Adhesion Inhibition of F1C-Fimbriated *Escherichia coli* and *Pseudomonas aeruginosa* PAK and PAO by Multivalent Carbohydrate Ligands

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In order to evaluate their inhibition of bacterial adhesion, the carbohydrate sequences GalNAcβ1→4Gal and GalNAcβ1→4Galβ1→4Glc were synthesized. The disaccharide was conjugated to dendrons based on the 3,5-di-(2-aminoethoxy)-benzoic acid branching unit to yield di- and tetravalent versions of these compounds. A divalent compound was also prepared that had significantly longer spacer arms. Relevant monovalent compounds were prepared for comparison. Their anti-adhesion properties against F1C-fimbriated uropathogenic Escherichia coli *were evaluated in an ELISA-type assay by using a recombinant strain and also by using* Pseudomonas aeruginosa *strains PAO and PAK. Adhesion inhibition was observed in all cases, and multivalency effects of up to one order of magnitude were observed. The combination of spacer and multivalency effects led to a 38-fold increase in the potency of a divalent inhibitor with long spacer arms towards the PAO strain when compared with the free carbohydrate.*

Introduction

The increased antibiotic resistance of bacterial pathogens,[1] requires the development of alternative intervention methods.[2] Protein–carbohydrate interactions on the cell surface are often involved in the initial adhesion of many bacterial pathogens.[3–9] Bacterial lectin-like receptors bind to oligosaccharides present on the target cell surface. This binding is mediated by contacts involving the tissue carbohydrates in mono- and multivalent form that are matched by complementary bacterial binding sites and specifically oriented arrays of binding sites. There is an extensive list of pathogens for which the carbohydrate specificity is known, and that are therefore potential targets for the anti-adhesion approach.[10] Free oligosaccharides block adhesion, but the binding strength of these monovalent compounds is typically low (millimolar range) and they cannot effectively compete with the multivalent presentation of carbohydrates on cell surfaces either as glycolipids on membranes or as part of glycoproteins. It is becoming increasingly clear that multivalency is a powerful design approach to increase the binding strength of synthetic ligands.[11] Since strong binding is required for the practical application of this method, further research in this area is necessary. In the literature there are a few examples of the use of synthetic multivalent carbohydrates for the inhibition of bacterial adhesion. With *Streptococcus suis*, multivalency enhancements were observed by using relatively small tri- and tetravalent galabiose molecules, which were evaluated in haemagglutination inhibition experiments.[12] A divalent derivative was found to be 100 times more potent than a monovalent reference compound with the same linking moiety; this represents a relative potency of 50 per carbohydrate unit. Lindhorst et al. obtained affinity enhancements with multivalent mannose molecules in adhesion to type-1-fimbriated *E. coli* in a haemagglutination assay[13] and an ELISA-type assay.[14] The true multivalency enhancement is unclear, since comparisons were made to methyl α-D-mannoside while spacer effects play an important role in the binding to type-1 fimbriae. This conclusion was recently reached by Roy, Lee and co-workers.[15] who studied the adhesion inhibition of type-1-fimbriated *E. coli* by glycodendrimers and neoglycoproteins. Their study revealed that while the effects of the spacer were large (135-fold), multivalency effects were relatively modest (up to one order of magnitude); this was explained by the large distances between binding sites on different or the same fimbriae.

We describe here our synthesis of multivalent carbohydrates and their inhibition of adhesion of several bacterial targets. One of our targets was the F1C-fimbriated uropathogenic *E. coli*.

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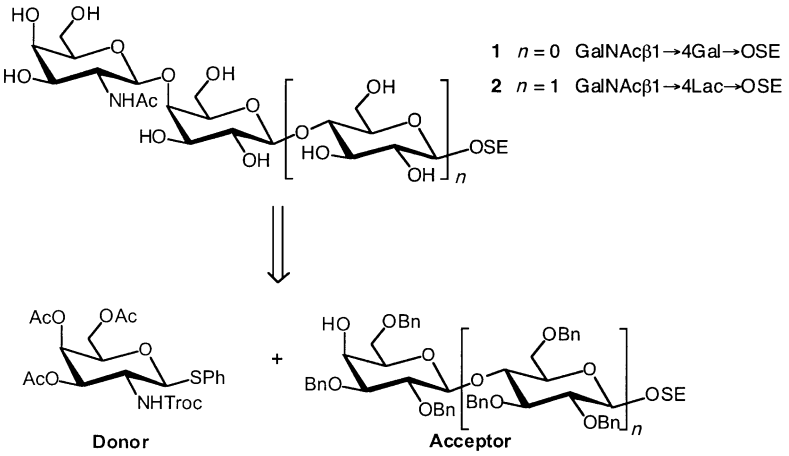
Urinary-tract infections in humans are strongly associated with *E. coli* that produce P, type 1, S, and F1C fimbriae.[16] The first three types are known to exhibit affinity for galabiose, mannose and sialic acid ligands, respectively. Recently the carbohydrate ligand of the F1C fimbria has been determined and appeared to be asialo-GM2 (GalNAcβ1 →4Galβ1 →4Glcβ1 →1Cer).[17, 18] Whether or not the terminal glucose residue in this sequence contributes to the binding is unclear. One of the aims of our work was to confirm the binding specificity of the F1C fimbria through the synthesis of the asialo-GM2 oligosaccharide. Furthermore, the role of the glucose moiety in asialo-GM2 had to be established. Finally, binding enhancement through multivalent linkage of the GalNAcβ1 →4Gal sequence to a multivalent scaffold was targeted. We used dendrons based on the 3,5-di-(2-aminoethoxy)-benzoic acid branching unit[19] as the multivalency scaffold. Lactose derivatives of this branching unit showed weak and strong multivalency effects with lectins and toxins, depending on the structural context.[20] Because the architecture of the F1C fimbria is not known and therefore no information is available on distances between carbohydrate binding sites, our molecules were prepared with both long and short spacer arms.



Scheme 1. *Retrosynthesis of monovalent target structures.*

Another bacterial target we used was *Pseudomonas aeruginosa*. Krivan et al. have shown that pulmonary pathogens such as those typically infecting cystic fibrosis (CF) patients (*P. aeruginosa*, *Haemophilus influenzae*, and *Staphylococcus aureus*) require GalNAcβ1 →4Gal as their minimal adhesion sequence.[21] This sequence was in fact shown to be present in greater abundance in CF-affected lung epithelia.[22] *P. aeruginosa* strains PAO and PAK were used in our studies.[23] A crystal structure of the PAK adhesin is available, based on which a model of the helical assembly of the individual adhesin molecules into fimbriae was proposed.[24] The fimbriae are 52 Å wide, and carbohydrate binding sites are only present at their tip. At the tip, five sites are exposed for binding; this suggests the possibility of simultaneous multivalent binding, whereas in previous models[25] this was deemed less likely.

