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X-ray Structures and Thermodynamics of the Interaction of PA-IIL from *Pseudomonas aeruginosa* with Disaccharide Derivatives

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Pseudomonas aeruginosa *is an opportunistic bacterium showing increasing resistance to antibiotics and consequently represents elevated threatening problems in hospital environments, particularly for cystic fibrosis patients. The use of glycomimetics as an anti-adhesive strategy against microorganisms may complement the use of antibiotics. PA-IIL lectin (LecB) from* P. aeruginosa *constitutes an appealing target for antibacterial agents, as it has been proposed to play a key role in binding to airway epithelia and/or to be involved in biofilm formation. The lectin has an unusually high affinity for L-fucose and related oligosaccharides. In the work presented herein, the disaccharide αFuc1-4GlcNAc is used as a scaffold toward the synthesis of a series of glycomimetic derivatives. Microcalorimetry and structural studies indicate that several of the derivatives are potent inhibitors of the lectin, with affinity in the same range as the best known natural ligand, Lewis a, and could represent interesting leads for the development of future antibacterial compounds.*

Introduction

Bacterial attachment to mucosal surfaces is the initial event in the pathogenesis of the majority of infectious diseases caused by bacteria in animals and humans. Amongst bacterial adhesins, lectins have been suggested to mediate bacterial adhesion to host cell surfaces and, for example, to respiratory mucins in cystic fibrosis (CF) patients.[1] The opportunistic pathogen *Pseudomonas aeruginosa* expresses two lectins, PA-IL and PA-IIL (also referred to as LecA and LecB), with specificity for D-galactose and L-fucose, respectively, that are produced with the virulence factors.[2] Although both *P. aeruginosa* lectins are found in large quantities in the cytoplasm,[3] they have also been located on the bacterial outer membrane.[4,5] Many roles have been suggested for these lectins, including cell toxicity by amplifying accompanying enzyme activity, biofilm formation, post-translational modification, and adhesion to airway mucins and glycoconjugates.[5–12]

The L-fucose binding lectin PA-IIL is characterized by an unusually high affinity towards its carbohydrate ligands, first reported by equilibrium dialysis[13] then by titration microcalorimetry.[14,15] L-Fucose is the monosaccharide ligand displaying the highest affinity to the lectin ($K_d = 3\times10^{-6}$ M)[15] and the crystal structure of the complex has unraveled the molecular basis for the particular interactions responsible for the high affinity.[14,16] The protein, a calcium dependent lectin, is rather promiscuous and also binds to other monosaccharides, albeit with lower affinity. The complexes with derivatives of L-galactose, D-arabinose, D-mannose, and D-fructose have been structurally characterized.[17,18]

Human milk can be a useful natural source of fucosylated oligosaccharides. Indeed, it has been reported to specifically inhibit the interaction of PA-IIL with erythrocytes[19] and is known to protect newborns from enteropathic bacteria and viruses.[20] Competitive binding assays against biotinylated polymeric L-fucose has indicated that, amongst human milk oligosaccharides (HMO) containing fucose, those containing the Lewis a (Le^a) epitope are better inhibitors than those containing Lewis x (Le^X) or H type epitopes.[15] This showed that PA-IIL binds more efficiently to fucose when this residue is linked to position four of D-GlcNAc than when present on position three of D-GlcNAc or position two of D-galactose. Enzyme-linked lectin

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assays (ELLA) conducted on a large panel of mammalian glycoconjugates confirmed the binding preference towards the Lewis a (Le^a) trisaccharide and the pentasaccharide (lacto-*N*-fucopentaose) containing the αFuc1-4GlcNAc linkage, over ABH blood group antigens that contain the αFuc1-2Gal linkage.[21] The highest affinity ligand for PA-IIL has been therefore identified as the Le^a trisaccharide with a K_d of 200 nM and the crystal structure of the PA-IIL/Le^a complex confirmed the additional contact established between the GlcNAc residue and the protein surface.[15]

The wealth of information that has been obtained in recent years on PA-IIL and its binding to monosaccharides and oligosaccharides[10] may now be used for the design of improved high affinity ligands that could act as competitors for the binding activity of the lectin. Indeed, successful treatment of *P. aeruginosa* airway infection with fucose-containing sugar solution has been reported in one case,[22] and recent in vitro assays involving fucose-containing dendrimers displayed high affinity for PA-IIL.[23] Glycomimetics are compounds that are designed using a bioactive oligosaccharide as reference to contain the essential functional groups that can mimic the active conformation of the parent structure.[24] In the present case, the aim is to obtain high affinity analogues whilst avoiding the synthesis of the complete Le^a trisaccharide. For this purpose, a number of derivatives have been synthesized using the simplified α-L-Fuc*p*-(1→4)-β-D-GlcNAc disaccharide scaffold as a promising lead candidate for development and a series of analogues was designed from the above disaccharide possessing a [1,2,3]-triazole moiety as the aglycone of the GlcNAc residue.

Results and Discussion

Binding of PA-IIL on glycan array

TFP-Alexa Fluor 488 labeled PA-IIL was tested on more than 250 oligosaccharide micro-plate assays and on the printed glycan arrays available from the Consortium for Functional Glycomics (http://www.functionalglycomics.org/fg/). The consolidated data are presented in Figure 1.

This new assay confirmed the preference of PA-IIL for Le^a oligosaccharides over H and AB epitopes and those containing Le^x. Sialyl Lewis a (SLe^a) is not a good ligand, but interestingly the lectin has high selectivity for the αFuc1-4GlcNAc disaccharide indicating that the galactose residue of the Le^a may not be crucial for binding. However, these data have been obtained for surface-linked sugars and their competition power in solution has yet to be confirmed.

Synthesis of disaccharide derivatives

The desired "lead" disaccharide α-L-Fuc*p*-(1→4)-β-D-GlcNAc **3**, bearing an aglyconic azide, was synthesized by glycosylation of dibenzoylated acceptor **2**[25] with perbenzylated donor **1**[26] under *N*-iodosuccinimide and triflic acid activation in chloroform at −15 °C (Scheme 1). Notably, only the α-anomer was obtained in 94 % yield ($\delta_{H\text{-}1B}$ = 4.87 ppm, $^3J_{1B\text{-}2B}$ = 3.3 Hz).

Glycosyl azide **3** was then coupled to a panel of alkynes, using different 1-3-dipolar cycloaddition conditions (Scheme 2). These chemoselective and high-yielding reactions, commonly referred to as "click chemistry" are of widespread application towards the preparation of families of analogues. The addition

