CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200800700

Glycomimetics and Glycodendrimers as High Affinity Microbial Anti-adhesins

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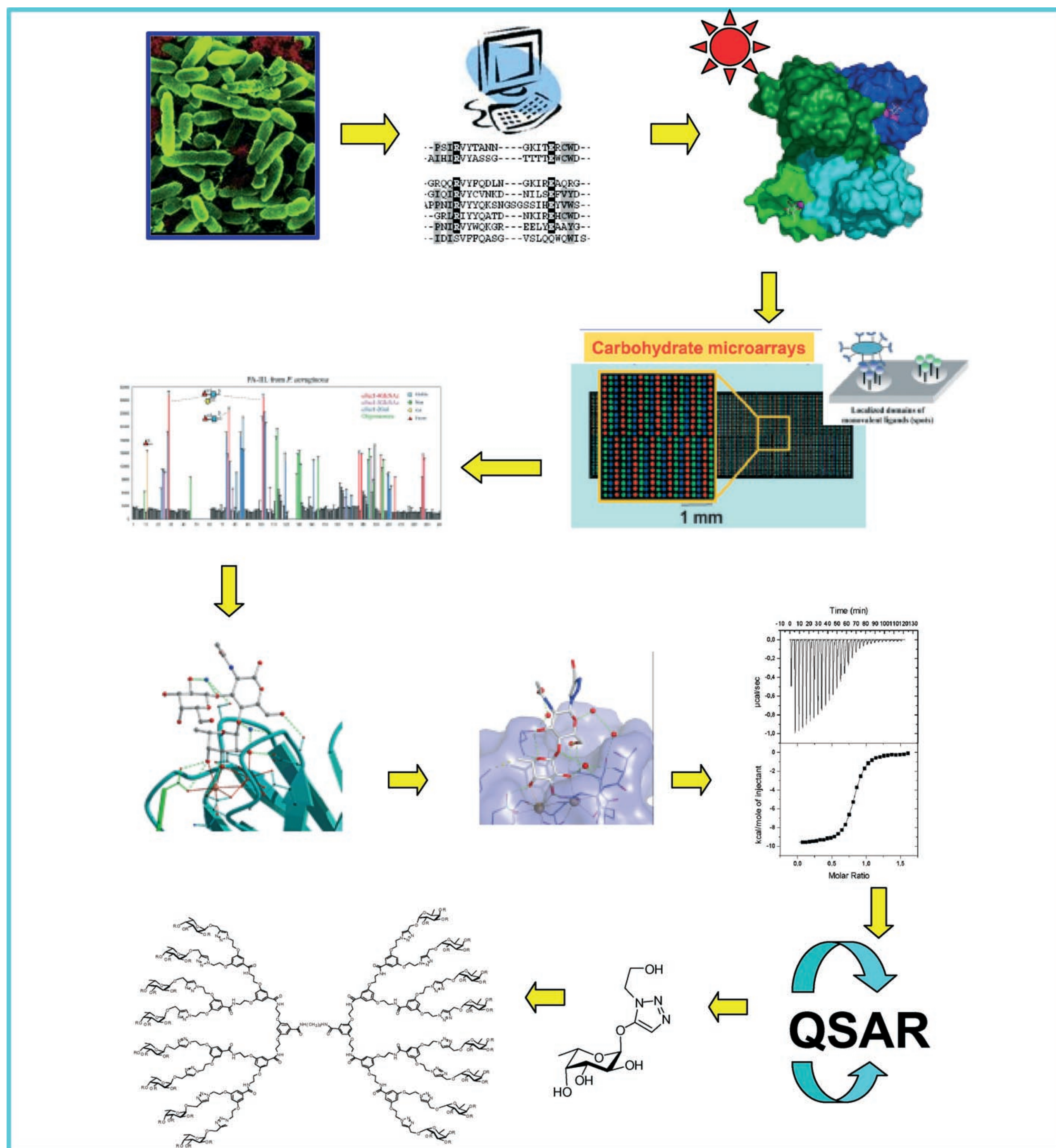

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Abstract: Adhesion to epithelial surface is often the first step in bacterial and viral infection. In this process, the microbes use a variety of proteins for interaction with host carbohydrates presented as glycoconjugates on cell surfaces. Crystal structures of adhesin and lectin binding sites in complexes with oligosaccharide open the route for design and synthesis of glycomimetics, glycodendrimers, and glycopolymers that are able to block infection at an early stage.

Keywords: adhesin · antiviral agents · dendrimers · glycomimetics · lectins

Introduction

Infection by pathogens is generally initiated by crucial steps of recognition and adhesion on host epithelia surfaces. Very frequently, the strategy used by micro-organisms involves the binding to host glycoconjugates by sugar-binding proteins, lectins, which are specific for the target tissue. The dependence between pathogen receptors and host glycans leads to the concept of "glycoecology".[1] In turn, the host immune system can also use lectins to identify and bind oligo- and polysaccharides on micro-organism surface, but in some cases the pathogens can reroute this process and use it for invasion. As we exemplify, these infection strategies involve interactions characterized by their high specificity and most of the time by multivalency. The biochemical and structural data that have been accumulated recently offer chemists the possibility to interfere in the infection process through molecules that mimic the natural oligosaccharidic ligands and effectively compete for attachment sites. Different strategies using modified oligosaccharides, glycomimetics, oligomers, dendrimers, or polymers have been developed to enhance the overall affinity of carbohydrate ligands.

Glycostrategies for Microbial Infection

Pathogens have a number of different types of lectin for targeting host sugars. In bacteria, lectins exist sometimes as domains of bacterial toxins and exploit adhesion to glycoconjugates as a mean of entering target cells. Different architectures are observed (Figure 1). The AB_5-type proteins of *Vibrio cholera,* enterotoxic *Escherichia coli*, and *Bordella pertussis* consist of one toxic ADP-ribosyltransferase and five lectin subunits that bind to gangliosides of gut or lung walls.[2] Clostridial neurotoxins involved in tetanus and botulism contain a lectin domain able to bind ganglioside GM1 on neurons helping in the attachment.

