constant in solution. In the present state, it is difficult to associate this phenomenon to flexibility difference or to an effect on solvent. This compound had the higher inhibitory power in ELLA assays, indicating that it may be more efficient on more concentrated proteins.

Trimeric glycocluster **21** could not cross-link PA-IIL in dilute solution. Nevertheless, this more rigid compound did not have any entropic cost upon binding, and the resulting affinity determined by ITC was almost as strong as that for the longest linear dimer **20**. The weak inhibitory potency observed in the ELLA test confirmed that the cross-linking was not efficient.

The multivalent glycoclusters studied here have not shown the ability to simultaneously bind to two different sites of one PA-IIL tetramer, and therefore no huge increase in affinity could be observed. Different strategies may be used in the future, such as chemically modifying the high affinity disaccharide or producing highly multivalent clusters. Nevertheless, the series of linear dimers **17**–**20** displayed an efficient cluster effect while keeping the natural high affinity of PA-IIL for the αFuc14GlcNAc ligand. The dissociation constant of 90 nM obtained for **20** made it the highest affinity ligand ever reported for this lectin. Since PA-IIL is associated to the outer membrane of *Pseudomonas aeruginosa*, we expect that high affinity cross-linking compounds will either precipitate the lectins away from the bacteria, or agglutinate bacteria themselves. The effect on biofilm formation and infection in animal models is currently under investigation.

Experimental

General methods

When needed, reactions were run under an atmosphere of dry nitrogen using oven-dried glassware and freshly distilled and dried solvents. THF was distilled from sodium benzophenone ketyl. All reagents were purchased from commercial suppliers and used without further purification. Analytical thin-layer chromatography (TLC) was performed using silica gel $60F_{254}$ precoated plates (0.2 mm thick) with a fluorescent indicator from Merck (Germany). Detection was done with molybdate solution or 5% H_2SO_4 in EtOH. Flash chromatography was performed using silica gel 60 Å (40–63 μm) from Silicycle Chemical division, Quebec. Chromatographic eluents are given as volume-to-volume ratios. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini300 NMR spectrometer at 300 and 75.5 MHz respectively. Routine spectra were referenced to TMS or to the residual proton or carbon signals of the solvent. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broadened (b). Some assignments were supported by 2D homonuclear chemical-shift correlation spectroscopy (COSY). Melting points are uncorrected. Optical rotations were measured at room temperature in quartz cells using a Perkin–Elmer JASCO P-1010 instrument in $CHCl_3$ or in the solvent indicated. ESI-MS analyses were carried out on a MICROMASS Quattro LC instrument.

General procedure for debenzoylation

The protected compound was dissolved in methanol at room temperature (or in a methanol–THF mixture, to allow complete solubilisation of the product). A catalytic amount of sodium methoxide (0.1 eq.) was added to this solution. The mixture was stirred at room temperature until completion of the reaction and neutralized with H^+ resin (amberlyst IR120). After filtration and concentration, the product was purified by column chromatography on silica gel.

General procedure for debenzylation

Benzyl ether was dissolved in methanol (0.02–0.05 M) and hydrogenolyzed on 20 wt% palladium hydroxide on carbon at room temperature. After completion of the reaction, filtration through Celite, evaporation and, if necessary, chromatography or filtration through a microfilter, gave the desired product.