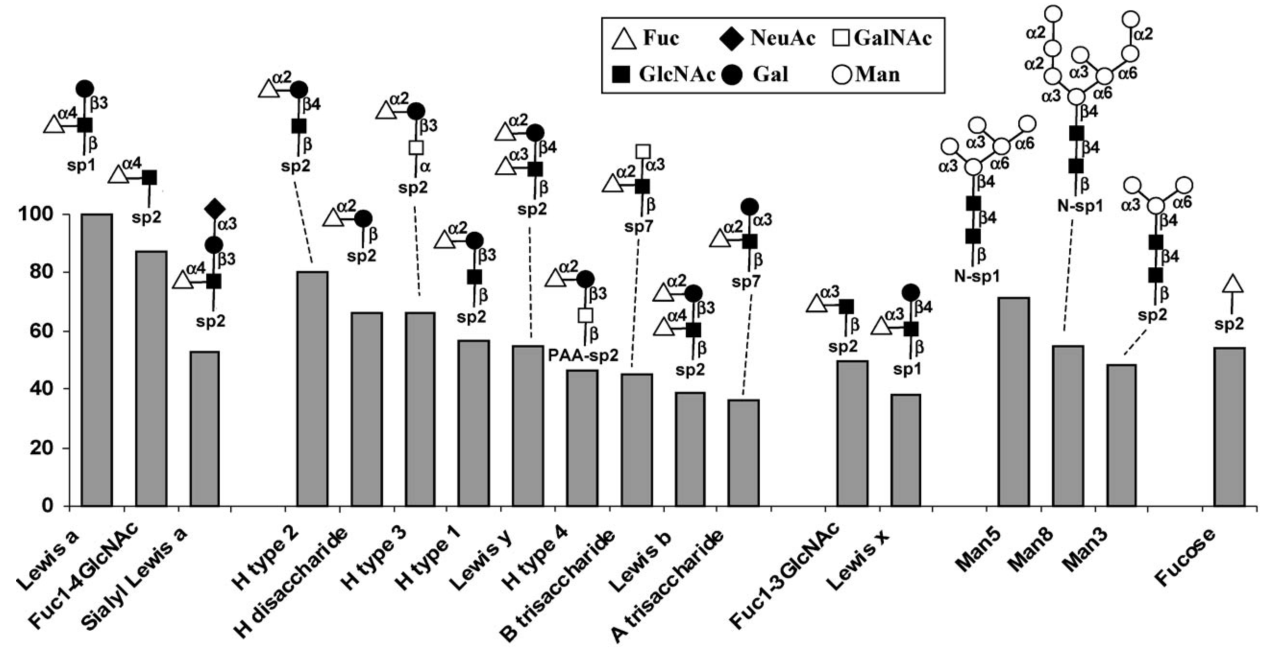
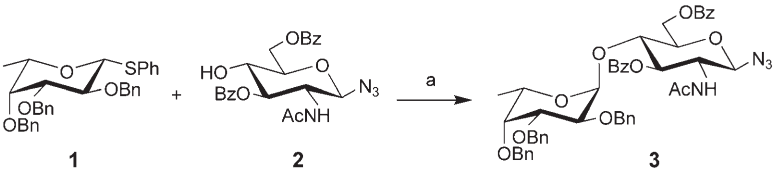
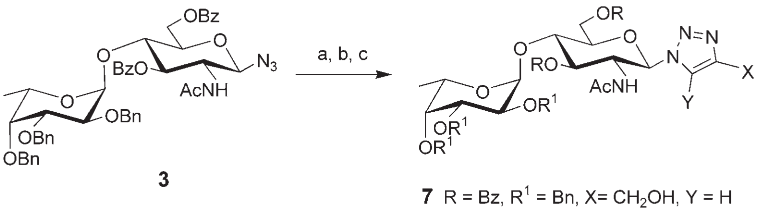


Figure 1. Relative binding affinity, calculated as relative to Le^a binding from consolidated data of a glycan array probed with PA-IIL-Alexa Fluor 488. The level of fluorescence was measured by averaging over three wells, after subtraction of the background signal. The 100 % level of the graph corresponds to the RFU signal of Le^a (32 900 ± 2200).



Scheme 1. Synthesis of the α-L-Fuc*p*-(1→ 4)-β-D-GlcNAc derivative: a) NIS, TfOH, CH_2Cl_2, MS 4 Å, −15 °C, 94 %.

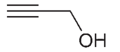
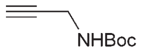
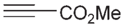


7 R = Bz, R^1 = Bn, X= CH_2OH, Y = H
8 R = Bz, R^1 = Bn, X = H, Y = CH_2OH
9 R = Bz, R^1 = Bn, X = CH_2NHBoc, Y = H
10 R = Bz, R^1 = Bn, X = CO_2Me, Y = H
11 R = R^1 = Y = H, X = CH_2OH
12 R = R^1 = X = H, Y = CH_2OH
13 R = R^1 = Y =H, X = CH_2NHBoc
14 R = R^1 = Y = H, X = CO_2Me
15 R = R^1 = Y = H, X = CO_2H

Scheme 2. Synthesis of the potential ligands a) for coupling conditions, see Table 1; b) MeONa, MeOH; c) H_2, $Pd(OH)_2/C$, MeOH.

of triazole group in the aglycon is of high interest as it opens the route towards the synthesis of multimeric compounds.

Table 1. Synthesis of the modified diasaccharide ligands.

Azide	Alkyne	Coupling conditions	Product	Yield [%]	Final[a]	Yield [%][b]
3		Toluene, 75 °C, 6 days	**7, 8**	45, 38	**11, 12**	84[b], 56[b]
3	4	$CuSO_4$, asc. ac., *t*BuOH/H_2O (*v*/*v* 1:1), RT, 18 h	**7**	80	**11**	84[b]
3		$CuSO_4$, asc. ac., *t*BuOH/H_2O (*v*/*v* 1:1), RT, 16 h	**9**	89	**13**	87[b]
3		$CuSO_4$, asc. ac., *t*BuOH/H_2O (*v*/*v* 1:1), 55 °C, 4 h	**10**	77	**14**	86[b]
2	4	$CuSO_4$, asc. ac., *t*BuOH/H_2O (*v*/*v* 1:1), RT, 18 h	**16**	69	**17**	quant.

[a] For structural formula see Figure 2. [b] For two steps: debenzoylation and debenzylation.

The classical reaction conditions described by Huisgen[27] involve thermal cycloaddition between an azide and an alkyne in an organic solvent (commonly toluene), resulting in the formation of 1,4 and 1,5-disubstituted 1,2,3-triazoles, which are both interesting for probing the PA-IIL–ligand interactions. We first applied these conditions and heated at reflux a mixture of the disaccharide **3** and propargyl alcohol **4** in toluene. Rapidly, the disaccharide decomposed. However, when performed at 75 °C, the reaction afforded as expected, a mixture of 1,4- **7** (45 %) and 1,5-regioisomer **8** (38 %), separable by silica gel column chromatography.

Next, the regioselective cycloadditions were done under the improved copper(I)-catalyzed conditions which allowed the clean formation of the 1,4-regioisomers.[28] Thus, addition of an aqueous solution of copper(II) sulfate-sodium ascorbate to a mixture of disaccharide **3** and alkynes **4**, **5**,[29] or **6** (1.2 equiv) in *tert*-butanol/water (*v*/*v* 1:1) at room temperature, resulted in the clean formation of the 1,4-regioisomers **7**, **9**, and **10**, respectively. As the reactions were carried out at room temperature, the reactions between **3** and propargyl alcohol **4** or *N*-Boc-propargylamine **5** were complete after 16–18 h, providing **7** and **9** in good yields (80 % and 89 %, respectively). However, coupling azide **3** with methyl propiolate **6** under the same reaction conditions occurred in only 48 % yield after 24 h whereas heating the mixture to 55 °C for 4 h afforded the expected 1,4-regioisomer **10** in 77 % yield. To confirm that the L-fucose moiety was essential for ligand recognition, the corresponding 1,2,3-triazole derivative of the *N*-acetylglucosamine was also synthesized to provide compound **17**.

The final products were obtained after two deprotection steps: a debenzoylation step using a catalytic amount of sodium methoxide in methanol followed, after purification, by a debenzylation step using catalytic hydrogenation with palladium hydroxide on carbon as the catalyst. The yields of the reactions are reported in Table 1. Methyl ester **14** was treated with lithium hydroxide to give, after treatment, the acid derivative **15** in 68 % yield.

Binding of disaccharide derivatives to PA-IIL

The various disaccharide derivatives were evaluated for their ability to inhibit the binding of biotinylated polymeric L-fucose to immobilized PA-IIL on the surface of microtiter plates, as previously described for the assays using milk oligosaccharides.[15] In the present study, a second assay with the polyacrylamide-fucose immobilized in the wells was made by measuring the inhibition of binding of soluble biotinylated PA-IIL. As at the present time it is not clear if the lectin acts as a mobile entity in the extracellular medium or as one fixed on the bacterial cell surface, these two methodologies allowed both situations to be simulated.

