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Comparison of Inhibitory Activities of *meta* and *para* Substituted *N*-aryl 3-Hydroxypyridin-4-one Mannosides Towards Type 1 Fimbriated *E. coli*

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THIS PAPER IS DEDICATED TO THE LOVING MEMORY OF IVANA WEYGAND-ĐURAŠEVIĆ (1952 – 2014)

Abstract: In uropathogenic *Escherichia coli*, mannose-specific adhesion is mediated by the FimH adhesin located at the tip of type 1 fimbriae. Novel mannosylated *N*-aryl substituted 3-hydroxypyridin-4ones with *meta* substituents on the aryl part of the molecule were prepared, and their inhibitory properties towards the adhesion of *E. coli* to guinea pig erythrocytes explored using the hemagglutination assay. These results were compared with inhibitory potencies of analogous *para* derivatives. The assays revealed greater preference of FimH towards *para* substituted compounds in general, with *p*-nitro and *p*-methoxy substituted substrates being much more effective then the hydrophobic *p*-methyl compound. When substituents are in *meta* position the positive affect on the binding of compounds in the FimH binding site was observed with all compounds tested but the structure with an alkyl group was shown to be the most effective one. This study provides guidelines for the rational design of novel, more effective series of FimH antagonists.

Keywords: *N*-aryl 3-hydroxypyridin-4-one mannosides, FimH antagonist, hemagglutination, *E. coli*.

INTRODUCTION

Research in the field of glycobiology has shown that the process of molecular recognition involving bacterial cell-surface proteins called lectins and complementary carbohydrates is of essentaial importance in many biological processes.[1,2] Adhesion of pathogenic organisms to host tissues is the initiation of the majority of infectious diseases and is often mediated by lectins present on the surface of infectious organisms which then combine with complementary sugars on the host surface.[3] Therefore, the concept of anti-adhesion therapy of microbial diseases based on blocking or inhibiting lectins by suitable carbohydrates has gradually emerged.[4–8] This concept seems to be an attractive and promising alternative to standard antibiotic therapy. Thus, the growing problem of bacterial multi-antibiotics resistance could be suppressed by using carbohydrates as anti-adhesion drugs for infectious diseases.[9] One of the best characterized enterobacterial surface lectins is mannose-specific type 1 fimbriae, a common bacterial adhesin involved in receptor-ligand interactions.[10,11] In uropathogenic *Escherichia coli* (UPEC), mannose-specific adhesion is mediated by the FimH adhesin located at the tip of type 1 fimbriae.[12,13] Several studies were reported with a large number of α-D-mannopyranosides as high-affinity ligands for *E. coli* type 1 adhesin. Recent research in this area has been focused on multivalent mannosides[14–18] which were found to bind with high affinity to FimH and thus prevent agglutination of red blood cells mediated by cross-linking of surface epitopes containing mannose. However, these high molecular weight structures are predicted to be poorly permeable to the gastrointestinal tract and probably not suitable for oral dosing because of their large molecular weight and high polarity.[19] X-ray studies revealed that UPEC lectin FimH perfectly accomodates one single α-mannosyl residue.[18–22] It was determined that the entrance of the binding site region of FimH is bordered with two aromatic residues belonging to Tyr48 and Tyr137 (so called "tyrosine gate").[23] Consequently, the introduction

of aromatic aglycon moiety induces π–π stacking interactions within the tyrosine gate enhancing the affinity of the relevant aromatic mannosides compared to alkyl mannosides.[24] Aryl mannoside inhibitors of hemagglutination were first reported many years ago[11,25] and several structure-activity relationship studies followed.[26–28] Further studies also showed that elongation of aglycon alkyl chains of synthetic FimH mannoside antagonists is of importance and that it enhances their potency.[21,22,24,29] Our previous reports were focused on the exploration of the influence of monovalent FimH mannoside antagonists with structurally different aglycons (lipophilic adamantane, aromatic ferrocene) on the resulting binding capacities.[30–32] Aromatic ferrocene mannosides showed better inhibitory potency in preventing the adhesion of *E. coli* to erythrocytes.[31,32] Since it is known that the potency of inhibition of hemagglutination can be further enhanced by extending the aglycon by a second aryl system[26] which is also capable of reaching the hydrophobic rim formed by Tyr48, Tyr137 and Ile52, we have explored the inhibitory potential of α-mannosides with *N*-aryl substituted 3-hydroxypyridin-4-ones (*N*-aryl 3,4-HPs) as aglycon parts of a molecule.[33] 3,4-HPs possess the needed structural characteristics since they are hydrophobic bicyclic compounds composed of the 3,4-HP core with an aryl extension. The 3,4-HPs, a family of heterocyclic compounds, have been extensively studied due to their broad field of application. They are used for therapeutical purposes and diagnosis, solvent extraction, and chemical analysis.[34–37] They also have excellent chelating properties toward 'hard' metals, such as Fe^{3+}, and represent a very promising structural pattern in the design of new chelating drugs.[34] Structural modifications of the heterocyclic ring at different positions including 3-hydroxyl oxygen can influence the various properties of 3,4-HPs.[37,38]

Previously, we have prepared and evaluated the mannosides **I**–**IV** (Figure 1) that contain *para* substituted *N*-aryl 3,4-HP moieties in aglycon part.[33] Of all compounds we have tested so far, they have shown the greatest potential as FimH antagonists. Therefore, we have extended our research on novel *N*-aryl 3,4-HP derivatives.

