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Monovalent and bivalent *N*-fucosyl amides as high affinity ligands for *Pseudomonas aeruginosa* PA-IIL lectin

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ABSTRACT

The adhesion of bacteria to human glycoconjugates can be inhibited by soluble glycomimetics that compete with the natural target. Four monovalent and one divalent α-fucosyl amides have been tested for their affinity for a fucose-binding lectin from *Pseudomonas aeruginosa*. Isothermal calorimetric titrations demonstrated that they bind to the lectin in the micromolar range, with highest affinity for the divalent ligand. Molecular modelling established that, compared to *O*-fucoside compounds, the glycomimetic amide group resulted in the loss of water-bridged hydrogen bonds that could be partially compensated by additional contact of the aglycone with the protein surface.

1. Introduction

N-Glycosyl amides are currently under intense scrutiny as potential effectors of carbohydrate-binding proteins.[1] Our group has reported on the synthesis of α-*N*-glycosyl amides as mimics of α-glycosides, potentially endowed with metabolic stability.[2–6] NMR analysis[4] revealed that these compounds appear to maintain the normal pyranose conformation of the monosaccharide involved and thus can be regarded as bona fide *structural* mimetics. Furthermore, we also showed[7] that an α-fucosyl amide anchor could be used to create a Lewis-x mimic capable of engaging the carbohydrate recognition domain (CRD) of DC-SIGN, a dendritic cell receptor with mannose and fucose specificities, which has been implicated in the onset of HIV infection.[8] This work established α-fucosyl amides as effective *functional* mimics of α-fucosides.

The CRD of DC-SIGN, however, has a typical large, shallow and solvent-exposed lectin binding site, which could be rather permissive in terms of structural requirements for bound ligands. *Pseudomonas aeruginosa* PA-IIL lectin, on the contrary, presents a much more demanding case. This soluble bacterial lectin binds with an unusually strong micromolar affinity to L-fucose in a tight binding site which requires two Ca^{2+} ions.[9–11] *P. aeruginosa* is the causative agent of lung infections that are the major cause of mortality for cystic fibrosis patients and PA-IIL has been proposed to be involved in adhesion to host glycan.[12] Preliminary clinical assays performed on human and mice with *P. aeruginosa* pneumonia demonstrated that administration of fucose or fucoside reduces bacterial load in the lungs.[13,14] The lectin therefore appears as an attractive target for therapeutic application and several groups have developed high affinity glycomimetics and glycodendrimers that can compete with host glycoconjugates and inhibit bacterial adhesion.[15–20]

A small group of α-fucosyl amides have been tested for their affinity for PA-IIL using isothermal calorimetric titrations and were found to bind in the micromolar range. These results constitute proof of principle that α-glycosyl amides can perform as effective mimics of α-fucosides also in high affinity, tight binding proteins.

2. Results and discussion

A group of 5 α-*N*-fucosyl amides **1–5** were considered in the present work (Table 1). The first three compounds, **1–3**, were obtained via modifications of the Lewis-x mimic previously described by our group.[7] Compound **4** represents a simple linear amide generated with β-alanine and compound **5,** a dimeric form of the latter. All ligands were synthesized using our modification[7] of DeShong's procedure (Scheme 1)[21] starting from the known 2,3,4-tri-*O*-acetyl-fucosyl azide, **7** (9:1 α/β mixture).[7] Thus, treatment of **7** with Ph_3P in nitroethane at reflux yielded the oxazoline **8**, which was acylated in situ with the pyridyl thioester of (1*S*,6*R*)-6-(benzyloxycarbonylamino) cyclohex-3-enecarboxylic acid[7] or with the pyridyl thioester of benzyloxycarbonyl-β-alanine,[22] to afford **9** and **10**, in 64% and 52% yield, respectively.

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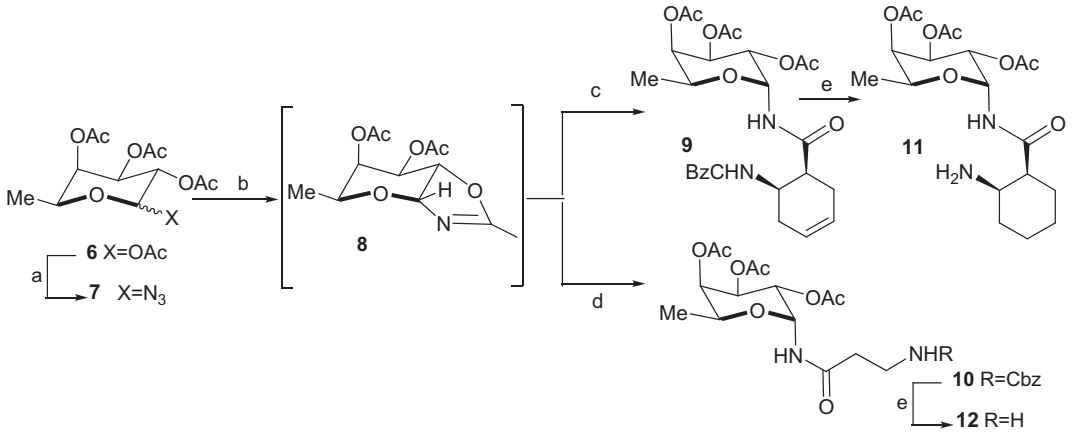


Scheme 1. Synthesis of α-*N*-fucosyl amides. Reagents and conditions: (a) TMSOTf 0.4 mol equiv, $TMSN_3$, DCM, rt, 94%; (b) Ph_3P, $EtNO_2$, reflux, 12 h; (c) pyridyl thioester of (1*S*,6*R*)-6-(benzyloxycarbonylamino) cyclohex-3-enecarboxylic acid, $CuCl_2$, 20 h, 40 °C; (d) pyridyl thioester of Cbz-β-alanine, $CuCl_2$, 20 h, 40 °C; (e) H_2, Pd–C.

The nitrogen-protecting group was removed to provide amines **11** and **12** which were functionalized with standard procedures, as shown in Scheme 2, to afford the final compounds **1**–**5**.

Compounds **1** to **5** were tested for their interaction with PA-IIL by titration microcalorimetry (Table 1). In all ITC binding experiments, the first injections resulted in large exothermic peaks, indicating an enthalpy-driven interaction (Fig. 1). After titration, no residual heat of binding was observed.

