Glycocluster tetrahydroxamic acids exhibiting unprecedented inhibition of *Pseudomonas aeruginosa* biofilms

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ABSTRACT. Opportunistic Gram-negative *P. aeruginosa* uses adhesins (e.g. LecA and LecB lectins, type VI pili and flagella) and iron to invade host cells with the formation of a biofilm: a thick barrier that protectsbacteria from drugs and host immune system. Hindering iron uptake and disrupting adhesins function could be relevant anti-pseudomonal strategy. To test this hypothesis we designed an α-L-fucocluster tetrahydroxamic acid iron-chelating calixarene to interfere with both iron sequestration and glycan recognition processes. Iron depletion led to increased production of the siderophore pyoverdine by *P. aeruginosa* to counteract the loss of iron uptake. Unprecedented strong biofilm inhibition was observed not only with the α-L-fucocluster (72%), but also with its α-D-*manno* (84%), and α-D-*gluco* (92%) counterparts used as negative controls. This unprecedented finding suggests that biofilm inhibition is not exclusively through deactivation of soluble lectins.

INTRODUCION

The increase in bacterial resistance to existing antibiotics presages a dramatic worldwide health disaster.[1,2] Consequently, new strategies to fight bacterial infections and resistance to drugs are urgently needed.[3,4,5] *Pseudomonas aeruginosa* is an opportunistic pathogen[6] that commonly infects immunocompromised individuals[7] and causes chronic, often fatal, lung infections in patients with cystic fibrosis (CF).[8] The intrinsic tolerance of *P. aeruginosa* to traditional drugs[9] is a result of several resistance mechanisms including growth as a multicellular community, or biofilm.[10] Inhibition of biofilm formation could protect against *P. aeruginosa* infection by enhancing antibiotic uptake and efficacy by unicellular planktonic cells.[11] Biofilm formation is a complex and highly regulated process that begins with bacterial attachment to host cells. The mechanisms involved in this early stage, are not fully understood, but in *P. aeruginosa* it is

thought to involve the soluble lectins (LecA and LecB)[12,13] and other adhesins such as type IV pili and flagella.[14,15] Molecules that efficiently inhibit biofilm formation could become useful antibiotic treatments, yet molecular biofilm inhibitors remain scarce. Recent research has focused on the design of multivalent molecules as host glycan mimics to inhibit the multivalent soluble lectins LecA and/or LecB.[16] To date only the polycationic fucosyl dendrimer **FD2** and its counterparts have shown favorable properties as lectin and biofilm inhibitors.[4,17] *P. aeruginosa* growth is also impacted by microelements such as iron.[18] The ferrous cation (Fe^{2+}) is the form involved in bacterial biochemical pathways but its scarcity under physiological conditions requires that *P. aeruginosa* indirectly exploits the more available ferric cation (Fe^{3+}) as a source. The recruitment of Fe^{3+} takes place efficiently via sophisticated siderophores such as the weakly iron-chelating pyochelin and the non-ribosomally synthesized pyoverdine (Pvd), a strong iron chelator containing two hydroxamic groups (HAGs) and a fluorescent dihydroxyquinoline chromophore.[19] Interfering with this process using artificial iron chelators is another promising strategy to stop bacterial growth.[20] This possible exogenous antibacterial treatment is likely to avoid resistance but is a still challenging approach as shown by small number of antimicrobial results obtained up to now.[21,2]

As a new anti-pseudomonal concept, we suggest drugs with dual iron chelating and adhesin inhibiting functions,. The candidates targeted for this preliminary investigation are clusters with multiple copies of hydroxamic acid group (HAG)[22], as bidentate ligands for Fe^{3+}-sequestration, and a suitable sugar terminus to interact with LecB lectin. A wide range of cluster-based α-L-fucose or α-D-mannose glycodendrimers have been studied, and the fucosylated derivatives typically had better affinities for LecB[23] than mannose derivatives.[24] We used α-L-fucose, the natural epitope of LecB, and α-D-mannose, a weak ligand of LecB, for our initial investigation.

As a versatile scaffold, we selected the pre-oriented *p*-tBu-calix[4]arene to improve both iron chelation and binding interactions with lectin. However, this advantage is often marred by the high hydrophobicity even when calixarene carry multiple copies of sugars which compromises biological assessment in aqueous media.[25] PEG-linkers,[26] anionic or cationic groups[27] have incorporated in the hybridized calixarene to improve water solubility but this problem was also solved when an alkyl spacer was linked to a peptide moiety to produce water soluble multivalent calixarenes with a high affinity for the specified lectin.[28] The latter solution was attractive for its potential to introduce functional groups in a modular faxhion. Furthermore the *N*-alkylated-HAG ligands[29] in a bridging position between the sugar and the scaffold were expected to further improve the water solubility of the calixarene glycoconjugate. We report herein a straightforward synthesis of two conformationally-locked calixglycoclusters **1** and **2** with four α-L-fucose and four α-D-mannose units respectively (Figure 1). We successively evaluated: i. their possible inherent antibacterial effect on PAO1 wild strain of *P. aeruginosa*; ii. their possible antibacterial effect in iron supplemented medium on both PAO1 producing siderophore and on siderophore-deficient PAD07 mutant strains of *P. aeruginosa*; iii. their interaction with LecB lectin and iv. their inhibition of the PAO1 biofilm formation. The synthesis of the glucocluster **3** and its evaluation against biofilm formation are also described. Our toxicity studies focused on the compounds' effects on the airway epithelial host cells from CF patients.

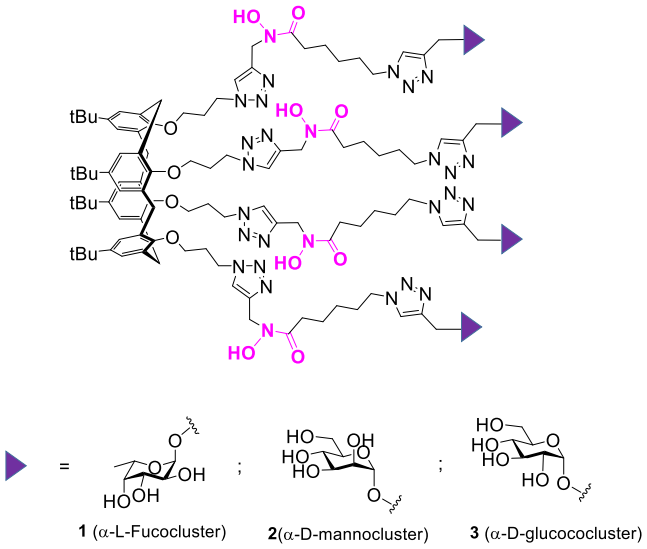
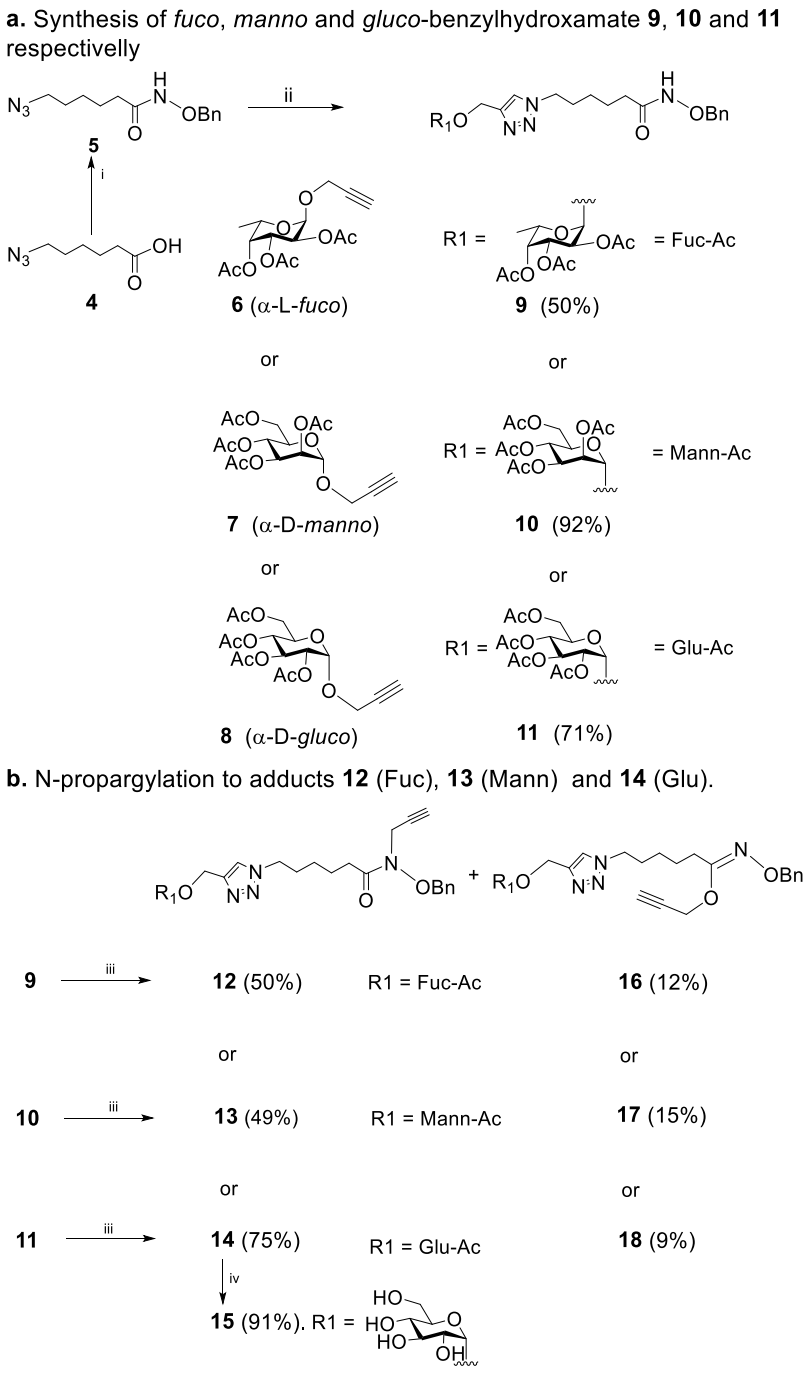


Figure 1. The targeted glycocluster-based calixarene **1** with α-L-fucose, **2** with α-D-mannose and **3** with α-D-glucose units.

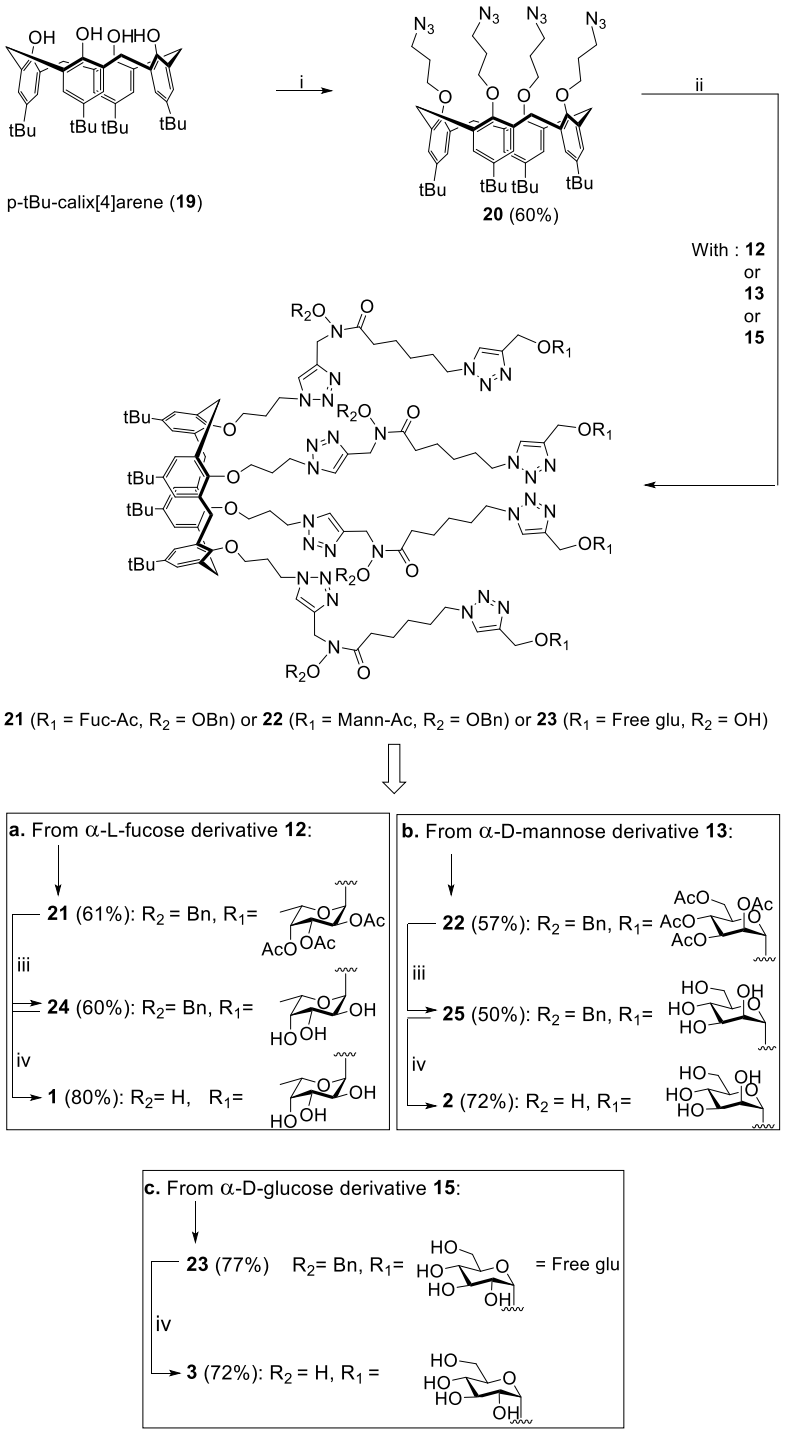
Results and discussion

Organic synthesis. A convergent strategy indicated in Schemes 1 and 2 was used to design the target clusters **1**, **2** and **3**. The key intermediates involved were *N*-propargylperacetylated benzylhydroxamates **12** with α-L-fucose, **13** with α-D-mannose and **14** with α-D-glucose as sugar derivatives (Scheme 1b). All were formed from masked hydroxamic acid **5** which was obtained in good yield (71%) from amidation of 6-azidohexanoic acid **4**[30] (Scheme 1a). Compound **5** was linked in turn to the propargylated sugars **6** (α-L-*fuco*), **7** (α-D-*manno*)[31] and **8** (α-D-*Gluco*) via click chemistry using CuI/DIPEA in acetonitrile to give intermediates **9** (50%), **10** (92%) and **11** (71%), respectively. The three *O*-glycosyl amide derivatives were transformed via *N*-propargylation reaction to their corresponding *N*-propargylated derivatives **12** (50%), **13** (49%) and **14** (75%) under microwave conditions (Schem 1b). As by-products, the three *O*-propargylated imidates **16** (*fuco*) (12%), **17** (*manno*) (15%) and **18** (*gluco*) (9%) (through amide-

imidate tautomerisation) were obtained. The *N*-propargylamide and the *O*-propargylimidate groups were distinguished in ^{13}C NMR spectroscopy by C=O and C=N chemical shifts at 175.6 and 163 ppm for the *fuco* derivatives **9** and **10**, 175.7 and 163.1 for the *manno* derivatives **15** and **16** and 176 and 154.5 ppm for the *gluco* derivatives **14** and **18** of the carbonyl and imidate carbon atoms, respectively. Fucocluster **1** was obtained through linking the azido-calixarene **20**[32] to alkyne *fuco*-derivative **12** via click chemistry. The tetrafuco benzylhydroxamate **21** (61%) obtained underwent deacetylation reaction to give free fucocluster **24** (60%). Hydrogenation with H_2 and Pd/C-10% produced iron chelating fucocluster **1** in good yield (80%). The same strategy was successfully applied to the synthesis of mannocluster **2** obtained from the reaction of *manno*-derivative **13** and **20**, and subsequent acetolysis, to give **25** as an intermediate. Hydrogenolysis gave the target cluster **2** in good overall yield. Finally, the synthesis of the glucocluster **3** was obtained in good overall yield using free sugar derivative **15** in the click reaction step, followed by hydrogenolysis. The three glycoclusters **1**, **2** and **3** were obtained in high purities by preparative HPLC on 40 µm C18 revese phase and a H_2O/CH_3CN gradient as the eluant. The purities were determined by LC-MS using C18 5µm reverse phase column and estimated from peak integrations (See SI, Figures S47, S49 and S51). Through the steps from **20** to **1**, **2** and **3**, the calix cone conformation was preserved ($J_{A,B}$ are approximately 12.3 and 12.6 Hz, respectively for the AB system of the bridged Ar$C\underline{H}_2$Ar). As expected, the three glycoclusters **1**, **2** and **3** were highly water-soluble, which made them suitable for biological studies in aqueous media. The free sugar units and hydroxamic acid moieties are required both for hydrophilicity as the corresponding *O*-benzylated clusters **23**, **24** and **25**, with only free sugar units, lose this fundamental property.



Scheme 1. Synthesis of *N*-propargyl hydroxamate bearing linker with α-L-fucose **12**, α-D-mannose **13** and α-D-glucose **14**/**15** for subsequent click chemistry (Scheme 2). Conditions: i. CuI, DIPEA, CH_3CN, ii. CuI, DIPEA, CH_3CN, microwave, 30 min, iii. Propargyl bromide 80% in toluene, MW, 30 min, iv. MeONa/MeOH.



Scheme 2. Synthesis of iron-chelating glycocluster-based calixarene **1** (with α-L-fucose units) and **2** (with α-D-mannose units) designed to stop bacterial growth and inhibit biofilm formation. Glucocluster **3**, designed to serve as a negative control, was synthesized by a modified strategy. Reagents and conditions: i. a: 1-bromo-3-chloropropane, NaH, DMF, 90 °C, 48 h; b: NaN_3,

DMF, 80 °C, 16 h; ii. $CuSO_4 \cdot 5H_2O$, sodium ascorbate, tBuOH/H_2O/THF, 65 °C, 16 h; iii. Et_3N, H_2O, MeOH, rt, 48 h; iv. H_2, Pd/C 10%, MeOH, rt, 24 h.

