DOI: 10.1002/cmdc.200900034

Synthetic Multimeric Heptyl Mannosides as Potent Antiadhesives of Uropathogenic *Escherichia coli*

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Urinary tract infections caused by uropathogenic *Escherichia coli* presents a serious communal and nosocomial health problem initiated by bacterial adhesion to the bladder cells. *E. coli* expresses fimbriae with a mannose-binding adhesin, FimH, at the tip. Heptyl α-D-mannoside (HM) is a nanomolar inhibitor of this lectin, preventing adhesion of type 1-piliated *E. coli* and reducing bacteria levels in a murine cystitis model. Herein, we described the synthesis of multimeric heptyl-mannosides with valencies ranging from one to four by copper-catalyzed azide alkyne cycloaddition (CuAAC). Biological evaluation of the multivalent compounds revealed an increase in potency compared to HM. Inhibition of bladder cell binding highlighted a promising tetravalent derivative with inhibitory concentrations 6000- and 64-fold lower than mannose and HM respectively.

The initial adhesion of pathogenic bacteria to mammalian tissues is mediated by weak carbohydrate–lectin interactions, typically with affinities in the millimolar range. Multivalent presentation of the sugar epitopes at the surface of the host cells allows the binding to become sufficiently strong for the attachment of microbes or viruses. The Gram-negative bacteria *Escherichia coli* can express several adhesive organelles such as pili or fimbriae, containing lectins termed adhesins, that interact with specific glycans on the host cells.[1] Although for many years the bacterium was simply considered to be a commensal organism of the large intestin, *E. coli* is also associated with a number of infectious diseases. Among them, urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. Women are particularly prone to UTIs because uropathogenic *E. coli* (UPEC) can reach the bladder more easily. Most uncomplicated UTIs can be treated with oral antibiotic such as nitrofurantoin or thimethoprim. However, chronic recurrence of the symptoms within months of the primary infection often occurs.[2] Once inside the bladder superficial umbrella cells, UPEC form biofilms protecting the colonies from the immune system of the host and antibiotics.[3] The bacteria can stay in the bladder for days prior to reinvade neighboring cells and re-establishing infection. The recurrence of UTIs and the emergence of antibiotic-resistant strains highlight the need for alternative treatments.

A promising approach is the development of glycomimetics as inhibitors preventing the crucial step of bacterial adhesion. This strategy is appealing because the emergence of resistance strains is unlikely, and a synergistic effect upon association with antibiotics is expectable. The most prevalent UPEC adhesin, expressed by a wide range of pathogenic strains is the monomeric FimH lectin, situated at the fibrillum tip. During the infection process, FimH binds to mannosides displayed by the glycoprotein uroplakin Ia abundantly present on uroepithelial cells. Previous studies have shown that FimH-mediated adhesion could be inhibited by natural or synthetic carbohydrates containing this sugar. Recently, heptyl-α-D-mannoside (HM) has been recognized as a strong binder to FimH with a affinity (K_d) of 5 nM, as determined by surface plasmon resonance (SPR) measurements.[4] Furthermore, this derivative inhibits both adhesion of type 1-piliated *E. coli*. on a bladder cell line and biofilm formation in vitro, and also reduces bacterial levels in a murine cystitis model.[5]

Extensive research has been carried out on multivalent inhibitors of pathogen adhesion, and a tremendous number of multimannosides based on different scaffolds have been synthesized.[6] Some of the corresponding glycoclusters with hydrophobic moieties were potent binders.[7] In principle it could be conceived that multivalent neoglycoconjugates bearing heptyl-mannosides epitopes could be efficient inhibitors with the ability to bind several FimH adhesins of UPEC simultaneously. This situation would approach that encountered in vivo where the bacteria binds to saccharides containing α-mannosides displayed in large numbers at the surface of the bladder cells. Herein, we describe the first synthesis of multiheptyl mannosides with oligo-ethylenglycol (EG) linkers by copper-catalyzed azide alkyne cycloaddition (CuAAC). To evaluate a potential "cluster effect" on the binding, we designed neoglycoconjugates **1**, **2**, **4** and **5** with valencies ranging from one to four, and similar distances between the binding epitopes. An additional divalent derivative **3**, based on a glucoside core, was also considered. Binding affinities of synthetic glycoclusters toward type-1-piliated *E. coli* were evaluated by inhibition of hemagglutination (HAI) and bladder binding assay (BBA).

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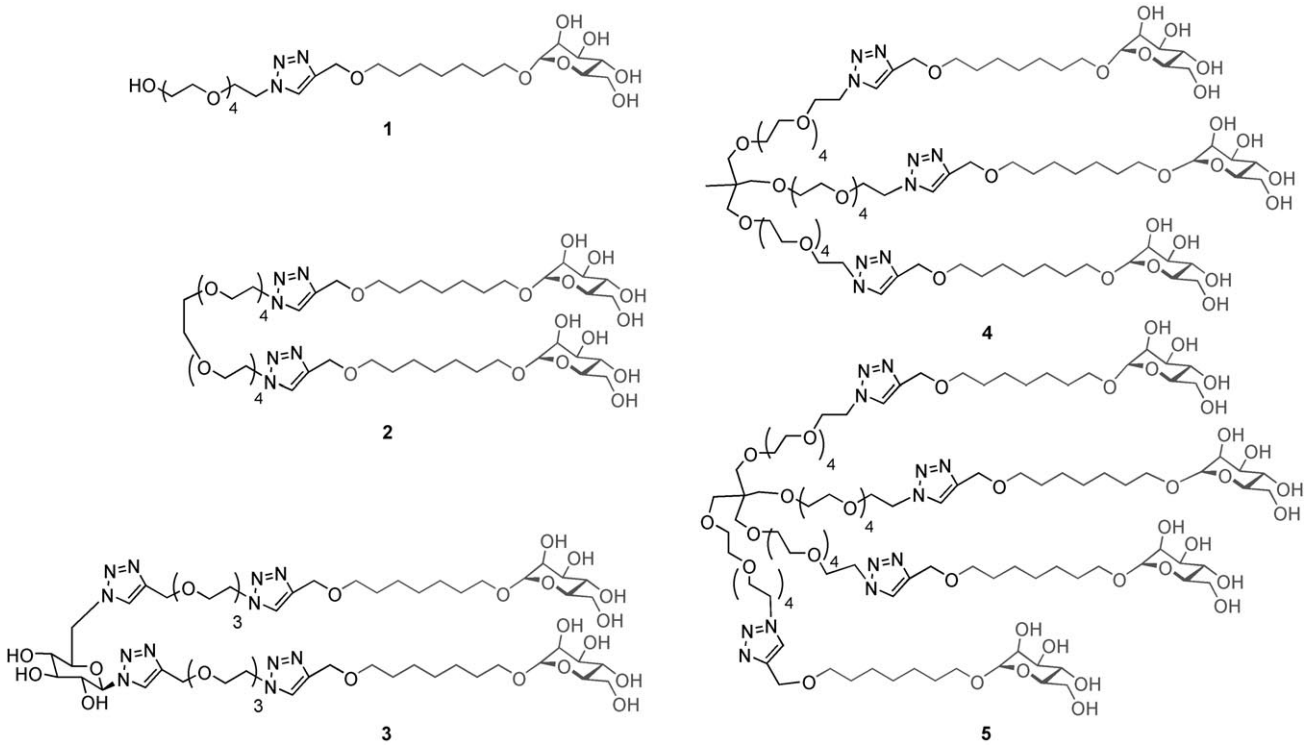
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200900034.

