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Cysteine-Based Mannoside Glycoclusters: Synthetic Routes and Antiadhesive Properties

Alexander Schierholt,[a] Mirja Hartmann,[a] Kirsten Schwekendiek,[a] and Thisbe K. Lindhorst*[a]

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Clustermannosides of different valency were synthesised based on cysteine. By employing the orthogonally protected amino acid as scaffold molecule, a variety of structurally varied products can be obtained according to different synthetic routes. Testing of the prepared glycoclusters as inhibitors of type 1 fimbriae mediated bacterial adhesion is reported, giving hints about the influence of sugar valency, sugar scaffolding, and the nature of the glycosidic aglycon in this testing system.

Introduction

Molecular recognition of carbohydrates is associated with numerous processes in cell biology, comprising cell adhesion and cell communication, signaling, inflammation, and various disease states, such as i.a. cancer and metastasis.[1] Because of the enormous structural diversity of the carbohydrates and the manyfold aspects of their supramolecular assembly on eukaryotic cell surfaces it is a major challenge to understand and investigate the molecular details of the mechanisms underlying glycobiology. In this context multivalent glycoconjugates and glycomimetics have been developed into a valuable class of molecular tools. Multivalent glycomimetics, such as glycodendrimers and glycoclusters, provide means to mimic and vary some of the complexity of the carbohydrate regime in order to study multivalency effects in carbohydrate–protein interactions and other binding effects in biological tests.[2]

Glycoproteins and glycopeptides have been shown to be involved in many important molecular recognition processes.[3] Consequently, synthetic glycopeptides, glycoamino acids as well as glycopeptide clusters have been designed and synthesised and were studied as ligands of carbohydrate-binding proteins such as the lectins.[4] Here a new approach is reported in which the amino acid cysteine is used as scaffold molecule (Figure 1). Both, the cysteine amino function and its carboxylic group can be addressed in peptide coupling reactions by employing suitably functionalised carbohydrates to give rise to divalent glycosides

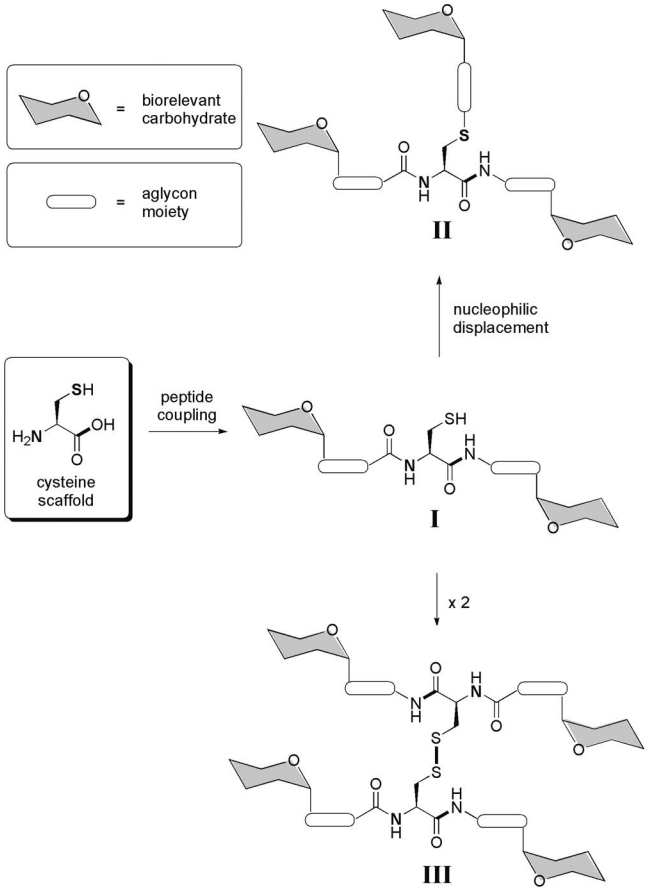

Figure 1. The amino acid cysteine can be used as scaffold molecule for the synthesis of glycoclusters with different valencies. When the carboxy group and the amino group of cysteine are addressed in peptide coupling reactions with appropriately functionalised glycosides, divalent glycoamino acids of type **I** can be obtained. A subsequent nucleophilic displacement step, involving the cysteine mercapto function, affords trivalent glycoclusters of type **II**. In addition, glycocysteine derivatives of type **I** enclose the option to access the respective tetravalent disulfide dimers of type **III**.