Biological assessments.

Impact of 1 and 2 on growth of PAO1 under Mueller-Hinton conditions[33] in iron-supplemented media. We first determined that fucocluster **1** and mannocluster **2** did not show any inherent antibiotic activity against wild-type *P. aeruginosa* PAO1 under standard Mueller-Hinton growth conditions[33] without iron supplementation (MIC > 512 µg/mL, see SI, Table S1). As no effective protocol exists for biofilm inhibition assays in iron-supplemented culture, we focused on growth in the presence of artificial iron chelators at various concentrations of iron following a well known protocol. We examined the growth of siderophore-producing PAO1 and the siderophore-deficient PAD07 strains in iron-supplemented succinate minimal medium (SMM)[34] in the presence of 8 µg/mL (50 µM) of $FeCl_3$ and increasing concentrations of **1** or **2** from 0.125 to 128 µg/mL (equivalent to a [**1**]/[$FeCl_3$] or [**2**]/[$FeCl_3$] ratio of 0.0625 to 128). Fluorescent pyoverdine-I (Pvd-I) is the main siderophore expressed by PAO1 to enhance growth under iron limitating conditions.[35] This pigment naturally fluoresces under iron limitating conditions and loses fluorescence in iron-rich media.[36] The presence of **1** or **2** did not influence PAO1 growth (See SI, Figure S57) but competed strongly with Pvd-I,[37] which was increasingly produced (indicated by green fluorescence in well plates, see SI, Figure S58) to counteract iron deficiency. This was corroborated by measurements of the Pvd-I-specific emission at 447 nm indicating that levels of iron-free Pvd-I increased with increasing concentrations of ligands **1** or **2** (Figure 2A). This iron-limitating effect, which could be a means of inhibiting biofilm formation, is directly related to the presence of hydroxamic acid groups, a conclusion supported by the absence of fluorescence for *O*-benzylated hydroxamate derivatives **24** and **25** (See SI, Figure

S58). No antibacterial effect was observed against PAO1; however, mannocluster **2** significantly disrupted growth of the siderophore-deficient PAD07 strain (Figure 2B). Fucocluster **1** had a weak effect on growth which supports the involvement of the sugar type in the growth-interfering activity. Interestingly, this result is also supportive of iron complexes derived from **1** and **2** not being recognized by bacterial siderophore receptors thus preventing their use by the pathogen to uptake iron as observed for enterobactin-mimics with *Escherichia coli*[38].

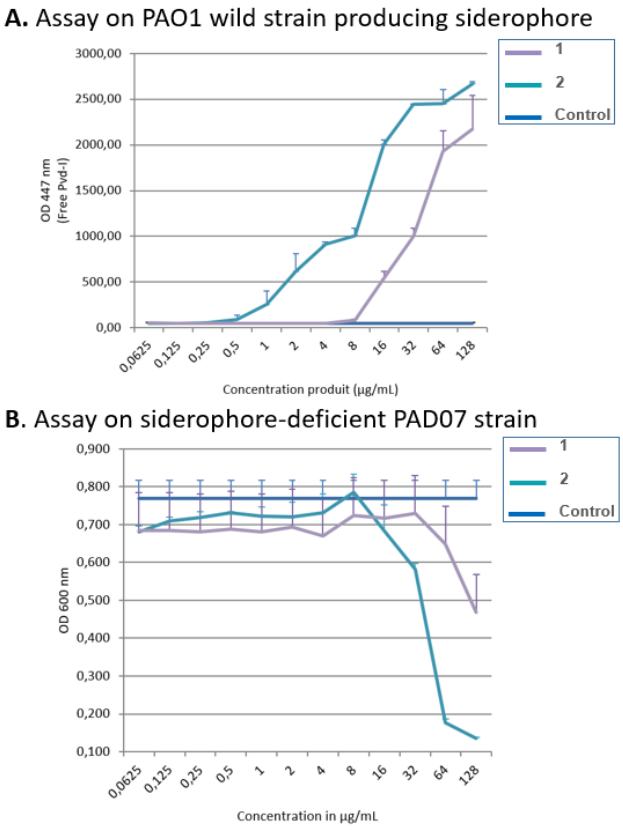


Figure 2. A. Iron-free Pvd-I fluorescence measurements at λ_{abs} = 400 nm and fluorescence recorded at λ_{em} = 447 nm in the presence of ligands. All points on the curves were obtained after incubation of well plates for 24h at 35 °C at the indicated concentrations. Baselines were made with solvent alone and with the same varying amounts of products **1** and **2** in the medium, without the bacterial inoculums, to assess the absorbance generated by our compounds.

Experiments were carried out in duplicate at least twice. Results are given as mean + Standard Deviation (SD). **B**. Growth of *P. aeruginosa* non-producing siderophore PAD07 in the presence of **1** or **2** in SMM supplemented with $FeCl_3$ (8 μg/mL). Results are displayed as means + standard deviation. *: $p \leq 0.05$ (Wilcoxon Mann Whitney test vs. control).

This apparently strong impact of **1** and, to a large extent, **2** on Pvd-I-production is probably related to the vectorization of the iron-complexes close to the bacterial membrane. Interactions may be with the recognition systems of PAO1 as the fucophilic LecB soluble lectin and/or other adhesin systems since mannose is usually weakly recognized by LecB. Consequently we assessed interaction of both ligands with LecB (*See below*).

Evaluation of LecB inhibition in presence of 1 or 2 using the fluorescence polarization technique.[39] Using the fluorescence polarization technique as described by Hauck et al.,[39] we performed a competitive binding assay with LecB to corroborate at least in part the vectorisation hypothesis. The experiments involved L-fucose, Me-α-L-fucoside[40] Me-α-D-mannoside, the monovalent carbohydrates **26** (*fuco*) and **27** (*manno*), and the multivalent glycoclusters **1** and **2** (Figure 3). Unexpectedly, **2** interact strongly with LecB illustrated by a decrease in fluorescence polarization amplification by factors of 220 and 236 compared to its monovalent derivative **27** and Me-α-D-mannoside respectively. This supports a strong positive effect of multivalency (β-avidities = 55 and 59 respectively, Table 1) never previously observed with a mannocluster. Surprisigly, only a weak relative potency (0.5 with **26** and 3.37 with Me-α-L-fucoside) with loss of binding interaction (β-avidities = 0.13 and 0.84 respectively) were observed with fucocluster **1** illustrating a negative effect of multivalency. Furthermore, the IC_{50} (664 nM) of **2** is only three times lower than that of **1** (294 nM). This finding contrasts with the monovalent Me-α-D-

mannoside, which is a 187-fold weaker inhibitor of LecB than Me-α-L-fucoside. It is striking that the cluster capped with D-mannose units is a better ligand for LecB than its *fuco*-counterpart although L-fucose is the natural epitope of this lectin. The most likely interaction here is via chelation, as previously suggested for LecB and blood group antigens bearing *N*-glycans.[41] This is supported by molecular mechanics (MMFF) which predicts the greatest separation between two mannose units of approximately 50 Å in a complex of **2** bound to sugar-free LecB (1OUX)[42] (Figure 4). This is enough to cover the shortest possible distance of 41 Å[43,44] between two LecB carbohydrate recognition domains (CRDs). Although this distance is also covered by the the *fuco*-counterpart **1 (**see SI, Figure 61), it was not enough for better recognition. We suggest that the orientation of mannose units, leading to complementary protein folding, and the existance of secondary interactions, due to the HAG-arms, contribute synergistically to the favorable recognition of multivalent mannocluster **2** by LecB.

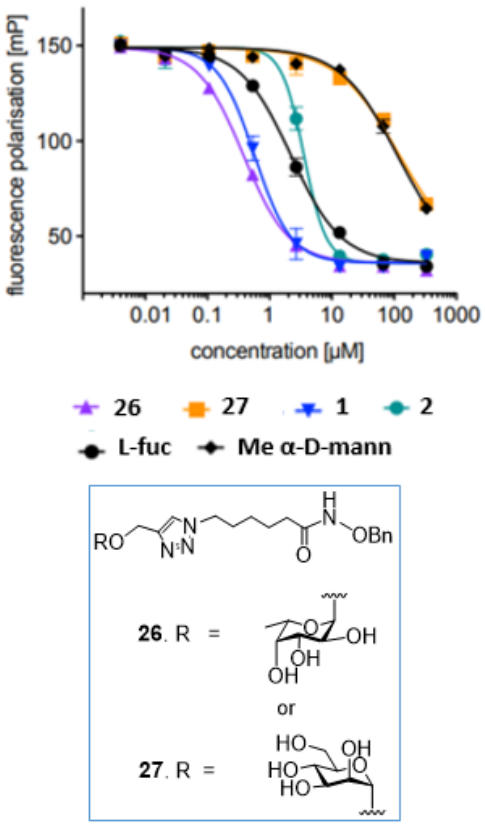


Figure 3. Evaluation of ligands **1**, **2**, **26** and **27** for competitive binding to LecB using a fluorescence polarization-based assay. Compounds **26** and **27** were obtained by traditional acetolysis (MeONa/MeOH) of compounds **9** and **10** respectivelly. Bottom and top plateaus were defined by the standard compounds L-fucose and methyl α-D-mannoside, respectively, and the data were reanalyzed with these values fixed. Three independent experiments were performed for each inhibitor and average IC_{50} values are given in Table 1.

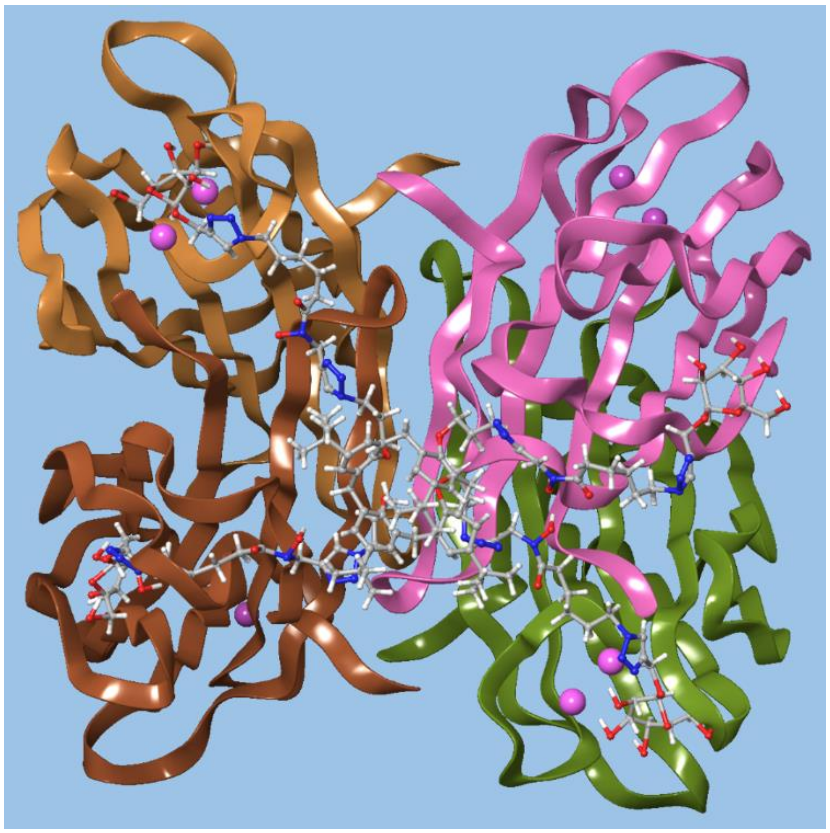


Figure 4. Computer generated views of **2** superimposed on the anti-parallel tetrameric lectin, LecB (1OUX);[42] We observe that the sugars in 1,3-position of calixarene can bind (See SI, Table S2 for output data) two CRDs that are normally separated by 41 Å.[43,44] For computer generated views of fucocluster **1** superimposed on LecB, see SI, Figure 61.

Table 1. Evaluation of monovalent and multivalent inhibitors in a competitive binding assay with LecB.

Entry	Compound	IC_{50} [μM]	s.d. [μM]	n[a]	Rel. IC_{50} [μM][b]	Rel. s.d. [μM]	Rel. Potency w.r.t. to corresponding monovalent **26** (*Fuco*)* or **27** (M*anno*)**	Rel. Potency w.r.t. to Me-fuc* or Me-man**[c]	β avidity = $IC_{50ligand}$ **1** or **2**/n
1	L-fucose	2.74[39]		1					
2	Me-α-D-mannoside	157[39]		1					
3	Me-α-L-fucoside	0.84[40]		1					
4	**1** (*Fuco*)	0.249	0.12	4	0.996	0.48	0.5*	3.37*	0.84
5	**2** (*Manno*)	0.664	0.170	4	2.658	0.68	220**	236**	59
6	**26** (*Fuco*)	0.50	0.3	1					
7	**27** (*Manno*)	146.3	24.40	1					

[a] Number of monosaccharide epitopes in the ligand; [b] relative IC_{50} = IC_{50}(ligand) x n; [c] relative potency (Rel. Pot.) = IC_{50}(Me-α-L-fuc)/IC_{50}(ligand) **1** and relative potency (Rel. Pot.) = IC_{50} (Me-α-D-man)/IC_{50}(ligand) for **2**. Three independent experiments were performed for each inhibitor, average IC_{50} values and standard deviations are given.

Due to this apparent strong inhibition of LecB and the well known relationship between LecB and biofilm control,[13,12] we hypothesized that **2** could be a much better inhibitor of PAO1 biofilm formation than **1** (*See below*).

Assessment of PAO1 biofilm inhibition by 1 and 2 and synergistic effect in presence of tobramycin (Tb) antibiotic using Diggle protocol.[45] We examined the ability of **1** and **2** to inhibit PAO1 biofilm formation using the protocol of Diggle et al.,[45] previously applied to the *fuco* dendrimer **FD2**[17] and used here as a positive control. The biofilm quantification was analysed using the WST-8 assay[46] to determine the number of viable cells in the biofilm. At a concentration of 50 µM, we determined that fucocluster **1** had no significant effect on PAO1 biofilm formation (Figure 5A and see SI, Figure S59 for well plates). Conversely, mannocluster **2** strongly reduced biofilm formation by 70% at 50 µM. This activity subsequently increased to 83% and at a lower concentration of 20 µM (Figure 5B). This is a surprising finding which contrasts with inactive calixarene mannoclusters reported in literature.[26] The lack of biofilm inhibition by fucocluster **1** was unexpected since fucose is the natural epitope of LecB. However, the unexpectedly potent interaction of mannocluster **2** with LecB requires caution regarding its link with biofilm inhibition at this stage as other undefined synergistic interactions involving both linker bearing HAG and sugar terminus remain possible.

The design of compound **2** was based on the anticipation of a synergetic interaction between two complementary antibiofilm moieties combined within the same structure: the inhibition of LecB and the hindrance of the bacterial iron uptake via iron sequestration. Although **2** exhibited an iron complexation property that induced a stress response of *P. aeruginosa*, it was not enough to cause death *via* iron depletion. The impact of iron depletion on the biofilm was not assessed, consequently, the observed biofilm inhibition wss attributed to the synergistic interaction

between the carbohydrate termini and the rest of the scaffold. To improve the anti-biofilm activity, we used **2** in combination with tobramycin (**Tb**) as another route to a synergistic effect. **Tb** is an antibiotic used to treat lung infections associated with cystic fibrosis.[47] While **Tb** is effective at eradicating *P. aeruginosa* in the airways of young patients, it is unable to completely clear chronic *P. aeruginosa* infections in older patients.[48] The normal action of **Tb** is intracellular; **Tb** binds to bacterial ribosomes and prevents translation leading to cell death. However, **Tb** also induces bacterial biofilm formation when used alone at sub-inhibitory concentrations (Sub-IC) of 0.1 µM.[49] Furthermore, combined with iron chelators (e.g. Deferasirox)[50] or with other antibiotics (e.g. Colistin,[51] Claritromycin[52] or fosfomycin[53]), **Tb** led to synergistic biofilm inhibition. Figure 5C shows the effect of **Tb** alone or in combination with **2** on biofilm formation. At doses greater than 0.15 µM of **Tb**, we observed its normal antibiotic role, and the planktonic cells were killed. From 0.15 µM to Sub-IC, biofilm formation occurs, in agreement with the literature.[49] Surprisingly, when **Tb** was administered with **2** at its Sub-IC, there was a total inhibition of biofilm formation without any toxic effects (Figure 6). This synergy could be related to the possible protonation of cationogenic **Tb**, as a base bearing five amino groups, by acidic cluster **2** with four hydroxamic acid groups transformed in turn in to their corresponding hydroxamate anions. The resulting cationic species is likely responsible for biofilm inhibition without any antimicrobial effects, as observed recently with small cationic molecules.[54] In this reaction between **2** and **Tb**, we not exclude the interference of this negatively charged hydroxamate species that acts following other mechanisms against the biofilm.