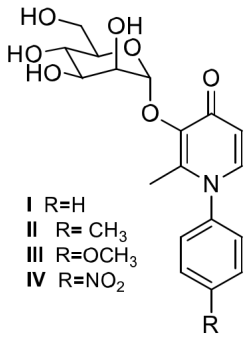


Figure 1. Previously evaluated *N*-aryl 3-hydroxypyridin-4-one mannosides.[33]

Here we report the synthesis of *meta* substituted *N*-aryl 3,4-HP mannosides, their evaluation and comparison with previously described *para* derivatives. Structure-acitivity relationship study using hemagglutination assay was performed in order to gain insight in structural patterns that influence the most binding affinities of *N*-aryl 3,4-HP mannosides towards FimH adhesin.

EXPERIMENTAL

Materials and Methods

Reagents and solvents for the synthesis of compounds were obtained from commercial sources (Sigma-Aldrich Corp. and J. T. Baker). When necessary solvents were further purified and/or dried using standard methods. Thin layer chromatography (solvents and ratios are given in the text) was performed on Fluka silica gel (60 F 254) plates (0.25 mm). Visualization was effected by use of UV light at 254 nm (UV Lamp Type 6, neoLab), iodine and/or charring with sulfuric acid. Column chromatography (solvents and ratios are also given in the text) was performed on Merck silica gel 60 (size 70–230 mesh ASTM). Melting points were determined in open capillaries using Büchi B-540 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with Bruker Avance spectrometer at room temperature in deuterated dimethyl sulfoxide at 600 MHz and 150 MHz, respectively. Chemical shifts (δ) are given in parts per million (ppm) downfield from tetramethylsilane as internal standard. Electrospray ionization mass spectrometry (ESI-MS) was performed using Agilent 6410 MS instrument. High resolution mass spectra (HRMS) were obtained by Agilent Q-TOF iFunnel LC/MS instrument.

The purity of the final compounds (**3a**–**3c**, 92.4 %, 93.0 % and 96.3 %, respectively based on the Area%) was obtained using HPLC at 254 nm. The instrument used was Agilent 1100 HPLC equipped with quaternary pump, thermostatted column compartment, an autosampler and DAD detector. The conditions of the analysis were as follows: Waters Symmetry Shield ™ RP 18 column (250 mm × 4.6 mm; 5 µm, column temperature 25 °C), 1.6 mL min^{-1} flow, acetonitrile : ultra pure water = 55 : 45, *v*/*v* mobile phase, isocratic elution, sample concentration 2 mg mL^{-1}, injection volume 7 µL.

General Procedure for Synthesis of Pyridinones 1a–1c

A mixture of 3-hydroxy-2-methyl-4-pyrone (maltol, 1 g, 7.93 mmol), appropriate aromatic amine (7.93 mmol) and *p*-toluenesulfonic acid (0.1500 g, 0.79 mmol) in water (20 mL)

was heated in a sealed thick-walled glass tube for 48 h at 150 °C. The crude product, obtained by cooling the reaction mixture to room temperature, was filtered off and recrystallized (from methanol). The purity of the products was monitored by TLC (ethyl acetate : methanol = 5 : 1, *v*/*v*).

3-HYDROXY-2-METHYL-1-(*m*-METHYLPHENYL)PYRIDIN-4-ONE (**1a**)
Yellow solid, 1.0201 g (60 %), mp 168.8–170.6 °C. ^{1}H NMR δ / ppm: 1.97 (s, 3H, CH_3), 2.38 (s, 3H, CH_3-Ar), 6.20 (d, 1H, J = 7.31 Hz, H-5), 7.24 (d, 1H, J = 7.80 Hz, H-Ar), 7.27 (s, 1H, H-Ar), 7.35 (d, 1H, J = 7.61 Hz, H-Ar), 7.44 (t, 1H, J = 7.65 Hz, H-Ar), 7.53 (d, 1 H, J = 7.31 Hz, H-6). ^{13}C NMR δ / ppm: 13.25, 20.64, 110.71, 123.88, 127.30, 129.30, 129.61, 128.50, 137.75, 139.41, 141.48, 144.94, 169.88. ESI-MS: *m*/*z* 216.2 $[M+H]^+$.

