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Bi- and trivalent glycopeptide mannopyranosides as inhibitors of type 1 fimbriae-mediated bacterial adhesion: variation of valency, aglycon and scaffolding

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Dedicated to Professor András Lipták on the occasion of his 75th birthday

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ABSTRACT

In order to test relevant structural parameters for effective inhibition of mannose-specific bacterial adhesion, bi- and trivalent glycopeptide α-D-mannopyranosides were synthesized that differ in their conformational properties as well as in the spatial arrangement of attached mannosyl residues. They were tested in an inhibition adhesion assay with fluorescent *Escherichia coli* bacteria and testing results were referenced to the inhibitory potency of methyl α-D-mannopyranoside. It was shown, that besides the nature of the mannoside aglycon moiety, scaffolding of α-D-mannopyranosides on a peptide backbone was important for the performance of the synthesized glycopeptides as inhibitors of bacterial adhesion.

1. Introduction

Adhesion of bacteria to the surface of their eukaryotic target cells is mediated by adhesive organelles projecting from the bacterial surface. These proteinaceous hair-like appendages are called fimbriae or, somewhat inaccurately, pili.[1] Mostly, bacterial adhesion is a prerequisite for infection such as in the case of uropathogenic *Escherichia coli* (UPEC), which cause urinary tract infections, one of the most common infections with millions of infected patients every year.[2]

E. coli bacteria possess α-mannoside-specific type 1 fimbriae, which have been identified as a major virulence factor in UPEC infections.[3,4] They have been thoroughly investigated, however, type 1 fimbriae-mediated bacterial adhesion in an in vivo-environment has not been fully understood until today.[5] Therefore, for many years, it has been our goal to synthesize and employ synthetic mannopyranosides as well as mannosidic glycomimetics to improve our understanding of type 1 fimbriae-mediated bacterial adhesion.[6,7] In addition, it has been appealing to design high-affinity ligands for the fimbrial lectin FimH in an attempt to find effective FimH antagonists that can be employed as specific antiadhesives.[8–11] Antiadhesive therapy could eventually be utilized to treat infections such as urinary tract infections.[12,13]

Design of ligands for the type 1 fimbrial lectin FimH should consider a number of known structural features as well as a number of unknowns: It is known from X-ray studies that the bacterial lectin FimH accommodates one single α-D-mannosyl residue with the aglycon portion sticking out of the carbohydrate binding site.[14–16] Furthermore, it has been clearly elucidated that the entrance of the FimH carbohydrate binding site is flanked by two aromatic tyrosine residues, those of Tyr48 and Tyr137, forming what has been called a 'tyrosine gate'.[17] Therefore, synthetic mannopyranosides with an aromatic aglycon show increased affinity for FimH due to π–π interactions of the mannosidic aglycon with the tyrosine gate at the entrance of FimH. This knowledge can be utilized when FimH antagonists are designed.[7,11] In addition, mannopyranosides with a rather extended aglycon moiety have often shown enhanced binding affinity,[18–20] but in this case no conclusive interpretation of structure–activity relationships has been achieved. The same is true for multivalency effects that have frequently been observed with miscellaneous cluster mannopyranosides as ligands for type 1 fimbriated bacteria, especially with bi- and trivalent glycoclusters.[21–25] A possible explanation, why multivalent mannopyranosides perform well as inhibitors of FimH, can be found in a statistical effect (basically an elevated local mannoside concentration),[26] on the other hand, it has been speculated that secondary

Abbreviations: Fmoc, fluorenylmethoxycarbonyl; Gly, L-glycine; Ser, L-serine; Lys, L-lysine; DPr, L-diaminopropionic acid; Ala, L-alanine; HATU, *O* -(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; DIPEA, *N*-ethyl-*N*-diisopropylamine; GFP, green fluorescent protein.

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binding sites on the fimbrial lectin might be occupied by multivalent mannnosides, hence resulting in an increased affinity of such ligands.[27]

On this account, a number of open questions about carbohydrate recognition of type 1 fimbriated *E. coli* bacteria have formed the basis of a study, in which bi- and trivalent low-molecular weight glycopeptides have been synthesized and tested as inhibitors of type 1 fimbriae-mediated bacterial adhesion to a mannan-coated surface.

2. Results and discussion

A collection of six mannosidic glycopeptides were prepared in order to vary three different structural parameters, which might influence their affinity as ligands for the bacterial lectin FimH: (i) carbohydrate valency, (ii) the glycoside aglycon, and particularly (iii) the spatial arrangement of the ligated mannopyranosides. Hence, the peptide backbone functions as a scaffold molecule and, in addition, may add favourably to glycopeptide–FimH interactions when employed in adhesion assays (Fig. 1).

2.1. Synthesis of glycopeptides

Four different peptides, pentapeptides **1**–**3** and the heptapeptide **4** were designed as oligoamines to allow attachment of carboxy-functionalized α-mannopyranosides via peptide coupling (Fig. 2). Peptides **1**–**4** were readily prepared by a standard SPPS (solid phase peptide synthesis) Fmoc protocol and obtained in good yields and high purity. Amino functional groups were either introduced as ε-amino groups of lysine (Lys) (**2**–**4**) or by employing the nonproteinogenic amino acid L-diaminopropionic acid (Dpr). When Dpr was used in SPPS a significantly less flexible scaffold peptide was obtained than when lysine was employed for the synthesis of analogous peptides. Consequently, glycopeptides derived from pentapeptide **1** might exhibit quite different properties than analogous peptides derived from **2**.

Carboxy-functionalized mannopyranosides **5** and **6** were selected as principal ligands for the FimH carbohydrate binding site (Fig. 2). They vary with respect to their aglycon moieties. In light of the structure of the fimbrial lectin FimH, it was anticipated that glycopeptides decorated with mannopyranoside **6**, carrying the aromatic aglycon moiety, would perform better as inhibitors of type 1 fimbriae-mediated bacterial adhesion than corresponding glycopeptides having mannopyranoside **5** attached. Both carboxy-functionalized mannopyranosides, **5** as well as **6**, could be obtained from mannose in four easy steps according to literature-known procedures.[28–30]

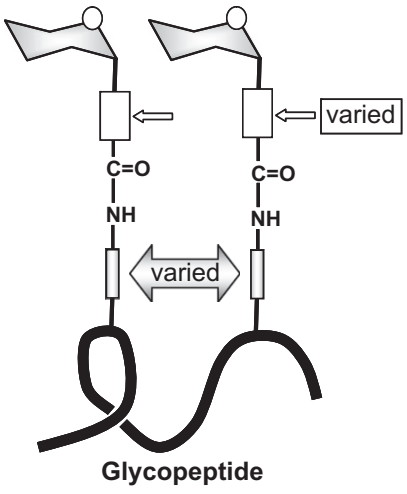


Figure 1. Mannopyranoside ligands for the bacterial lectin FimH with diverse aglycon moieties can be scaffolded on small peptides in order to vary the valency of the resulting glycopeptides, and the spatial arrangement of the ligated saccharides.

For the preparation of the target glycopeptides, HATU-mediated peptide coupling was employed. First pentapeptide **1** was reacted with the aliphatic carboxylic acid **5**, as well as with its benzylic analogue **6** to yield the bivalent glycopeptides **7** and **8**, respectively (Scheme 1). Likewise pentapeptide **2**, the di-lysine analogue of pentapeptide **1**, furnished the respective bivalent glycopeptides **9** and **10**. In all peptide coupling reactions a three-fold excess of the carboxy-functionalized mannoside was employed to drive the reaction to completion. Nevertheless, mono-coupling products were formed as side products in all cases, in addition to other side products, according to MS analysis. Unfortunately, purification of the obtained product mixtures was extremely tedious. Only a combination of MPLC, followed by gel chromatography (GPC) to remove low molecular weight impurities, and final HPLC purification gave pure products, in considerably lowered yields. Here, yields were not optimized, but the pure structurally varied glycopeptide mannopyranosides were employed in biological tests.