Synthesis of dimeric and trimeric compounds 16–21

Tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl azide 3. Thioglycoside acceptor **2**[28] (500 mg, 1.1 mmol) and donor **1**[29] (695.0 mg, 1.3 mmol, 1.2 eq.) were dissolved in dry chloroform (16.5 mL, 0.1 M) and stirred for 30 minutes in the presence of 4 Å molecular sieves at room temperature under a nitrogen atmosphere. The solution was then cooled to −15 °C before addition of *N*-iodosuccinimide (322 mg, 1.4 mmol, 1.3 eq.) and triflic acid (78 μL, 0.88 mmol, 0.8 eq.). After 1 h at −15 °C, the reaction mixture was neutralized with a few drops of triethylamine, filtered through Celite, and extracted with dichloromethane. The mixture was washed with 10% sodium thiosulfate solution and water. The aqueous layer was extracted twice with dichloromethane. The combined organic phases were dried over sodium sulfate and concentrated. The crude product was purified by column chromatography on silica gel (hexane–ethyl acetate 1 : 1) to give the disaccharide **3** (900.2 mg, 94%) as a white solid. Precipitation of the product in diethyl ether gave a white solid. m.p.: 164–166 °C. $[\alpha]_D^{22} = -68.3$ (*c* 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.64$ (d, 3H, $^3J_{6b-5b} = 6.6$ Hz, H-6b), 1.78 (s, 3H, CH_3CO), 3.48 (b, 1H, H-4b), 3.71 (m, 1H, H-5b), 3.85 (dd, 1H, $^3J_{3b-4b} = 2.5$ Hz, $^3J_{3b-2b} = 10.2$ Hz, H-3b), 3.90 (m, 1H, H-5a), 3.95 (dd, 1H, $^3J_{2b-1b} = 3.3$ Hz, H-2b), 4.01 (t, 1H, $^3J_{4a-3a} = {}^3J_{4a-5a} = 9.1$ Hz, H-4a), 4.17 (m, 1H, H-2a), 4.51 (d, 1H, $^2J = 11.5$ Hz, CH_2Ph), 4.63–4.74 (m, 4H, 2 × CH_2Ph, H-1a, H-6a), 4.78 (d, 1H, $^2J = 11.5$ Hz, CH_2Ph), 4.81 (d, 1H, $^2J = 12.1$ Hz, CH_2Ph), 4.84 (d, 1H, $^2J = 11.5$ Hz, CH_2Ph), 4.87 (d, 1H, H-1b), 4.96 (dd, 1H, $^3J_{6a-5a} = 2.2$ Hz, $^2J = 12.4$ Hz, H-6a), 5.41 (dd, 1H, $^3J_{3a-2a} = 8.5$ Hz, H-3a), 5.74 (d, 1H, $^3J_{NH-2a} = 9.3$ Hz, N*H*Ac), 7.18–7.98 (m, 21H, H-Ar), 7.97 (2 × bd, 2 × 2H, $^3J = 8.5$ Hz, H-Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta =$ 16.0, 23.1, 53.8, 62.8, 67.7, 72.7, 74.0, 74.3, 74.8, 75.2, 75.5, 75.5, 76.6, 79.2, 88.5, 100.00, 127.4, 127.5, 127.6, 127.8, 128.1, 128.3, 128.4, 128.4, 128.5, 129.0, 129.8, 129.9, 133.1, 133.6, 138.0, 138.3, 138.5, 166.0, 167.0, 170.3 ppm. ESI-MS: $m/z = 893.4$ $[M + Na]^+$.

Protected dimer 10. To a solution of the disaccharide **3** (150.0 mg, 0.17 mmol, 2.1 eq.) and bis-propargylated linker **4**[16] (20.0 mg, 0.082 mmol, 1.0 eq.) in a *tert*-butanol–water mixture (1 : 1, 0.04 M) were added copper sulfate (17.2 mg, 0.069 mmol, 0.8 eq.) and ascorbic acid (27.2 mg, 0.14 mmol, 1.6 eq.). The solution was white and milky. After 40 minutes at 55 °C, the reaction mixture, which now contained an orange precipitate, was extracted with ethyl acetate. The organic layer was washed successively with saturated sodium bicarbonate solution and with brine, dried over sodium sulfate and concentrated. The crude product was purified by column chromatography on silicagel (CH_2Cl_2–MeOH 30 : 1) to give the desired triazole **10** (134.2 mg, 83%) as a white, amorphous solid. $[\alpha]_D^{22} = -42.3$ (*c* 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.60$ (d, 6H, $^3J_{6b-5b} = 6.6$ Hz, 6 × H-6b), 1.52 (s, 6H, 2 × CH_3CO), 3.57 (b, 2H, 2 × H-4b), 3.77 (m, 2H, 2 × H-5b), 3.81–3.83 (m, 5H, CH_3O, 2 × H-3b), 3.97 (dd, 2H, $^3J_{2b-1b} = 3.0$ Hz, $^3J_{2b-3b} = 10.4$ Hz, 2 × H-2b), 4.16–4.28 (m, 4H, 2 × H-4a, 2 × H-5a), 4.48 (d, 2H, $^2J = 11.3$ Hz, CH_2Ph), 4.54–4.87 (m, 14H, 2 × H-2a, 2 × H-6a, 10 × CH_2Ph), 4.93 (d, 2H, 2 × H-1b), 5.04 (bd, 2H, $^2J = 11.8$ Hz, 2 × H-6a), 5.10 (b, 4H, × H-e), 5.80 (m, 2H, 2 × H-3a), 6.07 (bd, 2H, $^3J_{1a-2a} = 9.1$ Hz, 2 × H-1a), 6.39 (b, 2H, 2 × N*H*Ac), 6.68 (b, 1H, H-d), 7.13–7.61 (m, 44H, H-Ar, 2 × H-c), 7.90 (s, 2H, H-triazole), 7.98–8.06 (m, 8H, H-Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 15.9$, 22.6, 29.7, 52.2, 53.8, 61.9, 62.5, 67.8, 72.3, 74.4, 75.0, 75.8, 77.3, 79.3, 86.2, 100.9, 106.0, 106.7, 108.8, 127.4, 127.5, 127.8, 128.1, 128.2, 128.4, 128.5, 128.6, 128.7, 128.9, 129.6, 129.7, 129.9, 132.0, 133.2, 133.8, 137.8, 138.3, 138.5, 159.1, 165.8, 166.5, 167.3, 170.5 ppm. ESI-MS: $m/z = 2008.8$ $[M + Na]^+$.