[a] Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel,
Otto-Hahn-Platz 4, 24098 Kiel, Germany
Fax: +49-431-8807410
E-mail: tklind@oc.uni-kiel.de

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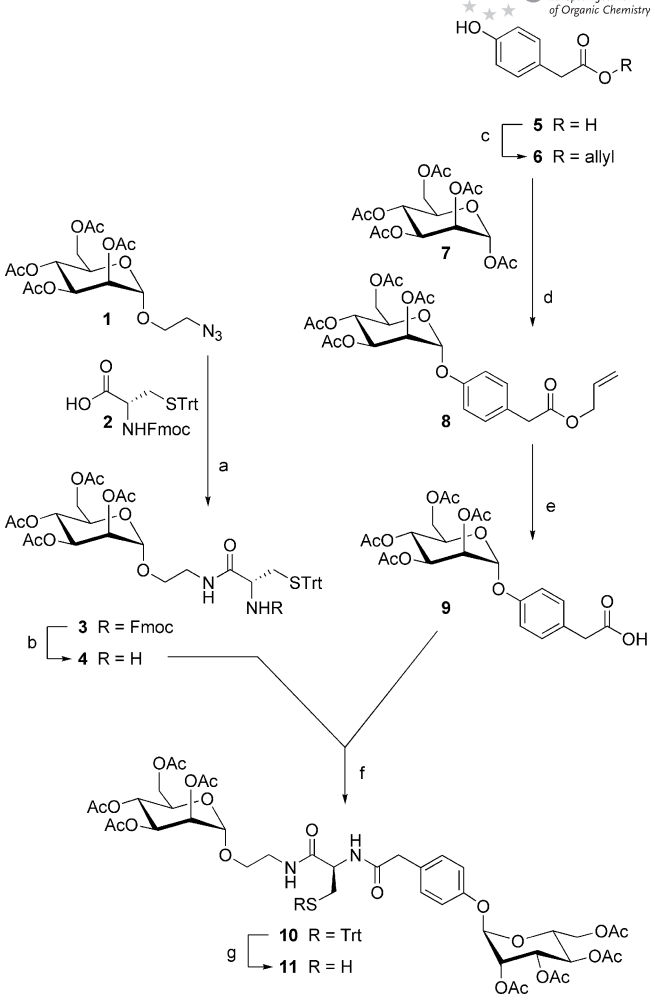
of type **I**. The sugar valency of the cysteine-based glycoclusters can be increased by functionalisation of the cysteine mercapto function, leading to type **II** glycoclusters, or by disulfide formation to achieve dimerised glycoclusters of type **III**. For a first evaluation of their ligand properties, mannose was employed in this account, and the respective cysteine-based mannose clusters were tested with type 1-fimbriated *Eschericha coli* bacteria.[5]

