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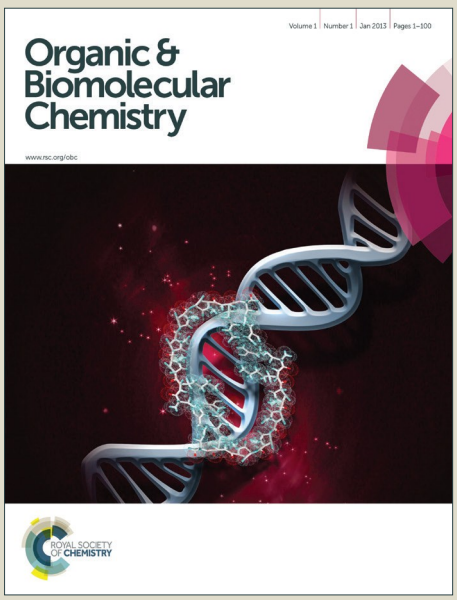

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Inhibition profiles of mono- and polyvalent FimH antagonists against 10 different *Escherichia coli* strains

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Mono- and polyvalent ligands with strong affinities for the mannose-binding adhesin FimH were synthesised, and their anti-adhesive properties against ten *E. coli* strains were compared in two cell-based assays. The compounds were assessed against the non-pathogenic *E. coli* K12 and nine strains isolated by coproculture or from patients with osteoarticular infections (OIs), Crohn's disease (CD) and urinary tract infections (UTIs). The results showed that the compounds could inhibit the whole set of bacterial strains but with marked differences in terms of effective concentrations. The relative inhibitory potency of the monovalent compounds was also conserved for the ten strains and in the two assays. These results clearly suggest that a potent monovalent anti-adhesive assessed on a single *E. coli* strain will probably be effective on a broad range of strains and may treat diverse *E. coli* infections (OIs, CD and UTIs). In contrast, the polyvalent compounds showed a significant strain-dependancy in preventing *E. coli* attachment to intestinal cells. The multivalent antiadhesive effect may therefore vary depending on the *E. coli* strain tested.

Introduction

Increasing bacterial resistance to antibiotics is a serious health problem, which is worsening with the constant identification of strains resilient to commonly available chemotherapeutic agents.[1] This is particularly true for Gram-negative bacteria such as *E. coli* coding for New Delhi metallo-beta-lactamase (NDM-1), conferring resistance to carbapenems.[2] Meanwhile, drug development programmes in the pharmaceutical industry for new antibiotics are stagnating despite the urgent need for antimicrobials with different mechanisms of action. Among the therapeutic alternatives developed at the academic level, the anti-adhesive strategy has seen a growing interest in the last 25 years.[3] The concept is to disrupt the lectin-mediated adhesion of the pathogen to eukaryotic cells. This therapeutic approach should be less prone to bacterial resistance and selection pressures as the pathogens are not killed during the decolonisation process.

Several relevant bacterial targets have been identified, including the mannose-binding lectin FimH, displayed at the tip of long proteinaceous *E. coli* organelles called pili. Since the pioneering work by Sharon and co-workers[4] showing that mannosides with anomeric phenylaglycons are potent FimH inhibitors, several groups have developed FimH antagonists with anomeric aglycons.[5] Monovalent FimH antagonists have been successfully developed to disrupt the attachment of uropathogenic *E. coli* to bladder cells *in vitro,*[6] and *in vivo* for the potential treatment of urinary tract infections (UTIs).[4,5] Recently, we extended the anti-adhesive concept to the potential treatment of Crohn's disease (CD) in which adherent-invasive *E. coli* (AIEC) have been shown to play a key role in the inflammation.[7] The multivalent strategy,[8–10] in which mannosides are grafted in multiple copies onto a common scaffold, was also developed as a complementary approach for the design of potent FimH antagonists.[11,12,13] In our group, we designed multivalent ligands based on heptylmannoside (HM), a nanomolar FimH inhibitor, and observed improvement in the *E. coli* anti-adhesive effect *in vitro*,[14,15] and *in vivo* in a murine cystitis model.[16] The synergetic effect was not due to a stronger affinity for FimH but to the ability of the multivalent compounds to form bacterial aggregates that are less prone to adhere to the host cells.

The development of FimH antagonists is an active field of research with most of the work centred on potential application to UTIs. Many studies have described the synthesis and biological evaluation of new FimH antagonists. The anti-adhesive cell-based assay reported has almost exclusively focused on the evaluation of the anti-adhesive effects against a single *E. coli* strain (generally *E. coli* K12 or UTI89). In this work, we compared the inhibition profiles of selected monovalent and polyvalent FimH inhibitors against the

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Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for all new compounds.

commensal *E. coli* K12 MG1655, *E. coli* 30195647 of faecal origin and eight clinically isolated *E. coli* strains from patients with urinary tract infections (UTI89), Crohn's disease (LF28, LF31, LF73, LF82, 7136) and osteoarticular infections (8012603 = total hip replacement, 8005515 = incomplete hip replacement) in two different cell-based assays. This will provide information on how a potent anti-adhesive candidate evaluated against a specific *E. coli* strain is susceptible to having a broad effect on different *E. coli* infections.