The stoichiometry of the interaction between PA-IIL and compounds **1** to **3** is close to one, in agreement with the crystal structure. Compound **4** displays a higher stoichiometry, close to 1.4, which could be indicative of some nonspecific binding. Interestingly, compound **5**, which is a dimeric form of **4**, has a stoichiometry lower than 1 and almost half of that of compound **4**, indicating that this compound is able to bind two PA-IIL binding sites.

For the compounds containing one *N*-fucosyl amide group, the dissociation constants vary from 1.2 to 2.1 μM, the higher affinity ligands being compound **1**. Such values are between those measured for α-methyl fucoside (K_D 0.38 μM) or previously obtained for fucose[11] (K_D 2.9 μM). Compound **5** that contains two *N*-fucosyl amide groups is the highest affinity ligand with a dissociation constant of 0.62 μM. Analysis of the thermodynamic contribution indicates that compounds **1** to **4** have a strong enthalpy of binding but also present favourable entropy contribution. Protein–carbohydrate interactions generally present unfavourable entropy contribution,[23] but the two-calcium lectins of PA-IIL family often display a favourable entropy contribution, which has been attributed to the release of the calcium-coordinating water molecules upon glycan binding.[24] The dimeric compound **5** presents very strong binding enthalpy that corresponds to twice that of compound **4** (Fig. 1 and Table 1). This term is partly counterbalanced by an unfavourable entropy term. Comparison of thermodynamic contribution of **4** and **5** clearly confirms that **5** is able to bind to two PA-IIL binding sites. The modest gain in affinity indicates that this compound does not bind to two sites of the same PA-IIL tetramer but more likely bridges two different lectins.

The data show that, despite its tight constraints, the PA-IIL binding site can accommodate an α-fucosyl amide moiety and form a complex with low micromolar affinity. To rationalize the decrease in affinity observed going from α-methyl fucoside to the amide in our series, a molecular docking study was performed for **1** and **2** in the binding site of PA-IIL. These two compounds were built in their low energy conformation, taking into account the information from 600 MHz spectra NMR for selecting the correct ring shape of the cyclohexane moiety.[7]

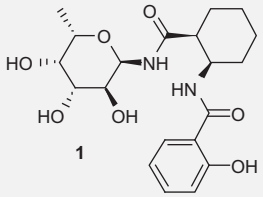
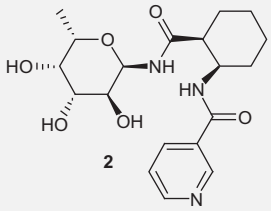
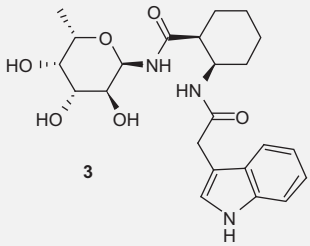
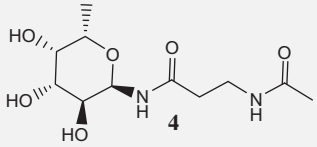
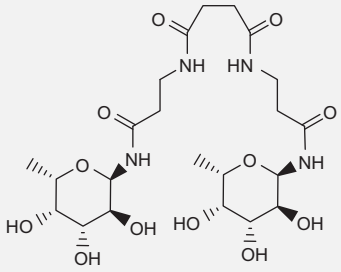
As opposed to α-*C*-fucosides,[25,26] α-fucosyl amides have been reported to adopt the native $^1C_4$(L) chair conformation.[4,7] The diagnostic large coupling constant value ($J_{2\text{-}3}$ = 10.6 Hz) between fucose H-2 and H-3 protons in **1**–**4** allows to confirm this feature for the compounds examined. The conformation of the *cis*-β-aminoacid fragment in **1** and **2** is also of concern. For these compounds, AMBER* calculations predicted a single chair conformation for the amino acid fragment, featuring the carboxy group in the equatorial position and the amino group in the axial position (Fig. 2b and c). Coupling constant analysis allowed for confirmation of the modelling results.

It was immediately clear that the water molecule that is conserved in all structures of PA-IL complexes cannot be maintained in the presence of *N*-fucosyl amides. As confirmed by molecular dynamics study,[27] this water molecule receives hydrogen bond from two amide nitrogens from the protein main chain and gives two hydrogen bonds to O-6 and O-1 of the fucose residue (Fig. 2a). The NH group at anomeric position perturbs this network and creates steric conflicts. The absence of this tightly bound water molecules rationalizes the differences observed between α-methyl fucoside and **4**, which is the simplest *N*-fucosyl amide in this study. The higher number of stabilizing contacts (hydrogen bonds) when the water is present explains the stronger enthalpy term (16 kJ/mol in favour of α-methyl fucoside), which is only partly compensated by the freezing of this water that is entropically unfavourable (12 kJ/mol in favour of *N*-fucosyl amine). The presence of the water molecule when binding *O*-fucose derivatives appears to be of strong influence on the affinity.

Except for this bridging water molecule, the *N*-fucosyl amides establish the same hydrogen bond network between fucose and PA-IIL binding site as observed in other fucosides (Fig. 2). The CO group at the glycosidic linkage establishes an additional hydrogen bond with Ser23. For compounds **1**–**3**, the CO of the other amide linkage is also predicted to establish hydrogen bond to this serine. Additional hydrophobic interactions can be established between the aromatic ring of these three compounds and the protein surface. The aglycon portions of compounds **4** and **5** are more flexible and it is not possible to predict the stabilizing interactions that could occur in the vicinity of the sugar binding site.

