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Quantitative Studies of the Binding of the Class II PapG Adhesin from Uropathogenic *Escherichia coli* to Oligosaccharides

Andreas Larsson,[a] Jörgen Ohlsson,[b] Karen W. Dodson,[c] Scott J. Hultgren,[c] Ulf Nilsson[b] and Jan Kihlberg[a,*]

[a]*Organic Chemistry, Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden*
[b]*Bioorganic Chemistry, Lund University, PO Box 124, SE-221 00 Lund, Sweden*
[c]*Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA*

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Abstract—Binding of the class II PapG adhesin, found at the tip of filamentous pili on *Escherichia coli*, to the carbohydrate moiety of globoseries glycolipids in the human kidney is a key step in development of pyelonephritis, a severe form of urinary tract infection. An assay based on surface plasmon resonance for quantification of the binding of the class II PapG adhesin to oligosaccharides has been developed. Using this assay dissociation constants ranging from 80 to 540 μM were determined for binding of the PapG adhesin to di-pentasaccharide fragments from the globoseries of glycolipids. A series of galabiose derivatives, modified at the anomeric position, O-2′ or O-3′, was also investigated. The anomeric position appeared to be the most promising for development of improved inhibitors of PapG-mediated adhesion of *E. coli*. *p*-Methoxyphenyl galabioside was found to be most potent ($K_d = 140$ μM), and binds to PapG almost as well as the Forssman pentasaccharide.

Introduction

Urinary tract infections (UTIs) belong to the most common bacterial infections, second in occurrence only to infections of the respiratory tract.[1] UTIs are more frequent in women than in men and one-third of the women in the United States have suffered from at least one UTI before the age of 65, with recurrent infections being common.[2] Recurrent bladder infections are associated with an increased risk of developing a more severe infection in the kidneys (pyelonephritis). *Escherichia coli* is the predominant causative agent of pyelonephritis,[3] and the ability of the bacterium to adhere to epithelial cells in the human kidney has been shown to be critical for development of disease.[4]

Adhesion to host tissue is an important virulence factor in many infections caused by bacteria, and glycoconjugates on the host cell surface often function as receptors for adhesive proteins expressed by bacteria and viruses.[5] An overwhelming majority of *E. coli* which cause pyelonephritis adhere to the globoseries of glycolipids found in the upper urinary tract. Several studies have shown that the disaccharide galabiose (Galα1-4Gal), which constitutes a part of these glycolipids, is required for binding of pyelonephritic *E. coli*.[6–10] Bacterial binding to the galabiose receptor is mediated by supramolecular protein appendages, termed P pili, which extend from the outer cell membrane of the *E. coli*.[11] P pili consist of a thin tip fibrillum that is joined to the distal end of a thicker pilus rod. Altogether the P pilus consists of a large number of proteins of six different types, with the galabiose-specific adhesin PapG located at the very tip of the pilus. Three different variants exist of the PapG adhesin (classes I–III), with the class II adhesin being particularly associated with development of pyelonephritis in humans.[12,13]

Most proteins with lectin-like functions bind naturally occurring carbohydrate ligands with low affinity ($K_d \approx 0.1$–1 mM). This constitutes a significant obstacle in attempts to develop carbohydrate based drugs which interfere with microbial attachment to host cell-surface glycoconjugates. However, some successful strategies to overcome this problem have been described recently. When microbial attachment occurs by use of multiple

*Corresponding author. Tel.: +46-90-7866890; fax: +46-90-138885; e-mail: jan.kihlberg@chem.umu.se

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copies of an adhesin, multivalent carbohydrate ligands have proven to be efficient inhibitors of attachment.[14,15] Another attractive approach to enhance the affinity of a ligand for a lectin is to use a small saccharide, which fulfils key polar interactions with the lectin, as a scaffold onto which substituents that enhance the affinity are added.[16,17]

As part of a program aimed at development of carbohydrate based inhibitors of pyelonephritic *E. coli* we now describe the development of an assay based on surface plasmon resonances for direct studies of the binding of the class II PapG adhesin to saccharide ligands. The assay was used to determine the dissociation constants for binding of PapG to the saccharide moieties of the globoseries of glycolipids and fragments thereof, as well as to a panel of substituted derivatives of the disaccharide galabiose.

Results and Discussion

Binding of the class II PapG adhesin to oligosaccharides

The oligosaccharide binding domain of the class II PapG adhesin, which consists of the N-terminal 196 amino acids, was recently cloned and expressed.[18] This PapGII truncate has now been covalently bound to the surface of a dextrane-coated sensor chip in order to investigate binding to receptor-active saccharides. Coupling was accomplished by using a standard BIACORE amine coupling procedure. Binding of saccharide moieties from the globoseries of glycolipids to the adhesin was then determined using surface plasmon resonance (Table 1, Fig. 1). The adhesin was found to bind to saccharides that contain a galabiose moiety (i.e., saccharides **1**–**6**) with K_d-values ranging from 80 to 540 μM, that is, in the range usually found for lectin-carbohydrate interactions. In agreement with previous studies using piliated bacteria, which carried different classes of PapG adhesins, saccharides which did not contain galabiose were not bound at all (cf. **7** and **8**).[6–10] The presence of a glucose moiety at the reducing end of the galabiose moiety led to a significant decrease in K_d, that is, it increased the affinity for the PapG adhesin (cf. **1** vs **2**, **3** vs **4**, and **5** vs **6**). In contrast, extension of the galabiose part with a mono- or disaccharide unit at O-3′ did not influence the dissociation constant (compare **5** with **3** and **1**). These results agree well with inhibitory powers determined for saccharides **1**–**8**, when used as inhibitors of haemagglutination caused by *E. coli* expressing the class II PapG adhesin, as well as adherence of the same bacteria to kidney tissue sections.[9]