Results and Discussion

We selected four different types of FimH antagonists for the present study and assessed the bacterial anti-adhesive properties of seven compounds (Figure 1). **HM** was included as a potent reference because this compound showed very low dissociation equilibrium constants (K_d) with FimH of 7 nM and 5 nM determined by isothermal titration calorimetry and surface plasmon resonance, respectively.[7,18]. We recently identified the thiazolylaminomannosides **5** and **6** (TazMans) as potent anti-adhesives of the *E. coli* reference strain LF82 that promotes inflammation in the ileum of CD patients.[7] Compound **6** was the most potent of the series in disrupting LF82 attachment to intestinal cells.[7] Finally, we designed polymeric HM ligands **1**-**4** with average valencies ranging from 23 to 902. HM was attached to biocompatible dextrans, a scaffold recently used to design polymeric iminosugars.[19]

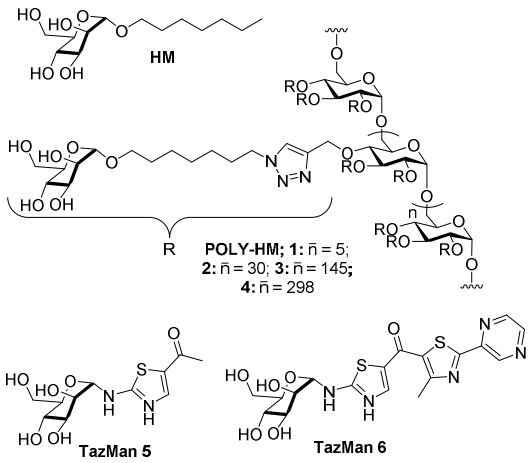
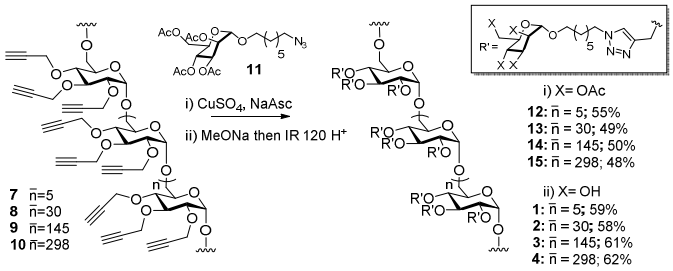


Figure 1. Structure of potent FimH antagonists **HM** and **1**-**6** assessed against 10 different *E. coli* strains.

The poly-alkynes **7**-**10**[19] were engaged with **11**[14] in a copper-catalysed azide-alkyne cycloaddition (CuAAc) under microwave irradiation. Residual copper was removed by adding excess EDTA to the solution. The polymers **12**-**15** were obtained with moderate yields (48-55%) after several purifications by precipitation. The complete and exclusive formation of the 1,4-substituted triazole regioisomers was evidenced by ^{13}C NMR where large $\Delta(\delta_{C\text{-}4}–\delta_{C\text{-}5})$ values (>20 ppm) were observed for the compounds **12**-**15**.[20] The acetates were removed with the basic Amberlite resin IRN 78 and the crude compounds **1**-**4** were purified by size exclusion chromatography on a Sephadex G-100 column.



Scheme 1. Synthesis of poly-HM **1**-**4**.

The anti-adhesive profiles of the seven compounds were first assessed by haemagglutination (HIA-Figure 2). Each *E. coli* can express hundreds of pili where the FimH adhesin, located at their tip, interacts with the mannosylated glycocalyx of guinea pig erythrocytes in the present case. These simultaneous interactions account for the formation of a bacteria-cells cross-linked matrix. The HIA assay gives the minimum inhibitory concentration (MIC) of compound required to prevent/disrupt gel formation. HIA is a particularly popular assay to estimate the relative potencies of a set of inhibitors. However, the absolute MIC values provided cannot be strictly compared with the results from previous experiments due to the heterogeneity of the blood samples. Visual detection also imposes an error of ± one well, which corresponds to a factor of two in the present case. The MIC values obtained for **HM** and compound **3** are presented in Figure 2. The results obtained for poly-HM **1**, **2**, and **4** were very similar to **3** and are presented Figure S1.