α-Fucosides are well known for their chemical instability and mimics of α-L-fucosides have been actively explored. Recently, both *C*-fucosides and simple α-L-fucoside ligands bearing various heterocycles in the aglycon portion were used to synthesize high affinity multivalent clusters bearing multiple copies of the L-fucose epitope.[28] The results we report here show that α-*N*-fucosyl

Table 1
Isothermal calorimetric titration of PA-IIL with **1–5**

Compound	*n*	K_A (10^4 M^{-1})	K_D (μM)	ΔG (kJ/mol)	Δ*H* (kJ/mol)	*T*ΔS (kJ/mol)
 **1**	1.02 (±0.02)	84 (±2)	1.2	−33.8	−27.1 (±0.1)	6.7
 **2**	1.11 (±0.01)	48 (±3)	2.1	−32.4	−22.8 (±0.1)	9.6
 **3**	1.13[a]	47.1	2.1	−32.3	−22.2	10.1
 **4**	1.39[a]	56.2	1.8	−32.8	−19.3	13.5
 **5**	0.80 (±0.01)	161 (±3)	0.62	−35.4	−38 (±1)	−2.6
α–Me-fucoside	1.05 (±0.01	261 (±4)	0.38	−36.6	−35.5 (±0.6)	1.1

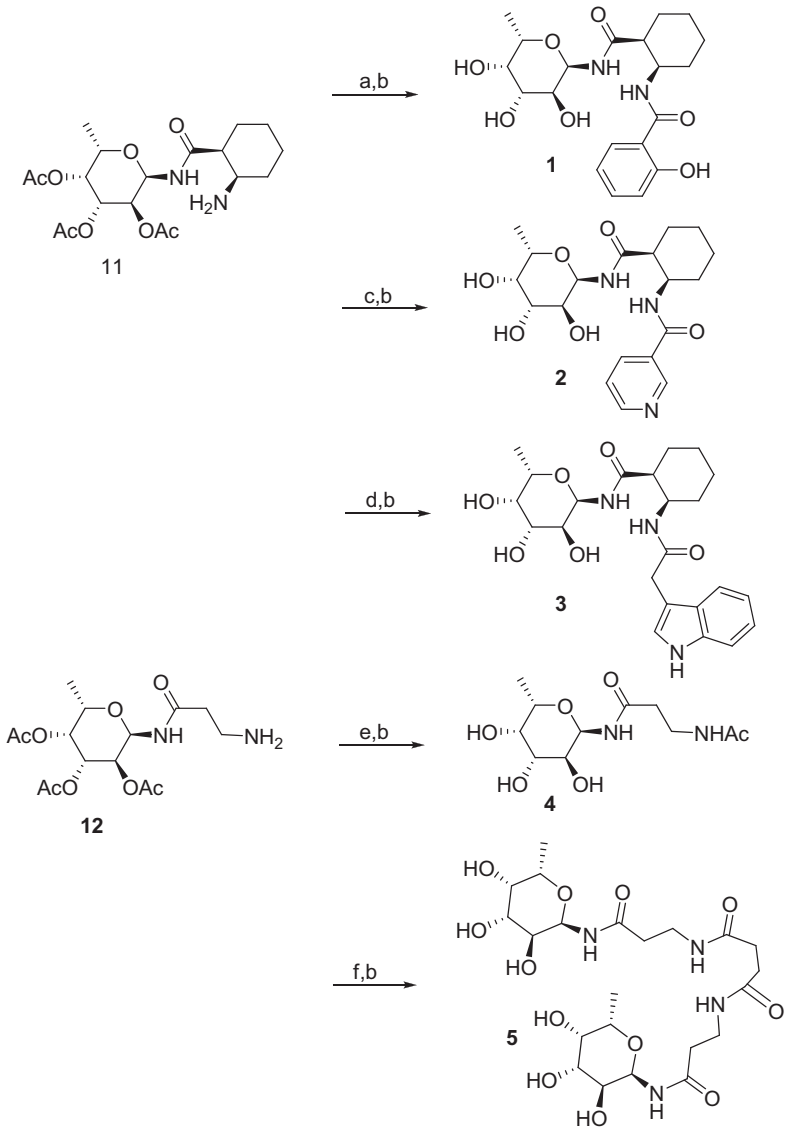
[a] Only one experiment performed.

amides can be used to the same effect. Although compared to *O*-fucosides, *N*-fucosyl amides appear to suffer from the presence of the NH group at the anomeric position, their activity can be rescued by additional interactions created by the amide side chain in the vicinity of the fucose binding site. In the future, it would be of interest to quantify this interaction by direct comparison with the corresponding glycoside or anomeric ester. At the same time α-*N*-fucosyl amides do not correspond to a known biological chemotype, and therefore, like *C*-glycosides, they ought to be stable to the action of hydrolytic enzymes. Information on how to design better inhibitors can now be gathered from the reported docking protocols, which can be used to prioritize in silico a series of possible side chains. The simple synthesis and easy functionalization of these compounds together with their chemical stability make them very interesting candidates for antagonism or modulation of fucose-binding lectins.

3. Experimental

3.1. General

Solvents were dried by standard procedures: CH_2Cl_2, MeOH and Et_3N were dried over calcium hydride; pyridine was dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under nitrogen. ^1H and ^{13}C and spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^1H and ^{13}C spectra are expressed in parts per million (ppm) relative to internal Me_4Si as a standard. Signals were abbreviated as s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a Bruker ion-trap Esquire 3000 apparatus (ESI ionization) or with a VG-Analytical Autospec Q mass spectrometer or with a FT-ICR Mass Spectrometer APEX II & Xmass software. Thin layer chromatography



Scheme 2. Functionalization of α-*N*-fucosyl amides. Reagents: (a) 2-acetoxy-benzoylchloride; (b) cat. MeONa, MeOH; (c) HBTU, nicotinic acid; (d) HBTU, indolacetic acid; (e) Ac_2O, pyridine; (f) succinic anhydride, THF, then PyBOP, Et_3N, CH_2Cl_2.

(TLC) was carried out with pre-coated Merck F_{254} silica gel plates. Flash chromatography (FC) was carried out with Macherey–Nagel Silica Gel 60 (230–400 mesh). Unless otherwise indicated, automated chromatography was carried out on silica gel with a Biotage System SP1 (KP-Sil cartridge). Compounds **9** and **11** were previously described.[7]

3.2. *N*-((1*S*,2*R*)-2-(2-Hydroxybenzamido)cyclohexanecarboxyl)-α-L-fucopyranosyl amine 1