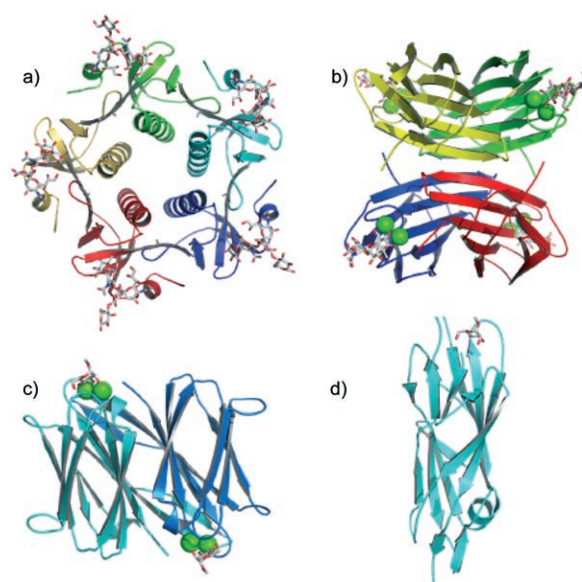

Figure 1. Different architectures observed in lectins from bacteria. a) Pentameric lectin of cholera toxin associated with GM1 ganglioside (3CHB[48]), b) tetrameric PA-IIL from *P. aeruginosa* complexed with Lewis a (1W8H[20]), c) dimeric BclA from *B. cenocepacia* complexed with methylmannoside (2 VNV[17]), and d) monomeric lectin domain from *E. coli* fimbrial FimH complexed with butylmannoside (1UWF[32]).

Soluble bacterial lectins from *Pseudomonas aeruginosa* and related gram-negative bacteria such as *Burkholderia cenocepacia* have been structurally characterized recently. They form dimers and tetramers that have the characteristic to bind sugar through bridging by calcium ions.[3] These soluble lectins are hypothesized to play a role in host recognition, but also in biofilm formation and cohesion.[4] Many bacteria are covered with pili, or fimbriae, that contain a very special class of lectin, referred to as adhesins, since they play a role in attachment to epithelial cells.[5] These lectins are monomeric and present only one binding site located at the tip of the pilus, but the large number of these organelles on the bacteria surface generates multivalency. The FimH adhesin, present on uropathogenic *E. coli* and specific for mannosylated structures, is the most studied member of the family.[6] Other type of lectin/adhesins are present in the outer membrane of bacteria, such as the BabA and the SabA adhesins, that binds Lewis b and sialylated epitopes, respectively, and play a role in gastric infection by *Helicobacter pylori*.[7]

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Many enveloped as well as non-enveloped human viruses also use human glycoconjugates as attachment points on epithelia. Several capsid proteins contain lectin domains, most of them with affinity for negatively charged carbohydrate such as sialic acid containing glycoconjugates and glycosaminoglycans. The virus–carbohydrate interactions are responsible for specific tissue tropism, such as the affinity of human influenza virus hemagglutinins for (α2–6)-linked sialic-containing glycans in airways.[8] Interestingly, the repartition of some viral infection such as acute gastroenteritis caused by the Norwald virus depends on the ABO status of the population with nonsecretor individuals, lacking αFuc1–2 in their mucins, being highly resistant to symptomatic infections with major strains of norovirus.[9]

The infection scheme can be reversed with human lectins interacting with glycans present either in cell-wall bacterial polysaccharides or in viral glycoproteins. A large number of lectins act as sentinels and present the trapped pathogens to other actors of the immune system. However, in some cases, the pathogens use lectins as entry points. The role of DC-SIGN, a C-type lectin of peripheral dendritic cells, in HIV transmission has been highlighted:[10] recognition of highly mannosylated gp120 by DC-SIGN leads to binding of virus and transmission to lymphal node. DC-SIGN is exploited by many other viruses and pathogens.[11]

Glycan Array: Tools for Screening Specificity

Screening for the best oligosaccharidic ligand for a given lectin can be a tedious and expensive task. This approach has been simplified in recent years by the availability of glycan arrays (glycochips) that were developed in different laboratories.[12–14] Each format differs in the type of glycans and the manner in which they are displayed. Some use non-covalent attachment to plastic or nitrocellulose membrane, and others use covalent attachment to plastic, gold, or glass. The Consortium for Functional Glycomics (www.functionalglycomics.org) proposes an ELISA-based microplate array that is comprised of a library of biotinylated synthetic and naturally occurring oligosaccharides attached by a spacer arm to streptavidin-coated microtiter plates (more than 240 glycans). The new format is a printed glycan microarray consisting of ligands (377 glycans) with amino linkers printed onto NHS-activated glass microscope slides.[15] Glycan arrays based on neoglycolipids were also shown to be successful for screening lectin specificity and new approaches were also developed in this area for ligation of the ligands.[16]

The information derived from glycan array experiments offer excellent tools for comparing specificity of similar lectins from related pathogens. For example PA-IIL from *P. aeruginosa* and BclA from *B. cenocepacia* are related lectins from opportunistic bacteria that have the particularity to contain two calcium ions in the carbohydrate binding site. Both lectins bind mannose in the same orientation, but PA-IIL also binds fucose, albeit in a different orientation.[17,18] Glycan array data indicate that although the lectins are closely related, BclA binds only to oligomannose glycans, while PA-IIL has a preference for fucosylated oligosaccharides (Figure 2).[17,19] The stronger binding is observed for αFuc1–4GlcNAc-containing oligosaccharides and indeed Lewis a trisaccharide (αFuc1–4(βGal1–3)GlcNAc) is the best natural ligand for PA-IIL with a dissociation constant of 210 nM.[20] The strict specificity of BclA for mannose-containing glycoconjugates can be rationalized from the comparison of the crystal structure of the complex BclA/mannoside[17] with those obtained for PA-IIL interaction with mannose and fucose.[18,21]

High Affinity Glycomimetics and Glycodendrimers Against Infection by Adhesion Competition

Efforts for blocking microbial adhesins and lectins have driven carbohydrate chemists towards the production of original molecules specifically designed for their anti-adhesion potency. For example, successful syntheses of such molecules have been obtained for cholera toxin,[22,23] shiga toxin,[24–26] or influenza virus.[27,28] In the present work, we will focus on the results recently obtained against two human pathogens: uropathogenic *E. coli* and *P. aeruginosa*.