Table 1. Dissociation constants for binding of the class II PapG adhesin to the saccharide moieties of the globoseries of glycolipids, and fragments thereof[a]

Compd		K_d (μM)
1	GalNAcα3GalNAcβ3Galα4Galβ4GlcβOTMSEt[b]	91
2	GalNAcα3GalNAcβ3Galα4GalβOTMSEt[b]	250
3	GalNAcβ3Galα4Galβ4GlcβOTMSEt[b]	84
4	GalNAcβ3Galα4GalβOTMSEt[b]	180
5	Galα4Galβ4GlcβOTMSEt[b]	78
6	Galα4GalβOTMSEt[b]	540
7	GalNAcα3GalNAcβ3GalαOMe	—[c]
8	Galβ4GlcβOTMSEt[b]	—[c]

[a]Determined by surface plasmon resonance using a Biacore 3000 instrument (cf. Experimental).
[b]TMSEt = 2-trimethylsilylethyl.
[c]Inactive, that is, no binding detected.

The crystal structure of the complex between globoside **3** and the oligosaccharide binding domain of the class II PapG adhesin was recently determined at 1.8 Å resolution (Fig. 2).[18] In the complex a large number of hydrogen bonds are formed between the adhesin and **3**, including both direct and water mediated hydrogen bonds. Four hydrogen bonds are formed between the adhesin and HO-4, O-5 and HO-6 of the GalNAc residue. The Galα moiety participates in the most extensive

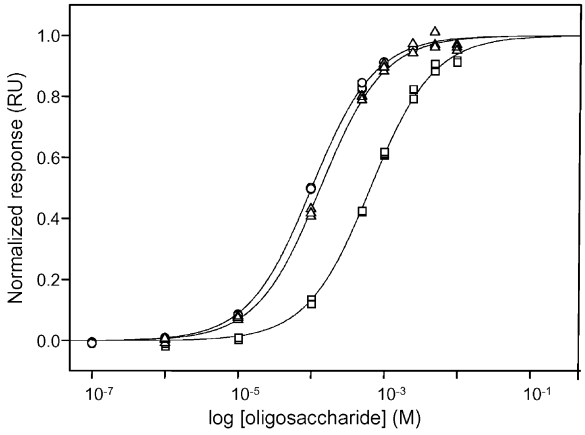


Figure 1. Binding isotherms with concentration-dependent responses at equilibrium for oligosaccharides **1** (○), **6** (□) and **11** (△) binding to immobilized PapGII adhesin on a CM5 surface plotted versus oligosaccharide concentration in triplicates. The binding responses at equilibrium were normalized against the maximum binding (R_{max}). Dissociation constants (K_d) were determined as the concentration of a saccharide that elicited half of the maximal response (R_{max}), that is, when 50% of the PapGII adhesin bound a ligand.

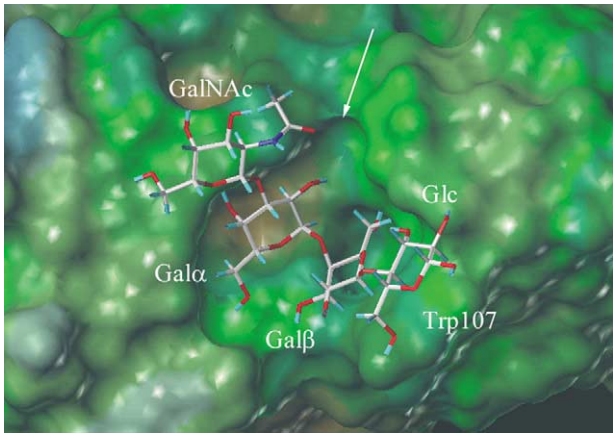


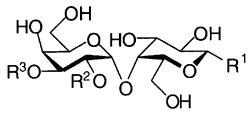
Figure 2. Structure of the crystalline complex between globoside **3** and the class II PapG adhesin.[18] The groove adjacent to HO-2′ of the galabiose moiety is indicated by an arrow. Polar parts of the PapG surface are blue, nonpolar parts are brown, and green represents intermediate polarity. The figure was generated with the program Sybyl.

hydrogen-bonding network, involving seven hydrogen bonds to HO-2, O-3, HO-4 and HO-6. For Galβ four hydrogen bonds to HO-3, O-5 and HO-6 are found, whereas Glc takes part in three hydrogen bonds to HO-2 and HO-3. In addition, the complex contains several hydrophobic contacts including H-1, H-2 and H-6 of Galα, H-1, H-3, H-4, H-5 and H-6 of Galβ, as well as H-2 and H-4 of Glc. The dissociation constants determined for oligosaccharides **1**–**8** provide additional insight into the importance of these interactions for the stability of the complex. Since attachment of further saccharides at O-3 of Galα in **5** does not influence the K_d-values (compare **5**, **3** and **1**) it can be concluded that the interactions between the GalNAc residue and the adhesin do not contribute to the stability of the complex. The critical role of the central galabiose moiety for binding is easily understood in view of the extensive contacts, including both hydrogen bonds and hydrophobic interactions, with the adhesin. The additional stabilization provided by the Glc residue could be due either to the hydrogen bonds formed with HO-2 and HO-3, or to hydrophobic contacts with Trp107, which is sandwiched below a non-polar region of the Glc and Galβ residues. Since attachment of aromatic aglycones to galabiose gives potent binders (cf. below) it is concluded that hydrophobic contacts play a significant role.