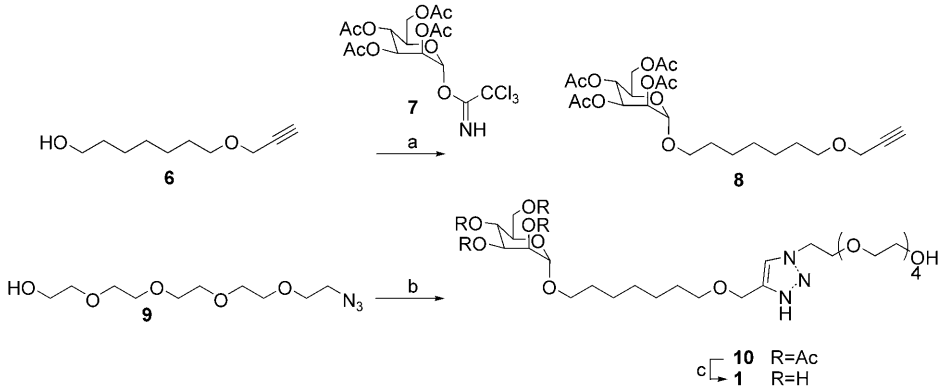


Results and Discussion

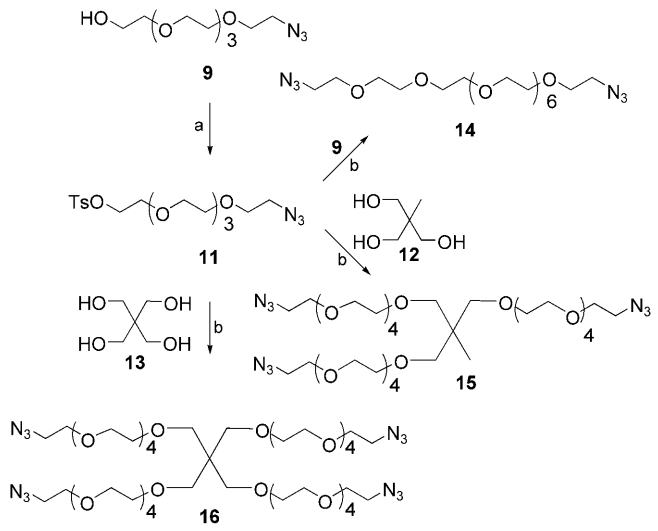
Synthesis

Flexible EG linkers were used to tether heptyl mannoside motifs in compounds **1** and **2** in order to ensure water solubility. Moreover, EG have been shown to be resistant to the non-specific adsorption of proteins.[8] Tri and tetravalent derivatives **4** and **5** were based on 1,1,1-tris(hydroxymethyl)ethane and pentaerythritol cores, respectively. These scaffolds have been successfully used recently for the design of multimeric glycoclusters.[9,10] Divalent glycoconjugate **3** was designed to evaluate the extend to which the nature of the core could influence the binding affinity for FimH. The efficient CuAAC, in terms of conversion and selectivity, was implemented to tether alkynyl armed heptyl mannosides to azide functionalized EG.[11] Standard glycosylation was first investigated to introduce the heptyl chain in the anomeric position of mannoside moieties.

Trials with mannose pentaacetate and compound **6** using Lewis acids such as $BF_3{\cdot}Et_2O$ or TMSOTf in excess, afforded the expected compound **8** with less than 20% yields (Scheme 1). Previous reports describe the synthesis of long chain alkyl glycosides with good yields by microwave-assisted solvent-free glycosylation.[12] However, in this case, no improvement was observed when this procedure was conducted with $ZnCl_2$ at 115 °C. Compound **8** could be isolated with slightly better yields by the Schmidt procedure, reacting trichloroacetamidate **7**[13] with **6** using $BF_3{\cdot}Et_2O$ at room temperature in CH_2Cl_2 (Scheme 1). CuAAC between compound **8** and the previously described azido-EG **9**[14] under microwave irradiation allowed a rapid conversion into the cycloadduct **10**, isolated in 73% yield after purification. These results corroborate preceding studies showing that microwaves could significantly improve the CuAAC reaction kinetics compared to classical heating.[15] Deacetylation under Zemplèn conditions (MeONa, MeOH) gave the unprotected monovalent derivative **1**. Azide functionalized cores presented in Scheme 2 were designed with a spacer long enough to allow a potential



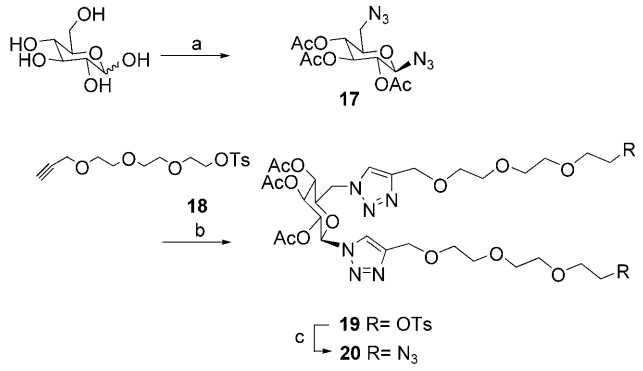
Scheme 1. *Reagents and conditions*: a) 1 equiv $BF_3{\cdot}Et_2O$, CH_2Cl_2, RT, 33%; b) 1 equiv **8**, 0.2 equiv $CuSO_4$, 0.4 equiv AscNa, MW, 70 °C, 20 min, 73%; c) MeONa, MeOH, 63%.