Protected dimer 11. To a solution of the disaccharide **3** (100.0 mg, 0.11 mmol, 2.0 eq.) and the bis-propargylated linker **5** (10.3 mg, 0.056 mmol, 1.0 eq.) in tetrahydrofuran (1.15 mL), were added copper iodide (6.6 mg, 0.035 mmol, 0.6 eq.) and diisopropylethylamine (20 μL, 0.11 mmol, 2.0 eq.). The reaction was stirred overnight at room temperature and then concentrated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 30 : 1) to give the dimer **11** (120.7 mg, quant.) as a slightly yellow oil. $[\alpha]_D^{21} = -37.5$ (*c* 0.65, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.66$ (d, 6H, $^3J_{6b-5b} =$ 6.4 Hz, 6 × H-6b), 1.55 (s, 6H, 2 × CH_3CO), 3.52–3.61 (m, 10H, 2 × H-4b, 8 × CH_2O), 3.80 (q, 2H, 2 × H-5b), 3.92 (dd, 2H, $^3J_{3b-4b} = 2.5$ Hz, $^3J_{3b-2b} = 10.3$ Hz, 2 × H-3b), 4.01 (dd, 2H, $^3J_{2b-1b} =$ 3.3 Hz, 2 × H-2b), 4.18 (m, 2H, 2 × H-5a), 4.28 (m, 2H, 2 × H-4a), 4.54 (d, 2H, $^2J = 11.4$ Hz, 2 × CH_2Ph), 4.56–4.78 (m, 12H, 2 × H-6a, 6 × CH_2Ph, 4 × O-CH_2-triazole), 4.83 (m, 2H, 2 × H-2a), 4.83 (d, 2H, $^2J = 11.8$ Hz, 2 × CH_2Ph), 4.87 (d, 2H, $^2J = 11.3$ Hz, 2 × CH_2Ph), 4.97 (d, 2H, $^3J_{1b-2b} = 3.4$ Hz, 2 × H-1b), 5.06 (bd, 2H, $^3J_{6a,6a'} = 11.1$ Hz, 2 × H-6a), 5.80 (m, 2H, 2 × H-3a), 6.14 (d, 2H, $^3J_{1a,2a} = 10.0$ Hz, 2 × H-1a), 6.74 (d, 2H, $^3J_{NH,2a} = 9.3$ Hz, 2 × N*H*Ac), 7.10–7.65 (m, 42H, H-Ar), 7.88 (s, 2H, 2 × H-triazole), 8.02–8.09 (m, 8H, H-Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 16.0$, 22.6, 53.8, 62.8, 64.2, 67.8, 69.5, 70.4, 72.6, 74.3, 74.8, 74.9, 75.4, 76.0, 77.7, 79.3, 86.0, 100.7, 121.8, 127.5, 127.7, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 129.3, 129.8, 129.8, 133.1, 133.6, 138.0, 138.5, 138.6, 145.3, 165.9, 167.0, 170.4 ppm.

Protected dimer 12. To a solution of the disaccharide **3** (100.0 mg, 0.11 mmol, 2.1 eq.) and the bis-propargylated linker **6** (12.4 mg, 0.055 mmol, 1.0 eq.) in tetrahydrofuran (1.15 mL) were added copper iodide (6.2 mg, 0.032 mmol, 0.6 eq.) and diisopropylethylamine (19 μL, 0.11 mmol, 2.0 eq.). The reaction was stirred overnight at room temperature and then concentrated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 25 : 1) to give the protected dimer **12** (108.6 mg, quant.) as an amorphous solid. $[\alpha]_D^{21} = -41.6$ (*c* 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.65$ (d, 6H, $^3J_{6b-5b} = 6.4$ Hz, 6 × H-6b), 1.58 (s, 6H, 2 × CH_3CO), 3.48–3.56 (m, 14H, 2 × H-4b, 12 × CH_2O), 3.76 (m, 2H, 2 × H-5b), 3.89 (dd, 2H, $^3J_{3b-4b} = 2.3$ Hz, $^3J_{3b-2b} = 10.4$ Hz, 2 × H-3b), 3.97 (dd, 2H, $^3J_{2b-1b} = 3.3$ Hz, 2 × H-2b), 4.07 (m, 2H, 2 × H-5a), 4.14 (m, 2H, 2 × H-4a), 4.52 (d, 2H, $^2J = 11.5$ Hz, 2 × CH_2Ph), 4.57–4.89 (m, 18H, 2 × H-6a, 10 × CH_2Ph, 4 × OCH_2-triazole, 2 × H-2a), 4.91 (m, 2H, $^3J_{1b-2b} = 3.3$ Hz, 2 × H-1b), 4.97 (bd, 2H, $^3J_{6a,6a'} = 11.1$ Hz, 2 × H-6a), 5.72 (m, 2H, 2 × H-3a), 6.10 (d, 2H, $^3J_{1a,2a} = 10.0$ Hz, 2 × H-1a), 6.45 (d, 2H, $^3J_{NH,2a} = 9.3$ Hz, 2 × N*H*Ac), 7.17–7.62 (m, 42H, H-Ar), 7.82 (s, 2H, 2 × H-triazole), 7.95–8.09 (m, 8H, H-Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 16.0$, 22.6, 53.9, 62.7, 64.2, 67.8, 69.5, 70.4, 70.5, 72.5, 74.3, 74.7, 75.0, 75.2, 76.0, 76.9, 77.6, 79.3, 86.1, 100.8, 121.7, 127.5, 127.7, 128.1, 128.1, 128.3, 128.3, 128.5, 128.6, 129.2, 129.7, 129.9,

