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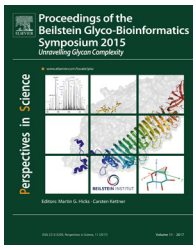

2-*C*-Branched mannosides as a novel family of FimH antagonists—Synthesis and biological evaluation☆

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Summary Urinary tract infections (UTIs), which are among the most prevalent bacterial infections worldwide, are mainly attributed to uropathogenic *Escherichia coli* (UPEC). Because of frequent antibiotic treatment, antimicrobial resistance constitutes an increasing therapeutic problem. Antagonists of the mannose-specific bacterial lectin FimH, a key protein mediating the adhesion of UPEC to human bladder cells, would offer an alternative anti-adhesive treatment strategy. In general, FimH antagonists consist of a mannose moiety and a wide range of lipophilic aglycones. Modifications of the mannose core led to a distinct drop in affinity. A visual inspection of the crystal structure of FimH revealed a previously unexplored cavity surrounded by Ile13, Phe142 and Asp140, which could be reached by functional groups in the equatorial 2-position of the mannose. Here, we describe the synthesis of 2-*C*-branched mannosides and evaluation of their pharmacodynamic properties. ITC experiments with the selected antagonists revealed a drastic enthalpy loss for all 2-*C*-branched antagonists, which, however, is partially compensated by an entropy gain. This supports the hypothesis that the target cavity is too small to accommodate 2-*C*-substituents.

Abbreviations: UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection; CRD, carbohydrate-recognition domain; IC_{50}, half maximal inhibitory concentration; ITC, isothermal titration calorimetry; K_D, dissociation constant.

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Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections affecting millions of people (Foxman et al., 2000). They are mainly associated with uropathogenic *Escherichia coli* (UPEC) (Roland, 2002). Currently, the first-line treatment involves antibiotics (Hooton et al., 2004; Fihn, 2003) which can induce resistance, especially when frequently applied (Sanchez et al., 2012). Therefore, novel and efficient non-antibiotic approaches are urgently needed.

In the first step of the infection cycle, UPEC attach to urothelial cells of the host by means of the bacterial adhesin called FimH, which is located at the tip of the approximately 300 bacterial type 1 pili (Mulvey et al., 2000; Schilling et al., 2001). This allows UPEC to evade elimination from the host organism by the bulk flow of the urine. FimH is composed of a lectin domain ($FimH_{LD}$) containing a carbohydrate recognition domain (CRD) and a pilin domain ($FimH_{PD}$) regulating the switch between the high and low affinity states of the CRD (Le Trong et al., 2010).

More than thirty years ago, Firon et al. (1982, 1983, 1987) reported on aryl α-D-mannosides abolishing FimH-mediated aggregation of UPEC with mannan-containing yeast cells (*Saccharomyces cerevisiae*) in *in vitro* assays. Over the course of the last few years, a range of highly potent monovalent antagonists consisting of a mannose moiety and a lipophilic aglycone was reported (Bouckaert et al., 2005; Sperling et al., 2006; Han et al., 2010; Klein et al., 2010; Cusumano et al., 2011; Han et al., 2012; Pang et al., 2012; Jiang et al., 2012; Schwardt et al., 2011; Kleeb et al., 2015; Brument et al., 2013; Jarvis et al., 2016; Chalopin et al., 2016). The various aglycones provide hydrophobic contacts or π–π stacking interactions to amino acids forming the entrance to the mannose binding pocket. This entrance called 'tyrosine gate' is composed of two tyrosines and one isoleucine. However, the pharmacokinetic properties, e.g., solubility and/or permeability, of most of the reported FimH antagonists are not suitable for an oral application. For physicochemical and pharmacokinetic reasons, the numerous reported multivalent FimH antagonists (Lindhorst et al., 1998; Nagahori et al., 2002; Appeldoorn et al., 2005; Patel and Lindhorst, 2006; Touaibia et al., 2007; Durka et al., 2011; Bouckaert et al., 2013) are rather suited for the therapy of *E. coli* induced colitis ulcerosa, a form of inflammatory bowel disease (Barnich et al., 2007; Carvalho et al., 2009).

When interacting with FimH, the mannose moiety establishes a perfect hydrogen bond network (Hung et al., 2002). Since every hydroxyl group of mannose is part of this network, the removal/replacement of individual various hydroxyl groups or the replacement of the whole mannose moiety by other hexoses (e.g., glucose, galactose, fructose) resulted in a significant loss of affinity (Bouckaert et al., 2005; Han et al., 2010; Old, 1972; Fiege et al., 2015). Moreover, recently reported 1-*C*-branched mannose derivatives bearing additional equatorial groups at the anomeric carbon also showed reduced activity compared to methyl α-D-mannoside (Gloe et al., 2015). In contrast, when the anomeric oxygen was replaced by carbon or nitrogen, nanomolar affinity could still be reached (Schwardt et al., 2011; Brument et al., 2013; Chalopin et al., 2016).

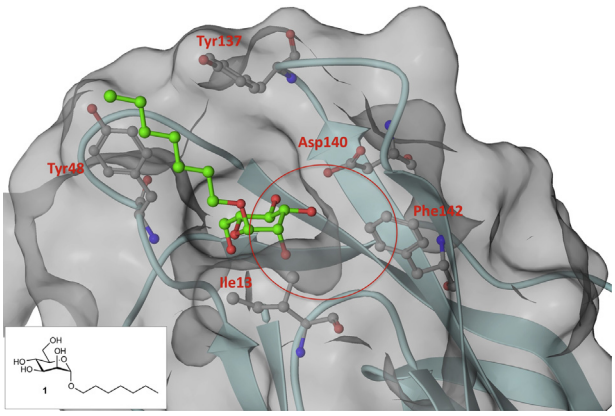


Figure 1 The crystal structure of $FimH_{LD}$ co-crystallized with *n*-heptyl α-D-mannopyranoside (**1**, PDB ID: 4BUQ) (Fiege et al., 2015). A mainly hydrophobic cavity formed by Ile13, Phe142 and Asp140 is located next to the entrance of the mannose binding site and can be reached by equatorial substituents in the 2-position of the mannose moiety.

A visual inspection of the crystal structure of $FimH_{LD}$ co-crystallized with *n*-heptyl α-D-mannoside (**1**, PDB ID: 4BUQ) (Fiege et al., 2015) revealed a previously unexplored hydrophobic cavity formed by Ile13, Phe142 and Asp140, which is located close to the entrance to the mannose-binding pocket (Fig. 1). By extending the 2-position of the mannose moiety with equatorial substituents (→ derivatives **2a**–**k**, Fig. 2), an interaction with the hydrophobic cavity should become possible.

