Carbohydrate Research 371 (2013) 22–31

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Are multivalent cluster glycosides a means of controlling ligand density of glycoarrays?

Johannes W. Wehner, Mirja Hartmann, Thisbe K. Lindhorst *

Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Otto-Hahn-Platz 3/4, 24098 Kiel, Germany

ARTICLE INFO

Article history:
Received 2 December 2012
Received in revised form 28 January 2013
Accepted 30 January 2013
Available online 8 February 2013

Keywords:
Bacterial adhesion
Glycoarrays
Cluster glycosides
Density control
Synthesis
Adhesion assays

ABSTRACT

Bacterial adhesion to the glycocalyx of human host cells is of biological and medicinal importance. This process is often initiated by the interaction of bacterial lectins and specific carbohydrate ligands. Thus, adhesion of bacterial cells to glycosylated surfaces is a suitable model system to study various parameters of lectin-mediated carbohydrate recognition. Glycoarrays have become important tools to study such lectin-mediated carbohydrate recognition. However, it is difficult to adjust the characteristics of a specific glycoarray regarding its carbohydrate density or the clustering of sugar ligands, respectively. Thus, we have made an attempt to use synthetic cluster glycosides of different valencies to vary carbohydrate density on a polystyrene surface. A series of mono-, di- and trivalent mannosides were synthesised for immobilisation on pre-functionalised polystyrene microtiter plates and the resulting glycoarrays were tested as adhesive surfaces in mannose-specific adhesion of *Escherichia coli*. Our measurements give first promising hints about the potential of this approach to alter ligand density of glycoarrays in a systematic way.

1. Introduction

Every eukaryotic cell is covered with a layer of complex glycoconjugates, the so-called glycocalyx, which plays a crucial role in processes like cell-cell communication, cellular adhesion, inflammation and signalling.[1–3] Due to the structural complexity of the glycoconjugates embedded into the cell membrane, elucidation of the molecular details of carbohydrate recognition is a demanding task. A specific scenario which is suited to test the details of carbohydrate recognition on a surface is bacterial adhesion to fabricated glycosylated surfaces and glycoarrays, respectively.[4–11] Bacteria utilise lectins, expressed as part of long protein appendages, called fimbriae, to attach to the glycosylated surface of their host cells.[12,13] This process facilitates bacterial colonisation of cell surfaces and biofilm formation and is connected to, i.a., severe inflammatory diseases of the host.

A key question in the investigation of carbohydrate recognition processes occurring on the cell surface is the composition of the glycocalyx. It is determined by the chemical nature of the carbohydrate constituents of the various glycoconjugates. But, moreover, the nature of the glycocalyx is regulated by additional parameters. These comprise (i) multivalency of sugar epitopes,[14–16] (ii) their orientation and conformational flexibility of their presentation[17–19] as well as (iii) ligand density and spacing of interaction partners.[20–23] Such parameters are difficult to adjust and to analyse and related research typically requires complex and demanding approaches. For example, carbohydrate spacing has been accomplished on DNA arrays,[24] and carbohydrate density on a surface has been varied using neoglycoproteins.[25] Indeed, glycoarrays offer an opportunity to address many important features of glycosylated surfaces but the effort to systematically vary and characterise their properties has remained high.[26–29]

Here, we report on a study where cluster glycosides of different valencies were used in an attempt to vary carbohydrate ligand density of a glycoarray in a facile way. It is known from surface chemistry, that the size of molecules used for surface decoration influences the density of the formed molecular layer as a consequence of steric hindrance during the immobilisation process.[30,31] Thus, we have made mono-, di- and trivalent (cluster) mannosides to functionalise polystyrene microtiter plates. Then, mannose-specific adhesion of bacterial cells to the respective glycoarrays was assayed in three parallel test series employing systematic concentration variation (Fig. 1). We were interested to investigate, which different influences concentration and valency of sugar ligands have on the 'stickiness' of a glycosylated surface. This was systematically studied with three different types of mannose-coated surfaces which were employed in a lectin-mediated bacterial adhesion assay.

2. Results and discussion

2.1. Synthesis of mannosides

A series of mono-, di- and trivalent cluster mannosides were synthesised in two variations. One set of mannosides was equipped

* Corresponding author. Tel.: +49 431 880 2023; fax: +49 431 880 7410.
E-mail address: tklind@oc.uni-kiel.de (T.K. Lindhorst).

0008-6215/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2013.01.023

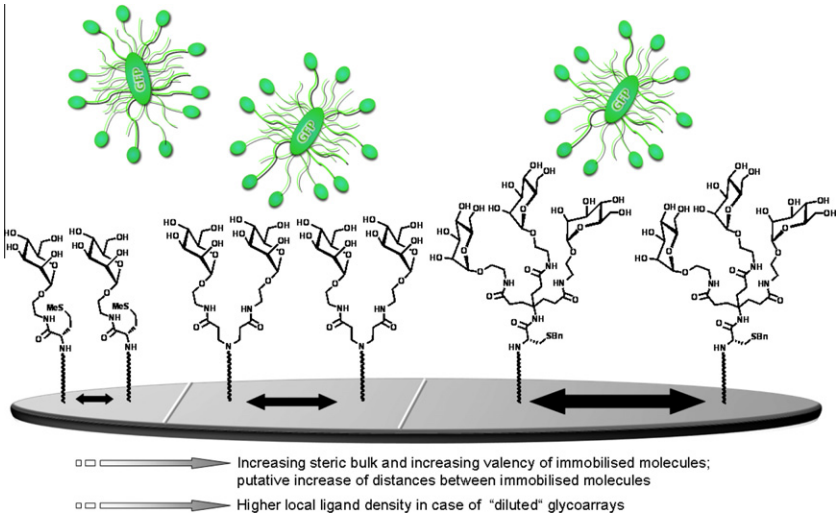


Figure 1. Bacterial adhesion of type 1 fimbriated *E. coli* to three different types of glycoarrays, fabricated from mono-, di- and trivalent (cluster) mannosides (from left to right). Hypothetically, each type of glycoarray could show different concentration dependencies in bacterial adhesion assays. When higher sample concentrations are used for immobilisation, density of exposed mannosyl ligands should be comparable in all three cases owing to steric circumstances. But in case of more 'diluted' glycoarrays, the surface that is decorated with cluster mannosides could be more adhesive then the simple mannoside-coated surface, because ligand clustering is maintained on the multivalent scaffold.

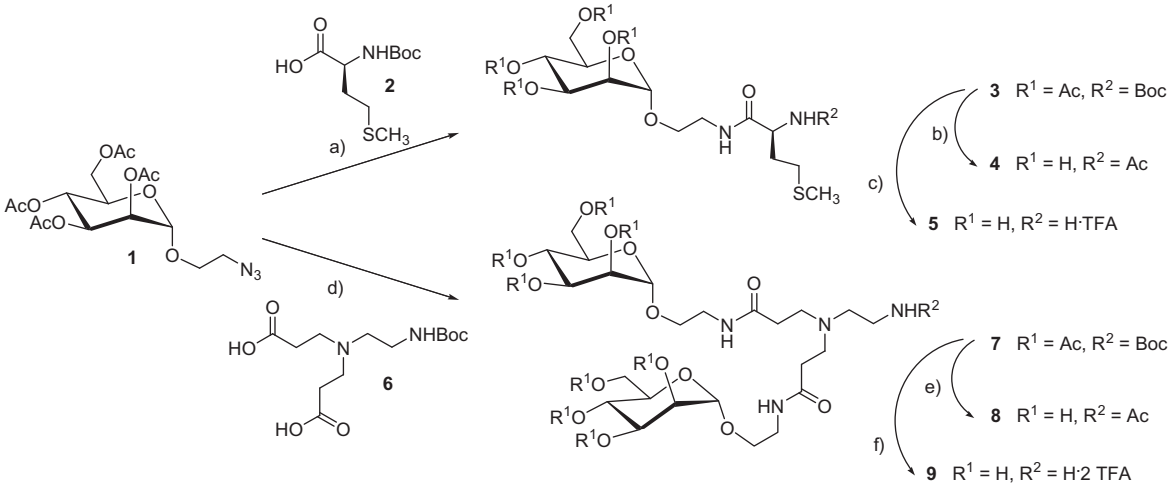
with a primary amino function to allow covalent immobilisation of pre-functionalised microtiter plates. A second set was prepared accordingly, but with the amino group in N-acetylated form. The latter mannosides were used as soluble inhibitors of bacterial adhesion to a mannan-coated surface to determine their IC_{50} values.

The synthetic routes started with azidoethyl mannoside **1**[32] which could be conjugated to the appropriate mono-, di- and trifunctional carboxylic acid derivatives **2**, **6** and **12** (Schemes 1 and 2) employing Staudinger ligation.[33]

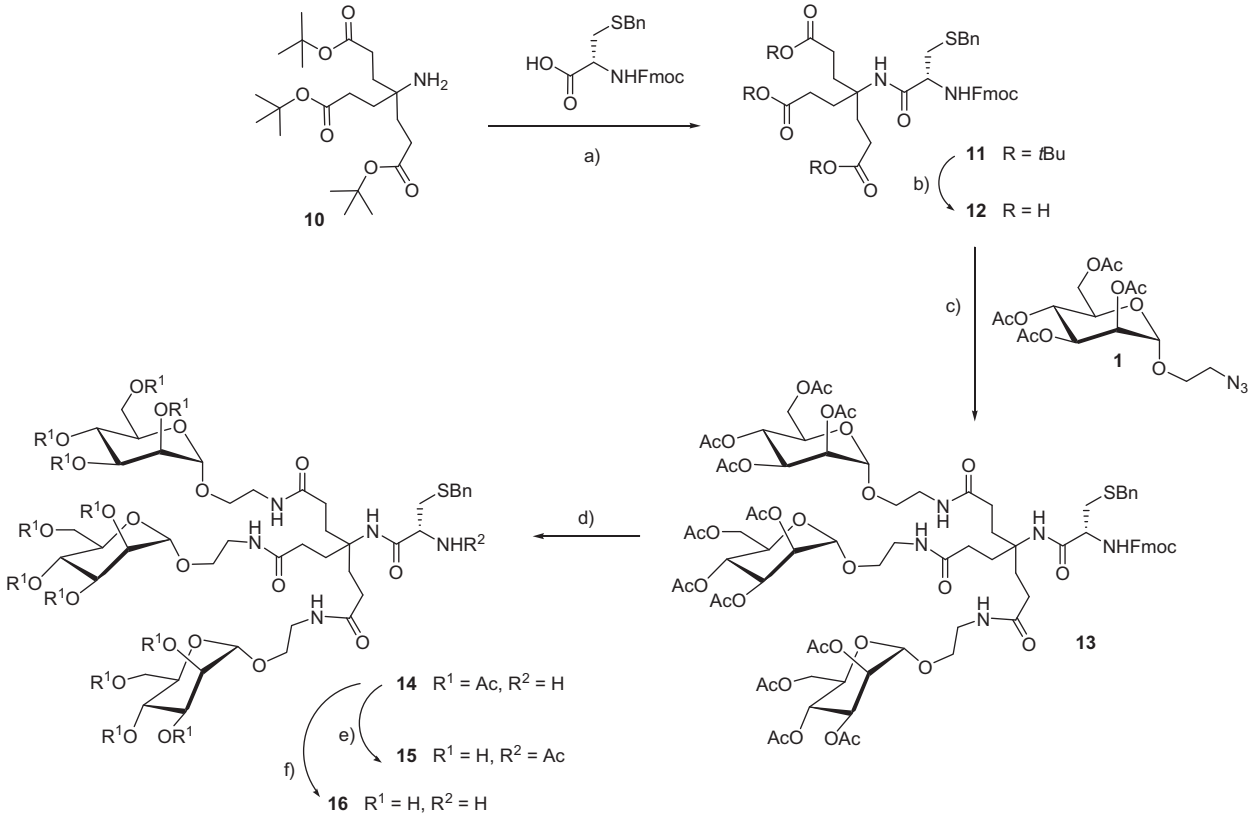
Mannoside **3** was obtained from reaction of **1** with commercially available *N*-Boc-protected methionine **2**. This fully protected mannoside was then treated with TFA to remove the *N*-Boc protecting group, followed by N,O-acetylation and de-O-acetylation according to Zemplén[34] to yield the OH-free N-acetylated derivative **4** in very good yield after purification by reversed phase chromatography (Scheme 1). The free amine **5** was obtained in the form of its TFA salt after de-O-acetylation of mannoside **3**, RP-MPLC of the intermediate and *N*-Boc removal applying TFA in dichloromethane in 97% overall yield.