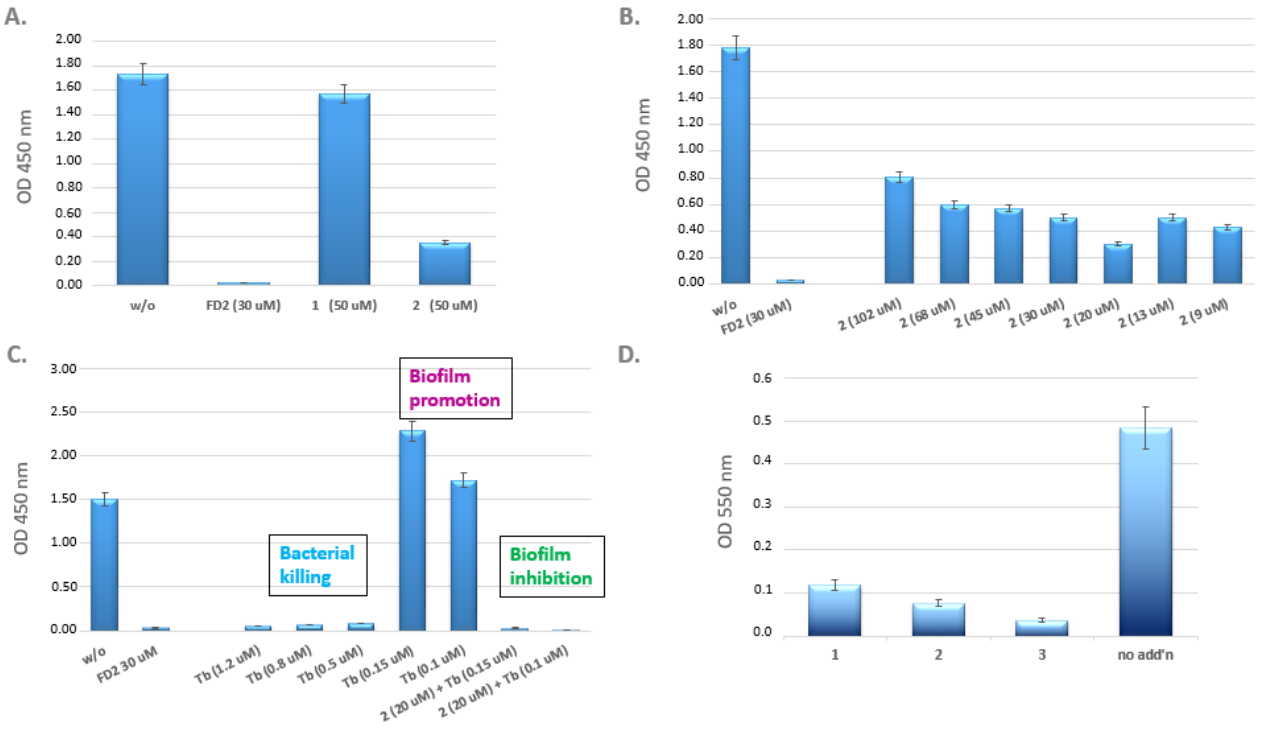


Figure 5. A. Biofilm formation assay using a 96-well dish microtiter plate assay to assess the efficacy of **1** and **2** at 50 µM for inhibition of the biofilm formed by *P. aeruginosa* PAO1. **B.** Biofilm inhibition assay with *P. aeruginosa* PAO1 treated with mannocalixarene **2** at different concentrations using 30 uM **FD2** as a reference. **C**. Biofilm inhibition assay with *P. aeruginosa* PAO1 treated with a mixture of **2** and a sub-inhibitory concentration (0.1 µM) of tobramycin (**Tb**). For experiments **A**, **B** and **C**, the biofilm was quantified using the WST-8 assay[46] to determine the number of viable cells in the biofilm. **D**. Biofilm inhibition assay with *P. aeruginosa* PAO1 treated with 20 µM of **1**, **2** and **3** glycoclusters respectively performed in M63 minimal medium supplemented with 0.4 % arginine and 1 mM magnesium sulfate, and incubated for 24 hours before staining with crystal violet prior to quantification.[55]

Assessment of PAO1 biofilm inhibition by 1, 2 and glucocluster 3 using Tool's method.[56] Observing the concentration dependent activities of **Tb** led us to revisit the potential use of fucocluster **1** as a biofilm inhibitor. We found that **1** had little impact on biofilm formation at 50 μM (Figure 5A), and decided to re-examine biofilm inhibition assays with 20 μM concentrations of **1**, **2** and added the glucocluster **3** as a negative control. Furthermore, to confirm result obtained using the Diggle method[45] for mannocluster **2** (Figure 5A), we used a second method described previously by O'Toole[56] to assess biofilm growth and inhibition. In fact, we found that mannocluster **2** efficiently inhibits 84% of biofilm formation at 20 μM (Figure 5D), in agreement with the previous assay using the Diggle method[45] (83% at 20 μM, Figure 4B). Surprisingly, fucocluster **1** prevented 72% biofilm formation at 20 μM, suggesting concentration dependent activity as hypothesized. Another intriguing result was the strong inhibition of biofilm formation with α-D-glucose-based cluster **3** (92% at 20 μM) used as negative control. In the best of our knowledge, it is the first glucocluster with efficient action against *P. aeruginosa* biofilm formation. This activity is likely to be independent of the ability to inhibit the function of the known soluble lectins. This supports the existence of other types of interactions with other virulence factors towards the bacterial membrane. We suggest the possibility of synergistic actions between the carbohydrate terminus and the anionogenic hydroxamic acids which interact with flagella of *P. aeruginosa* as do the negatively charged glycosaminoglycan heparane sulfates of host cell walls.[57]

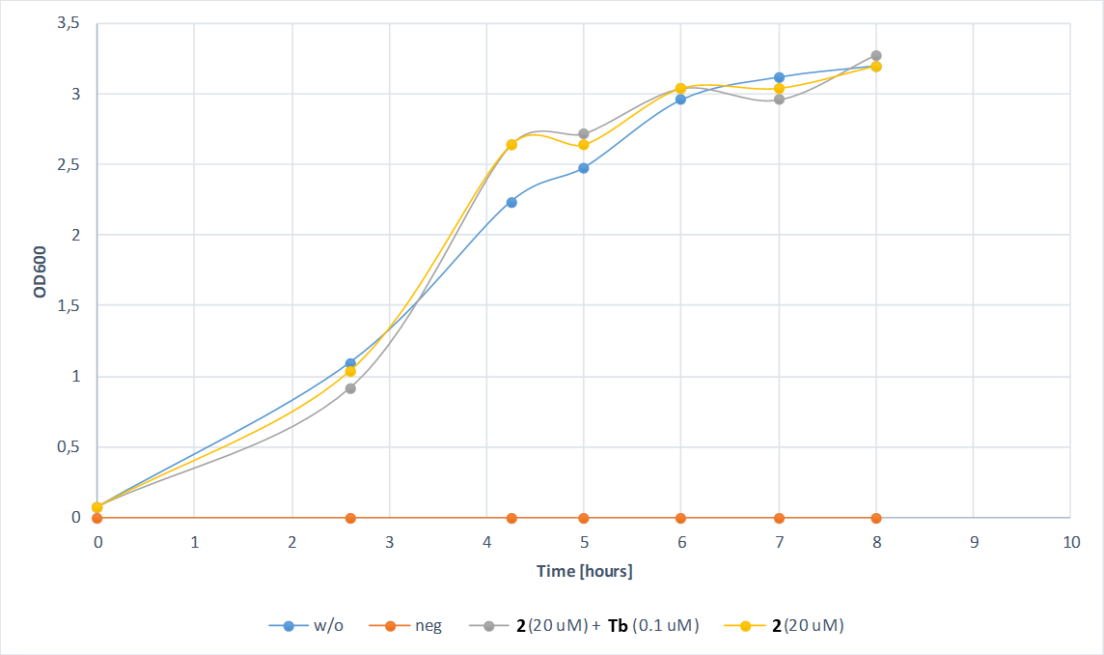


Figure 6. Bacterial growth curves of *P. aeruginosa* wild-type strain PAO1 in the presence of **2** (20 µM) alone and **2** (20 µM) + subinhibitory concentration of tobramycin (**Tb**).

Cytotoxicity on the airway epithelial host cells from CF patients (known as CFBEΔF cells). Although *P. aeruginosa* affects many tissues,[58,59] for toxicity evaluation we focused on the airway epithelial host cells from CF patients (CFBEΔF cells) by measuring lactate dehydrogenase (LDH) activity. No LDH activity[48,60,61] was detected in cells in the presence of **1**, **2** or **3**, as compared to cells treated with the negative control, minimal medium (MEM) alone (Figure 7). Complete lysis was detected when cells were treated with the surfactant Triton.[48] This result indicated that none of these ligands is toxic to CFBEΔF cells, supporting the potential of our research to improve the efficiency of ligands designed for potential antipseudomonal use.

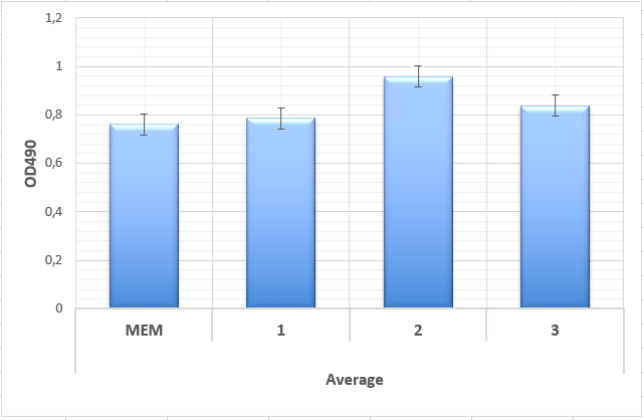


Figure 7. LDH assay of cytotoxicity of compounds **1**, **2**, and **3** on CFBEΔF cells.[48] The compounds were each set at a concentration of 20 µM in water. MEM is a minimal tissue culture medium.

CONCLUSION

In conclusion, we provide herein a straightforward convergent synthesis of unique, water-soluble calixarene-based iron-chelating glycoclusters designed to interfere with the iron uptake process to inhibit bacterial adhesins through glycan mimicry. Incorporating four mannose substituents and four hydroxamic acid groups showed mannocluster **2** to be a strong iron chelator through the production of the Pvd-I siderophore by PAO1 in response to iron depletion. Strong inhibition of biofilm was also observed when **2** was used alone. Complete biofilm eradication, without any toxic effect, was subsequently achieved synergistically through combination of **2** with sub-inhibitory concentrations of the antibiotic tobramycin. The unique inhibition of LecB at a nanomolar IC_{50} with 236-fold potency enhancement compared to the Me-α-D-mannoside, seemed to cause inhibition of the biofilm. This apparent relationship was revised when we discovered that the α-D-glucocluster counterpart **3** had a more potent action against biofilms. This unprecedented result suggests the alteration of another bacterial adhesin expressing a high

synergistic affinity to both alkylhydroxamic bearing linkers and sugar cap. The use of anti-biofilm drugs is an attractive therapy against *P. aeruginosa* infections, so this finding offers a real advance through the first cluster based D-glucose derivatibve that elicits inhibition of *P. aeruginosa* biofilm while preventing deactivation of fucophilic and/or mannophilic lectin of innate immune system.[62,63]

EXPERIMENTAL SECTION

Organic synthesis.

General methods. Syntheses under microwave irradiation were performed under pressure with a CEM Discover single-mode apparatus (2450 MHz) using an external reaction temperature sensor. All chemical reagents were purchased from Sigma, Fisher (France) or TCI-Europe. ^{1}H and ^{13}C NMR spectra were recorded on 300 and 600 MHz Bruker spectrometers in appropriate deuterated solvents; chemical shifts are reported on the δ scale. All ^{13}C NMR signals were assigned through C–H correlated HSQC spectra. TLC was performed on Silica Gel 60 F254, 230 mesh (E. Merck) with cyclohexane-EtOAc or EtOAc-MeOH, and spots were detected by vanillin–H_2SO_4 reagent. Preparative column chromatography was performed using 230–400 mesh Merck silica gel (purchased from Sigma). Flash chromatography was performed with Reveleris-Flash System apparatus with cartridge SiO_2-normal phase or C18-reverse phase. For purity estimation we used HPLC on Prevail C18, 5 μm column. In all cases the estimated purities was higher that required by *Journal of Medicinal Chemistry* (95%).

Optical rotations were determined with a Perkin Elmer Model 343 Polarimeter1 mL cell. Low-resolution electrospray mass spectra (ESI-MS) in the positive or negative ion mode were obtained on a Waters ZQ 4000 quadrupole instrument equipped with an electrospray (Z-spray) ion source. High resolution electrospray experiments (ESI-HRMS) were performed on a Waters Q-TOF Ultima Global hybrid quadrupole time-of-flight instrument, equipped with an electro-spray (Z-spray) ion source**.** Infrared spectra were recorded on an FTIR spectrometer.

***α*-L-*fuco*-*p*-tBu-calix[4]arene tetrahydroxamic acid 1.** To compound **24** (0.124 g; 0.0414 mmol) dissolved in MeOH (12 mL), was added Pd/C (10%) (0.040 g). The mixture was stirred at

room temperature under an atmosphere of hydrogen until the TLC monitoring (6/3/0.8: EtOAc/MeOH/H_2O) showed the total consumption of the starting material. The catalyst was then removed by filtration through Celite and washed with methanol. After evaporation of the solvent under reduced pressure, compound **1** was obtained as white solid after purification by preparative HPLC on 40 µM C18 revese phase with suitable gradient of H_2O/CH_3CN as eluant. Yield 0.085 g (80%); R_f (SiO_2, EtOAc/MeOH/H_2O: 6/3/0.8) = 0.13; ESI-MS: *m/z* = 1337.7 $[M-2Na^+]^{2+}$; $[\alpha]_D^{20}$ = -38 (c = 0.155 in MeOH). ^{1}H-NMR (600 MHz, MeOD, 25°C): δ = 8.01 (s, 4H, $CH_{triazole}$), 7.98 (s, 4H, $CH_{triazole}$), 6.83 (s, 8H, CH_{Ar}), 4.87 (m, 4H, H-1), 4.76 (d, *J* = 9.1 Hz, 4H, OCH_2triazole), 4.60 (m, 12H, OCH_2triazole/$CH_2N_{triazole}$), 4.36 (t, *J* =7.0 Hz, 8H, $CH_2N_{triazole}$), 4.31 (d, *J* =12.3 Hz, 4H, $ArCH_2Ar$), 3.95 (m, 4H, H-5), 3.88 (m, 8H, CH_2OAr), 3.75 (m, 8H , H-3/H-2), 3.13 (d, *J* = 12.3 Hz, 4H, $ArCH_2Ar$), 2.53 (m, 8H, CH_2), 2.47 (m, 8H, CH_2), 1.87 (m, 8H, CH_2), 1.61 (m, 8H, CH_2), 1.29 (m, 8H, CH_2), 1.18 (d, $J_{6,5}$ = 6.6 Hz, 12H, H-6), 1.08 (s, 36H, CH_3). ^{13}C-NMR (150 MHz, MeOD, 25°C): δ = 176.0 ($C{=}O_{amide}$), 154.2 ($ArCOCH_2$), 146.1 (ArCtBu), 145.6 ($C_{triazole}$), 144.2 ($C_{triazole}$), 134.8 ($ArCCH_2CAr$), 126.4 (CHAr), 125.3 ($CH_{triazole}$), 125.1 ($CH_{triazole}$), 100.1 (C-1), 73.5 (C-4), 73.0 (CH_2OAr), 71.5 (C-2), 69.9 (C-3), 67.7 (C-5), 61.7 (CH_2Otriazole), 51.1 (CH_2N), 34.7 ($C(CH_3)_3$), 32.9 (CH_2), 32.1 (CH_2), 32.0 (CH_3), 30.9 (CH_2), 27.0 (CH_2), 25.0 (CH_2), 24.0 (CH_2), 16.6 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+2Na]^{2+}$ calcd for $C_{128}H_{188}N_{28}O_{32}Na_2$ 1337.6870; found 1337.6852.

***α*-D-*manno*-*p*-tBu-calix[4]arene tetrahydroxamic acid 2**. To compound **25** (0.08 g; 0.026 mmol) dissolved in MeOH (8mL), was added Pd/C (10%) (0.023 g). The mixture was stirred at room temperature under an atmosphere of hydrogen until total consumption of the starting material. The catalyst was then removed by filtration through Celite and washed with methanol. After evaporation of the solvent under reduced pressure, compound **2** was obtained as white

solid after purification by preparative HPLC on 40 µM C18 revese phase with suitable gradient of H_2O/CH_3CN as eluant. Yield 0.051 g (72 %); ESI-MS: *m/z* = 1370.2 $[M-2Na^+]^{2+}$; $[\alpha]_D^{20}$ = +32 (c = 0.08 in MeOH). ^{1}H-NMR (600 MHz, MeOD, 25 °C): δ = 8.00 (s, 4H, C$\underline{H}_{triazole}$), 7.96 (s, 4H, C$\underline{H}_{triazole}$), 6.82 (s, 8H, $\underline{C}H_{Ar}$), 4.86 (m, 4H, H-1), 4.77 (d, *J* = 12.4 Hz, 4H, OC$\underline{H}_2$triazole), 4.61 (m, 12H, OC$\underline{H}_2$triazole/C$\underline{H}_2N_{triazole}$), 4.37-4.26 (m, 12H, C$\underline{H}_2N_{triazole}$/ArC$\underline{H}_2$Ar), 3.93-3.57 (m, 32H, H-6/H-5/H-4/H-3/H-2/C$\underline{H}_2$OAr), 3.12 (d, *J* = 12.3 Hz, 4H, ArC$\underline{H}_2$Ar), 2.53 (m, 8H, C$\underline{H}_2$), 2.46 (m, 8H, C$\underline{H}_2$), 1.86 (m, 8H, C$\underline{H}_2$), 1.59 (m, 8H, C$\underline{H}_2$), 1.29 (m, 8H, C$\underline{H}_2$), 1.07 (s, 36H, C$\underline{H}_3$). ^{13}*C*-NMR(75 MHz, MeOD, 25 °C): δ = 175.9 ($\underline{C}$=O_{amide}), 154.3 (Ar$\underline{C}OCH_2$), 146.1 (Ar$\underline{C}$tBu), 145.2 ($\underline{C}_{triazole}$), 144.2 ($\underline{C}_{triazole}$), 134.9 (Ar$\underline{C}CH_2\underline{C}$Ar), 126.4 ($\underline{C}H_{Ar}$), 125.3 ($\underline{C}H_{triazole}$), 100.7 (C-1), 74.9, 73.0 ($\underline{C}H_2$OAr), 72.4, 71.9, 68.6 (C-5, C-4, C-3, C-2), 62.9 (C-6), 60.7 ($\underline{C}H_2$Otriazole), 51.2 ($\underline{C}H_2N$), 44.5 ($\underline{C}H_2N$), 34.7 ($\underline{C}(CH_3)_3$), 32.9 ($\underline{C}H_2$), 32.1 ($\underline{C}H_3$), 30.9 ($\underline{C}H_2$), 27.1 ($\underline{C}H_2$), 25.0 ($\underline{C}H_2$). HRMS(ESI/Q-TOF) *m/z* : $[M+2Na]^{2+}$ calcd for $C_{128}H_{188}O_{36}N_{28}Na_2$ 1369.6763; found 1369.6700.