2.2. Adhesion inhibitions assays

The synthesized glycopeptide mannopyranosides **7**–**12** (Scheme 1) were investigated as inhibitors of type 1 fimbriae-mediated bacterial adhesion to the polysaccharide mannan on mannan-coated 96-well microtiter plates.[31] A GFP-tagged uropathogenic *E. coli* strain was employed, which expresses exclusively type 1 fimbriae. Serial dilutions of the respective inhibitor were incubated with fluorescent *E. coli* cells and inhibition curves were determined from which IC_{50} values were deduced for each tested glycopeptide. As the tested glycopeptides could never effect 100% inhibition of bacterial adhesion, even at their maximal concentrations employed (Table 1), IC_{50} values were defined as the inhibitor concentration, which causes 50% of the maximally achieved inhibition. On each individual test plate the standard inhibitor methyl α-D-mannopyranoside (MeMan) was tested in parallel. Then, the inhibitory potencies of tested glycopeptides were referenced to that determined for MeMan on the same plate, leading to relative inhibitory potencies (RIP values) for every tested compound. Thus, RIP values allow to compare the potencies of all tested glycopeptides as inhibitors of type 1 fimbriae-mediated bacterial adhesion, because they are consistently referenced.

Testing results are collected in Table 1. In addition to the measured IC_{50} values and deduced RIP values, valency-corrected RIP values, RIP_{vc}, are provided, in order to assess the inhibitory potency of a compound irrespective of its mannoside valency.

From the testing results collected in Table 1 it can be seen that phenyl mannopyranoside residues make a big difference when compared to alkyl mannopyranosides ligated to the same peptide. Hence, glycopeptides **8**, and **10**–**12** show enhanced inhibitory potencies in comparison to the standard glycoside for this assay, MeMan. Glycopeptides **7** and **9** on the other hand, do not significantly exceed the inhibitory potency of MeMan. Glycopeptide **9**, however, having two α-D-mannosyl residues attached on longer side chains than in **7**, has advantages (RIP = 2) over the less flexible molecule **7** (RIP = 1). When a phenyl moiety is included in the mannoside aglycone portion, the inhibitory potency of the respective glycopeptides is directly enhanced. This effect is more pronounced when **7** is changed into **8**, than when **9** is substituted by **10**. Strikingly, variation of the spatial exposition of mannoside ligands makes a big difference as well, as it can be seen by comparison of **10** (RIP = 20) and **11** (RIP = 8). This result is somewhat unexpected but quite instructive as it exemplifies the importance of the peptide scaffold that is chosen for an equal number of equal

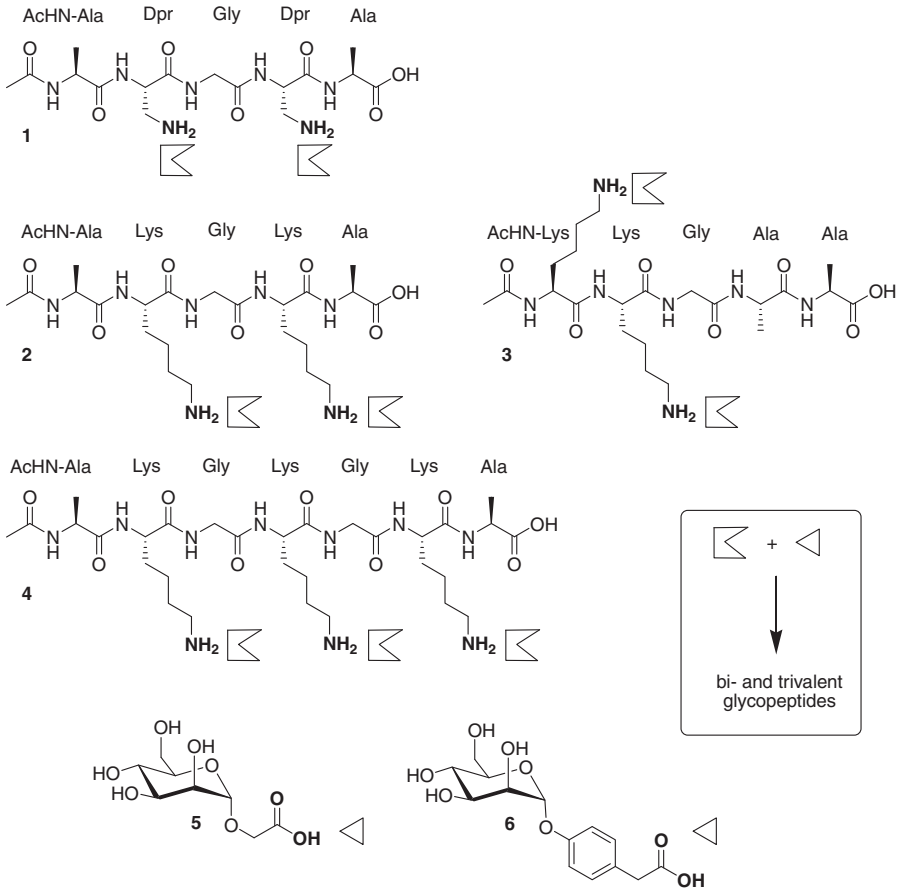


Figure 2. Pentapeptides **1**, **2**, and **3**, and the heptapeptide **4** were prepared by standard Fmoc SPPS and used as scaffold molecules for the synthesis of bi- and trivalent glycopeptide mannopyranosides by peptide coupling. Carboxy-functionalized α-D-mannopyranosides, **5** and **6** were employed in peptide coupling reactions in solution.

mannopyranosides. The best inhibitor tested was the trivalent glycopeptide **12**, exceeding the inhibitory potency of MeMan by 43-fold. On a valency-corrected basis, **12** (RIP_{vc} = 14) ranges together with the bivalent glycopeptide **8** (RIP_{vc} = 13.5).

3. Conclusions

From the results obtained in this account it can be concluded that the glycopeptide approach to inhibitors of type 1 fimbriae-mediated bacterial adhesion is a promising one. The structurally versatile system of mannopyranoside scaffolding on a peptide backbone[32] allows to optimize valency (cf. **12**), conformational flexibility (cf. **8**), and the spatial arrangement of mannoside ligands (cf. **10** vs **11**). Apparently, it is fundamental for optimization of inhibitory potencies of mannoside derivatives, to choose mannopyranosides with an aromatic aglycon portion, as this leads to favourable π–π interactions with the tyrosine gate at the entrance of the fimbrial lectin FimH. This result is in accordance with published work[16–20,24] and again confirmed with the herein synthesized, so far unknown glycopeptides.