By analogy to the synthesis of **4** and **5**, the preparation of the divalent mannosides **8** and **9** started with Staudinger ligation of mannoside **1** with diacid **6**.[35] This step initially led to cluster mannoside **7**. Then, *N*-Boc deprotection, N,O-acetylation and deprotection of the O-acetyl groups yielded the *N*-acetyl-protected mannoside **8**. The free amine **9** was obtained as bis-trifluoroacetate after Zemplén deprotection of **7** followed by acidic *N*-Boc removal applying TFA in dichloromethane.

For the preparation of the trivalent cluster mannosides **15** and **16**, the tris-carboxylic acid **12** was employed. This was prepared from the well-known trivalent wedge-type molecule **10**[36,37] which was coupled to Fmoc-Cys(Bn)-OH applying HBTU/DIPEA following a literature-known procedure[9] yielding **11** in 87% yield. Acidic cleavage of the ^{t}Bu-esters using formic acid gave **12**. Then, threefold Staudinger ligation of **12** and mannoside **1** was successfully accomplished to obtain **13** in fair yield (Scheme 2). For preparation



Scheme 1. Synthesis of mono- and divalent NHAc- and NH_2-functionalised mannosides **4**, **5**, **8** and **9**. Reagents and conditions: (a) P(tBu)$_3$, DIC, dry THF, 0 °C → rt, overnight, 72%, (b) (i) TFA, CH_2Cl_2, rt, 4 h; (ii) acetic anhydride, pyridine, rt, overnight; (iii) Na, dry MeOH, rt, overnight, 88% over three steps; (c) (i) Na, dry MeOH, rt, 4.5 h; (ii) TFA, CH_2Cl_2, rt, 1.5 h, 97% over two steps; (d) HOBt, P(tBu)$_3$, DIC, dry THF, 0 °C → rt, 40 h, 55%, (e) (i) TFA, CH_2Cl_2, rt, 9 h; (ii) acetic anhydride, pyridine, rt, overnight; (iii) Na, dry MeOH, rt, overnight, 86% over three steps. (f) (i) Na, dry MeOH, rt, overnight; (ii) TFA, CH_2Cl_2, rt, overnight, 99% over two steps.



Scheme 2. Synthesis of trivalent NHAc- and NH_2-functionalised cluster mannosides **15** and **16**. Reagents and conditions: (a) HBTU, DIPEA, dry DMF, rt, overnight, 87%; (b) formic acid, rt, overnight, quant.; (c) HOBt, $P(tBu)_3$, DIC, dry THF, 0 °C → rt, overnight, 62%, (d) piperidine, DMF, rt, 1.5 h, 98%; (e) (i) acetic anhydride, pyridine, rt, 2 h; (ii) Na, dry MeOH, rt, 2 h, 94% over two steps; (f) Na, dry MeOH, rt, overnight, 99%.

of the free amine **14**, the Fmoc protecting group was removed with piperidine in DMF. Next, the N-acetylated OH-unprotected cluster mannoside **15** was furnished in a sequence of N,O-acetylation and Zemplén deprotection of O-acetates. The unprotected free amine **16** was obtained after O-deprotection of **14** in quantitative yield.

2.2. Adhesion inhibition assay with 4, 8 and 15

Next, the synthesised *N*-acetylated mannosides **4**, **8** und **15** were tested as inhibitors of α-D-mannoside-specific adhesion of type 1-fimbriated *Escherichia coli* bacteria to a mannan-coated surface.[38] The employed *E. coli* strain PKL1162 contains the GFP (green fluorescing protein) gene and thus bacterial adhesion to the mannan-coated surface can be correlated to measured fluorescence intensity. Serial dilutions of the inhibitors were employed according to a published protocol.[38] This led to inhibition curves of which the respective IC_{50} values could be deduced. For a better comparison of inhibitors that were tested in different experiments, relative inhibitory potencies (RIP) were calculated by referencing the measured data to the IC_{50} value of methyl α-D-mannoside (MeMan), which was tested on the respective plate.

The results of the adhesion-inhibition assays show that the methionyl mannoside **4** has a very similar inhibitory potency as the standard inhibitor of this system, MeMan (Table 1). The divalent cluster mannoside **8** inhibits bacterial adhesion to mannan 2.5 times better than MeMan, and the trivalent cluster mannoside **15** was shown to be an approximately 6-times more potent inhibitor in comparison to simple MeMan. When these relative inhibitory potencies (RIP values) were valency-corrected, in other words, when the number of α-D-mannosyl residues per inhibitor molecule was taken into account, RIP_{vc} values resulted as depicted in Table 1. These RIP_{vc} values show that on a valency-corrected basis only the trivalent cluster glycoside has a significant advantage over MeMan with $RIP_{vc} = 2$. This small multivalency effect can most probably be attributed to a statistical advantage of the trivalent cluster mannoside over simple mannosides: as soon as one mannosyl residue departs from the FimH carbohydrate binding site, the next ligand is already pre-positioned on the same scaffold and immediately in place to complex with the lectin's carbohydrate recognition domain.

Based on the little differences seen in the RIP_{vc} values and with regard to many earlier control studies[13] we can assume that the different natures of the glycocluster scaffold moieties exert no relevant influence on the inhibitory potencies of the respective glycocluster. Thus, after the evaluation of mannosides **4, 8** and **15** as inhibitors of bacterial adhesion in solution, their amino-functionalised analogues **5**, **9** and **16** were used to fabricate the corresponding glycoarrays on appropriately activated polystyrene surfaces for biological comparison studies.

Table 1
Inhibitory potencies of mannosides **4**, **8** and **15** as determined in an adhesion-inhibition assay on mannan-coated microtiter plates

Inhibitor	IC_{50}[a] (μM)	IC_{50} MeMan[b] (μM)	RIP[c]	RIP_{vc}[c]
4 (Monovalent)	3616	5181	1.4	1.4
8 (Divalent)	7152	17667	2.5	1.25
15 (Trivalent)	879	5181	5.9	2.0

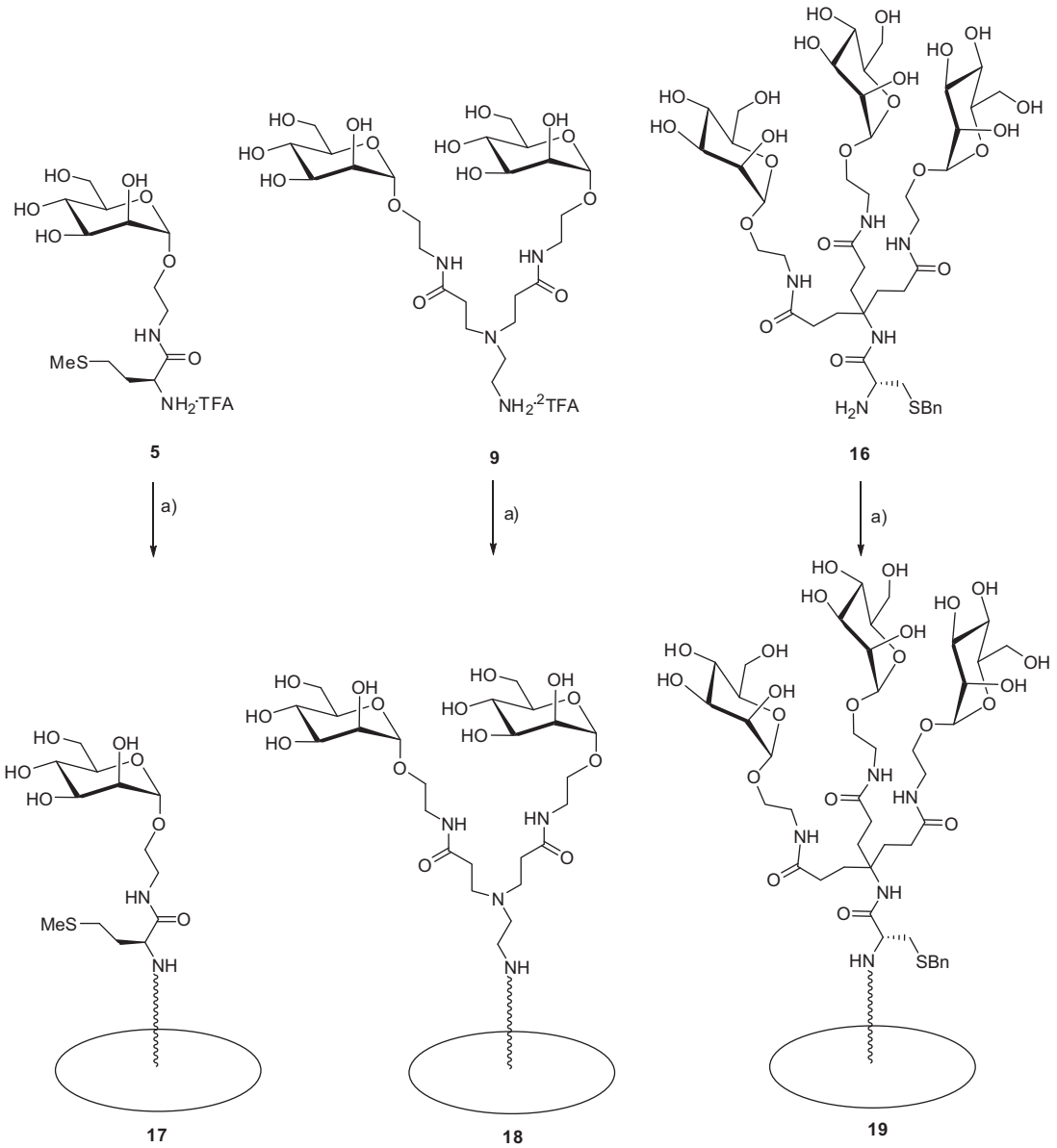
[a] IC_{50} values are average values from triplicate results.
[b] Tested on the same microtiter plate.
[c] RIP: relative inhibitory potency with IP (MeMan)≡1; RIP_{vc}: valency-corrected RIP.