3-HYDROXY-1-(*m*-METHOXYPHENYL)-2-METHYLPYRIDIN-4-ONE (**1b**)
Yellow solid, 0.2399 g (13 %), mp 240.0–241.7 °C. ^{1}H NMR δ / ppm: 1.99 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.20 (d, 1H, J = 7.30 Hz, H-5), 6.99–7.01 (m, 1H, H-Ar), 7.06 (s, 1H, H-Ar), 7.09–7.11 (m, 1H, H-Ar), 7.46 (t, 1H, J = 8.09 Hz, H-Ar), 7.54 (d, 1H, J = 7.30 Hz, H-6). ^{13}C NMR δ / ppm: 13.16, 55.47, 110.64, 112.60, 114.91, 118.90, 130.28, 128.47, 137.69, 142.55, 144.88, 159.83, 169.51. ESI-MS: *m*/*z* 232.1 $[M+H]^+$.

3-HYDROXY-2-METHYL-1-(*m*-NITROPHENYL)PYRIDIN-4-ONE (1c)
Yellow solid, 0.3418 g (18 %), mp 217.9–218.7 °C. ^{1}H NMR δ / ppm: 1.93 (s, 3H, CH_3), 6.25 (d, 1H, J = 7.37 Hz, H-5), 7.64 (d, 1H, J = 7.37 Hz, H-6), 7.83–7.88 (m, 1H, H-Ar), 7.98–8.00 (m, 1H, H-Ar), 8.38-8.40 (m, 2H, 2H-Ar). ^{13}C NMR δ / ppm: 13.31, 111.16, 122.42, 123.43, 130.91, 133.94, 128.43, 137.81, 142.08, 144.96, 148.16, 169.88. ESI-MS: *m*/*z* 247.2 $[M+H]^+$.

General Procedure for Synthesis of Acetylated Mannosides 2a–2c

Prepared pyridinones **1a**–**1c** were dissolved in dry DCM (4 mL). Collidine (1.17 equiv.) and silver trifluoromethanesulfonate (AgOTf, 1.17 equiv.) were added next and the mixtures were stirred in a dry ice bath. 2',3',4',6'-Tetra-*O*-acetyl-1-bromo-α-D-mannopyranose, prepared previously according to a known procedure[39], was dissolved in dry DCM (2 mL) and added gradually during 1 h to the reaction mixtures (2 equiv. for each reaction). After 4 h another 0.57 equivalent of AgOTf were added and the mixtures were stirred for 24 h. They were monitored by TLC (ethyl acetate : methanol = 5 : 1, *v*/*v*), filtered over a celite bed and washed with DCM. Organic layers were washed first with cold water, then with cold 3 % aqueous HCl solution and water again. They were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residues were purified by column chromatography on silica gel (ethyl acetate : methanol = 5 : 1, *v*/*v*) giving the corresponding mannosides **2a**–**2c**.

3-(2',3',4',6'-TETRA-*O*-ACETYL-α-D-MANNOPYRANOSYLOXY)-2-METHYL-1-(*m*-METHYLPHENYL)PYRIDIN-4-ONE (**2a**)
Red oil, 0.1131 g (19 %). ^{1}H NMR δ / ppm: 1.97 (s, 3H, CH_3), 1.98, 2.06, 2.10, 2.17 (s, 12H, 4 × CH_3, Ac), 2.40 (s, 3H, CH_3-Ar), 4.00 (m, 1H, H-6'$_b$), 4.15 (m, 1H, H-6'$_a$), 4.24 (br s, 1H, H-5'), 5.20 (t, 1H, J = 9.82 Hz, H-4'), 5.45–5.47 (m, 2H, H-2', H-3'), 6.33 (s, 1H, H-1'), 7.32–7.51 (m, 6H, H-5, H-6, 4 × H-Ar). ^{13}C NMR δ / ppm: 14.62, 20.30, 20.33, 20.34, 20.47, 20.62, 61.50, 65.11, 68.00, 68.31, 68.90, 94.33, 122.42, 125.75, 129.53, 130.52, 139.71, 142.10, 144.20, 153.88, 153.90, 169.35, 169.41, 169.46, 169.80. ESI-MS: *m*/*z* 546.2 $[M+H]^+$.