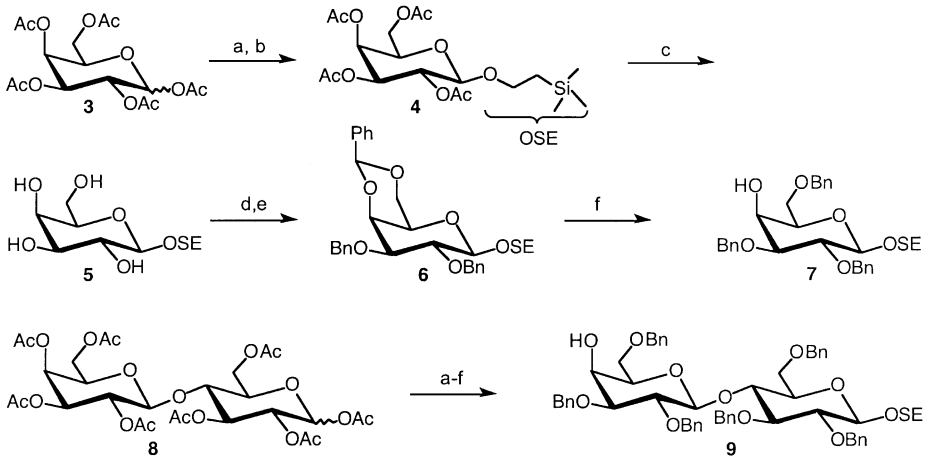
Results and Discussion

General carbohydrate design

For the synthesis of the target molecules we opted for a route using the monovalent oligosaccharide sequences GalNAcβ1 →4Gal →OSE (**1**) and GalNAcβ1 →4Lac →OSE (**2**) (Scheme 1). These di- and trisaccharides were approached by using a single glycosylation of a suitable galactosamine donor and a galactose- or lactose-based acceptor, along the lines of the early work of Lemieux et al.[26] and the more recent preparation of tri- and tetravalent versions by Jiao and Hindsgaul.[27] Although only one glycosylation reaction was to be performed, synthesis was expected to be hampered by the low reactivity of the 4-OH group of the galactose part of the acceptor molecules. To overcome this problem, we used strong activation of the donor by a thiophenyl group at C1 in combination with a suitable promoter. In addition, since a β-stereoselective glycosylation was desired, the donor molecule was equipped with a participating trichloroethoxycarbonyl (Troc) group on C-2.

Acceptor synthesis

Both galactose- and lactose-based acceptors, used for the syntheses of **1** and **2**, respectively, were synthesized according to the procedures of Magnusson et al.,[28] with some modifications (Scheme 2). For the galactose acceptor, the synthesis started with peracetylated galactose **3**, which was brominated followed by introduction of the 2-(trimethylsilyl)ethoxy (OSE) group to yield **4**. Next, **4** was deacetylated with NaOMe in MeOH

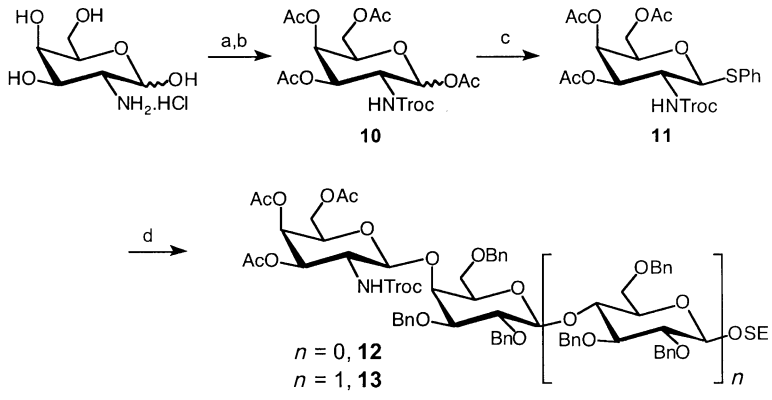


Scheme 2. *Galactose-based acceptor synthesis. Reagents and conditions: a) HBr/AcOH (8 equiv), CH_2Cl_2, 2 h; b) 2-TMSEtOH (3 equiv), collidine, AgOTf (1.2 equiv), CH_2Cl_2, 18 h, 72% (from **3**); c) NaOMe, MeOH, quant.; d) benzaldehyde dimethyl acetal (1.5 equiv), p-TsOH (cat.), acetonitrile, 3 Å molecular sieves; e) NaH, BnBr, DMF, 18 h, 74% (from **5**); f) $NaCNBH_3$ (9.2 equiv), HCl/Et_2O, THF, 3 h, 61%.*

to yield **5**. This reaction was followed by the introduction of a benzylidene ring.[29] The crude product was subsequently benzylated with benzylbromide and sodium hydride in DMF to give crystalline **6**. Selective opening of the benzylidene ring by sodium cyanoborohydride gave the desired galactose acceptor **7** with a free hydroxyl at C-4. This sequence was repeated for the preparation of the appropriate lactose acceptor **9**, starting from lactose hepta-acetate **8**.

Donor synthesis and coupling

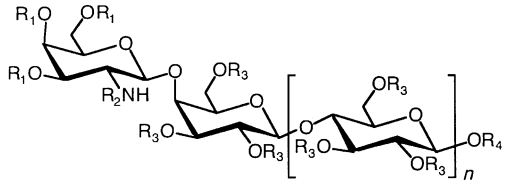
A donor molecule containing a Troc protecting group was employed for synthesis. This protecting group was reported to give rise to a significant increase in donor reactivity in coupling reactions, compared with its *N*-acetyl or *N*-phthaloyl counterparts, while effecting good β selectivity as well.[30, 31] The donor was prepared from galactosamine hydrochloride (Scheme 3), which was treated[32] with trichloroethoxycarbonyl chloride (TrocCl) and $NaHCO_3$ in water, followed by acetylation with acetic anhydride and pyridine to yield the *N*-Troc compound **10**. Then, for activation of C-1, a thiophenyl group was introduced[33] after reaction with thiophenol and ethereal borontrifluoride in dichloromethane to furnish donor molecule **11**. Coupling reactions were performed with *N*-iodosuccinimide (NIS) and triflic acid (TfOH) in dichloromethane, as described by van Boom et al.[34] Coupling reactions with donor **11** and both acceptors **7** and **9** led to the formation of only one isomer in both cases. This was concluded from the ^{1}H NMR spectra, which showed only one singlet at 0 ppm from the trimethylsilyl group. However, deducing the anomeric configuration from ^{1}H NMR spectra was difficult due to peak overlap of the benzylic CH_2 groups with the C-1 doublet. The solution was to measure a proton-coupled carbon spectrum. C-1 coupling constants for α bonds lie between 170 and 175 Hz, which is significantly higher than the reported values of 160 – 165 Hz for a β bond.[35, 36] For all C-1 signals (including C-1′ and C-1″) that can be found around 100 ppm, the recorded spectra showed coupling constants of approximately 160 Hz; this unambiguously proved the presence of the β isomer.