2.3. Fabrication of glycoarrays on polystyrene and testing of their adhesive properties using *E. coli* cells

The simple mannoside **5** and the di- and trivalent cluster mannosides **9** and **16** were immobilised on pre-activated 96 well microtiter plates to yield surfaces **17**, **18** and **19** (Scheme 3). For immobilisation, the plates were incubated with serial dilutions of the amino-functionalised carbohydrate derivatives in carbonate buffer (pH 9.6). Under these conditions the free amines are formed from the TFA salts and react with the microplate surface

Immobilisation was followed by several washing steps with buffer (PBST) to remove carbohydrates that were not covalently bound to the surface and unreacted surface groups were blocked with ethanolamine. Then, the so formed glycoarrays were incubated with fluorescent type 1 fimbriated *E. coli* (PKL1162), non-adhered microorganisms were washed away and adhesion was quantified by fluorescence read out. The results are depicted in Figure 2.

In case of glycoarray **17** fabricated from the simple mannoside **5**, a slight but significant decrease of adhesiveness was observed going from higher (15 mM) to lower concentrations (2 mM). This finding was expected and is not surprising, as more dilute glycoarrays present less ligands for fimbriae-mediated bacterial adhesion, which is consequently less strong. Interestingly, a reverse course of adhesiveness was observed with surfaces **18** and **19**, made from the di- and trivalent cluster mannosides **9** and **16**, respectively. Here, adhesiveness increased from left to right in the concentration window shown. (At higher concentrations, no significant differences between the three glycoarrays were seen, cf. Supplementary data.) In addition, the ratios of adhesiveness between the three surface types at one particular concentration also undergo a notable change. Whereas at higher concentrations, glycoarray **17** is slightly more adhesive than surfaces **18** and **19**, this situation is reversed in case of the more dilute glycoarrays.



Scheme 3. Preparation of glycoarrays **17**, **18** and **19** on pre-functionalised microtiter plates. Reagents and conditions: (a) carbonate buffer (pH 9.6), rt, incubation with microplates overnight.

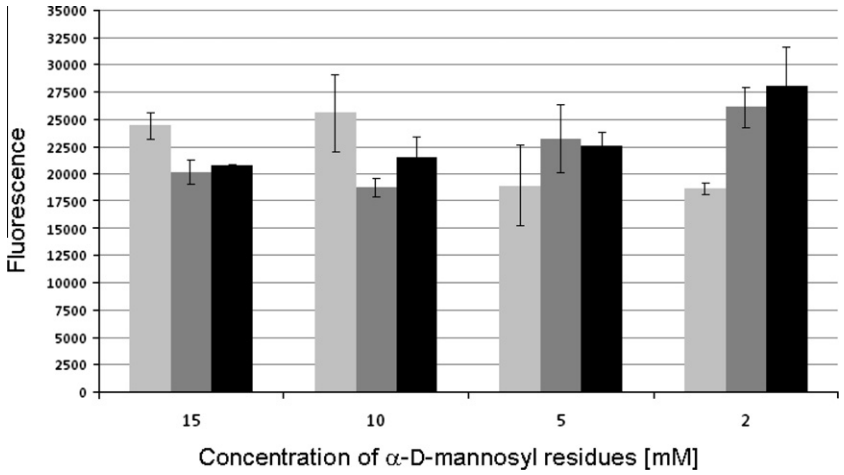


Figure 2. Adhesion of *E. coli* strain PKL1162 to the three different glycoarrays, **17** (bright grey columns), **18** (dark grey columns) and **19** (black columns), as determined by fluorescence read-out, with standard deviations (SD) indicated. For adequate interpretation, the depicted concentrations are valency-corrected, that is, given numbers refer to the concentrations of α-D-mannosyl moieties rather than to the sample concentration that was used for glycoarray fabrication (that means a two-fold difference for **18**, a three-fold difference for **19**).

3. Discussion

Our findings give a first hint that the hypothesis behind this study is promising. It has been suggested that cluster glycosides might be used to vary ligand density of glycoarrays, which can be rudimentally seen in our experiments. Apparently, concentration-dependent fabrication of glycoarrays using glycoclusters leads to different trends in adhesiveness depending on the cluster valency. Based on earlier experiments, we have no reason to assume, that the effectiveness of the immobilisation reaction on the pre-functionalised polystyrene plates is a limiting or otherwise critical factor in glycoarray fabrication. Thus, steric features of the employed molecules should be responsible for the stickiness of the resulting glycoarray. Steric bulk on one hand and clustering of mannosyl ligands on the other hand have to be weighed for an interpretation of the obtained data. Presumably, at higher concentrations, immobilisation of the simple mannoside **5** leads to a more adhesive surface than when the more bulky glycoclusters **9** and **16** are employed. But upon dilution of the solutions used for glycoarray fabrication, the glycoarrays **18** and **19** made from **9** and **16** show better adhesive properties than the more simple glycoarray **17** made from **5** (with valency correction taken into account). Thus, in the case of the more dilute glycoarrays, type 1 fimbriated *E. coli* cells find local high supply of α-D-mannosyl ligands only in case of surfaces **18** and **19**, which are glycocluster-functionalised. This turns out favourably for cellular adhesion. The simple glycoarray **17**, on the other hand, can apparently not provide an analogously advantageous situation in case of the sparsely covered microtiter plate.

In conclusion, it appears likely that the significant differences in relative trends of stickiness of the three tested surfaces can be attributed to varied ligand density arising from the specific architecture of the employed glycocluster. This is an interesting observation, which requires further biophysical studies to gain better understanding of how adhesive processes are governed by ligand density. We have commenced such work on other surfaces than polystyrene to allow spectroscopic characterisation of the glycosylated surface.

4. Experimental

4.1. General experimental methods

Commercially available starting materials and reagents were used without further purification unless otherwise noted. Anhydrous DMF was purchased, other solvents were dried for reactions or distilled for chromatography. Black Immobilizer Amino™ F96 MicroWell™ plates were purchased from Nunc (Thermo Fisher Scientific). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of nitrogen. 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). Preparative MPLC was performed on a BÜCHI apparatus using a LiChroprep RP-18 (40–60 μm, Merck) column for reverse phase and a LiChroprep Si 60 (40–60 μm, Merck) column for normal phase silica gel chromatography. ^{1}H and ^{13}C were recorded on Bruker DRX-500 and AV-600 machines. 2D NMR experiments (^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC) were performed for full assignment of the spectra. Chemical shifts were reported relative to the following shifts: TMS (^{1}H: δ 0.00 ppm); $CHCl_3$ (^{13}C: δ 77.00 ppm), MeOH (^{1}H: δ 3.31 ppm; ^{13}C: δ 49.00 ppm) or H_2O (δ 4.65 ppm). ESI MS measurements were performed on a Mariner ESI-ToF 5280 instrument (Applied Biosystems). MALDI-TOF mass spectra were recorded on a Bruker Biflex-III 19 kV instrument with Cl-CCA (4-chloro-α-cyanocinnamic acid) or DHB (2,5-dihydroxybenzoic acid) as matrix. Optical rotation was measured on a Perkin–Elmer polarimeter 341 (Na-D-line: 589 nm, length of cell 1 dm). IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR instrument. For sample preparation a Golden Gate diamond ATR unit with a saphire stamp was used. Elemental analyses were measured on a Euro-EA elemental analyser (EuroVector) at the Institute of Inorganic Chemistry (Christiana Albertina University of Kiel). For bacterial adhesion studies, a TECAN infinite 200 multifunction microplate reader was employed. The wavelengths of the band pass filters for excitation and emission were 485 and 535 nm, respectively. Compounds **1**[32] and **6**[35] were prepared according to literature-known procedures. Amberlyst A-21 was treated before usage as described by Srinivasan and co-workers.[39]

4.2. General procedure for Staudinger ligation

The corresponding azide-functionalised carbohydrate (1 equiv) and the carboxylic acid (1.8 equiv) were combined with HOBt (1.8 equiv) in a Schlenk flask and dried for more than 1 h in vacuo. This mixture was dissolved in dry THF under a nitrogen atmosphere and cooled to 0 °C. Then DIC (1.8 equiv) was added and

the solution was stirred for 10 min, followed by the addition of tri-*n*-butylphosphane (1–1.8 equiv) and stirring for 1 h at 0 °C. Then the reaction mixture was stirred at ambient temperature, diluted with water (50 mL) and extracted four times with dichloromethane (30 mL each). The combined organic phases were washed with brine, dried over $MgSO_4$, it was filtered and the filtrate concentrated under reduced pressure. The crude product was purified by MPLC.

4.3. General procedure for N,O-acetylation

The compound was dissolved in pyridine and acetic anhydride was added. The reaction mixture was stirred at ambient temperature (minimum 2 h, maximum overnight). Then the solution was concentrated under reduced pressure and the residue codistilled three times with toluene (5 mL each). The crude product was purified by MPLC.

4.4. General procedure for de-O-acetylation

The acetylated compound was dissolved in dry methanol and sodium (30–50 mg dissolved in 1 mL dry MeOH) was added under nitrogen. The reaction mixture was stirred at ambient temperature until the reaction was complete (minimum 1.5 h, maximum overnight), then it was neutralised by the addition of Amberlite IR-120 ion exchange resin, filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by MPLC where necessary.

4.5. General procedure for *N*-Boc-deprotection

The Boc-protected amine was dissolved in dichloromethane and TFA (118 μL-3 mL) was added. The solution was stirred at ambient temperature (minimum 1 h, maximum overnight). The solvent was removed under reduced pressure and the residue codistilled three times with toluene (5 mL each). Products used for functionalisation of the microtiter plates were used without further purification.

4.6. *N*-(*tert*-Butyloxycarbonyl)-L-methionine-[2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl]amide (3)

According to general procedure for Staudinger ligation azido-functionalised mannoside **1** (1.00 g, 2.40 mmol), Boc-Met-OH (**2**, 1.08 g, 4.32 mmol) and HOBt (584 mg, 4.32 mmol) were dried for 1 h in vacuo and dissolved in anhydrous THF (20 mL). DIC (670 μL, 4.32 mmol) and *n*-tributylphosphane (592 μL, 2.40 mmol) were added and it was stirred overnight. After a standard work-up, the crude product was subjected to MPLC (100 g, silica column, A: ethyl acetate, B: cyclohexane, A: 20% → 100%, 180 min) yielding the title compound (1.08 g, 1.73 mmol, 72%) as a colourless foam; R_f 0.63 (ethyl acetate); $[\alpha]_D^{23}$ +29.6 (*c* 0.5, methanol); ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 5.30 (m_c, 1H, H-3$_{Man}$), 5.25 (dd, ^3J = 1.9 Hz, ^3J = 3.2 Hz, 1H, H-2$_{Man}$), 5.24 (dd∼t, ^3J = 10.0 Hz, 1H, H-4$_{Man}$), 4.86 (br s, 1H, H-1$_{Man}$), 4.27 (dd, ^2J = 12.2 Hz, ^3J = 5.1 Hz, 1H, H-6a$_{Man}$), 4.17 (dd, ^3J = 5.0 Hz, ^3J = 8.4 Hz, 1H, H$_{Met,\alpha}$), 4.09 (dd, ^2J = 12.2 Hz, ^3J = 1.9 Hz, 1H, H-6b$_{Man}$), 4.06–4.01 (m, 1H, H-5$_{Man}$), 3.79 (m_c, 1H, O*C*H*H*CH$_2$NH), 3.60–3.54 (m, 1H, OCH*H*CH$_2$NH), 3.53–3.46 (m, 1H, OCH$_2$*CH*HNH), 3.44–3.38 (m, 1H, OCH$_2$CH*H*NH), 2.60–2.47 (m, 2H, CH$_2$*CH*$_2$SCH$_3$), 2.14 (s, 3H, OC(O)*CH*$_3$), 2.09 (s, 3H, CH$_2$CH$_2$S*CH*$_3$), 2.07 (s, 3H, OC(O)*CH*$_3$), 2.07–1.98 (m, 1H, *CH*HCH$_2$SCH$_3$), 2.04 (s, 3H, OC(O)*CH*$_3$), 1.96 (s, 3H, OC(O)*CH*$_3$), 1.91–1.82 (m, 2H, C*H*H*C*H$_2$SCH$_3$), 1.45 (s, 9H, C(*CH*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 175.0 (NH*C*(O)$_{Met}$), 172.4 (O*C*(O)CH$_3$), 170.6, 170.5, 170.5 (3 O*C*(O)CH$_3$), 157.8 (NH*C*(O)O), 98.9 (C-1$_{Man}$), 80.7 (*C*(CH$_3$)$_3$), 70.8 (C-2$_{Man}$), 70.6 (C-3$_{Man}$), 69.9 (C-5$_{Man}$), 67.7 (O*CH*$_2$CH$_2$NH), 67.2 (C-4$_{Man}$), 63.6 (C-6$_{Man}$), 55.2 (CH$_{Met,\alpha}$), 40.1 (OCH$_2$*CH*$_2$NH), 33.2 (*CH*$_2$CH$_2$SCH$_3$), 31.3 (CH$_2$*CH*$_2$SCH$_3$), 28.7 (C(*CH*$_3$)$_3$), 20.7, 20.7, 20.6, 20.6 (4 OC(O)*CH*$_3$), 15.3 (CH$_2$CH$_2$S*CH*$_3$) ppm; MALDI-ToF MS (DHB): calcd for $C_{26}H_{42}N_2NaO_{13}S$: *m*/*z* 645.23 [M+Na]$^+$; found: *m*/*z* 645.29 [M+Na]$^+$, calcd for $C_{26}H_{42}N_2KO_{13}S$: *m*/*z* 661.20 [M+K]$^+$; found: *m*/*z* 661.27 [M+K]$^+$; IR (ATR): $\tilde{\nu}$ = 3345, 2978, 2937, 2493, 1746, 1712, 1660, 1521, 1367, 1218, 1165, 1137, 1081, 1044, 978, 784 cm^{-1}.