Scheme 2. *Reagents and conditions*: a) Ag_2O, KI, *p*TsCl, 89%; b) NaH, THF, 70 °C, ~58% (**14**, **15**), 36% (**16**).

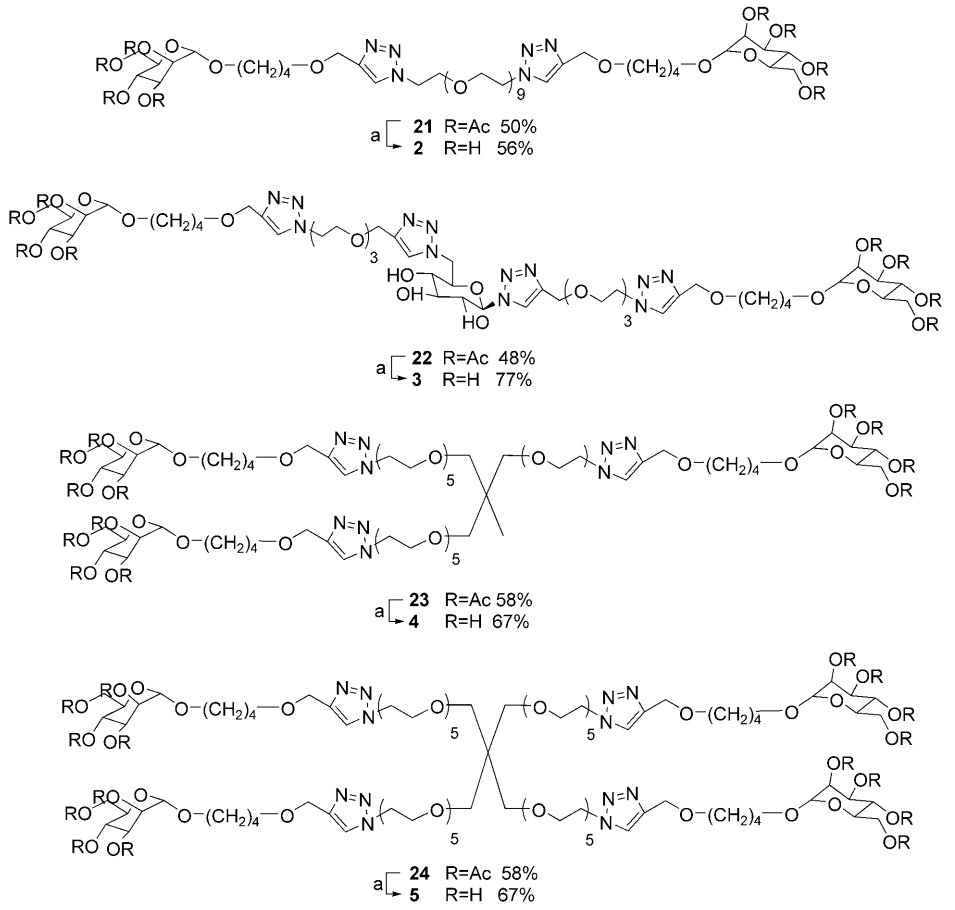
recruitment of several FimH receptor simultaneously. A tosyl group was introduced in compound **9** using *p*TsCl, KI and Ag_2O leading to **11** in 89% yield.[16] Subsequent reaction with compound **9** in the presence of NaH[17] allowed the isolation of diazido-EG **14** in 59% yield. A similar procedure was used for the synthesis of tri- and tetraazido derivatives **15** and **16** starting from 1,1,1-tris(hydroxymethyl)ethane **12** and pentaerythritol **13**, respectively. During the reactions, we observed the formation of diazido-EG **14** as a side product, which was particularly difficult to separate from compound **16** by flash chromatography.

Diazidation of D-glucose was then carried out following a direct "one pot" procedure that we previously described for a set of unprotected carbohydrates (Scheme 3).[18] Derivative **17** was obtained with $PPh_3/CBr_4/NaN_3$[19] as the azidation system and subsequent standard acetylation of the crude residue in pyridine and acetic anhydride. Microwave-assisted CuAAC conducted between compound **17** and the previously reported alkyne **18**[20] in the presence of $CuSO_4$ and sodium ascorbate allowed the incorporation of spacer arms to generate **19**, easily converted into **20** with NaN_3 in refluxing



Scheme 3. *Reagents and conditions*: a) See reference [18]; b) 0.5 equiv $CuSO_4$, 1 equiv AscNa, MW, 70 °C, 1 h, 40%; c) NaN_3, CH_3CN, reflux, 61%.

CH_3CN. The CuAAC procedure used in the synthesis of monovalent ligand **1** was repeated with azido-EG **14**, **15**, **16**, **20** and alkynyl-armed mannoside **8** to generate cycloadducts **21**, **22**, **23** and **24** in moderate yields. The CuAAC was highly regioselective yielding 1,4-disubstitued-1,2,3-triazoles identified by the large $\Delta(\delta_{C\text{-}4}-\delta_{C\text{-}5})$ values (19–22 ppm) observed in the ^{13}C NMR spectra for the different structures.[21] After Zemplèn deprotection, pure products **1**–**5** were obtained by preparative HPLC (Scheme 4).