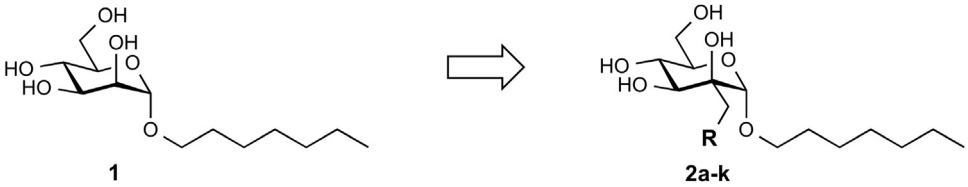
An adaption of the synthetic pathway of previously reported 2-*C*-branched mannose derivatives, in which the 2-position is modified at an early stage, lead to rather laborious approaches (Mitchell et al., 2007). We therefore planned a more convergent synthesis with a more flexible introduction of aglycones as well as equatorial substituents in the 2-position.

Result and discussion

The synthetic route to 2-*C*-branched FimH antagonists fulfils two requirements: The facile introduction of various aglycones as well as various equatorial *C*-substituents in the 2-*C*-position of the mannose moiety.

Synthesis

The synthesis of the 2-*C*-branched mannoside donor **5** is depicted in Scheme 1. The 2-*C*-modified D-mannofuranose **3** was synthesized according to a literature procedure starting from commercially available D-mannose (Witczak et al., 1984). Selective benzylation of the hydroxymethyl group using dibutyltin oxide (Malleron and David, 1998) followed by cleavage of the acetonides under acidic conditions yielded the 2-*C*-branched D-mannopyranose **4** (Waschke et al., 2011). For its perbenzoylation with benzoyl chloride in presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) in dry pyridine, elevated temperature



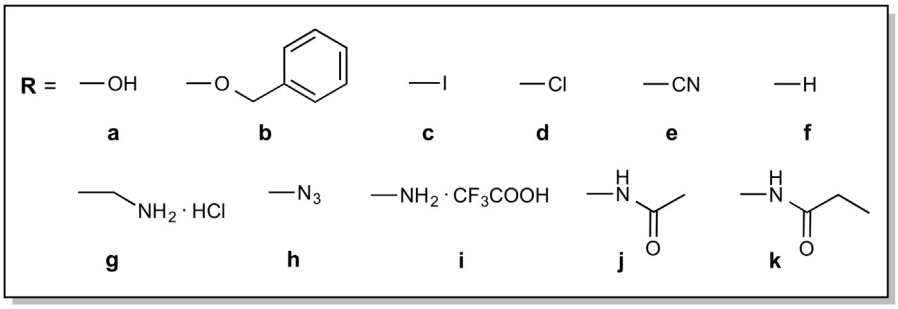


Figure 2 Modifications of the mannose moiety of *n*-heptyl α-D-mannopyranoside (**1**) by equatorial substituents in the 2-position.

(110—120 °C) had to be applied. Subsequently, the glycosyl donor **5** was obtained by reaction with thiophenol using $BF_3{\cdot}Et_2O$ as a promoter. To couple donor **5** with 1-heptanol, different promoters were tested. Whereas with NIS/TMSOTf or NIS/TfOH donor **5** was only partially consumed after 24 h, it reacted within minutes in the presence of commercially available *p*-nitrobenzenesulfenyl chloride (*p*-NO_2PhSCl) accompanied by silver triflate (AgOTf) (Crich et al., 2008). Apart from the desired α-anomeric mannoside **6** (32%), the 2-OH deprotected α-anomer **7** (32%) and the 2-OH deprotected β-anomer **8** (5%) were obtained as well. As silver triflate-mediated glycosylation has been reported to lead to partial transesterification affecting acetyl groups at the 2-*O*-position, low stereoselectivity of the glycosylation reaction was not unexpected (Ziegler et al., 1990; Nukada et al., 1999; Murakami et al., 2007).

To functionalize the equatorial substituent in the 2-*C*-position, **6** was debenzylated by catalytic hydrogenolysis to afford the primary alcohol **9**. However, attempts to mesylate its primary hydroxyl group failed. Since we attributed the low reactivity of the hydroxyl group in **9** to steric hindrance, we switched to mannoside **7** with an unprotected axial hydroxyl group in the 2-position.

Indeed, after hydrogenolysis of **7** (→**11**), we were able to selectively mesylate the primary hydroxyl groups (→**12**). However, displacement of the mesylate by fluoride using KF in aprotic solvent in presence of crown ether at elevated temperature afforded epoxide **14** instead of the desired fluoride **13**. Under these reaction conditions, the strongly basic fluoride is obviously deprotonating the axial hydroxyl group followed by conversion of mesylate **12** into the epoxide **14** by an intramolecular S_N2 mechanism. With an excess of LiCl, epoxide **14** could be opened, leading to the chloride **15** in 41% yield. By acting as Lewis acid, the large excess of lithium ions can facilitate opening of the epoxide. Apart from the epoxide route, substituents can be introduced directly by nucleophilic substitution (see Scheme 2). However, prevalence of one mechanism over the other may depend on the nucleophile, i.e., its nucleophilicity and basicity as well as temperature and concentration.

The synthesis of a series of 2-*C*-branched FimH antagonists is depicted in Scheme 2. Debenzoylation of **6** under Zemplén conditions (→**2b**) followed by $Pd(OH)_2$-catalyzed hydrogenolysis afforded test compound **2a**. The configuration at the anomeric carbon of deprotected derivative **2a** ($^1J_{H,C}$ = 169 Hz) was unambiguously confirmed by the $^{13}C—^1H$ coupling constant of the anomeric nuclei using undecoupled ^{13}C NMR. In general, the coupling constant for the equatorial anomeric proton amounts to ~170 Hz, while a value of ~160 Hz is indicative for an anomeric proton in axial orientation (Bubb, 2003).