It can be assumed that intramolecular interactions, such as π–π stacking interactions of phenyl mannopyranoside residues within one glycopeptide, might lead to diminished inhibitory potencies. This has been suggested earlier, after molecular dynamic studies which have shown, that clustering of phenyl mannopyranosides on a multivalent scaffold is not necessarily a successful approach to high affinity ligands for FimH.[33] However, here it was also seen, that a rather narrow assembly of phenyl mannopyranosides such as in case of glycopeptide **8** can be relatively successful, probably because intramolecular π–π stacking is hampered. In addition it should be stated that earlier on, lysine-based glycodendrimers have been shown to effectively inhibit adhesion of type 1 fimbriated adhesion of *E. coli*.[25]

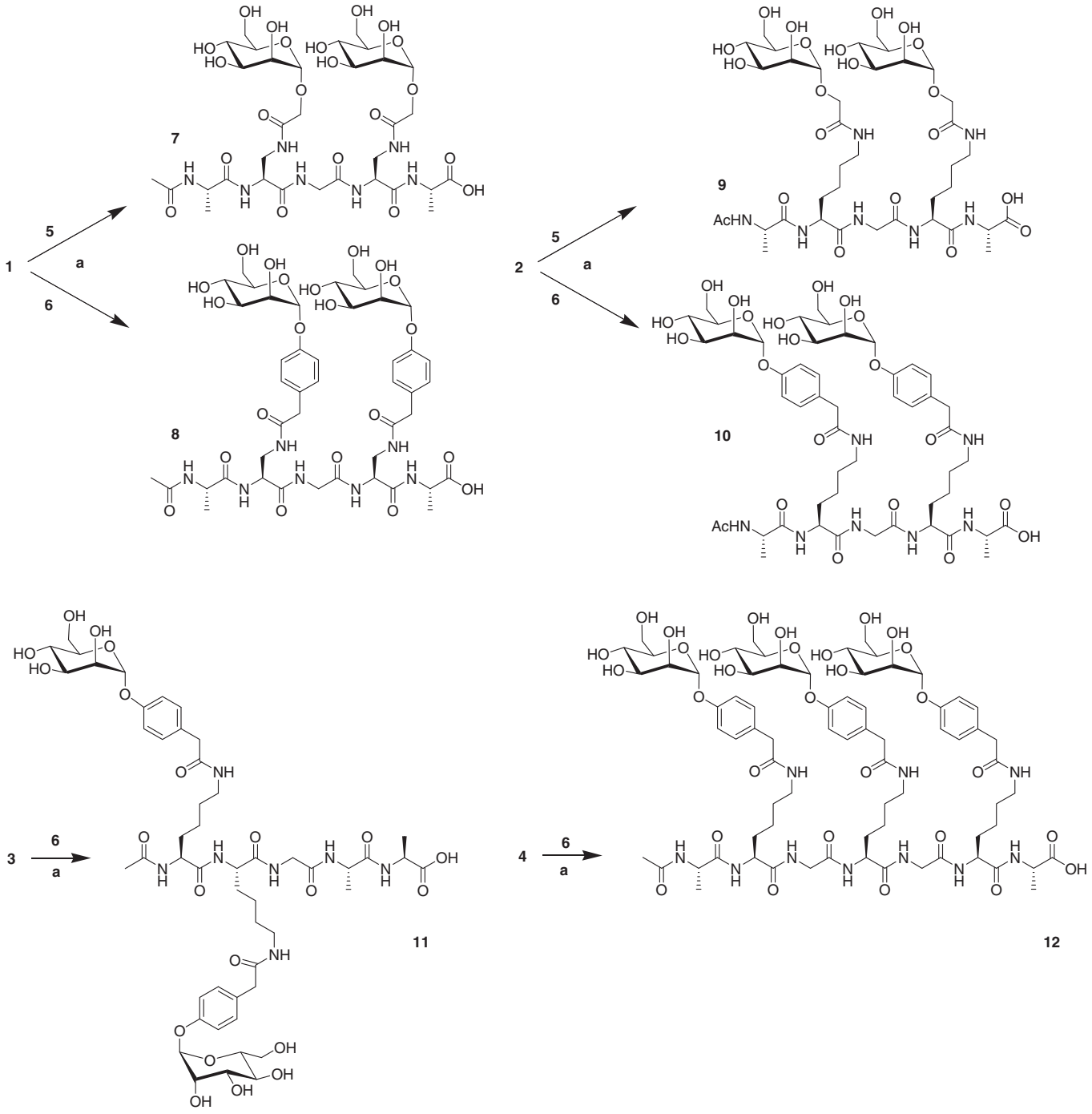
In future studies we will expand this work on the development of potent inhibitors of type 1 fimbriae-mediated bacterial adhesion by combining small peptide scaffold molecules with advanced mannoside residues.[34] This approach could lead to very effective antagonists of the bacterial lectin FimH, also useful in an in vivo-scenario.

4. Experimental

4.1. Synthesis

4.1.1. General methods

Thin layer chromatography was performed on silica gel plates (GF 254, Merck). Detection was effected by UV irradiation and subsequent charring with 10% sulfuric acid in EtOH followed by heat treatment. Flash chromatography was performed on Silica Gel 60 (230–400 mesh, particle size 0.040–0.063 mm, Merck) using distilled solvents. Optical rotations were measured on a Perkin–Elmer 241 polarimeter (Na-D-line: 589 nm, length of cell 1 dm). MALDI-MS measurements were recorded on a MALDI-ToF-MS-Biflex III (Bruker) instrument. Preparative MPLC was performed on an apparatus of BÜCHI Labortechnik GmbH using a LiChroprep RP-18 (40–60 μm, Merck) column for reversed-phase silica gel chromatography. Preparative HPLC was performed on a Shimadzu instrument with diode array detection and a LiChrosorb RP-8



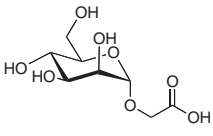
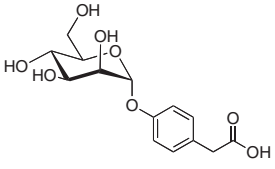
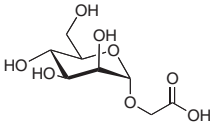
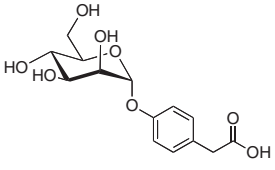
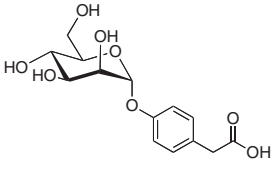
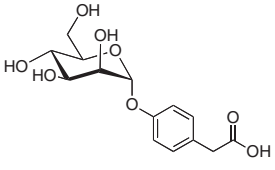
Scheme 1. Reagents and conditions: (a) peptide coupling: HATU, DIPEA, DMF, 0 °C to rt, overnight; **7**: 21%; **8**: 32%; **9**: 15%; **10**: 16%; **11**: 24%; **12**: 25%.

(7 μm) silica gel column (Merck). ^{1}H and ^{13}C spectra were recorded on Bruker DRX-500 and AV-600 spectrometers. 2D NMR techniques (^{1}H–^{1}H-COSY, ^{1}H–^{13}C-HSQC and ^{1}H–^{13}C-HMBC) were used for full assignment of the spectra. Chemical shifts were reported relative to internal tetramethylsilane (δ 0.00 ppm) or D_2O (δ 4.76 ppm). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of nitrogen. Commercial reagents were used without purification unless otherwise noted.