Scheme 4. *Reagents and conditions*: a) MeONa, MeOH.

Inhibition of hemagglutination (HAI)

Binding affinities of the synthetic glycoconjugates with type 1-piliated *E. coli* UTI89 clinical isolate were first evaluated in an HAI assay. Addition of bacteria to guinea pig erythrocytes in this case, produces a cross-linked matrix due to interaction of *E. coli* FimH adhesins with the glycocalyx of the red blood cells. Subsequent additions of twofold dilutions of the synthetic sugars into the wells ultimately prevent the agglutination reaction. The inhibition titer is defined as the lowest concentration of the glycoconjugate at which hemagglutination is still inhibited. Due to serial dilutions, the maximal error is ± 1 well, meaning a factor of two. The lead ligand heptyl mannoside (HM), recorded to be a more than 650-fold better inhibitor than mannose, was used as a reference compound. The monovalent derivative **1** (Table 1) appears to be a little less potent compared with HM. This could possibly be caused by less favorable interactions between the triazolyl substituent and the end of the active site of FimH. No significant differences were observed regarding the nature of the scaffold (compounds **2** and **3**). Potency increases with valency and the tetravalent compound **5** is eightfold better than monovalent analogue **1**, meaning a twofold improvement when reported on a mannose molar basis. The inhibitory effect of the glycoconjugates on bacteria-mediated red blood agglutination thus is rather small. This could be because the red blood cells display much larger surfaces with consequently much larger multivalency for clustering the bacteria than the competing glycoconjugates.

Table 1. HAI of HM and synthetic compound **1**–**5**.

Ligand	Titer [μM]	Ratio (HM/L)
1	15.6	0.5
2	7.8	1
3	7.8	1
4	3.9	2
5	1.9	4
HM	7.8	1

Bladder binding assay (BBA)

The ability of the glycoconjugate ligands to prevent UPEC bacterial adhesion to the human uroepithelium was measured in vitro using the human bladder cell line 5637. ATCC cell line HBT09 was seeded at 3×10^4 cells mL^{-1} in a 96-well plate in RPMI/10%FCS and allowed to grow to confluency. The UTI89 UPEC strain was supplemented with a twofold dilution of the synthetic glycoconjugates **1**–**5**, or HM, in a concentration range from 100 μM to 100 pM. Upon bacterial adhesion, the bladder cells were washed, released from the plate, lysed and diluted for bacterial counting.[5] The minimal concentration of each of the sugars still causing a significant reduction in bacterial adhesion (tenfold reduction in colony forming units) is reported in Figure 1 (in grey). The valency-corrected relative binding potencies expressed in terms of molarity of mannopyranosyl residue relative to monovalent derivative **1** is also presented (in black). These results indicate strong differences in the inhibition trends as a function of valency. The experimental data indicate that compound **1** is slightly more potent than HM. The divalent derivatives **2** and **3** exhibited a remarkable cluster effect, with an inhibition efficiency 13-fold better than HM and a 3.2-fold enhancement on a molar basis relative to compound **1**. The identical affinity recorded for both divalent saccharides (**2** and **3**) corroborate HAI results showing that the nature of the scaffold do not play a significant role. Going from divalent to trivalent arrangements (**2** and **3** to **4**) led to higher potencies and valency-corrected molecular values also increased from 3.2 to 5.1. A similar trend was observed for glycoconjugate **5** bearing four sugar haptens. The last dilution of compound **5** that still inhibits is 12.2 nM, compared with 780 nM recorded for HM. Multivalent glycoconjugate **5** is thus 64-fold more potent than the best inhibitor evaluated to date in the present assay.

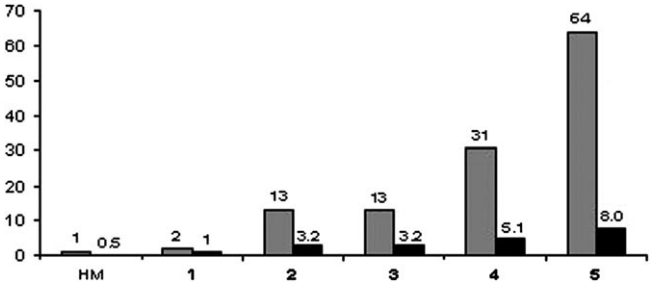


Figure 1. Binding bladder assay: Maximal dilution of sugar still causing inhibition of bacterial binding (■). Valency-corrected inhibitory potency (gain due to the cluster effect for each mannopyranosyl residues) compared to the monovalent heptyl mannose ligand **1** (■).

Conclusions

In summary, we described a short stepwise strategy for the design of glycoconjugate with valencies ranging from one to four. The crucial step of coating the scaffolds with alkynyl-armed heptyl mannosides proceeded cleanly by click chemistry to give the target saccharides. Compounds **1**–**5** obtained after Zemplèn deprotection were highly soluble in water due to the sugar moieties and hydrophilic EG linkers. Evaluation for their capacity to inhibit the binding of *E. coli* to erythrocytes in vitro revealed that multimers were more potent than heptyl α-D-mannoside. No significant differences were observed with regard to the nature of the scaffold. Unprecedented adhesion inhibitions of piliated *E. coli* to human bladder cells were recorded with the multimers. The cluster effect displayed in the BBA illustrate that the use of multivalent heptyl mannosides is a justifiable strategy to prevent bacterial adhesion and could be validated in a biological setting such as the urinary tract infection mouse model.[22] With an inhibition of bacterial bladder cell binding at 12.2 nM (~6000- and 64-fold lower than mannose[5] and HM, respectively), tetravalent compound **5** is currently one of the most promising antiadhesive drugs for the treatment of urinary tract infections under development.