***α*-D-*gluco*-*p*-*t*Bu-calix[4]arene tetrahydroxamic acid 3**. To compound **23** (0.2g; 0.061 mmol) dissolved in MeOH (18 mL), was added Pd/C (10%) (0.1 g). The mixture was stirred at room temperature under an atmosphere of hydrogen until total consumption of the starting material. The catalyst was then removed by filtration under Celite and washed with methanol. After evaporation of the solvent under reduced pressure, compound **3** was obtained after purification by preparative HPLC on 40 µM C18 revese phase with suitable gradient of H_2O/CH_3CN as eluant. Yield 0.092 g (72 %); ESI-MS: *m/z* = 1370.2 $[M-2Na^+]^{2+}$; $[\alpha]_D^{20}$ = +15.2 (c = 0.5 in MeOH). ^{1}H-RMN (600 MHz, MeOD, 25°C): δ = 8.02 (s, 4H, C$\underline{H}_{triazole}$), 7.98 (s, 4H, C$\underline{H}_{triazole}$), 6.83 (s, 8H, $\underline{C}$HAr), 4.1 (d, *J* = 3.5Hz, 4H, H-1), 4.72-4.52 (m, 12H, OC$\underline{H}_2$triazole/H-3), 4.46-4.24 (m, 12H, OC$\underline{H}_2$triazole/C$\underline{H}_2Nt_{riazole}$), 3.96-3.76 (m, 12H, H-2/ ArC$\underline{H}_2$Ar), 3.73-3.56

(m, 12H, H-6a/H-4), 3.46 **(**dd, *J* = 3.6 Hz, *J* = 9.7 Hz, 4H, H-6b), 3.37-3.27 **(**m, 4H, H-5), 3.13 **(**d, *J*= 12.7 Hz, 4H, ArC$\underline{H}_2$Ar), 2.5 (m, 8H, C$\underline{H}_2$), 1.87 (m, 8H, C$\underline{H}$2), 1.62 (m, 8H, C$\underline{H}_2$), 1.31 (m, 8H, C$\underline{H}_2$), 1.08 (s, 36H, C$\underline{H}_3$). ^{13}C-RMN (150 MHz, MeOD, 25°C): δ = 175.8 ($\underline{C}$=O), 154.3 (Ar$\underline{C}$OCH$_2$), 145.1 (Ar$\underline{C}$tBu), 145.4 ($\underline{C}_{triazole}$), 144.3 ($\underline{C}_{triazole}$), 134.9 (Ar$\underline{C}$CH$_2\underline{C}$Ar), 126.4 ($\underline{C}$HAr), 125.3 ($\underline{C}$H $_{triazole}$), 99.5 (C-1), 75.0, 74.0, 73.4, 73.0 ($\underline{C}$H$_2$OAr), 71.8 (C-5, C-4, C-3, C-2), 62.6 (C-6), 61.4 ($\underline{C}$H$_2$Otriazole), 51.2 ($\underline{C}$H$_2$N)**,** 44.5 ($\underline{C}$H$_2$N$_{calix}$), 34.8 ($\underline{C}$(CH$_3$)$_3$), 32.9 ($\underline{C}$H$_2$), 32.0 ($\underline{C}$H$_3$), 30.9 ($\underline{C}$H$_2$), 27.1 ($\underline{C}$H$_2$), 25.0 ($\underline{C}$H$_2$). HRMS(ESI/Q-TOF) *m/z*: [M+Na]$^+$ calcd for $C_{128}H_{188}O_{36}N_{28}$ 1369.6763, found, 1369.6700.

6-Azido-*N*-benzyloxyhexanamide (5). A stirred solution of 6-azido hexanoïc acid (**4**)[64] (2 g, 12 mmol) and *O*-benzylhydroxylamine (1.58 g, 15.2 mmol) in CH_2Cl_2 (66 mL) was cooled to 0°C. A solution of DCC (3.154 g, 15.28 mmol) in CH_2Cl_2 (40 mL) was added dropwise over 5 min and the solution was allowed to warm to room temperature, with stirring continued for 3h. The mixture was filtered and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc) afforded 6-azido-*N*-benzyloxyhexanamide **5** as a colorless oil. Yield 2.387 g (71%); R_f (SiO_2, cyclohexane/EtOAc: 4/3) = 0.32; ESI-MS: *m/z* = 284.9 [M+Na]$^+$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 10.24 (s, 1H, NH), 7.45-7.29 (m, 5H, CH_{Bn}), 4.84 (s, 2H, CH_{2Bn}), 3.15 (t, *J* = 6.8 Hz, 2H, CH_2), 2.05 (t, *J* = 6.6 Hz, 2H, CH_2), 1.59-1.46 (m, 4H, CH_2), 1.28 (m, 2H, CH_2).^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ = 170.8 (C=O), 135.5 (C_{Bn}), 128.9 (CH $_{Bn}$), 128.4 (CH_{Bn}), 128.3 (CH_{Bn}), 77.9 (CH_{2Bn}), 51.1 (CH_2N_3), 32.6 (CH_2), 28.4 (CH_2), 25.8 (CH_2), 24.8 (CH_2). HRMS(ESI-TOF) *m/z* : [M+Na]$^+$ calcd for $C_{13}H_{18}N_4O_2Na$ 285.1327; found 285.1324.

Propargyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside (6). Peracetylated fucose (0.4 g, 1.203 mmol), boron trifluoride diethyl etherate (0.684 g, 4.814 mmol) and propargyl alcohol (0.269 g,

4.814 mmol) in CH_2Cl_2 (4mL), were irradiated under pressure at 100 W, 70 °C during 10 min with stirring. After acetylation of the crude product and standard workup, purification by flash chromatography (cyclohexane/EtOAc) afforded α-anomer compound **6** as a yellow solid. Yield 0.256 g (70%); R_f (SiO_2, cyclohexane/EtOAc: 4/2) = 0.44. ESI-MS: *m/z* = 350.9 $[M+Na]^+$; $[\alpha]_D^{20}$ = -164 (c = 0.2 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ = 5.23 (dd, $J_{3,2}$ = 10.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.18 (dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-4), 5.13 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 5.03 (dd, $J_{2,1}$ = 3.8 Hz, $J_{2,3}$ = 10.8 Hz, 1H, H-2), 4.15 (d, *J* = 2.4 Hz, 2H, C<u>H</u>$_2$C≡CH), 4.09 (qd, $J_{5,6}$ = 6.6 Hz, $J_{5,4}$ = 1.1Hz, 1H, H-5), 2.39 (t, *J* = 2.4 Hz, 1H, CH_2C≡C<u>H</u>), 2.05 (s, 3H, CH_3), 1.97 (s, 3H, CH_3),1.87 (s, 3H, CH_3), 1.03 (d, $J_{6,5}$ = 6.6 Hz, 3H, H-6). ^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ = 170.3 (C=O), 170.1 (C=O), 169.7 (C=O), 94.9 (C-1), 78.5 (CH_2C≡<u>C</u>H), 74.8 (CH_2<u>C</u>≡CH), 70.9 (C-4), 67.7 (C-2, C-3), 64.8 (C-5), 55.0 (<u>C</u>H_2C≡CH), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 15.6 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{15}H_{20}O_8Na$ 351.1056; found 351.1051.

6''-[4'-(2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyloxy-methyl)-1'H-1',2',3'-triazole-1'-yl]-*N*-benzyloxy-hexanamide (9). To a stirred solution of compounds **6** (0.49 g, 1.49 mmol) and **5** (0.58 g, 2.23 mmol) in THF/tBuOH (5 mL, 7.5 mL) was added a freshly prepared solution of Cu(I) (from $CuSO_4.5H_2O$ (0.18 g, 0.746 mmol) and sodium ascorbate (0.295 g, 1.49 mmol) in H_2O (7.5 mL)). The mixture was stirred for 3h at 65 °C. The solvent was evaporated and the crude product was purified by flash chromatography (cyclohexane/EtOAc) to afford **9** as the main product as white powder. Yield 0.567 g (64%); R_f (SiO_2, cyclohexane/EtOAc: 1/9) = 0.30. ESI-MS: *m/z* = 613.0 $[M+Na]^+$; $[\alpha]_D^{20}$ = -86 (c = 0.25 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.50 (s, 1H, $CH_{triazole}$), 7.33 (m, 5H, CH_{Bn}), 5.31 (dd, $J_{3,2}$ = 10.6, $J_{3,4}$ = 3.3 Hz,1H, H-3), 5.24 (dd, $J_{4,3}$ = 3.3 Hz, $J_{4,5}$ = 10.5 Hz, 1H, H-4), 5.12 (d, $J_{1,2}$ = 3.5 Hz, 1H,H-1), 5.07 (dd, $J_{2,1}$

= 3.5 Hz, $J_{2,3}$ = 10.6 Hz, 1H, H-2), 4.91- 4.73 (m, 3H,CH_{2Bn}/OCH_2triazole), 4.59 (d, *J* = 12.3 Hz, 1H, OCH_2triazole), 4.29 (t, *J* = 6.6 Hz, 2H, CH_2), 4.14 (dq, $J_{5,4}$ = 10.5 Hz et $J_{5,6}$ = 6.5 Hz, 1H, H-5), 2.12 (s, 3H, CH_3), 2.03 (t, 2H, CH_2), 1.98 (s,3H, CH_3), 1.93 (s, 3H, CH_3), 1.86 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.24 (m, 2H, CH_2), 1.09 (d, $J_{6,5}$ = 6.5 Hz, 3H, H-6). ^{13}C-NMR(75 MHz, $CDCl_3$, 25°C): δ = 170.5 (C=O), 170.3 (C=O), 170.0 (C=O), 143.7 ($C_{triazole}$), 135.4 (C_{Bn}), 129.1 (CH_{Bn}), 128.4 (CH_{Bn}), 122.8 ($CH_{triazole}$), 95.4 (C-1), 78.0 (CH_{2Bn}), 71.1 (C-4), 68.0 (C-2), 67.9 (C-3), 64.6 (C-5), 61.0 (OCH_2triazole), 49.9 (CH_2N), 32.5 (CH_2), 29.7 (CH_2), 25.7 (CH_2), 24.4 **(**CH_2), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 15.7 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{28}H_{38}O_{10}N_4Na$ 613.2486; found 613.2470.

6''-[4'-(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl-oxymethyl)-1'*H*-1',2',3'-triazole-1'-yl]-*N*-benzyloxy-hexanamide (10). To a stirred solution of compounds **7** (0.8 g, 2.07 mmol) and **5** (0.814 g, 3.105 mmol) in THF/tBuOH (8 mL/12 mL) was added a freshly prepared solution of Cu(I) (from $CuSO_4.5H_2O$ (0.258g, 1.035 mmol) and sodium ascorbate (0.41 g, 2.07 mmol)) in H_2O (12 mL)). The mixture was stirred for 3h at 65°C. The solvent was evaporated and the crude product was purified by column chromatography (cyclohexane/EtOAc : 1/9) to afford **10** as a white powder. Yield 1.237 g (92%); R_f (SiO_2, cyclohexane/EtOAc: 1/9) = 0.12; ESI-MS: *m/z* = 671.3 $[M+Na]^+$; $[\alpha]_D^{20}$ = +31 (c = 0.125 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.55 (s, 1H, $CH_{triazole}$), 7.32 (m, 5H, CH_{Bn}), 5.36-5.12 (m, 3H, H-4/ H-3/ H-2), 4.94-4.70 (m, 4H, H-1, CH_{2Bn}/CH_2O), 4.62 (d, *J* = 12.3 Hz, 1H, CH_2O), 4.26 (m, 3H, CH_2N/H-6_b), 4.06 (m, 2H, H-6_a/H-5), 2.09 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.92 (s, 3H, CH_3), 1.93-1.75(m, 2H, CH_2), 1.70-1.45 (m, 2H, CH_2), 1.34-1.09 (m, 2H, CH_2). ^{13}C-NMR(75 MHz, $CDCl_3$, 25°C): δ = 170.7 (C=O), 170.1 (C=O), 170.1 (C=O), 169.7 (C=O), 143.4 (C_{Bn}), 129.1 (CH_{Bn}), 128.5 (CH_{Bn}), 123.0 ($CH_{triazole}$), 96.8 (C-1), 78.0 (CH_{2Bn}), 69.5, 69.1, 68.7, 66.0 (C-2, C-3, C-4, C-5), 62.4

(C-6), 61.0 (CH_2O), 50.1 (CH_2), 32.6 (CH_2), 29.8 (CH_2), 25.8 (CH_2), 24.51 (CH_2), 20.8 (CH_3), 20.7 (CH_3), 20.7 (CH_3). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{30}H_{40}O_{12}N_4Na$ 671.2540; found 671.2548.

6''-[4'-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyloxymethyl)-1'*H*-1',2',3'-triazole-1'-yl]-*N*-benzyloxyhexanamide (11). To a stirring solution of compound **8** (1 g, 2.58 mmol) with compound **5** (1.06 g, 4.1 mmol) in THF/tBuOH (6 mL/9 mL) a freshly prepared solution of Cu(I) ($CuSO_4.5H_2O$(0.37g) and sodium ascorbate (0.58 g) in H_2O (9 mL)) was added. The mixture was stirred for 3h at 65°C. The solvent was evaporated and the residue was purified by column chromatography (cyclohexane/EtOAc : 1/9) to offord **11** as a white powder. Yield 1.2 g (71 %); R_f (SiO_2, Cyclohexane/EtOAc: 1/9) = 0.30. ESI-MS: *m/z* = 671.3 $[M+Na]^+$; $[\alpha]_D^{20}$ = +23.8 (c = 0.5 in CH_2Cl_2). ^{1}H-RMN (400 MHz, $CDCl_3$, 25°C): δ = 7. 53 (s, 1H, C$\underline{H}_{triazole}$), 7.34 (m, 5H, C$\underline{H}_{benzyle}$), 5.45 (t, *J*= 10.3, *J*= 9.3 Hz, 1H, H-3), 5.15 (d, *J*=3.7 Hz, 1H, H-1), 5.09-4.99 (m, 1H, H-4), 4.90- 4.75 (m, 4H, H-2, C$\underline{H}_{2benzyl}$/CH_2O), 4.64 (d, 1H, C$\underline{H}_2O$, *J* = 12.4 Hz), 44.36-4.27 (m, 2H, C$\underline{H}_2N$), 4.24 (m, 1H, H-6_a), 4.14-3.96 (m, 2H,H6_b/H_5), 2.06 (s, 3H, C$\underline{H}_3$), 2.01 (s, 3H, C$\underline{H}_3$), 1.98 (s, 3H, C$\underline{H}_3$), 1.97 (s, 3H, C$\underline{H}_3$), 1.91-1.85(m, 2H, C$\underline{H}_2$), 1.68-1.61 (m,2H, C$\underline{H}_2$), 1.32-1.19 (m, 2H, C$\underline{H}_2$). ^{13}C-RMN (100 MHz, $CDCl_3$, 25°C): δ = 170.5 (C=O), 169.9 (C=O), 170.1 (C=O),169.3 (C=O), 143.1 (C$_{benzyl}$), 128.9 (CH$_{benzyl}$), 128.3 (CH$_{benzyl}$), 122.6 (CH$_{triazole}$), 94.8 (C-1), 77.8 (CH$_{2benzyl}$), 70.4, 69.8, 68.2, 67.2 (C-2,C-3, C-4, C-5), 61.5 (C-6), 61.0 (C$\underline{H}_2$N), 49.9 (C$\underline{H}_2$), 32.4 (C$\underline{H}_2$), 29.6 (C$\underline{H}_2$), 25.5 (C$\underline{H}_2$), 24.1 (C$\underline{H}_2$), 20.5 (C$\underline{H}_3$), 20.4 (C$\underline{H}_3$), 20.4 (C$\underline{H}_3$), 20.3 (C$\underline{H}_3$). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{30}H_{40}O_{12}N_4Na$ 671.2540 found: 671.2548.

6''-[4'-(2,3,4-Tri-O-acetyl-α-L-fucopyranosyloxy-methyl)-1'H-1',2',3'-triazole-1'-yl]-N-benzyloxy-N-(prop-2-yn-1-yl)hexanamide (12). Compound **9** (1.240 g, 2.1 mmol), K_2CO_3

(0.580 g, 4.198 mmol) and propargyl bromide (0.749 g, 6.298 mmol) were suspended in CH_3CN (37 mL). The mixture was stirred at 70°C overnight. The solvent was removed under reduced pressure and the residue was purified by flash chromatography to afford **12** and **16. 12** is obtained as yellow oil. Yield 0.652 g (50%) yield (0.652 g); R_f (SiO_2, cyclohexane/EtOAc: 1/2) = 0.18. ESI-MS: *m/z* = 650.8 $[M+ Na]^+$; $[\alpha]_D^{20}$ = - 91(c = 0.15 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.48 (s, 1H, $CH_{triazole}$), 7.35 (m, 5H, CH_{Bn}), 5.31 (dd, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.24 (dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.1, 1H, H-4), 5.13 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.07 (dd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.7 Hz, 1H, H-2), 4.92 (s, 2H, CH_{2Bn}), 4.78 (d, *J* = 12.4 Hz, 1H, OCH_2triazole), 4.60 (d, *J* = 12.4 Hz, 1H, OCH_2triazole), 4.31 (d, *J* = 2.5 Hz, 2H, $C\underline{H}_2C{\equiv}CH$), 4.28 (t, 2H, CH_2), 4.21-4.11 (m, 1H, H-5), 2.32 (t, *J* = 7.2 Hz, 2H, CH_2), 2.24 (t, *J* = 2.5 Hz, 1H, $CH_2C{\equiv}C\underline{H}$), 2.10 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.91 (s, 3H, CH_3),1.85 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.27 (m, 2H, CH_2), 1.09(d, $J_{6,5}$ = 6.5 Hz, 3H, H-6). ^{13}C-NMR(75 MHz, $CDCl_3$, 25°C): δ = 175.6 ($C{=}O_{amide}$), 170.5 (C=O), 170.2 (C=O), 169.9 (C=O), 143.8 ($C_{triazole}$), 134.4 (C_{Bn}), 129.4 (CH_{Bn}), 129.0 (CH_{Bn}), 128.71(CH_{Bn}), 122,6 ($CH_{triazole}$), 95.6 (C-1), 78.2 ($CH_2\underline{C}{\equiv}CH$), 77.5 ($CH_{2Bn}$), 72.0 ($CH_2C{\equiv}\underline{C}H$), 71.1 (C-4), 67.9 (C-2), 67.94 (C-3), 64.6 (C-5), 61.3 (OCH_2triazole), 50.0 (CH_2N), 36.7 (CH_2), 31.9 (CH_2), 30.0 (CH_2), 25.9 (CH_2), 23.3 (CH_2), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3)**,** 15.8 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{31}H_{40}O_{10}N_4Na$ 651.2642; found 651.2632.