Targeting the fimbrial adhesins of *E. coli*: Adhesins mediate the interaction of enterotoxic and uropathogenic *E. coli* strains with the host in a tissue-dependant way. Flexible F17 fimbriae of enterotoxigenic *E. coli* are capped with GafD adhesins specific for GlcNAc-terminated glycoconjugates. Uropathogenic *E.coli* bind to different parts of the human urinary tract. The long and flexible P fimbriae expose PapG adhesins specific for galabiose (αGal1–4Gal), while shorter type 1 pili are terminated by FimH, which binds oligomannose.[29] The three fimbrial lectins, GafD, FimH, and PapG, share similar beta-barrel folds, but display different ligand-binding regions and disulfide bond patterns.

Surface plasmon resonance (SPR) studies of live-bacteria adhesion on a galabiose surface have been used for testing the inhibiting properties of a set of synthetic mono- and multivalent galabiose compounds.[30] An octavalent compound appeared to be the most effective inhibitor, but in this particular case, the anti-inhibition potency was only moderately affected by the valency.

It has been established by Sharon et al. that aryl mannosides bearing varied substitution patterns had decreasing IC_{50}'s toward the inhibition of binding of *E. coli* to epithelial cells.[31] Hence, it was early recognized that hydrophobic aglycones had beneficial effect in the binding affinity of mannoside derivatives. Based on these observations, Bouckaert et al. prepared a series of ω-alkyl α-D-mannopyranosides from methyl to octyl (**1–8**) and measured their K_d's using SPR and isolated FimH attached to the gold chip by an anti-FimH antibody (Table 1).[32] The K_d's steadily decreased to reach an optimum value at heptylmannoside **7** ($n=6$). The authors were able to obtain crystal data for the

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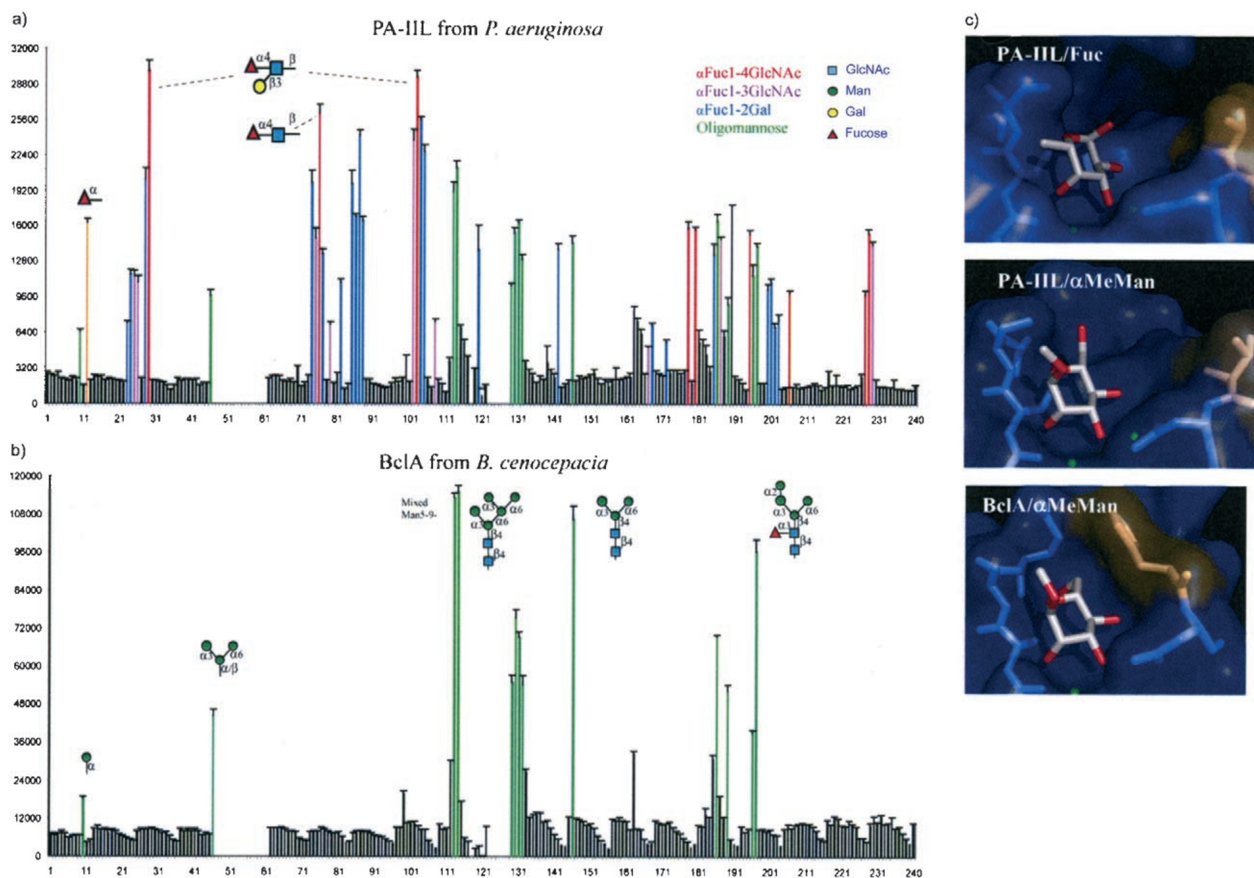

Figure 2. Comparison of glycan array data (fluorescence unit) obtained with a) lectin PA-IIL from *P. aeruginosa* and b) lectin BcLA from *B. cenocepacia* (b). Lectins were labeled with Alexafluor 488 and tested on the Plate Array v3.8 from Consortium for Functional Glycomics. Color coding indicates the type of oligosaccharide (oligomannose in green) and the fucose linkage when present (black lines for low fluorescence or for mixed glycans). All details about oligosaccharides and spacers are available at www.functionalglycomics.org. c) Binding sites from the crystal structure of PA-IIL and BclA complexed with monosaccharides are displayed with key amino acids in yellow.

butylmannoside (**4**), which confirmed the hydrophobic character of the active site near the aglycone portion and therefore demonstrated the existence of a "tyrosine gate" (Tyr48-Tyr137) that closes upon binding (Figure 3). Using these data, Touaibia et al. prepared a library of approximately hundred mannoside analogues, from which new candidates such as **12** and **17** (Scheme 1) had K_d's at low nanomolar values (Table 1).[33] Interestingly, G(0)-derived glycodendrimer **18** showed the lowest known K_d value observed so far (K_d = 0.45 nM (1.4 nM per Man residue)).