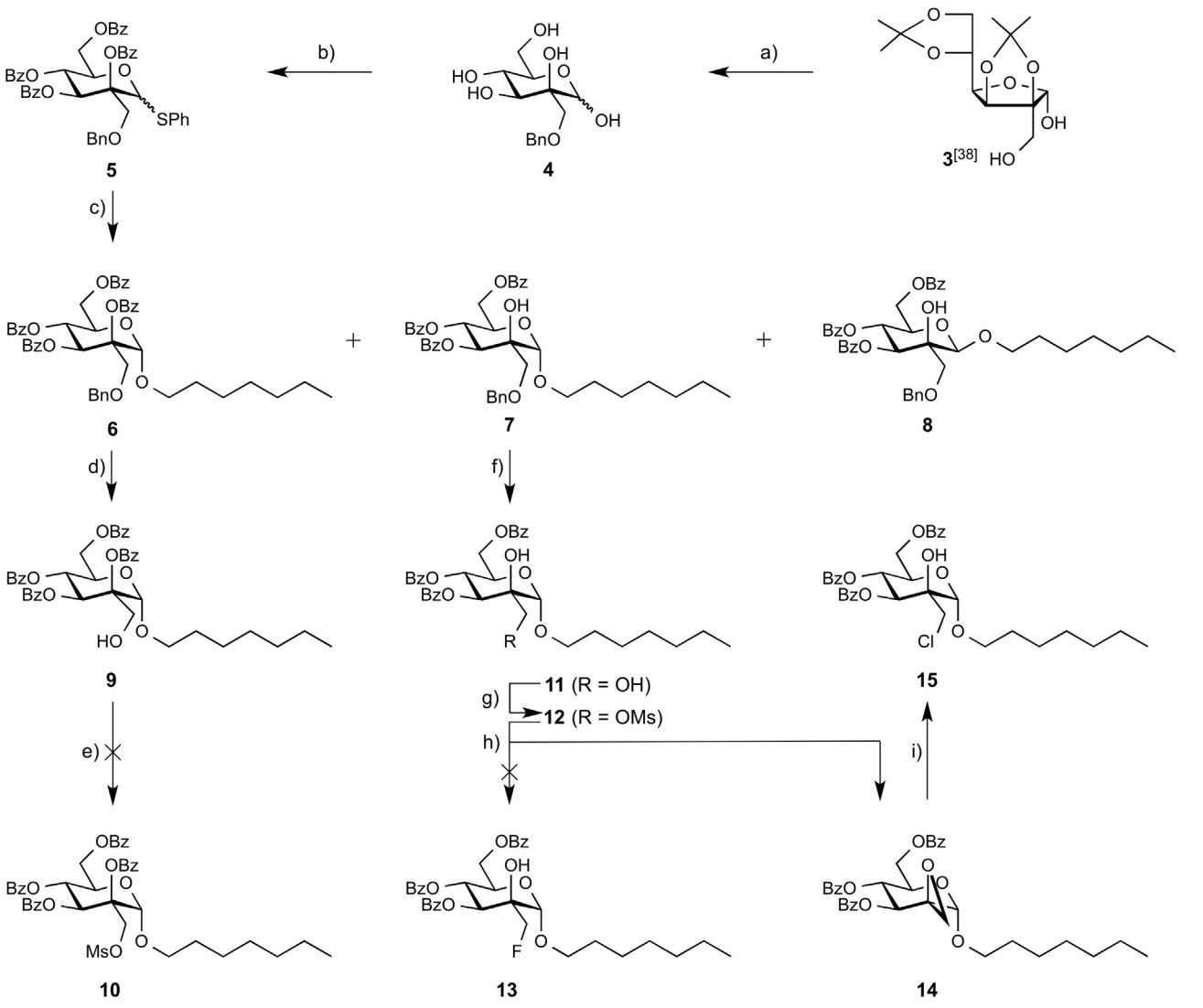
Upon mesylation of **11**, chloride was introduced using LiCl followed by deprotection of the intermediate with sodium methoxide to afford derivative **2d**. Direct introduction of chloride starting from mesylate was faster and gave a higher yield than the already discussed opening of epoxide **14**. Since only traces of epoxide were observed by TLC control, S_N2 reaction seems to be the prevailing mechanism in this particular case. However, more basic nucleophiles may lead to different results.

Using an identical synthetic approach, iodide **2c**, cyanide **2e** and azide **2h** were obtained with NaI, KCN and NaN_3 as nucleophiles. Hydrogenation of iodide **2c,** cyanide **2e** and azide **2h** in presence of $Pd(OH)_2$ on carbon yielded the methyl derivative **2f** and amine derivatives **2g** and **2i**, respectively. In addition, when **2h** was hydrogenated and subsequently acylated with acetyl chloride or propionyl chloride followed by deacetylation under Zemplén conditions, amides **2j** and **2k** were obtained.

To evaluate the β-anomeric derivative as well in the biological assay, β-mannoside **8** was debenzoylated (→**16b**, Scheme 3) followed by hydrogenolysis to yield **16a** ($^1J_{H,C}$ = 159 Hz).

Affinity and thermodynamic profile

The affinities of the 2-*C*-branched mannosides were determined in a cell-free competitive binding assay (Table 1) (Rabbani et al., 2010). The assay uses $FimH_{LD}$-Th-His_6 (Th: thrombin cleavage site) as a target protein and a