6''-[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-oxymethyl)-1'H-1',2',3'-triazole-1'-yl]-*N*-benzyloxy-N-(prop-2-yn-1-yl)hexanamide (13). Compound **10** (0.8 g, 1.23 mmol), K_2CO_3 (0.34 g, 2.46 mmol) and propargyl bromide (0.44 g, 3.69 mmol) were suspended in CH_3CN (24 mL). The mixture was stirred at 60°C overnight. The solvent was removed under

reduced pressure and the residue was purified by flash chromatography to afford **13** and **17** as byproduct.

Compound **13** was obtained as a yellow oil. Yield 0.416 g (49%); R*f* (SiO_2, cyclohexane/EtOAc: 1/2) = 0.25; ESI-MS: *m/z* = 710.1 $[M+Na]^+$; $[\alpha]_D^{20}$ = +29 (c = 0.1 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ = 7.55 (s, 1H, $CH_{triazole}$), 7.41-7.32 (m, 5H, CH_{Bn}), 5.33-5.19 (m, 3H, H-4/H-3/H-2), 4.96-4.90 (m, 3H, $CH_{2\ Bn}$ /H-1), 4.82 (d, *J* = 12.3 Hz, 1H, CH_2O), 4.63 (d, *J* = 12.3 Hz, 1H, CH_2O), 4.36-4.21 (m, 5H, C<u>H</u>$_2$C≡CH /C<u>H</u>$_2$N/ <u>H</u>-6_b), 4.13-3.96 (m, 2H, H-5/H-6_a), 2.35 (t, *J* = 7.2 Hz, 2H, CH_2), 2.25 (t, *J* = 2.5 Hz, 1H, $CH_2C≡C$<u>H</u>), 2.11 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.95-1.78 (m, 2H, CH_2), 1.66-1.51 (m, 2H, CH_2), 1.34-1.21 (m, 2H, CH_2). ^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ = 175.7 ($C=O_{amide}$), 170.7 (C=O), 170.0 (C=O), 169.6 (C=O), 169.7 (C=O), 143.4 ($C_{triazole}$), 134.4 (C_{Bn}), 128.4 (CH_{Bn}), 128.1 (CH_{Bn}), 127.7 (CH_{Bn}), 122.9 ($CH_{triazole}$), 96.9 (C-1), 78.2 ($CH_2C≡CH$), 77.3 (CH_{2Bn}), 72.0 ($CH_2C≡CH$), 69.5, 69.1, 68.7, 66.1 (C-2, C-3, C-4, C-5), 62.4 (C-6), 61.1 (CH_2O), 50.2 (CH_2N), 36.8 ($CH_2C≡CH$), 32.0 (CH_2), 29.9 (CH_2), 26.1 (CH_2), 23.4 (CH_2), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{33}H_{42}O_{12}N_4Na$ 709.2697; found:709.2690.

6''-[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyloxymethyl)-1'*H*-1',2',3'-triazole-1'-yl]-*N*-benzyloxy-*N*-(prop-2-yn-1-yl)hexanamide (14). A mixture of compound **11** (0.8 g, 1.23 mmol), K_2CO_3 (0.34 g, 2.46 mmol) and propargyl bromide (0.44 g, 3.69 mmol) in CH_3CN (24 mL) was irradiated under pressure with 100 W power microwaves for 40 min at 70°C under stirring. After cooling for 5 min, the solvent was removed under reduced pressure and the residue was purified by column chromatography (cyclohexane/EtOAc : 2/4) to afford **14** and **18**. **14** was obtained as yellow oil. Yield 0.637 g (75%). R_f (SiO_2, EtOAc/cyclohexane: 3/1) = 0.49; ESI-MS: *m/z* = 709.27 $[M+Na]^+$; $[\alpha]_D^{20}$ = +78 (c = 0.5 in CH_2Cl_2). ^{1}H-RMN (400 MHz, $CDCl_3$, 25 °C): δ

= 7.51 (s, 1H, C<u>H</u>$_{triazole}$), 7.42-7.32 (m, 5H, CH$_{benzyl}$), 5.44 (t, *J* =10.8 Hz, 1H, H-3) 5.33-4.96 (m, 3H, H-4/ H-2/ H-1), 4.96 (m, 2H, CH$_{2benzyl}$), 4.63 (dd, *J* = 12.3, *J* = 1.9 Hz ,1H, CH$_2$O), 4.39-4.17 (m, 4H, C<u>H</u>$_2$C≡CH /CH$_2$N), 4.15-3.96 (m, 2H, H-5/H-6$_a$), 3.44 (dd, *J* =7.0, *J* = 2.4 Hz, 1H, H-6$_b$), 2.34 (t, *J* = 7,2 Hz, 2H, CH$_2$), 2.25 (t, *J* = 2.4 Hz, 1H, CH$_2$C≡C<u>H</u>), 2.11 (s, 3H, C<u>H</u>$_3$), 2.06 (s, 3H, C<u>H</u>$_3$), 2.00 (s, 3H, C<u>H</u>$_3$), 1.98 (s, 3H, C<u>H</u>$_3$), 1.91-1.78 (m, 2H, C<u>H</u>$_2$), 1.68-1.52 (m, 2H, C<u>H</u>$_2$), 1.36-1.18 (m, 2H, C<u>H</u>$_2$). ^{13}C-RMN (100 MHz, CDCl$_3$, 25°C): δ = 176.0 (C=O $_{amide}$), 171 (C=O), 170.4 (C=O), 169.6 (C=O), 143.7 (C$_{triazole}$), 134.7 (C$_{benzyl}$), 129.7 (CH$_{benzyl}$), 129.4 (CH$_{benzyl}$), 129.1 (CH$_{benzyl}$), 123.1 (CH$_{triazole}$), 95.4 (C-1), 78.5 (CH$_2$C≡<u>C</u>H), 77.9 (CH$_{2benzyl}$), 72.3 (CH$_2$<u>C</u>≡CH), 70.9, 70.4, 68.8, 67.8 (C-2, C-3, C-4, C-5), 66.1 (C-6), 62.1 (CH$_2$O), 50.4 (CH$_2$N), 37.1 (<u>C</u>H$_2$C≡CH), 32.3 (<u>C</u>H$_2$), 30.4 (<u>C</u>H$_2$), 26.3 (<u>C</u>H$_2$), 23.7 (<u>C</u>H$_2$), 21.1 (<u>C</u>H$_3$), 21.0 (<u>C</u>H$_3$), 20.9 (<u>C</u>H$_3$). HRMS(ESI/Q-TOF) *m/z* : [M+Na]$^+$ calcd for $C_{33}H_{42}N_4NaO_{12}$ 709.2697, found 709.2689.

6''-[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyloxymethyl)-1'*H*-1',2',3'-triazole-1'-yl]N-benzyloxy-*N*-(prop-2-yn-1-yl)hexanamide (15). Compound **14** (0.6 g, 0.87 mmol) was dissolved in MeOH (35 mL) and catalytic amount of sodium (Na^+) was added. The reaction was then stirred overnight at room temperature. The base was neutralized with Amberlite IR-120. The resine was then removed by filtration and the solvent evaporated *in vacuo* to afford **15** as colourless oil. Yield 0.41 g (91%); ESI-MS: *m/z* = 541.3 $[M+ Na]^+$; $[\alpha]_D^{20}$ = + 56 (c = 0.5 in MeOH). ^{1}H-RMN (400 MHz, MeOD, 25°C): δ = 8. 03 (s, 1H, C<u>H</u>$_{triazole}$), 7.52-7.34 (m, 5H, CH$_{benzyl}$), 5.02 (m, 2H, CH$_{2benzyl}$), 4.94 (d, *J*=3.7 Hz, 1H, H-1), 4.87 (s, 2H, CH$_2$O), 4.51-4.31 (m, 4H, C<u>H</u>$_2$C≡CH /CH$_2$N), 3.89 (m 1H, H-6$_a$), 3.77-3.57, (m, 3H, H-2/H-3/H-4), 3.44 (dd, *J*= 9.7, *J*= 3.8 Hz, 1H, H-6$_b$), 3.39-3.25 (m, 1H, H-5), 2.71 (t, *J* = 2.5 Hz , 1H, CH$_2$C≡C<u>H</u>), 2.37 (t, 2H, C<u>H</u>$_2$ *J*= 7.3 Hz), 1.95-1.72 (m, 2H, C<u>H</u>$_2$), 1.66-1.52 (m, 2H, C<u>H</u>$_2$), 1.37-1.19 (m, 2H, C<u>H</u>$_2$). ^{13}C-RMN (100 MHz, MeOD, 25°C): δ =177.9 (C=O $_{amide}$), 146.0 (C$_{triazole}$), 136.6 (C$_{benzyl}$), 131.4

(CH_{benzyl}), 130.7 (CH_{benzyl}), 130.3 (CH_{benzyl}), 125.8 ($CH_{triazole}$), 100.2 (C-1), 79.7 ($CH_2C{\equiv}\underline{C}H$), 77.7 ($CH_{2benzyl}$), 75.6 ($CH_2\underline{C}{\equiv}CH$), 74.6, 74.1, 73.9, 72.4 (C-2, C-3, C-4, C-5), 63.3 (C-6), 62.0 (CH_2O), 51.8 (CH_2N), 33.7 ($\underline{C}H_2C{\equiv}CH$), 33.6 ($\underline{C}H_2$), 31.5 ($\underline{C}H_2$), 27.5 ($\underline{C}H_2$), 25.3 ($\underline{C}H_2$). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{33}H_{42}O_{12}N_4Na$ 709.2697, found: 709.2690.

6''-[4'(2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl-oxymethyl)-1'H-1',2',3'-triazole-1'-yl]-*O*-(prop-2-yn-1-yl)-N-benzyloxyacetimidate (16). Obtained as a yellow oil. Yield 0.158 g (12%). R_f (SiO_2, cyclohexane/EtOAc: 1/2) = 0.28 $[\alpha]_D^{20}$ = -77 (c = 0.215 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.40(s,1H,$CH_{triazole}$),7.24 (m, 5H,CH_{Bn}), 5.28(dd, 1, H-3), 5.21(dd, 1H, H-4), 5.13-4.99(m,2H, H-1/H-2),4.86 (s, 2H, $CH_{2\ Bn}$), 4.76-4.41 (m, 4H, OCH_2triazole/$C\underline{H}_2C{\equiv}CH$), 4.21 (m, 3H, CH_2), 4.13 (m,1H,H-5), 2.31 (t, 2H, CH_2), 2.17 (m, 2H, CH_2), 2.07 (s, 3H, CH_3), 1.94 (t, 3H, CH_3), 1.85 (t, 3H, CH_3), 1.79 (t, 2H, CH_2), 1.52 (t, 2H, CH_2), 1.23 (t, 2H, CH_2), 1.05 (d, 3H, H-6). ^{13}C-NMR (75 MHz, $CDCl_3$, 25 °C): δ =170.4 (C=O), 170.2 (C=O), 169. 9 (C=O), 163.0 (N=C-O), 143.7 ($C_{triazole}$), 137.7 (C_{Bn}), 128.4 (CH_{Bn}), 128.1 (CH_{Bn}), 122.4 ($CH_{triazole}$), 95.5 (C-1), 78.5 ($CH_2\underline{C}{\equiv}CH$), 75.8 ($CH_2C{\equiv}\underline{C}H$), 74.6 ($CH_{2\ Bn}$), 71.1 (C-4), 67.9 (C-2), 67.9 (C-3), 64.6 (C-5), 61.2 (OCH_2triazole), 54.0 ($\underline{C}H_2C{\equiv}CH$), 50.0 ($CH_2N$), 29.8 ($CH_2$), 26.6 ($CH_2$), 25.8 ($CH_2$), 24.2 ($CH_2$), 20.7 ($CH_3$), 20.5 ($CH_3$), 20.4 ($CH_3$), 15.8 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{31}H_{40}O_{10}N_4Na$ 651.2642; found 651.2657.

6''-[4'(2,3,4-Tri-*O*-acetyl-α-D-mannopyranosyl-oxymethyl)-1'H-1',2',3'-triazole-1'-yl]-*O*-(prop-2-yn-1-yl)-*N*-benzyloxyacetimidate (17). Compound **17** was obtained as a yellow oil. Yield 0.128 g (15%); R_f (SiO_2, cyclohexane/EtOAc: 1/2) = 0.37; $[\alpha]_D^{20}$ = +31(c = 0.175 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ = 7.47 (s, 1H, $C\underline{H}_{triazole}$), 7.35-7.15 (m, 5H, $C\underline{H}_{Bn}$), 5.32-5.13(m, 3H, H-4/ H-3/ H-2), 4.91 (d, *J* = 1.5 Hz, 1H, H-1), 4.85 (s, 2H, $C\underline{H}_{2Bn}$), 4.79 (d, *J* = 12.2 Hz, 1H, $C\underline{H}_2O$),4.62 (d, *J* = 12.2 Hz, 1H, $C\underline{H}_2O$), 4.53 (d, *J* =2.4 Hz, 2H,

C$\underline{H}_2$C≡CH), 4.24 (m, 3H, C$\underline{H}_2$N/ H-6$_b$), 4.04-3.99 (m, 2H, H-5/H-6$_a$), 2.45 (t, *J* = 2.4 Hz, 1H, CH$_2$C≡C$\underline{H}$), 2.35 (t, *J* = 7.2 Hz, 2H, C$\underline{H}_2$), 2.10 (s, 3H, C$\underline{H}_3$), 2.07 (s, 3H, C$\underline{H}_3$), 1.98 (s, 3H, C$\underline{H}_3$), 1.93 (s, 3H, C$\underline{H}_3$), 1.89-1.78 (m, 2H, C$\underline{H}_2$), 1.58-1.47 (m, 2H, C$\underline{H}_2$), 1.31-1.16 (m, 2H, C$\underline{H}_2$). ^{13}C-NMR(75 MHz, $CDCl_3$, 25°C): δ = 170.6 ($\underline{C}$=O), 169.9 ($\underline{C}$=O), 169.8 ($\underline{C}$=O), 169.6 ($\underline{C}$=O), 163.1 ($\underline{C}$=N$_{imidate}$), 143.3 ($\underline{C}_{triazole}$), 137.8 ($\underline{C}_{Bn}$), 128. 4 ($\underline{C}H_{Bn}$), 128.2 ($\underline{C}H_{Bn}$), 127. 7 ($\underline{C}H_{Bn}$), 122. 7 ($\underline{C}H_{triazole}$), 96.7 (C-1), 78.5 (CH$_2$C≡$\underline{C}$H), 75.9 ($\underline{C}H_{2Bn}$), 74. 8 (CH$_2\underline{C}$≡CH), 69.4, 69.0, 68.6, 66.0 (C-2, C-3, C-4, C-5), 62.0 (C-6), 61.0 ($\underline{C}H_2$O), 54.0 ($\underline{C}H_2$C≡CH), 50.1 ($\underline{C}H_2$N), 29.6 ($\underline{C}H_2$), 26.3 ($\underline{C}H_2$), 25.8 ($\underline{C}H_2$), 24.1 ($\underline{C}H_2$), 20.8 ($\underline{C}H_3$), 20.7 ($\underline{C}H_3$), 20.6 ($\underline{C}H_3$). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{33}H_{42}O_{12}N_4Na$ 709.2697, found 709.2681.