Experimental Section

General Procedures: All purchased materials were used without further purification. CH_2Cl_2 was distilled over CaH_2 and THF over Na and benzophenone. Analytical TLC was carried out on DC-Alufolien Kieselgel 60 F_{254} (Merck). Flash chromatography (FC) was performed on GEDURAN SI 60, 0.040–0.060 mm pore size using distilled solvents. ^1H, and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz with a Bruker AC-300 spectrometer, respectively, and chemical shifts are reported in parts per million (ppm) relative to TMS or the residual solvent peak ($CHCl_3$: ^1H: $\delta = 7.26$, ^{13}C: $\delta = 77.2$). Peak multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), sextet (s), multiplet (m), and broad (br). HRMS where obtained by electrospray ionization (ESI) on a Micromass-Waters Q-TOF Ultima Global. Optical rotations were measured on a 343 PERKIN ELMER at 20 °C in a 1 cm cell in the stated solvent; $[\alpha]_D^{20}$ values are given in 10^{-1} deg cm^2 g^{-1} (concentration *c* given as g/100 mL). Microwave irradiation was performed in a CEM Discover apparatus (300 W). Preparative reversed-phase HPLC was accomplished on a Waters PREP LC 4000 chromatography system with a (DEDL) PL-ELS 1000 photodiode array detector. All HPLC samples were purified on a preparative Prevail C-18 column (2.2× 25 cm). The mobile phase was H_2O (solvent A) and MeOH (solvent B). The gradient consisted of 5% A for 5 min to 100% B in 45 min (22.0 mL min^{-1} flow rate).

Chemistry

General procedure for CuAAc: Compounds **10, 21**–**24** were synthesized using this procedure. A solution of alkyne **8** (100 mg, 200 μmol) and azido derivative **9** (53 mg, 200 μmol) in a dioxane/H_2O mixture (4:1, 3.5 mL) was treated with $CuSO_4$ (6 mg, 38 μmol) and AscNa (16 mg, 81 μmol) and the mixture was irradiated for 20 min at 70 °C in a sealed vessel. The mixture was poured into H_2O (5 mL) and extracted with EtOAc (3×5 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed in vacuo. The residue was purified by FC (MeOH/EtOAc, 1:4) to give **10** (111 mg, 73% yield) as a colorless oil.

4-[9-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-oxanonyl)]-1-[14-hydroxy-3,6,9,12-tetraoxatetradecyl]-[1,2,3]-triazol (10): $[\alpha]_D^{20} = +5$ ($c = 0.4$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 7.65$ (1H, s, CHTri), 5.22 (1H, dd, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.15 (1H, t, $J_{4,5}$ 9.6 Hz, H-4), 5.11 (1H, s, H-2), 4.69 (1H, d, $J_{1,2}$ 1.1 Hz, H-1), 4.50 (2H, s, OC*H*$_2$Tri), 4.43 (2H, t, *J* 5.0 Hz, CH_2CH_2N), 4.23 (1H, dd, $J_{5,6a}$ 5.3 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.05 (1H, dd, $J_{5,6b}$ 2.4 Hz, H-6b), 3.90 (1H, ddd, H-5), 3.77 (2H, t, *J* 5.0 Hz, C*H*$_2$CH$_2$N), 3.60–3.47 (17H, br, 8×CH_2O, SugOCH*H*CH$_2$), 3.40 (2H, t, *J* 6.6 Hz, C*H*$_2$OCH$_2$Tri), 3.30 (1H, br, SugOC*H*HCH$_2$), 2.90 (1H, br, OH), 2.04, 1.98, 1.93, 1.88 (12H, each s, CH_3), 1.47 (4H, br, 2 CH_2), 1.23 ppm (6H, br, 3 CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.5$, 169.9, 169.7, 169.6 (CO), 145.0 (*C*qTri), 123.6 (*CH*Tri), 97.4 (C-1), 72.5, 70.5, 70.4, 70.3, 70.1, 69.5, 69.4 (*C*H$_2$OCCH, OCH$_2$CH$_2$O, C-2, C-3), 68.3 (CH_2OSug), 68.2 (C-5), 66.1 (C-4), 64.1 (OCH$_2$Tri), 62.4 (C-6), 61.5 (CH_2OH), 50.1 (CH_2N), 29.5, 29.1, 25.9 (CH_2), 20.8, 20.6 ppm (CH_3); HRMS (ES+): *m/z* calcd for $C_{39}H_{48}N_9O_9Na$: 786.3575, found 786.3600.

Bis-1,29-[4-(9-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-oxanonyl)triazol-1-yl]-3,6,9,12,15,18,21,24,27-nonaoxanonacosane (21): $[\alpha]_D^{20} = +26$ ($c = 0.5$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 7.65$ (2H, br, CHTri), 5.25 (2H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.18 (2H, t, $J_{4,5}$ 9.6 Hz, H-4), 5.17 (2H, dd, H-2), 4.75 (2H, d, $J_{1,2}$ 1.5 Hz, H-1), 4.51 (8H, br, OCH$_2$Tri, CH$_2$C*H*$_2$N), 4.23 (2H, dd, $J_{5,6a}$ 5.3 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.05 (2H, dd, $J_{5,6b}$ 2.3 Hz, H-6b), 3.90 (2H, ddd, H-5), 3.84 (4H, t, *J* 5.0 Hz, 2×C*H*$_2$CH$_2$N), 3.60–3.47 (38H, br, 16×C*H*$_2$O, 2×SugOCH*H*CH$_2$, 2×C*H*$_2$OCH$_2$Tri), 3.30 (2H, br, 2×SugOC*H*HCH$_2$), 2.10, 2.05, 1.99, 1.94 (24H, each s, CH_3), 1.57–1.47 (8H, br, CH_2), 1.30 ppm (12H, br, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): ^{13}C NMR (75 MHz, DMSO): $\delta = 170.0$, 169.7, 169.5 (CO), 144.0 (*C*qTri), 124.1 (*CH*Tri), 96.5 (C-1), 69.8, 69.6, 69.5, 68.8, 67.9, 67.4, 65.4, 63.3 (OCH_2Tri, *C*H$_2$OCCH, *C*H$_2$Osug, OCH_2CH$_2$O, (C-2, C-3, C-4, C-5) M = Mannose), 62.0 (C-6), 49.3 (CH_2N), 29.1, 28.6, 25.5 (CH_2), 20.6, 20.5, 20.4 ppm (CH_3); HRMS (ES+): *m/z* calcd for $C_{64}H_{108}N_{12}O_{29}Na$: 1531.7243, found 1531.7253.