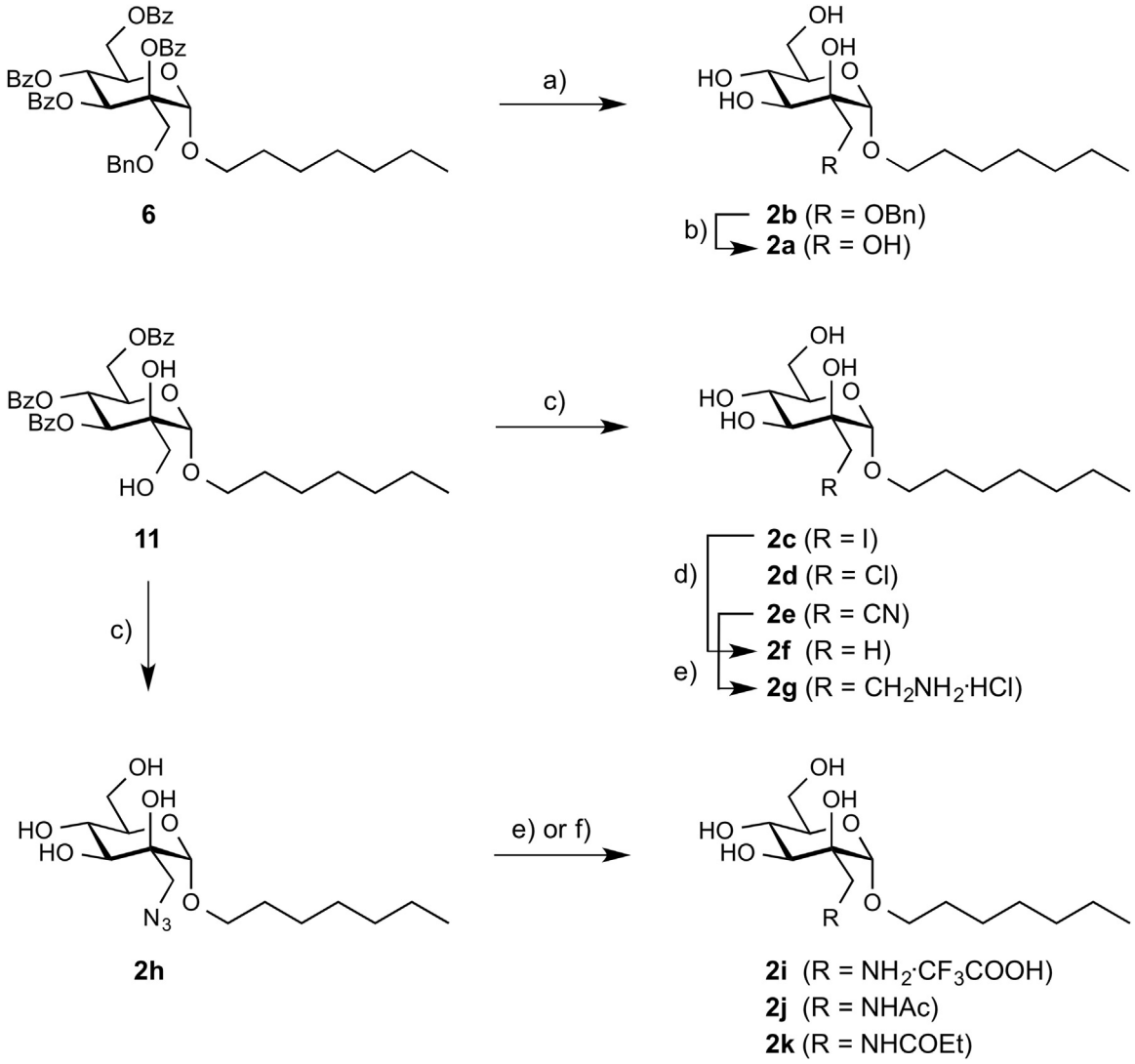


Scheme 1 (a) i. BnBr, Bu_2SnO, TBAB, toluene, 120 °C → 80 °C, 24 h; ii. Amberlyst-15 (H^+), EtOH, H_2O, 50 °C, 43 h, 90%; (b) i. BzCl, DMAP, pyridine, 120 °C, 48 h; ii. PhSH, $BF_3{\cdot}Et_2O$, DCM, 0 °C → rt, 24 h, 42%; (c) 1-heptanol, *p*-NO_2PhSCl, AgOTf, DCM, 4Å MS, 0 °C → rt, 5 h, 32% for **6**, 32% for **7**, 5% for **8**; (d) $Pd(OH)_2/C$, H_2, EtOH, AcOH, rt, 22 h, 86%; e) MsCl, TEA, DCM, 0 °C → 65 °C, 7 h, 0%; (f) $Pd(OH)_2/C$, H_2, EtOH, rt, 5 h, 93%; (g) MsCl, TEA, DCM, 0 °C → rt, 6 h, 86%; (h) KF, Kryptofix 2.2.2, DMSO, 100 °C, 2 h, 85%; i) LiCl, DMF, 90 °C, 7 h, 41%.

biotinylated polyacrylamide glycopolymer as competitor. Conjugation of biotin with streptavidin-horseradish peroxidase allows quantification of the bound polymer and therefore the determination of the IC_{50}. The activity of all antagonists was measured twice in duplicates. The antagonist *n*-heptyl α-D-mannopyranoside (**1**) was used as a reference compound and tested in parallel to ensure comparability. The affinities are referred to the activity of **1** as rIC_{50}.

In addition to the competitive binding assay, ITC experiments were performed with mannosides **1**, **2a** and **2f** to reveal a thermodynamic fingerprint of mannose-modified FimH antagonists (Table 2). ITC enables direct measurement of the dissociation constant (K_D) and the change in enthalpy (ΔH°), which are further used to calculate the changes in free energy (ΔG°) and entropy (ΔS°) (Chen and Wadsö, 1982; Freire et al., 1990).

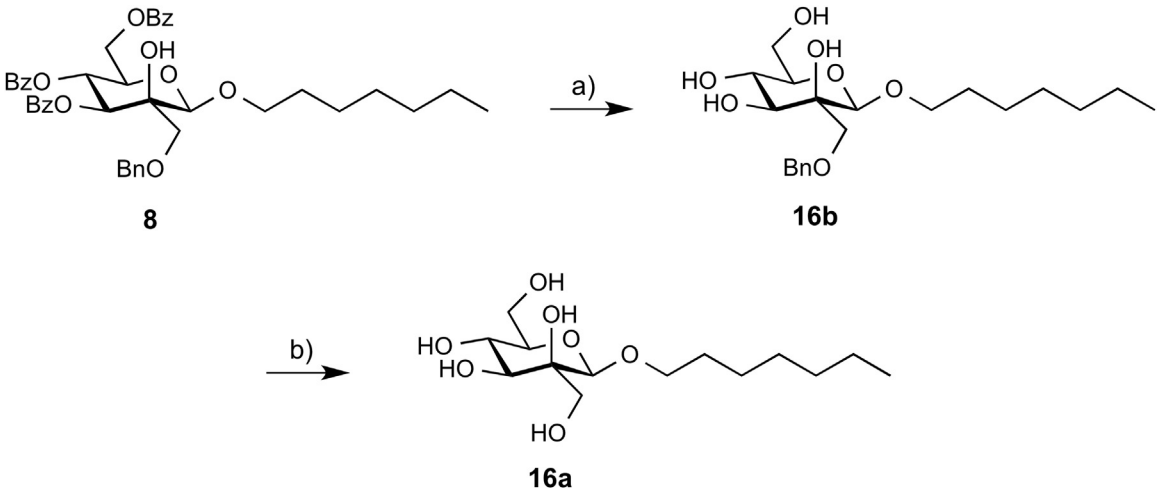
Unfortunately, all 2-*C* modifications proved to be detrimental to the affinity. Already the smallest substituent, a methyl group (→**2f**), resulted in a 2.8-fold higher IC_{50} value. A comparable 4.1-fold drop in activity was observed in ITC. This finding might be explained by an unexpected unfavourable steric clash of Ile13 and/or Phe142 with the methyl group already too big to fit to the targeted cavity. This hypothesis is supported by the considerably improved entropy term ($-T\Delta\Delta S^\circ$ −17.2 kJ/mol) compared to the reference **1**, indicating an increased conformational flexibility of the ligand. The resulting disruption of the hydrogen bond network within the pocket is also reflected by a substantial decline of enthalpy ($\Delta\Delta H^\circ$ +20.6 kJ/mol). A further reduction in affinity to the micromolar level for larger substituents, e.g., iodomethyl and chloromethyl (→**2c** and **2d**, Table 1) is in full agreement with this argumentation.