6''-[4'-(2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyloxymethyl)-1'*H*-1',2',3'-triazole-1'-yl]-*O*-(prop-2-yn-1-yl)-*N*-benzyloxyacetimidate (18). Obtained as yellow oil. Yield 0.087 g (9%); R_f (SiO_2, cyclohexane /EtOAc : 1/2) = 0.27. ^{1}H-RMN (400 MHz, $CDCl_3$, 25 °C): δ = 7.41 (s, 1H, C$\underline{H}_{triazole}$), 7.31-7.14 (m, 5H, CH$_{benzyl}$), 5.38 (t, *J* = 9.8 Hz , 1H, H-3), 5.10 (t, *J* = 3.8 Hz, 1H, H-1) 4.98 (t, *J*= 9.7 Hz, 1H, H-4), 4.86 (m, 2H, CH$_{2benzyl}$), 4 .83-4.69 (m, 4H, CH$_2$O /C$\underline{H}_2$C≡CH), 4.57 (d, *J* =12.3 Hz, 1H, H-6$_a$) 4.28-4.10 (m, 3H, H-2 /CH$_2$N), 4.05-3.9 (m, 2H, H-5/H-6$_b$), 2.46 (t, *J* = 2.4 Hz, 1H, CH$_2$C≡C$\underline{H}$), 2.15 (t, *J* = 7.3 Hz, 2H, CH$_2$), 2 (s, 3H, C$\underline{H}_3$), 1.94 (s, 3H, C$\underline{H}_3$), 1.93 (s, 3H, C$\underline{H}_3$), 1.92 (s, 3H, C$\underline{H}_3$), 1.81-1.78 (m, 2H, C$\underline{H}_2$), 1.6-1.43 (m, 2H, C$\underline{H}_2$), 1.35-1.17 (m, 2H, C$\underline{H}_2$). ^{13}C-RMN (100 MHz, $CDCl_3$, 25°C): δ = 170.6 ($\underline{C}$=O), 170.0 ($\underline{C}$=O), 169.8 ($\underline{C}$=O), 169.5 ($\underline{C}$=O), 154.5 ($\underline{C}$=O$_{iminol}$), 143.8 ($\underline{C}_{triazole}$), 137.7 ($\underline{C}_{benzyl}$), 128.2 ($\underline{C}H_{benzyl}$), 128.1 ($\underline{C}H_{benzyl}$), 127.7 ($\underline{C}H_{benzyl}$), 122.3 ($\underline{C}H_{triazole}$), 94.8 (C-1), 78.2 (CH$_2$C≡$\underline{C}$H), 75.8 ($\underline{C}H_{2benzyl}$), 75. 5 (CH$_2\underline{C}$≡CH), 70.37, 69.81, 68.25, 67.23 (C-2, C-3, C-4, C-5), 61.51 (C-6), 61.16 ($\underline{C}H_2$O), 57.57 ($\underline{C}H_2$C≡CH), 59.42 ($\underline{C}H_2$N), 29.80 ($\underline{C}H_2$), 29.63 ($\underline{C}H_2$), 25.44 ($\underline{C}H_2$), 24.80 ($\underline{C}H_2$), 20.48 ($\underline{C}H_3$),

20.40 ($\underline{C}H_3$), 20.34 ($\underline{C}H_3$). HRMS(ESI/Q-TOF) *m/z* : $[M+Na]^+$ calcd for $C_{33}H_{42}O_{12}N_4Na$ 709.2697, found 709.2690.

Tetra-azidopropyloxy-*p*-tBu-calix[4]arene 20. Synthesized following the reported procedure[65]

Peracetylated fuco-*p*-tBu-calixarene benzyl-hydroxamate 21. To a stirred solution of compounds **20** (0.185 g, 0.189 mmol) and **12** (0.523 g, 0.832 mmol) in a mixture of THF/tBuOH (7.5 mL/15 mL) as solvent, was added a freshly prepared aqueous solution of Cu(I) (from $CuSO_4.5H_2O$ (0.094 g) and sodium ascorbate (0.149 g) in H_2O (15mL)). The mixture was stirred at 65°C for 3h. After removing the solvent, the residue was purified by column chromatography (acetone/ EtOAc/cyclohexane: 3/3/1.5) to afford **21** as a yellow solid. Yield 0.407 g (61%); R_f (SiO_2, acetone/EtOAc/cyclohexane: 3/3/1.5) = 0.18; ESI-MS: *m/z* = 1769.6 $[M+2Na^+]^{2+}$; $[\alpha]_D^{20}$ = -54 (c = 0.155 in CH_2Cl_2). ^{1}H-NMR (600 MHz, $CDCl_3$, 25 °C): δ = 7.56 (s, 4H, $CH_{triazole}$), 7.44 (s, 4H, $CH_{triazole}$), 7.23 (s, 20H, CH_{Bn}), 6.66 (s, 8H, CH_{Ar}), 5.28 (dd, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$= 3.3 Hz, 4H, H-3), 5.21 (dd, $J_{4,3}$ = 3.3 Hz, $J_{4,5}$ = 1.0 Hz, 4H, H-4), 5.11 (d, $J_{1,2}$ = 3.7 Hz, 4H, H-1), 5.05 (dd, $J_{2,3}$ = 10.7 Hz, $J_{2,1}$ = 3.7 Hz, 4H, H-2), 4.82 (s, 8H, CH_2N_{amide}), 4.80 (s, 8H, CH_{2Bn}), 4.79 (d, *J* = 12.4 Hz, 4H, OCH_2triazole), 4,62 (d, *J* = 12.4 Hz, 4H, OCH_2triazole), 4.41 (t, 8H, $CH_2N_{triazole}$), 4.26 (t, 8H, $CH_2N_{triazole}$), 4.18 (m, 8H, $ArCH_2Ar$ / H-5), 3.79 (t, *J* = 6.2 Hz, 8H, OCH_{2calix}), 3.01 (d, *J* = 12.7 Hz, 4H, $ArCH_2Ar$), 2.42 (t, 8H, $CH_2CH_2CH_{2calix}$), 2.23 (t, *J* = 7.1 Hz, 8H, CH_2), 2.08 (s, 12H, CH_3), 1.94 (s, 12H, CH_3), 1.89 (s, 12H, CH_3), 1.76 (m, 8H, CH_2), 1.48 (m, 8H, CH_2), 1.19 (m, 8H, CH_2), 1.06 (d, $J_{6,5}$ = 6.7 Hz, 12H, H-6), 0.98 (s, 36H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$, 25°C): δ = 174.9 ($C=O_{amide}$), 170.6 (C=O), 170.4 (C=O), 170.0 (C=O), 152.7 ($Ar\underline{C}O\underline{C}H_2$), 145.1 ($Ar\underline{C}tBu$), 143.8 ($C_{triazole}$), 143.1 ($C_{triazole}$), 134.4 ($C_{Bn}$), 133.3 ($Ar\underline{C}CH_2\underline{C}Ar$), 129.5 ($CH_{Bn}$), 129.0 ($CH_{Bn}$), 128.7 ($CH_{Bn}$), 125.2 ($CH_{Ar}$), 123.6 ($CH_{triazole}$), 122.6 ($CH_{triazole}$), 95.7 (C-1), 76.8 ($CH_{2\ Bn}$), 71.7 ($CH_2OAr$), 71.1 (C-4), 68 .0 (C-3), 68.0 (C-2), 64.7

(C-5), 60.4 (CH_2Otriazole), 50.1 (CH_2N), 47.7 (CH_2N), 42.2 (CH_2N_{amide}), 33.9 ($\underline{C}(CH_3)_3$), 32.0 (CH_2), 31.4 (CH_3), 31.0 (CH_2), 30.9 (CH_2), 30.1 (CH_2), 26.1 (CH_2), 23.6 (CH_2), 20.8 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 15.9 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+2Na]^{2+}$ calcd for $C_{180}H_{236}N_{28}O_{44}Na_2$ 1769.8442; found: 1769.8457.

Peracetylated *manno-p*-tBu-calix[4]arene benzylhydroxamate 22. To a stirred solution of compounds **20** (0.123 g, 0.125 mmol) and **13** (0.38 g, 0.55 mmol) in a mixture THF/tBuOH (5 mL/7.5 mL) mixture as solvent, a freshly prepared solution of Cu(I) (from $CuSO_4.5H_2O$ (0.062 g) and sodium ascorbate (0.099 g)) in H_2O (7.5mL)) was added. The mixture was stirred for 3h at 65°C and solvent was evaporated. The crude product was purified by column chromatography (acetone/EtOAc/cyclohexane: 3/3/1) to afford **22** as a yellow solid. Yield 0.268 g (57%); R_f(SiO_2, acetone/EtOAc/cyclohexane: 3/3/1) = 0.57. ESI-MS: *m/z* = 1886.8 $[M+2Na^+]^{2+}$; $[\alpha]_D^{20}$ = +20 (c = 0.095 in CH_2Cl_2). ^{1}H-NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.57 (s, 4H, $CH_{triazole}$), 7.50 (s, 4H, $CH_{triazole}$), 7.23 (m, 20H, CH_{Bn}), 6.66 (s, 8H, CH_{Ar}), 5.31-5.13 (m, 12H, H-3/H-4/H-2), 4.93-4.71 (m, 24H, H-1/CH_{2Bn}/ CH_2N_{amide}/ OCH_2triazole), 4.59 (d, *J* = 12.1 Hz, 4H, OCH_2triazole), 4.42 (m, 8H, $CH_2N_{triazole}$), 4.23 (m, 12H, $CH_2N_{triazole}$ /H-6_b), 4.15 (d, *J* = 12.1 Hz, 4H, $ArCH_2Ar$), 4.06-3.97 (m, 8H, H-6_a/H-5), 3.79 (m, 8H, OCH_2calix), 3.01 (d, *J* = 12.1 Hz, 4H, $ArCH_2Ar$), 2.43 (m, 8H, CH_2), 2.24 (m, 8H, CH_2), 2.07 (s, 12H, CH_3), 2.04 (s, 12H, CH_3), 1.95 (s, 12H, CH_3), 1.90 (s, 12H, CH_3), 1.78 (m, 8H, CH_2), 1.28 (m, 8H, CH_2), 1.50 (m, 8H, CH_2), 0.98 (s, 36H, CH_3). ^{13}C-NMR(75 MHz, $CDCl_3$, 25°C): δ = 174.8 ($C=O_{amide}$), 170.6 (C=O), 170.0 (C=O), 169.8 (C=O), 169.7 (C=O), 152.7 ($Ar\underline{C}OCH_2$), 145.0 ($Ar\underline{C}_{tBu}$), 143.3 ($C_{triazole}$), 143.1 ($C_{triazole}$), 134.4 (C_{Bn}), 133.3 ($Ar\underline{C}CH_2\underline{C}Ar$), 129.5 ($CH_{Bn}$), 128.9 ($CH_{Bn}$), 128.6 ($CH_{Bn}$), 125.2 ($CH_{Ar}$), 123.6 ($CH_{triazole}$), 122.9 ($CH_{triazole}$), 96.8 (C-1), 77.1 ($\underline{C}H_{2Bn}$), 71.5 ($\underline{C}H_2OAr$), 69.4, 68 .9, 68.7, 66.0 (C-5, C-4, C-3, C-2), 62.4 (C-6), 61.0 ($\underline{C}H_2$Otriazole), 50.2 ($\underline{C}H_2N$), 47.7

(CH$_2$N), 42.3 (CH$_2$N$_{amide}$), 33.8 (C(CH$_3$)$_3$), 32.0 (CH$_2$), 31.3 (CH$_3$), 30.9 (CH$_2$), 30.0 (CH$_2$), 29.6 (CH$_2$), 26.1 (CH$_2$), 23.6 (CH$_2$), 20.8 (CH$_3$), 20.8 (CH$_3$), 20.7 (CH$_3$). HRMS(ESI/Q-TOF) *m/z* : [M+Na]$^+$ calcd for $C_{188}H_{244}O_{52}N_{28}Na_2$ 1885.8547; found 1885.8490.

Synthesis of α-D-glucocluster based *p-t*Bu-calix[4]arene 23. To a stirring solution of compound **15** (0.35 g, 0.68 mmol) with calix **20** (0.13 g, 0.13 mmol) in acetonitrile (3mL), copper iodide (0.0127 g, 0.069 mmol) and diisopropylethylamine (0.086 g, 0.67 mmol) were added. The mixture was placed under pressure and irradiated at 150 W in a microwave with stirring for 30 min at 80°C. After cooling for 5 min, the solvent was removed under reduced pressure and the residue was purified by reverse-phase chromatography with eluent gradient of H_2O/CH_3CN to afford **23** as white powder. Yield 0.286g (67%); R_f (C_{18}, H_2O/CH_3CN : 8/2) = 0.36; ESI-MS: *m/z* = 1550.8 $[M+2Na]^{2+}/2$; $[\alpha]_D^{20}$ = + 38 (c = 0.5 in MeOH). ^{1}H-RMN (400 MHz, MeOD, 25 °C): δ = 7.99 (s, 8H, CH$_{triazole}$), 7.42- 7.24(m, 20H, CH$_{benzyl}$), 6.79 (s, 8H, CHAr), 5.04-4.44 (m, 36H, CH$_{2benzyl}$/ H-1/ CH$_{2Namide}$/ OCH$_{2sucre}$ /CH$_2$N$_{triazole}$), 4.37-4.12 (m, 12H, CH$_2$N $_{triazole}$ / ArCH$_2$Ar), 3.89-3.23 (m, 24H, H-6a/H-6b/H-5/H-4/H-3/H-2), 3.04 (d, *J* = 12.6 Hz, 4H, ArCH$_2$Ar), 2.51 (t, *J* =7.18H, CH$_2$), 2.34 (m, 8H, CH$_2$), 1.77 (m, 8H, CH$_2$), 1.50 (m, 8H, CH$_2$), 1.24 (m, 8H, CH$_2$), 1.10 (s, 36H, CH$_3$). ^{13}C-RMN (100 MHz, MeOD, 25 °C): δ = 177.2 (C=O$_{amide}$), 154.6 (ArCOCH$_2$), 146.5 (ArCtBu), 145.8 (C$_{triazole}$), 144.8 (C$_{triazole}$), 136.4 (C$_{benzyl}$), 135.2 (ArCCH$_2$CAr), 131.2 (CH$_{benzyl}$), 130.5 (CH$_{benzyl}$), 130.1 (CH$_{benzyl}$), 126.8 (CHAr), 125.6 (CH$_{triazole}$), 100.2 (C-1), 78.6 (CH$_{2benzyl}$), 75.4, 74.4, 73.9 (CH$_2$OAr), 73.4, 72.2 (C-5,C-4,C-3, C-2), 63.1 (C-6), 61.9 (CH$_2$Otriazole), 51.5 (CH$_2$N), 35.2 (CH$_2$N), 33.5 (C(CH$_3$)$_3$), 32.4 (CH$_2$), 32.0 (CH$_3$), 27.4 (CH$_2$), 26.9 (CH$_2$), 25.2 (CH$_2$). HRMS(ESI/Q-TOF) *m/z*: $[M+2Na]^{2+}$ calcd for $C_{157}H_{216}O_{36}N_{28}$ 1549.7701, found: 1549.7739

***Fuco-p*-tBu-calix[4]arene benzylhydroxamate 24.** Compound **21** (0.135 g, 0.0386 mmol) was dissolved in distilled MeOH, ultra-pure water and triethylamine (7.5 mL/0.64 mL/0.64 mL). The mixture was stirred under argon at room temperature until TLC (EtOAc/MeOH/H_2O: 6/2/0.8) monitoring showed the total disappearance of the starting material. Solvents were evaporated and co-evaporated with toluene until dryness. The crude product was purified by column chromatography (EtOAc/MeOH/H_2O: 6/2/0.6) to afford **24** as white solid. Yield 0.069 g (60%); R_f(SiO_2, EtOAc/MeOH/H_2O: 6/2/0.8) = 0.23; ESI-MS: *m/z* = 1518.6 $[M+2Na^+]^{2+}$. $[\alpha]_D^{20}$ = -39 (c = 0.150 in MeOH). ^{1}H-NMR (600 MHz, MeOD, 25°C): δ = 7.97 (s, 4H, $CH_{triazole}$), 7.96 (s, 4H, $CH_{triazole}$), 7.32 (m, 20H, CH_{Bn}), 6.77 (s, 8H, CH_{Ar}), 4.94 (s, 8H, CH_2N_{amide}), 4.89 (m, 12H, H-1/CH_{2Bn}), 4.77 (d, *J* = 12.5 Hz, 4H, OCH_2triazole), 4.63 (d, *J* = 12.5 Hz, 4H, OCH_2triazole), 4.53 (t, *J* = 6.6 Hz, 8H, $CH_2N_{triazole}$), 4.29 (t, *J* = 7.1 Hz, 8H, $CH_2N_{triazole}$), 4.20 (d, *J* = 12.4 Hz, 4H, $ArCH_2Ar$), 3.95 (m, 4H, H-5), 3.77 (m, 16H , H-3/H-2/CH_2OAr), 3.66 (m, 4H, H-4), 3.02 (d, *J* = 12.4 Hz, 4H, $ArCH_2Ar$), 2.49 (m, 8H, CH_2), 2.31 (m, 8H, CH_2), 1.76 (m, 8H, CH_2), 1.48 (m, 8H, CH_2), 1.19 (d, $J_{6,5}$ = 6.2 Hz, 12H, H-6), 1.07 (s, 36H, CH_3). ^{13}C-NMR (150 MHz, MeOD, 25°C): δ = 176.5 ($C{=}O_{amide}$), 154.2 ($ArCOCH_2$), 146.0 (ArCtBu), 145.6 ($C_{triazole}$), 144.3 ($C_{triazole}$), 136.1 (C_{Bn}), 134.8 ($ArCCH_2CAr$), 130.8 (CH_{Bn}), 129.8 (CH_{Bn}), 126.4 (CHAr), 125.3 ($CH_{triazole}$), 125.0 ($CH_{triazole}$), 100.1 (C-1), 77.6 (CH_{2Bn}), 73.5 (C-4), 72.9 (CH_2OAr), 71.5 (C-2), 69.9 (C-3), 67.8 (C-5), 61.7 (OCH_2triazole), 51.1 (CH_2N), 34.7 ($C(CH_3)_3$), 32.0 (CH_3), 30.9 (CH_2), 26.9 (CH_2), 24.7 (CH_2), 16.7 (C-6). HRMS(ESI/Q-TOF) *m/z* : $[M+2Na]^{2+}$ calcd for $C_{156}H_{212}N_{28}O_{32}Na_2$ 1517.7809; found: 1517.7837.