133.1, 133.6, 138.0, 138.5, 138.6, 145.4, 165.8, 167.1, 170.4 ppm. ESI-MS: $m/z = 1991.4$ $[M + Na]^+$.

Protected dimer 13. To a solution of the disaccharide **3** (100.0 mg, 0.11 mmol, 2.1 eq.) and the bis-propargylated linker **7** (14.8 mg, 0.055 mmol, 1.0 eq.) in tetrahydrofuran (1.15 mL), were added copper iodide (6.2 mg, 0.032 mmol, 0.6 eq.) and diisopropylethylamine (19 μL, 0.11 mmol, 2.0 eq.). The reaction was stirred overnight at room temperature and then concentrated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 20 : 1) to give the protected dimer **13** (89.6 mg, 81%) as an amorphous solid. $[\alpha]_D^{21} = -41.1$ (*c* 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.64$ (d, 6H, $^3J_{6b-5b} = 6.3$ Hz, 6 × H-6b), 1.59 (s, 6H, 2 × CH_3CO), 3.53–3.69 (m, 18H, 2 × H-4b, 16 × CH_2O), 3.82 (m, 2H, 2 × H-5b), 3.93 (bd, 2H, $^3J_{3b-2b} = 10.5$ Hz, 2 × H-3b), 4.03 (dd, 2H, $^3J_{2b-1b} = 3.3$ Hz, 2 × H-2b), 4.25–4.38 (m, 4H, 2 × H-4a, 2 × H-5a), 4.54 (d, 2H, $^2J = 11.4$ Hz, 2 × CH_2Ph), 4.56–4.92 (m, 18H, 2 × H-6a, 10 × CH_2Ph, 4 × OCH_2-triazole, 2 × H-2a), 5.00 (d, 2H, $^3J_{1b-2b} = 3.3$ Hz, 2 × H-1b), 5.11 (bd, 2H, $^3J_{6a,6a'} = 12.4$ Hz, 2 × H-6a), 5.90 (m, 2H, 2 × H-3a), 6.17 (d, 2H, $^3J_{1a,2a} = 9.9$ Hz, 2 × H-1a), 6.77 (d, 2H, $^3J_{NH,2a} = 9.9$ Hz, 2 × N*H*Ac), 7.12–6.68 (m, 42H, H-Ar), 7.89 (s, 2H, 2 × H-triazole), 8.03–8.15 (m, 8H, H-Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 16.0$, 22.6, 53.9, 62.6, 64.2, 67.8, 69.5, 70.4, 70.5, 72.4, 74.3, 74.7, 75.0, 76.0, 76.9, 77.6, 79.4, 86.1, 100.9, 121.5, 127.4, 127.5, 127.7, 128.1, 128.1, 128.3, 128.3, 128.4, 128.4, 128.5, 128.6, 129.1, 129.7, 129.8, 129.8, 129.9, 133.1, 133.6, 137.9, 138.4, 138.5, 145.4, 165.8, 167.2, 170.4 ppm. ESI-MS: $m/z = 2035.5$ $[M + Na]^+$.