The different derivatives of the αFuc1-4GlcNAc disaccharides have rather similar inhibition properties in the ELLA tests (Figure 2). Some differences were observed between the assay methods (that is, depending if the lectin or the polymeric ligand were fixed in the wells), but overall the observed IC_{50} values are in the same range as those obtained with the use of Le^a trisaccharide (0.6 μM and 0.7 μM with the two ELLA methods). Overall, these ligands show the highest affinity determined until now for PA-IIL. The strict requirement for the binding to the L-fucoside moiety was also confirmed as compound

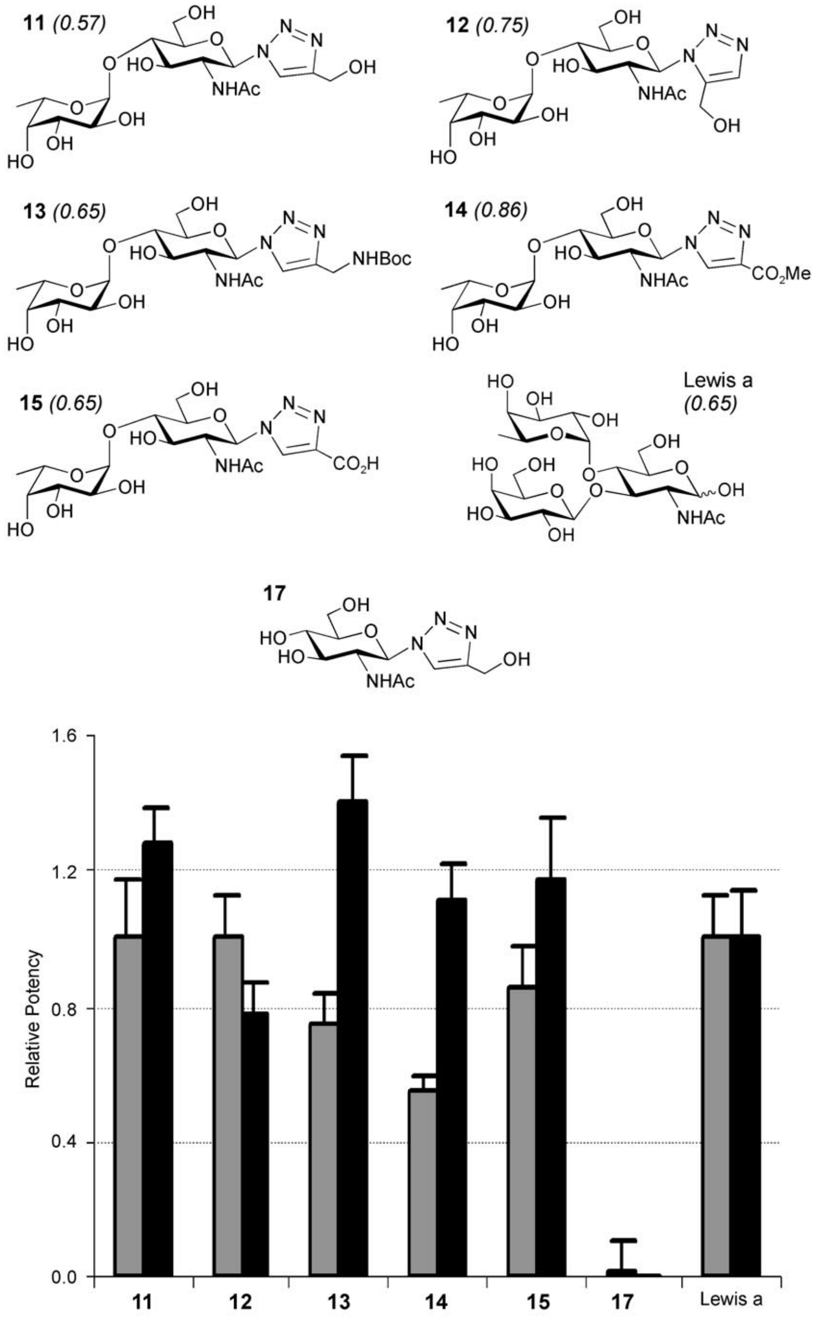


Figure 2. Different disaccharide derivatives with IC_{50} (μм averaged between two methods) indicated in italics. The lower graph displays the relative inhibitory potency (relative to Le^a trisaccharide with an IC_{50} of 0.65 μм) of several disaccharide derivatives towards the PA-IIL–fucose interaction. Grey bars: wells coated with fucose, black bars: wells coated with PA-IIL.

17 showed no binding activity. These experiments therefore confirm that the entire Le^a trisaccharide does not constitute a stringent requirement for optimal binding and that the αFuc1-4GlcNAc brings sufficient affinity to serve as the basis for further design of glycocompetitors.

Thermodynamics of the interaction with PA-IIL

The affinity of the best disaccharide ligand **11** and also of compound **14** towards PA-IIL was confirmed by microcalorimetry (Figure 3 and Table 2). For compound **11**, averaging over three experiments, an association constant of $3.2\times10^{6}\,\text{м}^{-1}$ ($K_d = 310$ nм) was determined and compound **14** also displayed high affinity. The affinity of the disaccharide derivatives is therefore in the same range as the K_d of 210 nм previously measured for the entire trisaccharide Le^a interacting with PA-IIL, which is about 14 times higher than the one for fucose.[15]

For all ligands listed in Table 2, the interactions are clearly enthalpy driven, with large exothermic values. Compound **11** has the largest negative enthalpy ($\Delta H = -43.4\,\text{kJ}\,\text{mol}^{-1}$) with an unfavorable entropy contribution ($T\Delta S = -6.3\,\text{kJ}\,\text{mol}^{-1}$), a behavior classically observed in protein–carbohydrate interactions. This situation is in fact rather different from the binding of Le^a which displays a favorable entropy of binding ($T\Delta S = +3.1\,\text{kJ}\,\text{mol}^{-1}$). Compound **14** has an intermediate behavior with no entropic contribution, as was previously observed for fucose. The difference in thermodynamic behavior between the different compounds is likely due to their difference in flexibility (see discussion below).

Structure of PA-IIL in complex with inhibitors

Crystals of PA-IIL complexed with L-fucose were obtained as described previously, then soaked in solutions containing inhibitors **11** or **14**. The resulting crystals diffracted to 1.1 Å resolution and are isomorphous to the PA-IIL/fucose crystals previously described of space group $P2_1$.[16] Diffraction data were collected at ESRF on beamline ID14-2 and the structures determined by molecular replacement and subsequently refined (Table 3). The disaccharide derivative **11** was observed with full occupancy in three sites of the asymmetric unit tetramer (Figure 4a). Analysis of the packing indicates that access to the fourth site is blocked by interactions with the neighboring tetramer.

The disaccharide derivatives **11** and **14** bind similarly and good quality electron density could be observed in three bind-

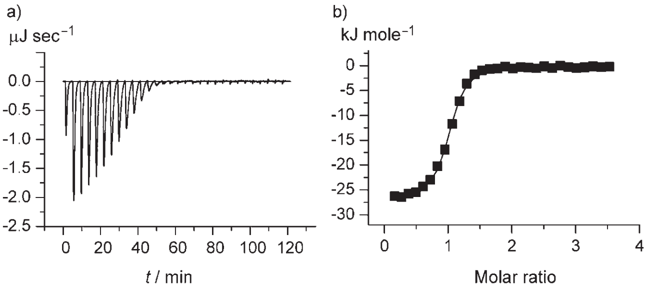


Figure 3. Titration microcalorimetry results of compound **11** (0.28 mM) binding to PA-IIL (0.018 mM) at 25 °C. a) Data obtained for 30 automatic injections, 10 μL each, of **11** into PA-IIL in the cell. b) Plot of the total heat released as a function of total ligand concentration for the titration shown above (squares). The solid line represents the best least-square fit for the obtained data.

Table 2. ITC data for different ligands interacting with PA-IIL[a]

ligand	K_a [10^6 M^{-1}]	K_d [10^{-9} M]	n	$-\Delta G$ [kJ mol^{-1}]	$-\Delta H$ [kJ mol^{-1}]	$-T\Delta S$ [kJ mol^{-1}]
L-Fuc[b]	0.34	2900	0.96	31.5	31.2	−0.3
Le^{a}[b]	4.7	210	1.08	38.1	34.9	−3.1
11	3.2	310	0.98	37.1	43.4	6.3
14	3.4	290	0.80	37.3	36.7	−0.6

[a] Standard deviations are lower than 10% in all cases. [b] Values taken from ref. [15].

ing sites of PA-IIL for both ligands (Figure 4b). The most distal atoms (hydroxymethyl group of **11** and methyl ester of **14**) have weaker electron density (reflected in higher *B*-factors), indicating some flexibility.