We also explored the anti-adhesive properties of **HM** and compounds **1**-**6** in an *in vitro* adhesion assay, using intestinal epithelial cells (ICS-Figure 3). In CD, AIEC bacteria adhere to the mannosylated CEACAM6 protein, abnormally expressed by the ileal mucosa of patients. We previously set up an *in vitro* assay mimicking these conditions.[7] In this assay, the intestinal epithelial cells T84, highly expressing CEACAM6, were infected with the different bacterial strains previously incubated for 1 h with the inhibitors at different concentrations. The amount of bacteria attached to the cells was determined in the presence and absence of inhibitors (reference normalised to 100%). Inhibitory effects on the bacterial adhesion for compounds **HM** and **5** at a concentration of 10 µM, and for compound **6** at 1 µM are presented in Figure 3. We tested **6** at a 10-fold lower concentration because of its much higher anti-adhesive activity.

The inhibitory effects of polymeric compounds **1**-**4** were assessed by ICS against three representative *E. coli* strains (LF82, UTI89 and 8012603) and are presented Figure 4.

The seven compounds were shown to be active at the concentrations tested against the ten *E. coli* strains, both in HIA and ICS. The only exception was **HM**, which was inactive at 10 µM against the non-pathogenic *E. coli* K12 (ICS). This

indicates that a given FimH antagonist can potentially be used to treat diverse pathologies involving specific *E. coli* strains such as UTIs, CD and OIs. In HIA, low MIC values ranging from 1.5 nM to 25 µM were measured, while in ICS, the minimal percentage of bacteria remaining attached to the cells was 21%. In both cases, the lower values were obtained with TazMan **6,** by far the most potent compound of the series. Its outstanding MIC value of 1.5 nM (Figure 2) with the CD reference strain LF82 is 100-fold lower than that of the potent FimH antagonist HM (MIC = 195 nM). Strikingly, this gap in MIC values was preserved for the different *E. coli* strains, and the same tendency was observed for compound **5**. In other words, the relative inhibitory potency of the monovalent compounds, which is **6**>>**5**≈**HM**, was unchanged for the ten different strains both in HIA and ICS. Therefore, the relative potencies observed when inhibitors are assessed against a single *E. coli* strain, as classically reported in the literature, is probably indicative of a general inhibitory trend for *E. coli* species. Poly-HM **1**-**4** did not exhibit a significant multivalent effect compared to **HM** in HIA (Figure S1). This is surprising, considering that we previously showed that diverse multi- and poly-HM structures were significantly more potent than monovalent HM references.[14–16,21]

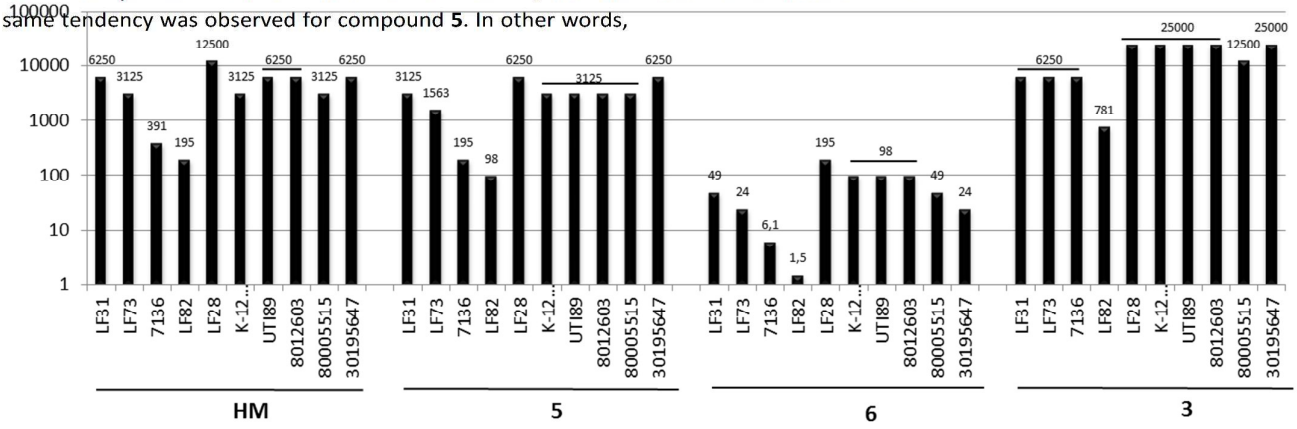


Figure 2. Minimum inhibitory concentration (MIC value) of **HM**, monovalent TazMans **5** and **6**, and poly-HM **3** to inhibit the haemagglutination process between ten different *E. coli* strains and guinea pig erythrocytes (Y scale logarithmic, in nanomolar). Due to serial dilution, the error is ± 1 well or a factor of 2.