3-(2',3',4',6'-TETRA-*O*-ACETYL-α-D-MANNOPYRANOSYLOXY)-1-(*m*-METHOXYPHENYL)-2-METHYLPYRIDIN-4-ONE (**2b**)
Colourless oil, 0.2537 g (47 %). ^{1}H NMR δ / ppm: 1.96 (s, 3H, CH_3), 1.98, 2.06, 2.10, 2.17 (s, 12H, 4 × CH_3, Ac), 3.82 (s, 3H, OCH_3), 4.00 (dd, 1H, J = 12.12 Hz, J = 2.10 Hz, H-6'$_b$), 4.14 (m, 1H, H-6'$_a$), 4.23–4.27 (m, 1H, H-5'), 5.20 (app t, 1H, J = 9.99 Hz, J = 9.97 Hz, H-4'), 5.38–5.45 (m, 2H, H-2', H-3'), 6.35 (s, 1H, H-1'), 7.06–7.08 (m, 1H, H-Ar), 7.15–7.19 (m, 2H, 2 × H-Ar), 7.23 (d, 1H, J = 6.47 Hz, H-5), 7.34 (d, 1H, J = 6.47 Hz, H-6), 7.51 (t, 1H, J = 8.03 Hz, J = 7.97 Hz, H-Ar). ^{13}C NMR δ / ppm: 14.53, 20.33, 20.36, 20.49, 55.61, 61.57, 65.13, 68.05, 68.43, 68.83, 94.12, 111.14, 112.75, 115.73, 117.35, 122.34, 130.58, 143.34, 144.21, 153.73, 158.44, 159.89, 169.40, 169.46, 169.53, 169.83. ESI-MS: *m*/*z* 562.3 $[M+H]^+$.

3-(2',3',4',6'-TETRA-*O*-ACETYL-α-D-MANNOPYRANOSYLOXY)-2-METHYL-1-(*m*-NITROPHENYL)PYRIDIN-4-ONE (**2c**)
Yellow oil, 0.2610 g (21 %). ^{1}H NMR δ / ppm: 1.97 (s, 3H, CH_3), 1.98, 2.06, 2.10, 2.17 (s, 12H, 4 × CH_3, Ac), 4.02 (dd, 1H, J = 12.28 Hz, J = 2.32 Hz, H-6'$_b$), 4.15 (m, 1 H, H-6'$_a$), 4.23–4.26 (m, 1H, H-5'), 5.21 (app t, 1H, J = 10.11 Hz, J = 10.09 Hz, H-4'), 5.41–5.45 (m, 2H, H-2', H-3'), 6.37 (s, 1H, H-1'), 7.28 (d, 1H, J = 6.46 Hz, H-5), 7.42–7.43 (m, 1H, H-6), 7.91 (t, 1H, J = 8.13 Hz, H-Ar), 8.05–8.06 (m, 1H, H-Ar), 8.45–8.47 (m, 1H, H-Ar), 8.49–8.50 (m, 1H, H-Ar). ^{13}C NMR δ / ppm: 14.52, 20.23, 20.27, 20.39, 61.50, 65.11, 68.02, 68.35, 68.83, 94.16, 121.13, 124.68, 131.15, 132.29, 142.52, 144.26, 148.12, 154.19, 169.27, 169.34, 169.41, 169.73. ESI-MS: *m*/*z* 577.2 $[M+H]^+$.

General Zemplén Deacetylation Procedure

Acetylated compounds **2a**–**2c** were dissolved in dry methanol (2 mL). A solution of sodium methoxide in methanol (25 %, 0.5 equiv.) was added next to each

mixture. Reaction mixtures were stirred at room temperature for 1 h and monitored by TLC (acetonitrile : H_2O = 5 : 1, *v*/*v*). They were purified first by flash chromatography using methanol as solvent. The organic layers were then concentrated *in vacuo*. The remaining residues were purified by column chromatography on silica gel (acetonitrile : H_2O : methanol = 5 : 1 : 1, *v*/*v*/*v*) giving deacetylated mannosides **3a**–**3c**.

3-(α-D-MANNOPYRANOSYLOXY)-2-METHYL-1-(*m*-METHYLPHENYL)PYRIDIN-4-ONE (3a)

Red oil, 0.0793 g (24 %). ^{1}H NMR δ / ppm: 2.06 (s, 3H, CH_3), 2.40 (s, 3H, CH_3-Ar), 3.43–3.62 (m, 4H, H-4', H-5', H-6'$_a$, H-6'$_b$), 3.81 (dd, 1H, *J* = 9.02 Hz, *J* = 3.21 Hz, H-3'), 3.92 (s, 1H, H-2'), 4.53 (br s, 1H, OH), 5.18 (br s, 3H, 3 × OH), 5.75 (d, 1H, *J* = 1.75 Hz, H-1'), 7.14 (d, 1H, *J* = 6.58 Hz, H-5), 7.20–7.36 (m, 4H, H-6, 3 × H-Ar), 7.51 (app t, 1H, *J* = 7.82 Hz, *J* = 7.86 Hz, H-Ar). ^{13}C NMR δ / ppm: 14.52, 20.59, 60.92, 66.94, 69.66, 70.46, 75.12, 98.21, 110.31, 122.48. 122.86, 125.81, 129.43, 130.26, 139.61, 141.74, 142.40, 156.27, 158.11. ESI-MS: *m*/*z* 378.2 $[M+H]^+$. HRMS: calcd. for $C_{19}H_{23}NO_7$ $[M+H]^+$ 378.1553; found at *m*/*z* 378.1546.