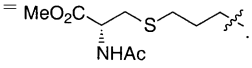
Binding of galabiose derivatives to the class II PapG adhesin. The binding studies performed with saccharides **1**–**8**, and the crystal structure of the complex between **3** and the class II adhesin,[18] suggest that substituents could be added at several positions of a galabiose core in efforts to develop improved PapG binders. The role of the anomeric position is highlighted by the additional stabilization provided by the presence of a glucose residue (cf. **6** vs **5**). Since the GalNAc residue is tolerated at O-3′ of the galabiose moiety it could well be possible to increase the affinity by attachment of substituents at this position. In addition, the crystal structure of the complex reveals the presence of a groove adjacent to HO-2′ (Fig. 2), which potentially could be filled by addition of substituents to O-2′ of galabiose.

The role of substituents at the anomeric position of galabiose was probed with different *O*-alkyl (**6**, **9**, and **10**), *O*-aryl (**11** and **12**), benzamido (**13**), and *S*-aryl (**14**) groups (Table 2, Fig. 1). The 2-trimethylsilylethyl glycoside **6**, as well as benzamide **13** and thioglycoside **14**, bound with lower affinity than the simple methyl glycoside **9**. More potent ligands were obtained when *O*-aryl groups were located at the anomeric position, with the *p*-methoxyphenyl glycoside **11** being the most potent binder ($K_d = 140\ \mu M$). It should be pointed out that **11**[19] binds almost as well as pentasaccharide **1** (Fig. 1),[20] which is considerably more demanding to prepare. In view of the high potency of **11**, the *p*-methoxyphenyl group was maintained at the anomeric position during preliminary studies with substituents at O-2′ and O-3′. The extended pocket in the adhesin adjacent to O-2′ was probed with compounds **15**–**17**. Attachment of a methyl (**15**) or propyl (**16**) group at O-2′ gave very poor binders. In contrast, use of a methoxymethyl group (**17**) gave a significantly improved binder, as compared to **15** and **16**. Substituents at O-2′ thus have large, and differing, influences on the binding strength, which leads to the speculation that selection of a suitable substituent at this position might yield more potent ligands for the adhesin. At O-3′, the presence of an allyl group (**18**), or a substituent obtained by addition of a protected cystein to the allyl group (**19**), either did not affect or led to an increase in the K_d-value. However, attachment of a *m*-nitrobenzyl group at O-3′ (cf. **20**) resulted in a slight lowering of the K_d value, indicating the possibility that appropriate substituents at this position could have a beneficial influence on binding.

Table 2. Dissociation constants for binding of the class II PapG adhesin to substituted derivatives of galabiose (Galα4Galβ)[a]



Compd	R^1	R^2	R^3	K_d (μM)
6	TMSEtO[b]	H	H	540
9	MeO	H	H	340
10	Cyclohexyl-O	H	H	260
11	*p*MeOPhO	H	H	140
12	2-Naphthyl-O	H	H	200
13	Ph(CO)NH	H	H	>1000
14	*p*MeOPhS	H	H	490
15	*p*MeOPhO	Me	H	>1500
16	*p*MeOPhO	*n*Pr	H	>1000
17	*p*MeOPhO	$MeOCH_2$	H	490
18	*p*MeOPhO	H	Allyl	140
19	*p*MeOPhO	H	X[c]	290
20	*p*MeOPhO	H	*m*NO_2Bn	120

[a]Determined by surface plasmon resonance using a Biacore 3000 instrument (cf. Experimental).
[b]TMSEt = 2-trimethylsilylethyl.
[c]X = 

In an attempt to understand the reasons for the improved binding of *p*-methoxyphenyl galabioside **11** to PapG, as compared to for example, alkyl galabiosides **6** and **9**, galabioside **11** was docked into the active site of the class II PapG adhesin. This was done by manual superimposition of **11** onto the galabiose moiety of tetrasaccharide **3** in the crystalline complex with PapG,[18] followed by energy minimization using Macromodel. In the resulting complex (Fig. 3) all interactions between the galabiose moiety and PapG that were found in the crystalline complex of PapG and tetrasaccharide **3** are maintained. In addition, the *p*-methoxyphenyl group is located in a pocket formed by Trp107 and Arg170 of PapG. Most likely this stabilizes the complex by hydrophobic interactions with the aromatic side chain of Trp107. Additional stabilization appears to be obtained through a π-cation interaction between the *p*-methoxyphenyl group and Arg170, thus explaining why galabiosides with aromatic aglycons bind better than those having aliphatic aglycones.