4.7. *N*-(Acetyl)-L-methionine-[2-(α-D-mannopyranosyloxy) ethyl]amide (4)

Mannoside **3** (135 mg, 217 μmol) was dissolved in dichloromethane (4 mL), TFA (200 μL) was added and the reaction mixture was refluxed for 4 h. Then, it was diluted with dichloromethane (5 mL), Amberlyst A-21 ion exchange resin (2.00 g) was added and the mixture was stirred for 30 min. The resin was filtered off and it was washed with dichloromethane/methanol (1:1, 5 mL). The solvents were removed to yield an intermediate with the amino group deprotected (110 mg, 210 μmol, 97%) as a colourless syrup; R_f 0.27 (methanol/dichloromethane, 1:9). Part of this material (90.0 mg, 172 μmol) was acetylated according to the general procedure for N,O-acetylation using pyridine (3 mL) and acetic anhydride (500 μL) overnight. Then volatile components were removed in vacuo and the residue was codistilled with toluene. The crude product was subjected to purification by repeated MPLC (first: 100 g, silica column, A: methanol, B: dichloromethane, A: 1% → 10%, 60 min; second: 50 g, silica column, A: ethyl acetate, B: methanol, A: 100% → 90%) to yield a fully N,O-acetylated intermediate (93.0 mg, 165 μmol, 96%) as a colourless syrup; R_f: 0.60 (methanol/dichloromethane, 1:8). Part of this material (48 mg, 88.6 μmol) was de-O-acetylated overnight according to the general procedure. The crude product was subjected to purification by MPLC (60 g, RP-18 column, A: water, B: methanol, A: 99% → 60%, 120 min) yielding title compound (32 mg, 80.7 μmol, 95%; 88% over three steps) as a colourless lyophylisate; R_f 0.14 (methanol/ethyl acetate, 1:3); $[\alpha]_D^{23}$ +27.2 (*c* 0.25, methanol); ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 4.76 (d, ^3J = 1.7 Hz, 1H, H-1$_{Man}$), 4.42 (dd, ^3J = 5.4 Hz, ^3J = 8.9 Hz, 1H, H$_{Met,\alpha}$), 3.84 (dd, ^2J = 11.8 Hz, ^3J = 2.3 Hz, 1H, H-6a$_{Man}$), 3.80 (dd, ^3J = 1.7 Hz, ^3J = 3.4 Hz. 1H, H-2$_{Man}$), 3.76 (m_c, 1H, O*CH*HCH$_2$NH), 3.71 (dd, ^3J = 3.4 Hz, ^3J = 9.8 Hz, 1H, H-3$_{Man}$), 3.71–3.67 (m, 1H, H-6b$_{Man}$), 3.58 (dd∼t, ^3J = 9.8 Hz, 1H, H-4$_{Man}$), 3.55–3.49 (m, 2H, H-5$_{Man,}$ OCH$_2$*CH*HNH), 3.48–3.42 (m, 1H, OCH$_2$*CH*HNH), 3.40–3.34 (m, 1H, OCH$_2$CH*H*NH), 2.59–2.46 (m, 2H, CH$_2$*CH*$_2$SCH$_3$), 2.09 (s, 3H, CH$_2$CH$_2$S*CH*$_3$), 2.08–2.01 (m, 1H, *CH*HCH$_2$SCH$_3$), 2.00 (s, 3H, NHC(O)*CH*$_3$), 1.94–1.85 (m, 1H, *CH*HCH$_2$SCH$_3$) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 174.2 (NH*C*(O)$_{Met}$), 173.5 (NH*C*(O)CH$_3$), 101.7 (C-1$_{Man}$), 74.8 (C-5$_{Man}$), 72.6 (C-3$_{Man}$), 72.1 (C-2$_{Man}$), 68.8 (C-4$_{Man}$), 67.0 (O*CH*$_2$CH$_2$NH), 63.0 (C-6$_{Man}$), 54.1 (CH$_{Met,\alpha}$), 40.3 (OCH$_2$*CH*$_2$NH), 32.6 (*CH*$_2$CH$_2$SCH$_3$), 31.2 (CH$_2$*CH*$_2$SCH$_3$), 22.5 (NHC(O)*CH*$_3$), 15.3 (CH$_2$CH$_2$S*CH*$_3$) ppm; MALDI-ToF MS (Cl-CCA): calcd for $C_{15}H_{28}N_2NaO_8S$: *m*/*z* 419.15 [M+Na]$^+$; found: *m*/*z* 419.19 [M+Na]$^+$, calcd for $C_{15}H_{28}KN_2O_8S$: *m*/*z* 435.12 [M+K]$^+$; found: *m*/*z* 435.19 [M+K]$^+$; HR-ESI MS: calcd for $C_{15}H_{28}NaN_2O_8S$: *m*/*z* 419.1459 [M+Na]$^+$; found: *m*/*z* 419.1439 [M+Na]$^+$; IR (ATR): $\tilde{\nu}$ = 3272, 2919, 1644, 1538, 1429, 1373, 1292, 1132, 1054, 1031, 974, 915, 880, 807 cm^{-1}; EA: calcd for $C_{15}H_{32}N_2O_{10} \times 2\ H_2O$: C 41.66; H 7.46; N 6.48; S 7.41; found: C 41.95; H 6.88; N 6.35; S 7.09.

4.8. L-Methionine-[2-(α-D-mannopyranosyloxy)ethyl]amide trifluoroacetate (5)

According to the general procedure mannoside **3** (700 mg, 1.12 mmol) was de-O-acetylated over 4.5 h and the resulting prod-

uct purified by MPLC (60 g, RP-18 column, A: water, B: methanol, A: 50% → 0%, 120 min) to yield the OH-unprotected intermediate (499 mg, 1.10 mmol, 98%) as a colourless syrup; R_f 0.24 (RP-18, water/methanol, 3:1); R_f 0.44 (methanol/ethyl acetate, 1:3). Part of this *N*-Boc-protected intermediate (240 mg, 528 μmol) was treated with TFA (3 mL) for 1.5 h according to the general procedure. After co-distillation, the residue was lyophilised to yield the title compound (247 mg, 527 μmol, 99%, 97% over two steps) as a colourless lyophilisate; $[\alpha]_D^{23}$ +41.1 (*c* 0.5, methanol); ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 4.77 (d, ^3J = 1.6 Hz, 1H, H-1_{Man}), 3.96 (dd∼t, ^3J = 6.7 Hz, 1H, $H_{Met,\alpha}$), 3.85 (dd, ^2J = 11.8 Hz, ^3J = 2.1 Hz, 1H, H-$6a_{Man}$), 3.83–3.77 (m, 2H, H-2_{Man}, O*C*HCH_2NH), 3.71–3.64 (m, 2H, H-3_{Man}, H-$6b_{Man}$), 3.62–3.55 (m, 1H, OCH_2*C*HNH), 3.57 (dd∼t, ^3J = 9.2 Hz, 1H, H-4_{Man}), 3.55–3.52 (m, 1H, H-5_{Man},), 3.52–3.48 (m, 1H, OCH_2*C*HNH), 3.45–3.38 (m, 1H, OCH_2C*H*NH), 2.58 (t, ^3J = 7.6 Hz, 2H, CH_2*C*H_2SCH_3), 2.18–2.06 (m, 2H, *C*$H_2CH_2SCH_3$), 2.13 (s, 3H, CH_2CH_2*S*CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 169.8 ($NHC(O)_{Met}$), 101.6 (C-1_{Man}), 75.0 (C-5_{Man}), 72.6 (C-3_{Man}), 72.0 (C-2_{Man}), 68.8 (C-4_{Man}), 67.0 (O*C*H_2CH_2NH), 63.1 (C-6_{Man}), 53.7 ($CH_{Met,\alpha}$), 40.6 (OCH_2*C*H_2NH), 32.1 (CH_2*C*H_2SCH_3), 29.9 (*C*$H_2CH_2SCH_3$), 15.1 (CH_2CH_2*S*CH_3) ppm; MALDI-ToF MS (DHB): calcd for $C_{13}H_{26}N_2NaO_7S$: *m*/*z* 377.14 $[M+Na]^+$; found: *m*/*z* 377.21 $[M+Na]^+$; calcd for $C_{13}H_{26}KN_2O_7S$: *m*/*z* 393.11 $[M+K]^+$; found: *m*/*z* 393.21 $[M+K]^+$; HR-ESI MS: calcd for $C_{13}H_{26}N_2NaO_7S$: *m*/*z* 377.1353 $[M+Na]^+$; found: *m*/*z* 377.1359 $[M+Na]^+$; IR (ATR): $\tilde{\nu}$ = 3288, 3091, 2924, 1667, 1542, 1430, 1292, 1184, 1130, 1092, 1054, 1032, 974, 916, 879, 837, 800 cm^{-1}.