This was rationalised by the formation of bacterial clusters promoted by the multi-HM, in which a significant proportion of FimH are trapped in the matrix and less prone to adhere to the cell-coated surface. HIA, however, is markedly different as the cells (erythrocytes) are not coated on the surface, but homogeneously dispersed with the bacteria in the solution. We hypothesise that this is much less favourable for the formation of "pure" bacterial clusters promoted by the poly-HM. In ICS, a clear multivalent effect was observed only against LF82 adhesion. Polyvalent **1, 4** were significantly more potent than **HM** at the same mannose concentration (Figure 4). In contrast, no multivalent effects were observed on the inhibition of UTI 89 and 8012603 adhesion in these experimental conditions. These first results suggests that the level of the multivalent effect may significantly vary in function of the *E. coli* strain tested.

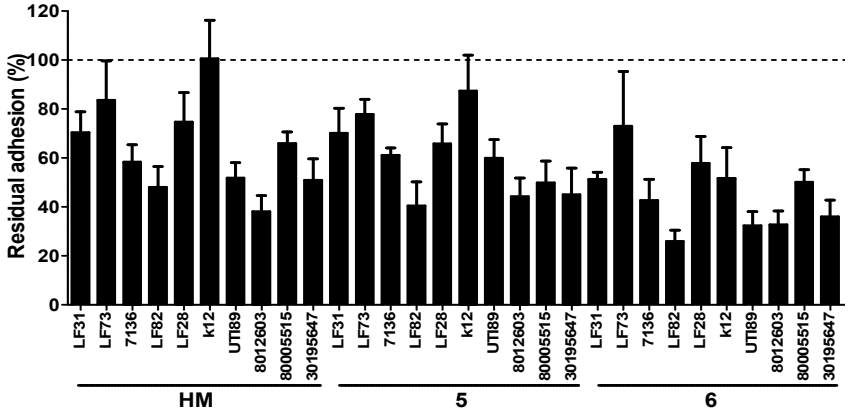


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Figure 3. Comparison of the inhibitory effects on the bacterial strain adhesion to T84 cells obtained with monovalent **HM**, **5**, and **6** at 10 µM mannose concentration for **HM** and **5,** and 1 µM for **6**. Results are quadruplicates and expressed in percentage of bacteria adhering to the cell in the presence of compounds (bacterial adhesion in the absence of any compound was considered 100%, represented by the dotted line).

Significantly different concentrations of mono- and polyvalent compounds were also required to disrupt the cell-attachment of the different *E. coli* strains in HIA and ICS. For example, the CD-associated strain LF82 required around two orders of magnitude lower concentrations of inhibitors compared to the IE-associated *E. coli* 30195647 in HIA. These differences can be ascribed to different levels of expression of the FimH adhesin and/or different binding strengths of the bacterial strains for the specific mannosylated carbohydrates at the surface of the erythrocytes. Coherently with the HIA results, the level of disruption of *E. coli* adhesion in ICS also varied significantly for the different strains. For example, 1 µM of **6** reduced the bacterial adhesion of LF73 and LF82 to 75 and 25%, respectively. In some cases, the inhibitory profiles or "preferences" of the compounds for specific strains differed in HIA and ICS. This can probably be explained by the different structures of the mannosylated ligands at the surface of the cells, favouring the adhesion of specific strains, which in return decreases the anti-adhesive potency of the compounds.

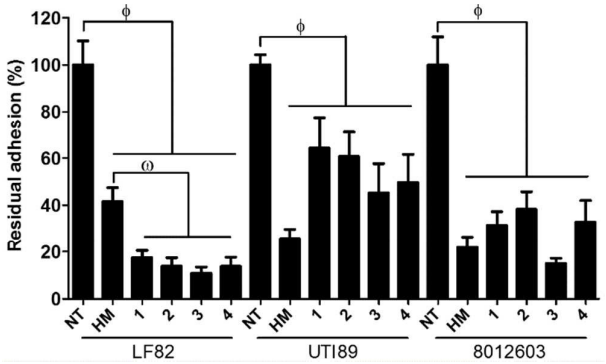

Figure 4. Comparison of the inhibitory effects on the bacterial strain adhesion to T84 cells obtained with **HM**, and poly-HM **1**-**4**, at 10 µM mannose concentration. Results are sextuplicates and expressed in percentage of bacteria adhering to the cell in the presence of compounds (bacterial adhesion in the absence of any compound was considered 100%, represented by the dotted line). Φ: $p<0.05$ compared to NT; ω: $p<0.05$ compared to HM treatment.