3-(α-D-MANNOPYRANOSYLOXY)-1-(*m*-METHOXYPHENYL)-2-METHYLPYRIDIN-4-ONE (3b)

Yellow oil, 0.0447 g (80 %). ^{1}H NMR δ / ppm: 2.07 (s, 3H, CH_3), 3.45–3.61 (m, 4H, H-4', H-5', H-6'$_a$, H-6'$_b$), 3.78 (s, 1H, H-3'), 3.81 (s, 3H, OCH_3), 3.91 (br s, 1H, H-2'), 4.54 (br s, 1H, OH), 5.12 (br s, 3H, 3 × OH), 5.75 (s, 1H, H-1'), 7.13–7.17 (m, 4H, H-5, 3 × H-Ar), 7.31 (d, 1H, *J* = 6.51 Hz, H-6), 7.51 (app t, 1H, *J* = 8.36 Hz, *J* = 8.05 Hz, H-Ar). ^{13}C NMR δ / ppm: 14.52, 55.62, 60.93, 66.71 69.67, 70.46, 75.19, 98.30, 110.16, 111.25, 115.63, 117.50, 123.09, 130.53, 141.75, 143.41, 156.43, 157.93, 159.88. ESI-MS: *m*/*z* 416.2 $[M+H]^+$. HRMS: calcd. for $C_{19}H_{23}NO_8$ $[M+H]^+$ 394.1502; found at *m*/*z* 394.1494.

3-(α-D-MANNOPYRANOSYLOXY)-2-METHYL-1-(*m*-NITROPHENYL)PYRIDIN-4-ONE (3c)

Brown oil, 0.0202 g (69 %). ^{1}H NMR δ / ppm: 2.07 (s, 3H, CH_3), 3.35–3.65 (m, 4H, H-4', H-5', H-6'$_a$, H-6'$_b$), 3.77 (dd, 1H, *J* = 9.04 Hz, *J* = 3.31 Hz, H-3'), 3.89–3.91 (m, 1H, H-2'), 4.54 (br s, 1H, OH), 5.19 (br s, 2H, 2 × OH), 5.79 (s, 1H, H-1'), 7.17 (d, 1H, *J* = 6.55 Hz, H-5), 7.35 (d, 1H, *J* = 6.55 Hz, H-6), 7.90 (t, 1H, *J* = 8.16 Hz, H-Ar), 8.00–8.08 (m, 1H, H-Ar), 8.44–8.48 (m, 2H, 2 × H-Ar). ^{13}C NMR δ / ppm: 14.56, 62.75, 66.57, 69.66, 70.42, 75.19, 98.22, 110.34, 121.24. 126.32, 126.61, 127.93, 131.20, 139.61, 142.73, 148.15, 156.80, 158.22. ESI-MS: *m*/*z* 431.1 $[M+Na]^+$. HRMS: calcd. for $C_{18}H_{20}N_2O_9$ $[M+H]^+$ 409.1247; found at *m*/*z* 409.1240.

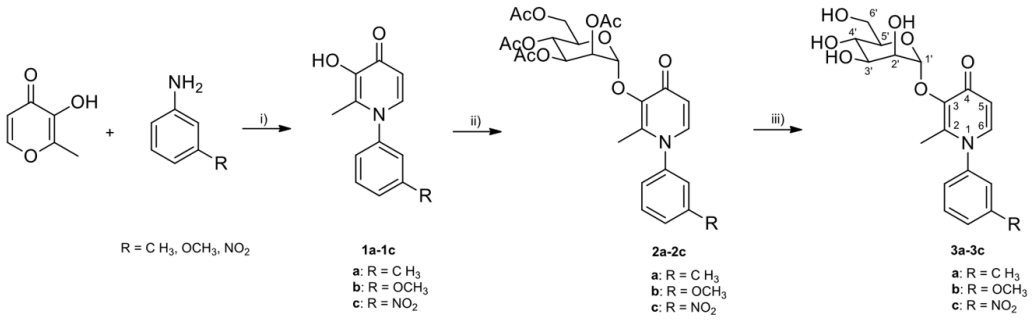
Inhibition Hemagglutination Test

A recombinant type 1 fimbriated *E. coli* strain, *E. coli* HB 101 (pPKl4), was used and cultured according to the protocol applied in our previous studies.[30–33] Guinea pig erythrocytes were isolated and used as described.[30,31,33] Mannosides **3a**–**3c** were dissolved in distilled deionized water, and 10 µL of stock sugar solutions (diluted solutions starting from 20 mM concentration) was mixed with 10 µL of bacteria suspension in wells in V-shaped 96-well microtiter plates (Nunc). After 10 min, guinea-pig erythrocytes (10 µL) were added. Erythrocyte agglutination was read after approximately 10 min at room temperature. The lowest sugar concentration that inhibited hemagglutination was determined visually as inhibition titer (IT). IT values were obtained from four independent tests.