4.9. *N*-[2-(*tert*-Butyloxycarbonylamino)ethyl]imino dipropionic acid *N*′,*N*″-di-[2-(2,3,4,6-tetra-*O*-acteyl-α-D-mannopyranosyloxy) ethyl] diamide (7)

According to the general procedure for Staudinger ligation, mannoside **1** (1.44 g, 3.45 mmol) was combined with the divalent acid **6** (520 mg, 1.71 mmol), HOBt (462 mg, 3.42 mmol) was added and the mixture was dried for 1 h in vacuo. Anhydrous THF (40 mL), DIC (533 μL, 3.42 mmol) and *n*-tributyl phosphane (1.26 mL, 5.04 mmol) were added and it was stirred for 40 h. Then, standard work-up gave the crude product which was subjected to MPLC purification (200 g, silica column, A: methanol, B: ethyl acetate, A: 0% → 20%, 240 min) to yield title compound (985 mg, 937 μmol, 55%) as a colourless foam; R_f 0.31 (methanol/ethyl acetate, 1:9); $[\alpha]_D^{23}$ +40.2 (*c* 0.5, methanol); ^{1}H NMR (600 MHz, CD_3OD, 298 K): δ = 5.29 (dd, ^3J = 10.1 Hz, ^3J = 3.4 Hz, 2H, H-3_{Man}), 5.26 (dd, ^3J = 3.4 Hz, ^3J = 1.6 Hz, 2H, H-2_{Man}), 5.24 (dd∼t, ^3J = 10.1 Hz, ^3J = 9.9 Hz, 2H, H-4_{Man}), 4.88 (d, ^3J = 1.6 Hz, 2H, H-1_{Man}), 4.24 (dd, ^2J = 12.2 Hz, ^3J = 5.3 Hz, 2H, H-$6a_{Man}$), 4.13 (dd, ^2J = 12.2 Hz, ^3J = 2.4 Hz, 2H, H-$6b_{Man}$), 4.04 (m_c, 2H, H-5_{Man}), 3.81 (m_c, 2H, O*C*HCH_2NH), 3.59 (m_c, 2H, OC*H*CH_2NH), 3.51–3.40 (m, 4H, OCH_2*C*H_2NH), 3.16 (t, ^3J = 6.1 Hz, 2H, NH*C*H_2CH_2N), 2.85–2.77 (m, 4H, N*C*H_2CH_2C(O)NH), 2.62–2.56 (m, 2H, $NHCH_2$*C*H_2N), 2.44–2.35 (m, 4H, NCH_2*C*H_2C(O)NH), 2.14, 2.07, 2.04, 1.96 (each s, each 6H, OC(O)*C*H_3), 1.44 (s, 9H, C(*C*H_3)$_3$) ppm; ^{13}C NMR (150 MHz, CD_3OD, 298 K): δ = 175.2 (NH*C*(O)), 172.4, 171.6, 171.6, 171.5 (8 O*C*(O)CH_3), 158.2 (NH*C*(O)O), 98.9 (C-1_{Man}), 80.2 (*C*$(CH_3)_3$), 70.8 (C-2_{Man}), 70.6 (C-3_{Man}), 70.0 (C-5_{Man}), 67.8 (O*C*H_2CH_2NH), 67.3 (C-4_{Man}), 63.6 (C-6_{Man}), 53.6 ($NHCH_2$*C*H_2N), 51.0 (N*C*H_2CH_2C(O)NH), 40.1 (OCH_2*C*H_2NH), 38.6 (NH*C*H_2CH_2N), 34.5 (NCH_2*C*H_2C(O)NH), 28.8 (C(*C*H_3)$_3$), 20.7, 20.7, 20.6, 20.6 (8 OC(O)*C*H_3) ppm; MALDI-TOF MS (DHB): calcd for $C_{45}H_{71}N_4O_{24}$: *m*/*z* 1051.45 $[M+H]^+$; found: *m*/*z* 1051.50 $[M+H]^+$; calcd for $C_{45}H_{70}NaN_4O_{24}$: *m*/*z* 1073.43 $[M+Na]^+$; found: *m*/*z* 1073.49 $[M+Na]^+$; ESI MS: calcd for $C_{45}H_{71}N_4O_{24}$: *m*/*z* 1051.445 $[M+H]^+$; found: *m*/*z* 1051.443 $[M+H]^+$; calcd for $C_{45}H_{70}NaN_4O_{24}$: *m*/*z* 1073.427 $[M+Na]^+$; found: *m*/*z* 1073.434 $[M+Na]^+$; IR (ATR): $\tilde{\nu}$ = 3323, 2976, 2940, 1743, 1659, 1528, 1432, 1367, 1275, 1216, 1169, 1135, 1081, 1043, 977, 764, 750 cm^{-1}.

4.10. *N*-[2-(Acetamido)ethyl]imino dipropionic acid *N*′,*N*″-di-[2-(α-D-mannopyranosyloxy)ethyl] diamide (8)

Glycocluster **7** (161 mg, 153 μmol) was treated according to the general procedure for *N*-Boc deprotection with TFA (118 μL) in dichloromethane (2 mL) for 9 h. After co-distillation, the residue was lyophilised yielding the *O*-acetylated free amine intermediate (163 mg, 153 μmol, quant.) as a colourless lyophilisate; R_f 0.21 (methanol/ethyl acetate, 1:3). Part of this intermediate (110 mg, 103 μmol) was treated according to the general procedure for N,O-acetylation with pyridine (5 mL) and acetic anhydride (157 μL, 155 μmol) overnight. Then volatile compounds were removed and the residue was codistilled. The crude product was dissolved in dichloromethane (5 mL) and washed three times with water (5 mL each). The organic layer was evaporated yielding the crude fully N,O-protected intermediate as slightly yellow foam. Then, according to the general procedure for de-O-acetylation, this intermediate was treated overnight with sodium in dry methanol (3 mL). After neutralisation, the crude product was subjected to MPLC purification (120 g, RP-18 column, A: water, B: methanol, A: 90% → 50%, 120 min) yielding the title compound (58 mg, 88.3 μmol, 86% over three steps) as a colourless foam; $[\alpha]_D^{23}$ +31.1 (*c* 0.25, methanol); ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 4.77 (d, ^3J = 1.7 Hz, 2H, H-1_{Man}), 3.84 (dd, ^2J = 11.8 Hz, ^3J = 2.3 Hz, 2H, H-$6a_{Man}$), 3.82 (dd, ^3J = 3.4 Hz, ^3J = 1.7 Hz, 2H, H-2_{Man}), 3.77 (m_c, 2H, O*C*HCH_2NH), 3.72–3.67 (m, 4H, H-3_{Man}, H-$6b_{Man}$), 3.60 (dd∼t, ^3J = 9.7 Hz, ^3J = 9.3 Hz, 2H, H-4_{Man}), 3.58–3.51 (m, 4H, OC*H*CH_2NH, H-5_{Man}), 3.48–3.42 (m, 2H, OCH_2*C*HNH), 3.39–3.33 (m, 2H, OCH_2C*H*NH), 3.26 (t, ^3J = 6.7 Hz, 2H, NH*C*H_2CH_2N), 2.80 (t, ^3J = 6.8 Hz, 4H, N*C*H_2CH_2C(O)NH), 2.59 (t, ^3J = 6.7 Hz, 2H, $NHCH_2$*C*H_2N), 2.37 (t, ^3J = 6.8 Hz, 4H, NCH_2*C*H_2C(O)NH), 1.96 (s, 3H, NHC(O)*C*H_3) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 175.2 (*C*(O) NH), 173.2 (NH*C*(O)CH_3), 101.7 (C-1_{Man}), 74.7 (C-5_{Man}), 72.6 (C-3_{Man}), 72.1 (C-2_{Man}), 68.7 (C-4_{Man}), 67.3 (O*C*H_2CH_2NH), 63.0 (C-6_{Man}), 53.3 ($NHCH_2$*C*H_2N), 51.0 (N*C*H_2CH_2C(O)NH), 40.3 (OCH_2*C*H_2NH), 39.9 (NH*C*H_2CH_2N), 34.6 (NCH_2*C*H_2C(O)NH), 22.7 (NHC(O)*C*H_3) ppm; HR-ESI MS: calcd for $C_{26}H_{49}N_4O_{15}$: *m*/*z* 657.3189 $[M+H]^+$; found: *m*/*z* 657.3199 $[M+H]^+$; IR (ATR): $\tilde{\nu}$ = 3289, 2939, 1630, 1555, 1430, 1376, 1202, 1130, 1095, 1056, 1032, 975, 838, 801, 722 cm^{-1}.

4.11. *N*-(2-Aminoethyl)imino dipropionic acid *N*′,*N*″-di-[2-(α-D-mannopyranosyloxy)ethyl] diamide bis-trifluoroacetate (9)

According to the general procedure for de-O-acetylation, the divalent cluster mannoside **7** (71 mg, 67.7 μmol) was treated overnight with sodium in dry methanol (3 mL). After neutralisation, the *N*-Boc-protected intermediate amine was obtained (48 mg, 67.2 μmol, 99%) as a colourless lyophilisate; R_f 0.32 (RP-18, water/methanol, 1:1). Part of this intermediate (35 mg, 49.0 μmol) was treated overnight according to the general procedure for *N*-Boc deprotection with TFA (300 μL) in dichloromethane (3 mL). After co-distillation the residue was lyophilised yielding the title compound (41 mg, 49.0 μmol, quant., 99% over two steps) as a colourless lyophylisate; $[\alpha]_D^{23}$ +33.6 (*c* 0.35, methanol); ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 4.77 (d, ^3J = 1.7 Hz, 2H, H-1_{Man}), 3.85 (dd, ^2J = 11.8 Hz, ^3J = 2.3 Hz, 2H, H-$6a_{Man}$), 3.81 (dd, ^3J = 3.3 Hz, ^3J = 1.7 Hz, 2H, H-2_{Man}), 3.77 (m_c, 2H, O*C*HCH_2NH), 3.72–3.65 (m, 4H, H-3_{Man}, H-$6b_{Man}$), 3.56 (dd∼t, ^3J = 9.7 Hz, ^3J = 9.2 Hz, 2H, H-4_{Man}), 3.58–3.51 (m, 4H, OC*H*CH_2NH, H-5_{Man}), 3.49–3.43 (m, 2H, OCH_2*C*HNH), 3.42–3.35 (m, 2H, OCH_2C*H*NH), 3.30 (br s, 2H, NH*C*H_2CH_2N), 3.13 (m_c, 6H, N*C*H_2CH_2C(O)NH, $NHCH_2$*C*H_2N), 2.59 (br s, 4H, NCH_2*C*H_2C(O)NH) ppm; ^{13}C NMR (125 MHz, CD_3OD,

300 K): δ = 174.2 (NHC(O)), 101.6 (C-1_{Man}), 74.9 (C-5_{Man}), 72.6 (C-3_{Man}), 72.0 (C-2_{Man}), 68.8 (C-4_{Man}), 67.2 (OCH_2CH_2NH), 63.1 (C-6_{Man}), 50.7 ($NCH_2CH_2C(O)NH$), 50.6 ($NHCH_2CH_2N$), 40.5 (OCH_2CH_2NH), 37.2 ($NHCH_2CH_2N$), 32.2 ($NCH_2CH_2C(O)NH$) ppm; MALDI-ToF MS (DHB): calcd for $C_{24}H_{47}N_4O_{14}^+$: *m*/*z* 615.31 $[M+H]^+$; found: *m*/*z* 615.31 $[M+H]^+$; HR-ESI MS: calcd for $C_{24}H_{47}N_4O_{14}$: *m*/*z* 615.3083 $[M+H]^+$; found: *m*/*z* 615.3087 $[M+H]^+$; IR (ATR): $\tilde{\nu}$ = 3294, 2934, 1669, 1562, 1428, 1344, 1138, 1127, 1092, 1054, 1028, 974, 915, 837, 799 cm^{-1}.