Conclusions

In this work, we synthesised and compared the inhibitory profiles of mono- and polyvalent ligands **HM**, **1**-**6** against ten *E. coli* strains, in two different cell-based assays. A great deal of interesting information can be extracted from the results. First, the evaluation and ranking of monovalent anti-adhesive inhibitors against a single *E. coli* strain, as classically performed, is representative of their general inhibitory trends for the *E. coli* species expressing FimH. This implies that the best monovalent FimH antagonist identified in a single *E. coli* strain assay will probably remain the best for other *E. coli* species, and could be considered to treat a wide range of infections such as hip infections, UTIs or CD. In contrast, our preliminary results obtained by ICS with polyvalent **1**-**5** suggests a strain-dependancy for the multivalent effect. Second, the compounds can present an anti-adhesive effect at very different concentrations depending on the strains tested, even if the strains are isolated from patients with the same pathology (i.e. LF82 and LF73). This probably reflects different binding capacities and levels of FimH expression. This tendency will complicate the determination of the dose required by preclinical candidates to treat *E. coli* infections. Finally, the poly-HM exhibited a significant multivalent effect against LF82 in ICS but not in HIA. These two cell-based assays are very different in nature with cells in solutions for HIA and coated on a surface for ICS. This further illustrates the crucial role of choosing the appropriate cell model, to account for a potential *in vivo* effect. In the present case, the poly-HM may be relevant to disrupt LF82 adhesion on the surface of intestinal cells but not in solution.

Experimental section

General information

NMR spectra were recorded at room temperature with a Bruker Avance 500 MHz or Bruker Avance HD 700 MHz spectrometer and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CDCl_3$: ^1H: δ=7.26, ^{13}C: δ=77.2; D_2O: ^1H: δ=4.79). Peak

multiplicity is reported as: singlet (s), multiplet (m), and broad (br). High-resolution mass spectra (HRMS) were obtained by Electrospray Ionisation (ESI) on a Micromass-Waters Q-TOF Ultima Global or with a Bruker Autoflex III SmartBeam spectrometer (MALDI). Low-resolution mass spectra (MS) were recorded with a Thermo electron DSQ spectrometer. All reagents were purchased from Acros Organics or Aldrich and were used without further purification. Column chromatography was conducted on silica gel Kieselgel SI60 (40-63 µm) from Merck. Gel filtration chromatography was conducted on Sephadex® G-100 from Sigma Aldrich. Reactions requiring anhydrous conditions were performed under argon. Dichloromethane was distilled from calcium hydride under nitrogen prior to use. Microwave experiments were conducted in sealed vials in commercial microwave reactors especially designed for synthetic chemistry (MultiSYNTH, Milestone). The instrument features a special shaking system that ensures high homogeneity of the reaction mixtures.

Synthetic procedures

General procedure for 1,3-dipolar cycloadditions (Method A):

Copper sulfate (26.5 mg, 0.10 mmol, 0.75 eq per alkyne function) and sodium ascorbate (42 mg, 0.21 mmol, 1.5 eq per alkyne function) were added to a solution of the alkyne dextran (13 mg, 47.1 µmol of monosaccharide unit) and **11** (96.5 mg, 0.20 mmol, 1.4 eq per alkyne function) in dioxane-H_2O (5 mL, 4–1). The mixture was irradiated at 80 °C for 2 h in a sealed vessel, with addition of 0.25 eq of copper sulphate and 0.5 eq of sodium ascorbate after 1 h. Residual copper was removed by adding EDTA (1.5 eq per alkyne function), and stirring for 1 hour. The mixture was poured into a NH_4Cl satd. solution (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude product was dissolved in a small amount of CH_2Cl_2 and the product was precipitated with Et_2O (50 mL). The precipitate was collected by filtration, washed with Et_2O (50 mL) and precipitated twice from Et_2O.

General procedure for acetate deprotection (Method B):

Compound **12** (38 mg, 21.7 µmol of monosaccharide unit) was dissolved in MeOH/H_2O (1:1, 5 mL). Amberlite resin IRN 78 1.25 meq/mL (2 g) was added to the solution, and the mixture was stirred for 48 h at rt. The resin was filtered off and washed with methanol and water. The solvent was evaporated under reduced pressure. The crude product was purified by Sephadex G-100 gel filtration, affording **1** (16 mg, 59%) as a pale yellow solid.

Compound 12: Obtained following method A in 55% yield (46 mg) as a yellow solid.
^{1}H NMR (700 MHz, $CDCl_3$) δ(ppm): 8.17-7.50 (br, 3H, H-8''), 5.35-5.28 (m br, 3H, H-3'), 5.28-5.22 (m br, 3H, H-4'), 5.22-5.16 (m br, 3H, H-2'), 5.07-4.60 (m br, 10H, 1*H-1, 3*H-1', 6*H-10''), 4.37-4.21 (m br, 9H, 3*H-6'$_A$, 6*H-7''), 4.11-4.03 (m br, 3H, 3*H-6'$_B$), 3.98-3.92 (s br, 3H, 3*H-5'), 3.88-3.70 (br, 3H, H-3,4,5), 3.70-3.60 (s, 4H, H-6$_A$, 3*H-1''$_A$), 3.51-3.32 (br, 5H, H-2, H-6$_B$, 3*H-1''$_B$), 2.13, 2.08, 2.02, 1.97 (4*s, 4*9H, O=CH_3) 1.93-1.82 (br, 6H, H-6''), 1.62-1.54 (br, 6H, H-2''), 1.40-1.25 (br, 18H, H-3'', 4'', 5''); ^{13}C NMR (176 MHz, $CDCl_3$) δ(ppm): 170.6, 170.0, 169.8, 169.7 (C=O), 144.9 (C-9''), 123.5 (C-8''), 97.5 (C-1,1'), 81.3 (C-3), 79.9 (C-2), 73.4 (C-4), 71.0 (C-5), 69.7 (C-2'), 69.1 (C-3'), 68.4 (C-5', C-1''), 66.8-65.5 and 64.1 (C-6, C-4', C-10''), 62.5 (C-6'), 50.2 (C-7''), 30.3 (C-6''), 29.2 (C-2''), 28.8, 26.5, 25.9 (C-3'', C-4'', C-5''), 20.9, 20.7 (CH_3).