Scheme 2 (a) MeONa/MeOH, rt, 4 h, 85%; (b) $Pd(OH)_2/C$, H_2, EtOH, rt, 6 h, 75%; (c) i. MsCl, TEA, DCM, 0 °C → rt, 1.5—6 h; ii. NaI, LiCl, KCN or NaN_3, DMF or DMSO, 70—100 °C, 3—64 h; iii. MeONa/MeOH, rt, 1—6 h, 39% for **2c**, 38% for **2d**, 11% for **2e**, 73% for **2h**; (d) $Pd(OH)_2/C$, H_2, EtOH, TEA, rt, 44 h, 91%; (e) i. $Pd(OH)_2/C$, H_2, MeOH or EtOH, rt, 3—11 h; ii. 0.1% TFA or 0.01 M HCl, H_2O, MeOH, 76% for **2g**, 86% for **2i**; (f) i. $Pd(OH)_2/C$, H_2, EtOH, rt, 7 h; ii. AcCl or CH_3CH_2COCl, pyridine, DCM, rt, 2.5—5.5 h; iii. MeONa/MeOH, rt, 1.5—4 h, 20% for **2j**, 38% for **2k**.

Unexpectedly, the benzyloxymethyl group in **2b**, despite its bulkiness, only slightly reduced the activity compared to the methyl substituent (→**2f**). Furthermore, **2b** performed better compared to the halogens **2c** and **2d**. This may result from a smaller van der Waals radius of oxygen compared to chloride or iodide. Moreover, a solvent exposed phenyl ring can be involved in non-specific hydrophobic interactions with the surface of the protein, attenuating the negative effect of the size of the 2-*C*-branch.

The antagonists bearing hydrogen bond donating groups, such as a hydroxyl group (→**2a**), an amine (→**2i**) or an amide (→**2j** and **2k**), were among the most active derivatives. The hydroxymethyl group (→**2a**) led to roughly a 5-fold drop in affinity in both, competitive binding assay and ITC. Compared to the methyl group (→**2f**), this substituent was expected to disrupt the hydrogen bond network even further due to its larger size and to cause additional enthalpy costs due to a desolvation penalty related to the hydroxyl group. However, the enthalpy loss in this case was smaller ($\Delta\Delta H^\circ_{2a-2f} = 6.1$ kJ/mol) implying additional beneficial interactions formed by **2a**. As a consequence, the entropy gain was limited ($-T\Delta\Delta S^\circ$ −10.4 kJ/mol) compared to **2f**. However, this beneficial effect was almost compensated by a loss in entropy. Furthermore, the structurally similar aminomethyl derivative **2i** was the most active compound within the series with only 2.1-fold lower affinity compared to reference **1**. Presumably, the hydroxymethyl (→**2a**) and aminomethyl (→**2i**) groups are involved in electrostatic interactions with a hydrogen bond acceptor, i.e., Asp140 or the backbone amide of Ile13. The improved affinity of **2i** may result from the fact that the ammonium group in **2i** can form a slightly stronger interaction (Lopes Jesus and Redinha, 2011). Finally, when we incorporated a longer linker between the nitrogen and the sugar moiety (→**2g**)



Scheme 3 (a) MeONa/MeOH, rt, 2 h, 68%; (b) $Pd(OH)_2/C$, H_2, EtOH, rt, 3 h, 93%.

Table 1 Affinity of FimH antagonists. The rIC_{50} values were calculated by dividing the IC_{50} of the compound of interest by the IC_{50} of the reference compound *n*-heptyl α-D-mannopyranoside (**1**). rIC_{50} values below 1.0 are obtained for antagonists more active than reference compound **1**, whereas rIC_{50} above 1.0 are obtained for antagonists less active than reference compound **1**.

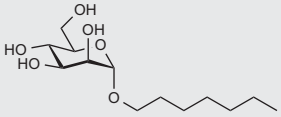
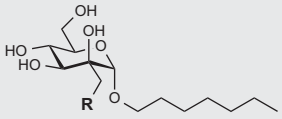
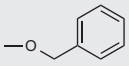
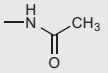
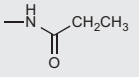
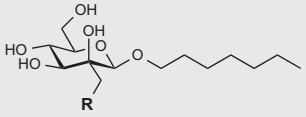
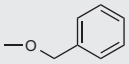
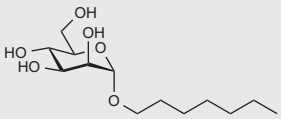
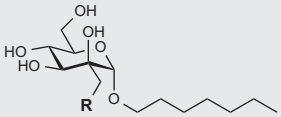
Entry	Compound	R	IC_{50} [nM]	rIC_{50}
1	**1**		48.9—65.2	1
				
2	**2a**	—OH	311.0	4.9
3	**2b**		340.9	5.4
4	**2c**	—I	>2000	38.9
5	**2d**	—Cl	>2000	304
6	**2e**	—CN	522.3	9.7
7	**2f**	—H	181.2	2.8
8	**2g**	$—CH_2NH_2 \cdot HCl$	>2000	37.1
9	**2h**	$—N_3$	437.0	6.9
10	**2i**	$—NH_2 \cdot CF_3COOH$	135.0	2.1
11	**2j**		182.5	3.4
12	**2k**		295.2	5.5
				
13	**16a**	—OH	446.4	7.0
14	**16b**		4400	69.4

Table 2 Thermodynamic profile of FimH antagonists in binding to $FimH_{LD}$-Th-His_6 at 25 °C and pH 7.4.

Entry	Compound	R	K_D [nM]	ΔG° [kJ/mol]	ΔH° [kJ/mol]	$-T\Delta S^\circ$ [kJ/mol]
						
1	**1**		28.9	−43.0	−50.3	7.3
						
2	**2f**	—H	118.6	−39.5	−29.7	−9.9
3	**2a**	—OH	154.0	−38.9	−35.8	−3.1

the affinity was lowered to the micromolar level. Similarly to the aminomethyl derivative **2i**, the amide **2j** had one of the best affinities in the series. However, elongation of the aliphatic chain attached to the amide (→**2k**) again resulted in reduction of potency.