Synthesis of oligosaccharides and galabiose derivatives. The synthesis of the saccharides used in the present study, except **15**–**17** and **19**, has been described previously

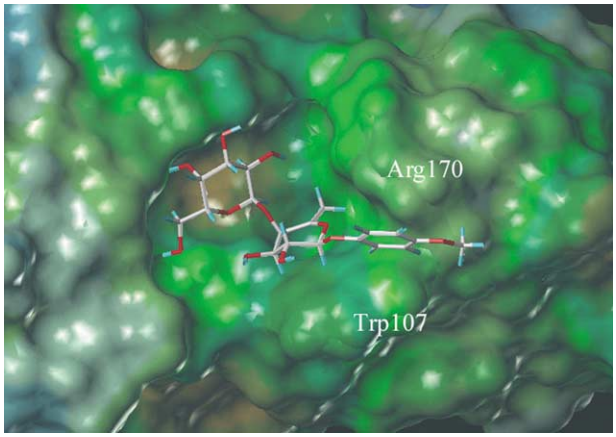
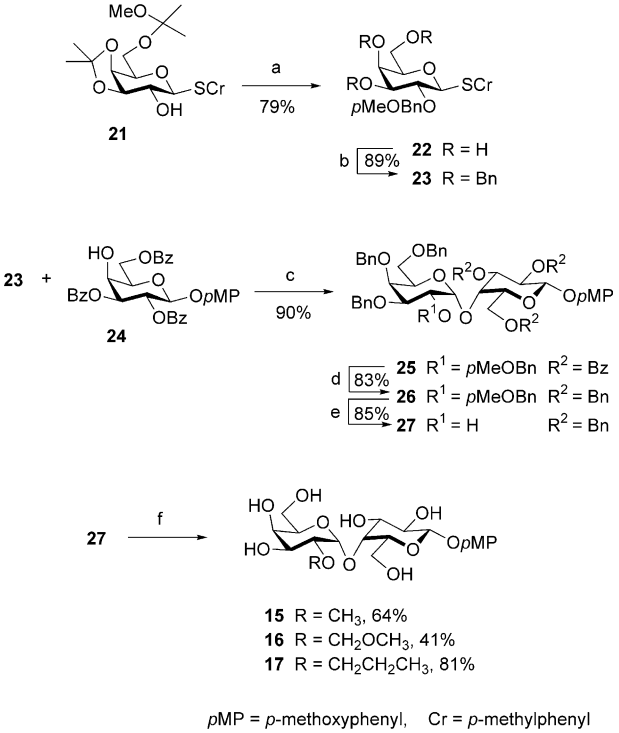
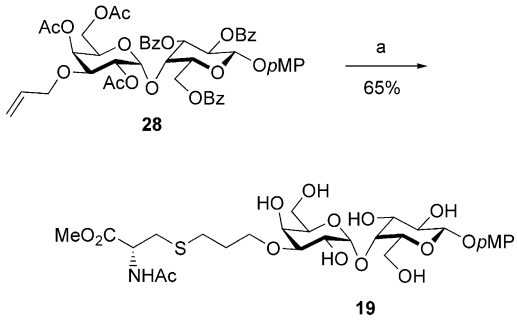


Figure 3. An energy-minimized model of the complex between galabioside **11** and the class II PapG adhesin. Polar parts of the PapG surface are blue, nonpolar parts are brown, and green represents intermediate polarity. The figure was generated with the program Sybyl.

(cf. references in the Experimental). For the preparation of the 2′-*O*-modified *p*-methoxyphenyl galabiosides (**15**–**17**), a benzylated galabioside with HO-2′ unprotected was required (Scheme 1). Synthesis of galactosyl donor **23** was achieved by protection of HO-2 of **21**[21] as a *p*-methoxybenzyl ether, followed by removal of the isopropylidene and 1-methoxyisopropyl groups (→ **22**, 79% yield), and subsequent benzylation (→ **23**, 89% yield). α-Galactosylation of acceptor **24**[22] with **23**, using *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate as promotor system,[23,24] gave galabioside **25**



Scheme 1. (a) (i) NaH, *p*MeOBnBr, DMF; (ii) 80% aqueous AcOH, 60 °C. (b) NaH, BnBr, DMF. (c) NIS, TMSOTf, CH_2Cl_2–Et_2O (1:1), −50 °C. (d) (i) NaOMe, MeOH; (ii) NaH, BnBr, DMF. (e) 2% TFA, CH_2Cl_2, 0 °C. (f) (i) NaH, alkyl halide, DMF; (ii) H_2 (1 bar), 10% Pd/C, MeOH.



Scheme 2. (a) (i) *N*-acetyl-L-cystein methyl ester, AIBN, hυ, EtOAc, 48 h; (ii) NaOMe, MeOH.

in 90% yield. As an alternative, a galactosyl donor with an allyl group instead of a *p*-methoxybenzyl group at HO-2 was prepared from **21** by a similar reaction sequence as for **23**. However, in the following α-glycosylation a modest yield of 68% was obtained and this route was not explored further. Conventional, base-catalyzed debenzoylation of **25** followed by benzylation gave **26** in 83% yield. Treatment of **26** with 2% trifluoroacetic acid in dichloromethane[25,26] then afforded galabioside **27**, which has HO-2 unprotected. The 2′-*O*-modified galabiosides (**15**–**17**) were prepared in 41–81% yield by treatment of **27** with NaH and either of methyl iodide, allyl bromide, or bromomethyl methyl ether, followed by hydrogenolys over Pd/C in glacial acetic acid. Galabioside **19** was prepared in two steps from the known **28**[19] (Scheme 2). UV-mediated radical addition of *N*-acetyl-L-cystein methyl ester to the allyl group at O-3′ of **28**, followed by conventional *O*-deacylation gave **21** in 65% overall yield.

Conclusions

An assay based on surface plasmon resonance that allows determination of dissociation constants for binding of the class II PapG adhesin of uropathogenic *E. coli* to oligosaccharides has been developed. The adhesin was found to bind to saccharides from the globoseries of glycolipids, which function as ligands for *E. coli* in the upper urinary tract, with K_d-values ranging from 80 to 540 μM. These values agree well with those usually found for interactions between lectins and carbohydrates. A series of galabiose derivatives, modified at the anomeric position, O-2′ or O-3′, was also investigated. The anomeric position appeared to be the most promising from the point of view of development of improved inhibitors of adhesion of *E. coli* to host tissue. *p*-Methoxyphenyl galabioside (**11**) was found to be a most potent galabioside ($K_d = 140$ μM), and binds to PapG almost as well as the Forssman pentasaccharide, which is considerably more difficult to synthesize.