Protected dimer 14. To a solution of the disaccharide **3** (100.0 mg, 0.11 mmol, 2.1 eq.) and the bis-propargylated linker **8** (17.1 mg, 0.054 mmol, 1.0 eq.) in tetrahydrofuran (1.15 mL) were added copper iodide (6.2 mg, 0.032 mmol, 0.6 eq.) and diisopropylethylamine (19 μL, 0.11 mmol, 2.0 eq.). The reaction was stirred overnight at room temperature and then concentrated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 20 : 1) to give the protected dimer **14** (115.6 mg, quant.) as an amorphous solid. $[\alpha]_D^{21} = -41.9$ (*c* 1.0, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.54$ (d, 6H, $^3J_{6b-5b} = 6.3$ Hz, 6 × H-6b), 1.50 (s, 6H, 2 × CH_3CO), 3.47–3.58 (m, 22H, 2 × H-4b, 20 × CH_2O), 3.73 (m, 2H, 2 × H-5b), 3.83 (bd, 2H, $^3J_{3b-2b} = 10.4$ Hz, 2 × H-3b), 3.93 (dd, 2H, $^3J_{2b-1b} = 3.3$ Hz, 2 × H-2b), 4.27–4.38 (m, 4H, 2 × H-4a, 2 × H-5a), 4.53 (d, 2H, $^2J = 11.4$ Hz, 2 × CH_2Ph), 4.56–4.92 (m, 18H, 2 × H-6a, 10 × CH_2Ph, 4 × OCH_2-triazole, 2 × H-2a), 5.00 (d, 2H, $^3J_{1b-2b} = 3.4$ Hz, 2 × H-1b), 5.12 (bd, 2H, $^3J_{6a,6a'} = 12.2$ Hz, 2 × H-6a), 5.91 (m, 2H, 2 × H-3a), 6.17 (d, 2H, $^3J_{1a,2a} = 10.0$ Hz, 2 × H-1a), 6.82 (d, 2H, $^3J_{NH,2a} = 9.6$ Hz, 2 × N*H*Ac), 7.16–6.69 (m, 42H, H-Ar), 7.89 (s, 2H, 2 × H-triazole), 8.04–8.13 (m, 8H, H-Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 15.9$, 22.6, 53.7, 62.5, 64.1, 67.7, 69.4, 70.3, 70.4, 70.4, 72.2, 74.3, 74.6, 74.8, 74.9, 75.8, 76.8, 77.3, 79.3, 86.1, 100.8, 121.5, 127.4, 127.5, 127.7, 128.1, 128.2, 128.3, 128.3, 128.4, 128.5, 128.5, 128.6, 129.0, 129.7, 129.9, 133.2, 133.7, 137.8, 138.3, 138.4, 145.3, 165.8, 167.2, 170.5 ppm. ESI-MS: $m/z = 2079.4$ $[M + Na]^+$.

Protected trimer 15. A solution of the linker **9** (14.2 mg, 0.044 mmol) and the disaccharide **3** (119.8 mg, 0.138 mmol) in tetrahydrofuran (1.8 mL, 0.024 M) was prepared. Copper iodide (7.7 mg, 0.051 mmol) and diisopropylethylamine (46 μL, 0.263 mmol) were added. The mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the crude was purified by flash chromatography (CH_2Cl_2–MeOH 20 : 1) to give the trimer **15** as a white solid (110.3 mg, 85%). m.p.: 170–173 °C. $[\alpha]_D^{21} = -37.8$ (*c* 0.8, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.61$ (d, 9H, $^3J_{6'-5'} = 6.3$ Hz, 9×H-6′), 1.25 (s, 9H, 3 × CH_3CO), 3.49 (b, 3H, 3 × H-4′), 3.76 (m, 3H, 3 × H-5′), 3.93 (b, 6H, 3 × H-2′, 3 × H-3′), 4.12 (b, 6H, 3 × H-2, 3 × H-5), 4.48–5.06 (m, 42H, 3 × H-1, 3 × H-3, 3 × H-4, 6 × H-6, 3 × H-1′, 18 × CH_2Ph, 6 × CH_2NH) 5.85, 6.20 (b, 6H, 3 × N*H*Ac, 3 × CH_2N*H*), 7.11–7.92 (m, 81H, 78 × H-Ar, 3 × H-triazole) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 16.0$, 22.5, 35.4, 67.7, 72.6, 74.2, 74.9, 75.5, 77.2, 79.3, 100.6, 127.4, 127.5, 127.6, 128.1, 128.2, 128.2, 128.3, 128.4, 129.4, 129.7, 133.2, 138.0, 138.4, 138.6, 166.1, 166.3, 171.2 ppm. ESI-MS: $m/z = 1478.1$ $[M + H + Na]^{2+}$.