Scheme 3. *Donor synthesis; reagents and conditions: a) TrocCl (1.5 equiv), $NaHCO_3$ (2.5 equiv), H_2O, 18 h; b) Ac_2O, pyridine, 18 h (88%, two steps); c) thiophenol (2 equiv), $BF_3 \cdot Et_2O$ (1.3 equiv), CH_2Cl_2, 18 h, 75%; d) **7** or **9** (1 equiv), NIS (1.3 equiv), TfOH (0.2 equiv), CH_2Cl_2, 4 Å molecular sieves, −70 °C to room temperature, yields: 72% (**12**) and 62% (**13**).*

Deprotection of monovalent antiadhesins

To determine the increase of affinity due to multivalency and spacer effects, fully deprotected monovalent reference compounds were also tested. To this end, compounds **12** and **13** were first stirred in acetic anhydride in the presence of excess zinc powder to convert the Troc protecting group into an acetyl function (**14** and **15**, Scheme 4).[37] These compounds were then



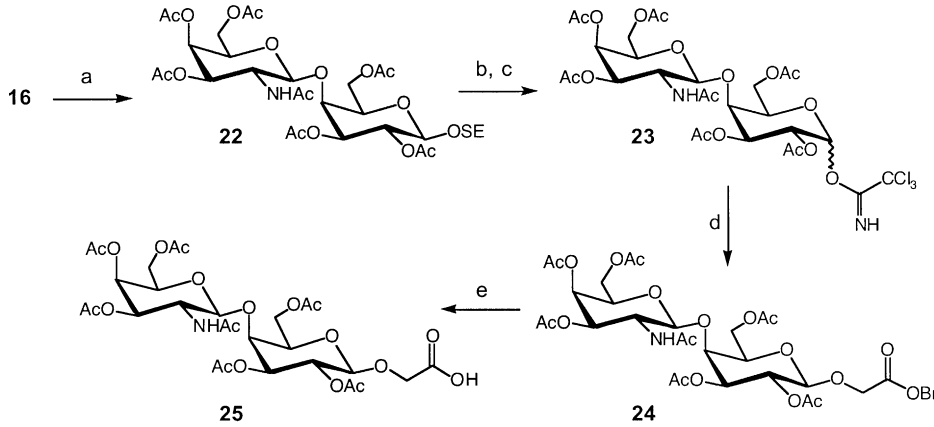
	n=0	*n*=1	R_1	R_2	R_3	R_4
a	**12**	**13**	Ac	Troc	Bn	OSE
b	**14**	**15**	Ac	Ac	Bn	OSE
c	**16**	**17**	Ac	Ac	OH	OSE
d	**18**	**19**	OH	Ac	OH	OSE
	20	**21**	OH	Ac	OH	OH

Scheme 4. *Deprotection of monovalent target compounds; reagents and conditions: a) zinc powder (excess), Ac_2O, 5 h, 73% for **14**, 72% for **15**; b) $Pd(OH)_2$ on carbon, H_2, THF, 18 h, 81% for **16**, 78% for **17**; c) NaOMe, MeOH, 3 h, 80% for **18**, 90% for **19**; d) TFA, CH_2Cl_2, 1.5 h, quant. for both **20** and **21**.*

hydrogenated under high pressure in THF, by using palladium hydroxide on carbon as a catalyst to yield **16** and **17**, and subsequently deacetylated with NaOMe to give **18** and **19**. In addition, part of the material of these compounds was treated with trifluoroacetic acid (TFA) in CH_2Cl_2 to give fully deprotected GalNAcβ1 → 4Gal (**20**) and GalNAcβ1 → 4Lac (**21**).

Linker attachment

Multivalency was studied with the GalNAcβ1 →4Gal sequence, which needed to be in a form suitable for attachment to the multivalent scaffold, dendrons in our case, and thus a spacer had to be attached. The linking moiety was introduced by benzyl glycolate as described by Dean et al.[38] First, all protecting groups of the carbohydrate were replaced by acetyl groups to facilitate deprotection when the carbohydrates were linked to a dendrimer backbone (Scheme 5). To achieve this, compound **16** was acetylated overnight to obtain **22** in quantitative yield. The next step was removal of the OSE protecting group as described above, followed by introduction[39] of a trichloroacetamidate group, for activation of C-1, by using trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 to give an α/β mixture of compound **23**. This compound was immediately converted to **24** by treatment with benzyl glycolate in dichloromethane at −70 °C, with TMSOTf as a catalyst. The final step before linkage to the dendrimer backbone



Scheme 5. *Linker attachment; reagents and conditions: a) Ac_2O, pyridine, 18 h, quant.; b) TFA, CH_2Cl_2, 1.5 h; c) Cl_3CCN, DBU, CH_2Cl_2; d) benzyl glycolate (3 equiv.), TMSOTf (cat.), CH_2Cl_2, $-70-0\,^{\circ}C$, 57% (from **22**); e) Pd/C, H_2, THF, 18 h, 93%.*

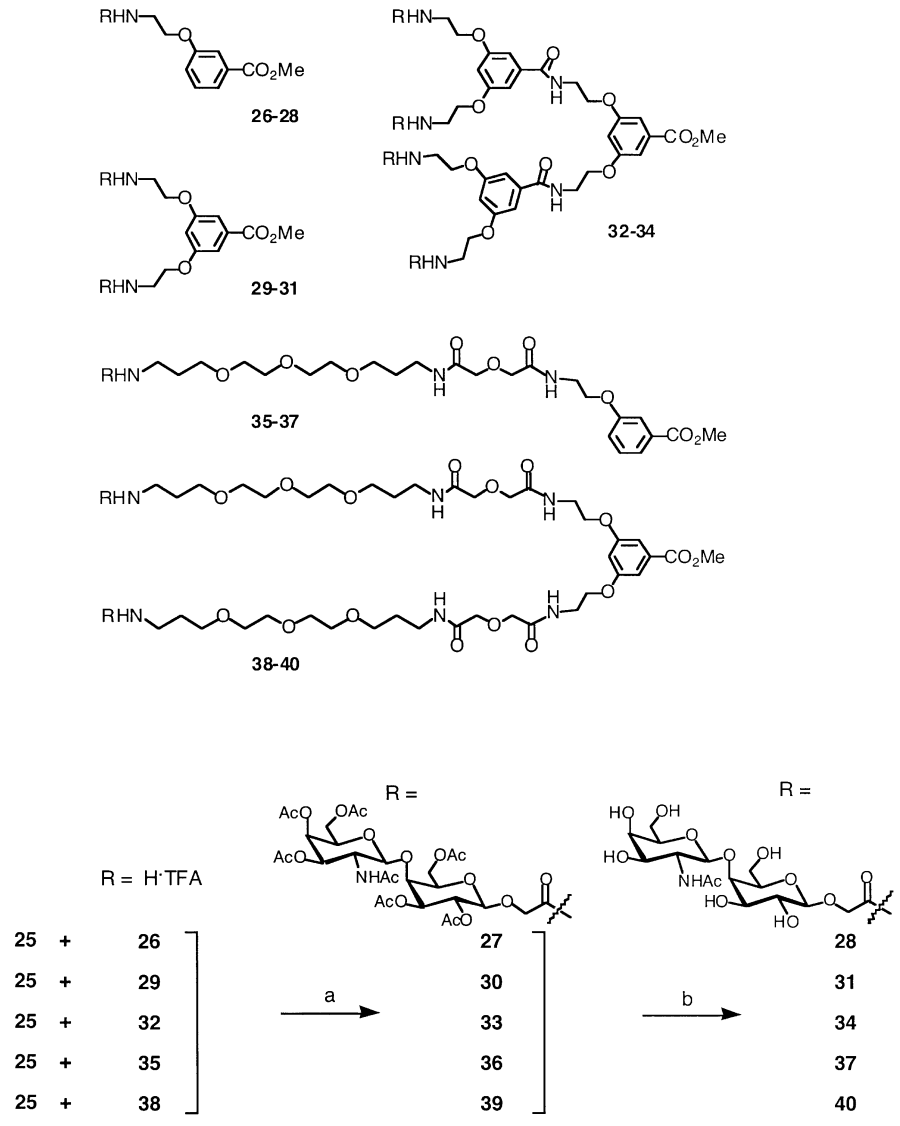
was the liberation of the spacer's carboxylic function; this was achieved through overnight hydrogenation with palladium on carbon as a catalyst in THF to provide compound **25**.