Compound 13: Obtained following method A in 49% yield (41 mg) as a yellow solid.
^{1}H NMR (700 MHz, $CDCl_3$) δ(ppm): 8.20-7.54 (br, 3H, H-8''), 5.36-5.28 (m br, 3H, H-3'), 5.28-5.22 (m br, 3H, H-4'), 5.22-5.17 (m br, 3H, H-2'), 5.00-4.63 (m br, 10H, 1*H-1, 3*H-1', 6*H-10''), 4.38-4.22 (m br, 9H, 3*H-6'$_A$, 6*H-7''), 4.12-4.06 (m br, 3H, 3*H-6'$_B$), 3.98-3.94 (s br, 3H, 3*H-5'), 3.87-3.72 (br, 3H, H-3,4,5), 3.69-3.62 (s, 4H, H-6$_A$, 3*H-1''$_A$), 3.50-3.36 (br, 5H, H-2, H-6$_B$, 3*H-1''$_B$), 2.13, 2.07, 2.02, 1.97 (4*s, 4*9H, O=CH_3) 1.94-1.80 (br, 6H, H-6''), 1.62-1.51 (br, 6H, H-2''), 1.40-1.26 (br, 18H, H-3'', 4'', 5''); ^{13}C NMR (176 MHz, $CDCl_3$) δ(ppm): 170.7, 170.2, 169.9 (C=O), 145.1 (C-9''), 123.1 (C-8''), 97.7 (C-1'), 96.9 (C-1), 81.3 (C-3), 80.2 (C-2), 73.1 (C-4), 70.9 (C-5), 69.8 (C-2'), 69.3 (C-3'), 68.5 (C-5', C-1''), 66.5-65.5 and 63.9 (C-6, C-4', C-10''), 62.6 (C-6'), 50.4 (C-7''), 30.4 (C-6''), 29.3 (C-2''), 28.9, 26.6, 26.1 (C-3'', C-4'', C-5''), 21.0, 20.9, 20.8 (CH_3).

Compound 14: Obtained following method A in 50% yield (42 mg) as a yellow solid.
^{1}H NMR (700 MHz, $CDCl_3$) δ(ppm): 8.12-7.60 (br, 3H, H-8''), 5.34-5.13 (m br, 9H, H-3', H-4', H-2'), 4.99-4.54 (m br, 10H, 1*H-1, 3*H-1', 6*H-10''), 4.38-4.15 (m br, 9H, 3*H-6'$_A$, 6*H-7''), 4.11-4.02 (m br, 3H, 3*H-6'$_B$), 3.98-3.90 (s br, 3H, 3*H-5'), 3.79-3.58 (br, 7H, H-3,4,5, 3*H-1''$_A$), 3.50-3.32 (br, 5H, H-2, H-6$_B$, 3*H-1''$_B$), 2.12, 2.07, 2.02, 1.96 (4*s, 4*9H, O=CH_3) 1.90-1.75 (br, 6H, H-6''), 1.63-1.48 (br, 6H, H-2''), 1.40-1.21 (br, 18H, H-3'', 4'', 5''); ^{13}C NMR (176 MHz, $CDCl_3$) δ(ppm): 170.7, 170.1, 170.0, 169.8 (C=O), 145.1 (C-9''), 123.8 (C-8''), 97.6 (C-1, C-1'), 81.4 (C-3), 79.9 (C-2), 73.0 (C-4), 70.1 (C-5), 69.7 (C-2'), 69.2 (C-3'), 68.5 (C-5', C-1''), 66.7-65.6 and 63.9 (C-6, C-4', C-10''), 62.6 (C-6'), 50.3 (C-7''), 30.4 (C-6''), 29.3 (C-2''), 29.0, 26.6, 26.1 (C-3'', C-4'', C-5''), 21.0, 20.9, 20.8 (CH_3).