Results and Discussion

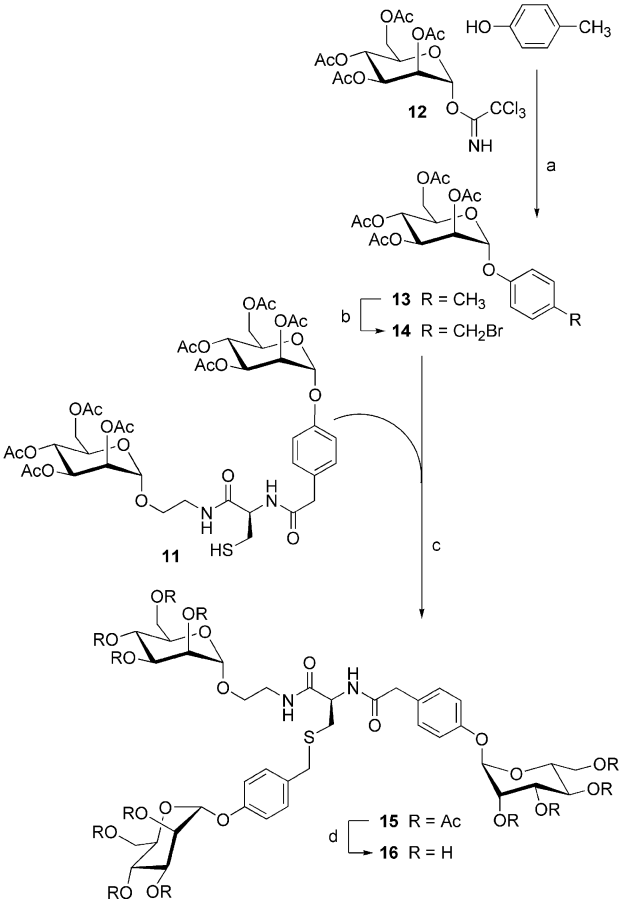
The synthesis of trivalent cluster mannosides of type **II** (Figure 1) was started by using *O*-protected mannosides. According to a synthetic route, which was published earlier,[6] the 2-azidoethyl mannoside **1**, which is available from mannose in three simple steps,[7] was conjugated to the orthogonally protected L-cysteine derivative Fmoc-Cys(Trt)-OH (**2**) in a Staudinger ligation. This led to the protected glycoamino acid **3** in high yield (Scheme 1). Then, cleavage of the Fmoc protecting group gave amine **4**, which was ready for the next peptide coupling step by using an appropriate carboxylic acid derivative such as the mannoside **9**. This was synthesised in three steps by starting with (4-hydroxyphenyl)acetic acid (**5**). Acid-catalysed treatment with allyl alcohol led to the allyl ester **6**,[8] which was treated with the mannose pentaacetate **7** in a Lewis acid catalysed glycosylation reaction to afford the fully protected mannoside **8**. The allyl ester protecting group was cleaved chemoselectively after isomerisation of the double bond to lead to the desired *O*-acetylated carboxylic acid mannoside **9**. This was subjected without extensive purification to peptide coupling with the amine **4** by using 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and diisopropyl(ethyl)amine (DIPEA) to afford the divalent glycoamino acid **10** in good yield. For subsequent functionalisation of the mercapto function deprotection of the *S*-trityl protecting group was performed by using TFA[9] to yield the thiol **11** in a quantitative reaction.



Scheme 1. Synthesis of the bis(mannosidic) L-cysteine derivative **11**. Reagents and conditions: (a) DIC, HOBt, nBu$_3$P, THF, 0 °C → room temp., overnight, 78%; (b) morpholine, DMF, room temp., 1.5 h, 83%; (c) allyl alcohol, H_2SO_4, 6 h, 100 °C, 84%; (d) BF_3–Et_2O, CH_2Cl_2, 0 °C → room temp., overnight, 62%; (e) Pd-$(PPh_3)_4$, morpholine, THF, room temp., 3 h, 69%; (f) HATU, DIPEA, DMF, 0 °C → room temp., overnight, 60%; (g) TFA/CH_2Cl_2 (1:1), TES, room temp., 2 h, quant.

In order to achieve a trivalent cluster glycoside, an appropriate mannoside was seeked to react with thiol **11** according to a Williamson-type thioether synthesis. Thus, *p*-cresol was mannosylated by employing the mannosyl trichloroacetimidate **12** as the glycosyl donor.[10] The Lewis acid catalysed stereospecific reaction gave the α-glycoside **13** in high yield, which was subsequently subjected to radical bromination by using azobis(isobutyronitrile) (AIBN) and dimethyldibromohydanthoin (DBDMH) to result in the benzyl bromide **14** (Scheme 2). The subsequent nucleophilic displacement reaction with **11** proceeded in acceptable yields when ultrasound was applied; no de-*O*-acetylation was observed in this step. The pure protected trivalent glycocluster **15** was obtained after laborious gel permeation chromatography (GPC). Deprotection under Zemplén conditions[11] gave the unprotected trivalent cluster mannoside **16**.