Multivalent antiadhesins

Compound **25** was coupled to the di- and tetravalent dendrons with short spacer arms **29** and **32**,[19] the divalent dendron with long spacer arms **38**[19d] and two monovalent reference compounds **26**[20a] and **35**.[19d] Standard BOP ((benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate)-coupling procedures were used and gave rise to the expected products with protected sugar moieties. Finally, the desired antiadhesion compounds were obtained by removing the *O*-acetyl protecting groups with NaOMe to furnish compounds **28**, **31**, **34**, **37** and **40** (Scheme 6). All compounds showed good mass spectra and well-resolved NMR spectra (see Supporting Information).

Biological testing

The compounds were tested in an adhesion assay in which biotinylated bacteria were incubated in wells with attached asialo-GM1. Inhibitors were incubated with the bacteria prior to this test. The number of attached bacteria was determined by using a chromogenic reaction involving horseradish perox-



Scheme 6. *Synthesis and deprotection of multivalent carbohydrates; reagents and conditions: a) BOP, DIPEA, CH_2Cl_2; b) NaOMe, MeOH.*

idase-conjugated streptavidine. The results of the inhibition experiments are shown in Tables 1 and 2, and examples of binding curves are shown in Figure 1. Inhibition of the adhesion of F1C-fimbriated recombinant *E. coli* by GalNAcβ1→4Gal was clearly observed and confirmed this recently identified sequence.[17] When comparing the monovalent derivatives, it became clear that the lipophilic OSE group at the reducing end of the disaccharide as in **18** ($IC_{50} = 70\,\mu M$) was beneficial for interaction. Replacing it with either a glucose–OSE group, as in **19**, or removing it altogether, as in **20**, led to large reductions in affinities and a less well-defined inhibition. The relatively polar spacer of **28** represents an in-between case with an affinity of roughly half that of the OSE derivative. The divalent version, compound **31**, exhibited sixfold stronger binding; this represents a threefold affinity increase per carbohydrate moiety. The tetravalent derivative **34** did not show a significant further increase in potency. Experiments with purified F1C fimbriae[17] instead of whole bacteria were also performed and showed inhibition of fimbriae binding to the asialo-GM1 surface by the compounds ($IC_{50} = 29\,\mu M$ for **34**, data not shown); this supports causative involvement in bacterial adhesion.

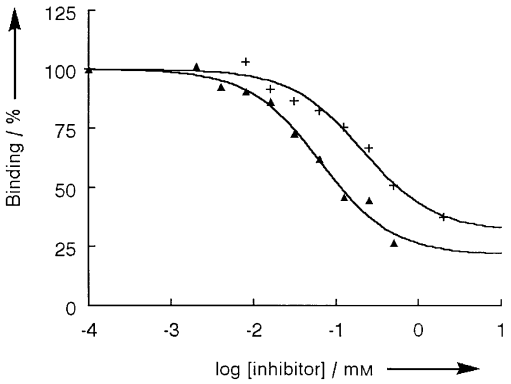


Figure 1. *Examples of inhibition curves of the inhibition of* E. coli *by the monovalent* ***28*** *(+) and the tetravalent* ***34*** *(▲).*

Table 1. *Inhibition potencies of mono- and multivalent carbohydrates towards* E. coli *binding to asialo-GM1.*

Compound	IC_{50} [μM], ± s.d.[a]	
Monovalent compounds		
18 GalNAcβ1→4Galβ→OSE	70 ± 13	
19 GalNAcβ1→4Galβ1→4Glcβ→OSE	669 ± 408	
20 GalNAcβ1→4Gal	438 ± 197	
Mono- vs. multivalent compounds		Rel. potency[b]
28 Monovalent, short arms	115 ± 17	1 (1)
31 Divalent, short arms	19 ± 4	6 (3)
34 Tetravalent, short arms	15 ± 4	8 (2)

[a] s.d. = standard deviation. [b] Potency relative to monovalent **28**; the relative potency per sugar is given in parenthesis, that is, the relative potency divided by the number of sugar units present in the molecule.

P. aeruginosa strains PAO and PAK also showed good adhesion inhibition. Of the monovalent compounds, the disaccharide containing the OSE group (**18**) was again the most potent. The observation that binding is enhanced with increasing lipophilic substitution at or near the reducing end of the disaccharide was consistent with previous studies with PAK.[40] For PAO inhibition, this effect was also seen but was less pronounced. The presence of an additional glucose moiety, as in **19** and **21**, was not beneficial for either PAK or PAO inhibition. Multivalency effects were seen in several cases. For PAK inhibition, the divalent **31** was ten times more potent than its monovalent reference compound **28** in the series with short spacer arms. Surprisingly, the tetravalent **34** was less potent than the divalent **31**; this suggests steric hindrance caused by the additional substituents. In the short-spacer-arm series, the trends in PAO inhibition are the same as for PAK inhibition. For the compounds with longer spacers this is not the case, the IC_{50} for the divalent **40** was 13 times lower than for **37**, whereas with PAK the relative potencies per carbohydrate moiety of the two compounds are identical. For PAO, comparison of the IC_{50} of the parent sugar **20** (266 μM) with the best inhibitor **40** (7 μM) shows a 38-fold improvement in inhibition due to a combination of spacer and multivalency effects.

Conclusion

Multivalent versions of the carbohydrate sequence GalNAcβ1→4Gal were prepared along with relevant monovalent

Table 2. *Inhibition potencies of mono- and multivalent carbohydrates towards* P. aeruginosa *binding to asialo-GM1.*

Inhibitor	Strain: PAK IC_{50} [μM], ± s.d.[a]		Strain: PAO IC_{50} [μM], ± s.d.[a]	
Monovalent compounds				
18 GalNAcβ1→4Galβ→OSE	33 ± 11		88 ± 20	
19 GalNAcβ1→4Galβ1→4Glcβ→OSE	86 ± 28		215 ± 56	
20 GalNAcβ1→4Gal	49 ± 15		266 ± 46	
21 GalNAcβ1→4Galβ1→4Glc	124 ± 35		162 ± 28	
Mono- vs. multivalent compounds		Rel. potency[b]		Rel. potency[b]
28 Monovalent, short arms	210 ± 39	1 (1)	124 ± 23	1 (1)
31 Divalent, short arms	21 ± 6	10 (5)	15 ± 4	8 (4)
34 Tetravalent, short arms	65 ± 11	3 (0.8)	29 ± 7	4 (1)
37 Monovalent, long arms	127 ± 44	1 (1)	89 ± 24	1 (1)
40 Divalent, long arms	62 ± 18	2 (1)	7 ± 2	13 (7)

[a] s.d. = standard deviation.[b] Potency relative to the monovalent reference compound containing the same spacer arm, the relative potency per sugar is given in parenthesis, that is, the relative potency divided by the number of sugar units present in the molecule.