Based on these premises, glycochemists have designed several families of mannodendrimers.[34] Amongst these, the early hyperbranched L-lysine scaffold, prepared by solid-phase peptide and Fmoc chemistry, was elongated with *N*-chloroacetylglycylglycine and efficiently coupled to an *N*-acryloylated *para*-aminophenyl α-D-mannopyranoside[35] to provide octamer **19** (IC_{50} 2.8 nM (22.4 nM/Man)) (Scheme 2). It was found that a competitive binding assay measuring the binding of ^{125}I-labeled, highly mannosylated neoglycoprotein (BSA) to the type 1 fimbriated *Escherichia coli* (K12) strain in suspension gave much lower IC_{50} values than the equivalent values obtained by hemagglutination or in assays that

Table 1. Relative affinity of mannosides for *Escherichia coli* K-12 isolated FimH as measured by surface plasmon resonance (SPR).[32,33]

	K_d SPR [nM]	$\Delta G°$ SPR [Kcal mol^{-1}]	Relative affinity	Relative potency[a]
mannose	2.3×10^3	−7.6	0.96	
MeαMan (**1**)	2.2×10^3	−7.7	1.00	1
ethylαMan (**2**)	1.2×10^3	−8.1	1.8	
propylαMan (**3**)	300	−8.9	7.3	
butylαMan (**4**)	151	−9.3	15	
pentylαMan (**5**)	25	−10.4	88	
hexylαMan (**6**)	10	−10.9	220	
heptylαMan (**7**)	5	−11.3	440	
octylαMan (**8**)	22	−10.4	100	
PNPαMan (**9**)	44	−10.0	50	70
10	–	–	–	150
11	–	–	–	240
12	4.8	−11.3	458	
13	–	–	–	970
MeUmbαMan (**14**)	20	−10.5	110	1010
15	113	−9.5	19	
16	55	−9.9	40	
17	0.65[b]	−12.3	3385	
18	0.45 (1.8/Man)	−12.5	4889	

[a] Relative potency for the inhibition of binding of *E. coli* to epithelial cells.[27] [b] Such sub-nanomolar Kds are subject to large standard deviation due to the software limitation.

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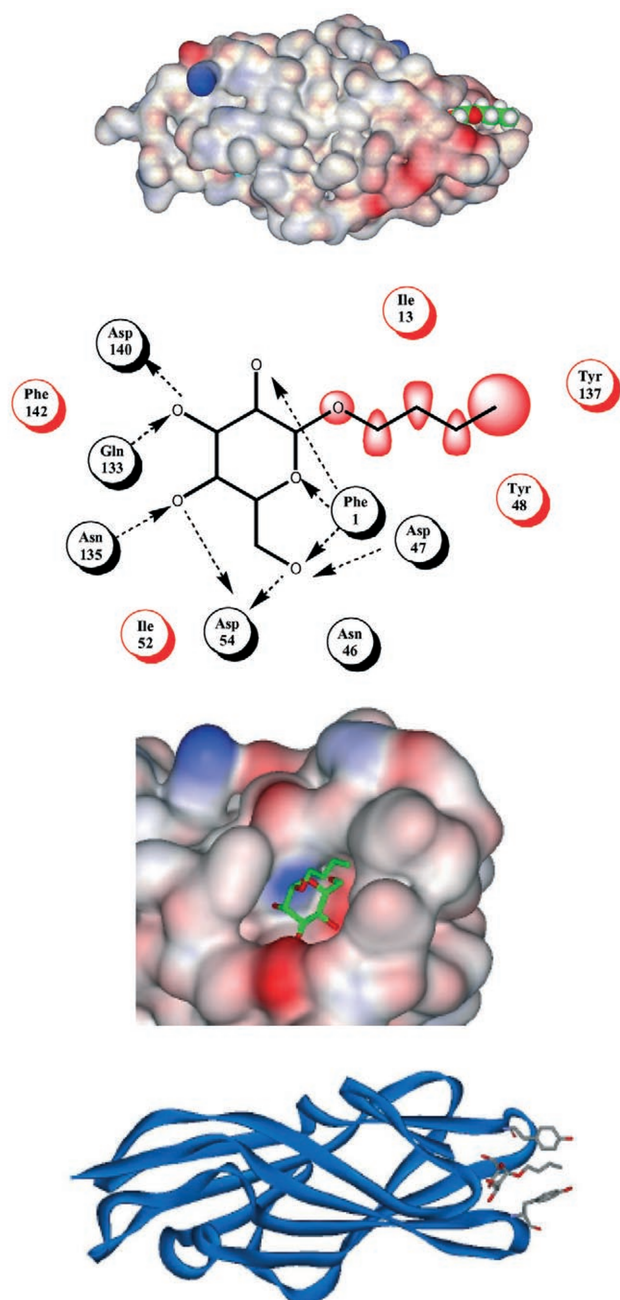
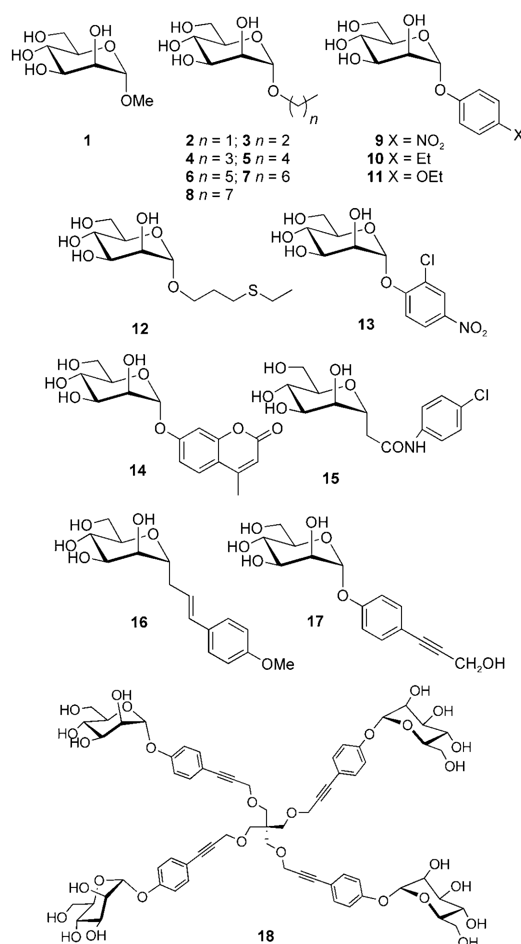