Compound 15: Obtained following method A in 48% yield (40 mg) as a yellow solid.
^{1}H NMR (700 MHz, $CDCl_3$) δ(ppm): 8.09-7.63 (br, 3H, H-8''), 5.35-5.28 (m br, 3H, H-3'), 5.28-5.22 (m br, 3H, H-4'), 5.22-5.16 (m br, 3H, H-2'), 5.03-4.64 (m br, 10H, 1*H-1, 3*H-1', 6*H-10''), 4.41-4.21 (m br, 9H, 3*H-6'$_A$, 6*H-7''), 4.12-4.04 (m br, 3H, 3*H-6'$_B$), 3.99-3.92 (s br, 3H, 3*H-5'), 3.80-3.62 (br, 3H, H-3,4,5,3*H-1''$_A$), 3.51-3.36 (br, 5H, H-2, H-6$_B$, 3*H-

1''$_B$), 2.13, 2.07, 2.02, 1.96 (4*s, 4*9H, O=CH$_3$) 1.93-1.79 (br, 6H, H-6''), 1.62-1.50 (br, 6H, H-2''), 1.41-1.26 (br, 18H, H-3'', 4'', 5''); ^{13}C NMR (176 MHz, $CDCl_3$) δ(ppm): 170.7, 170.2, 170.0, 169.9 (C=O), 145.0 (C-9''), 123.2 (C-8''), 97.6 (C-1,1'), 81.4 (C-3), 79.8 (C-2), 73.1 (C-4), 70.9 (C-5), 69.8 (C-2'), 69.3 (C-3'), 68.5 (C-5', C-1''), 66.6-65.4 and 63.7 (C-6, C-4', C-10''), 62.6 (C-6'), 50.4 (C-7''), 30.4 (C-6''), 29.3 (C-2''), 28.9, 26.6, 26.1 (C-3'', C-4'', C-5''), 21.0, 20.9, 20.8 (CH_3).

Compound 1: Obtained following method B in 59% yield (16 mg) as a pale yellow solid.
^{1}H NMR (700 MHz, D_2O) δ(ppm): 8.25-7.86 (br, 3H, H-8''), 4.95-4.85 (s br, 10H with a part under solvent peak, H-1, 3*H-1', 6*H-10''), 4.36 (s br, 6H, H-7''), 4.00-3.53 (m br, 27H, H-2, H-3, H-4, H-5, H-6, H-2', H-3', H-4', H-5', H-6', 3*H-1''$_A$), 3.47 (s br, 3H, 3*H-1''$_B$), 1.82 (s br, 6H, H-6''), 1.53 (s br, 6H, H-2''), 1.25 (s br, 18H, H-3'', H-4'', H-5''); ^{13}C NMR (176 MHz, D_2O) δ(ppm): 144.3 (C-9''), 124.4 (C-8''), 99.8 (C-1'), 96.3 (C-1), 79.5 (C-3), 76.8 (C-2), 72.8 (C-4, C-4'), 70.8 (C-5, C-3'), 70.2 (C-2'), 67.6 (C-1''), 66.7 (C-5'), 65.3 and 63.5 (C-6, C-10''), 60.9 (C-6'), 50.3 (C-7''), 29.7 (C-6''), 28.7 (C-2''), 28.3, 25.8, 25.4 (C-3'', C-4'', C-5'').

Compound 2: Obtained following method B in 58% yield (15.5 mg) as a pale yellow solid.
^{1}H NMR (700 MHz, D_2O) δ(ppm): 8.20-7.94 (br, 3H, H-8''), 5.98-4.82 (s br, 10H with a part under solvent peak, H-1, 3*H-1', 6*H-10''), 4.38 (s br, 6H, H-7''), 3.98-3.56 (m br, 27H, H-2, H-3, H-4, H-5, H-6, H-2', H-3', H-4', H-5', H-6', 3*H-1''$_A$), 3.48 (s br, 3H, 3*H-1''$_B$), 1.84 (s br, 6H, H-6''), 1.54 (s br, 6H, H-2''), 1.27 (s br, 18H, H-3'', H-4'', H-5''); ^{13}C NMR (176 MHz, D_2O) δ(ppm): 144.2 (C-9''), 124.5 (C-8''), 99.8 (C-1'), 96.3 (C-1), 79.3 (C-3), 77.8 (C-2), 72.8 (C-4'), 72.4 (C-4), 70.9 (C-3'), 70.3 (C-2'), 69.8 (C-5), 67.6 (C-1''), 66.7 (C-5'), 65.2 and 63.4 (C-6, C-10''), 60.9 (C-6'), 50.3 (C-7''), 29.7 (C-6''), 28.7 (C-2''), 28.3, 25.8, 25.4 (C-3'', C-4'', C-5'').