In fact, when looking at the details of the binding between the ligands and the proteins, the triazole ring and its substituent do not establish any contact with the binding site (Figure 4c and 4d). There is therefore no significant difference in the binding modes of the two disaccharide derivatives and only the interaction of **11** with PA-IIL will be further discussed. The three binding sites that are occupied by the analogues are very similar. In all cases, the L-fucose residue participates in the coordination of the two calcium ions through hydroxy groups O2, O3, and O4, as previously described.[14,16] The L-fucose moiety also establishes the previously described network of hydrogen bonds with the side chain of several amino acid residues as well as with the C-terminal carboxyl group of glycine (G114*) and of the neighboring monomer (Figure 4c). The ring oxygen forms a hydrogen bond to the main chain NH group of Ser 23. One water molecule, conserved in all structures of PA-IIL complexed with mono- or oligosaccharides,[14–17] bridges the L-fucose O2 and O1 oxygens to the main chain nitrogen atoms of Thr 98 and Asp 99. Hydrophobic contacts are limited to the interaction of the fucose C6 methyl group with the side chain of the Thr 45 residue.

The GlcNAc residue establishes two additional hydrogen bonds, one between O3 and the hydroxy group of Ser 23 and one between the O6 hydroxymethyl group and the side chain of Asp 96. The resulting orientation of the GlcNAc residue is significantly different to that observed in the crystal structure of the PA-IIL–Le^{a} complex as described below.

Correlation between structural and thermodynamic data

The observation that PA-IIL exhibits as high an affinity for derivatives of the αFuc1-4GlcNAc disaccharide as for the trisaccharide Le^{a} is rather surprising as these two molecules have very different conformational behaviors. Le^{a}, as for other branched histo-blood group antigens, only adopts one conformation in solution because of the steric hindrance between the fucose and the galactose residues.[30,31] The conformation of Le^{a} observed in the crystal structure of the complex with PA-IIL indeed corresponds closely to that predicted by molecular mechanics calculations. Disaccharides, however, are much more flexible molecules with more conformational freedom originating from possible rotation around the glycosidic linkage. Whereas rotation around the C1–O1 bond (Φ) is restricted by the stereoelectronic effect referred to as the *exo*-anomeric effect, the other bond (O1–Cx) (Ψ) is more flexible with energy minima corresponding to the staggered orientations of attached groups.

Conformational studies on αFuc1-4GlcNAc have demonstrated that the solution behavior analyzed by NMR data can be explained only when taking into account a mixture of different conformations.[32] Compound **11** adopts very similar conformations in the three binding sites of PA-IIL with average values of $\Phi_{(O5-C1-O1-C4)} = -80°$ and $\Psi_{(C1-O1-C4-C5)} = -123°$. As Figure 5 shows, this conformation is markedly different to the Le^{a} complex ($\Phi = -75°$ and $\Psi = -100°$) and results in a different orientation of the GlcNAc ring in the binding site, with a modified hydrogen bond network. Whereas GlcNAc in the PA-IIL/Le^{a} complex establishes only one hydrogen bond between O6 and a main chain oxygen, the orientation in the PA-IIL/αFuc1-4GlcNAc complex allows hydrogen bonds to be established between O6 and the carboxyl group of Asp 96 and also between O3 and the hydroxy group of Ser 23. The difference in hydrogen bonding partner for O6 of GlcNAc is due to both change of conformation at the glycosidic linkage and change in orientation of the C6 hydroxymethyl group. This latter adopts a usual *gauche-trans* (*gt*) orientation (O6 *anti* to C4) in Le^{a} and a *gauche-gauche* (*gg*) orientation in the disaccharide derivative **11**.

As expected for the binding of all disaccharides, a significant unfavorable entropy contribution ($T\Delta S = -6.3$ kJ mol^{-1}) is observed upon the binding of compound **11** to PA-IIL, due to the flexibility of its carbohydrate linkage in solution. However, the strong hydrogen bond network generated with compound **11** results in strong enthalpy of binding compensating the entropy cost, and the resulting affinity is in the same range as the rigid Le^{a} trisaccharide.

Table 3. Data collections and refinement statistics.

Ligand	**11**	**14**
Data collection		
Resolution (Å)	22.3–1.10 (1.13–1.10)[a]	22.3–1.10 (1.13–1.10)
Space group	$P2_1$	$P2_1$
Unit cell	$a = 52.55$ Å $b = 72.83$ Å $c = 54.37$ Å $\beta = 94.28°$	$a = 52.63$ Å $b = 73.09$ Å $c = 54.50$ Å $\beta = 94.36°$
Matthews coef (Å^3/DA)	2.2	2.2
Solvent (%)	44	45
Observed reflections	827 162	834 551
Unique reflections	158 737 (10 947)	16 5425 (11 449)
Multiplicity	5.2 (3.6)	5.0 (3.5)
Completeness (%)	96.3 (92.78)	99.4 (97.6)
Average $I/\sigma I$	22.6 (9.4)	18.7 (6.5)
R merge (%)	0.050 (0.12)	0.054 (0.18)
Wilson Factor (Å^2)	9.8	9.3
Refinement		
R_{crys} (observations)	0.107 (153 917)	0.113 (157 059)
R_{free} (observations)	0.131 (4790)	0.134 (8139)
Number of atoms: Hetero/Protein/Solvent	117/3257/735	123/3421/623
Average B factor (Å^2) Protein/Sugar Ligand/Calcium/Water	7.4/14.4/5.0/25.6	7.8/15.4/5.2/22.5
RMS deviation deviations from ideality		
Bond length (Å)	0.019	0.017
Angles (°)	1.77	1.69
Ramachandran plot	99.7% (395) residues in most favourable and additionally allowed regions. None in disallowed regions.	99.7% (395) residues in most favourable and additionally allowed regions. None in disallowed regions.

[a] Values between parentheses refer to the highest resolution shell.

Conclusions

Five compounds containing the disaccharide αFuc1-4GlcNAc were synthesized bearing different (1,2,3)-triazole groups in the aglycon. All are strong ligands to *Pseudomonas aeruginosa* fucose-specific lectin as the PA-IIL affinity towards them is ten times stronger than that observed for fucose. Titration microcalorimetry established that the affinity for **11** is 310 nM, in the same range as that measured for the entire trisaccharide Le^a, which is, up to now, the natural ligand with highest affinity for PA-IIL. Nevertheless, the binding mechanism of PA-IIL towards **11** and Le^a is rather different as the first one displays an unfavorable entropy of binding whereas the second one has a favorable entropy contribution. This could be attributable to the conformational behavior of the ligand as the disaccharide is flexible in solution whilst the branched trisaccharide is fairly rigid. Crystal structures of PA-IIL complexed with disaccharide derivatives **11** and **14** illustrate the hydrogen bonding network that rationalizes the high enthalpic contribution and confirms that the triazole ring did not interact directly with the protein surface. Toxicity and cost of production of these derivatives have yet to be evaluated. The present study demonstrates that it is not necessary to synthesize the whole trisaccharide moiety to obtain high affinity ligands, and that the presence of triazole group in the aglycon does not affect the overall binding, therefore opening the route toward the synthesis of multimeric compounds that could be of therapeutic interest. Work is in progress on this aspect and the results will be reported in due course.

Experimental Section

General Methods. All reactions were run under an atmosphere of dry nitrogen using oven-dried glassware and freshly distilled and dried solvents. Tetrahydrofuran and diethyl ether were distilled from sodium benzophenone ketyl. Pyridine was distilled from calcium hydride or calcium hydroxide. Dichloromethane was distilled from phosphorus pentoxide. All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. 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, iodine, 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 (*v*/*v*). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 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 in a Perkin–Elmer JASCO P-1010, at the concentration indicated, in Chloroform or in the solvent indicated. ESI-MS analyses were carried out on a MICROMASS Quattro LC.