reference compounds. In the synthesis of the disaccharide, the Troc protecting group on the nitrogen atom of the GalNAc donor was crucial for stereoselective coupling. After removal of the anomeric blocking group and linker attachment, peptide-coupling procedures were used to attach the disaccharides to multivalent scaffold molecules with either long or short spacer arms. Adhesion inhibition by these compounds was observed in an ELISA-type assay for F1C-fimbriated *E. coli* and the *P. aeruginosa* strains PAO and PAK. It was clear that lipophilic spacers are beneficial in all cases. Multivalency effects of up to one order of magnitude were observed and, due to a combination of spacer and multivalency effects, a maximum overall enhancement by a factor of 38 was observed when comparing the divalent **40** to the parent sugar **20**. Despite the differences between the *E. coli* and *Pseudomonas* adhesins, relatively similar results were obtained.[41] The inhibition results of *E. coli* were fully consistent with a recent study in which the specificity of the F1C fimbriae was reported for the first time.[17] However, in another recent report, binding to the GalNAcβ1→4Gal epitope was not observed.[42] Structural information on the adhesin was available only for the PAK system. The possibility of simultaneous multivalent binding at the tip of the PAK fimbriae could not be determined from the structure.[24, 25] Our results suggest that it is indeed a possibility, judging from a tenfold affinity increase in the divalent **31** over the monovalent **28**. The distance between the sites is likely to be relatively small considering the reduced enhancement of the long-armed **40** over its monovalent counterpart **37**. The fact that the tetravalent **34** was relatively ineffective may be a result of the helical orientation of the adhesin proteins exposed at the fimbrial tip, which may require a specific orientation of carbohydrate ligands and which may not tolerate additional substituents. More research will be needed to further decipher the delicate spacer and multivalency effects that have the potential to enhance the design and synthesis of newer generations of anti-adhesion compounds with the required potencies. Structural studies of the relevant proteins will guide these endeavours.

Experimental Section

General remarks: Chemicals were obtained from commercial sources and used without further purification unless stated otherwise. The solvents CH_2Cl_2 and MeOH were purchased from Biosolve, The Netherlands, and were stored on 4 and 3 Å molecular sieves, respectively. Activated molecular sieves were prepared by flame-drying under vacuum followed by a nitrogen flush. The base DIPEA (*N*-ethyldiisopropylamine) was distilled from ninhydrin and KOH. Column chromatography was performed on Merck Kieselgel 60 (40–63 μm). Dowex 50 × 8 (H^+ form; 20–50 mesh, Fluka), was used for neutralization. ^{13}C NMR spectra were recorded on a Varian G-300 spectrometer at 75.4 MHz in $CDCl_3$ (referenced to $CDCl_3$ at 77.0 ppm) and in 5% CD_3OD/D_2O (referenced to CD_3OD at 49.0 ppm). Electrospray ionisation (ESI) mass spectrometry was carried out with a Shimadzu LCMS QP-8000 single-quadrupole bench-top mass spectrometer (*m/z* range < 2000), coupled with a QP-8000 data system. Compounds **13**, **15**, **17**, **19** and **21** were prepared according to the same procedures and obtained in similar yields to those of **12**, **14**, **16**, **18** and **20**, respectively. The (poly)amine scaffolds **26**, **29** and **32** were reported previously.[20] Compounds **35** and **38** were prepared as described in the Supporting Information. All spectroscopic data of the prepared compounds can also be found in the Supporting Information.

2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (4): Compound **3** (3.9 g, 10 mmol) was dissolved in dichloromethane (50 mL) and stirred at 0 °C. A hydrogen bromide solution (33% in AcOH, 13.7 mL, 80 mmol) was added, and the mixture was left to stir for 2 h at 0 °C. The progress of the reaction was monitored by TLC analysis (10% $MeOH/CH_2Cl_2$). After completion, the mixture was poured into a beaker containing ice-water (100 mL), neutralized with solid $NaHCO_3$, washed with H_2O (2 × 50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give the corresponding α-acetobromogalactose in quantitative yield. The bromide was dissolved in CH_2Cl_2 (75 mL), and, after activated ground molecular sieves (4 Å) and 2-(trimethylsilyl)ethanol (4.3 mL, 30 mmol) had been added, the mixture was stirred for 3 h. The reaction mixture was then cooled to 0 °C, collidine (1.2 mL, 9.0 mmol) and silver trifluoromethanesulfonate (4.9 g, 19 mmol) were added, and the mixture was stirred in darkness for 18 h. Progress of the reaction was monitored by TLC analysis with EtOAc/hexane (1:1) as eluent. After completion of the reaction, solids were removed by filtration over celite. The filtrate was washed with H_2O (2 × 75 mL), $KHSO_4$ (1 N, 75 mL) and brine (75 mL) and then dried with Na_2SO_4 and concentrated in vacuo to give a yellow oil. To facilitate purification of crude **4**, excess 2-(trimethylsilyl)ethanol was acetylated overnight by using acetic anhydride (20 mL) and pyridine (30 mL). Silica gel column chromatography with EtOAc/hexane (1:3) as eluent was performed to obtain purified compound **4** as a clear oil; yield: 72%.

2-(Trimethylsilyl)ethyl β-D-galactopyranoside (5): Compound **4** (2.1 g, 4.8 mmol) was dissolved in methanol (30 mL), sodium methoxide (30% *w/w* in MeOH, 0.6 mL) was added, and the mixture was stirred overnight. TLC analysis (5% $MeOH/CH_2Cl_2$) revealed that the product was completely deacetylated. The reaction mixture was neutralized with Dowex/H^+ and evaporated to dryness under reduced pressure. Yield: quantitative (white solid).

2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (6): Compound **5** (1.28 g, 4.56 mmol) was suspended in dry acetonitrile (10 mL). Molecular sieves (3 Å) and *p*-toluene sulfonic acid monohydrate (26 mg, 0.14 mmol) were added, and the mixture was stirred for 10 min. Finally, benzaldehyde dimethyl acetal (1.0 mL, 6.8 mmol) was added; this immediately turned the milky white suspension into a clear solution. After 45 min, TLC analysis (EtOAc/MeOH, 15:1) revealed the reaction was complete. The mixture was neutralized (Et_3N), filtered over celite and concentrated to give a yellow oil. Sodium hydride (60% in mineral oil, 0.78 g, 19.6 mmol) was added to a stirred solution of the crude product in DMF (50 mL). After 10 min, benzyl bromide (0.66 mL, 5.6 mmol) was added, and the mixture was stirred for 1 h at 0 °C, and then at 60 °C for 30 min. The reaction was monitored by TLC analysis (EtOAc/hexane, 1:1) and stopped with methanol (4 mL) when benzylation was complete. The mixture was taken up in CH_2Cl_2 (250 mL), washed several times with H_2O (4 × 75 mL), dried (Na_2SO_4) and concentrated to give a yellow syrup. The product was crystallized from ethanol (white needles). The product remaining in the mother liquor was obtained pure as a white solid by silica gel column chromatography with EtOAc/hexane (1:3) as eluent. Yield: 74%.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (7): Et_2O saturated with HCl was added dropwise to a stirred mixture of **6** (0.79 g, 1.44 mmol), sodium cyanoborohydride (0.83 g, 13.3 mmol) and ground molecular sieves (4 Å) in dry THF (20 mL) until bubbling ceased. The reaction was monitored by TLC analysis

(5% MeOH/CH_2Cl_2). When completed, solid $NaHCO_3$ ($\approx$1 g), CH_2Cl_2 (20 mL) and saturated aqueous $NaHCO_3$ (5 mL) were added, and the mixture was filtered over celite. The organic phase was washed with aqueous $NaHCO_3$ (sat., 2×50 mL), H_2O (50 mL) and dried with Na_2SO_4. After concentration, product **7** was purified by silica gel column chromatography, initially with CH_2Cl_2 and after elution of impurities with 2% MeOH/CH_2Cl_2 as eluent. Yield: 61% (clear oil).