4.1.2. General procedure for Fmoc SPPS

The glycopeptide was assembled manually by using a fritted glass reaction vessel according to a standard Fmoc SPPS protocol. Fmoc-Ala-Wang resin was swollen in DMF (4 mL) for 2 h before the synthesis and then deprotected with 20% piperidine solution in DMF (2 × 15 min). The next Fmoc-protected amino acid (4 equiv), HBTU (3.6 equiv) and HOBt (4 equiv) were dissolved in DMF (3 mL), it was shaken for 5 min, then DIPEA (4 equiv) was added and this mixture shaken for another 2 min before it was transferred to the reaction vessel which contained the resin. The reaction mixture was shaken for 4 h or overnight at rt, filtered and washed with DMF (5 × 5 mL). For diaminopropionic acid coupling, Fmoc-Dpr(Boc)-OH (2 equiv) and HATU (2 equiv) were dissolved in DMF (3 mL), shaken for 5 min and then DIPEA (2 equiv) was added. This mixture was shaken for another 2 min and then

Table 1
The potencies of tested glycopeptides as inhibitors of *E. coli* adhesion to mannan were referenced to MeMan, which was tested on the same microtitre plate.

Glycopeptide	Structure of scaffolded mannopyranoside moiety (valency)	Maximal concentration applied on test plate (mM)	Maximal inhibition achieved (%)	RIP (SD)	RIP_{vc}
7	 (2)	31.0	69	1 (0.2)	0.5
8	 (2)	26.2	75	27 (2)	13.5
9	 (2)	30.9	61	2 (0.3)	1
10	 (2)	32.8	66	20 (7)[a]	10
11	 (2)	22.8	78	8 (0.8)	4
12	 (3)	16.7	48	43 (14)[a]	14

RIP values are listed together with their standard deviations (SD).
[a] Standard deviations in these cases are rather high, as one experiment gave outlier values.

transferred to the reaction vessel. The reaction mixture was shaken overnight at rt, filtered and washed with DMF (5 × 5 mL). Any unreacted amino groups are capped as acetamides by treatment of the resin with a solution of Ac_2O (160 μL) and DIPEA (280 μL) in 3 mL DMF (1 × 60 min, 1 × 30 min). After coupling of the *N*-terminal amino acid, the peptide was treated first with piperidine to remove the terminal Fmoc-group and capped afterwards. Cleavage of the peptide from the resin was achieved with TFA/CH_2Cl_2 (5:1) (1 × 15 min, 1 × 10 min), followed by washing with EtOH. The solvent was removed in vacuo and the crude product subjected to lyophilization in order to yield a fluffy product, which can be easily handled.

4.1.3. General procedure for peptide coupling to achieve target glycopeptides

The carboxy-functionalized mannoside (3 equiv for each amino function) and HATU (2.7 equiv for each amino function) were dissolved in dry DMF under nitrogen atmosphere and the solution cooled to 0 °C. DIPEA (3 equiv for each amino group) was added and the reaction mixture shaken for 2 min. Then, the glycopeptide was dissolved in dry DMF and added dropwise at 0 °C. After 4 h the cooling was removed and the reaction mixture stirred overnight at rt. The solvent was removed in vacuo and the crude product purified by MPLC (acetonitrile/water, 1:1), followed by GPC on Sephadex LH-20 (eluent: methanol) and final RP-HPLC employing a LiChrosorb RP-8 column at a flow rate of 10 mL/min (linear gradient of 20% acetonitrile to 80% acetonitrile over 100 min).

4.1.4. *N*-Acetyl-L-alanyl-L-diaminopropionyl-glycyl-L-diaminopropionyl-L-alanine (1)

The glass reaction vessel was loaded with Fmoc-Ala-Wang resin (220 mg, 176 μmol, 1 equiv). According to the general procedure for Fmoc SPPS, Fmoc-Dpr(Boc)-OH (150 mg, 0.351 mmol, 2 equiv), Fmoc-Gly-OH (210 mg, 0.706 mmol, 4 equiv), Fmoc-Dpr(Boc)-OH (150 mg, 0.351 mmol, 2 equiv), and Fmoc-Ala-OH (220 mg, 0.707 mmol, 4 equiv) were reacted successively and cleaved from the resin to yield the title compound as a white lyophilisate (62 mg, 0.14 mmol, 82%). ^{1}H NMR (600 MHz, D_2O): δ = 4.80 (m, 2H, C*H*CH_2, hidden under water peak), 4.40 (q, 1H, $^3J_{H\text{-}\alpha,H\text{-}\beta}$ 7.2 Hz, H-$\alpha_{(Ala)}$), 4.26 (q, 1H, $^3J_{H\text{-}\alpha,H\text{-}\beta}$ 7.2 Hz, H-$\alpha_{(Ala)}$), 4.02 (s, 2H, H-$\alpha_{(Gly)}$), 3.51 (m_c, 2H, CHC*H*H), 3.34–3.26 (m, 2H, CHCH*H*), 2.02 (s, 3H, NHAc), 1.42 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.3 Hz, H-$\beta_{(Ala)}$), 1.39 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.3 Hz, H-$\beta_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 178.1–166.8 (*C*OOH, 4*C*ONH, *C*OCH_3), 52.4 (2C-*C*HCH_2)

52.0, 50.4 (2C-$\alpha_{(Ala)}$), 44.1 (C-$\alpha_{(Gly)}$), 41.8 (2C–CH_2), 23.4 ($COCH_3$), 18.0 (C-$\beta_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{16}H_{29}N_7O_7+H]^+$: 432.22; found *m/z* 433.22; calcd for $[C_{16}H_{29}N_7O_7+Na]^+$: 454.20; found *m/z* 454.21.

4.1.5. *N*-Acetyl-L-alanyl-L-lysyl-glycyl-L-lysyl-L-alanine (2)

The glass reaction vessel was loaded with Fmoc-Ala-Wang resin (220 mg, 176 μmol, 1 equiv). According to the general procedure for Fmoc SPPS, Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv), Fmoc-Gly-OH (210 mg, 0.706 mmol, 4 equiv), Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv), and Fmoc-Ala-OH (220 mg, 0.707 mmol, 4 equiv) were reacted successively and cleaved from the resin to yield the title compound as a white lyophilisate (83 mg, 0.16 mmol, 91%). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.30–8.26 (m, 1H, NH), 8.15–8.09 (m, 1H, NH), 8.06–8.02 (m, 1H, NH), 7.96–7.92 (m, 1H, NH), 4.31–4.14 (m, 4H, H-$\alpha_{(Lys,Ala)}$), 3.72 (m_c, 2H, H-$\alpha_{(Gly)}$), 2.78–2.70 (m, 4H, H-ε), 1.84 (s, 3H, NHAc), 1.72–1.61 (m, 2H, H-βa_{Lys}), 1.58–1.47 (m, 6H, H-$\beta b_{(Lys)}$, H-δ), 1.36–1.29 (m, 4H, H-γ), 1.27 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.4 Hz, H-$\beta_{(Ala)}$), 1.18 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.4 Hz, H-$\beta_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 174.0–168.5 (*C*OOH, 4*C*ONH, *C*OCH_3), 52.4–47.5 (4C-$\alpha_{(Lys,Ala)}$), 42.0 (C-$\alpha_{(Gly)}$), 38.6 (2C-ε), 30.8 (2C-$\beta_{(Lys)}$), 26.5 (2C-δ), 22.5 ($COCH_3$), 22.0 (2C-γ), 17.9, 17.0 (C-$\beta_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{22}H_{41}N_7O_7+H]^+$: 516.31; Found *m/z* 516.96; calcd for $[C_{22}H_{41}N_7O_7+Na]^+$: 538.29; found *m/z* 538.92.