Figure 3. Crystal structure of *E. coli* K12 FimH incorporating the potent inhibitor butyl αMan **4** (PDB 1UWF). Bottom: cartoon showing the mannose ligand flanked by two tyrosines Y48 and Y137.

involve microplate immobilization. Two important factors that strongly influence the affinity to *E. coli* adhesin were: 1) the presence of an α-oriented aglycone bearing a hydrophobic group, and 2) the presence of multiple mannosyl residues that can span a distance of 20 nm or longer. The two best inhibitors were a highly mannosylated neoglycoprotein with the longest linking arm between a mannose and protein amino group and a hexadecameric mannosylated dendrimer (fourth generation, IC_{50} 0.9 nM (14.4 nM/Man)).[36]

Ligands for PA-IIL, a calcium-dependent lectin from *P. aeruginosa*: The opportunistic pathogen *P. aeruginosa* is a causative agent of respiratory-track infections and is the main cause of mortality for cystic fibrosis (CF) patients. Two soluble lectins, PA-IL and PA-IIL (also referred to as LecA and LecB), with specificity for D-galactose and L-fucose, respectively, are produced together with the virulence factors.[37] PA-IIL has been demonstrated to have micromolar affinity for fucose, an unusually strong affinity for a lectin–monosaccharide interaction that has been correlated to the presence of two calcium ions in the binding site.[18] Since human milk oligosaccharides are known for their high rate of fucosylation and their protective properties against microbial infection,[38] they were tested for the presence of these high affinity ligands. Based on these assays and glycan array screening (Figure 2), the Lewis a trisaccharide, αFuc1–4(βGal1–3)-GlcNAc has been identified with a K_d of 210 nM.[20]

The first generation of glycomimetics was designed with the aim to obtain high affinity analogues, whilst avoiding the synthesis of the complete Lewis a trisaccharide. Several compounds containing the αFuc1–4GlcNAc disaccharide and bearing different (1,2,3)-triazole groups were synthesized.[19] When tested for inhibition of binding biotinylated



Scheme 1. Library of α-D-mannopyranoside analogues providing low K_d upon binding to *E. coli* FimH.

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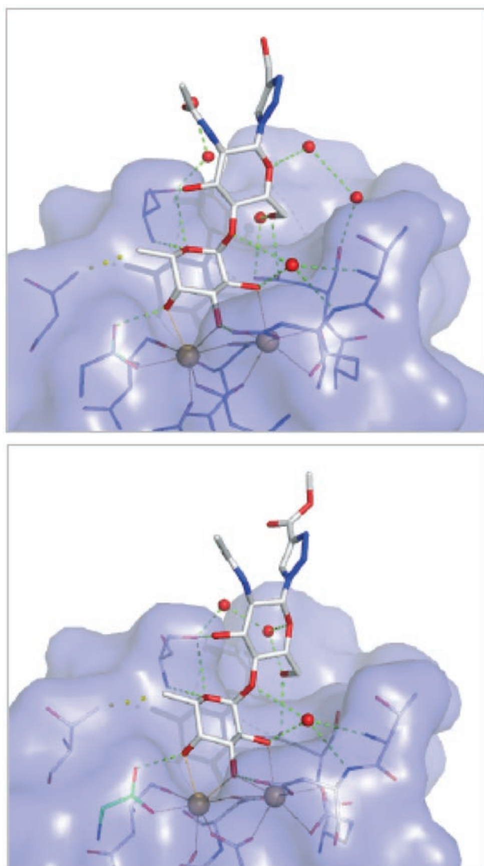
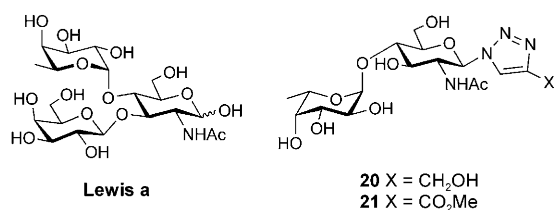


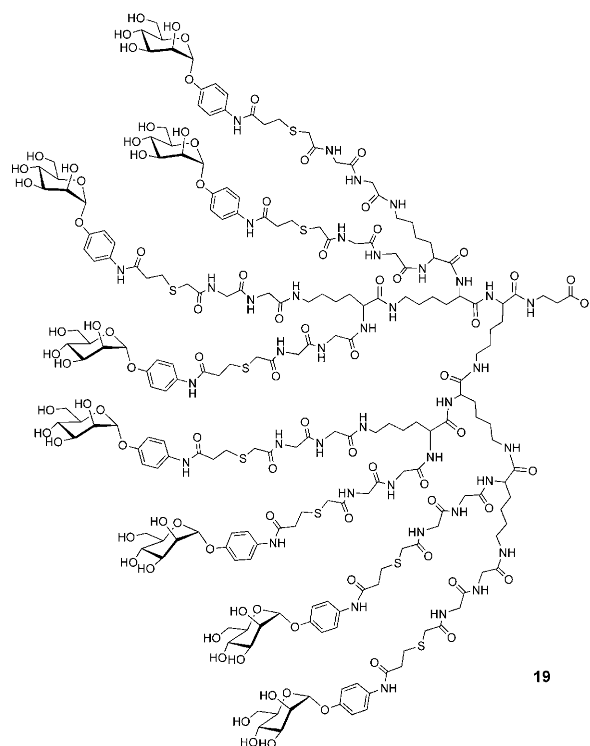
Figure 4. Crystal structures of complexes of PA-IIL with glycomimetic compounds **20** (top) and **21** (bottom). The protein accessible surface is colored in blue. Calcium ions and water molecules are represented as brown and red spheres, respectively. Hydrogen bonds are displayed as green dotted lines.