Quite surprisingly, the β-anomeric derivative **16a** (Table 1, entry 13) performed only slightly worse than its α-anomeric analogue **2a**. However, when a benzyloxymethyl group was introduced (→**16b**, entry 14) the affinity was almost 13-fold lower than for its α-anomeric counterpart **2b**, according to docking studies (data not shown) due to a steric clash with Ile13.

Conclusions

A new family of mannose-based FimH antagonists equipped with equatorial substituents at the 2-position of the sugar moiety was designed and synthesized to target a cavity located close to the entrance of FimH-CRD. Only when the axial 2-hydroxyl group was unprotected (→**11**), the otherwise unsuccessful substitution at the equatorial 2-position of the mannose moiety could be performed, leading to the test compounds **2c**—**k**. In one case, the intermediate epoxide **14** could be isolated, indicating two possible reaction pathways; one via direct S_N2-substitution and one via the epoxide **14**. The resulting epoxide intermediate could be opened, however, affording only a moderate yield.

The activities of the 2-*C*-branched FimH antagonists were evaluated in a cell-free competitive binding assay and compared to the reference compound *n*-heptyl α-D-mannopyranoside (**1**). None of the modifications proved to be advantageous for binding to FimH-CRD. The loss of affinity is probably related to steric hindrance as it was already observed upon introduction of the smallest substituent, a methyl group (→**2f**). With hydrogen bond donating substituents (→**2a**, **2i**—**k**), affinity could be partially regained. Unexpectedly, the β-anomer **16a** performed only slightly worse than its α-anomeric counterpart **2a**. However, as already experienced in the α-series, a larger benzyloxymethyl substituent (→**16b**) severely compromised affinity.

Finally, ITC experiments with the selected antagonists **1**, **2a** and **2f** revealed a drastic enthalpy loss for the 2-*C*-branched antagonists, which, however, is partially compensated by an entropy gain. This supports the hypothesis that the target cavity is too small to accommodate 2-*C*-substituents. However, with larger, hydrogen bond donating substituents the enthalpy loss could be substantially reduced.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pisc.2016.10.002.

References

Appeldoorn, C.C.M., Joosten, J.A.F., el Maate, F.A., Dobrindt, U., Hacker, J., Liskamp, R.M.J., Khan, A.S., Pieters, R.J., 2005. Novel multivalent mannose compounds and their inhibition of the adhesion of type 1 fimbriated uropathogenic *E. coli*. Tetrahedron: Asymmetry 16, 361—372, http://dx.doi.org/10.1016/j.tetasy.2004.11.014.

Barnich, N., Carvalho, F.A., Glasser, A.-L., Darcha, C., Jantscheff, P., Allez, M., Peeters, H., Bommelaer, G., Desreumaux, P., Colombel, J.-F., Darfeuille-Michaud, A., 2007. CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. J. Clin. Invest. 117, 1566—1574, http://dx.doi.org/10.1172/jci30504.

Bouckaert, J., Berglund, J., Schembri, M., De Genst, E., Cools, L., Wuhrer, M., Hung, C.-S., Pinkner, J., Slättergard, R., Zavialov, A., Choudhury, D., Langermann, S., Hultgren, S.J., Wyns, L., Klemm, P., Oscarson, S., Knight, S.D., Greve, H.D., 2005. Receptor binding studies disclose a novel class of high-affinity inhibitors of the *Escherichia coli* FimH adhesin. Mol. Microbiol. 55, 441—455, http://dx.doi.org/10.1111/j.1365-2958.2004.04415.x.

Bouckaert, J., Li, Z., Xavier, C., Almant, M., Caveliers, V., Lahoutte, T., Weeks, S.D., Kovensky, J., Gouin, S.G., 2013. Heptyl α-D-mannosides grafted on a β-cyclodextrin core to interfere with *Escherichia coli* adhesion: an

in vivo multivalent effect. Chem. Eur. J. 19, 7847–7855, http://dx.doi.org/10.1002/chem.201204015.

Brument, S., Sivignon, A., Dumych, T.I., Moreau, N., Roos, G., Guérardel, Y., Chalopin, T., Deniaud, D., Bilyy, R.O., Darfeuille-Michaud, A., Bouckaert, J., Gouin, S.G., 2013. Thiazolylaminomannosides as potent antiadhesives of type 1 piliated *Escherichia coli* isolated from Crohn's disease patients. J. Med. Chem. 56, 5395–5406, http://dx.doi.org/10.1021/jm400723n.

Bubb, W.A., 2003. NMR spectroscopy in the study of carbohydrates: characterizing the structural complexity. Concepts Magn. Reson. A 19A, 1–19, http://dx.doi.org/10.1002/cmr.a.10080.

Carvalho, F.A., Barnich, N., Sivignon, A., Darcha, C., Chan, C.H.F., Stanners, C.P., Darfeuille-Michaud, A., 2009. Crohn's disease adherent-invasive *Escherichia coli* colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM. J. Exp. Med. 206, 2179–2189, http://dx.doi.org/10.1084/jem.20090741.

Chalopin, T., Alvarez Dorta, D., Sivignon, A., Caudan, M., Dumych, T.I., Bilyy, R.O., Deniaud, D., Barnich, N., Bouckaert, J., Gouin, S.G., 2016. Second generation of thiazolylmannosides, FimH antagonists for *E. coli*-induced Crohn's disease. Org. Biomol. Chem. 14, 3913–3925, http://dx.doi.org/10.1039/c6ob00424e.

Chen, A.-t., Wadsö, I., 1982. Simultaneous determination of ΔG, ΔH and ΔS by an automatic microcalorimetric titration technique. Application to protein ligand binding. J. Biochem. Biophys. Methods 6, 307–316, http://dx.doi.org/10.1016/0165-022X(82)90012-4.

Crich, D., Cai, F., Yang, F., 2008. A stable, commercially available sulfenyl chloride for the activation of thioglycosides in conjunction with silver trifluoromethanesulfonate. Carbohydr. Res. 343, 1858–1862, http://dx.doi.org/10.1016/j.carres.2008.03.002.

Cusumano, C.K., Pinkner, J.S., Han, Z., Greene, S.E., Ford, B.A., Crowley, J.R., Henderson, J.P., Janetka, J.W., Hultgren, S.J., 2011. Treatment and prevention of urinary tract infection with orally active FimH inhibitors. Sci. Transl. Med. 3, 109ra115, http://dx.doi.org/10.1126/scitranslmed.3003021.

