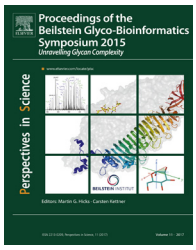
Perspectives in Science (2017) 11, 53—61

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/pisc



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

Wojciech Schönemann, Marcel Lindegger, Said Rabbani, Pascal Zihlmann, Oliver Schwardt, Beat Ernst*

Institute of Molecular Pharmacy, Pharmacenter, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Received 5 July 2016; accepted 9 September 2016
Available online 20 October 2016

KEYWORDS
Urinary tract infections;
Uropathogenic *Escherichia coli*;
FimH antagonists;
Carbohydrate-recognition domain;
2-*C*-Branched carbohydrates

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.

* Corresponding author. Fax: +41 61 267 15 52.
E-mail address: beat.ernst@unibas.ch (B. Ernst).

http://dx.doi.org/10.1016/j.pisc.2016.10.002

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).

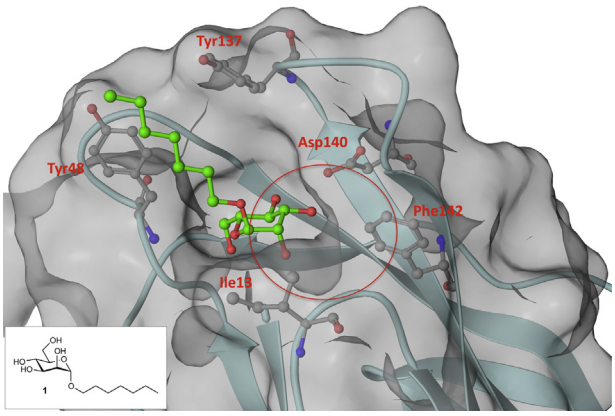


Figure 1 The crystal structure of $FimH_{LD}$ co-crystallized with *n*-heptyl α-D-mannopyranoside (**1**, PDB ID: 4BUQ) (Fiege et al., 2015). A mainly hydrophobic cavity formed by Ile13, Phe142 and Asp140 is located next to the entrance of the mannose binding site and can be reached by equatorial substituents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