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**[25] (500 mg, 1.1×10^{-3} mol) and donor **1**[26] (695.0 mg, 1.3×

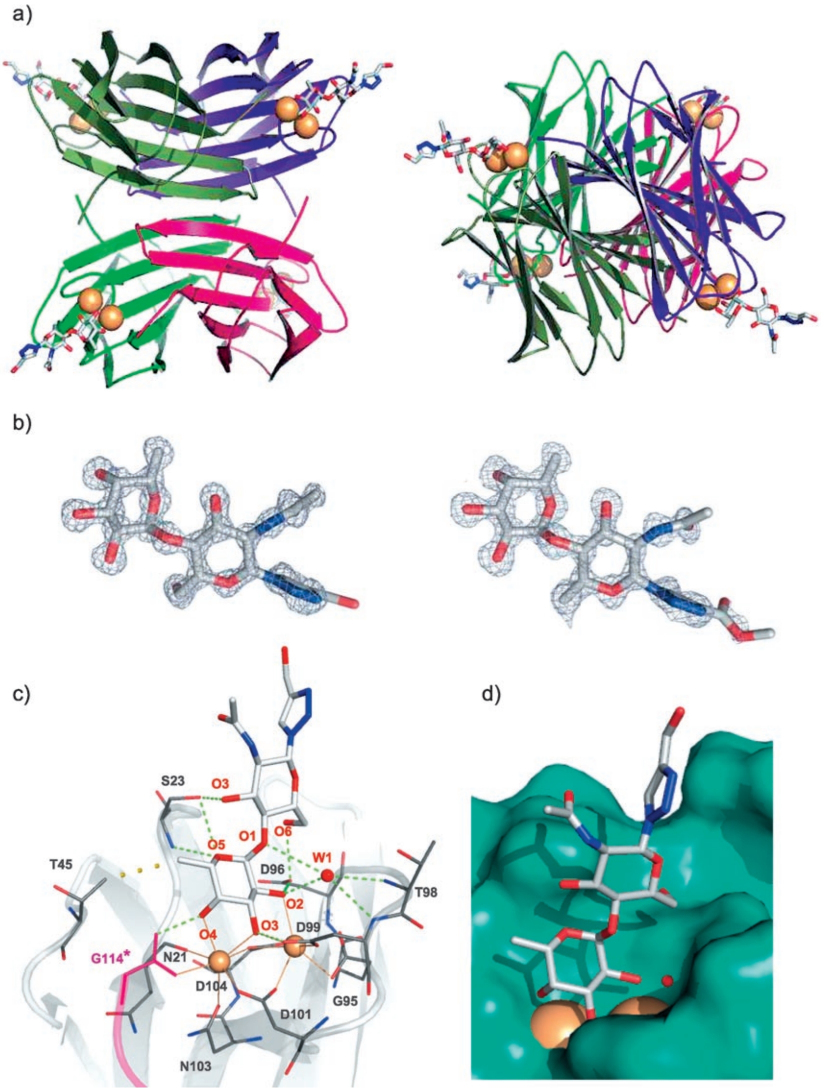


Figure 4. a) Orthogonal views of the complex of PA-IIL with **11**. Protein is represented as ribbon, calcium as spheres, and ligands as sticks. b) Diagram showing the high quality of the electron density (contoured at 1.0 σ) for the two disaccharide derivatives **11** and **14** in complex with PA-IIL. c) Details of the contact with hydrogen bonds represented by green dashed lines, coordination bonds by orange solid lines, and hydrophobic contacts by yellow dotted lines. d) Connolly surface of the protein with calcium ion as spheres and ligand as sticks.

10^{-3} mol, 1.2 equiv) were dissolved in dry chloroform (16.5 mL, 0.1 M) and stirred for 30 min in the presence of molecular sieves 4 Å at room temperature under nitrogen atmosphere. The solution was then cooled at $-15\,^{\circ}$C before addition of *N*-iodosuccinimide (322 mg, 1.4×10^{-3} mol, 1.3 equiv) and triflic acid (78 µL, 8.8×10^{-4} mol, 0.8 equiv). After 1 h at $-15\,^{\circ}$C, the reaction mixture was neutralized with a few drops of triethylamine, filtered through celite, and extracted with dichloromethane. The mixture was washed with a 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. mp: 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, $^3J_{6b\text{-}5b}=6.6$ Hz, H-6b), 1.78 (s, 3H, CH$_3$CO), 3.48 (b, 1H, H-4b), 3.71 (m, 1H, H-5b), 3.85 (dd, 1H, $^3J_{3b\text{-}4b}=2.5$ Hz, $^3J_{3b\text{-}2b}=10.2$ Hz, H-3b), 3.90 (m, 1H, H-5a), 3.95 (dd, 1H, $^3J_{2b\text{-}1b}=3.3$ Hz, H-2b), 4.01 (t, 1H, $^3J_{4a\text{-}3a}={}^3J_{4a\text{-}5a}=9.1$ Hz, H-4a), 4.17 (m, 1H, H-2a), 4.51 (d, 1H, $^2J=11.5$ Hz, CH$_2$-Ph), 4.63–4.74 (m, 4H, 2×CH$_2$-Ph, H-1a, H-6a), 4.78 (d, 1H, $^2J=11.5$ Hz, CH$_2$-Ph), 4.81 (d, 1H, $^2J=12.1$ Hz, CH$_2$-Ph), 4.84 (d, 1H, $^2J=11.5$ Hz, CH$_2$-Ph), 4.87 (d, 1H, H-1b), 4.96 (dd, 1H, $^3J_{6a\text{-}5a}=2.2$ Hz, $^2J=12.4$ Hz, H-6a), 5.41 (dd, 1H, $^3J_{3a\text{-}2a}=8.5$ Hz, H-3a), 5.74 (d, 1H, $^3J_{NH\text{-}2a}=9.3$ Hz, NHAc), 7.18–7.98 (m, 21H, H-Ar), 7.97 ppm (2× bd, 2×2H, $^3J=8.5$ Hz, H-Ar). ^{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: $m/z=893.4$ $[M+\mathrm{Na}]^+$.

[1-(tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]methanol 7. Copper sulfate (2.3 mg, 9.2×10^{-6} mol, 0.2 equiv) and ascorbic acid (3.6 mg, 1.8×10^{-5} mol, 0.4 equiv) was added to a solution of the disaccharide **3** (40.0 mg, 4.6×10^{-5} mol) and propargyl alcohol **4** (3.5 µL, 5.5×10^{-5} mol, 1.2 equiv) in a mixture *tert*-butanol/water (*v*/*v* 1:1, 0.04 M). The solution was milky white. After 18 h at room temperature, the reaction mixture was slightly yellow with an orange precipitate. The mixture was extracted with dichloromethane. The organic layer was washed successively with a saturated sodium bicarbonate solution and with brine, dried over sodium sulfate, and concentrated. The crude product was purified by column chromatography on silicagel (dichloromethane/methanol 20:1) to give the desired triazole **7** (34.1 mg, 80%) as a slightly yellow oil (even after treatment with charcoal). $[\alpha]_D^{22}=-49.8$ ($c=1.0$, chloroform). ^{1}H NMR (300 MHz, CDCl$_3$, 25 °C): $\delta=0.57$ (d, 3H, $^3J_{6b\text{-}5b}=6.4$ Hz, H-6b), 1.44 (s, 3H, CH$_3$CO), 3.50 (b, 1H, H-4b), 3.72 (m, 1H, H-5b), 3.85 (dd, 1H, $^3J_{3b\text{-}4b}=2.3$ Hz, $^3J_{3b\text{-}2b}=10.3$ Hz, H-3b), 3.95 (dd, 1H, $^3J_{2b\text{-}1b}=3.3$ Hz, H-2b), 4.13 (m, 1H, H-5a), 4.17 (m, 1H, H-2a), 4.46 (d, 1H, $^2J=11.4$ Hz, CH$_2$-Ph), 4.58–4.71 (m, 7H, H-4a, H-6a, 3×CH$_2$-Ph, 2×CH$_2$OH), 4.77, 4.80 (2×d, 2×1H, $^2J=11.4$ Hz, CH$_2$-Ph), 4.89 (d, 1H, H-1b), 4.96 (bd, 1H, $^2J=12.4$ Hz, H-6a), 5.71 (m, 1H, H-3a), 6.03 (d, 1H, $^3J_{1a\text{-}2a}=9.9$ Hz, H-1a), 6.74 (d, 1H, $^3J_{NH\text{-}2a}=9.4$ Hz, NHAc), 7.10–7.60 (m, 21H, H-Ar), 7.79 (s, 1H, H-triazole), 7.94, 8.00 ppm (2×bd, 2×2H, $^3J=7.2$ Hz, H-Ar). ^{13}C NMR (75 MHz, CDCl$_3$, 25 °C): $\delta=15.9$, 22.6, 53.7, 56.3, 62.5, 67.7, 72.4, 74.4, 74.5, 74.9, 75.0, 75.7, 76.7, 77.3, 79.3, 86.2, 100.7, 127.4, 127.5, 127.5, 127.8, 128.1, 128.2, 128.4, 128.5, 128.5, 128.6, 128.9, 129.6, 129.7,