RESULTS AND DISCUSSION

We report the synthesis of novel *N*-aryl 3,4-HP mannosides with methyl, methoxy and nitro substituents in the *meta* position of the aryl part. The aim of our work was to examine the inhibitory potential of prepared α-mannosides with the *N*-aryl 3,4-HP aglycon toward the adhesion of *E. coli* to erythrocytes and to study the influence of substituents at the *m*- and *p*-aryl position on the FimH affinity. Target compounds **3a**–**3c** were prepared as shown in Scheme 1.

Novel compounds **1a**–**1c** were prepared following the previously published procedure for analogous *para* derivatives.[33] Aryl parts of the pyridinones **1a**–**1c** were



Scheme 1. Preparation of pyridinone mannosides **3a–3c**: i) autoclave, *p*-TsOH, H_2O, 150 °C, 48 h; ii) AgOTf, collidine, acetobromomannose, dry DCM, –78 °C, 24 h; ii) NaOMe, dry MeOH, 1 h.

meta substituted phenyls: *m*-methylphenyl (**1a**), *m*-methoxyphenyl (**1b**) and *m*-nitrophenyl (**1c**). Their structures were confirmed using NMR spectroscopy and mass spectrometry techniques. Acetylated glycoconjugates **2a**–**2c** were synthesized by the Königs-Knorr method[2,33] using acetobromomannose as the glycosyl donor and *meta* substituted *N*-aryl 3,4-HPs **1a**–**1c** as acceptors in the presence of AgOTf. Because of participating acetyl group at the 2-position on the sugar molecule, Königs-Knorr reaction resulted with stereospecific formation of 1,2-*trans* glycosides, acetylated α-mannosides **2a**–**2c** in 19–47 % yields. Compounds **2a**–**2c** were deprotected using Zemplén deacetylation.[2,11] The efficacy of deacetylation was confirmed by the absence of the characteristic signals in ^{1}H and ^{13}C NMR spectra belonging to acetyl groups. Target α-mannosides **3a**–**3c** were obtained in 24–80 % yields.

In vitro structure-activity relationship study, using hemagglutination assay (HA), was performed on *para*[33] and *meta* substituted *N*-aryl 3,4-HP mannosides. These compounds were tested as antagonists of FimH-mediated bacterial adhesion. The minimal concentration of a tested *meta* substituted compound required to prevent *E. coli* FimH from agglutinating guinea pig erythrocytes was determined (expressed as IT value) and compared to that of a reference inhibitor, methyl α-D-mannoside (MeMan) and previously described 3,4-HP mannosides (**I**–**IV**). The results are shown in Figure 2.

All tested compounds showed better inhibitory potency than the reference MeMan. Determined IT values for compounds **3a**–**3c** were 2.6, 3.0, 3.3 mM, respectively. These results show that α-mannosides **3a**–**3c** are 6–7 times better inhibitors of the hemagglutination than MeMan. Previously described mannoside **IV** showed a very

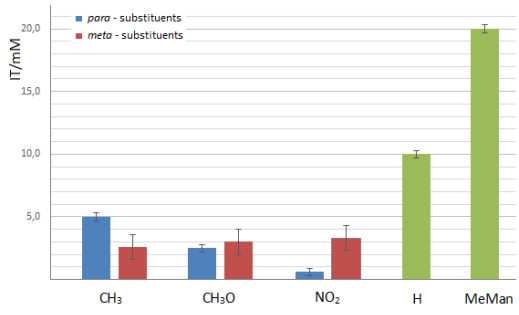


Figure 2. Comparison of IT values of *N*-aryl 3,4-HP mannosides **3a**-**3c** towards type 1 fimbriated *E. coli*. The minimal concentration of a compound required to inhibit hemagglutination (IT value) was obtained from four independent tests for *m*-substituted compounds; **3a** 2.6 mM, **3b** 3.0 mM, **3c** 3.3 mM and compared with *p*-substituted compounds; **I** 10 mM, **II** 5 mM, **III** 2.5 mM, **IV** 0.6 mM. Methyl α-D-mannopyranoside (MeMan) is used as reference inhibitor in 20 mM concentration.