Durka, M., Buffet, K., Iehl, J., Holler, M., Nierengarten, J.-F., Taganna, J., Bouckaert, J., Vincent, S.P., 2011. The functional valency of dodecamannosylated fullerenes with *Escherichia coli* FimH—towards novel bacterial antiadhesives. Chem. Commun. 47, 1321–1323, http://dx.doi.org/10.1039/c0cc04468g.

Fiege, B., Rabbani, S., Preston, R.C., Jakob, R.P., Zihlmann, P., Schwardt, O., Jiang, X., Maier, T., Ernst, B., 2015. The tyrosine gate of the bacterial lectin FimH: a conformational analysis by NMR spectroscopy and X-ray crystallography. ChemBioChem 16, 1235–1246, http://dx.doi.org/10.1002/cbic.201402714.

Fihn, S.D., 2003. Acute uncomplicated urinary tract infection in women. N. Engl. J. Med. 349, 259–266, http://dx.doi.org/10.1056/nejmcp030027.

Firon, N., Ofek, I., Sharon, N., 1982. Interaction of mannose-containing oligosaccharides with the fimbrial lectin of *Escherichia coli*. Biochem. Biophys. Res. Commun. 105, 1426–1432, http://dx.doi.org/10.1016/0006-291x(82)90947-0.

Firon, N., Ofek, I., Sharon, N., 1983. Carbohydrate specificity of the surface lectins of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. Carbohydr. Res. 120, 235–249, http://dx.doi.org/10.1016/0008-6215(83)88019-7.

Firon, N., Ashkenazi, S., Mirelman, D., Ofek, I., Sharon, N., 1987. Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated *Escherichia coli* to yeast and intestinal epithelial cells. Infect. Immun. 55, 472–476.

Foxman, B., Barlow, R., D'Arcy, H., Gillespie, B., Sobel, J.D., 2000. Urinary tract infection: self reported incidence and associated costs. Ann. Epidemiol. 10, 509–515, http://dx.doi.org/10.1016/s1047-2797(00)00072-7.

Freire, E., Mayorga, O.L., Straume, M., 1990. Isothermal titration calorimetry. Anal. Chem. 62, 950A–959A, http://dx.doi.org/10.1021/ac00217a002.

Gloe, T.-E., Stamer, I., Hojnik, C., Wrodnigg, T.M., Lindhorst, T.K., 2015. Are D-manno-configured Amadori products ligands of the bacterial lectin FimH? Beilstein J. Org. Chem. 11, 1096–1104, http://dx.doi.org/10.3762/bjoc.11.123.

Han, Z., Pinkner, J.S., Ford, B., Obermann, R., Nolan, W., Wildman, S.A., Hobbs, D., Ellenberger, T., Cusumano, C.K., Hultgren, S.J., Janetka, J.W., 2010. Structure-based drug design and optimization of mannoside bacterial FimH antagonists. J. Med. Chem. 53, 4779–4792, http://dx.doi.org/10.1021/jm100438s.

Han, Z., Pinkner, J.S., Ford, B., Chorell, E., Crowley, J.M., Cusumano, C.K., Campbell, S., Henderson, J.P., Hultgren, S.J., Janetka, J.W., 2012. Lead optimization studies on FimH antagonists: discovery of potent and orally bioavailable *ortho*-substituted biphenyl mannosides. J. Med. Chem. 55, 3945–3959, http://dx.doi.org/10.1021/jm300165m.

Hooton, T.M., Besser, R., Foxman, B., Fritsche, T.R., Nicolle, L.E., 2004. Acute uncomplicated cystitis in an era of increasing antibiotic resistance: a proposed approach to empirical therapy. Clin. Infect. Dis. 39, 75–80, http://dx.doi.org/10.1086/422145.

Hung, C.-S., Bouckaert, J., Hung, D., Pinkner, J., Widberg, C., DeFusco, A., Auguste, C.G., Strouse, R., Langermann, S., Waksman, G., Hultgren, S.J., 2002. Structural basis of tropism of *Escherichia coli* to the bladder during urinary tract infection. Mol. Microbiol. 44, 903–915, http://dx.doi.org/10.1046/j.1365-2958.2002.02915.x.

Jarvis, C., Han, Z., Kalas, V., Klein, R., Pinkner, J.S., Ford, B., Binkley, J., Cusumano, C.K., Cusumano, Z., Mydock-McGrane, L., Hultgren, S.J., Janetka, J.W., 2016. Antivirulence isoquinolone mannosides: optimization of the biaryl aglycone for FimH lectin binding affinity and efficacy in the treatment of chronic UTI. ChemMedChem 11, 367–373, http://dx.doi.org/10.1002/cmdc.201600045.

Jiang, X., Abgottspon, D., Kleeb, S., Rabbani, S., Scharenberg, M., Wittwer, M., Haug, M., Schwardt, O., Ernst, B., 2012. Antiadhesion therapy for urinary tract infections—a balanced PK/PD profile proved to be key for success. J. Med. Chem. 55, 4700–4713, http://dx.doi.org/10.1021/jm300192x.

Kleeb, S., Pang, L., Mayer, K., Eris, D., Sigl, A., Preston, R.C., Zihlmann, P., Sharpe, T., Jakob, R.P., Abgottspon, D., Hutter, A.S., Scharenberg, M., Jiang, X., Navarra, G., Rabbani, S., Smieško, M., Lüdin, N., Bezençon, J., Schwardt, O., Maier, T., Ernst, B., 2015. FimH antagonists: bioisosteres to improve the in vitro and in vivo PK/PD profile. J. Med. Chem. 58, 2221–2239, http://dx.doi.org/10.1021/jm501524q.

Klein, T., Abgottspon, D., Wittwer, M., Rabbani, S., Herold, J., Jiang, X., Kleeb, S., Lüthi, C., Scharenberg, M., Bezençon, J., Gubler, E., Pang, L., Smieško, M., Cutting, B., Schwardt, O., Ernst, B., 2010. FimH antagonists for the oral treatment of urinary tract infections: from design and synthesis to in vitro and in vivo evaluation. J. Med. Chem. 53, 8627–8641, http://dx.doi.org/10.1021/jm101011y.

Le Trong, I., Aprikian, P., Kidd, B.A., Forero-Shelton, M., Tchesnokova, V., Rajagopal, P., Rodriguez, V., Interlandi, G., Klevit, R., Vogel, V., Stenkamp, R.E., Sokurenko, E.V., Thomas, W.E., 2010. Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like β sheet twisting. Cell 141, 645–655, http://dx.doi.org/10.1016/j.cell.2010.03.038.