Compound 3: Obtained following method B in 61% yield (17 mg) as a pale yellow solid.
^{1}H NMR (700 MHz, D_2O) δ(ppm): 8.21-7.94 (br, 3H, H-8''), 5.08-4.83 (s br, 10H with a part under solvent peak, H-1, 3*H-1', 6*H-10''), 4.37 (s br, 6H, H-7''), 3.97-3.58 (m br, 27H, H-2, H-3, H-4, H-5, H-6, H-2', H-3', H-4', H-5', H-6', 3*H-1''$_A$), 3.47 (s br, 3H, 3*H-1''$_B$), 1.83 (s br, 6H, H-6''), 1.53 (s br, 6H, H-2''), 1.26 (s br, 18H, H-3'', H-4'', H-5''); ^{13}C NMR (176 MHz, D_2O) δ(ppm): 144.2 (C-9''), 124.4 (C-8''), 99.8 (C-1'), 96.3 (C-1), 80.8 (C-3), 79.6 (C-2), 72.8 (C-4'), 72.4 (C-4), 70.9 (C-3'), 70.3 (C-5, C-2'), 67.6 (C-1''), 66.7 (C-5'), 65.3 and 63.4 (C-6, C-10''), 60.9 (C-6'), 50.3 (C-7''), 29.7 (C-6''), 28.7 (C-2''), 28.3, 25.9, 25.5 (C-3'', C-4'', C-5'').

Compound 4: Obtained following method B in 62% yield (17.5 mg) as a pale yellow solid.
^{1}H NMR (700 MHz, D_2O) δ(ppm): 8.20-7.96 (br, 3H, H-8''), 5.08-4.82 (s br, 10H with a part under solvent peak, H-1, 3*H-1', 6*H-10''), 4.40 (s br, 6H, H-7''), 3.97-3.59 (m br, 27H, H-2, H-3, H-4, H-5, H-6, H-2', H-3', H-4', H-5', H-6', 3*H-1''$_A$), 3.48 (s br, 3H, 3*H-1''$_B$), 1.86 (s br, 6H, H-6''), 1.55 (s br, 6H, H-2''), 1.29 (s br, 18H, H-3'', H-4'', H-5''); ^{13}C NMR (176 MHz, D_2O δ(ppm): 144.2 (C-9''), 124.5 (C-8''), 99.8 (C-1'), 96.4 (C-1), 79.3 (C-3), 77.6 (C-2), 72.8 (C-4'), 72.4 (C-4), 70.9 (C-3'), 70.3 (C-2'), 69.7 (C-5), 67.6 (C-1''), 66.7 (C-5'), 65.6 and 63.5 (C-6, C-10''), 60.9 (C-6'), 50.4 (C-7''), 29.6 (C-6''), 28.7 (C-2''), 28.2, 25.8, 25.4 (C-3'', C-4'', C-5'').

Haemagglutination

Guinea pig blood was purchased from Eurogentec. Interaction of *E. coli* FimH adhesins with the glycocalyx of guinea pig erythrocytes forms a cross-linked network in the wells. Glycoconjugates, added in a 2-fold dilution series, prevent the agglutination reaction. The inhibition titre is defined as the lowest concentration of the glycoconjugate at which haemagglutination is still inhibited. *E. coli* strains were grown statically overnight in LB at 37 °C, washed three times in ice-cold phosphate-buffered saline, and redissolved. A 2-fold dilution of glycoconjugates with a starting concentration of 1 mM was prepared in 25 µL of 20 mM HEPES pH 7.4 with 150 mM NaCl. Importantly, the pipette tip was changed at every dilution step to avoid carry-over. Next, the bacterial solution (25 µL) was added to the 2-fold dilution series of the compound. Finally, 50 µL of guinea pig red blood cells, washed and diluted in the buffer to 5% of the blood volume, was added to reach 100 µL. The plates were left on ice for one night before read-out.

Adhesion assays of *E. coli* strains on intestinal epithelial cells T84

Bacteria were grown overnight at 37 °C in Luria–Bertani (LB) broth. The human intestinal cell line T84, purchased from the American Type Culture Collection (ATCC, CCL-248), was maintained in an atmosphere containing 5% CO_2 at 37 °C in the culture medium recommended by ATCC. T84 cells were seeded in 48-well tissue culture plates at a density of 1.5 × 10^5 cells/well and incubated at 37 °C for 48 h. Before infection, bacteria were incubated for 1 h with **HM**, **3**, and **5** at final concentrations of 10 µM, or with **6** at 1 µM. Epithelial cells were then infected in the presence of inhibitory compounds with the different strains for 3 h at a multiplicity of infection (MOI) of 10 bacteria per cell (1.5 × 10^6 bacteria/well). Monolayers were washed four times with PBS and lysed with 1% Triton X-100 (Sigma) in deionised water. Samples were diluted and plated onto LB agar plates to determine the number of colony-forming units (CFU). For each *E. coli* strain, the percentages of residual adhesion were determined by normalising the number of adherent bacteria in the presence of inhibitory compounds by the number of adherent bacteria in the absence of any compounds.

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Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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