For the preparation of cluster glycosides of type **III** (cf. Figure 1), a synthetic approach was envisioned in which *O*-protection is dispensable. Thus, the OH-free 2-aminoethyl mannoside **17**[12] was employed in a peptide coupling reaction with the cysteine derivative **2** (Scheme 3). Standard conditions by using HATU and DIPEA led to the desired glycoamino acid **18** in very good yield. Removal of the Fmoc protecting group gave the free amine **19**, which was further treated in two different reactions. Firstly, chemoselective *N*-acetylation was performed to achieve the model compound **20**, which was used to elaborate thiol deprotection in order to obtain the target disulfide glycoclusters. Removal of the *S*-trityl protection group and concurrent oxidation to the disulfide dimer **21** was possible in only 15 min by using iodine in methanol.[13] Purification of **21** by silica gel chromatography was very convenient in this case. Enouraged by these promising results, synthesis of the tetravalent glycocluster disulfide **24** was tackled next. The amine **19** was conjugated to the carboxy-functionalised mannoside **22**,[14] again by using standard peptide coupling

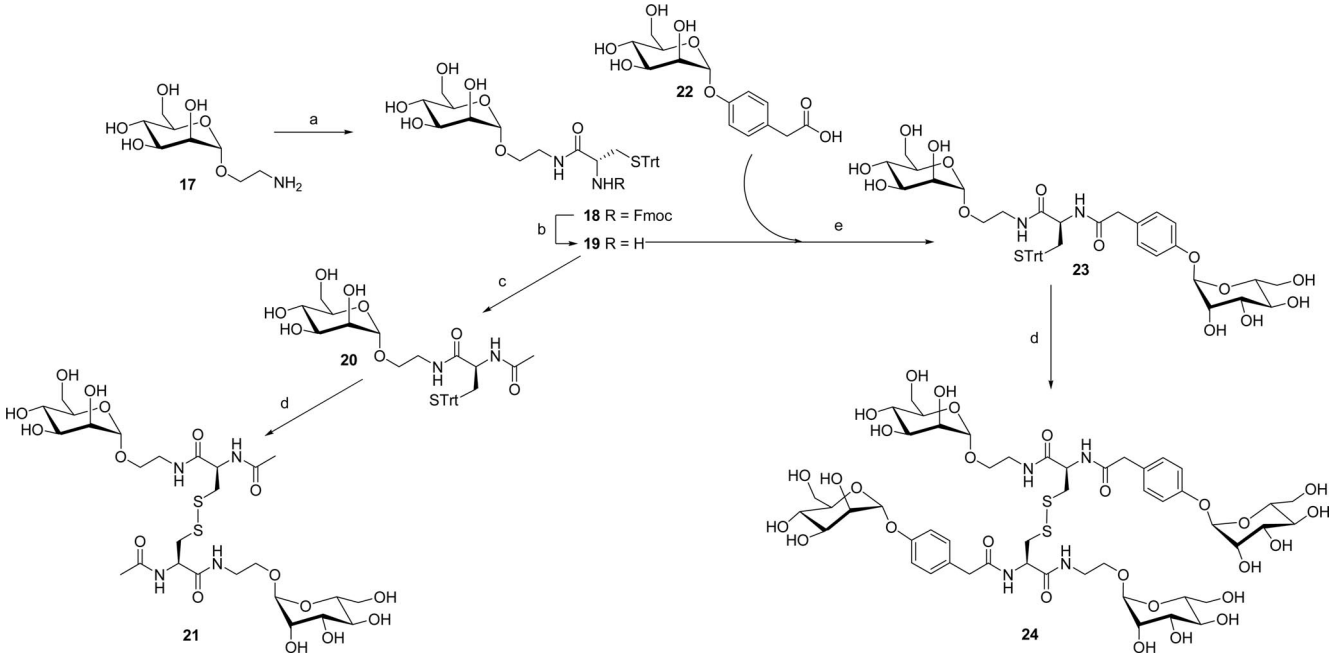


Scheme 2. Synthesis of the trivalent cluster mannoside **16**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, room temp., overnight, 81%; (b) AIBN, DBDMH, benzene, hν, 1.5 h, reflux, 80%; (c) NaH, NaI, DMF, ultrasound, room temp., 3 h, 34%; (d) NaOMe, MeOH, room temp., 3 h, 98%.

conditions, to yield the *S*-trityl-protected bis(mannoside) **23** in moderate yield. Application of the approved oxidising deprotection conditions, iodine in methanol, indeed led to the cluster mannoside **24**. However, in this case, purification was problematic and required a combination of reverse-phase medium-pressure chromatography (RP-MPLC) and subsequent GPC, thus lowering the yield considerably.