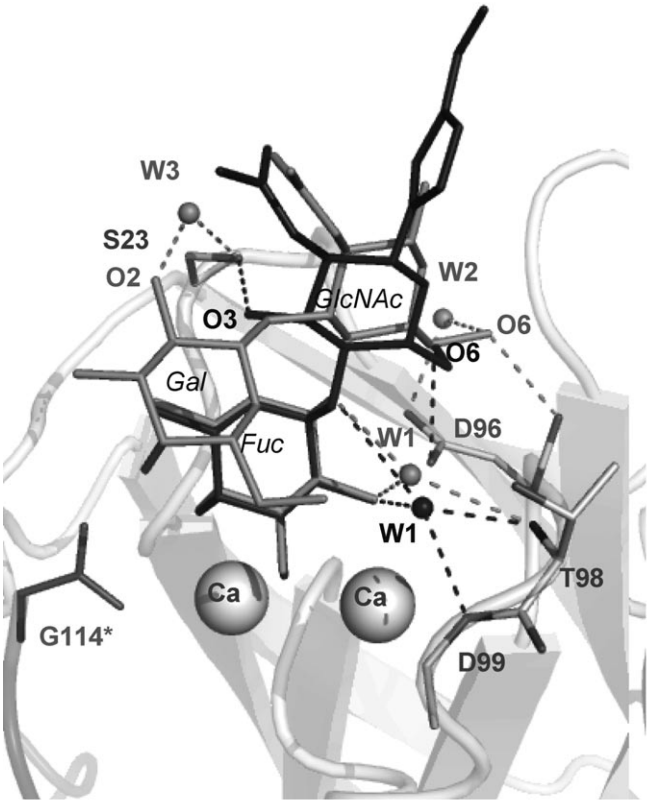


Figure 5. Graphical representation of the superimposition of **11** (dark grey) and Le^a trisaccharide (light grey, PDB code 1W8H) in the binding site of PA-IIL.

129.9, 133.2, 133.7, 137.8, 138.3, 138.5, 165.9, 167.1, 170.7 ppm. ESI-MS: $m/z = 950.4$ $[M+Na]^+$.

[1-(tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]methanol 7 and [3-(tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-3*H*-1,2,3-triazol-4-yl]methanol 8. A solution of the disaccharide **3** (150.0 mg, 1.7×10^{-4} mol) and propargyl alcohol **4** (11 μL, 1.9×10^{-4} mol, 1.1 equiv) in toluene was stirred 6 days at 75 °C in a sealed tube. After completion of the reaction, the mixture was concentrated. The regioisomers synthesized were separated by column chromatography on silicagel (dichloromethane/methanol 20:1) to give 57.5 mg of 1,5-disubstituted 1,2,3-triazole **8** (38 %) and 67.9 mg of the 1,4-regioisomer **7** (45 %) as colorless, amorphous solids. Analysis for **8**: $[\alpha]_D^{21} = -48.5$ ($c = 0.71$, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.63$ (d, 3 H, $^3J_{6b\text{-}5b} = 6.4$ Hz, H-6b), 1.63 (s, 3 H, CH_3CO), 3.61 (b, 1 H, H-4b), 3.81 (m, 1 H, H-5b), 3.88 (dd, 1 H, $^3J_{3b\text{-}4b} = 2.5$ Hz, $^3J_{3b\text{-}2b} = 10.4$ Hz, H-3b), 4.00–4.08 (m, 2 H, H-2b, CH_2OH), 4.21 (m, 1 H, H-5a), 4.33 (m, 1 H, H-4a), 4.49 (d, 1 H, $^2J = 11.6$ Hz, CH_2-Ph), 4.53 (d, 1 H, $^2J = 11.3$ Hz, CH_2-Ph), 4.61 (d, 1 H, $^2J = 11.6$ Hz, CH_2-Ph), 4.71–4.94 (m, 6 H, 3×CH_2-Ph, H-6a, 2×CH_2OH), 5.01–5.10 (m, 3 H, H-6a, H-2a, H-1b), 5.83 (dd, 1 H, $^3J_{3a\text{-}4a} = 9.0$ Hz, $^3J_{3a\text{-}2a} = 10.5$ Hz, H-3a), 6.16 (d, 1 H, $^3J_{1a\text{-}2a} = 10.0$ Hz, H-1a), 6.69 (d, 1 H, $^3J_{NH,2a} = 9.7$ Hz, N*H*Ac), 7.16–7.68 (m, 21 H, H-Ar), 7.66 (s, 1 H, H-triazole), 8.03–8.14 ppm (m, 4 H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 15.9$, 22.6, 52.6, 53.6, 62.0, 67.8, 71.9, 74.1, 74.5, 74.7, 75.0, 75.7, 77.2, 77.3, 79.4, 87.7, 101.0, 127.3, 127.4, 127.5, 127.8, 128.1, 128.2, 128.3, 128.4, 128.6, 128.7, 128.7, 129.5, 129.7, 130.0, 133.4, 134.0, 135.3, 137.1, 137.7, 138.3, 138.4, 165.9, 167.6, 171.5 ppm. ESI-MS: $m/z = 950.3$ $[M+Na]^+$.

***tert*-Butyl [1-(tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]methylcarbamate 9**. Copper sulfate (8.6 mg, 3.4×10^{-5} mol, 0.2 equiv) and ascorbic acid (13.6 mg, 6.9×10^{-5} mol, 0.4 equiv) was added to a solution of the disaccharide **3** (150.0 mg, 1.7×10^{-4} mol) and *N*-Boc-protected propargylamine **5**[29] (32.1 mg, 2.1×10^{-4} mol, 1.2 equiv) in a mixture *tert*-butanol/water (*v*/*v* 1:1, 0.04 M). The solution was milky white. After 16 h at room temperature, the reaction mixture was slightly blue with an orange precipitate. The mixture was extracted with ethyl acetate. The organic layer was washed successively with a saturated sodium bicarbonate solution and with brine, dried over sodium sulfate, and concentrated. The crude product was purified by column chromatography on silicagel (dichloromethane/methanol 20:1) to give the desired triazole **9** (157.2 mg, 89 %) as a colorless oil. $[\alpha]_D^{22} = -50.3$ ($c = 1.0$, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.59$ (d, 3 H, $^3J_{6b\text{-}5b} =$ 6.3 Hz, H-6b), 1.38 (s, 9 H, *t*Bu), 1.54 (s, 3 H, CH_3CO), 3.59 (b, 1 H, H-4b), 3.77 (m, 1 H, H-5b), 3.87 (bd, 1 H, $^3J_{3b\text{-}2b} = 10.7$ Hz, H-3b), 3.98 (dd, 1 H, $^3J_{2b\text{-}1b} = 3.6$ Hz, H-2b), 4.20–4.30 (m, 2 H, H-4a, H-5a), 4.30–4.40 (m, 2 H, CH_2NHBoc), 4.48, 4.52 (2×d, 2×1 H, $^2J = 11.5$ Hz, CH_2-Ph), 4.60 (d, 1 H, $^2J = 11.8$ Hz, CH_2-Ph), 4.67–4.85 (m, 5 H, 3×CH_2-Ph, H-2a, H-6a), 4.95 (d, 1 H, H-1b), 5.06 (bd, 1 H, $^2J = 12.4$ Hz, H-6a), 5.13 (m, 1 H, N*H*Boc), 5.85 (m, 1 H, H-3a), 6.11 (d, 1 H, $^3J_{1a\text{-}2a} = 9.9$ Hz, H-1a), 6.93 (b, 1 H, N*H*Ac), 7.11–7.62 (m, 21 H, H-Ar), 7.78 (s, 1 H, H-triazole), 8.00–8.07 ppm (m, 4 H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 15.9$, 22.6, 28.2, 36.0, 53.6, 62.3, 67.7, 72.1, 74.3, 74.6, 74.9, 75.7, 76.8, 77.2, 79.4, 86.1, 100.8, 120.5, 127.4, 127.4, 127.7, 128.0, 128.1, 128.3, 128.5, 128.6, 128.6, 128.8, 129.6, 129.7, 129.9, 133.2, 133.8, 137.7, 138.3, 138.4, 145.8, 155.5, 165.7, 167.4, 170.3 ppm. ESI-MS: $m/z = 1027.4$ $[M+H]^+$.