4.12. 3-Cascade:N-(fluorenylmethoxycarbonyl)-S-(benzyl)-L-cysteinyl-aminomethane[3]:propionic acid tert-butyl ester (11)

Fmoc-Cys(Bn)-OH (728 mg, 1.68 mmol), the trivalent ester **10** (350 mg, 842 μmol) and HBTU (637 mg, 1.68 mmol) were dried for 1 h in vacuo. Then dry DMF (20 mL) and DIPEA (577 μmol, 3.37 mmol) were added and it was stirred overnight at ambient temperature. Solvents were removed under reduced pressure and the crude product was dissolved in dichloromethane (25 mL). It was washed twice with water and brine (25 mL each), volatile compounds were removed under reduced pressure and the crude product was subjected to column chromatography (ethyl acetate/cyclohexane, 1:4) yielding the title compound (609 mg, 733 μmol, 87%) as a colourless solid; R_f 0.34 (ethyl acetate/cyclohexane, 1:3); ^{1}H NMR (500 MHz, $CDCl_3$, 300 K) δ = 7.76 (d, ^3J = 7.5 Hz, 2H, $H_{aryl,Fmoc}$), 7.62–7.59 (m, 2H, $H_{aryl,Fmoc}$), 7.39 (m_c, 2H, $H_{aryl,Fmoc}$), 7.37–7.28 (m, 6H, $H_{aryl,Fmoc}$, $H_{aryl,benzyl}$), 7.26–7.22 (m, 1H, $H_{aryl,benzyl}$), 6.32 (br s, 1H, NH), 5.61 (br s, 1H, NH), 4.44 (m_c, 1H, C*H*H_{Fmoc}), 4.38 (br s, 1H, CH*H*$_{Fmoc}$), 4.23 (t, ^3J = 7.1 Hz, 1H, CH_{Fmoc}), 4.12 (br s, 1H, $H_{Cys,\alpha}$), 3.82–3.72 (m, 2H, $CH_{2,benzyl}$), 2.88–2.80 (m, 1H, $CH_{2,Cys,\ \beta a}$), 2.71 (m_c, 1H, $CH_{2,Cys,\ \beta b}$), 2.20 (m_c, 6H, C*CH*$_2$CH$_2$C(O)), 1.95 (m_c, 6H, C*CH*$_2$CH$_2$C(O)), 1.42 (s, 27H, C(*CH*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 300 K): δ = 172.7 (*O*C(O)), 169.0 (NH*C*(O)), 158.2 (O*C*(O)NH), 143.7 ($C_{aryl,benzyl}$), 141.3, 141.3 ($C_{aryl,Fmoc}$), 129.0, 128.7 ($CH_{aryl,Fmoc}$), 127.7, 127.3, 127.1 ($CH_{aryl,benzyl}$), 125.1, 120.0 ($CH_{aryl,Fmoc}$), 80.7 (*C*(CH$_3$)$_3$), 67.2 ($CH_{2,Fmoc}$), 58.0 (NH*C*(CH$_2$CH$_2$)$_3$), 54.5 ($CH_{Cys,\alpha}$), 47.5 (CH_{Fmoc}), 36.8 ($CH_{2,benzyl}$), 34.2 ($CH_{2,Cys,\beta}$), 29.9 (CCH$_2$*CH*$_2$C(O)), 29.7 (C*CH*$_2$CH$_2$C(O)), 28.1 (C(*CH*$_3$)$_3$)ppm; ESI MS: calcd for $C_{47}H_{62}N_2NaO_9S$: *m*/*z* 854.073 $[M+Na]^+$; found: *m*/*z* 854.452 $[M+Na]^+$.

4.13. 3-Cascade:N-(fluorenylmethoxycarbonyl)-S-(benzyl)-L-cysteinyl-aminomethane[3]:propionic acid (12)

The cysteinylated triester **11** (609 mg, 733 μmol) was dissolved in formic acid (20 mL) and it was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and it was codistilled three times with toluene (10 mL each). Then it was lyophilised leading to the title compound (485 mg, 733 μmol, quant.) as a colourless lyophilisate. ^{1}H NMR (500 MHz, CD_3OD, 300 K) δ = 7.78 (d, ^3J = 7.5 Hz, 2H, $H_{aryl,Fmoc}$), 7.68–7.64 (m, 2H, $H_{aryl,Fmoc}$), 7.37 (t, ^3J = 7.4 Hz, 2H, $H_{aryl,Fmoc}$), 7.34–7.27 (m, 7H, $H_{aryl,Fmoc}$, $H_{aryl,benzyl}$), 4.43 (dd, ^3J = 6.9 Hz, ^2J = 10.3 Hz, 1H, C*H*H_{Fmoc}), 4.35 (dd, ^3J = 7.3 Hz, ^2J = 10.3 Hz, 1H, CH*H*$_{Fmoc}$), 4.24–4.22 (m, 2H, $H_{Cys,\alpha}$, CH_{Fmoc}), 3.77 (s, 2H, $CH_{2,benzyl}$), 2.74 (dd, ^3J = 7.2 Hz, ^2J = 13.5 Hz, 1H, $CH_{2,Cys,\beta a}$), 2.60 (dd, ^3J = 7.3 Hz, ^2J = 13.5 Hz, 1H, $CH_{2,Cys,\beta b}$), 2.32–2.29 (m, 6H, C*CH*$_2$CH$_2$C(O)), 2.04–2.00 (m, 6H, C*CH*$_2$CH$_2$C(O)) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 177.0 (HO*C*(O)), 172.4 (NH*C*(O)), 158.2 (O*C*(O)NH), 145.4 ($C_{aryl,benzyl}$), 145.2 (C-aryl), 142.6, 142.6 ($C_{aryl,Fmoc}$), 130.1, 129.6 ($CH_{aryl,Fmoc}$), 128.8, 128.2, 128.1 ($CH_{aryl,benzyl}$), 126.2, 120.9 ($CH_{aryl,Fmoc}$), 68.1 ($CH_{2,Fmoc}$), 59.0 (NH*C*(CH$_2$CH$_2$)$_3$), 56.3 ($CH_{Cys,\alpha}$), 48.4 (CH_{Fmoc}), 37.0 ($CH_{2,benzyl}$), 33.8 ($CH_{2,Cys,\beta}$), 30.5 (CCH$_2$*CH*$_2$C(O)), 29.2 (C*CH*$_2$CH$_2$C(O)) ppm; HR-ESI MS: calcd for $C_{35}H_{39}N_2O_9S$: *m*/*z* 663.2376 $[M+H]^+$; found: *m*/*z* 663.2384 $[M+H]^+$.

4.14. 3-Cascade:N-(fluorenylmethoxycarbonyl)-S-(benzyl)-L-cysteinyl-aminomethane[3]: N′-[2-(2,3,4,6-tetra-O-α-D-mannopyranosyloxy)ethyl] propionic acid amide (13)

According to the general procedure for Staudinger ligation mannoside **1** (1.31 g, 3.02 mmol), triacid **12** (384 mg, 580 μmol) and HOBt (408 mg, 3.02 mmol) were dried for 1 h in vacuo and dissolved in anhydrous THF (25 mL). DIC (468 μL, 3.02 mmol) and *n*-tributylphosphane (754 μL, 3.02 mmol) were added and it was stirred overnight. After a standard work-up, the crude product was obtained and subjected to MPLC (150 g, silica column, A: ethyl acetate, B: cyclohexane, A: 90% → A: 100%, 105 min; A: ethyl acetate, B: methanol, A: 100% → 85%, 135 min) followed by size exclusion chromatography (LH-20, methanol) yielding the title compound (640 mg, 359 μmol, 62%) as a colourless foam; R_f 0.55 (methanol/ethyl acetate, 1:9); $[\alpha]_D^{23}$ +32.6 (*c* 0.475, chloroform); ^{1}H NMR (500 MHz, $CDCl_3$, 300 K): δ = 7.74 (d, ^3J = 7.6 Hz, 2H, $H_{aryl,Fmoc}$), 7.60 (m_c, 2H, $H_{aryl,Fmoc}$), 7.38 (dt, ^4J = 2.1 Hz, ^3J = 7.5 Hz, 2H, $H_{aryl,Fmoc}$), 7.32–7.25 (m, 6H, $H_{aryl,Fmoc}$, $H_{aryl,benzyl}$), 7.24–7.19 (m, 1H, $H_{aryl,benzyl}$), 7.14 (br s, 1H, NH), 6.56 (br s, 3H, *H*NCH$_2$CH$_2$O), 5.94 (d, ^3J = 6.7 Hz, 1H, NH), 5.31 (dd, ^3J = 10.1 Hz, ^3J = 3.5 Hz, 3H, H-3_{Man}), 5.26 (dd, ^3J = 3.5 Hz, ^3J = 1.7 Hz, 3H, H-2_{Man}), 5.24 (dd∼t, ^3J = 10.1 Hz, ^3J = 10.0 Hz, 3H, H-4_{Man}), 4.80 (d, ^3J = 1.7 Hz, 3H, H-1_{Man}), 4.40 (q, ^3J = 10.6 Hz, ^3J = 7.3 Hz, 1H, C*H*H_{Fmoc}), 4.26 (t, ^3J = 7.3 Hz, 1H, CH*H*$_{Fmoc}$), 4.23 (dd, ^2J = 12.3 Hz, ^3J = 5.5 Hz, 3H, H-$6a_{Man}$), 4.22–4.18 (m, 2H, CH_{Fmoc}, $H_{Cys,\alpha}$), 4.10 (m_c, 3H, H-$6b_{Man}$), 3.98 (m, 3H, H-5_{Man}), 3.78–3.67 (m, 5H, $CH_{2,benzyl}$, HNCH$_2$*CH*HO), 3.52–3.44 (m, 6H, HNCH$_2$C*H*HO, HN*CH*HCH$_2$O), 3.33–3.25 (m, 3H, HNC*H*HCH$_2$O), 2.85–2.73 (m, 2H, $CH_{2,Cys,\beta}$), 2.20 (m_c, 6H, C*CH*$_2$CH$_2$C(O)), 2.11,2.07 (s, 18H, OC(O)*CH*$_3$), 2.01–1.94 (m, 6H, CCH$_2$*CH*$_2$C(O)), 2.00, 1.95 (s, 18H, OC(O)*CH*$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 300 K): δ = 173.5 (3 NH*C*(O)), 170.7, 170.2, 170.2 (9 OC(O)CH$_3$), 169.9 (NH*C*(O)), 169.7 (3 OC(O)CH$_3$), 157.9 (O*C*(O)NH), 143.8, 141.2 ($C_{aryl,Fmoc}$), 137.8 ($C_{aryl,benzyl}$), 129.0, 128.7, 127.3 ($CH_{aryl,benzyl}$), 127.8, 127.1, 125.1 120.0 ($CH_{aryl,Fmoc}$), 97.6 (C-1_{Man}), 69.4 (C-3_{Man}), 69.2 (C-2_{Man}), 68.7 (C-5_{Man}), 67.1 ($CH_{2,Fmoc}$), 67.0 (HNCH$_2$*CH*$_2$O), 66.1 (C-4_{Man}), 62.5 (C-6_{Man}), 58.8 (NH*C*(CH$_2$CH$_2$)$_3$), 54.7 ($CH_{Cys,\alpha}$), 47.1 (CH_{Fmoc}), 38.8 (HN*CH*$_2$CH$_2$O), 36.5 ($CH_{2,benzyl}$), 33.5 ($CH_{2,Cys,\beta}$), 31.3 (CCH$_2$*CH*$_2$C(O)), 30.8 (C*CH*$_2$CH$_2$C(O)), 20.9, 20.7, 20.7, 20.7 (12 OC(O)*CH*$_3$) ppm; MALDI-ToF MS (DHB): calcd for $C_{83}H_{107}N_5NaO_{36}S$: *m*/*z* 1804.63 $[M+Na]^+$; found: *m*/*z* 1804.64 $[M+Na]^+$; calcd for $C_{83}H_{107}KN_5O_{36}S$: *m*/*z* 1820.60 $[M+K]^+$; found: *m*/*z* 1820.61 $[M+K]^+$; IR (ATR): $\tilde{\nu}$ = 3326, 2942, 1743, 1652, 1531, 1451, 1368, 1218, 1136, 1083, 1044, 977, 762, 744 cm^{-1}.