2-Acetoxybenzoic acid (14 mg, 0.08 mmol) was refluxed in 1 mL of toluene in the presence of oxalyl chloride (3 mmol) during 3 h. The solution was then evaporated and added to a solution of **11** (30 mg, 0.072 mmol) and Et_3N (2 equiv) in 1 mL of THF. The solution was stirred at room temperature for 18 h, the solvent was evaporated and the crude dissolved with CH_2Cl_2. The solution was washed with a satd aq $NaHCO_3$, 10% citric acid and an aqueous saturated NaCl solution. The solvent was evaporated under vacuum and the residue was purified by automated chromatography using a hexane–EtOAc gradient to yield 50% (21 mg, 0.038 mmol) of *N*-((1*S*,2*R*)-2-(2-acetoxybenzamido)cyclohexanecarboxyl)-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.68 (d, 3H, $J_{5\text{-}6}$ = 6.3 Hz, H_6), 1.32–2.05 (m, 8H, CH_{2cycl}), 1.89, 1.94, 2.09, 2.25 (4 × s, 12H, 4×–C(O)CH_3), 2.77 (m, 1H, H_9), 3.60 (m, 1H, H_5), 4.26 (m, 1H, H_{10}), 4.99 (s, 1H, H_4), 5.24–5.32 (m, 2H, H_2, H_3), 5.82 (dd, 1H, $J_{1\text{-}2}$ = 5.2 Hz, $J_{1\text{-NH}}$ = 8.7 Hz, H_1), 7.04, 7.23, 7.27, 7.80 (4 × m, 5H, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ (ppm) = 16.1 (C_6), 20.9, 21.5 (4×–C(O)*C*H_3), 22.0, 24.7, 27.8, 29.2 (4×CH_{2cycl}), 45.0 (C_9), 48.8 (C_{10}), 65.8 (C_5), 66.6 (C_2), 68.3 (C_3), 71.0 (C_4), 74.5 (C_1), 123.4, 126.7 (2 × C_{Ar}), 128.3 (C_{ipso}), 130.1, 132.3 (2 × C_{Ar}), 148.2 (C_{ipso}), 165.7 (C_{12}), 169.7, 169.9, 170.6, 170.97 (4 × –*C*(O)CH_3), 174.8 (C_8); ESI-MS: *m*/*z* = 599 $[M+Na]^+$, 100%; R_f = 0.30 (hexane–EtOAc 1:1).

Deprotection under Zemplen's conditions afforded **1** in quantitative yield. ^{1}H NMR (D_2O): δ (ppm) = 0.78 (d, 3H, $J_{5\text{-}6}$ = 6.5 Hz, H_6), 1.48–2.05 (m, 8H, CH_{2cycl}), 3.00 (m, 1H, H_9), 3.61 (dq, 1H, $J_{5\text{-}6}$ = 6.5 Hz, $J_{4\text{-}5}$ <1 Hz, H_5), 3.67 (dd, 1H, $J_{3\text{-}4}$ = 3.4 Hz, H_4), 3.83 (dd, 1H, $J_{2\text{-}3}$ = 10.6 Hz, H_3), 4.00 (dd, 1H, $J_{1\text{-}2}$ = 5.6 Hz, H_2), 4.56 (m, 1H, H_{10}), 5.48 (d, 1H, H_1), 7.08, 7.49, 7.84 (3 × m, 4H, H_{Ar}); ^{13}C NMR (D_2O): δ (ppm) = 16.1 (C_6), 21.6, 23.9, 24.2, 30.4 (4 × CH_{2cyl}), 45.6 (C_9), 49.6 (C_{10}), 66.8 (C_2), 67.9 (C_5), 70.4 (C_3), 72.3 (C_4), 77.7 (C_1), 118.0 (C_{Ar}), 118.2 (C_{ipso}), 121.3, 130.4, 135.0 (3 × C_{Ar}), 157.3 (C_{ipso}),

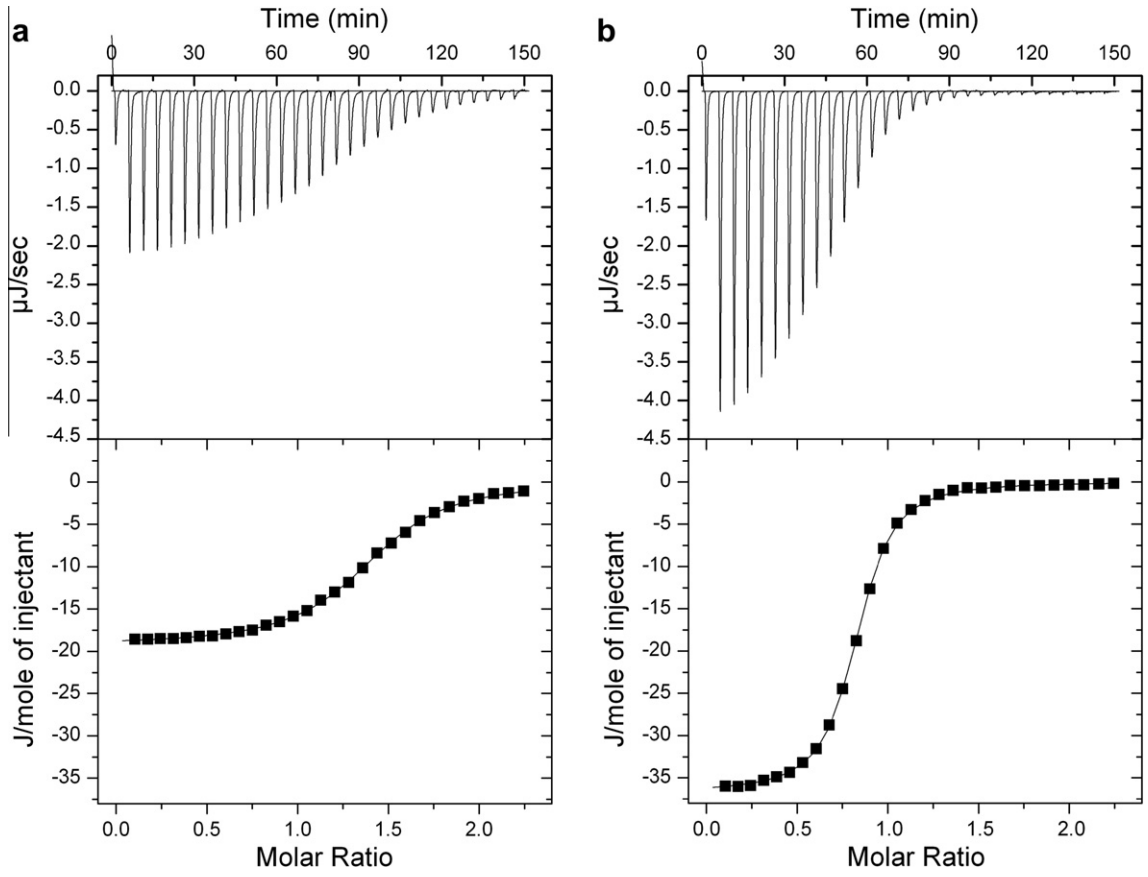


Figure 1. Titration microcalorimetry of PA-IIL by **4** (a) and **5** (b) with titration curves (top) and integration of heat released with the solid line representing the best least-squares fit (bottom). Titration performed at 25° by 30 automatic injections of 10 µL compound (0.5 mM) added every 300 s to PA-IIL containing cell (0.05 mM).