Experimental

Synthetic saccharides (1–20). The saccharides used in the present study, except **15**–**17** and **19**, were synthesized as described: **1**,[20] **2**,[20] **3** and **4**;[27] **5**,[28] **6**,[29] **7**,[20] **8**,[29] **9**,[30] **10**,[31] **11**,[19] **12**–**14**,[31] **18**,[19] **20**.[19]

General synthetic procedures. All non-aqueous reactions were run in septum-capped, oven-dried flasks under Ar (1 atm). CH_2Cl_2 was dried by distillation from CaH_2 and Et_2O was distilled from Na. Concentration of organic solutions were made using rotary evaporation with a bath temperature at or below 40 °C. Flash chromatography was performed on Grace Amicon Silica gel 60 (35–70 μm) and TLC was performed on Kieselgel 60 F_{254} plates (Merck). NMR spectra were recorded with a Bruker DRX-400 or a Bruker ARX-300 instrument for solutions in $CDCl_3$ or MeOH-d_4 (residual $CHCl_3$ or CD_2HOD were used as internal references at 7.27 and 3.31 ppm, respectively). ^{1}H NMR spectral assignments were made based on COSY spectra.

4-Methylphenyl 2-*O*-(*p*-methoxybenzyl)-1-thio-β-D-galactopyranoside (22). Sodium hydride (55 mg, 1.34 mmol, 60% in mineral oil) was added to a solution of **21**[21] (410 mg, 1.03 mmol) in DMF (8 mL). The mixture was stirred for 15 min, cooled to 0 °C, and then *p*-methoxybenzyl chloride (193 μL 1.43 mmol) was added dropwise. The mixture was stirred at room temperature for 4 h then methanol (1 mL) was added. The mixture was diluted with CH_2Cl_2 (30 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 3:1 heptane–EtOAc, 2% Et_3N) to give a fully protected galactoside as intermediate. To the fully protected galactoside was added 80% aqueous acetic acid and the solution was stirred at 60 °C for 2 h. The mixture was concentrated, co-concentrated with toluene, and the residue was purified by flash chromatography (SiO_2, 3:1 toluene–acetone, 2% MeOH) to give **22** (327 mg, 79%): $[\alpha]_D^{23}$ −3 (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD): δ 7.44 (m, 2H, S*Ph*Me), 7.37 (m, 2H, OCH_2*Ph*OMe), 7.11 (m, 2H, S*Ph*Me), 6.87 (m, 2H, OCH_2*Ph*OMe), 4.72 (s, 2H, OCH_2PhOMe), 4.56 (d, 1H, *J* = 9.2 Hz, H-1), 3.88 (dd, 1H, *J* = 0.8, 3.3 Hz, H-4), 3.78–3.68 (m, 5H, H-6, OMe), 3.64–3.55 (m, 2H, H-2, H-3), 3.50 (dt, 1H, *J* = 0.8, 5.4 Hz, H-5), 2.30 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 160.9, 138.5, 132.7, 132.6, 132.1, 131.1, 130.7, 114.7, 89.7, 80.6, 79.5, 76.6, 76.1, 70.9, 62.7, 55.8, 21.2; HRMS calcd for $C_{21}H_{26}O_6SNa$ (M + Na): 429.1348, found: 429.1351.

4-Methylphenyl 3,4,6-tri-*O*-benzyl-2-*O*-(*p*-methoxybenzyl)-1-thio-β-D-galactopyranoside (23). Sodium hydride (24 mg, 0.60 mmol, 60% in mineral oil) was added to a solution of **22** (68 mg, 0.17 mmol) in DMF (2 mL). The mixture was stirred for 15 min and then cooled to 0 °C. Benzyl bromide (77 μL, 0.65 mmol) in DMF (1 mL) was added dropwise and the resulting mixture was stirred at room temperature for 2 h after which methanol (0.5 mL) was added. The mixture was diluted with CH_2Cl_2 (10 mL), washed with saturated aqueous $NaHCO_3$ (5 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (SiO_2, 5:1 heptane–EtoAc) to give **23** (101 mg, 89%): $[\alpha]_D^{23}$ +4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.53–7.50 (m, 2H, S*Ph*Me), 7.41–7.30 (m, 17H, Ar–H), 7.04 (m, 2H, S*Ph*Me), 6.90 (m, 2H, OCH_2*Ph*OMe), 5.01 and 4.65 (ABq, 1H each, *J* = 11.5 Hz, OCH_2Ph), 4.80–4.69 (m, 4H), 4.63 (d, 1H, *J* = 9.5 Hz, H-1), 4.51 and 4.47 (ABq, 1H each, *J* = 11.7 Hz, OCH_2Ph), 4.01 (d, 1H, *J* = 2.4 Hz, H-4), 3.94 (t, 1H, *J* = 9.4 Hz, H-2), 3.85 (s, 3H, OMe), 3.70 (m, 2H, H-6), 3.64–3.61 (m, 2H, H-3, H-5), 2.33 (s, 3H, Me); ^{13}C NMR ($CDCl_3$): δ 159.7, 139.2, 138.8, 138.3, 137.6, 132.6, 131.0, 130.7, 130.4, 130.0, 128.9, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 114.2, 88.5, 84.7, 75.7, 74.8, 74.0, 74.00, 73.2, 69.2, 55.7, 21.5; HRMS calcd for $C_{42}H_{44}O_6SNa$ (M + Na): 699.2756, found: 699.2773.