***Manno-p*-tBu-calix[4]arene benzylhydroxamate 25.** Compound **22** (0.217 g, 0.057 mmol) was dissolved in distilled MeOH, ultra-pure water and ultra-pure triethylamine (8 mL, 1.29 mL, 1.29 mL). The mixture was stirred under argon at room temperature until total consumption of the

starting material. Solvents were evaporated and co-evaporated with toluene until dryness. The crude product was purified by reverse-phase chromatography with eluent gradient of H_2O/CH_3CN to afford **25** as yellow solid. Yield 0.088 g (50 %); R_f (C_{18}, H_2O/CH_3CN: 8/2) = 0.26; ESI-MS: *m/z* = 1550.8 $[M+2Na^+]^{2+}$; $[\alpha]_D^{20}$= +32 (c = 0.06 in MeOH). ^{1}H-NMR (300 MHz, MeOD, 25°C): δ = 7.98 (s, 8H, C$\underline{H}_{triazole}$), 7.41-7.21(m, 20H, C$\underline{H}_{Bn}$), 6.78 (s, 8H, C$\underline{H}_{Ar}$), 5.05-4.49 (m, 36H, C$\underline{H}_{2Bn}$/ H-1/ C$\underline{H}_2N_{amide}$/ OC$\underline{H}_{2sugar}$ /C$\underline{H}_2N_{triazole}$), 4.38-4.11 (m, 12H, C$\underline{H}_2N_{triazole}$ /ArC$\underline{H}_2$Ar), 3.99-3.53 (m, 24H, H-6_a/H-6_b/H-5/H-4/H-3/H-2), 3.04 (d, *J* = 12.6 Hz, 4H, ArC$\underline{H}_2$Ar), 2.5 (m, 8H, C$\underline{H}_2$), 2.32 (m, 8H, C$\underline{H}_2$), 1.77 (m, 8H, C$\underline{H}_2$), 1.48 (m, 8H, C$\underline{H}_2$), 1.19 (m, 8H, C$\underline{H}_2$), 1.08 (s, 36H, C$\underline{H}_3$). ^{13}*C*-NMR(75 MHz, MeOD, 25 °C): δ = 176.7 (C$\underline{=}O_{amide}$), 154.2 (Ar$\underline{C}OCH_2$), 146.1 (Ar$\underline{C}$tBu), 145.1 ($\underline{C}_{triazole}$), 144.0 ($\underline{C}_{triazole}$), 135.9 ($\underline{C}_{Bn}$), 134.8 (Ar$\underline{C}CH_2\underline{C}$Ar), 130.8 ($\underline{C}H_{Bn}$), 130.0 ($\underline{C}H_{Bn}$), 129.7 (C$\underline{H}_{Bn}$), 126.4 ($\underline{C}H_{Ar}$), 125.3 ($\underline{C}H_{triazole}$), 100.7 (C-1), 77.6 ($\underline{C}H_{2Bn}$), 74.9, 72.9 ($\underline{C}H_2$OAr), 72.5, 72.0, 68.6 (C-5,C-4,C-3, C-2), 62.9 (C-6), 60.7 ($\underline{C}H_2$Otriazole), 51.1 ($\underline{C}H_2$N), 48.7 ($\underline{C}H_2$N), 34.7 ($\underline{C}(CH_3)_3$), 33.0 ($\underline{C}H_2$), 32.0 ($\underline{C}H_3$), 30.9 ($\underline{C}H_2$), 26.9 ($\underline{C}H_2$), 24.5 ($\underline{C}H_2$). HRMS(ESI/Q-TOF) *m/z* : $[M+2Na]^{2+}$ calcd for $C_{156}H_{212}O_{36}N_{28}Na_2$ 1549.7701; found 1549.7639.

6''-(4'-(α-L-fucopyranosyloxymethyl)-1'H-1',2',3'-triazole-1'-yl]-*N*-benzyloxy-hexanamide (26). Obtained by conventional acetolysis of **9** with MeONa in MeOH at rt and reaction was monitored by TLC on silica gel with 6/1: EtOAc/MeOH as eluant until completion. After workup **26** was obtained quantitavely as a white solid. R_f (SiO_2, EtOAc/MeOH: 6/1) = 0.24; ESI-MS: *m/z* = 487.0 $[M+Na]^+$; $[\alpha]_D^{20}$ = -59 (c 0.16, MeOH). *^{1}H*-NMR (MeOD, 300 MHz), *δ* en ppm: 7.96 (s, 1H, C$\underline{H}_{triazole}$), 7.43- 7.28 (m, 5H, C$\underline{H}_{benzyl}$), 4.98-4.54 (m, 5H, H-1/C$\underline{H}_{2benzyl}$/ OC$\underline{H}_{2triazole}$), 4.36 (t,2H, C$\underline{H}_2$N, *J* = 7.0 Hz), 3.95 (m, 1H, H-5), 3.75 (s, 2H, H-2/ H-3), 3.66 (s, 1H, H-4),2.05 (t, 2H,$_\alpha$C$\underline{H}_2$, *J* = 7.3 Hz), 1.89 (m, 2H,$_\delta$C$\underline{H}_2$), 1,61 (m, 2H, $_\beta$C$\underline{H}_2$), 1,28 (m,

2H,$_{\gamma}$C$\underline{H}_2$), 1,19 (d, 3H, H-6, $J_{6,5}$ = 6.4Hz). *^{13}C*-NMR (MeOD, 75 MHz), δ en ppm: 172.2 ($\underline{C}$=O), 136.6 ($\underline{C}_{benzyl}$), 130.0 (CH_{benzyl}), 129.4($\underline{C}H_{benzyl}$), 129.2 ($\underline{C}H_{benzyl}$), 124.9 ($\underline{C}H_{triazole}$), 99.8 (C-1), 78.7 ($\underline{C}H_{2benzyl}$), 73.2 (C-4), 71.3, 69.7 (C-2/ C-3), 67.5 (C-5), 61.6 (O$\underline{C}H_2$triazole), 50.9 ($\underline{C}H_2$N), 33.2 ($_{\alpha}CH_2$), 30.6 ($_{\delta}CH_2$), 26.5 ($_{\gamma}CH_2$), 25.6 ($_{\beta}CH_{2)}$, 16.5 (C-6). HRMS(ESI/Q-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{22}H_{32}N_4O_7Na$ 487.2271; found 487.2162.

6''-(4'-(α-D-mannopyranosyloxymethyl)-1'H-1',2',3'-triazole-1'-yl]-*N*-benzyloxy-hexanamide (27). Compound **10** (0.1 g, 0.154 mmol) was dissolved in MeOH (8 mL) and a catalytic amount of sodium was added. The reaction was then stirred overnight at room temperature. After workup, **27** was obtained quantitaively as a yellow powder. ESI-MS: *m/z* = 503.3 $[M+Na]^+$. ^{1}H-NMR (300 MHz, MeOD, 25 °C): δ = 8.05 (s, 1H, $CH_{triazole}$), 7.46-7.37 (m, 5H, CH_{Bn}), 4.99-4.79 (m, 7H, H-4/ H-3/ H-2/ H-1, CH_{2Bn}/CH_2O), 4.68 (d, *J* = 12.3 Hz, 1H, CH_2O), 4.42 (t, *J* = 7.0 Hz, 2H, CH_2N), 3.90 (dd, *J* = 11.7 Hz, *J* = 5.4 Hz, 1H, H-6_b), 3.84 (m, 1H, H-5), 3.77 (dd, *J* = 11.7 Hz, *J* = 5.4 Hz, 1H, H-6_a), 2.09 (t, *J* = 7.3 Hz, 2H, CH_2), 1.93 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 1.29 (m, 4H, CH_2). ^{13}C-NMR(75 MHz, MeOD, 25°C): δ = 171.1 ($C{=}O_{amide}$), 143.8 (C_{Bn}), 135.6 ($C_{triazole}$), 128.9 (CH_{Bn}), 128.2 (CH_{Bn}),128.1(CH_{Bn}), 123.9 ($CH_{triazole}$), 99.3 (C-1), 77.5 (CH_{2Bn}), 73.5, 71.1, 70.6, 67.2 (C-2, C-3, C-4, C-5), 61.5 (C-6), 59.3 (CH_2O), 49.7 (CH_2N), 32.0 (CH_2), 29.4 (CH_2), 25.3 (CH_2), 24.4 (CH_2). HRMS(ESI/Q-TOF) m/z: $[M+Na]^+$ calcd for $C_{22}H_{32}O_8N_4Na$ 503.2118, found 503.2115.

Biologycal assessment

In vitro determination of Minimum Inhibitory Concentrations (MICs). *P. aeruginosa* DSM 1117 was grown overnight at 35°C in Tryptic Soy Broth and streaked on Tryptic Soy Agar (TSA) (AES, Bruz, France). From these isolation plates, inoculums were prepared according to

CLSI recommendations and the broth microdilution technique carried out in cation-adjusted Mueller-Hinton broth using drug concentrations ranging from 0.250 to 512 $\mu g.ml^{-1}$.[33] Ciprofloxacin and deferoxamine mesylate (DFO) were purchased from Sigma-Aldrich (Saint Quentin-Fallavier, France) and used in each series of experiments as controls for antibiotics and iron chelators, respectively. MICs were determined as the highest dilution at which wells remained visually clear (i.e. devoid of bacterial growth). The growth of *P. aeruginosa* PAO1 and PAD07[34] was monitored in Succinate Minimum Medium (SMM)[66] supplemented with a fixed amount of Fe^{3+} through addition of iron trichloride (Sigma-Aldrich). Products **1** and **2** were added to the culture medium in varying amounts (final [product]/[iron] ratios from 1:128 to 8:1). A stock solution (3200 $\mu g.mL^{-1}$) of each product in sterile DMSO (Sigma-Aldrich) was prepared and two-fold serially diluted in the same solvent down to 25 $\mu g.mL^{-1}$. These product concentrations correspond to 0.0078 to 8 times the amount theoretically required for chelating the amount of iron introduced in the SMM culture medium. In a 96-well plate, 5µL of each product dilution were distributed to which 5µL of the $FeCl_3$ solution in sterile water were added. Each well was then filled with 220 µL of SMM and 20 µL of a bacterial suspension of the required strain at 0.5 McFarland. A growth control was added to each series of experiments in which 5 µL of pure DMSO was added in place of the 5 µL of either product **1** or **2** dilutions. Plates were incubated for 24h at 37°C. Absorbance (OD) was then read on a Multiskan EX multiplate reader (Thermo Labsystems, Issy les Moulineaux, France) at 600 nm to monitor bacterial growth after a 24h incubation at 35°C and at 405 nm to assess the amount of free pyoverdin in PAO1 cultures.[67] The amount of iron-free pyoverdin was also evaluated by fluorescence measurement (FP-8500 Spectrofluorometer, JASCO, Bouguenais, France) in each well (λ_{exc} = 400 nm; λ_{em} = 447 nm).[29] Baselines were made with solvent alone and with the same

varying amounts of products **1** and **2** in the medium, without the bacterial inoculums, to assess the absorbance generated by our compounds. Experiments were carried out at least in duplicate. Results are given as mean + Standard Deviation (SD).

Statistical analysis. The statistical analysis was carried out using the Mann-Whitney signed rank test page of Vassarstats website.[68] A p value inferior to 0.05 was considered as significant.

LecB interaction assay. *Material and methods.* *Recombinant expression and purification of LecB.* The protein was expressed and purified as described previously.[69] *E. coli* BL21(DE3) carrying the plasmid were grown in 1 L LB supplemented with ampicillin (100 µg/mL) to an OD600 = 0.5-0.6 at 37 °C and 180 rpm. The expression was induced with IPTG (0.5 mM final concentration) and bacteria were cultured for 4 h at 30 °C and 180 rpm. The cells were then harvested by centrifugation (3000 x g, 10 min) and the pellet was washed with PBS. The cells were resuspended in 20 mL TBS/Ca (20 mM Tris, 137 mM NaCl, 2.6 mM KCl at pH 7.4 supplemented with 100 µM CaCl2), PMSF (1 mM) and lysozyme (0.4 mg/mL) and subsequently disrupted using a sonicator on ice (5 cycles of 10 s). Cell debris was removed by centrifugation (10 min, 10000 x g) and the supernatant was loaded on mannosylated sepharose CL-6B.[70] The column was washed with TBS/Ca and LecB was eluted by addition of 100 mM D-mannose to the buffer. The eluted fractions were extensively dialyzed against distilled water and then, the protein was lyophilized. The protein was dissolved in TBS/Ca before use and the concentration was determined by UV spectroscopy at 280 nm using a molar extinction coefficient of 6990 M-1 cm-1.[71]

Fluorescence polarization assay. The assay was performed as described by Hauck *et al.*[39] Typically, to 20 µL of a stock solution of LecB (225 nM) and fluorescent reporter ligand (1.5 nM) in TBS/Ca were added 10 µL serial dilutions of testing compounds in TBS/Ca in triplicates. After addition of the reagents, the microtiter plates were incubated for 4-21 h at room temperature with shaking. The fluorescence was measured on a BMG Labtech PHERAstar FS plate reader (BMG Labtech, Germany) with excitation filters at 485 nm and emission filters at

535 nm in black 384-well microtiter plates (Greiner Bio-One, Germany, cat no 781900). The measured intensities were reduced by buffer values. The data were analyzed with the MARS 2.41 software package (BMG Labtech, Germany) and fitted according to the four parameter variable slope model. Bottom and top plateaus were defined by the standard compounds L-fucose and methyl α-D-mannoside respectively and the data was reanalyzed with these values fixed.

Biofilm inhibition assays. *Material and methods*

First method reported by Diggle et al.[45] A modified version of the method described by Diggle et al. was employed. 96-well sterile, U-bottomed polystyrene microtiter plates (TPP Switzerland) were prepared by adding 200 μL of sterile deionized water to the peripheral wells to decrease evaporation from test wells. Aliquots of 180 μL of culture medium (0.25% (w/v) nutrient broth No. 2, Oxoid) containing desired concentration of the test compound were added to the internal wells. Inoculum of *P. aeruginosa* strain PAO1 was prepared from 5 mL overnight culture grown in LB broth. Aliquots of 20 μL of overnight cultures, pre-washed in 0.25% (w/v) nutrient broth and normalized to an OD_{600} of 1, were inoculated into the test wells. Plates were incubated in a humid environment for 24-25 hours at 37 °C. Wells were washed twice with 200 μL sterile deionized water before staining with 200 μL 0.25% (w/v) nutrient broth containing 0.5 mM WST-8 and 20 μM phenazine ethosulfate for 3 hours at 37 °C. Afterwards, the well supernatants were transferred to a polystyrene flat bottomed 96-well plate (TPP Switzerland) and the absorbance was measured at 450 nm with a plate reader (SpectraMax250 from Molecular Devices). For OD600 measurements, the 200 μL of the supernatant prior to washing were added to 5 mL of LB medium and shaked at 37°C for 7-8 hours. The OD600 was measured and compared to the w/o supernatant. For CFU plating, the 200 μL of the supernatant was added to the first well of a 96-wells-F-bottomed microtiter plate (TPP, untreated) and diluted serially by ½

(raw 1 to 6) in 180 μL NaCl 0.9%. 4 μL of the raw 6 were plated on LB agar and incubated at 37°C for 24 hours. It is for the toxicity determination under the biofilm conditions.

Second method reported by O'Toole.[56] PAO1 biofilm inhibition at 20 μM with **1**, **2** and **3**. Biofilm Assays were performed in 96 well plates as described previously. Briefly, *P. aeruginosa* PAO1 was grown in M63 minimal medium supplemented with arginine (0.4%) and magnesium sulfate (1 mM). The indicated compounds were tested at 20 uM, or an equal volume of the vehicle control (water) was added as a control. The indicated compounds were added at the time of inoculation of the medium, and the biofilm assays were then incubated for 8 hrs at 37^{o}C. At the end of the incubation period, 100 ul of the medium was removed from the biofilm assay plate to a fresh flat bottom, 96 well plate and the wells measured at 550 nm to determine bacterial growth. The original biofilm plate was stained with crystal violet (0.1%) and the biofilm quantified as described previously.

Cytotoxicity on host cells. Cytotoxicity of the CF-derived bronchial epithelial (CFBEΔF cells) cells was determined as reported previously.[48] Briefly, the indicated compounds were applied at 20 uM to a monolayer of CBFE cells and incubated for 24 hrs. An equal volume of the vehicle control (water) was added to a set of wells to serve as a control. After the incubation, an aliquot of the medium overlaying the CFBE cells was removed and cytotoxicity was assessed as a function of released lactate dehydrogenase (LDH) activity. LDH was measured with the CytoTox 96 Non-radioactive Cytotoxicity Kit (Promega).

Molecular mechanics (MMFF) prediction. The distance of 50 Å between two discarded mannose was determined using molecular mechanics (MMFF) in presence of LecB pdb with frozen atoms (from protein data bank (pdb)).[42] Two orthogonal CRD sites are separated by 40 Å.[43] For the ouput data see Tables S2 and S3 of SI.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information including: NMR spectra, LC-MS data, Minimum inhibitory concentrations (MIC) table, well plates showing Pvd-I fluorescence at high concentration of **1** or **2**, well plates of inhibition of PAO1 biofilm assays, MMFF calcualtions, is available free of charge on the ACS Publications website at DOI:

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

HAG, hydroxamic acid; Pvd-I, pyoverdine I; CF, cystic fibrosis; CFBE, cystic fibrosis derived bronchial epithelial; LDH, lactate dehydrogenase; Tb, tobramycin; MMFF, molecular mecanics force fields; MBL, mannose binding ligand; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; pdb, protein data bank.

REFERENCES

1. Butler, M. S.; Cooper, M. A. Antibiotics in the clinical pipeline in 2011. *J. Antibiol.* **2011,** 64, 413-425.

2. Bilitewski, U.; Blodgett, J. A.; Duhme-Klair, A. K.; Dallavalle, S.; Laschat, S.; Routledge, A.; Schobert, R. Chemical and Biological Aspects of Nutritional Immunity—Perspectives for New Anti-Infectives that Target Iron Uptake Systems. *Angew. Chem. Int. Ed.* **2017,** 56, 14360-14382.

3. Mills, J.; Robinson, K.; Zehnder, T.; Pierce, J. G. Synthesis and Biological Evaluation of the Antimicrobial Natural Product Lipoxazolidinone A. *Angew.Chem.* **2018,** 130, 8818-8822.

4. Calvert, M. B.; Jumde, V. R.; Titz, A. Pathoblockers or antivirulence drugs as a new option for the treatment of bacterial infections. *Beilstein J. Org. Chem.* **2018,** 14, 2607-2617.

5. Klahn, P.; Brönstrup, M. Bifunctional antimicrobial conjugates and hybrid antimicrobials. *Nat. Prod. Rep.* **2017,** 34, 832-885.