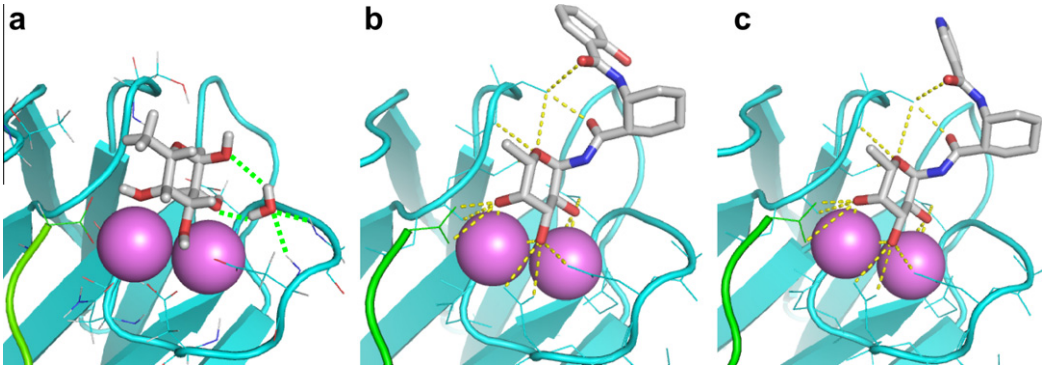


Figure 2. Complex of PA-IIL with fucose (a), **1** (b) and **2** (c). Complex with fucose and hydrogen bond network (yellow dotted lines) of crystalline water (green dotted lines) is adapted from.[10] Complexes with **1** and **2** result from the docking study. For (b) and (c) hydrogen atoms are not represented for clarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

169.1, 178.6 (2 × C=O); ESI-MS: *m/z* = 431.5 $[M+Na]^+$, 30%; FT-ICR (ESI) calcd. for $C_{20}H_{28}N_2O_7Na$ $[M+Na]^+$: 431.17942, found: 431.17927; R_f = 0.75 ($CHCl_3$–MeOH 4:1).

3.3. *N*-((1*S*,2*R*)-2-(3-Pyridinecarboxamido)cyclohexanecarboxyl)-α-L-fucopyranosyl amine 2

To a solution of **11** (30 mg, 0.072 mmol) in 0.7 mL of CH_2Cl_2, Et_3N (0.26 mmol, 3 equiv), nicotinic acid (13.2 mg, 0.08 mmol, 1.5 equiv) in 0.7 mL of CH_2Cl_2 were added. Subsequently, HBTU (0.08 mmol, 1.5 equiv) was added and the reaction mixture was stirred at room temperature. After 18 h, 10 mL of CH_2Cl_2 was added to the reaction mixture and the organic phase was washed with 0.5 M NaOH (10 mL), 1 M $KHSO_4$ (10 mL), water (10 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated under vacuum. The residue was purified via automated chromatography (EtOAc) to yield 77% (22.8 mg, 0.035 mmol) of *N*-((1*S*,2*R*)-2-(3-pyridinecarboxamido)cyclohexanecarboxyl)-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine.

^{1}H NMR ($CDCl_3$): δ (ppm) = 0.89 (d, 3H, $J_{5\text{-}6}$ = 6.3 Hz, H_6), 1.36–1.48 (m, 3H, $H_{4'}$, $H_{5'}$), 1.61–1.72 (m, 3H, $H_{3'ax}$, $H_{4'}$, $H_{5'}$, $H_{6'ax}$), 1.92 (m, 1H, $H_{6'eq}$), 2.06 (m, 1H, $H_{3'eq}$), 1.93, 1.96, 2.08 (2 × s, 3H, 2 × –(O)CCH_3), 2.79 (m, 1H, $H_{1'}$), 3.87 (m, 1H, $H_{5'}$), 4.30 (m, 1H, $H_{2'}$), 5.13 (m, 1H, H_4), 5.24 (dd, 1H, $J_{2\text{-}3}$ = 11.0 Hz, $J_{3\text{-}4}$ = 3.1 Hz,

H_3), 5.31 (dd, 1H, $J_{1\text{-}2}$ = 5.2 Hz, $J_{2\text{-}3}$ = 11.0 Hz, H_2), 5.85 (dd, 1H, $J_{1\text{-}2}$ = 5.2 Hz, $J_{1\text{-}7}$ = 8.1 Hz, H_1), 7.18 (d, 1H, $J_{1\text{-}7}$ = 8.1 Hz, H_7), 7.35 (d, 1H, $J_{2'\text{-}9}$ = 9.2 Hz, H_9), 7.38 (dd, 1H, $J_{14\text{-}15}$ = 4.9 Hz, $J_{15\text{-}16}$ = 7.9 Hz, H_{15}), 8.0 (d, 1H, $J_{15\text{-}16}$ = 7.9 Hz, H_{16}), 8.72 (d, 1H, $J_{14\text{-}15}$ = 4.9 Hz, H_{14}), 9.10 (pseudo-s, 1H, H_{12}); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 16.0 (C_6), 20.5, 20.6, 20.7 (3 × –(O)CCH_3), 22.3, 23.3 ($C_{4'}$, $C_{5'}$), 27.4 ($C_{6'}$), 29.2 ($C_{3'}$), 44.58 ($C_{1'}$), 48.62 ($C_{2'}$), 65.6 (C_5), 66.2 (C_2), 68.1 (C_3), 70.5 (C_4), 74.2 (C_1), 123.4 (C_{15}), 134.8 (C_{16}), 148.3 (C_{12}), 152.1 (C_{14}), 165.1 (C_{10}), 169.3, 170.4, 170.6 (3 × –(O)CCH_3), 174.8 (C_8); ESI-MS: m/z = 542 $[M+Na]^+$, 83%; R_f = 0.30 (EtOAc).