Unprotected dimer 16. The debenzoylation step of dimer **10** with sodium methoxide (133.8 mg, 0.067 mmol) gave after column chromatography on silica gel (CH_2Cl_2–MeOH 15 : 1) **16** (83.4 mg, 79%). The debenzylation step, using 78.7 mg (0.050 mmol) of this product, by catalytic hydrogenation, gave the desired product as a white, amorphous solid (45.6 mg, 96%). $[\alpha]_D^{22} = -115.9$ (*c* 1.0, methanol). ^{1}H NMR (300 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta =$ 1.17 (d, 6H, $^3J_{6b-5b} = 6.1$ Hz, 6 × H-6b), 1.69 (s, 6H, 2 × CH_3CO), 3.75–3.05 (m, 19H, 2 × H-3a, 2 × H-4a, 2 × H-5a, 4 × H-6a, 2 × H-2b, 2 × H-3b, 2 × H-4b, CH_3O), 4.27 (m, 2H, 2 × H-2a), 4.38 (m, 2H, 2 × H-5b), 5.02 (d, 2H, $^3J_{1b-2b} = 3.2$ Hz, 2 × H-1b), 5.29 (b, 4H, 4 × H-e), 5.85 (d, 2H, $^3J_{1a-2a} = 9.8$ Hz, 2 × H-1a), 6.90 (b, 1H, H-d), 7.29 (m, 2H, 2 × H-c), 8.29 (s, 2H, H-triazole) ppm. ^{13}C NMR (75 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 15.9$, 22.1, 53.5, 56.3, 60.3, 61.8, 61.9, 67.7, 68.7, 70.1, 72.6, 73.0, 77.2, 79.0, 86.9, 100.4, 108.8, 110.1, 124.6, 132.5, 143.9, 159.1, 169.0, 174.5 ppm. ESI-MS: $m/z = 1051.1$ $[M + Na]^+$.

Unprotected dimer 17. The debenzoylation step of the dimer **11** (66.0 mg, 0.034 mmol) with sodium methoxide gave after column chromatography on silica gel (CH_2Cl_2–MeOH 10 : 1) the disaccharide (50.8 mg, 98%). The debenzylation step, using 48.0 mg (0.032 mmol) of this product, by catalytic hydrogenation, gave the desired product **17** as a white, amorphous solid (29.9 mg, 97%). $[\alpha]_D^{22} = -107.1$ (*c* 1.0, methanol). ^{1}H NMR (300 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 1.18$ (d, 6H, $^3J_{6b-5b} = 6.6$ Hz, 6 × H-6b), 1.81 (s, 6H, 2 × CH_3CO), 3.67 (b, 8H, 8 × CH_2O), 3.77–4.03 (m, 16H, 2 × H-3a, 2 × H-4a, 2 × H-5a, 4 × H-6a, 2 × H-2b, 2 × H-3b, 2 × H-4b), 4.28 (m, 2H, 2 × H-2a), 4.40 (m, 2H, 2 × H-5b), 4.69 (m, 4H, 4 × OCH_2-triazole), 5.02 (d, 2H, $^3J_{1b-2b} = 3.3$ Hz, 2 × H-1b), 5.86 (d, 2H, $^3J_{1a-2a} = 9.7$ Hz, 2 × H-1a), 8.26 (s, 2H, 2 × H-triazole) ppm. ^{13}C NMR (75 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 15.9$, 22.3, 56.5, 60.3, 63.5, 67.7, 68.7, 69.4, 70.1, 70.1, 72.6, 72.9, 77.2, 79.0, 86.9, 100.4, 124.4, 144.7, 174.7 ppm. ESI-MS: $m/z = 989.4$ $[M + NH_4]^+$.

Unprotected dimer 18. The debenzoylation step of the dimer **12** (95.7 mg, 0.049 m mol) with sodium methoxide gave after column chromatography on silica gel (CH_2Cl_2–MeOH 10 : 1) the disaccharide (35.6 mg, 55%). The debenzylation step, using 32.0 mg (0.021 mmol) of this product, by catalytic hydrogenation, gave the desired product **18** as a white, amorphous solid (21.1 mg,

quant.). $[\alpha]_D^{22} = -80.5$ (c 1.0, methanol). ^{1}H NMR (300 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 1.18$ (d, 6H, $^3J_{6b-5b} = 6.5$ Hz, 6 × H-6b), 1.82 (s, 6H, 2 × CH_3CO), 3.68 (b, 12H, 12 × CH_2O), 3.75–4.03 (m, 16H, 2 × H-3a, 2 × H-4a, 2 × H-5a, 4 × H-6a, 2 × H-2b, 2 × H-3b, 2 × H-4b), 4.28 (m, 2H, 2 × H-2a), 4.39 (m, 2H, 2 × H-5b), 4.69 (m, 4H, 4 × OCH_2-triazole), 5.01 (d, 2H, $^3J_{1b-2b} =$ 3.1 Hz, 2 × H-1b), 5.86 (d, 2H, $^3J_{1a-2a} = 9.7$ Hz, 2 × H-1a), 8.25 (s, 2H, 2 × H-triazole) ppm. ^{13}C NMR (75 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 15.9$, 22.3, 56.5, 60.3, 63.5, 67.7, 68.7, 69.4, 70.1, 70.1, 70.2, 72.6, 72.9, 77.2, 79.0, 86.9, 100.4, 124.4, 144.7, 174.7 ppm. ESI-MS: $m/z = 1033.4$ [M + Na]$^+$.