6. Rice, L. B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. In The University of Chicago Press: **2008**.

7. Franzetti, F.; Grassini, A.; Piazza, M.; Degl'Innocenti, M.; Bandera, A.; Gazzola, L.; Marchetti, G.; Gori, A. Nosocomial bacterial pneumonia in HIV-infected patients: risk factors for adverse outcome and implications for rational empiric antibiotic therapy. *Infect. Immun.* **2006,** 34, 9-16.

8. Nair, N.; Biswas, R.; Götz, F.; Biswas, L. Impact of Staphylococcus aureus on pathogenesis in polymicrobial infections. *Infect. Immun.* **2014,** 82, 2162-2169.

9. European Centre of Disease Prevention and Control. Antimicrobial resistance surveillance in Europe. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm, E., **2011**.

10. Antoniu, S. Novel inhaled combined antibiotic formulations in the treatment of *Pseudomonas aeruginosa* airways infections in cystic fibrosis. *Exp. Rev. Anti-Infect. Ther.* **2015,** 13, 897-905.

11. Bjarnsholt, T.; Ciofu, O.; Molin, S.; Givskov, M.; Høiby, N. Applying insights from biofilm biology to drug development—can a new approach be developed? *Nat. Rev. Drug Discov.* **2013,** 12, 791-808.

12. Mitchell, E.; Houles, C.; Sudakevitz, D.; Wimmerova, M.; Gautier, C.; Pérez, S.; Wu, A. M.; Gilboa-Garber, N.; Imberty, A. Structural basis for oligosaccharide-mediated adhesion of *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients. *Nat. Struct. Mol. Biol.* **2002,** 9, 918-921.

13. Tielker, D.; Hacker, S.; Loris, R.; Strathmann, M.; Wingender, J.; Wilhelm, S.; Rosenau, F.; Jaeger, K.-E. *Pseudomonas aeruginosa* lectin LecB is located in the outer membrane and is involved in biofilm formation. *Microbiol.* **2005,** 151, 1313-1323.

14. O'toole, G. A.; Kolter, R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* **1998,** 30, 295-304.

15. Chemani, C.; Imberty, A.; de Bentzmann, S.; Pierre, M.; Wimmerová, M.; Guery, B. P.; Faure, K. Role of LecA and LecB lectins in *Pseudomonas aeruginosa*-induced lung injury and effect of carbohydrate ligands. *Infect. Immun.* **2009,** 77, 2065-2075.

16. Cecioni, S.; Imberty, A.; Vidal, S. Glycomimetics versus multivalent glycoconjugates for the design of high affinity lectin ligands. *Chem. Rev.* **2014,** 115, 525-561.

17. Michaud, G.; Visini, R.; Bergmann, M.; Salerno, G.; Bosco, R.; Gillon, E.; Richichi, B.; Nativi, C.; Imberty, A.; Stocker, A. Overcoming antibiotic resistance in *Pseudomonas aeruginosa* biofilms using glycopeptide dendrimers. *Chem. Sci.* **2016,** 7, 166-182.

18. Gray, R. D.; Duncan, A.; Noble, D.; Imrie, M.; O'Reilly, D. S. J.; Innes, J. A.; Porteous, D. J.; Greening, A. P.; Boyd, A. C. Sputum trace metals are biomarkers of inflammatory and suppurative lung disease. *Chest* **2010,** 137, 635-641.

19. Banin, E.; Vasil, M. L.; Greenberg, E. P. Iron and *Pseudomonas aeruginosa* biofilm formation. *PNAS USA* **2005,** 102, 11076-11081.

20. Ammons, M. C.; Ward, L. S.; Dowd, S.; James, G. A. Combined treatment of *Pseudomonas aeruginosa* biofilm with lactoferrin and xylitol inhibits the ability of bacteria to respond to damage resulting from lactoferrin iron chelation. *Int. J. Antimicrob. Agents* **2011,** 37, 316-23.

21. Zhou, T.; Chen, K.; Kong, L.-M.; Liu, M.-S.; Ma, Y.-M.; Xie, Y.-Y.; Hider, R. C. Synthesis, iron binding and antimicrobial properties of hexadentate 3-hydroxypyridinones-terminated dendrimers. *Bioorg. Med. Chem. Lett.* **2018**.

22. Hannauer, M.; Barda, Y.; Mislin, G. L.; Shanzer, A.; Schalk, I. J. The ferrichrome uptake pathway in Pseudomonas aeruginosa involves an iron release mechanism with acylation of the siderophore and recycling of the modified desferrichrome. *J. Bacteriol.* **2010,** 192, 1212-1220.

23. Berthet, N.; Thomas, B.; Bossu, I.; Dufour, E.; Gillon, E.; Garcia, J.; Spinelli, N.; Imberty, A.; Dumy, P.; Renaudet, O. High affinity glycodendrimers for the lectin LecB from Pseudomonas aeruginosa. *Bioconjug. Chem.* **2013,** 24, 1598-611.

24. Wu, A. M.; Gong, Y.-P.; Li, C.-C.; Gilboa-Garber, N. Duality of the carbohydrate-recognition system of Pseudomonas aeruginosa-II lectin (PA-IIL). *FEBS Lett.* **2010,** 584, 2371-2375.

25. Morbioli, I.; Porkolab, V.; Magini, A.; Casnati, A.; Fieschi, F.; Sansone, F. Mannosylcalix [n] arenes as multivalent ligands for DC-SIGN. *Carbohydr. Res.* **2017,** 453, 36-43.

26. Boukerb, A. M.; Rousset, A.; Galanos, N.; Mear, J.-B.; Thepaut, M.; Grandjean, T.; Gillon, E.; Cecioni, S.; Abderrahmen, C.; Faure, K. Antiadhesive properties of glycoclusters against Pseudomonas aeruginosa lung infection. *J. Med. Chem.* **2014,** 57, 10275-10289.

27. Ryu, E.-H.; Zhao, Y. Efficient synthesis of water-soluble calixarenes using click chemistry. *Org. Lett.* **2005,** 7, 1035-1037.

28. Roy, R.; Kim, J. M. Amphiphilic p-tert-butylcalix [4] arene scaffolds containing exposed carbohydrate dendrons. *Angew. Chem. Int. Ed.* **1999,** 38, 369-372.

29. Hannauer, M.; Barda, Y.; Mislin, G. L.; Shanzer, A.; Schalk, I. J. The ferrichrome uptake pathway in *Pseudomonas aeruginosa* involves an iron release mechanism with acylation of the siderophore and recycling of the modified desferrichrome. *J. Bacteriol.* **2010,** 192, 1212-1220.

30. Grandjean, C.; Boutonnier, A.; Guerreiro, C.; Fournier, J. M.; Mulard, L. A. On the preparation of carbohydrate-protein conjugates using the traceless Staudinger ligation. *J. Org. Chem.* **2005,** 70, 7123-32.

31. Kiviniemi, A.; Virta, P.; Lönnberg, H. Utilization of intrachain 4′-C-azidomethylthymidine for preparation of oligodeoxyribonucleotide conjugates by click chemistry in solution and on a solid support. *Bioconjug. Chem.* **2008,** 19, 1726-1734.

32. Pomecko, R.; Asfari, Z.; Hubscher-Bruder, V.; Bochenska, M.; Arnaud-Neu, F. Anion recognition by phosphonium calix [4] arenes: synthesis and physico-chemical studies. *Supramol. Chem.* **2010,** 22, 275-288.

33. Wikler, M. *Clinical and Laboratory Standards Institute Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard*; Report: **2009**.

34. Takase, H.; Nitanai, H.; Hoshino, K.; Otani, T. Impact of Siderophore Production onPseudomonas aeruginosa Infections in Immunosuppressed Mice. *Infect. Immun.* **2000,** 68, 1834-1839.

35. Hannauer, M.; Schäfer, M.; Hoegy, F.; Gizzi, P.; Wehrung, P.; Mislin, G. L.; Budzikiewicz, H.; Schalk, I. J. Biosynthesis of the pyoverdine siderophore of *Pseudomonas aeruginosa* involves precursors with a myristic or a myristoleic acid chain. *FEBS Lett.* **2012,** 586, 96-101.

36. Barrero, J.; Morino-Bondi, M.; Pérez-Conde, M.; Cámara, C. A biosensor for ferric ion. *Talanta* **1993,** 40, 1619-1623.

37. Albrecht-Gary, A.-M.; Blanc, S.; Rochel, N.; Ocaktan, A. Z.; Abdallah, M. A. Bacterial iron transport: coordination properties of pyoverdin PaA, a peptidic siderophore of *Pseudomonas aeruginosa*. *Inorg. Chem.* **1994,** 33, 6391-6402.

38. Yadav, R.; Kikkeri, R. Carbohydrate functionalized iron (iii) complexes as biomimetic siderophores. *Chem. Commun.* **2012,** 48, 1704-1706.

39. Hauck, D.; Joachim, I.; Frommeyer, B.; Varrot, A.; Philipp, B.; Möller, H. M.; Imberty, A.; Exner, T. E.; Titz, A. Discovery of two classes of potent glycomimetic inhibitors of *Pseudomonas aeruginosa* LecB with distinct binding modes. *ACS Chem. Biol.* **2013,** 8, 1775-1784.

40. Sommer, R.; Exner, T. E.; Titz, A. A biophysical study with carbohydrate derivatives explains the molecular basis of monosaccharide selectivity of the *Pseudomonas aeruginosa* Lectin LecB. *PLoS One* **2014,** 9, e112822.

41. Sommer, R.; Wagner, S.; Varrot, A.; Nycholat, C. M.; Khaledi, A.; Häussler, S.; Paulson, J. C.; Imberty, A.; Titz, A. The virulence factor LecB varies in clinical isolates: consequences for ligand binding and drug discovery. *Chem. Sci.* **2016,** 7, 4990-5001.

42. Loris, R.; Tielker, D.; Jaeger, K. E.; Wyns, L. Structural basis of carbohydrate recognition by the lectin LecB from *Pseudomonas aeruginosa*. *J Mol Biol* **2003,** 331, 861-70.

43. Buffet, K.; Nierengarten, I.; Galanos, N.; Gillon, E.; Holler, M.; Imberty, A.; Matthews, S. E.; Vidal, S.; Vincent, S. P.; Nierengarten, J. F. Pillar [5] arene-Based Glycoclusters: Synthesis and Multivalent Binding to Pathogenic Bacterial Lectins. *Chem. -A Eur. J.* **2016,** 22, 2955-2963.

44. Pieters, R. J. Maximising multivalency effects in protein–carbohydrate interactions. *Org. Biomol. Chem.* **2009,** 7, 2013-2025.

45. Diggle, S. P.; Stacey, R. E.; Dodd, C.; Cámara, M.; Williams, P.; Winzer, K. The galactophilic lectin, LecA, contributes to biofilm development in *Pseudomonas aeruginosa*. *Environ. Microbiol.* **2006,** 8, 1095-1104.

46. Ishiyama, M.; Miyazono, Y.; Sasamoto, K.; Ohkura, Y.; Ueno, K. A highly water-soluble disulfonated tetrazolium salt as a chromogenic indicator for NADH as well as cell viability. *Talanta* **1997,** 44, 1299-1305.

47. Shteinberg, M.; Elborn, J. S. Use of inhaled tobramycin in cystic fibrosis. *Adv. Ther.* **2015,** 32, 1-9.

48. Price, K. E.; Orazi, G.; Ruoff, K. L.; Hebert, W. P.; O'Toole, G. A.; Mastoridis, P. Mannitol does not enhance tobramycin killing of *Pseudomonas aeruginosa* in a cystic fibrosis model system of biofilm formation. *PloS one* **2015,** 10, e0141192.

49. Hoffman, L. R.; D'argenio, D. A.; MacCoss, M. J.; Zhang, Z.; Jones, R. A.; Miller, S. I. Aminoglycoside antibiotics induce bacterial biofilm formation. *Nat.* **2005,** 436, 1171-1175.

50. Moreau-Marquis, S.; O'toole, G. A.; Stanton, B. A. Tobramycin and FDA-approved iron chelators eliminate *Pseudomonas aeruginosa* biofilms on cystic fibrosis cells. *Am. J. Respir. Cell Mol. Biol.* **2009,** 41, 305-313.

51. Herrmann, G.; Yang, L.; Wu, H.; Song, Z.; Wang, H.; Høiby, N.; Ulrich, M.; Molin, S.; Riethmüller, J.; Döring, G. Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm *Pseudomonas aeruginosa*. *J. Infect. Dis.* **2010,** 202, 1585-1592.

52. Thellin, O.; Zorzi, W.; Jolois, O.; Elmoualij, B.; Duysens, G.; Cahay, B.; Streel, B.; Charif, M.; Bastin, R.; Heinen, E. In vitro approach to study the synergistic effects of tobramycin and clarithromycin against *Pseudomonas aeruginosa* biofilms using prokaryotic or eukaryotic culture media. *Int. J. Antimicrob. Agents* **2015,** 46, 33-38.

53. McCaughey, G.; McKevitt, M.; Elborn, J. S.; Tunney, M. M. Antimicrobial activity of fosfomycin and tobramycin in combination against cystic fibrosis pathogens under aerobic and anaerobic conditions. *J. Cyst. Fibros.* **2012,** 11, 163-172.

54. de la Fuente-Núñez, C.; Korolik, V.; Bains, M.; Nguyen, U.; Breidenstein, E. B.; Horsman, S.; Lewenza, S.; Burrows, L.; Hancock, R. E. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. *Antimicrob. agents Chemother.* **2012,** 56, 2696-2704.

55. Merritt, J. H.; Kadouri, D. E.; O'Toole, G. A. Growing and analyzing static biofilms. *Curr. Protoc. Microbiol.* **2011,** 22, 1B. 1.1-1B. 1.18.

56. O'Toole, G. A. Microtiter dish biofilm formation assay. *Journal of visualized experiments: JoVE* **2011**, 2437.

57. Bucior, I.; Pielage, J. F.; Engel, J. N. *Pseudomonas aeruginosa* pili and flagella mediate distinct binding and signaling events at the apical and basolateral surface of airway epithelium. *PLoS Pathog.* **2012,** 8, e1002616.

58. Gellatly, S. L.; Hancock, R. E. *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog. Dis.* **2013,** 67, 159-173.

59. Fothergill, J. L.; Walshaw, M. J.; Winstanley, C. Transmissible strains of Pseudomonas aeruginosa in cystic fibrosis lung infections. *Eur. Respir. J.* **2012,** 40, 227-238.

60. Kaja, S.; Payne, A. J.; Singh, T.; Ghuman, J. K.; Sieck, E. G.; Koulen, P. An optimized lactate dehydrogenase release assay for screening of drug candidates in neuroscience. *J. Pharmacol. Toxicol. Methods* **2015,** 73, 1-6.

61. Legrand, C.; Bour, J.; Jacob, C.; Capiaumont, J.; Martial, A.; Marc, A.; Wudtke, M.; Kretzmer, G.; Demangel, C.; Duval, D. Lactate dehydrogenase (LDH) activity of the number of dead cells in the medium of cultured eukaryotic cells as marker. *J. Biotech.* **1992,** 25, 231-243.

62. Feinberg, H.; Taylor, M. E.; Razi, N.; McBride, R.; Knirel, Y. A.; Graham, S. A.; Drickamer, K.; Weis, W. I. Structural basis for langerin recognition of diverse pathogen and mammalian glycans through a single binding site. *J. Mol. Biol.* **2011,** 405, 1027-1039.

63. Geng, J.; Mantovani, G.; Tao, L.; Nicolas, J.; Chen, G.; Wallis, R.; Mitchell, D. A.; Johnson, B. R.; Evans, S. D.; Haddleton, D. M. Site-directed conjugation of "clicked" glycopolymers to form glycoprotein mimics: binding to mammalian lectin and induction of immunological function. *J. Am. Chem. Soc.* **2007,** 129, 15156-15163.

64. Grandjean, C.; Boutonnier, A.; Guerreiro, C.; Fournier, J.-M.; Mulard, L. A. On the preparation of carbohydrate− protein conjugates using the traceless Staudinger ligation. *J. Org. Chem.* **2005,** 70, 7123-7132.

65. Taouai, M.; Chakroun, K.; Vallin-Butruille, A.; Cézard, C.; Romdhani, M. New synthesis of heteroglycoclusters from p-t-butylcalix [4] arene tetraalkoxyheterohalides as key intermediates. *Arkivoc* **2018**, 186-200.

66. Meyer, J. a.; Abdallah, M. The fluorescent pigment of Pseudomonas fluorescens: biosynthesis, purification and physicochemical properties. *Microbiol.* **1978,** 107, 319-328.

67. Baysse, C.; De Vos, D.; Naudet, Y.; Vandermonde, A.; Ochsner, U.; Meyer, J.-M.; Budzikiewicz, H.; Schäfer, M.; Fuchs, R.; Cornelis, P. Vanadium interferes with siderophore-mediated iron uptake in *Pseudomonas aeruginosa. Microbiol.* **2000,** 146, 2425-2434.

68. Lowry, R. Concepts and applications of inferential statistics. **2014**.

69. Mitchell, E. P.; Sabin, C.; Šnajdrová, L.; Pokorná, M.; Perret, S.; Gautier, C.; Hofr, C.; Gilboa-Garber, N.; Koča, J.; Wimmerová, M. High affinity fucose binding of *Pseudomonas aeruginosa* lectin PA-IIL: 1.0 Å resolution crystal structure of the complex combined with thermodynamics and computational chemistry approaches. *Proteins: Struct. Funct. Bioinf.* **2005,** 58, 735-746.

70. Fornstedt, N.; Porath, J. Characterization studies on a new lectin found in seeds of Vicia ervilia. *FEBS Lett.* **1975,** 57, 187-191.

71. Gasteiger, E.; Hoogland, C.; Gattiker, A.; Wilkins, M. R.; Appel, R. D.; Bairoch, A. Protein identification and analysis tools on the ExPASy server. In *Proteomic. protocols handbook*, Springer: **2005**; pp 571-607.

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