4.15. 3-Cascade:S-(benzyl)-L-cysteinyl-aminomethane[3]: N′-[2-(2,3,4,6-tetra-O-α-D-mannopyranosyloxy)ethyl] propionic acid amide (14)

The Fmoc-protected glycocluster **13** (629 mg, 353 μmol) was dissolved in DMF (9 mL) and piperidine (1 mL) was added. The reaction mixture was stirred at ambient temperature for 90 min. All volatile compounds were removed under reduced pressure and the crude product was subjected to MPLC (50 g, silica column, A: ethyl acetate, B: methanol, A: 100% → 90%) yielding the free amine (541 mg, 347 μmol, 98%) as a colourless syrup; R_f 0.31 (methanol/ethyl acetate, 1:5); $[\alpha]_D^{23}$ +41.7 (*c* 0.5, methanol); ^{1}H NMR (500 MHz, $CDCl_3$, 300 K): δ = 7.44 (br s, 1H, NH), 7.29–7.25 (m, 4H, $H_{aryl,benzyl}$), 7.22–7.18 (m, 1H, $H_{aryl,benzyl}$), 6.56 (t, ^3J = 5.5 Hz, 3H, *H*NCH$_2$CH$_2$O), 5.27 (dd, ^3J = 9.9 Hz, ^3J = 3.4 Hz, 3H, H-3_{Man}), 5.25–5.20 (m, 6H, H-2_{Man}, H-4_{Man}), 4.79 (d, ^3J = 1.5 Hz, 3H, H-1_{Man}), 4.23 (dd, ^2J = 12.3 Hz, ^3J = 5.5 Hz, 3H, H-$6a_{Man}$), 4.10–4.05 (m, 3H, H-$6b_{Man}$,), 3.96 (m, 3H, H-5_{Man}), 3.75–3.67 (m, 5H, $CH_{2,benzyl}$, HNCH$_2$*CH*HO), 3.52–3.42 (m, 7H, $H_{Cys,\alpha}$, HNCH$_2$C*H*HO, HN*CH*HCH$_2$O), 3.35–3.28 (m, 3H, HNC*H*HCH$_2$O), 2.83 (dd,

^3J = 5.3 Hz, ^2J = 13.4 Hz, $CH_{2,Cys,\beta a}$), 2.73 (dd, ^3J = 7.4 Hz, ^2J = 13.4 Hz, $CH_{2,Cys,\beta b}$), 2.19 (t, ^3J = 7.9 Hz, 6H, $CCH_2CH_2C(O)$), 2.11, 2.06 (each s, 18H, $OC(O)CH_3$), 2.02–1.92 (m, 6H, $CCH_2CH_2C(O)$), 2.00, 1.94 (each s, 18H, $OC(O)CH_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 300 K): δ = 173.5 (3 NHC(O)), 171.1 (NHC(O)), 170.7, 170.2, 170.2, 169.7 (12 OC(O)CH_3), 137.9 ($C_{aryl,benzyl}$), 128.9, 128.7, 127.3 ($CH_{aryl,benzyl}$), 97.6 (C-1_{Man}), 69.4 (C-3_{Man}), 69.2 (C-2_{Man}), 68.6 (C-5_{Man}), 67.0 ($HNCH_2CH_2O$), 66.0 (C-4_{Man}), 62.4 (C-6_{Man}), 58.3 ($NHC(CH_2CH_2)_3$), 53.9 ($CH_{Cys,\alpha}$), 38.9 ($HNCH_2CH_2O$), 36.5 ($CH_{2,benzyl}$), 36.1 ($CH_{2,Cys,\beta}$), 31.2 ($CCH_2CH_2C(O)$), 30.7 ($CCH_2CH_2C(O)$), 20.8, 20.7, 20.7, 20.6 (12 OC(O)CH_3) ppm; MALDI-ToF MS (DHB): calcd for $C_{68}H_{97}N_5NaO_{34}S$: m/z 1582.56 $[M+Na]^+$; found: m/z 1586.68 $[M+Na]^+$; calcd for $C_{68}H_{97}KN_5O_{34}S$: m/z 1598.54 $[M+K]^+$; found: m/z 1598.64 $[M+K]^+$; ESI MS: calcd for $C_{68}H_{98}N_5O_{34}S$: m/z 1560.581 $[M+H]^+$; found: m/z 1560.586 $[M+H]^+$; IR (ATR): $\tilde{\nu}$ = 3302, 2944, 1740, 1651, 1538, 1432, 1368, 1217, 1135, 1082, 1044, 978, 897, 692 cm^{-1}.

4.16. 3-Cascade:N-(acetyl)-S-(benzyl)-L-cysteinyl-aminomethane[3]: N′-[2-α-D-mannopyranosyloxy)ethyl] propionic acid amide (15)

According to the general procedure for acetylation the amine **14** (124 mg, 79.5 μmol) was treated with pyridine (3 mL) and acetic anhydride (300 μL) for 2 h at ambient temperature. Then volatile compounds were removed and after co-distillation the crude product was dissolved in dichloromethane (5 mL) and washed three times with water (5 mL each). The organic layer was evaporated and the crude product was subjected to de-O-acetylation according to the general procedure using sodium in dry methanol (5 mL) for 2 h. After neutralisation, purification of the product by RP-MPLC (120 g, RP-18 column, A: water, B: methanol, A: 25% → 50%, 90 min) gave the pure title compound (82 mg, 74.7 μmol, 94% over two steps) as a colourless lyophilisate; R_f 0.57 (RP-18, water/methanol, 1:1); $[\alpha]_D^{23}$ +43.8 (*c* 0.5, methanol); ^1H NMR (500 MHz, CD_3OD, 300 K): δ = 7.37–7.27 (m, 4H, $H_{aryl,benzyl}$), 7.26–7.22 (m, 1H, $H_{aryl,benzyl}$), 4.77 (d, ^3J = 1.5 Hz, 3H, H-1_{Man}), 4.36 (t, ^3J = 7.2 Hz, 1H, $H_{Cys,\alpha}$, 3.84 (dd, ^2J = 11.9 Hz, ^3J = 2.4 Hz, 3H, H-$6a_{Man}$), 3.83–3.80 (m, 5H, H-2_{Man}, $CH_{2,benzyl}$), 3.79–3.73 (m, 3H, $HNCH_2CHHO$), 3.73–3.66 (m, 6H, H-3_{Man}, H-$6b_{Man}$, 3.60 (dd∼t, ^3J = 9.8 Hz, ^3J = 9.2 Hz, 3H, H-4_{Man}), 3.57–3.50 (m, 6H, H-5_{Man}, $HNCH_2CHHO$), 3.46–3.39 (m, 3H, $HNCHHCH_2O$), 3.38–3.32 (m, 3H, $HNCHHCH_2O$), 2.80 (dd, ^3J = 6.8 Hz, ^2J = 13.5 Hz, $CH_{2,Cys,\beta a}$), 2.67 (dd, ^3J = 7.7 Hz, ^2J = 13.5 Hz, $CH_{2,Cys,\beta b}$), 2.25–2.19 (m, 6H, $CCH_2CH_2C(O)$), 2.01 (s, 3H, NHC(O)CH_3), 2.00–1.96 (m, 6H, $CCH_2CH_2C(O)$) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 176.0 (3 NHC(O)), 172.2 (NHC(O)), 139.5 ($C_{aryl,benzyl}$), 130.2, 129.6, 128.2 ($CH_{aryl,benzyl}$), 101.7 (C-1_{Man}), 74.8 (C-5_{Man}), 72.6 (C-3_{Man}), 72.1 (C-2_{Man}), 68.8 (C-4_{Man}), 67.3 ($HNCH_2CH_2O$), 63.0 (C-6_{Man}), 59.6 ($NHC(CH_2CH_2)_3$), 55.2 ($CH_{Cys,\alpha}$), 40.4 ($HNCH_2CH_2O$), 37.0 ($CH_{2,benzyl}$), 36.0 ($CH_{2,Cys,\beta}$), 31.9 ($CCH_2CH_2C(O)$), 31.3 ($CCH_2CH_2C(O)$), 22.6 (NHC(O)CH_3) ppm; MALDI-ToF MS (Cl-CCA): calcd for $C_{46}H_{75}N_5NaO_{23}S$: m/z 1120.45 $[M+Na]^+$; found: m/z 1120.41 $[M+Na]^+$; HR-ESI MS: calcd for $C_{46}H_{75}N_5NaO_{23}$: m/z 1120.4466 $[M+Na]^+$; found: m/z 1120.4476 $[M+Na]^+$; IR (ATR): $\tilde{\nu}$ = 3282, 2932, 1634, 1539, 1430, 1373, 1245, 1132, 1055, 1032, 969, 881, 806 cm^{-1}; EA: calcd for $C_{46}H_{75}N_5O_{23}S \times 4\ H_2O$: C 47.21; H 7.15; N 5.98; found: C 47.20; H 6.99; N 5.94.

4.17. 3-Cascade:S-(benzyl)-L-cysteinyl-aminomethane[3]: N′-[2-α-D-mannopyranosyloxy)ethyl] propionic acid amide (16)

According to the general procedure for deacetylation the amine **14** (186 mg, 119 μmol) was treated with sodium in dry methanol (3 mL) overnight and it was neutralised. The crude product was washed three times with dichloromethane (5 mL each) yielding the title compound (124 mg, 118 μmol, 99%) as a colourless lyophilisate; $[\alpha]_D^{23}$ +45.2 (*c* 0.5, methanol); ^1H NMR (500 MHz, CD_3OD, 300 K): δ = 7.40–7.29 (m, 4H, $H_{aryl,benzyl}$), 7.27–7.23 (m, 1H, $H_{aryl,benzyl}$), 4.77 (d, ^3J = 1.6 Hz, 3H, H-1_{Man}), 3.85 (dd, ^2J = 12.0 Hz, ^3J = 2.3 Hz, 3H, H-$6a_{Man}$), 3.84–3.81 (m, 5H, $CH_{2,benzyl}$, H-2_{Man}), 3.77–3.73 (m, 3H, $HNCH_2CHHO$), 3.73–3.66 (m, 7H, H-3_{Man}, H-$6b_{Man}$, $H_{Cys,\alpha}$, 3.61 (dd∼t, ^3J = 9.7 Hz, ^3J = 9.2 Hz, 3H, H-4_{Man}), 3.57–3.51 (m, 6H, H5_{Man}, $HNCH_2CHHO$), 3.46–3.40 (m, 3H, $HNCHHCH_2O$), 3.38–3.32 (m, 3H, $HNCHHCH_2O$), 2.89 (dd, ^3J = 6.1 Hz, ^2J = 13.6 Hz, $CH_{2,Cys,\beta a}$), 2.75 (dd, ^3J = 7.5 Hz, ^2J = 13.4 Hz, $CH_{2,Cys,\beta b}$), 2.27–2.17 (m, 6H, $CCH_2CH_2C(O)$), 2.01 (t, ^3J = 8.3 Hz, 6H, $CCH_2CH_2C(O)$) ppm; ^{13}C NMR (125 MHz, CD_3OD, 300 K): δ = 176.1 (NHC(O)), 176.0 (3 NHC(O)), 139.3 ($C_{aryl,benzyl}$), 130.1, 129.7, 128.3 ($CH_{aryl,benzyl}$), 101.7 (C-1_{Man}), 74.8 (C-5_{Man}), 72.6 (C-3_{Man}), 72.1 (C-2_{Man}), 68.7 (C-4_{Man}), 67.3 ($HNCH_2CH_2O$), 63.0 (C-6_{Man}), 59.6 ($NHC(CH_2CH_2)_3$), 55.0 ($CH_{Cys,\alpha}$), 40.4 ($HNCH_2CH_2O$), 37.3 ($CH_{2,benzyl}$), 36.0 ($CH_{2,Cys,\beta}$), 31.8 ($CCH_2CH_2C(O)$), 31.3 ($CCH_2CH_2C(O)$) ppm; MALDI-ToF MS (Cl-CCA): calcd for $C_{44}H_{74}N_5O_{22}S$: m/z 1056.45 $[M+H]^+$; found: m/z 1056.44 $[M+H]^+$; calcd for $C_{44}H_{73}N_5NaO_{22}S$: m/z 1078.44 $[M+Na]^+$; found: m/z 1078.45 $[M+Na]^+$; ESI MS: calcd for $C_{44}H_{74}N_5O_{22}S$: m/z 1056.454 $[M+H]^+$; found: m/z 1056.455 $[M+H]^+$; IR (ATR): $\tilde{\nu}$ = 3292, 2929, 1634, 1424, 1244, 1202, 1132, 1056, 1028, 969, 806 cm^{-1}.