Unprotected dimer 19. The debenzoylation step of the dimer **13** (80.0 mg, 0.040 mmol) with sodium methoxide gave after column chromatography on silica gel (CH_2Cl_2–MeOH 20 : 1) the disaccharide (52.6 mg, 83%). The debenzylation step, using 47.0 mg (0.029 mmol) of this product, by catalytic hydrogenation, gave the desired product **19** as a white, amorphous solid (29.6 mg, 95%). $[\alpha]_D^{22} = -80.3$ (c 1.0, methanol). ^{1}H NMR (300 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 1.18$ (d, 6H, $^3J_{6b-5b} = 6.5$ Hz, 6 × H-6b), 1.82 (s, 6H, 2 × CH_3CO), 3.65–3.70 (b, 16H, 16 × CH_2O), 3.77–4.03 (m, 16H, 2 × H-3a, 2 × H-4a, 2 × H-5a, 4 × H-6a, 2 × H-2b, 2 × H-3b, 2 × H-4b), 4.28 (m, 2H, 2 × H-2a), 4.39 (m, 2H, 2 × H-5b), 4.69 (m, 4H, 4 × OCH_2-triazole), 5.01 (d, 2H, $^3J_{1b-2b} =$ 3.5 Hz, 2 × H-1b), 5.86 (d, 2H, $^3J_{1a-2a} = 9.8$ Hz, 2 × H-1a), 8.26 (s, 2H, 2 × H-triazole) ppm. ^{13}C NMR (75 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 15.9$, 22.3, 56.5, 60.3, 63.5, 67.7, 68.7, 69.4, 70.1, 70.1, 70.2, 72.6, 72.9, 77.2, 79.0, 86.9, 100.4, 124.4, 144.7, 174.7 ppm. ESI-MS: $m/z = 1077.4$ [M + Na]$^+$.

Unprotected dimer 20. The debenzoylation step of the dimer **14** (78.5 mg, 0.038 mmol) with sodium methoxide gave after column chromatography on silica gel (CH_2Cl_2–MeOH 15 : 1) the disaccharide (39.1 mg, 62%). The debenzylation step, using 32.6 mg (0.029 mmol) of this product, by catalytic hydrogenation, gave the desired product **20** as a white, amorphous solid (20.6 mg, 95%). $[\alpha]_D^{22} = -96.1$ (c 1.0, methanol). ^{1}H NMR (300 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 1.18$ (d, 6H, $^3J_{6b-5b} = 6.1$ Hz, 6 × H-6b), 1.82 (s, 6H, 2 × CH_3CO), 3.63–3.75 (b, 20H, 20 × CH_2O), 3.77–4.03 (m, 16H, 2 × H-3a, 2 × H-4a, 2 × H-5a, 4 × H-6a, 2 × H-2b, 2 × H-3b, 2 × H-4b), 4.28 (m, 2H, 2 × H-2a), 4.38 (m, 2H, 2 × H-5b), 4.70 (m, 4H, 4 × OCH_2-triazole), 5.02 (d, 2H, $^3J_{1b-2b} =$ 3.5 Hz, 2 × H-1b), 5.87 (d, 2H, $^3J_{1a-2a} = 9.8$ Hz, 2 × H-1a), 8.26 (s, 2H, 2 × H-triazole) ppm. ^{13}C NMR (75 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 15.9$, 22.3, 56.5, 60.3, 63.5, 67.7, 68.7, 69.4, 70.1, 70.1, 70.2, 72.6, 72.9, 77.2, 79.0, 86.9, 100.4, 124.4, 144.7, 174.7 ppm. ESI-MS: $m/z = 1121.4$ [M + Na]$^+$.

Unprotected trimer 21. Compound **15** (109.1 mg, 0.037 mmol) was dissolved in MeOH–THF (0.01 M, 1 : 1) and the solution was treated with a catalytic amount of 1 M NaOMe/MeOH. After being stirred at rt for 2 days, the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. Methyl benzoate, resulting from the deprotection, was removed by column chromatography (CH_2Cl_2–MeOH 9 : 1) to afford the debenzoylated trimer (61.4 mg, 71%). The resulting trimer (44.3 mg, 0.019 mmol) was dissolved in MeOH (5 mL) and the debenzylation was accomplished with an excess of $Pd(OH)_2/C$ (20 wt%). After one night under H_2 atmosphere, the solution was filtered through Celite and concentrated. The purification of the product by column chromatography (CH_3CN–H_2O 90 : 10 to 70 : 30) afforded the fully deprotected trimer **21** as a white solid (18.7 mg, 65%). $[\alpha]_D^{21} = -62.0$ (c 0.6, DMSO). ^{1}H NMR (300 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 1.15$ (d, 9H, $^3J_{6'-5'} = 6.6$ Hz, 9 × H-6′), 1.75 (s, 9H, 3 × CH_3CO), 3.78–3.95 (m, 24H, 3 × H-3, 3 × H-4, 3 × H-5, 6 × H-6, 3 × H-2′, 3 × H-3′, 3 × H-4′), 4.25 (app t, 3H, $^3J_{2-1} = 9.6$ Hz, 3 × H-2), 4.37 (m, 3H, 3 × H-5′), 4.68 (s, 6H, 3 × CH_2N), 4.99 (d, 3H, $^3J_{1'-2'} = 3.3$ Hz, 3 × H-1′), 5.81 (d, 3H, $^3J_{1-2} = 9.6$ Hz, 3 × H-1), 8.18 (s, 3H, 3 × H-triazole), 8.31 (s, 3H, H-Ar) ppm. ^{13}C NMR (75 MHz, D_2O–acetone 600 : 1, 25 °C): $\delta = 15.9$, 22.2, 35.6, 56.4, 60.3, 67.7, 68.7, 70.0, 72.6, 72.9, 77.2, 79.0, 86.9, 100.4, 123.3, 129.9, 135.3, 169.2, 174.6 ppm. ESI-MS: $m/z = 1498.6$ [M + H]$^+$.