significant 32-fold improvement in potency relative to MeMan. Even more improved inhibitory potencies were also observed with mannosides that possess biaryl aglycons.[24,25] Furthermore, all derivatives with substituents in either *meta* or *para* position on the phenyl ring showed stronger activity compared to compound **I** with no substituents in the aryl part of the molecule, indicating the importance of phenyl ring substitution for binding capacity. Mannosides **3b** and **3c**, with methoxy and nitro substituents in *meta* position, respectively, showed higher IT value compared to corresponding *para* derivatives. This result implies that *para* substituent is relevant for the binding affinity. Based on obtained results and on molecular modeling study performed on *para* compounds[33] which indicated the hydrogen bond between *p*-methoxy or *p*-nitro moiety and hydroxyl group of Tyr137 residue in FimH binding pocket, one can conclude that displacement of the groups from *para* to *meta* position influences unfavorably on hydrogen bonding with Tyr137 and consequently on effectiveness of binding to FimH. On the other hand, analogue **3a** with methyl substituent in *meta* position showed two-times better inhibitory activity than mannoside **II**. This suggests that hydrophobic substituent in *meta* position positively affects the binding of 3,4-HP mannosides in FimH binding site. Obtained results will be considered in our future studies in the design of novel 3,4-HP mannosides as FimH antagonists. Preparation and evaluation of disubstituted *N*-aryl 3,4-HP derivatives which have alkyl substituents in *meta* position and such groups in *para* position which can act as hydrogen bond acceptors in hydrogen bond with hydroxyl group of Tyr137 residue could be one promising route.

CONCLUSION

Novel *meta* substituted (methyl-, methoxy-, nitro-) *N*-aryl 3-hydroxypyridin-4-one mannosides were synthesized and their inhibitory potencies as FimH antagonists were evaluated and compared to previously prepared *para* substituted 3,4-HP α-mannosides. The hemagglutination assays revealed greater preference of FimH towards the *para* substituted *N*-aryl 3,4-HP mannosides, especially those with -OCH_3 and -NO_2 substituents. This study provides guidelines for rational design of novel, more effective FimH antagonists. In the continuation of our research on 3,4-HP derived FimH antagonists we plan further chemical transformations on aryl moiety. Based on obtained results, we conclude that the most promising route for the preparation of more efficient 3,4-HP derived *E. coli* anti-adhesives is introduction of alkyl substituents in *meta* position and substituents which can form hydrogen bond with Tyr137 hydroxyl group in *para* position of aryl part.