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (9): Lactose-based acceptor **9** was prepared from compound **8** as described above for the galactose-based acceptor. Overall yield: 20%.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-D-galactopyranoside (10): Trichloroethoxycarbonyl chloride (5.47 mL, 39.5 mmol) was added under ice-cooling to a solution of D-(+)-galactosamine hydrochloride (5.0 g, 23.2 mmol) and $NaHCO_3$ (5.52 g, 65.7 mmol) in H_2O (120 mL). The mixture was stirred overnight. Progression of the reaction was checked by TLC analysis (EtOAc/AcOH/MeOH/H_2O, 4:3:3:2). Crude 2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-D-galactopyranoside (white solid) was collected ($2\times$) by filtration, washed with ice-cold H_2O and dried, after which it was acetylated overnight by using acetic anhydride (9.3 mL) and pyridine (15 mL). Standard work-up procedures were used to obtain pure **10**. More material was isolated from the filtrates, which were concentrated in vacuo and were then also acetylated overnight. After concentration, the mixture was dissolved in CH_2Cl_2 (75 mL), washed with H_2O (75 mL), aqueous $KHSO_4$ (1 M, 50 mL), $NaHCO_3$ (sat. 50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. If TLC analysis (5% MeOH/CH_2Cl_2) showed purification to be necessary, silica-gel column chromatography was performed. Combined yield: 88% (white foam), α/β-mixture (1:1.2).

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (11): A solution of **10** (7.07 g, 13.5 mmol) in dry CH_2Cl_2 (115 mL) was prepared, to which thiophenol (2.76 mL, 27.0 mmol) and boron trifluoride ethyl etherate (2.23 mL, 17.3 mmol) were successively added under an argon atmosphere. The mixture was stirred overnight, after which TLC analysis (EtOAc/hexane 1:2) revealed the reaction to be complete. The mixture was washed with H_2O (75 mL) and aqueous $NaHCO_3$ (sat., 2×75 mL), dried (Na_2SO_4) and concentrated in vacuo. Purified **11** was obtained after performing silica-gel column chromatography (EtOAc/hexane 1:2). Yield: 75% (white foam).

2-(Trimethylsilyl)ethyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino))-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (12): Donor **11** (104 mg, 0.18 mmol) and acceptor **7** (100 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (2.5 mL), and the mixture was stirred under an argon atmosphere in the presence of activated crushed 4 Å molecular sieves. After 1.5 h, the mixture was cooled to $-70\,^{\circ}C$, and *N*-iodosuccinimide (58 mg, 0.24 mmol) and a catalytic amount of triflic acid were added. The reaction was stirred in darkness, and the temperature was allowed to rise to 20 °C. Progression of the reaction was checked by TLC analysis (EtOAc/hexane 1:1). After 5 h, the reaction mixture was filtered over celite and washed with aqueous $NaHCO_3$ (sat., 10 mL), Na_2SO_3 (sat., 10 mL) and H_2O (10 mL). The organic phase was dried with Na_2SO_4 and concentrated in vacuo. The purified product was obtained after silica-gel column chromatography with EtOAc/hexane (2:5) as eluent. Yield: 72%.

2-(Trimethylsilyl)ethyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (14): Compound **12** (0.91 g, 0.9 mmol) was dissolved in acetic anhydride, excess zinc powder (11.8 g, 180 mmol) was added, and the mixture was vigorously stirred for 6 h. TLC analysis (2% MeOH in CH_2Cl_2) showed all starting material had reacted to give compound **14**. The mixture was filtered over celite and subsequently coevaporated with toluene, EtOH and CH_2Cl_2. Purified **14** was obtained after silica-gel column chromatography with EtOAc/hexane (1:1) as eluent. Yield: 73%.

2-(Trimethylsilyl)ethyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside (16): Compound **14** (80 mg, 0.09 mmol) in THF (5 mL) was hydrogenated overnight with $Pd(OH)_2$/C as catalyst by using a Parr apparatus. After disappearance of the starting material was confirmed by TLC (5% MeOH in CH_2Cl_2), the mixture was filtered over celite and concentrated under reduced pressure. Impurities were removed with silica-gel column chromatography (6% MeOH in CH_2Cl_2). Yield: 81% (white foam).

2-(Trimethylsilyl)ethyl (2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside (18): Purified **16** was dissolved in MeOH (5 mL), sodium methoxide (30% *w*/*w* in MeOH, 0.1 mL) was added and the mixture was stirred for 3 h. The reaction mixture was then neutralized with Dowex/H^+, filtered, concentrated in vacuo and lyophilized to give compound **18**. Yield: 80%.

2-Deoxy-2-acetamido-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (20): Product **20** was obtained after stirring compound **18** (14.6 mg, 23 μL) for 2 h in a mixture of TFA (0.5 mL) and CH_2Cl_2 (0.25 mL). Reagents were removed by coevaporating with EtOAc and toluene, followed by dissolving the residue in water and lyophilization. Yield: quantitative (α/β mixture).

2-(Trimethylsilyl)ethyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (22): Compound **16** (0.40 g, 0.66 mmol) was acetylated by stirring it overnight in a mixture of acetic anhydride (6 mL) and pyridine (10 mL). Reagents were removed by coevaporating with toluene, EtOH and then CH_2Cl_2, and drying at high vacuum. Yield: quantitative.

(3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate (23): Compound **22** (80.5 mg, 0.09 mmol) was treated with TFA (0.93 mL) in CH_2Cl_2 (0.46 mL) for 2 h to free C-1. The reaction was monitored by TLC analysis (5% MeOH in CH_2Cl_2). After standard work-up procedures, the intermediate was dissolved in dry CH_2Cl_2 (1.4 mL) and stirred under an argon atmosphere. The solution was cooled to 0 °C, and trichloroacetonitrile (285 μL, 2.84 mmol) and DBU (11 μL, 0.07 mmol) were added. The progress of the reaction was checked by TLC analysis (5% MeOH in CH_2Cl_2). After 1.5 h, conversion was complete, and the reaction mixture was coevaporated with toluene. A small portion was purified by silica-gel column chromatography (EtOAc/hexane 5:1) for analytical purposes, while the remaining portion was immediately used for coupling to benzyl glycolate as described below.

Benzyloxycarbonylmethyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (24): Compound **23** (60 mg, 0.077 mmol) and benzyl glycolate (38.3 mg, 0.23 mmol) were dissolved in CH_2Cl_2 (3 mL). The solution was stirred under an argon atmosphere in the presence of activated ground 4 Å molecular sieves for 30 min. Then the mixture was cooled to $-70\,^{\circ}C$, and one drop of TMSOTf was added. The pH was checked and if it was not acidic (caused by residual DBU), more TMSOTf was added. The mixture was allowed to warm up to room temperature. After 18 h, the reaction mixture was filtered over celite, taken up in CH_2Cl_2 (10 mL) and washed with $NaHCO_3$ (sat., 10 mL), $KHSO_4$ (1 M, 10 mL) and H_2O (10 mL). The organic phase was dried with Na_2SO_4 and concentrated. Purification of **24** was realized by

using silica-gel column chromatography with EtOAc/hexane (4:1) as eluent. Yield over three steps: 57% (from **22**).