Methyl 1-(tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-carboxylate 10. Copper sulfate (2.9 mg, 1.1×10^{-5} mol, 0.2 equiv) and ascorbic acid (4.5 mg, 2.3×10^{-5} mol, 0.4 equiv) was added to a solution of the disaccharide **3** (50.0 mg, 5.7×10^{-5} mol) and methyl propiolate **6** (6.1 μL, 6.9×10^{-5} mol, 1.2 equiv) in a mixture *tert*-butanol/water (*v*/*v* 1:1, 0.04 M). The solution was milky white. After 4 h at 55 °C, the reaction mixture was slightly blue with an orange precipitate. The mixture was extracted with ethyl acetate. The organic layer was washed successively with a saturated sodium bicarbonate solution and with brine, dried over sodium sulfate, and concentrated. The crude product was purified by column chromatography on silicagel (hexane/ethyl acetate 1:1) to give the desired triazole **10** (42.0 mg, 77 %) as an colorless oil. $[\alpha]_D^{22} = -59.0$ ($c = 1.0$, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 0.58$ (d, 3 H, $^3J_{6b\text{-}5b} = 6.3$ Hz, H-6b), 1.58 (s, 3 H, CH_3CO), 3.60 (b, 1 H, H-4b), 3.74–3.80 (m, 1 H, H-5b), 3.86 (dd, 1 H, $^3J_{3b\text{-}4b} = 2.5$ Hz, $^3J_{3b\text{-}2b} = 10.2$ Hz, H-3b), 3.88 (s, 3 H, CH_3O), 3.98 (dd, 1 H, $^3J_{2b\text{-}1b} = 3.6$ Hz, H-2b), 4.27 (m, 1 H, H-4a), 4.48, 4.53 (2×d, 2×1 H, $^2J = 11.5$ Hz, CH_2-Ph), 4.60 (d, 1 H, $^2J =$ 11.3 Hz, CH_2-Ph), 4.69–4.84 (m, 6 H, H-2a, H-5a, H-6a, 3×CH_2-Ph), 4.95 (d, 1 H, H-1b), 5.08 (bd, 1 H, $^2J = 12.4$ Hz, H-6a), 5.83 (m, 1 H, H-3a), 6.13 (d, 1 H, $^3J_{1a\text{-}2a} = 9.9$ Hz, H-1a), 6.54 (b, 1 H, N*H*Ac), 7.12–8.07 (m, 25 H, H-Ar), 8.37 ppm (s, 1 H, H-triazole). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta = 15.9$, 22.6, 52.2, 53.9, 62.3, 67.8, 72.1, 74.3, 74.4, 74.5, 75.0, 75.7, 77.2, 79.4, 86.5, 101.0, 126.5, 127.4, 127.5, 127.8, 128.1, 128.2, 128.3, 128.4, 128.7, 129.6, 129.7, 129.9, 133.3, 134.0, 137.7, 138.3, 138.3, 140.2, 160.6, 165.7, 167.5, 170.5 ppm. ESI-MS: $m/z = 978.3$ $[M+Na]^+$.

General procedure for debenzoylation. The protected compound was dissolved in methanol (0.1 M) at room temperature (or in a mixture methanol/THF to allow complete solubilization of the product). A catalytic amount of sodium methoxide (0.1 equiv) 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 silicagel.

General procedure for debenzylation. Benzyl ether was dissolved in methanol (0.02–0.05 M) and hydrogenolyzed on 20 wt% palladium dihydroxide on carbon at room temperature. After completion of the reaction, successive steps of filtration through celite, evaporation, and, if necessary, chromatography or filtration through microfilter, gave the desired product.

[1-(α-L-fucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]methanol 11. The two deprotection steps occurs with 84% yield, giving **11** as a white, amorphous solid. $[\alpha]_D^{22}=-114.7$ ($c=1.0$, methanol). ^{1}H NMR (300 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=1.18$ (d, 3H, $^3J_{6b\text{-}5b}=6.0$ Hz, H-6b), 1.83 (s, 3H, CH_3CO), 3.77–4.02 (m, 8H, H-3a, H-4a, H-5a, 2×H-6a, H-2b, H-3b, H-4b), 4.29 (m, 1H, H-2a), 4.40 (m, 1H, H-5b), 4.72 (s, 2H, CH_2O), 5.02 (d, 1H, $^3J_{1b\text{-}2b}=3.0$ Hz, H-1b), 5.86 (d, 1H, $^3J_{1a\text{-}2a}=9.6$ Hz, H-1a), 8.20 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=15.9$, 22.2, 55.2, 56.4, 60.3, 67.7, 68.7, 70.1, 72.6, 72.9, 77.2, 79.0, 86.9, 100.4, 123.3, 147.7, 174.8 ppm. ESI-MS: $m/z=471.3$ $[M+Na]^+$.

[3-(α-L-fucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3*H*-1,2,3-triazol-4-yl]methanol 12. The two deprotection steps occurs with 50% yield, giving **12** as a white, amorphous solid. $[\alpha]_D^{22}=-114.7$ ($c=1.0$, methanol). ^{1}H NMR (300 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=1.18$ (d, 3H, $^3J_{6b\text{-}5b}=6.5$ Hz, H-6b), 1.81 (s, 3H, CH_3CO), 3.76–4.04 (m, 8H, H-3a, H-4a, H-5a, 2×H-6a, H-2b, H-3b, H-4b), 4.35–4.50 (m, 2H, H-2a, H-5b), 4.84 (s, 2H, CH_2O), 5.01 (d, 1H, $^3J_{1b\text{-}2b}=3.0$ Hz, H-1b), 5.88 (d, 1H, $^3J_{1a\text{-}2a}=9.7$ Hz, H-1a), 7.76 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=15.9$, 22.3, 52.6, 56.1, 60.4, 67.7, 68.7, 70.1, 72.6, 73.0, 77.4, 79.1, 85.4, 100.4, 133.9, 133.9, 134.0, 138.9, 174.7 ppm. ESI-MS: $m/z=471.3$ $[M+Na]^+$.

***tert*-Butyl [1-(α-L-fucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]methylcarbamate 13**. The two deprotection steps occurs with 87% yield, giving **13** as a white, amorphous solid. $[\alpha]_D^{22}=-103.2$ ($c=1.0$, methanol). ^{1}H NMR (300 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=1.18$ (d, 3H, $^3J_{6b\text{-}5b}=6.6$ Hz, H-6b), 1.41 (s, 9H, *t*Bu), 1.82 (s, 3H, CH_3CO), 3.76–4.00 (m, 8H, H-3a, H-4a, H-5a, 2×H-6a, H-2b, H-3b, H-4b), 4.27 (m, 1H, H-2a), 4.33 (m, 2H, CH_2NH), 4.39 (m, 1H, H-5b), 5.01 (d, 1H, $^3J_{1b\text{-}2b}=3.6$ Hz, H-1b), 5.83 (d, 1H, $^3J_{1a\text{-}2a}=9.9$ Hz, H-1a), 8.08 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=15.9$, 22.3, 28.2, 35.9, 56.4, 60.3, 67.7, 68.7, 70.1, 72.6, 73.0, 77.2, 79.0, 86.8, 100.4, 122.9, 158.6, 174.7 ppm. ESI-MS: $m/z=570.4$ $[M+Na]^+$.