4-Methoxyphenyl 3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (25). CH_2Cl_2 (1.5 mL) and Et_2O (3.0 mL) were added to a mixture of **24**[22] (63 mg, 0.106 mmol), **23** (100 mg, 0.148 mmol) and *N*-iodosuccinimide (38 mg, 0.170 mmol), and the mixture was cooled to −55 °C. Trimethylsilyl trifluoromethanesulfonate (4 μL, 20 μmol) was added and the mixture was stirred for 1 h. Triethylamine (0.1 mL) was then added and the mixture was stirred for 0.5 h at −55 °C. The mixture was allowed to obtain room temperature, and was diluted with CH_2Cl_2 (10 mL), washed with 10% aqueous $Na_2S_2O_3$ (2 mL) and saturated aqueous $NaHCO_3$ (2 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (SiO_2, 3:1 heptane–EtOAc) to give **25** (110 mg, 90%): $[\alpha]_D^{23}$ +69 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.11–7.97 (m, 6H, Ar–H), 7.67–7.27 (m, 26H, Ar–H), 7.01 (m, 2H, O*Ph*OMe), 6.72 (m, 4H, O*Ph*OMe, OCH_2*Ph*OMe), 6.04 (dd, 1H, *J* = 7.8, 10.5 Hz, H-2), 5.32 (dd, 1H, *J* = 2.9, 10.6 Hz, H-3), 5.19 (d, 1H, *J* = 7.8 Hz, H-1), 4.94–4.80 (m, 7H), 4.68 (A-part of ABq, 1H, *J* = 11.4 Hz,), 4.53 (B-part of ABq, 1H, *J* = 11.1 Hz,), 4.49 (d, 1H, *J* = 2.6 Hz, H-4), 4.42 (dd, 1H, *J* = 5.0, 9.0 Hz, H-5′), 4.25–4.14 (m, 5H), 4.10 (dd, 1H, *J* = 3.4, 10.2 Hz, H-6), 3,76 (s, 3H, OMe), 3.68 (s, 3H, OMe), 3.45 (t, 1H, *J* = 8.8 Hz, H-6′), 3.01 (dd, 1H, *J* = 5.0, 8.4 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 166.9, 166.5, 165.8, 159.5, 155.9, 151.7, 139.3, 139.2, 138.8, 133.8, 133.6, 133.6, 130.9, 130.4, 130.4, 130.3, 130.2, 130.1, 130.0, 129.5, 128.9, 128.9, 128.8, 128.8, 128.7, 128.5, 128.4, 128.0, 127.9, 127.8, 127.8, 119.2, 114.8, 114.1, 101.8, 101.4, 79.4, 76.1, 76.0, 75.4, 75.2, 74.6, 74.1, 73.6, 73.4, 73.0, 70.3, 70.0, 67.9, 63.3, 56.0, 55.5; HRMS calcd for $C_{69}H_{66}O_{16}Na$ (M + Na): 1173.4249, found: 1173.4250.

4-Methoxyphenyl 3,4,6-tri-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (26). NaOMe (50 μL, 1 M in MeOH) was added to a solution of **25** (110 mg, 96 μmol) in a mixture of methanol (4 mL) and $CHCl_3$ (1 mL), and the solution was stirred over night. Methanolic acetic acid (10%) was added until a neutral reaction on moist pH-paper was obtained. The resulting mixture was concentrated and the residue was purified by flash chromatography (SiO_2, 2:1 toluene–acetone). Sodium hydride (15 mg, 0.37 mmol, 60% in mineral oil) was added to the debenzoylated product in DMF (3 mL). The mixture was stirred for 15 min and then cooled to 0 °C. Benzyl bromide (51 μL, 0.43 mmol) was added dropwise and the resulting mixture was stirred at room temperature for 2 h, then methanol (0.5 mL) was added. Concentration and purification of the residue by flash

chromatography (SiO_2, 3:1 heptane–EtOAc) gave **26** (88 mg, 83%): $[\alpha]_D^{23}$ +39 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.23–7.00 (m, 32H, Ar–H), 6.88 (m, 2H, O*Ph*OMe), 6.62 (m, 2H, O*Ph*OMe), 6.56 (m, 2H, OCH_2*Ph*OMe), 4.83–4.62 (m, 9H), 4.45–4.37 (m, 3H), 4.28 (dd, 1H, *J* = 4.4, 8.8 Hz, H-5′), 4.07–3.91 (m, 7H), 3.88 (d, 1H, *J* = 2.8 Hz, H-4), 3.82–3.73 (m, 2H, H-2, H-6), 3.58 (s, 3H, OMe), 3.53 (s, 3H, OMe), 3.48–3.38 (m, 3H, H-5, H-6, H-6′), 3.29 (dd, 1H, *J* = 2.8, 9.9 Hz, H-3), 3.10 (dd, 1H, *J* = 4.9, 8.4 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 159.4, 155.5, 152.1, 139.4, 139.2, 139.0, 139.0, 138.6, 138.5, 131.4, 130.1, 128.8, 128.8, 128.7, 128.6, 128.5, 128.3, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 118.8, 114.9, 114.0, 103.5, 101.1, 81.3, 79.6, 79.0, 77.2, 76.7, 75.7, 75.4, 75.2, 75.1, 74.5, 73.9, 73.7, 73.6, 72.8, 72.7, 69.9, 68.8, 68.5, 56.1, 55.6; HRMS calcd for $C_{69}H_{72}O_{13}Na$ (M + Na): 1131.4871, found: 1131.4860.