1-{2,3,4-Tri-*O*-acetyl-6-deoxy-6-[4-[4-(9-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-3,6,9-trioxadecyl]-triazol-1-yl]-β-D-glucopyranos-1-yl}-4-{4-((9-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-3,6,9-trioxadecyl}-[1,2,3]-triazol (22): $[\alpha]_D^{20} = +24$ ($c = 0.2$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 7.78$, 7.72, 7.71, 7.53 (4H, each s, C*H*Tri), 5.85 (1H, d, $J_{1,2}$ 8.6 Hz, H-1 βG), 5.44 (1H, t, $J_{1,2} = J_{2,3}$ 8.6 Hz, H-2 G), 5.39 (1H, t, $J_{3,4}$ 9.5 Hz, H-3 G), 5.29 (2H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, 2×H-3 M), 5.21 (2H, t, $J_{4,5}$ 9.6 Hz, 2×H-4 M), 5.20 (2H, s, 2×H-2 M), 5.02 (1H, t, $J_{4,5}$ 9.5 Hz, H-4 G), 4.79 (2H, d, $J_{1,2}$ 1.5 Hz, 2×H-1 M), 4.66–4.50 (10H, br, 4×OCH_2Tri, 2×H-6b G), 4.28 (3H, br, 2×H-6 aM, H-6a G), 4.10 (2H, dd, $J_{5,6b}$ 2.4 Hz, 2×H-6b M), 3.95 (2H, ddd, 2×H-5 M), 3.87 (4H, 2×C*H*$_2$CH$_2$N), 3.67–3.50 (27H, m, 12×C*H*$_2$O, 2×ManOCH*H*CH$_2$, H-5G), 3.40 (2H, br, 2×ManOC*H*HCH$_2$), 2.15, 2.10, 2.04, 1.98 (24H, 4×s, 8×CH_3CO M), 2.11, 2.03, 1.85 (9H, 3×s, 3×CH_3CO G), 1.57 (8H, br, CH_2), 1.23 ppm (12H, br, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.8$, 170.2, 169.9, 169.7 (CH_3CO), 146.1, 145.5 (CTri), 124.1, 123.8, 121.3 (CHTri), 97.7 (C-1 M), 85.5 (C-1 G), 72.6, 70.9, 70.6, 70.2, 70.0, 69.8, 69.6, 69.3, 69.1, 68.6, 68.5 (CH_2O, C-2 to C-6 G, C-2, C-3 M, C-5 M), 66.4 (C-4 M, 64.6 (OCH$_2$Tri), 62.6 (C-6 M), 50.7, 50.5 (C-6 G, CH_2N), 29.7, 29.3, 26.1 (CH_2), 21.0, 20.8, 20.6, 20.3ppm (*C*H$_3$CO); HRMS (ES+): *m/z* calcd for $C_{78}H_{118}N_{12}O_{35}$: 1805.7744, found 1805.7767.

1.1.1-Tris-[16-(4-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-2,5,8,11,14,-pentaoxahexadecyl]ethane (23): $[\alpha]_D^{20} = +26$ ($c = 0.7$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 5.30$ (3H, dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.21 (3H, t, $J_{4,5}$ 9.6 Hz, H-4), 5.18 (3H, br, H-2), 4.74 (3H, s, $J_{1,2}$ 1.5 Hz, H-1), 4.61 (6H, br, 3×CH$_2$C*H*$_2$N), 4.20 (3H, dd, $J_{5,6a}$ 5.2 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.04 (3H, dd, $J_{5,6b}$ 2.3 Hz, H-6b), 3.90 (9H, br, H-5, 3×C*H*$_2$CH$_2$N), 3.60–3.47 (60H, br, 24×C*H*$_2$O, 3×C*H*$_2$OCH$_2$Tri, 3×SugOCH$_2$CH$_2$), 3.24 (6H, s, CH$_3$C(C*H*$_2$)$_3$O), 2.10, 2.04, 1.99, 1.94 (36H, each s, CH_3), 1.57–1.47 (12H, br, CH_2), 1.30 (18H, br, CH_2), 0.86 ppm (3H, s, CH_3); ^{13}C NMR (75 MHz, DMSO): $\delta = 170.0$, 169.7, 169.5 (CO), 144.0 (CTri), 124.1 (CHTri), 96.5 (C-1), 73.0, 69.8, 69.5, 68.8, 67.8, 65.4 (*C*H$_2$OCCH, OCH$_2$CH$_2$O, OCH$_2$Tri, (C-2, C-3, C-4, C-5) M), 62.0 (C-6), 49.3 (CH_2N), 29.1, 28.6, 25.5 (CH_2), 20.4 ppm (*C*H$_3$CO), 17.2 (CH_3); HRMS (ES+): *m/z* calcd for $C_{107}H_{175}N_9O_{48}Na$: 2379.1584, found 2379.1616.

Tetrakis-[16-(4-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-2,5,8,11,14,-pentaoxahexadecyl]methane (24): $[\alpha]_D^{20} = +23$ ($c = 0.6$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 7.70$ (br, C*H*Tri), 5.30 (4H, dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.20 (4H, t, $J_{4,5}$ 9.6 Hz, H-4), 5.19 (4H, s, H-2), 4.76 (4H, s, $J_{1,2}$ 1.5 Hz, H-1), 4.61 (8H, br, 4×CH$_2$C*H*$_2$N), 4.21 (4H, dd, $J_{5,6a}$ 5.2 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.04 (4H, dd, $J_{5,6b}$ 2.3 Hz, *H*-6b), 3.90 (4H, br, *H*-5), 3.83 (8H, br, 4×C*H*$_2$CH$_2$N), 3.60–3.30 (96H, br, 36×C*H*$_2$O, 4×C*H*$_2$OCH$_2$Tri, 4×SugOCH$_2$CH$_2$, C(C*H*$_2$)$_4$), 2.11, 2.06, 2.01, 1.95 (48H, each s, CH_3), 1.57–1.47 (16H, br, C*H*$_2$), 1.30 ppm (24H, br, C*H*$_2$); ^{13}C NMR (75 MHz, DMSO): $\delta = 170.0$, 169.7, 169.5 (CO), 144.0 (CTri), 124.1 (CHTri), 96.5 (*C*-1), 70.5, 69.8, 69.6, 69.5, 68.8, 68.7, 67.9, 67.4, 65.5, 63.3

(CH_2OCCH, OCH_2Tri, OCH_2CH_2O, (C-2, C-3, C-4, C-5) M, CH_2OSug), 62.0 (C-6), 49.3 (CH_2N), 45.1 ($C(CH_2O)_4$), 29.1, 28.7, 28.6, 25.5 (CH_2), 20.6, 20.5, 20.4 ppm (CH_3CO); HRMS (ES+): *m/z* calcd for $C_{141}H_{230}N_{12}O_{64}Na$ requires 3140.5166, found 3140.5246.