(3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl glycolic acid (25): Compound **24** (50 mg, 0.06 mmol) was dissolved in THF (5 mL), Pd/C (10%, 100 mg) was added, and the mixture was stirred overnight under an H_2 atmosphere. The mixture was filtered over celite and concentrated in vacuo, after which the crude product was purified with silica gel column chromatography (20% MeOH in CH_2Cl_2). Yield: 93%.

Synthesis of 27, 30 and 33: The (poly)amine TFA salts **26, 29** and **32** were mixed with **25** (1 equiv per amino group), BOP (1.1 equiv per amino group) and DIPEA (3.3 equiv per amino group) in dry CH_2Cl_2 and stirred under an argon atmosphere for 14 h. The reaction mixture was taken up in EtOAc and washed with $KHSO_4$, NaOH (1 N) and H_2O. The organic phase was dried with Na_2SO_4, concentrated in vacuo and purified by silica gel column chromatography. Yields: 71% (**27**), 64% (**30**) and 56% (**33**).

Synthesis of 36 and 39: The (poly)amine TFA salts **35** and **38** were mixed with **25** (1 equiv per amino group), BOP (1.1 equiv per amino group) and DIPEA (3.3 equiv per amino group) in dry CH_2Cl_2 and stirred under an argon atmosphere for 14 h. Purification was carried out by using silica-gel column chromatography followed by chromatography on SPE (gradient 0–50% CH_3CN in H_2O) to remove residual hexamethylphosphotriamide (HMPA). Yields: 62% (**36**) and 30% (**39**).

General deprotection procedure: The protected conjugates were dissolved in MeOH, sodium methoxide (30% *w/w* in MeOH, 3 μL) was added, and the mixture was stirred overnight. TLC analysis (5% MeOH/CH_2Cl_2) revealed complete deacetylation. The reaction mixture was neutralized with Dowex/H^+ and concentrated in vacuo. Yield: quantitative for **28**, **31**, **34**, **37** and **40**.

Bacterial strains and growth conditions: The *E. coli* K-12 strain SE5000 used in this study was transformed with the plasmid pPIL110-54 (pACYC184 plasmid vector containing the complete *foc* gene cluster cloned from the uropathogenic *E. coli* strain AD110).[43] Recombinant bacteria were grown overnight (with shaking) in liquid broth supplemented with the appropriate antibiotic (50 μg of chloramphenicol per mL). *P. aeruginosa* strains PAO and PAK[44] were kindly provided by Prof. Burkhard Tuemmler (Klinische Forschergruppe, Medizinische Hochschule Hannover, Germany). PAO and PAK strains were grown on liquid broth agar plates.

Purification of F1C fimbriae: F1C fimbriae were harvested from recombinant strain SE5000 (pPIL110-54) by using an Omnimixer commercial blender (Waring) and purified essentially as described by Khan and Schifferli.[45]

Biotinylation of bacteria: Overnight cultures of bacteria were pelleted at 4000 rpm for 15 min. After washing the bacterial pellet three times with phosphate-buffered saline solution, bacteria were biotinylated essentially as described by Khan et al.[17]

Solid-phase binding and binding inhibition assay: The procedure used here was essentially the same as described previously.[17] Briefly, poly(vinyl chloride) plates (Falcon, Becton Dickinson) were coated with glycolipid asialo-GM1 (0.5 μg per well; Sigma, Deisenhofen, Germany) in chloroform/methanol (1:9, *v/v*). The solvent was allowed to evaporate overnight at room temperature, and the wells were blocked with 3% BSA–phosphate-buffered saline solution (PBS; pH 7.37) either for 2 h at room temperature or for 1 h at 37 °C and washed three times with PBS. For glycolipid receptor asialo-GM1, a serial dilution of *P. aeruginosa* strain PAO or PAK and F1C-fimbriated *E. coli* SE5000 (pPIL110-54), control strain SE5000 (pACYC184) or purified F1C fimbriae, was first tested to determine the numbers of bacteria and the quantity of purified fimbriae required to obtain 50% binding. The number of fimbriated *P. aeruginosa* strains PAO or PAK and SE5000 (pPIL110-54) and nonfimbriated control strain SE5000 (pACYC184) was determined by measuring absorbance at a wavelength of 550 nm against a standardized chart correlating absorbance with viable counts. After removal of the blocking solution and washing, serially diluted biotinylated bacterial suspensions (100 μL; 1.6×10^8 bacteria in first well) or purified fimbrial solution (100 μL; 10 μg fimbriae in first well) in PBS/1% BSA, were added to each well and incubated either for 2 h at room temperature or 90 min at 37 °C. After washing wells as previously, horseradish peroxidase-conjugated streptavidine (Pierce Chemical) in PBS/1% BSA (1:1500) or, to wells with fimbriae, anti-F1C rabbit antibody (polyclonal; 1:2000), was added for 1 h 25 min at 37 °C (100 μL per well). Following another washing step in the case of the fimbriae assay, peroxidase-conjugated goat anti-rabbit IgG (Dako, Hamburg, Germany) in PBS/1% BSA (1:2000) was added for 1 h 25 min at 37 °C (100 μL per well). In all cases, following a final wash, the bound enzyme was detected by the addition of substrate (100 μL per well; Pierce ImmunoPure TMB Substrate Kit) for 5–30 min. The reaction was stopped by adding H_2SO_4 (2 M, 100 μL per well). Absorbance was measured at a wavelength of 450 nm with an ELISA reader. The control wells were treated in the same manner except that blank control wells had no bacteria or fimbriae.

For the binding inhibition assays, *P. aeruginosa* strains PAK (1×10^8 bacteria per well), PAO (1×10^8 bacteria per well) and *E. coli* (2×10^8 bacteria per well) or purified F1C fimbriae (0.4 μg per well) giving 50% binding were preincubated with twofold serially diluted carbohydrates for 10 min at room temperature. The mixtures were transferred to the asialo-GM1-containing microtiter plates, and bacterial or fimbrial bindings were determined as described above. Percentages of binding were calculated by the following equation: percent binding $= \{(A_{450}$ of the test well $- A_{450}$ of the blank control well$)/(A_{450}$ of the 100% binding control well $- A_{450}$ of the blank control well$)\} \times 100$. For this calculation, the 100% binding control wells had no carbohydrate inhibitors and the blank control well had no bacteria, purified fimbriae or inhibitors. Each strain was tested in, on average, four separate experiments, and in each experiment two or three determinations of bacterial or fimbrial adherence were performed in parallel and the results were averaged. IC_{50} values were derived by nonlinear curve fitting to a sigmoid function by using the SlideWrite Plus program.

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[1] S. B. Levy, *Sci. Am.* March **1998**, 32.
[2] H. Breithaupt, *Nat. Biotechnol.* **1999**, *17*, 1165.
[3] N. Sharon, *FEBS Lett.* **1987**, *217*, 145.
[4] K.-A. Karlsson, *Mol. Microbiol.* **1998**, *29*, 1.