4-Methoxyphenyl 3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (27). Trifluouroacetic acid (0.36 mL) was added to a solution of **26** (365 mg, 0.355 mmol) in CH_2Cl_2 (18 mL) at 0 °C and the resulting solution was stirred for 40 min. *n*-Propylacetate (4 mL) was added and the solution was concentrated and then co-concentrated with toluene. The residue was purified by flash chromatography (SiO_2, 3:1 heptane–EtOAc) to give **27** (308 mg, 95%): $[\alpha]_D^{23}$ +53 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.30–7.06 (m, 30H, Ar–H), 6.88 (m, 2H, O*Ph*OMe), 6.67 (m, 2H, O*Ph*OMe), 4.95 (d, 1H, *J* = 3.9 Hz, H-1′), 4.87–4.59 (m, 7H), 4.48–4.42 (m, 3H), 4.35–4.29 (m, 2H), 4.10–4.03 (m, 4H), 3.99 (m, 1H, H-4′), 3.81 (t, 1H, *J* = 8.4 Hz, H-6), 3.71 (dd, *J* = 7.6, 9.8 Hz, H-2), 3.66 (dd, *J* = 2.6, 10.1 Hz, H-3′), 3.21 (s, 3H, OMe), 3.55–3.46 (m, 3H, H-5, H-6, H-6′), 3.35 (dd, 1H, *J* = 2.8, 9.9 Hz, H-3), 3.18 (dd, 1H, *J* = 4.9, 8.4 Hz, H-6), ^{13}C NMR ($CDCl_3$): δ 155.3, 151.6, 138.9, 138.5, 138.5, 138.4, 138.4, 138.0, 137.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.7, 127.7, 127.7, 127.6, 127.5, 127.4, 127.0, 118.5, 114.6, 103.3, 100.5, 80.4, 79.3, 78.7, 75.3, 74.9, 74.1, 73.5, 73.4, 73.3, 72.8, 72.3, 72.1, 69.7, 68.1, 67.3, 55.6; HRMS calcd for $C_{61}H_{64}O_{12}Na$ (M + Na): 1011.4295, found: 1011.4277.

4-Methoxyphenyl 2-*O*-methyl-α-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (15). NaH (11 mg, 0.26 mmol, 60% in mineral oil) was added to a solution of **27** (125 mg, 0.132 mmol) in DMF (2 mL). The resulting mixture was stirred at room temperature for 15 min and was then cooled to 0 °C. MeI (16 μL, 0.26 mmol) was added and the resulting mixture was allowed to obtain room temperature and was stirred overnight. MeOH (1 mL) was added and the mixture was concentrated, co-concentrated with toluene, and the residue was purified by flash chromatography (SiO_2, 4:1 heptane–EtOAc) to give 2′-*O*-methylated galabioside. This was dissolved in AcOH (2 mL) and hydrogenolyzed (H_2, 1 bar, 10% Pd/C, 20 mg) for 2 h. The solution was filtered through Celite, concentrated, and the residue was purified by flash chromatography (CH_2Cl_2—MeOH–H_2O, 6:1:0.1→4:1:0.1) to give **15** (36 mg, 64%): ^1H NMR (CD_3OD): δ 7.05 (m, 2H, Ar–H), 6.83 (m, 2H, Ar–H), 5.17 (d, 1H, *J* = 3.7 Hz, H-1′), 4.77 (d, 1H, *J* = 7.4 Hz, H-1), 4.20 (t, 1H, *J* = 5.6 Hz, H-5′), 4.02 (d, 1H, *J* = 2.7 Hz, H-4), 3.93–3.71 (m, 11H), 3.62–3.52 (m, 5H); ^{13}C NMR (CD_3OD): δ 156.8, 153.2, 119.5, 115.6, 104.2, 100.7, 80.7, 80.6, 76.5, 75.0, 73.2, 72.7, 71.3, 70.6, 62.8, 61.9, 59.7, 56.2; HRMS calcd for $C_{20}H_{30}O_{12}Na$ (M + Na): 485.1635, found: 485.1645.

4-Methoxyphenyl 2-*O*-propyl-α-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (16). Compound **16** was prepared from **27** and allyl bromide as described for **15** in 81% yield. Compound **16** had: ^1H NMR (CD_3OD): δ 7.05 (m, 2H, Ar–H), 6.88 (m, 2H, Ar–H), 5.13 (d, 1H, *J* = 3.7 Hz, H-1′), 4.78 (d, 1H, *J* = 7.5 Hz, H-1), 4.19 (t, 1H, *J* = 5.4 Hz, H-5′), 4.01 (d, 1H, *J* = 2.2 Hz, H-4), 3.93–3.58 (m, 15H), 1.65 (m, 2H, CH_2CH_3), 0.94 (t, 3H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (CD_3OD): δ 156.7, 153.1, 119.4, 115.4, 104.0, 101.2, 80.8, 79.0, 76.4, 75.0, 73.2, 72.6, 71.4, 70.6, 68.1, 62.8, 62.0, 56.0, 24.2, 10.7; HRMS calcd for $C_{22}H_{34}O_{12}Na$ (M + Na): 513.1948, found: 513.1941.