PA-IIL to immobilized polymeric fucose, compounds **20** and **21** were as effective as the Lewis a trisaccharide. Titration calorimetry confirmed the high affinity for the *Pseudomonas*



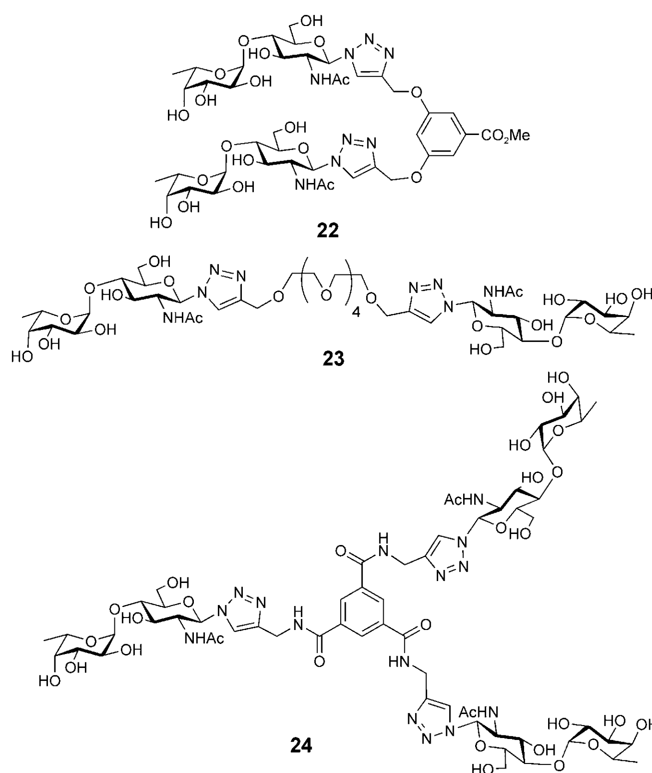
lectin (K_d of 310 and 290 nM). The αFuc1–4GlcNAc derivatives are significantly more flexible than the branched Lewis a trisaccharide, and this resulted in an elevated entropic cost for the binding. Nevertheless, the strong affinity can be reached, due to the high number of hydrogen bonds between the ligand and the lectins as proven by the crystal structures of the complexes (Figure 4).

The second generation of ligands (**22**–**24**) contain 2 or 3 high affinity αFuc1–4GlcNAc disaccharides connected



Scheme 2. Arylated glycomannoside bearing 8 residues based on a polylysines core. This construct showed an IC_{50} of 2.8 nM against *E. coli* K12.

through linkers of various lengths and geometries.[39] Among them, the linear and flexible compound **23** displays the highest affinity (K_d = 90 nM), as observed from titration microca-



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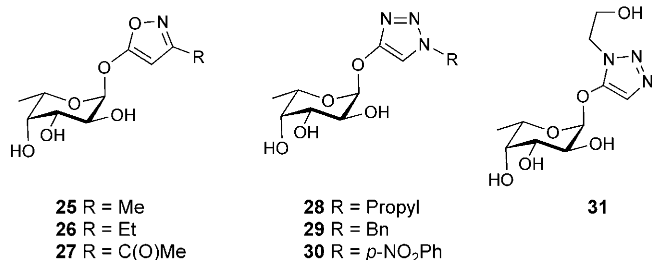
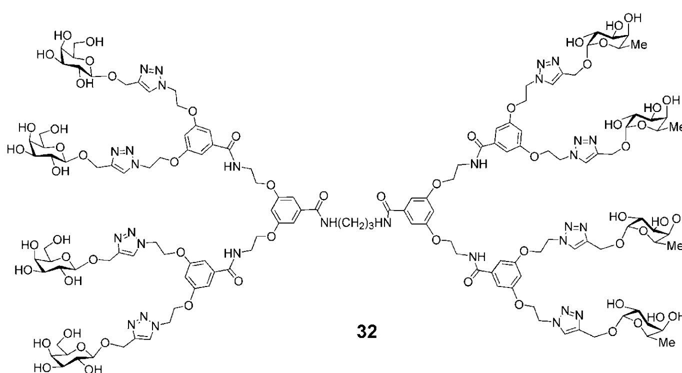
lorimetry. At low concentration of the ligand, a PA-IIL/ligand stoichiometry of 2:1 is observed, followed by precipitation at higher concentration, indicating that this divalent ligand is an efficient clustering agent. Another divalent compound with a more rigid linker (**22**) is very efficient for inhibition of binding to immobilized fucose, but is twice less efficient in solution when tested by ITC, illustrating that the competition can be significantly different when tested in solution or on surface-bound compounds. Interestingly, the corresponding trivalent cluster **24** displayed similar affinity (K_d = 100 nM) to that of dimer **23**, thus indicating that one additional branch had no beneficial effect upon binding. This situation is analogous to the one previously observed for a trimeric mannoside cluster built on the same 1,3,5-tribenzoic acid scaffold using the phytohemagglutinin ConA from *Canavalia ensiformis*.[40]

The encouraging results described above prompted us to prepare a small library of even simpler α-L-fucoside ligands bearing varied heterocycles in the aglyconic portion with the aim to conserve high affinity while removing the *N*-acetylglucosaminide residue. To this end, a series of isoxazoles (**25–27**) and 1,4-trisubstituted 1,2,3-triazoles (**28–30**), together with the 1,5-disubstituted triazole (**31**), were synthesized (Béha et al. unpublished results). These Lewis a mimetics were all 7–10-fold better ligand than L-fucose taken alone and compound **27** was equipotent to Lewis a itself, thus supporting the hypothesis.

Multivalency was next explored by using different scaffolds. At the present time, only fucose-containing multivalent compounds have been synthesized, while keeping in mind, as shown above, that the use of terminating αFuc1–4GlcNAc disaccharide could increase the affinity further. A series of fucodendrimers with valencies between two and sixteen were prepared by using the partly optimized 1,4-disubstituted 1,2,3-triazoles described above.[41] Using nephelometry, a method based on light scattering by particles, these glycodendrimers showed fast and reversible precipitation of the PA-IIL lectin as anticipated, thus confirming the cross-linking abilities of such nanometric architectures. To further prevent the adhesion behavior of *P. aeruginosa* onto the lung's epithelial lining, heterobifunctional glycodendrimers bearing both D-galactoside (PA-IL) and L-fucoside (PA-IIL) lectins ligands were constructed.[41] This novel strategy to tackle two lectins from the same organism was validated by using microprecipitation experiments. Each lectin showed strong binding to dendrimer **32** (Scheme 3), while PA-IIL had higher affinity for the fucoside portion.