General procedure for deacetylation: Compounds **1**–**5** were synthesized using this procedure. Cycloadduct **10** (40 mg, 52 μmol) was dissolved in dry MeOH (2.5 mL), treated with a solution of MeONa in MeOH (100 μL, 1 M) and the mixture was stirred under N_2 at RT for 3 h. Amberlyst IR120 (H^+) was added and the mixture stirred until it reached pH 5. The resin was filtered off and the solution was concentrated. Preparative HPLC purification (see General Methods) gave the unprotected product **1** (21 mg, 67% yield).

4-[9-(α-D-mannopyranosyl)-2-oxanonyl)]-1-[14-hydroxy-3,6,9,12-tetraoxatetradecyl]-[1,2,3]-triazol (1): $[\alpha]_D^{20} = +31$ ($c = 0.2$, H_2O); ^{1}H NMR (300 MHz, D_2O) $\delta = 8.08$ (1H, s, CHTri), 4.86 (1H, s, $J_{1,2}$ 1.6 Hz, *H*-1), 4.66 (4H, s, OCH_2Tri, CH_2CH_2N), 4.56–3.42 (30H, H-2 to H-6, $SugOCH_2$, CH_2OCH_2Tri, CH_2-EG), 1.59 (4H, br, 2 CH_2), 1.32 ppm (6H, br, $3CH_2$); ^{13}C NMR (75 MHz, D_2O) $\delta = 144.1$ (CqTri), 125.5 (CHTri), 99.6 (C-1), 72.7, 71.7, 70.6, 70.4, 70.1, 69.6, 69.5, 69.4, 68.8, 67.9, 66.7 (OCH_2CH_2O, C-2,3,4,5, CH_2OSug), 62.6 (OCH_2Tri), 60.9 (C-6), 60.3 (CH_2OH), 50.0 (CH_2N), 28.4, 28.1, 25.3, 25.1 ppm (CH_2); HRMS (ES+): *m/z* calcd for $C_{26}H_{49}N_3O_{12}Na$: 618.3214, found 618.3200.

Bis-1,29-[4-(9-(α-D-mannopyranosyl)-2-oxanonyl)triazol-1-yl]-3,6,9,12,15,18,21,24,27-nonaoxanonacosane (2): $[\alpha]_D^{20} = +25$ ($c =$ 0.9, H_2O); ^{1}H NMR (300 MHz, D_2O) $\delta = 8.13$ (2H, s, C*H*Tri), 4.86 (1H, s, $J_{1,2}$ 1.4 Hz, H-1), 4.65 (8H, s,$2\times OCH_2Tri$, $2\times CH_2CH_2N$), 4.00–3.49 (60H, H-2 to H-6, $SugOCH_2$, CH_2OCH_2Tri, CH_2-EG), 1.59 (8H, br, $4\times$ CH_2), 1.32 ppm (12H, br, $6\times CH_2$); ^{13}C NMR (75 MHz, D_2O) $\delta = 99.6$ (C-1), 72.7, 70.7, 70.4, 70.1, 69.6, 68.8, 67.8, 66.7 (CH_2OCCH, OCH_2CH_2O, *C*-2,3,4,5, CH_2OSug), 62.7 (OCH_2Tri), 60.9 (C-6), 50.1 (CH_2N), 28.5, 28.2, 25.3, 25.1 ppm (CH_2); HRMS (ES+): *m/z* calcd for $C_{52}H_{96}N_6O_{23}Na_2$: 1195.6425, found 1195.6404.

1-{6-deoxy-6-[4-[4-(9-(α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-3,6,9-trioxadecyl]triazol-1-yl]-β-D-glucopyranos-1-yl}-4-{4-((9-(α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-3,6,9-trioxadecyl}-[1,2,3]-triazol (3): $[\alpha]_D^{20} = +28$ ($c = 0.1$, D_2O); ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 8.19$, 8.03, 8.02, 7.90 (4H, s, CHTri), 5.70 (1H, d, $J_{1,2}$ 9.1 Hz, H-1 βG), 4.83 (4H, s, $J_{1,2} < 1$ Hz, H-1 M), 4.70–4.54 (8H, br, $4\times OCH_2Tri$), 4.20 (2H, ddd, $2\times$H-5 G), 3.90–3.40 (br, CH_2OPEG, $ManOCH_2CH_2$, CH_2OCH_2Tri, H-2,3,4 M, H-6 M, H-2 M to H-6 M), 1.51 (8H, br, CH_2), 1.24 ppm (12H, br, CH_2); ^{13}C NMR (75 MHz, D_2O): $\delta =$ 144.3, 144.1, 143.8 (CqTri), 125.9, 125.4, 124.1 (CHTri), 99.6 (C-1 M), 87.3 (C-1G), 76.4, 75.6, 72.7, 72.1, 70.6, 70.3, 70.1, 69.7, 69.5, 69.1, 68.8, 68.7, 67.8, 66.7 (CH_2O, C-2 G to C-6 G, (C-2, C-3, C-5) M), 63.0, 62.6 (OCH_2Tri), 60.8 (C-6 M), 50.7, 50.0 (C-6 G), 28.5, 28.4, 28.1, 25.3, 25.1 ppm (CH_2); HRMS (ESI+): *m/z* calcd for $C_{56}H_{96}N_{12}O_{24}Na$: 1343.6558, found 1343.6526.