Methyl 1-(α-L-fucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-carboxylate 14. The two deprotection steps occurs with 86% yield, giving **14** as a white, amorphous solid. $[\alpha]_D^{22}=-97.9$ ($c=0.66$, methanol). ^{1}H NMR (300 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=1.18$ (d, 3H, $^3J_{6b\text{-}5b}=6.5$ Hz, H-6b), 1.83 (s, 3H, CH_3CO), 3.80–4.04 (m, 11H, CH_3O, H-3a, H-4a, H-5a, 2×H-6a, H-2b, H-3b, H-4b), 4.28 (m, 1H, H-2a), 4.41 (m, 1H, H-5b), 5.02 (d, 1H, $^3J_{1b\text{-}2b}=3.0$ Hz, H-1b), 5.93 (d, 1H, $^3J_{1a\text{-}2a}=9.8$ Hz, H-1a), 8.82 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=15.9$, 22.2, 53.4, 56.6, 60.3, 67.7, 68.7, 70.1, 72.6, 72.8, 77.1, 79.1, 87.2, 100.4, 128.8, 140.0, 162.7, 174.8 ppm. ESI-MS: $m/z=499.3$ $[M+Na]^+$.

1-(α-L-fucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-carboxylic acid 15. A solution of methyl ester **14** 13.4 mg (2.8×10^{-5} mol) in a mixture methanol/water (*v/v* 2:1) was treated with lithium hydroxyle (1.3 mg, 5.6×10^{-5} mol, 2 equiv). After 1 h, the reaction mixture was neutralized with amberlyst IR120 resin, filtrated, and evaporated. The residue was purified by column chromatography on silicagel (dichloromethane/methanol 2:1) and filtrated through a microfilter to give the carboxylic acid derivative **15** (8.8 mg, 68%) as a white solid. $[\alpha]_D^{22}=-106.7$ ($c=0.45$, methanol). ^{1}H NMR (300 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=1.17$ (d, 3H, $^3J_{6b\text{-}5b}=6.6$ Hz, H-6b), 1.83 (s, 3H, CH_3CO), 3.79–4.02 (m, 8H, H-3a, H-4a, H-5a, 2×H-6a, H-2b, H-3b, H-4b), 4.31 (m, 1H, H-2a), 4.41 (m, 1H, H-5b), 5.01 (d, 1H, $^3J_{1b\text{-}2b}=3.6$ Hz, H-1b), 5.89 (d, 1H, $^3J_{1a\text{-}2a}=9.8$ Hz, H-1a), 8.41 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=15.9$, 22.3, 56.4, 60.3, 67.7, 68.7, 70.1, 72.6, 72.9, 77.2, 79.0, 79.1, 86.8, 100.4, 126.7, 145.6, 167.8, 174.5 ppm. ESI-MS: $m/z=485.3$ $[M+Na]^+$.

(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl methanol 16. Copper sulfate (42.3 mg, 1.7×10^{-4} mol, 0.2 equiv) and ascorbic acid (88.8 mg, 4.5×10^{-4} mol, 0.6 equiv) was added to a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (260.0 mg, 7.0×10^{-4} mol) and propargyl alcohol 4 (49 μL, 8.4×10^{-4} mol, 1.2 equiv) in a mixture *tert*-butanol/water (*v/v* 1:1, 3 mL/3 mL). The mixture was stirred at 50 °C overnight. The mixture was extracted with ethyl acetate. The organic layer was washed successively with a saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and concentrated. The crude product was purified by column chromatography on silicagel (dichloromethane/Methanol 20:1 to 15:1) to give the desired triazole **16** (206.9 mg, 69%) as a white compound. mp: 214–216 °C. $[\alpha]_D^{30}=-27.1$ ($c=0.5$, chloroform). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta=1.69$ (s, 3H, CH_3CO), 2.06 (s, 9H, 3×CH_3CO), 4.14 (m, 3H, H-6a, H-5, OH), 4.28 (dd, 1H, $^2J=12.8$ Hz, $^3J_{5\text{-}6}=4.7$ Hz, H-6b), 4.62 (dd, 1H, $^3J_{2\text{-}1}={}^3J_{2\text{-}3}={}^3J_{2\text{-}NH}=9.9$ Hz, H-2), 4.74 (b, 2H, CH_2OH), 5.26 (app. t, 1H, $^3J=9.8$ Hz, H-4), 5.54 (app. t, 1H, $^3J=9.9$ Hz, H-3), 6.17 (d, 1H, $^3J=9.9$ Hz, H-1), 7.45 (d, 1H, $^3J=9.0$ Hz, NHAc), 7.98 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): $\delta=20.5$, 20.6, 20.7, 22.6, 53.3, 55.8, 61.7, 68.1, 72.3, 74.6, 85.7, 121.3, 148.0, 169.4, 170.6, 170.8, 171.2 ppm. ESI-MS: $m/z=451.1$ $[M+Na]^+$.

(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl methanol 17. The debenzoylation occurs quantitatively, giving **17** as a white solid. mp: 226–228 °C (decomp.). $[\alpha]_D^{30}=+5.9$ ($c=0.5$, water). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): $\delta=1.78$ (s, 3H, CH_3CO), 3.58 (m, 2H, H-4, H-5), 3.73 (m, 2H, H-3, H-6a), 3.90 (d, 1H, $^2J=12.1$ Hz, H-6b), 4.25 (app. t, 1H, $^3J_{2\text{-}1}={}^3J_{2\text{-}3}={}^3J_{2\text{-}NH}=9.9$ Hz, H-2), 4.65 (s, 2H, CH_2OH), 5.79 (d, 1H, $^3J=9.9$ Hz, H-1), 8.10 ppm (s, 1H, H-triazole). ^{13}C NMR (75 MHz, D_2O/Acetone (*v/v* 600:1), 25 °C): $\delta=22.3$, 55.2, 56.0, 61.1, 70.0, 74.2, 79.6, 87.0, 123.4, 147.7, 174.9 ppm. ESI-MS: $m/z=325.1$ $[M+Na]^+$.

Preparation of PA-IIL. Recombinant PA-IIL was purified from *Escherichia coli* BL21(DE3) containing the plasmid pET25pa2l as described previously.[14] Biotinylation of PA-IIL was performed as described in the literature.[33] 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 (DMF) containing 4.5 mM biotin for 2 h at room temperature, under agitation. After dialysis against 0.15 M of NaCl solution then water, the lectin was lyophilized. For the carbohydrate microarray assay carried out by the Consortium for Functional Genomics, the protein was labeled by Alexa Fluro 488 TFP ester (Molecular Probes, Inc, Eugene, Or) following the manufacturer instructions.

ELLA (enzyme-linked lectin assay) experiments. First test (plate coated with fucose-PAA): 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. 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. Sugars and proteins were dissolved in the same buffer of 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 rev. min^{-1}. 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:

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

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

Crystallization. Complexes of PA-IIL/inhibitor were obtained by soaking fucose-containing crystals of PA-IIL in a solution of 10 mM of inhibitor during one night at room temperature. Fucose-containing crystals were obtained by hanging drop vapor diffusion from conditions consisting of 1.75 M ammonium sulfate in 0.1 M Tris HCl (pH 8.5) and stored at 20 °C as described previously.[14]

Data collection and structure solution. Crystals were cryocooled at 100 K after soaking them for as short a time as possible in 25 % (*v/v*) glycerol in the precipitant solution. All data images were recorded on an ADSC Q4R CCD detector (Quantum Corp.) at a wavelength of 0.933 Å on beamline ID14-2 at ESRF (Grenoble, France). Data were processed with MOSFLM[34] and scaled and converted to structure factors using the CCP4 program suite.[35] Phasing of the PA-IIL/inhibitor complex was performed by molecular replacement, using the crystal structure of the PA-IIL-fucose complex determined previously at 1.0 Å resolution as the search probe.[14] An initial structure was built automatically using ARP/warp.[36] The resulting electron density maps showed clear features corresponding to the inhibitor ligands. As a result of crystal packing, only three of the four binding sites of the tetramer are complexed with inhibitors, the last binding site (monomer A) still containing a fucose monosaccharide. Cycles of iterative manual rebuilding and refinement were performed using the programs Coot[37] and REFMAC.[38] All graphical representations were prepared with Pymol (Delano Scientific LLC, South San Francisco).

Coordinates of the final structures (see refinement statistics in Table 3) have been deposited in the Protein Data Bank under codes 2JDH and 2JDK for complexes with compound **11** and compound **14**, respectively.

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