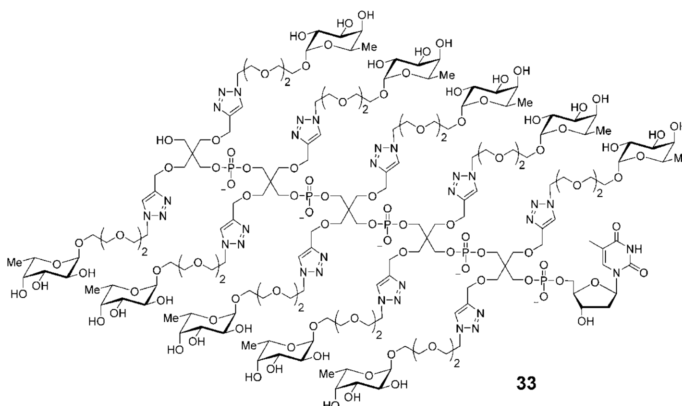


Linear triazole-bearing phosphodiester oligomers built on a pentaerythritol scaffold were recently prepared by using a



Scheme 3. Heterobifunctional dendrimer exposing both D-galactoside and L-fucoside residues for simultaneous binding to *P. aeruginosa* lectin PA-IL and PA-IIL, respectively.

DNA synthesizer and phosphoramidite chemistry. An azido fucoside triethylene glycol derivative was treated under "click" chemistry conditions to construct a series of glycosylated clusters bearing 4, 6, 8, and 10 (**33**) L-fucoside residues. Binding to *P. aeruginosa* lectin (PA-IIL) was determined by an enzyme-linked lectin competition assay. The IC_{50} values measured were 10–20 times better than the monovalent L-fucose with a modest twofold increase on a per saccharide basis.[42]



Similarly, a large (390625 members) neoglycopeptide dendrimer library[43,44] ending with C-linked fucoside derivatives was initially synthesized by using solid-phase combinatorial variation. The strongest binding with *P. aeruginosa* PA-IIL was observed with a tetravalent dendrimer **35** (Scheme 4, IC_{50} 0.6 μM), which combined multivalency with the presence of a positive guanidine charge in proximity to the carbohydrate residue, in comparison to the divalent analogue **34** lacking the N-terminal lysine residues (IC_{50} 5.0 μM) (Table 2).

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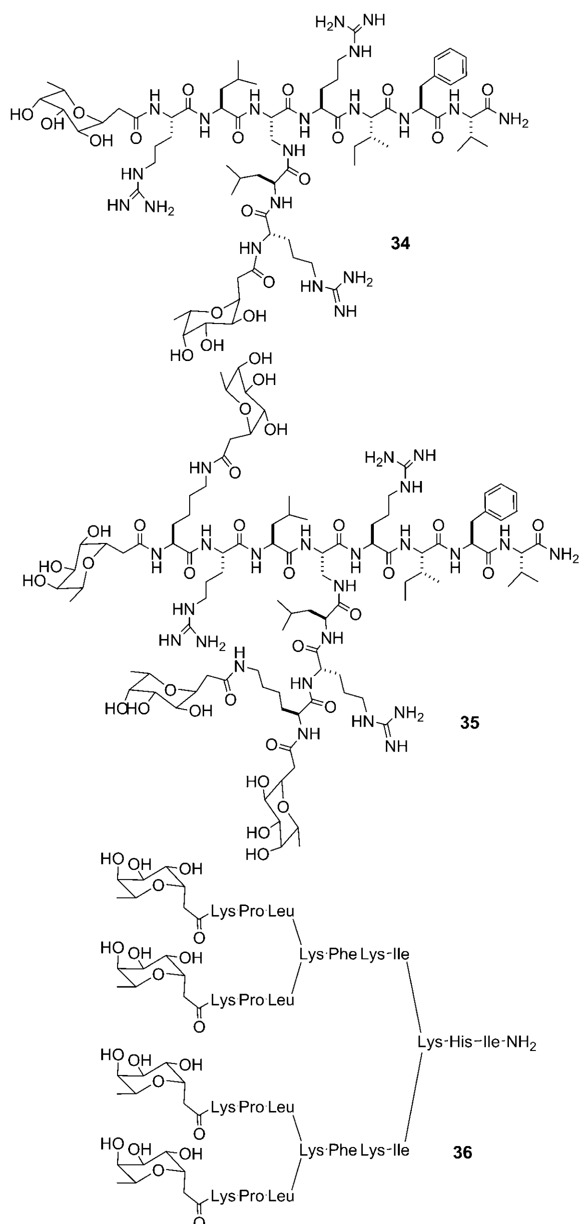

Scheme 4. Best ligand candidates for the PA-IIL lectin of *P. aeruginosa* selected from a large chemical library of glycopetidomimetics.[43,44]

An improved peptide dendrimer combinatorial library with 15625 members was analogously constructed.[44] Dendrimer **36** (Scheme 4, Fuc-α-CH_2CO-Lys-Pro-Leu)$_4$(Lys-Phe-Lys-Ile)$_2$Lys-His-Ile-NH_2 was identified as a potent ligand against *Ulex europaeus* lectin UEA-I (IC_{50} 11 μM) and *P. aeruginosa* lectin PA-IIL (IC_{50} 0.14 μM).

Several other relevant glycomimetics and some of their multivalent counterparts against PapG adhesins of *E. coli*, *Streptoccocus suis* (galabiose ligand - Galα1–4Gal), and *P. aeruginosa* adhesin of type IV pili (GalNAcβ1–4Gal ligand) have also been reviewed recently.[45,46] Scheme 5 illustrates some relevant structures. When anchored to a G(1)-PAMAM dendrimer, octameric galabioside derivative

Table 2. Binding data for PA-IIL interacting with different carbohydrates, glycomimetics, and glycopolymers.

