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.

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Abbreviations: UPEC, uropathogenic Escherichia coli; UTI, urinary tract infection; CRD, carbohydrate-recognition domain; IC₅₀, half maximal inhibitory concentration; ITC, isothermal titration calorimetry; Kₑq, 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 adhesion 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 (FimH₅₀) containing a carbohydrate recognition domain (CRD) and a pilin domain (FimH₂₀) 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 mannann-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 mannone 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; Schwart 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 mannone 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; Appeldorn 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 mannone moiety establishes a perfect hydrogen bond network (Hung et al., 2002). Since every hydroxyl group of mannone is part of this network, the removal/replacement of individual various hydroxyl groups or the replacement of the whole mannone 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 mannone 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).

A visual inspection of the crystal structure of FimH₅₀ co-crystallized with n-heptyl α-D-mannopyranoside (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 of the mannone binding site and can be reached by equatorial substituents in the 2-position of the mannone moiety.

An adaption of the synthetic pathway of previously reported 2-C-branched mannone derivatives, in which the Z-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 mannone moiety.

Synthesis

The synthesis of the 2-C-branched mannone donor 5 is depicted in Scheme 1. The 2-C-modified α-mannofuranose 3 was synthesized according to a literature procedure starting from commercially available α-mannose (Witzczak et al., 1984). Selective benzylation of the hydroxymethyl group using dibutyltin oxide (Malleron and David, 1998) followed by cleavage of the acetones under acidic conditions yielded the 2-C-branched α-mannopyranose 4 (Waschke et al., 2011). For its perbenzoylation with benzoyl chloride in presence of a catalytic amount of 4-dimethylamino-pyridine (DMAP) in dry pyridine, elevated temperature
(110–120 °C) had to be applied. Subsequently, the glycosyl donor 5 was obtained by reaction with thiophenol using 
BF3·Et2O 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-nitrobenzenesulfonyl chloride (p-NO2PhSCl) 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 acyl 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, preva-

ence 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. Debenzylation of 6 under 
Zemplén conditions (→2b) followed by Pd(OH)_2-catalyzed hydrogenolysis afforded test compound 2a. The configur-
ation at the anomeric carbon of deprotected derivative 2a (^1J_H,C = 169 Hz) was unambiguously confirmed by the ^13C−^1H 
coupling constant of the anomeric nuclei using undecoupled ^13C NMR. In general, the coupling constant for the equato-
rial anomeric proton amounts to ~170 Hz, while a value of ~160 Hz is indicative for an anomeric proton in axial orienta-
tion (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 
metath 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 condi-
tions, amides 2j and 2k were obtained.

To evaluate the β-anomeric derivative as well in the biological assay, β-mannoside 8 was debenzyolated (→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_Lo-Th-His_6 
(Th: thrombin cleavage site) as a target protein and a
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 mannoses 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 (∆H^\circ) which are further used to calculate the changes in free energy (∆G^\circ) and entropy (∆S^\circ) (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∆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 (∆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.
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 ($\Delta H^\circ_{\text{2f-2j}} = 6.1 \text{ kJ/mol}$) implying additional beneficial interactions formed by 2a. As a consequence, the entropy gain was limited ($\Delta S^\circ = -10.4 \text{ kJ/mol}$) 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, H₂, EtOH, rt, 3 h, 93%.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>IC₅₀ [nM]</th>
<th>rIC₅₀</th>
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<tbody>
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<td>1</td>
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<td>-</td>
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</tr>
<tr>
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<td>-OH</td>
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<td>2b</td>
<td>-</td>
<td>340.9</td>
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<td>4</td>
<td>2c</td>
<td>-I</td>
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</tr>
<tr>
<td>5</td>
<td>2d</td>
<td>-Cl</td>
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</tr>
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</tr>
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<td>2g</td>
<td>-CH₂NH₂-HCl</td>
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<tr>
<td>10</td>
<td>2i</td>
<td>-NH₂-CF₃COOH</td>
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<td>2.1</td>
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<tr>
<td>11</td>
<td>2j</td>
<td>-</td>
<td>182.5</td>
<td>3.4</td>
</tr>
<tr>
<td>12</td>
<td>2k</td>
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<td>7.0</td>
</tr>
<tr>
<td>14</td>
<td>16b</td>
<td>-</td>
<td>4400</td>
<td>69.4</td>
</tr>
</tbody>
</table>
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 lle13.

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 Sn2-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.


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