4.1.6. *N*-Acetyl-L-lysyl-L-lysyl-glycyl-L-alanyl-L-alanine (3)

The glass reaction vessel was loaded with Fmoc-Ala-Wang resin (220 mg, 176 μmol, 1 equiv). According to the general procedure for Fmoc SPPS, Fmoc-Ala-OH (220 mg, 0.707 mmol, 4 equiv), Fmoc-Ala-OH (220 mg, 0.707 mmol, 4 equiv), Fmoc-Gly-OH (210 mg, 0.706 mmol, 4 equiv), Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv), and Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv) were reacted successively and cleaved from the resin to yield the title compound as a white lyophilisate (81 mg, 0.15 mmol, 89%). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.31–8.26 (m, 1H, NH), 8.17–8.10 (m, 1H, NH), 8.07–8.02 (m, 1H, NH), 7.95–7.90 (m, 1H, NH), 4.29–4.15 (m, 4H, H-$\alpha_{(Lys,Ala)}$), 3.72 (m_c, 2H, H-$\alpha_{(Gly)}$), 2.77–2.69 (m, 4H, H-ε), 1.84 (s, 3H, NHAc), 1.72–1.61 (m, 2H, H-$\beta a_{(Lys)}$), 1.57–1.48 (m, 6H, H-$\beta b_{(Lys)}$, H-δ), 1.38–1.29 (m, 4H, H-γ), 1.27 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.2 Hz, H-$\beta_{(Ala)}$), 1.19 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.0 Hz, H-$\beta_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 174.0–168.5 (*C*OOH, 4*C*ONH, *C*OCH_3), 52.5, 52.4, 47.7, 47.5 (4C-$\alpha_{(Lys,Ala)}$), 42.1 (C-$\alpha_{(Gly)}$), 38.6 (2C-ε), 31.0 (2C-$\beta_{(Lys)}$), 26.5 (2C-δ), 22.5 ($COCH_3$), 22.1 (2C-γ), 17.9, 17.0 (C-$\beta_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{22}H_{41}N_7O_7+H]^+$: 516.31; found *m/z* 517.07; calcd. for $[C_{22}H_{41}N_7O_7+Na]^+$: 538.29; found *m/z* 539.05.

4.1.7. *N*-Acetyl-L-alanyl-L-lysyl-glycyl-L-lysyl-glycyl-L-lysyl-L-alanine (4)

The glass reaction vessel was loaded with Fmoc-Ala-Wang resin (220 mg, 176 μmol, 1 equiv). According to the general procedure for Fmoc SPPS, Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv), Fmoc-Gly-OH (210 mg, 0.706 mmol, 4 equiv), Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv), Fmoc-Gly-OH (210 mg, 0.706 mmol, 4 equiv), Fmoc-Lys(Boc)-OH (330 mg, 0.704 mmol, 4 equiv), and Fmoc-Ala-OH (220 mg, 0.707 mmol, 4 equiv) were reacted successively and cleaved from the resin to yield the title compound as a white lyophilisate (92 mg, 0.13 mmol, 74%). ^{1}H NMR (600 MHz, D_2O): δ = 4.34–4.23 (m, 3H, H-$\alpha_{(Lys,Ala)}$), 4.23–4.11 (m, 2H, H-$\alpha_{(Lys,Ala)}$), 4.00–3.86 (m_c, 4H, H-$\alpha_{(Gly)}$), 2.97–2.90 (m, 6H, H-ε), 1.97 (s, 3H, NHAc), 1.81–1.68 (m, 4H, H-$\beta a_{(Lys)}$), 1.68–1.59 (m, 8H, H-$\beta b_{(Lys)}$, H-δ), 1.46–1.39 (m, 6H, H-γ), 1.38 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.3 Hz, 3H, H-$\beta_{(Ala)}$), 1.34 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.2 Hz, 3H, H-$\beta_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O): δ = 178.2–172.8 (*C*OOH, 4*C*ONH, *C*OCH_3), 55.6, 55.2, 51.3, 50.5 (5C-$\alpha_{(Lys,Ala)}$), 43.0 (C-$\alpha_{(Gly)}$), 41.2 (3C-ε), 32.3 (2C-$\beta_{(Lys)}$), 28.2 (2C-δ), 24.0 ($COCH_3$), 23.6 (2C-γ), 18.0, 17.8 (C-$\beta_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{30}H_{56}N_{10}O_9+H]^+$: 701.43; found *m/z* 702.08; calcd for $[C_{30}H_{56}N_{10}O_9+Na]^+$: 723.41; found *m/z* 724.05.

4.1.8. *N*-Acetyl-L-alanyl-L-diaminopropionyl-N^β-{(α-D-mannopyranosyloxy)-acetamido}-glycyl-L-diaminopropionyl-N^β-{(α-D-mannopyranosyloxy)-acetamido}-L-alanine (7)

According to the general procedure for the peptide coupling in solution, mannoside **5** (98 mg, 0.41 mmol, 6 equiv), HATU (141 mg, 0.370 mmol, 5.4 equiv), DIPEA (70 μL, 0.41 mmol, 6 equiv) and peptide **1** (30 mg, 69 μmol, 1 equiv) were reacted in DMF and purified. The title compound was obtained as a white lyophilisate (13 mg, 14 μmol, 21%). HPLC: t_R = 9.3 min [A = waterf, B = acetonitrile + 1% TFA, 20% B→80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ +24.5 (*c* 0.6, MeOH). ^{1}H NMR (600 MHz, D_2O/H_2O, 1:11): δ = 8.44–8.32 (m, 3H, NH), 8.28–8.26 (m, 1H, NH), 8.17–8.12 (m, 1H, NH), 8.05–7.96 (m, 2H, NH), 4.80 (d, 2H, $J_{1,2}$ 1.5 Hz, H-1), 4.46–4.40 (m, 2H, C*H*CH_2), 4.27–4.25 (m, 2H, H-$\alpha_{(Ala)}$), 4.13–4.11 (m, 2H, OC*H*H), 3.98–3.96 (m, 2H, OCH*H*), 3.80 (dd, 2H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.66–3.62 (m, 2H, H-6b), 3.62–3.52 (m, 8H, H-2, H-4, H-5, H-$\alpha_{(Gly)}$), 3.45 (dd, 2H, $J_{2,3}$ 3.9 Hz, $J_{3,4}$ 9.7 Hz, H-3), 3.07 (m_c, 4H, CHC*H*$_2$), 1.92 (s, 3H, NHAc), 1.30 (t, 3H, *J* 7.2 Hz, H-$\beta_{(Ala)}$), 1.22 (d, 3H, $J_{H\text{-}\alpha,H\text{-}\beta}$ 7.2 Hz, H-$\beta_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O/H_2O, 1:11): δ = 174.4, 174.2, 172.5, 172.3, 171.9, 171.2 (6*C*ONH, *C*OCH_3) 101.1 (2C-1), 73.2 (2C-5), 72.5 (2C-2), 71.3 (2C-3), 68.3 (O*C*H_2) 66.6 (2C-4), 60.9 (2C-6), 52.5 (*C*HCH$_2$) 50.0, 49.0 (2C-$\alpha_{(Ala)}$), 46.6 (CH*C*H$_2$) 39.9 (C-$\alpha_{(Gly)}$), 21.8 ($COCH_3$), 16.5, 16.3 (C-$\beta_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{32}H_{53}N_7O_{21}+Na]^+$: 894.31; found *m/z* 894.91; calcd for $[C_{32}H_{53}N_7O_{21}+K]^+$: 910.29; found *m/z* 910.90.