1.1.1-Tris[16-(4-(α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-2,5,8,11,14,-pentaoxahexadecyl]ethane (4): $[\alpha]_D^{20} = +25$ ($c = 0.7$, H_2O); ^{1}H NMR (300 MHz, D_2O) $\delta = 8.09$ (3H, s, CHTri), 4.84 (3H, s, $J_{1,2}$ 1.4 Hz, H-1), 4.63 (12H, s, $3\times OCH_2Tri$, $3\times CH_2CH_2N$), 3.98–3.54 (96H, H-2 to H-6, $SugOCH_2$, CH_2OCH_2Tri, CH_2-EG), 3.38 (6H, s, $CH_3C(CH_2)_3O$), 1.58 (12H, br, CH_2), 1.30 (18H, br, CH_2), 0.91 ppm (3H, s, CH_3); ^{13}C NMR (75 MHz, D_2O) $\delta = 99.6$ (C-1), 73.5, 72.7, 70.7, 70.6, 70.3, 70.1, 69.7, 68.8, 67.8, 66.7 (CH_2OCCH, OCH_2CH_2O, C-2, C-3, C-4, C-5, CH_2OSug), 62.7 (OCH_2Tri), 60.9 (C-6), 50.1 (CH_2N), 40.5 (CH_3C), 28.6, 28.5, 28.2, 25.3, 25.2 (CH_2), 16.8 ppm (CH_3). HRMS (ES+): *m/z* calcd for $C_{83}H_{151}N_9O_{36}Na$: 1875.0316, found 1875.0344.

Tetrakis-[16-(4-(α-D-mannopyranosyl)-2-oxanonyl)-triazol-1-yl)-2,5,8,11,14,-pentaoxahexadecyl]methane (5): $[\alpha]_D^{20} = +26$ ($c = 0.6$, H_2O); ^{1}H NMR (300 MHz, D_2O) $\delta = 8.10$ (4H, s, C*H*Tri), 4.87 (4H, s, $J_{1,2}$ 1.5 Hz, H-1), 4.64 (16H, s, $4\times OCH_2Tri$, $4\times CH_2CH_2N$), 3.99–3.49 (120H, *H*-2 to *H*-6, $4\times SugOCH_2$, $4\times CH_2OCH_2Tri$, $36\times CH_2$-EG, C-$(CH_2)_4O$), 1.59 (16H, br, CH_2), 1.30 ppm (24H, br, CH_2); ^{13}C NMR (75 MHz, D_2O) $\delta = 144.0$ (CqTri), 125.4 (C*H*Tri), 99.7 (C-1), 72.7, 70.6, 70.4, 70.1, 69.7, 68.8, 67.8, 66.7 (CH_2OCCH, OCH_2CH_2O, C-2,3,4,5, CH_2OSug), 62.7 (OCH_2Tri), 60.9 (C-6), 50.1 (CH_2N), 45.1 ($C(CH_2O)_4$), 28.5, 28.3, 25.4, 25.2 ppm (CH_2) ; HRMS (ES+): *m/z* calcd for $C_{109}H_{198}N_{12}O_{48}Na_2$: 2468.3477, found 2468.3440.

Biology

Inhibition of Hemagglutination (HAI): Inhibition of guinea pig red blood cell hemagglutination by the type-1-piliated uropatogenic *E. coli* strain UTI89 by the newly synthesized glycoconjugates **1**–**5**. A twofold dilution of glycoconjugates was prepared, starting at 1 mM, in 25 μL phosphate-buffered saline (PBS: 150 mM NaCl, 15 mM Na/KH_2PO_4, pH 7.4). UTI89 cells were grown statically overnight in LB, washed and diluted to OD_{600nm} 0.5 in PBS. The bacterial solution (50 μL) were added to the test compound solutions. Finally 25 μL of guinea pig red blood cells, washed in PBS and diluted to 5%, were added. The negative control (−) contained PBS instead of bacteria and sugar ligand, the positive control (+) contained PBS instead of sugar.

Bladder binding assay (BBA): HM and glycoconjugates **1**–**5** were diluted in a twofold series from 100 μM to 100 pM concentrations in a 96-well plate. 2×10^6 bacteria mL^{-1} were added per well, together with the sugar composing 100 μL to be added on top of the monolayer of 5637 bladder cells. Bacteria were allowed to bind for 0.5 h under shaking conditions (150 rpm). Bladder cells were washed in PBS, released from the plate using trypsin and titrated to determine the output bacterial count (as described in reference [5]).

Acknowledgements

This work was carried out with financial support from the Centre National de la Recherche Scientifique, the Ministère Délégué à l'Enseignement Supérieur et à la Recherche and the Conseil Régional de Picardie. Julie Bouckaert was a postdoctoral fellow for the FWO.

Keywords: antiadhesive drugs · antibiotics · *E. coli* · click chemistry · multivalency

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Received: January 27, 2009
Published online on ■■ ■■, 2009

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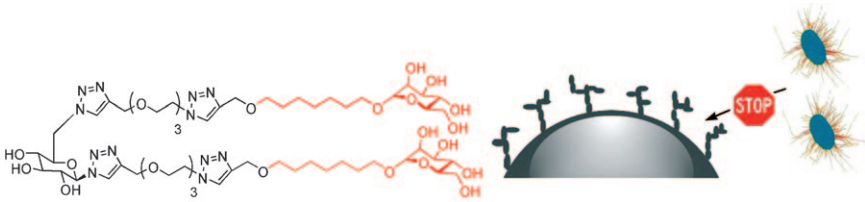
Synthetic Multimeric Heptyl Mannosides as Potent Antiadhesives of Uropathogenic *Escherichia coli*



Sweet medicine: Multimeric glycoconjugates with valencies ranging from one to four were synthesized by click chemistry. Unprecedented adhesion inhibitions of piliated *E. coli* to human bladder cells were recorded with the multimers; a tetravalent derivative showed inhibitory concentrations 6000- and 64-fold lower than mannose and heptyl α-D-mannoside, respectively.