Deprotection under Zemplen's conditions afforded **2** in 95% yield. ^{1}H NMR (D_2O): δ (ppm) = 0.70 (3H, d, $J_{5\text{-}6}$ = 6.5 Hz, H_6), 1.35–1.91 (m, 8H, CH_{2CYCL}), 2.91 (m, 1H, H_9), 3.55 (m, 1H, H_5), 3.36 (pseudo-d,1H, $J_{4\text{-}5}$ = 3.4 Hz, H_4), 3.77 (dd, 1H, $J_{2\text{-}3}$ = 10.6 Hz, H_3), 3.92 (dd, 1H, $J_{1\text{-}2}$ = 5.7 Hz, H_2), 4.43 (m, 1H, H_{10}), 5.43 (d, 1H, H_1), 7.52 (dd, 1H, J = 5.0 Hz, J = 7.7 Hz, H_{Ar}), 8.05 (d, 1H, J = 8.0 Hz, H_{Ar}), 8.69 (m, 2H, H_{Ar}); ^{13}C NMR (D_2O): δ (ppm) = 16.2 (C_6), 20.6, 23.6, 30.3 (4 × CH_{2CYCL}), 45.6 (C_9), 49.7 (C_{10}), 66.7 (C_2), 67.9 (C_5), 70.3 (C_3), 72.1 (C_4), 77.5 (C_1), 137.1, 148.2, 152.4 (4 × C_{Ar}), 169.1 (C_{12}), 178.6 (C_8); ESI-MS: m/z = 394.4 $[M+H]^+$, 43%; FT-ICR (ESI) calcd for $C_{19}H_{28}N_3O_6$ $[M+H]^+$: 394.19781, found: 394.19801; R_f = 0.33 ($CHCl_3$–MeOH 4:1).

3.4. *N*-((1*S*,2*R*)-2-(3-Indolacetamido)cyclohexanecarboxyl)-α-L-fucopyranosyl amine 3

Using the same procedure described for **2**, compound **11** (30 mg, 0.072 mmol) and 3-indolacetic acid (13.9 mg, 0.08 mmol) were condensed using HBTU (1.5 equiv) to yield 68% (28 mg, 0.049 mmol) of *N*-((1*S*,2*R*)-2-(3-indolacetamido)cyclohexanecarboxyl)-2,3,4-tri-*O*-acetyl-α-L-fucopyranosylamine. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.86 (d, $J_{5\text{-}6}$ = 6.4 Hz, H_6), 1.31–2.10 (m, 8H, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$), 1.99, 2.00, 2.14 (3 × s, 3H, 3 × –C(O)CH_3), 2.84 (m, 1H, $H_{1'}$), 3.68 (m, 2H, H_{11}), 3.83 (m, 1H, H_5), 4.22 (m, 1H, $H_{2'}$), 5.14 (m, 1H, H_4), 5.34 (dd, 1H, $J_{1\text{-}2}$ = 5.2 Hz, $J_{2\text{-}3}$ = 11.2 Hz, H_2), 5.43 (dd, 1H, $J_{2\text{-}3}$ = 11.2 Hz, $J_{3\text{-}4}$ = 3.2 Hz, H_3), 5.90 (dd, 1H, $J_{1\text{-}2}$ = 5.2 Hz, $J_{1\text{-}7}$ = 8.4 Hz, H_1), 6.59 (d, 1H, H_9), 7.07–7.51 (m, 5H, H_{Ar}), 7.89 (d, 1H, $J_{1\text{-}7}$ = 8.4 Hz, H_7), 8.75 (s, 1H, H_{14}); ^{13}C NMR ($CDCl_3$): δ (ppm) = 16.10 (C_6), 20.7, 20.8, 20.9 (3 × –(O)CCH3), 22.4, 22.7 ($C_{4'}$, $C_{5'}$), 26.9 ($C_{6'}$), 29.2 ($C_{3'}$), 33.5 (C_{11}), 44.9 ($C_{1'}$), 47.5 ($C_{2'}$), 65.6 (C_5), 66.2 (C_2), 68.2 (C_3), 70.7 (C_4), 74.3 (C_1), 108.6 (C_{12}), 111.5, 118.4, 122.5, 123.5 (C_{16}, C_{17}, C_{18}, C_{19}), 126.9 (C_{20}), 136.2 (C_{15}), 169.5, 170.5, 170.7 (3 × –(O)CCH_3), 171.7 (C_{10}), 174.4 (C_8); ESI-MS: m/z = 594 $[M+Na]^+$, 100%; R_f = 0.68 (EtOAc).

Deprotection under Zemplen's conditions afforded **3** in quantitative yield. ^{1}H NMR (CD_3OD): δ (ppm) = 1.14 (d, 3H, $J_{5\text{-}6}$ = 6.4 Hz, H_6), 1.30–1.84 (m, 8H, CH_{2cycl}), 2.84 (m, 1H, H_9), 3.74–3.91 (m, 5H, CH_{2INDOL}, H_3, H_4, H_5), 4.07 (dd, 1H, $J_{1\text{-}2}$ = 5.7 Hz, $J_{2\text{-}3}$ = 10.6 Hz, H_2), 4.39 (m, 1H, H_{10}), 5.56 (d, 1H, H_1), 7.24–7.39 (m, 3H, H_{Ar}), 7.61 (d, 1H, J = 8.2 Hz, H_{Ar}), 7.70 (d, 1H, J = 7.9 Hz, H_{Ar}); ^{13}C NMR (CD_3OD): δ (ppm) = 16.5 (C_6), 21.0, 23.6, 23.8, 30.5 (4 × CH_{2cyl}), 33.2 (CH_{2INDOL}), 45.7 (C_9), 48.8 (C_{10}), 66.8 (C_2), 68.0 (C_5), 70.3 (C_3), 72.2 (C_4), 77.5 (C_1), 108.4 ($C_{ipso\text{-}14}$), 112.7, 119.2, 120.3, 122.9, 125.6 (5 × C_{Ar}), 128.6, 137.1 (2 × C_{ipso}), 174.8 (C_{12}), 178.4 (C_8); ESI-MS: m/z = 446.3 $[M+H]^+$, 100%; FT-ICR (ESI) calcd for $C_{23}H_{32}N_3O_6$ $[M+H]^+$: 446.22911, found: 446.22895; R_f = 0.11 ($CHCl_3$–MeOH 4:1).