[5] D. Zopf, S. Roth, *Lancet* **1996**, *347*, 1017.
[6] I. Ofek, N. Sharon, *Cell. Mol. Life Sci.* **2002**, *59*, 1666.
[7] R. T. Irvin, D. L. Bautista, *Nat. Biotechnol.* **1999**, *17*, 20.
[8] T. Oelschlaeger, A. S. Khan, C. Meier, J. Hacker, *Nova Acta Leopold. NF75* **1997**, *301*, 195.
[9] A. S. Khan, N. C. Johnston, H. Goldfine, D. M. Schifferli, *Infect. Immun.* **1996**, *64*, 3688.
[10] K. F. Johnson, *Glycoconjugate J.* **1999**, *16*, 141.
[11] a) M. Mammen, S.-K. Chio, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754; b) L. L. Kiessling, L. E. Strong, J. E. Gestwicki, *Annu. Rep. Med. Chem.* **2000**, *35*, 321; c) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555; d) T. K. Lindhorst, *Top. Curr. Chem.* **2002**, *218*, 201; e) R. Roy, *Curr. Opin. Struct. Biol.* **1996**, *6*, 692; f) R. T. Lee, Y. C. Lee, *Glycoconjugate J.* **2000**, *17*, 543; g) W. B. Turnbull, J. F. Stoddart, *Rev. Mol. Biotechnol.* **2002**, *90*, 231; h) M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742.
[12] H. C. Hansen, S. Haataja, J. Finne, G. Magnusson, *J. Am. Chem. Soc.* **1997**, *119*, 6974.
[13] T. K. Lindhorst, C. Kieburg, U. Krallmann-Wenzel, *Glycoconj. J.* **1998**, *15*, 605.
[14] a) T. K. Lindhorst, M. Dubber, U. Krallmann-Wenzel, S. Ehlers, *Eur. J. Org. Chem.* **2000**, 2027; b) T. K. Lindhorst, S. Kötter, U. Krallmann-Wenzel, S. Ehlers, *J. Chem. Soc. Perkin Trans. 1* **2001**, 823.
[15] N. Nagahori, R. T. Lee, S.-I. Nishimura, D. Pagé, R. Roy, Y. C. Lee, *ChemBioChem* **2002**, *3*, 836.
[16] A. S. Khan, I. Mühldorfer, V. Demuth, U. Wallner, T. K. Korhonen, J. Hacker, *Mol. Gen. Genet.* **2000**, *263*, 96.
[17] A. S. Khan, B. Kniep, T. A. Oelschlaeger, I. van Die, T. Korhonen, J. Hacker, *Infect. Immun.* **2000**, *68*, 3541.
[18] A. S. Khan, J. Hacker, *Adv. Exp. Med. Biol.* **2000**, *485*, 213.
[19] a) S. J. E. Mulders, A. J. Brouwer, R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 3085; b) S. J. E. Mulders, A. J. Brouwer, P. G. J. van der Meer, R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 631; c) A. J. Brouwer, S. J. E. Mulders, R. M. J. Liskamp, *Eur. J. Org. Chem.* **2001**, 1903; d) I. Vrasidas, PhD thesis, Utrecht University (The Netherlands), **2003**.
[20] a) I. Vrasidas, N. J. de Mol, R. M. J. Liskamp, R. J. Pieters, *Eur. J. Org. Chem.* **2001**, 4685; b) S. André, R. J. Pieters, I. Vrasidas, H. Kaltner, I. Kuwabara, F.-T. Liu, R. M. J. Liskamp, H.-J. Gabius, *ChemBioChem* **2001**, *2*, 822; c) I. Vrasidas, S. André, P. Valentini, C. Böck, M. Lensch, H. Kaltner, R. M. J. Liskamp, H.-J. Gabius, R. J. Pieters, *Org. Biomol. Chem.* **2003**, *1*, 803.
[21] H. C. Krivan, D. D. Roberts, V. Ginsburg, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 6157.
[22] L. Imundo, J. Barasch, A. Prince, Q. Al-Awqati, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3019.
[23] H. B. Sheth, K. K. Lee, W. Y. Wong, G. Srivastava, O. Hindsgaul, R. S. Hodges, W. Paranchych, R. T. Irvin, *Mol. Microbiol.* **1994**, *11*, 715.
[24] D. W. Keizer, C. M. Slupsky, M. Kalisiak, A. P. Campbell, M. P. Crump, P. A. Sastry, B. Hazes, R. T. Irvin, B. D. Sykes, *J. Biol. Chem.* **2001**, *276*, 24186.
[25] B. Hazes, P. A. Sastry, K. Hayakawa, R. J. Read, R. T. Irvin, *J. Mol. Biol.* **2000**, *299*, 1005.
[26] S. Sabesan, K. Bock, R. L. Lemieux, *Can. J. Chem.* **1984**, *62*, 1034.
[27] H. Jiao, O. Hindsgaul, *J. Carbohydr. Chem.* **1999**, *18*, 499.
[28] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, *J. Org. Chem.* **1988**, *53*, 5629.
[29] A. K. Misra, N. Roy, *J. Carbohydr. Chem.* **1998**, *17*, 1047.
[30] Use of a donor containing a phthaloyl protecting group led to a mixture of inseparable isomers.
[31] a) Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734; b) S. Bhattacharyya, B. G. Magnusson, U. Wellmar, U. J. Nilsson, *J. Chem. Soc. Perkin Trans. 1* **2001**, 886.
[32] K. Higashi, H. Susaki, *Chem. Pharm. Bull.* **1992**, *40*, 2019.
[33] M. Nitz, D. R. Bundle, *J. Org. Chem.* **2000**, *65*, 3064.
[34] G. H. Veeneman, S. H. van Leeuwen, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 1331.
[35] K. Bock, I. Lundt, C. Pedersen, *Tetrahedron Lett.* **1973**, *13*, 1073.
[36] K. Bock, C. Pedersen, *J. Chem. Soc. Perkin Trans. 2* **1974**, 293.
[37] W. Dullenkopf, J. C. Castro-Palomino, L. Manzoni, R. R. Schmidt, *Carbohydr. Res.* **1996**, *296*, 135.
[38] B. Dean, H. Oguchi, S. Cai, E. Otsuji, K. Tashiro, S.-I. Hakomori, T. Toyokuni, *Carbohydr. Res.* **1993**, *245*, 175.
[39] M. Wilstermann, L. O. Kononov, U. Nilsson, A. K. Ray, G. Magnusson, *J. Am. Chem. Soc.* **1995**, *117*, 4742.
[40] F. Schweizer, H. Jiao, O. Hindsgaul, W. Y. Wong, R. T. Irvin, *Can. J. Microbiol.* **1998**, *44*, 307.
[41] The adhesin of the F1C fimbriae has not yet been identified but none of the eight genes required for its biogenesis (see ref. [17]) has significant homology to the *Pseudomonas* adhesins.
[42] F. Bäckhed, B. Alsén, N. Roche, J. Ångström, A. von Euler, M. E. Breimer, B. Westerlund-Wikström, S. Teneberg, A. Richter-Dahlfors, *J. Biol. Chem.* **2002**, *277*, 18198.
[43] I. van Die, I. B. van Geffen, W. Hoekstra, H. Bergmans, *Gene* **1985**, *34*, 187.
[44] C. Kiewitz, B. Tummler, *J. Bacteriol.* **2000**, *182*, 3125.
[45] A. S. Khan, D. M. Schifferli, *Infect. Immun.* **1994**, *62*, 4233.

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