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REFERENCES

[1] M. E. Taylor, K. Drickamer, *Introduction to Glycobiology*, *2nd ed.*, Oxford University Press, New York, **2006**, p. 105.
[2] T. K. Lindhorst, *Essentials of Carbohydrate Chemistry and Biochemistry*, *2nd ed.*, WileyVCH, Weinheim, **2003**, p. 175.
[3] N. Sharon, *Biochim. Biophys. Acta* **2006**, *1760*, 527.
[4] D. Zopf, S. Roth, *Lancet* **1996**, *347*, 1017.
[5] N. Sharon, I. Ofek, *Glycoconj. J.* **2000**, *17*, 659.
[6] I. Ofek, D. L. Hasty, N. Sharon, *FEMS Immunol. Med. Microbiol*. **2003**, *38*, 181.
[7] R. J. Pieters, *Med. Res. Rev*. **2007**, *27*, 796.
[8] M. Hartmann, T. K. Lindhorst, *Eur. J. Org. Chem*. **2011**, 3583.
[9] S. B. Levy, *Adv. Drug Delivery Rev*. **2005**, *57*, 1446.
[10] K. Lindhorst, C. Kieburg, U. Krallmann-Wenzel, *Glycoconj. J.* **1998**, *15*, 605.
[11] T. K. Lindhorst, S. Kötter, J. Kubisch, U. Krallmann-Wenzel, S. Ehlers, V. Kren, *Eur. J. Org. Chem.* **1998**, *15*, 1669.
[12] D. Choudhury, A. Thompson, V. Stojanoff, S. Langermann, J. Pinkner, S. J. Hultgren, S. D. Knight, *Science* **1999**, *285*, 1060.
[13] P. Aprikian, V. Tchesnokova, B. Kidd, O. Yakovenko, V. Yarov Yarovoy, E. Trinchina, V. Vogel, W. Thomas, E. Sokurenko, *J. Biol. Chem*. **2007**, *282*, 23437.
[14] Kötter, U. Krallmann-Wenzel, S. Ehlers, T. K. Lindhorst, *J. Chem. Soc. Perkin Trans*. **1998**, *1*, 2193.
[15] T. K. Lindhorst, C. Kieburg, U. Krallmann-Wenzel, *Glycoconj. J.* **1998**, *15*, 605.
[16] M. Touaibia, R. Roy, *Mini-Rev. Med. Chem*. **2007**, *7*, 1270.
[17] M. Almant, V. Moreau, J. Kovensky, J. Bouckaert, S. G. Gouin, *Chem. Eur. J.* **2011**, *17*, 10029.
[18] A. Schierholt, M. Hartmann, T. K. Lindhorst, *Carbohydr. Res*. **2011**, *346*, 1519.
[19] I. Ofek, D. L. Hasty, N. Sharon, *FEMS Immunol. Med. Microbiol.* **2003**, *38*, 181.
[20] C. S. Hung, J. Bouckaert, D. Hung, J. Pinkner, C. Widberg, A. Defusco, C. G. Auguste, R. Strouse, S. Langermann, G. Waksman, S. J. Hultgren, *Mol. Microbiol.* **2002**, *44*, 903.
[21] J. Bouckaert, J. Berglund, M. Schembri, E. De Genst, L. Cools, M. Wuhrer, C. S. Hung, J. Pinkner, R. Slättegård, A. Zavialov, D. Choudhury, S. Langermann, S. J. Hultgren, L. Wyns, P. Klemm, S. Oscarson, S. D. Knight, H. De Greve, *Mol. Microbiol*. **2005**, *55*, 441.
[22] A. Wellens, C. Garofalo, H. Nguyen, N. Van Gerven, R. Slättegård, J.-P. Hernalsteens, L. Wyns, S. Oscarson, H. De Greve, S. Hultgren, J. Bouckaert, *PLoS One* **2008**, *3*, 1.
[23] S. D. Knight, J. Bouckaert, *J. Top. Curr. Chem*. **2009**, *288*, 67.
[24] Z. Han, J. S. Pinkner, B. Ford, R. Obermann, W. Nolan, S. A. Wildman, D. Hobbs, T. Ellenberger, C. K. Cusumano, S. J. Hultgren, J. W. Janetka, *J. Med. Chem.* **2010**, *53*, 4779.
[25] N. Firon, S. Ashkenazi, D. Mirelman, I. Ofek, N. Sharon, *Infect. Immun*. **1987**, *55*, 472.
[26] T. Klein, D. Abgottspon, M. Wittwer, S. Rabbani, J. Herold, X. Jiang, S. Kleeb, Christine L., M. Scharenberg, J. Bezençon, E. Gubler, L. Pang, M. Smiesko, B. Cutting, O. Schwardt, B. Ernst, *J. Med. Chem.* **2010**, *53*, 8627.
[27] O. Sperling, A. Fuchs, T. K. Lindhorst, *Org. Biomol. Chem*. **2006**, *4*, 3913.
[28] O. Sperling, A. Fuchs, T. K. Lindhorst, *Infect. Immun*. **1987**, *55*, 472.
[29] S. Rabbani, X. Jiang, O. Schwardt, B. Ernst, *Anal. Biochem.* **2010**, *407*, 188.
[30] R. Ribić, M. Kovačević, V. Petrović Peroković, I. Gruić-Sovulj, V. Rapić, S. Tomić, *Croat. Chem. Acta* **2010**, *83*, 421.
[31] M. Kovačević, L. Barišić, R. Ribić, V. Petrović Peroković, S. Tomić, V. Rapić, *Appl. Organometal. Chem.* **2012**, *26*, 74.
[32] V. Kovač, R. Ribić, V. Petrović Peroković, S. Tomić, L. Barišić, *Appl. Organometal. Chem.* **2016**, *30*, 524.
[33] Ž. Car, T. Hrenar, V. Petrović Peroković, R. Ribić, M. Seničar, S. Tomić, *Chem. Biol. Drug Des*. **2014**, *84*, 393.
[34] M. A. Santos, S. M. Marques, S. Chaves, *Coord. Chem. Rev*. **2012**, *256*, 240.
[35] Y. Ma, T. Zhou, X. Kong, R. C. Hider, *Curr. Med. Chem*. **2012**, *19*, 2816.
[36] C. Queiros , M. J. Amorim, A. Leite, M. Ferreira, P. Gameiro, B. De Castro, K. Biernacki, A. Magalhaes, J. Burgess, M. Rangel, *Eur. J. Inorg. Chem*. **2011**, 131.
[37] D. E. Green, M. L. Bowen, L. E. Scoot, T. Storr, M. Merkel, K. Böhmerle, K. H. Thompson, B. O. Patrick, H. J. Schugar, C. Orvig, *Dalton Trans.* **2010**, *39*, 1604.
[38] E. L. Scott, M. Telpoukhovskaia, C. Rodriguez-Rodriguez, M. Merkel, M. L. Bowen, B. D. G. Page, D. E. Green, T. Storr, F. Thomas, D. D. Allen, P. R. Lockman, B. O. Patrick, M. J. Adam, C. Orvig, *Chem. Sci.* **2011**, *2*, 642.
[39] K. P. R. Kartha, H. J. Jennings, *J. Carbohydr. Chem*. **1990**, *9*, 777.