Preparation of PA-IIL protein

Recombinant PA-IIL was purified from *Escherichia coli* BL21(DE3) containing the plasmid pET25pa2l as described previously.[30] Biotinylation of PA-IIL was performed as described in the literature.[31] Briefly, PA-IIL (200 μM) diluted in buffer containing 0.1 M $NaHCO_3$ and 0.2 M NaCl was mixed with dimethyl formamide solution containing 4.5 mM biotin for 2 hours at room temperature, with agitation. After dialysis against 0.15 M of NaCl solution followed by water, the lectin was lyophilized.

Interaction studies with PA-IIL lectin

ELLA (enzyme-linked lectin assay) experiments. First test (plate coated with polyacrylamide–fucose): ELLA experiments were conducted using 96-well microtitre plates (Nunc Maxisorb) coated with polymeric α-L-fucose (5 μg mL^{-1}; Lectinity Holding, Inc., Moscow) diluted in carbonate buffer, pH 9.6 (100 μL) for 1 h at 37 °C. After blocking at 37 °C for 1 h with 100 μL per well of 3% (w/v) BSA in PBS, plates were incubated at 37 °C for 1 h with 100 μL of biotinylated PA-IIL at 0.1 μg mL^{-1} in the presence of serial dilutions of inhibitors. After washing with T-PBS (PBS containing 0.05% Tween), 100 μL of streptavidin–peroxidase conjugate (dilution 1 : 10 000; Boehringer-Mannheim) was added and left for 1 h at 37 °C. The color was developed using 100 μL per well of 0.05 M phosphate/citrate buffer containing *O*-phenylenediamine dihydrochloride (0.4 mg mL^{-1}) and urea hydrogen peroxide (0.4 mg mL^{-1}) (Sigma-Aldrich). The reaction was stopped by the addition of 50 μL of 30% H_2SO_4. The absorbance was read at 490 nm using a microtitre plate reader (Bio-Rad; model 680). Second test (plate coated with PA-IIL): The same protocol as above was used, but in this case, plates were coated with PA-IIL (5 μg mL^{-1}), and a biotinylated polymeric α-L-fucose (5 μg mL^{-1}; Lectinity Holding, Inc.) was used for competition with serial dilutions of inhibitors.

ITC (isothermal titration microcalorimetry) analysis. ITC experiments were performed with a VP-ITC isothermal titration calorimeter (Microcal). The experiments were carried out at 25 °C. Ligands and proteins were dissolved in the same buffer (0.1 M Tris with 0.03 mM $CaCl_2$) at pH 7.5. The protein concentration in the microcalorimeter cell (1.4 mL) varied from 17.8 to 20 μM. A total of 30 injections of 13 μL of sugar solution at concentrations varying from 0.08 to 0.21 mM were added at intervals of 5 min whilst stirring at 310 rpm. Control experiments performed by injection of buffer into the protein solution yielded

insignificant heats of dilution. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal, with ΔH (enthalpy change), K_a (association constant) and n (number of binding sites per monomer) as adjustable parameters. ΔG (free energy change) values and entropy contributions were determined from the standard equation:

$$\Delta G = \Delta H - T\Delta S$$

where T is the absolute temperature. All experiments were performed with c values $100 < c < 200$ ($c = K_a M$, where M is the initial concentration of the macromolecule).

Molecular modeling. All molecular editing was performed with the Sybyl software (Tripos, St Louis). Linkers were generated in their extended conformations, and geometry optimization was performed with the Tripos force-field. Disaccharide conformation was taken from the protein data bank,[32] using the crystal structure of the complex between PA-IIL and a derivative of αFuc14GlcNAc disaccharide[17] (PDB code 2JDK). Graphical representations were drawn using PyMol software (Delano Scientific LCC, San Francisco).

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