4-Methoxyphenyl 2-*O*-methoxymethyl-α-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (17). Compound **17** was prepared from **27** and bromomethyl methyl ether as described for **15** in 41% yield. Compound **17** had: ^1H NMR (CD_3OD): δ 7.05 (m, 2H, Ar–H), 6.82 (m, 2H, Ar–H), 5.09 (d, 1H, *J* = 3.3 Hz, H-1′), 4.82–4.74 (m, 3H, H-1, CH_2), 4.20 (t, 1H, *J* = 6.5 Hz, H-5′), 4.02 (d, 1H, *J* = 2.8 Hz, H-4), 3.95–3.68 (m, 12H), 3.60 (dd, 1H, *J* = 2.9, 10.1, H-3), 3.43 (s, 3H, CH_2OCH_3); ^{13}C NMR (CD_3OD): δ 156.7, 153.1, 119.4, 115.4, 104.1, 101.9, 98.5, 80.7, 76.9, 76.6, 74.9, 73.2, 72.6, 71.2, 70.3, 62.7, 61.9, 56.2, 56.0; HRMS calcd for $C_{21}H_{32}O_{13}Na$ (M + Na): 515.1741, found: 515.1740.

4-Methoxyphenyl 3-*O*-[3-(*S*-2-acetamido-2-methoxycarbonyl-ethylthio)-propyl]-α-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (19). *N*-Acetyl-L-cystein methyl ester (29 mg, 0.16 mmol) and AIBN (cat.) were added to a solution of **28**[19] (30 mg, 32 μmol) in EtOAc (0.5 mL), and Ar was bubbled through the mixture for 15 min. The reaction flask was sealed with a septum and irradiated with UV light for 48 h using a water-cooled Original Hanau 70 W mercury high-pressure lamp. Concentration and purification of the residue with flash chromatography (SiO_2, 2:1→1:2, heptane–EtOAc gradient) gave protected **19** which was de-*O*-acylated in methanolic NaOMe (10 mM, 1.5 mL) for 5 h. Methanolic acetic acid (10%) was added until a neutral reaction on moist pH paper was obtained. The resulting mixture was concentrated and the residue was purified by flash chromatography (SiO_2, 10:1:0→4:1:0.2, CH_2Cl_2–MeOH–H_2O gradient) to give **21** (14 mg, 65%): ^1H NMR (CD_3OD): δ 7.07 (m, 2H, Ar–H), 6.85 (m, 2H, Ar–H), 5.01 (d, 1H, *J* = 4.0 Hz, H-1′), 4.82 (d, 1H, *J* = 7.4 Hz, H-1), 4.62 (dd, 1H, *J* = 5.5, 8.0 Hz, C*H*NHAc), 4.31 (t, 1H, *J* = 6.3 Hz, H-5′), 4.14 (d, 1H, *J* = 2.1 Hz, H-4′), 4.07 (d, 1H, *J* = 2.9 Hz, H-4), 3.91–3.55 (m, 15H), 3.00 (ddd, 1H, *J* = 1.3, 5.2, 13.8 Hz, SCH_2CHNHAc), 2.85 (ddd, 1H, *J* = 1.1, 8.1, 13.8 Hz, SCH_2CHNHAc), 2.70 (m, 2H, $SCH_2CH_2CH_2O$), 1.99 (s, 3H, NHAc), 1.89 (m, 2H, $SCH_2CH_2CH_2O$); HRMS calcd for $C_{28}H_{43}O_{15}NSSiNa$ (M + Na): 688.2251, found: 688.2246.

Surface plasmon resonance studies of binding of compounds 1–20 to the class II PapG adhesin. The binding of carbohydrates **1**–**20** to the class II PapG adhesin was determined by surface plasmon resonance using a BIACORE 3000 instrument. The adhesin was immobilized on a sensor chip CM5 using a standard BIACORE amine coupling procedure. This involved activation of the carboxylic acid moieties of the dextrane surface of the sensor chip with *N*-ethyl-*N*-(dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). The PapGII 196 truncate[18] (1.2 mg/mL in 20 mM MES buffer, pH 5.8) was then reacted with the derivatized dextrane surface. Immobilization levels of 4000–6000 RU were obtained. Derivatized dextrane reacted with ethanolamine in one channel was used as reference surface. Each of carbohydrates **1**–**20** were diluted in BIA certified HBS-EP running buffer (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20) in concentration series ranging from 0.001 to 10 mM. The compounds were injected over the sensor chip (flow rate: 10 μL/min at 25 °C) in triplicates and binding to immobilized PapGII was observed in real time. After injection of each compound the surface of the sensor chip was regenerated by dissociation in running buffer for 18 min. Dissociation constants (K_d) were determined as the concentration of a saccharide that elicited half of the maximal response, that is, when 50% of the PapGII adhesin bound a ligand.

Prediction of the binding of galabioside 11 to the class II PapG adhesin. *para*-Methoxyphenyl galabioside **11** was docked manually into the active site of the class II PapG adhesin using Sybyl 6.3.[32] Docking was performed by superimposition of **11** on the galabiose moiety of the tetrasaccaride GalNacβ3Galα4Galβ4GlcβOTMSET (**3**) in the crystalline complex with PapGII.[18] The complex was energy minimized to relieve strain caused by addition of hydrogen atoms to the complex, and to allow **11** to assume a favorable conformation. This was performed on an SGI R12000 Octane computer using the batchmin program from Macromodel 6.5,[33] together with the Amber all-atom force field. The values for shell and core were set to 10 and 8 Å, respectively. The gradient convergence was 0.1 and an iteration limit of 10 000 was used. No solvation model was employed.

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