4.1.9. *N*-Acetyl-L-alanyl-L-diaminopropionyl-N^β-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-glycyl-L-diaminopropionyl-N^β-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-L-alanine (8)

According to the general procedure for the peptide coupling in solution, mannoside **6** (130 mg, 0.413 mmol, 6 equiv), HATU (141 mg, 0.370 mmol, 5.4 equiv), DIPEA (70 μL, 0.41 mmol, 6 equiv) and peptide **1** (30 mg, 69 μmol, 1 equiv) were reacted in DMF and purified. The title compound was obtained as a white lyophilisate (23 mg, 22 μmol, 32%). HPLC: t_R = 12.1 min [A = water, B = acetonitrile + 1% TFA, 20% B→80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ +11.8 (*c* 0.5, MeOH). ^{1}H NMR (600 MHz, D_2O/H_2O, 1:11): δ = 8.36–8.12 (m, 3H, NH), 8.06–7.98 (m, 1H, NH), 7.92–7.79 (m, 3H, NH), 7.14 (d, 4H, *J* 8.7 Hz, H-Ar), 7.00 (d, 4H, *J* 8.7 Hz, H-Ar), 5.48 (br s, 2H, H-1), 4.40–4.31 (m, 2H, H-$\alpha_{(Ala)}$), 4.16–3.88 (m, 6H, C*H*CH_2, H-2, H-3), 3.74–3.69 (m, 4H, C*H*$_2$-Ar), 3.67–3.57 (m, 8H, H-4, H-5, H-6a, H-6b), 3.55 (bs, 2H, H-$\alpha_{(Gly)}$), 3.43–3.31 (m, 4H, CHC*H*$_2$), 1.83 (s, 3H, NHAc), 1.24–1.13 (m, 6H, H-$\beta_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O/H_2O, 1:11): δ = 173.5, 173.3, 171.8, 171.6, 171.0, 170.9 (6*C*ONH, *C*OCH_3) 154.4, 130.7, 130.5, 117.3 (12C-Ar), 98.2 (2C-1), 73.3 (2C-5), 70.5 (2C-2), 70.0 (2C-3), 66.7 (2C-4), 60.7 (2C-6), 54.4 (2C-$\alpha_{(Ala)}$), 50.1 (2*C*HCH$_2$), 42.6 (2CH*C*H$_2$), 41.4 (2*C*H$_2$-Ar), 40.1 (C-$\alpha_{(Gly)}$), 21.7 ($COCH_3$), 16.3, 16.2 (C-$\beta_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{44}H_{61}N_7O_{21}+Na]^+$: 1046.38; found *m/z* 1046.22; calcd for $[C_{38}H_{65}N_7O_{21}+K]^+$: 1062.35; found *m/z* 1062.20.

4.1.10. *N*-Acetyl-L-alanyl-L-lysyl-N^ε-{(α-D-mannopyranosyloxy)-acetamido}-glycyl-L-lysyl-N^ε-{(α-D-mannopyranosyloxy)-acetamido}-L-alanine (9)

According to the general procedure for the peptide coupling in solution, mannoside **5** (125 mg, 0.525 mmol, 6 equiv), HATU (179 mg, 0.470 mmol, 5.4 equiv), DIPEA (91 μL, 0.525 mmol,

6 equiv) and peptide **2** (45 mg, 87 μmol, 1 equiv) were reacted in DMF and purified. The title compound was obtained as a white lyophilisate (13 mg, 13 μmol, 15%). HPLC: t_R = 10.6 min [A = water, B = acetonitrile + 1% TFA, 20% B→80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ +6.3 (*c* 0.4, MeOH). ^{1}H NMR (600 MHz, D_2O/H_2O, 1:11): δ = 4.94 (d, 2H, $J_{1,2}$ 1.7 Hz, H-1), 4.43–4.31 (m, 4H, H-$\alpha_{(Lys,Ala)}$), 4.26 (d, 2H, $J_{OCHH,OCHH}$ 15.1 Hz, OC*H*H), 4.15 (d, 2H, $J_{OCHH,OCHH}$ 15.1 Hz, OCH*H*), 4.10 (dd, 2H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.4 Hz, H-2), 3.99 (br s, 2H, H-$\alpha_{(Gly)}$), 3.94–3.90 (m, 4H, H-3, H-6a), 3.81 (dd, 2H, HH, $J_{5,6b}$ 5.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.72 (dd∼t, 2H, *J* 9.8 Hz, H-4), 3.68–3.64 (m, 2H, H-5), 3.30 (dd∼t, 4H, *J* 6.7 Hz, H-ε), 2.07 (s, 3H, NHAc), 1.93–1.85 (m, 2H, H-βa$_{(Lys)}$), 1.84–1.75 (m, 2H, H-βb$_{(Lys)}$), 1.64–1.57 (m, 4H, H-δ), 1.49–1.43 (m, 7H, H-γ, H-β$_{(Ala)}$), 1.42 (d, 3H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.2 Hz, H-β$_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O/H_2O, 1:11): δ = 174.9, 174.1, 173.6, 173.0, 170.9, 170.5 (5*C*ONH, *C*OCH$_3$) 99.5 (2C-1), 72.7 (2C-5), 69.9 (2C-3), 69.2 (2C-2), 66.1 (2C-4), 65.4 (O*C*H$_2$), 60.3 (2C-6), 53.9, 53.3, 53.1, 52.9 (4C-$\alpha_{(Lys,Ala)}$), 41.9 (C-$\alpha_{(Gly)}$), 38.2 (2C-ε), 29.8 (2C-β$_{(Lys)}$), 27.4 (2C-δ), 21.8 (2C-γ), 21.1 (CO*C*H$_3$), 16.0, 15.8 (C-β$_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{38}H_{65}N_7O_{21}+Na]^+$: 978.41; found *m/z* 978.95; calcd for $[C_{38}H_{65}N_7O_{21}+K]^+$: 994.38; found *m/z* 994.97.

4.1.11. *N*-Acetyl-L-alanyl-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-glycyl-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-L-alanine (10)

According to the general procedure for the peptide coupling in solution, mannoside **6** (164 mg, 0.522 mmol, 6 equiv), HATU (179 mg, 0.470 mmol, 5.4 equiv), DIPEA (89 μL, 0.522 mmol, 6 equiv) and peptide **2** (45 mg, 87 μmol, 1 equiv) were reacted in DMF and purified. The title compound was obtained as a white lyophilisate (16 mg, 14 μmol, 16%). HPLC: t_R = 11.3 min [A = water, B = acetonitrile + 1% TFA, 20% B→80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ +14.0 (*c* 0.5, MeOH). ^{1}H NMR (600 MHz, D_2O/H_2O, 1:11): δ = 8.40–8.36 (m, 1H, NH), 8.35–8.28 (m, 2H, NH), 8.26–8.22 (m, 2H, NH), 8.02–7.93 (m, 2H, NH), 7.32–7.24 (m, 4H, H-Ar), 7.18–7.11 (m, 4H, H-Ar), 5.60 (br s, 2H, H-1), 4.34 -4.24 (m, 4H, H-$\alpha_{(Lys,Ala)}$), 4.20–4.16 (m, 2H, H-2), 4.07–4.04 (m, 2H, H-3), 3.93 (m$_c$, 2H, H-$\alpha_{(Gly)}$), 3.81–3.70 (m, 8H, H-4, H-5, H-6), 3.52 (s, 4H, C*H*$_2$C$_{Ar}$), 3.17 (m$_c$, 4H, H-ε), 2.01 (s, 3H, NHAc), 1.84–1.76 (m, 2H, H-βa$_{(Lys)}$), 1.75–1.66 (m, 2H, H-βb$_{(Lys)}$), 1.54–1.46 (m, 4H, H-δ), 1.41–1.37 (m, 4H, H-γ), 1.35 (d, 6H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.2 Hz, H-β$_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O/H_2O, 1:11): δ = 178.9 (*C*OOH), 177.1, 176.3, 175.8 (5*C*ONH, *C*OCH$_3$) 156.2, 132.0, 131.3, 130.5, 119.0 (12C-Ar), 99.9 (2C-1), 75.1 (2C-5), 72.2 (2C-3), 71.7 (2C-2), 68.4 (2C-4), 62.5 (2C-6), 55.7, 51.5 (4C-$\alpha_{(Lys,Ala)}$), 43.4 (C-$\alpha_{(Gly)}$), 41.6 (2*C*H$_2$-C-Ar), 40.9 (2C-ε), 32.0 (2C-β$_{(Lys)}$), 29.5 (2C-δ), 23.9 (2C-γ), 23.4 (CO*C*H$_3$), 18.3, 18.0 (C-β$_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{50}H_{73}N_7O_{21}+Na]^+$: 1130.47; found *m/z* 1130.70.