	K_d [nM]	-Δ*G* [Kcal mol^{-1}]	Potency (ITC)	Potency (ELLA)	Potency per fucose	Ref.
L-Fuc	2.9×10^3	−7.5	1			[20]
Me-α-Man	71×10^3	−5.7	0.04			[49]
Me-α-Fuc	430	−8.7	6.7			[49]
Lewis a	210	−9.1	13.8			[20]
20	310	−8.9	9.3			[19]
21	290	−8.9	10			[19]
22	170	−9.2	17	40	8.5 –20[a]	[39]
23	90	−9.6	32		16	[39]
24	100	−9.5	29		9.7	[39]
33				22.4	2.2	[42]
34				3.5	1.7	[43]
35				29	7.2	[43]
36				80	20	[44]

[a] Values from ITC and ELLA, respectively.

shown above had a minimal inhibitory concentration (MIC) of 0.3 nM in an inhibition of hemagglutination assay of the Gram-positive pathogen *S. suis*.[47] This value represents a 256-fold improvement over the monovalent galabioside (32/Galabiose).

Conclusion

In this paper we report the efforts for understanding molecular basis of strategies used by microbes for adhering to host glycans, and the related progresses in producing glycomimetics and glycodendrimers that could block the adhesion. Because of the fine specificity of pathogens for oligosaccharide structures present on only few species, tissues or cell types, it has been generally possible to design specific glycomimetics. Multivalency of the receptors, due to oligomerization of lectin domains or multipresentation on pathogen surface, has been utilized for the synthesis of high avidity glycodendrimers.

Acknowledgements

The glycan array resources were provided by the Consortium for Functional Glycomics, grant number GM62116. A.I. was supported by the CNRS and financial help is gratefully acknowledged from Vaincre la Mucoviscidose and GDR Pseudomonas. R.R. is grateful to a Canadian Research Chair in Therapeutic Chemistry and to NSERC financial support. Y.M.C. is thankful to FQRNT for a postdoctoral fellowship.

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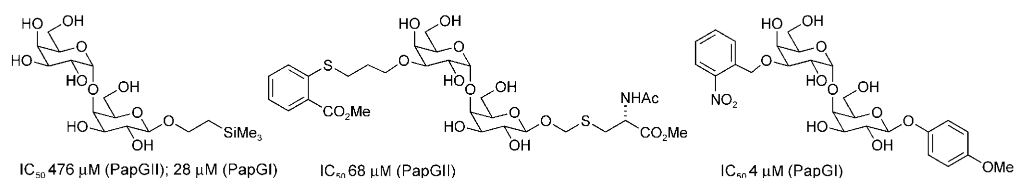
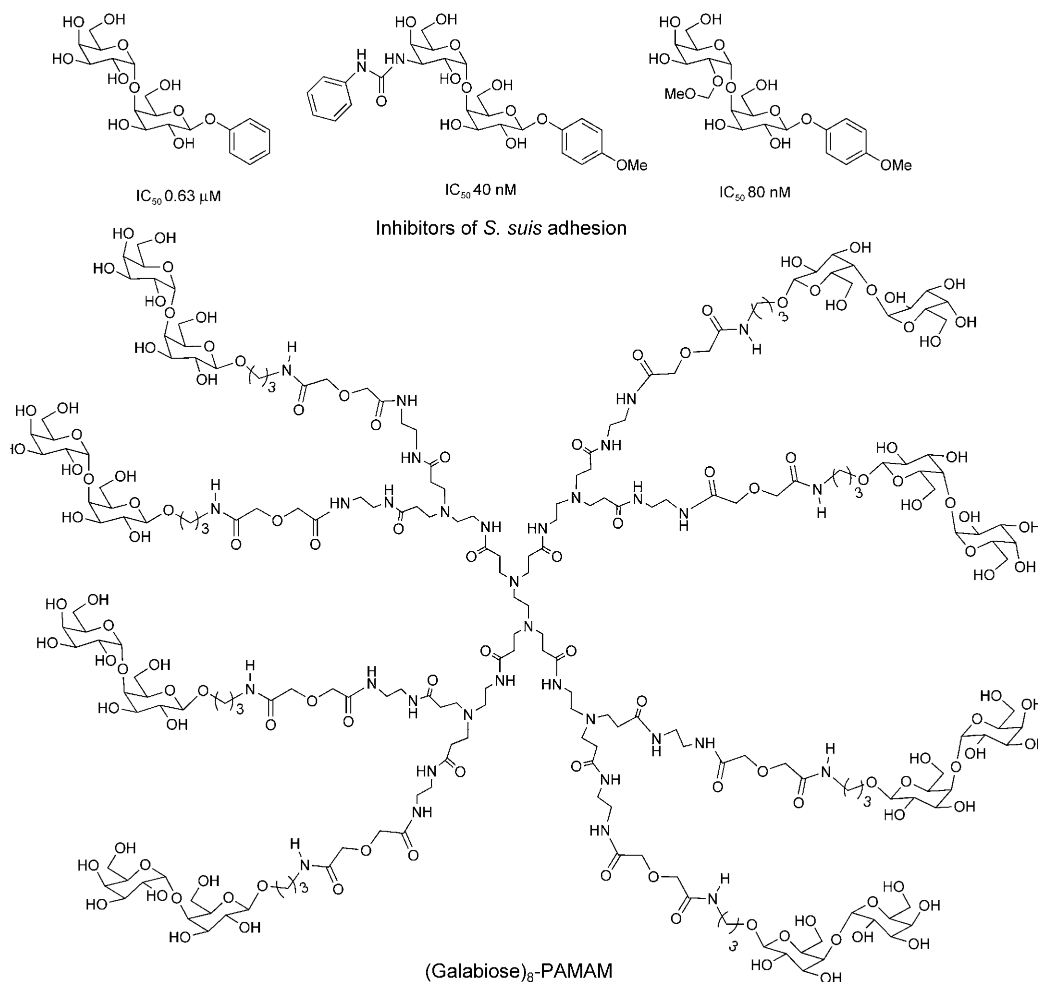


Inhibitors of the PapG adhesins of *E. coli*



Scheme 5. Galabiose mimetics and a potent dendritic galabiose ligand having improved affinities toward PapG adhesins of *E. coli* and *Streptoccocus suis*, respectively.

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Published online: July 10, 2008

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