3.5. *N*-(β-Alanyl)-2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl amine 12

To a solution of fucosyl azide **7** (0.113 g, 0.358 mmol; 1 equiv) in dry $EtNO_2$ (5 mL), grounded activated molecular sieves (4 Å) were added. PPh_3 (0.103 g, 0.394 mmol; 1.1 equiv) dissolved in $EtNO_2$ (5 mL) was added and the mixture was refluxed for 18 h. The reaction was monitored by TLC ($CHCl_3$/EtOAc = 1/1) to observe disappearance of the starting material and appearance of the oxazoline **8**. The reaction mixture was used directly in the next step without isolation. In a separate vessel, the pyridyl thioester of β-alanine[22] (0.147 g, 0.465 mmol; 1.3 equiv) and $CuCl_2$ H_2O (0.079 g, 0.465 mmol; 1.3 equiv) were dissolved in 1 mL of $EtNO_2$ and added to the solution of **8**. The reaction mixture was heated to 40 °C and monitored by TLC ($CHCl_3$/EtOAc, 1/1). After 20 h, the mixture was filtered through a Celite pad, and Celite was washed abundantly with EtOAc. The filtrate was washed with an aqueous solution of NH_3–NH_4Cl (pH 9), then with water to neutral pH. The organic phase was dried over Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by automated chromatography (hexane–EtOAc gradient 7:3→1:1) to obtain 0.092 g (0.186 mmol) of *N*-(*N'*-benzyloxycarbonyl-β-alanyl)-2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl amine **10** (52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.13 (d, 3H, $J_{6\text{-}5}$ = 6.4 Hz, H_6), 2.01–2.19 (3 s, 9H, 3 × –C(O)CH_3), 2.55 (br s, 2H, C(O)CH_2CH$_2$NHAc), 3.53 (br dd, 2H, J_1 = 6.4 Hz, J_2 = 6.8 Hz C(O)CH$_2$CH_2NHAc), 3.95 (dq, 1H, $J_{5\text{-}6}$ = 6.4 Hz, $J_{5\text{-}4}$ <1 Hz, H_5), 5.08–5.17 (m, 2H, H_3, H_4), 5.25 (br s, 2H, C(O)OCH_2Ph), 5.39 (dd + br s, 2H, $J_{2\text{-}3}$ = 11.0 Hz, $J_{2\text{-}1}$ = 5.6 Hz, H_2, C(O)CH$_2$CH$_2$N*H*Ac), 5.89 (dd, 1H, $J_{1\text{-}2}$ = 5.6 Hz, $J_{1\text{-}NH}$ = 7.6 Hz, H_1), 6.85 (d, 1H, $J_{1\text{-}NH}$ = 7.6 Hz, α-NHC(O)); R_f = 0.35 ($CHCl_3$–MeOH 50:1).

To a solution of **10** (0.1 g, 0.2 mmol) in MeOH (3 mL), 10% Pd–C (0.015 g) was added and the suspension was stirred for 18 h under a H_2 atmosphere. The catalyst was filtered through a Celite pad and the solvent evaporated. The crude *N*-(β-alanyl)-2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl amine **12** was used without further purification for the synthesis of **4** and **5**. An analytical sample was purified by automated chromatography (KP-NH cartridge) and characterized. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.09 (d, 3H, $J_{6\text{-}5}$ = 6.4 Hz, H_6), 1.93–2.11 (3 s, 9H, 3 × –C(O)CH_3), 2.33 (t, 2H, J = 4.4 Hz, C(O)CH_2CH$_2$NHAc), 2.93–3.05 (m, 2H, C(O)CH$_2$CH_2NHAc), 3.93 (dq, 1H, $J_{5\text{-}6}$ = 6.4 Hz, $J_{5\text{-}4}$ <1 Hz, H_5), 5.13 (dd, 1H, $J_{3\text{-}2}$ = 11.2 Hz, $J_{3\text{-}4}$ = 3.2 Hz, H_3), 5.20 (dd, 1H, $J_{4\text{-}3}$ = 3.2 Hz, $J_{4\text{-}5}$ <1 Hz, H_4), 5.31 (dd, 1H, $J_{2\text{-}3}$ = 11.2 Hz, $J_{2\text{-}1}$ = 5.2 Hz, H_2), 5.83 (dd, 1H, $J_{1\text{-}2}$ = 5.2 Hz, $J_{1\text{-}NH}$ = 8.2 Hz, H_1), 9.40 (d, 1H, $J_{1\text{-}NH}$ = 8.2 Hz, α-NHC(O)); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ (ppm) = 16.2 (C_6), 20.72, 20.73, 20.75 (3 × –(O)CCH_3), 37.5 (CH_2), 38.0 (CH_2), 65.7 (CH), 66.3 (CH), 68.3 (CH), 70.7 (CH), 74.2 (CH), 170.8, 172.1; ESI-MS: m/z = 361.0 $[M+H]^+$, 100%.

3.6. *N*-(*N'*-Benzyloxycarbonyl-β-alanyl)-α-L-fucopyranosyl amine 4

The crude amine was dissolved in CH_2Cl_2 (1 mL), pyridine (0.02 mL, 1.2 equiv) and Ac_2O (0.025 mL, 1.2 equiv) were added and the solution was stirred overnight. The mixture was diluted with CH_2Cl_2, extracted with water, diluted HCl, water. The organic phase was evaporated and the crude purified by flash chromatography (CH_2Cl_2–MeOH 20:1) to yield 73 mg (90% over the two steps) of acetamide as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.18 (d, 3H, $J_{6\text{-}5}$ = 5.6 Hz, H_6), 2.01–2.19 (4 s, 12H, 3 × –C(O)CH_3 + NHC(O)CH_3), 2.57 (br t, 2H, C(O)CH_2CH$_2$NHAc), 3.50–3.62 (m, 2H, C(O)CH$_2$CH_2NHAc), 4.10 (br q, 1H, $J_{5\text{-}6}$ = 5.6 Hz, H_5), 5.28 (br s, 2H, H_4, H_3), 5.37–5.42 (m, 1H, H_2), 5.94 (dd, 1H, $J_{1\text{-}2}$ = 5.6 Hz, $J_{1\text{-}NH}$ = 7.6 Hz, H_1), 6.24 (br s, 1H, C(O)CH$_2$CH$_2$N*H*Ac), 7.07 (d, 1H, $J_{1\text{-}NH}$ = 7.6 Hz, α-NHC(O)).