4.1.12. *N*-Acetyl-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-glycyl-L-alanyl-L-alanine (11)

According to the general procedure for the peptide coupling in solution, mannoside **6** (164 mg, 0.522 mmol, 6 equiv), HATU (179 mg, 0.470 mmol, 5.4 equiv), DIPEA (89 μL, 0.51 mmol, 6 equiv) and peptide **3** (45 mg, 87 μmol, 1 equiv) were reacted in DMF and purified. The title compound was obtained as a white lyophilisate (23 mg, 20 μmol, 24%). HPLC: t_R = 12.6 min [A = water, B = acetonitrile + 1% TFA, 20% B→80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ +16.5 (*c* 0.5, MeOH). ^{1}H NMR (600 MHz, D_2O, 1:11): δ = 8.57–8.54 (m, 2H, NH), 8.41–8.37 (m, 1H, NH), 8.28–8.24 (m, 2H, NH), 8.02–7.94 (m, 1H, NH), 7.25 (d, 4H, *J* 8.5 Hz, H-Ar), 7.11 (d, 4H, *J* 8.5 Hz, H-Ar), 5.59 (bs, 2H, H-1), 4.39–4.25 (m, 4H, H-$\alpha_{(Lys,Ala)}$), 4.19–4.16 (m, 2H, H-2), 4.06 (dd, 2H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 8.9 Hz, H-3), 3.82–3.71 (m, 8H, H-4, H-5, H-6), 3.70–3.65 (m, 2H, H-$\alpha_{(Gly)}$), 3.54 (s, 4H, CH_2–C-Ar), 3.16, 3.00 (bs, 4H, H-ε), 2.02 (s, 3H, NHAc), 1.92–1.85 (m, 2H, H-βa$_{(Lys)}$), 1.78–1.72 (m, 2H, H-βb$_{(Lys)}$), 1.70–1.62 (m, 4H, H-δ), 1.49–1.43 (m, 7H, H-γ, H-β$_{(Ala)}$), 1.42 (m$_c$, 3H, H-β$_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O/H_2O, 1:11): δ = 179.9 (*C*OOH), 176.0, 175.8, 175.5, 174.2, 173.4 (5*C*ONH, *C*OCH$_3$) 154.4, 130.4, 128.1, 117.2 (12C-Ar), 99.3 (2C-1), 73.3 (2C-5), 70.5 (2C-3), 70.0 (2C-2), 66.7 (2C-4), 60.7 (2C-6), 54.3, 50.7 (4C-$\alpha_{(Lys,Ala)}$), 43.2 (C-$\alpha_{(Gly)}$), 42.5 (2*C*H$_2$–C-Ar), 39.4 (2C-ε), 30.4 (2C-β$_{(Lys)}$), 26.2 (2C-δ), 21.9 (2C-γ), 21.6 (CO*C*H$_3$), 16.5, 16.2 (C-β$_{(Ala)}$) ppm. MALDI-ToF-MS: calcd for $[C_{50}H_{73}N_7O_{21}+Na]^+$: 1130.47; found *m/z* 1130.82; calcd for $[C_{50}H_{73}N_7O_{21}+K]^+$: 1146.44; found *m/z* 1146.78.

4.1.13. *N*-Acetyl-L-alanyl-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-glycyl-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-glycyl-L-lysyl-N$^{\varepsilon}$-{4-[(α-D-mannopyranosyloxy)-phenyl]-acetamido}-L-alanine (12)

According to the general procedure for the peptide coupling in solution, mannoside **6** (134 mg, 0.426 mmol, 10 equiv), HATU (148 mg, 0.389 mmol, 9 equiv), DIPEA (73 μL, 0.42 mmol, 10 equiv) and peptide **4** (30 mg, 42 μmol, 1 equiv) were reacted in DMF and purified. The title compound was obtained as a white lyophilisate (17 mg, 10 μmol, 25%). HPLC: t_R = 11.6 min [A = water, B = acetonitrile + 1% TFA, 20% B→80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ +5.5 (*c* 0.4, MeOH). ^{1}H NMR (600 MHz, D_2O/H_2O, 1:11): δ = 8.47–8.42 (m, 1H, NH), 8.36–8.26 (m, 2H, NH), 8.25–8.20 (m, 1H, NH), 8.12–8.02 (m, 1H, NH), 7.99–7.89 (m, 3H, NH), 7.24 (d, 6H, *J* 8.4 Hz, H-Ar), 7.11 (d, 6H, *J* 8.4 Hz, H-Ar), 5.58 (br s, 3H, H-1), 4.32–4.20 (m, 5H, H-$\alpha_{(Lys,Ala)}$), 4.16 (dd, 3H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.3 Hz, H-2), 4.04 (dd, 3H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 9.3 Hz, H-3), 3.91 (m$_c$, 4H, H-$\alpha_{(Gly)}$), 3.77 (dd∼t, 3H, *J* 9.4 Hz, H-4), 3.76–3.67 (m, 9H, H-5, H-6a, H-6b), 3.51 (s, 6H, C*H*$_2$C$_{Ar}$), 3.19–3.12 (m, 6H, H-ε), 2.00 (s, 3H, NHAc), 1.84–1.75 (m, 3H, H-βa$_{(Lys)}$), 1.74–1.64 (m, 3H, H-βb$_{(Lys)}$), 1.47 (m$_c$, 6H, H-δ), 1.41–1.36 (m, 6H, H-γ), 1.34 (d, 6H, $J_{H\text{-}\alpha(Ala),H\text{-}\beta(Ala)}$ 7.1 Hz, H-β$_{(Ala)}$) ppm. ^{13}C NMR (150 MHz, D_2O/H_2O, 1:11): δ = 177.1, 176.3, 175.8, 175.1, 173.1 (9*C*ONH, *C*OCH$_3$) 156.2, 132.3, 131.3, 119.0 (18C-Ar), 99.9 (3C-1), 75.1 (3C-5), 72.3 (3C-3), 71.7 (3C-2), 68.4 (3C-4), 62.5 (3C-6), 55.7, 51.5 (5C-$\alpha_{(Lys,Ala)}$), 44.3 (2C-$\alpha_{(Gly)}$), 43.4 (3*C*H$_2$–C-Ar), 40.9 (3C-ε), 32.1 (3C-β$_{(Lys)}$), 29.5 (3C-δ), 23.9 (3C-γ), 23.4 (CO*C*H$_3$), 18.3, 18.1 (C-β$_{(Ala)}$) ppm. MALDI-ToF-MS: Calcd for $[C_{72}H_{104}N_{10}O_{30}+Na]^+$: 1611.38; found *m/z* 1611.91.