Lindhorst, T.K., Kieburg, C., Krallmann-Wenzel, U., 1998. Inhibition of the type 1 fimbriae-mediated adhesion of *Escherichia coli* to erythrocytes by multiantennary d-mannosyl clusters: the effect of multivalency. Glycoconj. J. 15, 605–613, http://dx.doi.org/10.1023/a:1006920027641.

Lopes Jesus, A.J., Redinha, J.S., 2011. Charge-assisted intramolecular hydrogen bonds in disubstituted cyclohex-

ane derivatives. J. Phys. Chem. A 115, 14069—14077, http://dx.doi.org/10.1021/jp206193a.

Malleron, A., David, S., 1998. A preparation of protected 2-deoxy-2-hydroxymethyl-D-mannose and -D-glucose derivatives not involving organometallic reagents. Carbohydr. Res. 308, 93—98, http://dx.doi.org/10.1016/s0008-6215(98)00080-9.

Mitchell, D.A., Jones, N.A., Hunter, S.J., Cook, J.M.D., Jenkinson, S.F., Wormald, M.R., Dwek, R.A., Fleet, G.W.J., 2007. Synthesis of 2-*C*-branched derivatives of D-mannose: 2-*C*-aminomethyl-d-mannose binds to the human C-type lectin DC-SIGN with affinity greater than an order of magnitude compared to that of D-mannose. Tetrahedron: Asymmetry 18, 1502—1510, http://dx.doi.org/10.1016/j.tetasy.2007.06.003.

Mulvey, M.A., Schilling, J.D., Martinez, J.J., Hultgren, S.J., 2000. Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. Proc. Natl. Acad. Sci. U. S. A. 97, 8829—8835, http://dx.doi.org/10.1073/pnas.97.16.8829.

Murakami, T., Hirono, R., Sato, Y., Furusawa, K., 2007. Efficient synthesis of ω-mercaptoalkyl 1,2-*trans*-glycosides from sugar peracetates. Carbohydr. Res. 342, 1009—1020, http://dx.doi.org/10.1016/j.carres.2007.02.024.

Nagahori, N., Lee, R.T., Nishimura, S.-L., Pagé, D., Roy, R., Lee, Y.C., 2002. Inhibition of adhesion of type 1 fimbriated *Escherichia coli* to highly mannosylated ligands. ChemBioChem 3, 836—844, http://dx.doi.org/10.1002/1439-7633(20020902)3:9<836:aid-cbic836>3.0.co;2-2.

Nukada, T., Berces, A., Whitfield, D.M., 1999. Acyl transfer as a problematic side reaction in polymer-supported oligosaccharide synthesis. J. Org. Chem. 64, 9030—9045, http://dx.doi.org/10.1021/jo990712b.

Old, D.C., 1972. Inhibition of the interaction between fimbrial haemagglutinins and erythrocytes by D-mannose and other carbohydrates. J. Gen. Microbiol. 71, 149—157, http://dx.doi.org/10.1099/00221287-71-1-149.

Pang, L., Kleeb, S., Lemme, K., Rabbani, S., Scharenberg, M., Zalewski, A., Schädler, F., Schwardt, O., Ernst, B., 2012. FimH antagonists: structure-activity and structure-property relationships for biphenyl α-D-mannopyranosides. ChemMedChem 7, 1404—1422, http://dx.doi.org/10.1002/cmdc.201200125.

Patel, A., Lindhorst, T.K., 2006. A modular approach for the synthesis of oligosaccharide mimetics. Carbohydr. Res. 341, 1657—1668, http://dx.doi.org/10.1016/j.carres.2006.01.024.

Rabbani, S., Jiang, X., Schwardt, O., Ernst, B., 2010. Expression of the carbohydrate recognition domain of FimH and development of a competitive binding assay. Anal. Biochem. 407, 188—195, http://dx.doi.org/10.1016/j.ab.2010.08.007.

Roland, A., 2002. The etiology of urinary tract infection: traditional and emerging pathogens. Am. J. Med. 113 (Suppl. 1A), 14S—19S, http://dx.doi.org/10.1016/s0002-9343(02)01055-0.

Sanchez, G.V., Master, R.N., Karlowsky, J.A., Bordon, J.M., 2012. *In vitro* antimicrobial resistance of urinary *Escherichia coli* isolates among U.S. outpatients from 2000 to 2010. Antimicrob. Agents Chemother. 56, 2181—2183, http://dx.doi.org/10.1128/aac.06060-11.

Schilling, J.D., Mulvey, M.A., Hultgren, S.J., 2001. Structure and function of *Escherichia coli* type 1 pili: new insight into the pathogenesis of urinary tract infections. J. Infect. Dis. 183 (Suppl. 1), S36—S40, http://dx.doi.org/10.1086/318855.

Schwardt, O., Rabbani, S., Hartmann, M., Abgottspon, D., Wittwer, M., Kleeb, S., Zalewski, A., Smieško, M., Cutting, B., Ernst, B., 2011. Design, synthesis and biological evaluation of mannosyl triazoles as FimH antagonists. Bioorg. Med. Chem. 19, 6454—6473, http://dx.doi.org/10.1016/j.bmc.2011.08.057.

Sperling, O., Fuchs, A., Lindhorst, T.K., 2006. Evaluation of the carbohydrate recognition domain of the bacterial adhesin FimH: design, synthesis and binding properties of mannoside ligands. Org. Biomol. Chem. 4, 3913—3922, http://dx.doi.org/10.1039/b610745a.

Touaibia, M., Wellens, A., Shiao, T.C., Wang, Q., Sirois, S., Bouckaert, J., Roy, R., 2007. Mannosylated G(0) dendrimers with nanomolar affinities to *Escherichia coli* FimH. ChemMedChem 2, 1190—1201, http://dx.doi.org/10.1002/cmdc.200700063.

Waschke, D., Thimm, J., Thiem, J., 2011. Highly efficient synthesis of ketoheptoses. Org. Lett. 13, 3628—3631, http://dx.doi.org/10.1021/ol2012764.

Witczak, Z.J., Whistler, R.L., Daniel, J.R., 1984. Synthesis of 3-*C*-(hydroxymethyl)erythritol and 3-*C*-methylerythritol. Carbohydr. Res. 133, 235—245, http://dx.doi.org/10.1016/0008-6215(84)85201-5.

Ziegler, T., Kováč, P., Glaudemans, C.P.J., 1990. Transesterification during glycosylation promoted by silver trifluoromethanesulfonate. Eur. J. Org. Chem., 613—615, http://dx.doi.org/10.1002/jlac.1990199001115.