Deprotection under Zemplen's conditions afforded **4** in 75% yield. ^{1}H NMR (CD_3OD): δ (ppm) = 1.90 (d, 3H, $J_{6\text{-}5}$ = 5.6 Hz, H_6), 1.93 (s, 3H, NHC(O)CH_3), 2.50 (t, 2H, J = 6 Hz, C(O)CH_2CH$_2$NHAc), 3.43 (t, 2H, J = 6 Hz, C(O)CH$_2$CH_2NHAc), 3.65 (br s, 1H, H_4), 3.73–3.80 (br s, 2H, H_5, H_3), 3.93–4.10 (m, 1H, H_2), 5.55 (dd, 1H, $J_{1\text{-}2}$ = 5.6 Hz, $J_{1\text{-}NH}$ = 7.6 Hz, H_1), 8.05 (br s, 1H, C(O)CH$_2$CH$_2$N*H*Ac), 8.35 (d, 1H, $J_{1\text{-}NH}$ = 7.6 Hz, α-NHC(O)); ^{13}C NMR (CD_3OD): δ (ppm) = 15.5 (C_6), 21.15 (NHC(O)CH_3), 35.1 (C(O)CH_2CH$_2$NHAc), 35.4 (C(O)CH$_2$CH_2N-

HAc), 66.5 (C_2), 77.2, 70.0, 72.3 (C_4), 77.1 (C_1), 172.0, 173.6; ESI-MS: m/z = 277 $[M+H]^+$, 100%.

3.7. Dimer 5

To a solution of **10** (0.07 g, 0.14 mmol) in MeOH (2 mL), 10% Pd–C (0.01 g) was added and the suspension was stirred for 18 h under a H_2 atmosphere. The catalyst was filtered through a Celite pad and the solvent evaporated. The crude amine was dissolved in 2 mL of dry THF and succinic anhydride (0.007 g, 0.07 mmol, 0.5 equiv) was added to the ice-cooled solution. After 30 min, a solution of Py-BOP (0.054 g, 0.1 mmol, 0.75 equiv) in CH_2Cl_2 (1 mL) was added dropwise and the mixture was stirred at room temperature overnight. The solvent was evaporated; the residue dissolved in CH_2Cl_2 and was washed with a satd aq $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4 and the solvent evaporated. The crude was purified by flash chromatography (CH_2Cl_2/MeOH gradient, from 20:1→9:1) to yield 35 mg (63%) of protected dimer. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 1.12 (d, 6H, $J_{6\text{-}5}$ = 6.4 Hz, 2 × H_6), 2.01–2.19 (3 s, 18H, 6 × –C(O)CH_3), 2.45 (s, 4H, C(O)CH_2CH_2C(O)), 2.52 (t, 4H, J = 6.4 Hz, 2 × C(O)CH_2CH$_2$NHAc), 3.46 (td, 4H, J_1 = 6.4 Hz, J_2 = 2.8 Hz, 2 × C(O)CH$_2$CH_2NHAc), 4.07 (dq, 2H, $J_{5\text{-}6}$ = 6.4 Hz, $J_{5\text{-}4}$ <1 Hz, 2 × H_5), 5.23 (dd, 2H, $J_{2\text{-}1}$ = 5.6 Hz, $J_{2\text{-}3}$ = 11.2 Hz, 2 × H_2), 5.30 (br d, $J_{4\text{-}3}$ = 3.4 Hz, 2 × H_4), 5.49 (dd, 2H, $J_{3\text{-}4}$ = 3.4 Hz, $J_{3\text{-}2}$ = 11.2 Hz, 2 × H_3), 5.85 (d, 2H, $J_{1\text{-}2}$ = 5.6 Hz, 2 × H_1).

Deprotection under Zemplen's conditions afforded the dimer **5** in 78% yield. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 1.19 (d, 6H, $J_{6\text{-}5}$ = 6.8 Hz, 2 × H_6), 2.42–2.56 (m, 8H, 2 × C(O)CH_2CH$_2$NHAc, C(O)CH_2CH_2C(O)), 3.39–3.52 (m, 4H, 2 × C(O)CH$_2$CH_2NHAc), 3.67 (br d, 2H, $J_{4\text{-}3}$ = 2.8 Hz, 2 × H_4), 3.79–3.82 (m, 4H, 2 × H_3 + 2 × H_5), 3.97 (dd, 2H, $J_{2\text{-}3}$ = 10.0 Hz, $J_{2\text{-}1}$ = 5.4 Hz, 2 × H_2), 5.58 (d, 2H, $J_{1\text{-}2}$ = 5.4 Hz, 2 × H_1); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) = 15.5 (C_6), 30.7, 35.3, 35.5, 66.6 (C_2), 67.3, 70.0, 71.9 (C_4), 77.0 (C_1), 173.5, 173.8; TOF MS ES$^+$: m/z = 551.3 $[M+H]^+$, 573.2 $[M+Na]^+$, 100%; HRMS (TOF ES$^+$): calculated for $C_{22}H_{39}N_4O_{12}$: 551.2564, found 551.2572.

3.8. 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 PA-IIL 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) was set to 50 μM. A total of 30 injections of 10 μL of sugar solution at concentrations of 0.5 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. Free energy change (ΔG) and entropy contributions ($T\Delta S$) 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 $100 < c < 200$ ($c = K_aM$, where M is the initial concentration of the macromolecule).

3.9. Docking calculations

Three-dimensional structure of **1** and **2** were built using the Sybyl software editor (Tripos inc) starting from the fucose structure available from the monosaccharide database (Glyco3D, CERMAV), and information from high resolution NMR. Partial charges were derived by MNDO calculations. High resolution crystal structure of PA-IIL/fucose complex (pdb code 1GZT) was used as the template for docking study with inclusion of all hydrogen atoms and proper partial charges and parameters for the calcium ions as previously described.[29] Fucose moiety of compounds **1** and **2** were superimposed on the position of fucose in the crystalline complex. Conformational analysis of rotatable bond of the aglycon was performed with CO–NH linkage maintained in a *trans* conformation. Conformations with non-steric conflicts were energy minimized using the Tripos force-field[30] with PIM energy parameters[31] for the fucose moiety, and geometry optimisation of the ligand and side chains in the binding site. The docking approach was performed in the presence or absence of the crystalline water molecule present in the binding site.

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Supplementary data

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