4.2. Adhesion inhibition assay

4.2.1. GFP-based bacterial adhesion assay[27]

Black 96-well plates were filled with a solution of mannan from *Saccharomyces cerevisiae* (1.2 mg/mL in carbonate buffer, pH 9.5; 100 μL solution per well) and allowed to dry in at 37 °C overnight. The plates were washed with PBST (3 × 150 μL/well) and stored at 4 °C. Before use, the wells were blocked with BSA (5% in PBS, 120 μL/well) for 2 h at 37 °C and then washed with PBST (3 × 150 μL/well). Serial dilutions of the examined glycopeptide inhibitor were prepared in the mannan-coated, BSA-blocked 96-well plates. The bacteria suspension (2 mg/mL in PBS buffer, pH 7.2; 50 μL solution per well) was added and the plates were agitated (80 rpm) and incubated for 45 min at 37 °C. After washing with PBS (3 × 150 μL/well), the wells were filled with PBS (100 μL/well) and the fluorescence intensity (485 nm/535 nm) was determined. All assays were performed, using at least duplicate samples of each well. LB: lysogeny broth; rpm: revolutions per minute; PBS: phosphate buffered saline; PBST: PBS + 0.05% Tween 20.

4.2.2. Bacteria culture

E. coli bacteria of strain PKL1162 were grown in LB-media + AMP + CAM (100 mg ampicillin, 50 mg chloramphenicol/L) at 37 °C under slight agitation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.04.023.

References

1. Klemm, P.; Schembri, M. *Int. J. Med. Microbiol.* **2000**, *290*, 27–35.
2. Osrin, D.; Vergnano, S.; Costello, A. *Curr. Opin. Infect. Dis.* **2004**, *17*, 217–224.
3. Ohlsen, K.; Oelschlaeger, T. A.; Hacker, J.; Khan, A. S. *Top. Curr. Chem.* **2009**, *288*, 109–120.
4. Kau, A. L.; Hunstad, D. A.; Hultgren, S. J. *Curr. Opin. Microbiol.* **2005**, *8*, 54–59.
5. Le Trong, I.; Aprikian, P.; Kidd, B. A.; Forero-Shelton, M.; Tchesnokova, V.; Rajagopal, P.; Rodgriguez, V.; Interlandi, G.; Klevit, R.; Vogel, V.; Stenkamp, R. E.; Sokurenko, E. V.; Thomas, W. E. *Cell* **2010**, *141*, 645–655.
6. For example: Dubber, M.; Sperling, O.; Lindhorst, T. K. *Org. Biomol. Chem.* **2006**, *4*, 3901–3912.
7. For example: Sperling, O.; Fuchs, A.; Lindhorst, T. K. *Org. Biomol. Chem.* **2006**, *4*, 3913–3922.
8. Ofek, I.; Hasty, D. L.; Sharon, N. *FEMS Immunol. Med. Microbiol.* **2003**, *38*, 181–191.
9. Pieters, R. J. *Med. Res. Rev.* **2007**, *27*, 796–816.
10. Sivick, K. E.; Mobley, H. L. T. *Infect. Immun.* **2010**, *78*, 568–585.
11. 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. *J. Med. Chem.* **2010**, *53*, 4779–4792.
12. Klein, T.; Abgottspon, D.; Wittwer, M.; Rabbani, S.; Herold, J.; Jinag, X.; Kleeb, S.; Lüthi, C.; Scharenberg, M.; Bezencon, J.; Gubler, E.; Pang, L.; Smiesko, M.; Cutting, B.; Schwardt, O.; Ernst, B. *J. Med. Chem.* **2010**, *53*, 8627–8641.
13. Durka, M.; Buffet, K.; Lehl, J.; Holler, M.; Nierengarten, J.-F.; Taganna, J.; Bouckaert, J.; Vincent, S. P. *Chem. Commun.* **2011**, *47*, 1321–1323.
14. 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. *Mol. Microbiol.* **2002**, *44*, 903–918.
15. Bouckaert, J.; Berglund, J.; Schembri, M.; De Genst, E.; Cools, L.; Wuhrer, M.; Hung, C. S.; Pinkner, J.; Slättegård, R.; Zavialov, A.; Choudhury, D.; Langermann, S.; Hultgren, S. J.; Wyns, L.; Klemm, P.; Oscarson, S.; Knight, S. D.; De Greve, H. *Mol. Microbiol.* **2005**, *55*, 441–455.
16. Wellens, A.; Garofalo, C.; Nguyen, H.; Van Gerven, N.; Slättegård, R.; Hernalsteens, J.-P.; Wyns, L.; Oscarson, S.; De Greve, H.; Hultgren, S.; Bouckaert, J. *PloS ONE* **2008**, *3*, 1–13.
17. Knight, S. D.; Bouckaert, J. *Top. Curr. Chem.* **2009**, *288*, 67–107.
18. Lahmann, M. *Top. Curr. Chem.* **2009**, *288*, 17–65.
19. Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165–393.
20. Touaibia, M.; Roy, R. *Mini-Rev. Medicin. Chem.* **2007**, *7*, 1270–1283.
21. Lindhorst, T. K.; Kieburg, C.; Krallmann-Wenzel, U. *Glycoconjugate J.* **1998**, *15*, 605–613.
22. Lindhorst, T. K.; Dubber, M.; Krallmann-Wenzel, U.; Ehlers, S. *Eur. J. Org. Chem.* **2000**, 2027–2034.
23. Schierholt, A.; Hartmann, M.; Schwekendiek, K.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2010**, 3120–3128.
24. Touaibia, M.; Wellens, A.; Shiao, T. C.; Wang, Q.; Sirois, S.; Bouchaert, J.; Roy, R. *ChemMedChem* **2007**, *2*, 1190–1201.
25. Nagahori, N.; Lee, R. T.; Nishimura, S.-I.; Pagé, D.; Roy, R.; Lee, Y. C. *ChemBioChem* **2002**, *3*, 836–844.
26. Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem. Int. Ed.* **2006**, *45*, 2348–2368.
27. Lindhorst, T. K.; Bruegge, K.; Fuchs, A.; Sperling, O. *Beilstein J. Org. Chem.* **2010**, *6*, 801–809.
28. Derrien, D.; Midoux, P.; Petit, C.; Negre, E.; Mayer, R.; Monsigny, M.; Roche, A.-C. *Glycoconjugate J.* **1989**, *6*, 241–255.
29. Maity, S. K.; Dutta, S. K.; Banerjee, A. K.; Achari, B.; Singh, M. *Tetrahedron* **1994**, *50*, 6965–6974.
30. Cheaib, R.; Listkowski, A.; Chambert, S.; Doutheau, A.; Queneau, Y. *Tetrahedron: Asymmetry* **2008**, *19*, 1919–1933.
31. Hartmann, M.; Horst, A. K.; Klemm, P.; Lindhorst, T. K. *Chem. Commun.* **2010**, *46*, 330–332.
32. Compare: Euzen, R.; Reymond, J.-L. *Mol. BioSyst.* **2011**, *7*, 411–421.
33. von der Lieth, C.-W.; Frank, M.; Lindhorst, T. K. *Rev. Mol. Biotech.* **2002**, *90*, 311–337.
34. Grabosch, C.; Hartmann, M.; Schmidt-Lassen, J.; Lindhorst, T. K. *ChemBioChem.* **2011**, *12*, 1066–1074.