4.18. Biological assays

4.18.1. Media, buffer solutions, bacteria

LB-medium (+AMP,+CAM) (PKL1162): Tryptone (10.0 g), sodium chloride (10.0 g) and yeast extract (5.00 g) were dissolved in bidest. water (1.00 L); after sterilisation, ampicillin (100 mg) and chloramphenicol (50.0 mg) were added; *PBS buffer solution (pH 7.2)*: Sodium chloride (8.00 g), potassium chloride (200 mg), sodium hydrogen phosphate-dihydrate (1.44 g) and potassium dihydrogen phosphate (200 mg) were dissolved in bidest. water (1.00 L); *PBST buffer solution (pH 7.2)*: PBS buffer + 0.05% v/v Tween® 20. *pH-values* were adjusted with 0.1 M HCl or 0.1 M NaOH, respectively. *Carbonate buffer solution (pH 9.6)*: Sodium carbonate (10.6 g) and sodium hydrogen carbonate (8.40 g) were dissolved in bidest. water. *Bacteria culture: E. coli* bacteria (PKL1162)[38,40] were used and grown in LB-media + AMP + CAM (100 mg ampicillin, 50 mg chloramphenicol/L) at 37 °C under slight agitation.[38]

4.18.2. Covalent surface functionalisation

Serial dilutions of the amino-functionalised carbohydrate in carbonate buffer (100 mM, pH 9.6, 60 μL) were pipetted into 96 well Black Immobilizer Amino™ F96 MicroWell™ plates (Thermo Fisher Scientific, Nunc). Immobilisation was carried out overnight at room temperature under gentle agitation. The wells were washed with PBST three times (150 μL/well) and blocked by incubation with ethanolamine solution (10 mM in PBS, 100 mM pH 7.2; 100 μL/well) for 2 h at room temperature under slight agitation.

4.18.3. Bacterial adhesion assay on microtiter plates

After washing the plate with PBST three times (150 μL/well) a suspension of the bacteria (60 μL, 1 mg/mL in PBS) was added to all wells and the plate was agitated for 60 min at 37 °C. The wells were washed three times with PBS (150 μL/well). PBS (100 μL/well) was added to the wells and the surface bound bacteria were detected via fluorescence readout (excitation wavelength, 485 nm, emission wavelength 535 nm).

4.18.4. Mannan coating

Black 96-well plates (Nunc Maxisorp) were filled with a solution of mannan from *Saccharomyces cerevisiae* (1.2 mg/mL in carbonate buffer, pH 9.6; 120 μL solution per well) and allowed to

dry at 37 °C overnight. The plates were washed with PBST and PBS (150 μL/well each), blocked with BSA (5% in PBS, 150μL/well) for 2 h at 37 °C and then washed with PBS (3 × 150 μL/well). Then they were stored at 4 °C overnight.

4.18.5. Adhesion-inhibition assay

Serial dilutions of the examined inhibitor were prepared and 50 μL transferred into each well of a mannan-coated, BSA-blocked test plate. The bacterial suspension in PBS buffer (2 mg/mL) was added (50 μL/well) and the plates were agitated (120 rpm) and incubated for 1 h 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.

Acknowledgements

Funding of this work by the DFG (collaborative network SFB677) and by the Evonik Foundation (stipend for J.W.W.) is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.01.023.

References

1. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
2. Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357–2364.
3. Haltiwanger, R. S.; Lowe, J. B. *Annu. Rev. Biochem.* **2004**, *73*, 491–537.
4. Feizi, T.; Chai, W. G. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 582–588.
5. Laurent, N.; Voglmeir, J.; Flitsch, S. L. *Chem. Commun.* **2008**, 4400–4412.
6. Love, K. R.; Seeberger, P. H. *Angew. Chem.* **2002**, *114*, 3733–3736. *Angew. Chem., Int. Ed.* **2002**, *41*, 3583–3586.
7. Disney, M. D.; Seeberger, P. H. *Chem. Biol.* **2004**, *11*, 1701–1707.
8. Chabre, Y. M.; Giguère, D.; Blanchard, B.; Rodrigue, J.; Rocheleau, S.; Neault, M.; Rauthu, S.; Papadopoulos, A.; Arnold, A. A.; Imberty, A.; Roy, R. *Chem. Eur. J.* **2011**, *17*, 6545–6562.
9. Weissenborn, M. J.; Castangia, R.; Wehner, J. W.; Šardzík, R.; Lindhorst, T. K.; Flitsch, S. L. *Chem. Commun.* **2012**, *48*, 4444–4446.
10. Grabosch, C.; Kolbe, K.; Lindhorst, T. K. *ChemBioChem* **2012**, *13*, 1874–1879.
11. Wehner, J. W.; Weissenborn, M. J.; Hartmann, M.; Gray, C. J.; Šardzík, R.; Eyers, C. E.; Flitsch, S. L.; Lindhorst, T. K. *Org. Biomol. Chem.* **2012**, *10*, 8919–8926.
12. Connell, H.; Agace, W.; Klemm, P.; Schembri, M.; Marild, S.; Svanborg, C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9827–9832.
13. Hartmann, M.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2011**, 3583–3609.
14. Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem.* **1998**, *110*, 2908–2953. *Angew. Chem., Int. Ed*. **1998**, *37*, 2754–2794.
15. Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem.* **2006**, *118*, 5418–5422. *Angew. Chem., Int. Ed. Engl*. **2006**, *45*, 2348–2368.
16. Fasting, C.; Schalley, C.; Weber, M.; Seitz, O.; Hecht, S.; Koksch, B.; Dernedde, J.; Graf, C.; Knapp, E.; Haag, R. *Angew. Chem.* **2012**, *124*, 10622–10650. *Angew. Chem., Int. Ed.* **2012**, *51*, 10472–10498.
17. André, S.; CejasOrtega, P. J.; AlaminoPerez, M.; Roy, R.; Gabius, H.-J. *Glycobiology* **1999**, *9*, 1253–1261.
18. André, S.; Pieters, R. J.; Vrasidas, J.; Kaltner, H.; Kuwabara, I.; Liu, F.-T.; Liskamp, R. M. J.; Gabius, H.-J. *ChemBioChem* **2001**, *2*, 822–830.
19. Kilcoyne, M.; Gerlach, J. Q.; Kane, M.; Joshi, L. *Anal. Methods* **2012**, *4*, 2721–2728.
20. Branderhorst, H. M.; Ruijtenbeek, R.; Liskamp, R. M. J.; Pieters, R. J. *ChemBioChem* **2008**, *9*, 1836–1844.
21. Pera, N. P.; Branderhorst, H. M.; Kooij, R.; Maierhofer, C.; van der Kaaden, M.; Liskamp, R. M. J.; Wittmann, V.; Ruijtenbeek, R.; Pieters, R. J. *ChemBioChem* **2010**, *11*, 1896–1904.
22. Dam, T. K.; Brewer, C. F. *Glycobiology* **2010**, *20*, 1061–1064.
23. Zhu, X.-Y.; Holtz, B.; Wang, Y.; Wang, L.-X.; Orndorff, P. E.; Guo, A. *J. Am. Chem. Soc.* **2009**, *131*, 13646–13650.
24. Gorska, K.; Huang, K.-T.; Chaloin, O.; Winssinger, N. *Angew. Chem.* **2009**, *121*, 7831–7836. *Angew. Chem*., Int. Ed. **2009**, *48*, 769–7700.
25. Zhang, Y.; Li, Q.; Rodriguez, L. G.; Gildersleeve, J. C. *J. Am. Chem. Soc.* **2010**, *132*, 9653–9662.
26. Krishnamoorthy, L.; Mahal, L. K. *ACS Chem. Biol.* **2009**, *4*, 715–732.
27. Song, X.; Lasanajak, Y.; Xia, B.; Smith, D. F.; Cummings, R. D. *ACS Chem. Biol.* **2009**, *4*, 741–750.
28. Dhayal, M.; Ratner, D. M. *Langmuir* **2009**, *25*, 2181–2187.
29. Liang, C.-H.; Wang, S.-K.; Lin, C.-W.; Wang, C.-C.; Wong, C.-H.; Wu, C.-I. *Angew. Chem.* **2011**, *123*, 1646–1650. *Angew. Chem., Int. Ed.* **2011**, *50*, 1608–1612.
30. Gillard, R. E.; Raymo, F. M.; Stoddart, J. F. *Chem. Eur. J.* **1997**, *3*, 1933–1940.
31. Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005**, *105*, 1103–1170.
32. Kleinert, M.; Röckendorf, N.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2004**, 3931–3940.
33. Schierholt, A.; Shaikh, H. A.; Schmidt-Lassen, J.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2009**, 3783–3789.
34. Zemplén, G.; Pacsu, E. *Ber. Dtsch. Chem. Ges. B* **1929**, *62*, 1613–1614.
35. Krist, P.; Vannucci, L.; Kuzma, M.; Man, P.; Sadalapure, K.; Patel, A.; Bezouška, K.; Pospíšil, M.; Petruš, L.; Lindhorst, T. K.; Křen, V. *ChemBioChem* **2004**, *5*, 445–452.
36. Newkome, G. R.; Behera, R. K.; Moorefield, C. N.; Baker, G. R. *J. Org. Chem.* **1991**, *56*, 7162–7167.
37. Akpo, C.; Weber, E.; Reiche, U. *New J. Chem.* **2006**, *30*, 1820–1833.
38. Hartmann, M.; Horst, A. K.; Klemm, P.; Lindhorst, T. K. *Chem. Commun.* **2010**, *46*, 330–332.
39. Srinivasan, N.; Yurek-George, A.; Ganesan, A. *Mol. Diversity* **2005**, *9*, 291–293.
40. Reisner, A.; Haagensen, J. A. J.; Schembri, M. A.; Zechner, E. L.; Molin, S. *Mol. Microbiol.* **2003**, *48*, 933–946.