Testing Ligand Properties

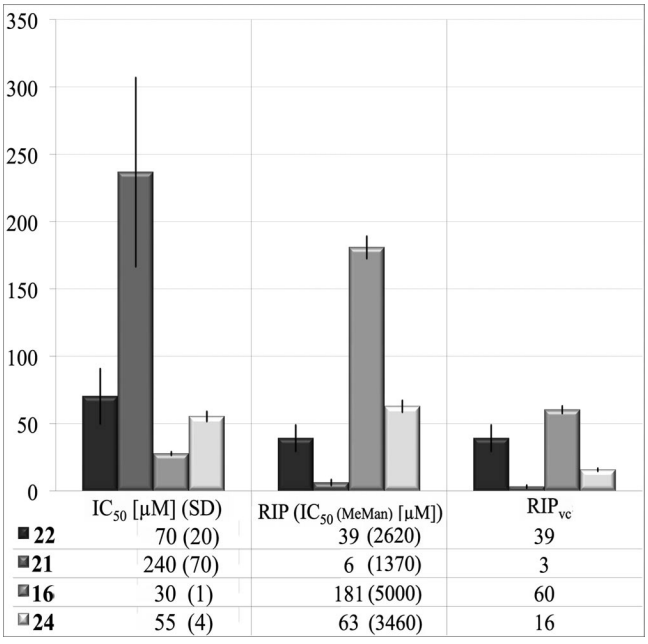
It was shown that structurally varied glycoclusters can be easily obtained based on cysteine as the scaffold molecule. For a first evaluation of their properties as lectin ligands, a collection of the herein described cluster mannosides were tested as inhibitors of type 1-fimbriated bacterial adhesion by employing uropathogenic *Escherichia coli* cells.[5] This important adhesion system is well described, and the X-ray structure of the participating lectin, FimH, has been solved.[15] It has been shown that sugar valency of a carbohydrate ligand and the nature of the glycosidic aglycon moieties have an influence on type 1 fimbriae mediated bacterial binding.[16] Therefore, the mono- to tetravalent glycoclusters and mannosides **22**, **21**, **16**, and **24** were checked for their inhibitory potencies in an inhibition adhesion assay. Serial dilutions of the respective inhibitor were incubated with fluorescent *E. coli* cells in mannan-coated 96-well microtiter plates.[17] Inhibition curves were determined for each tested glycocluster, from which IC_{50} values were deduced. IC_{50} values in this case reflect the inhibitor concentration, which causes 50% inhibition of bacterial binding to mannan. On each individual test plate methyl α-D-mannoside (MeMan), which is typically used as standard inhibitor in this assay, was tested in parallel. Hence, the IC_{50} values obtained for the tested glycoclusters could be



Scheme 3. Synthesis of disulfide glycoclusters **21** and **24**. Reagents and conditions: (a) **2**, HATU, DIPEA, DMF, 0 °C → room temp., overnight, 85%; (b) morpholine, DMF, room temp., quant.; (c) Ac_2O, DIPEA, room temp., 4 h, quant.; (d) I_2, MeOH, room temp., 15 min, 60% for **21**, 39% for **24**; (e) HATU, DIPEA, DMF, 0 °C → room temp., overnight, 45%.

referenced to the IC_{50} value that was determined for MeMan on the same plate leading to a relative inhibitory potency (RIP) for every tested compound. Thus, RIP values allow to compare the inhibitory potencies of all tested inhibitors, even when they were not examined in the same experiment, because, they are consistently referenced.

Testing results are collected in Figure 2. In addition to the measured IC_{50} values and obtained RIP values, valency-corrected RIP_{vc} values are depicted, in order to assess the inhibitory potency of a compound irrespective of its mannoside valency. This approach translates the RIP value of 6 measured for the divalent cluster mannoside **21** into a RIP_{vc} value of 3, for example.



	IC_{50} [μM] (SD)	RIP (IC_{50} (MeMan) [μM])	RIP_{vc}
22	70 (20)	39 (2620)	39
21	240 (70)	6 (1370)	3
16	30 (1)	181 (5000)	60
24	55 (4)	63 (3460)	16

Figure 2. Inhibitory potencies of mannoside **22** and the di- to tetravalent clusters **21**, **16**, and **24** obtained from triplicate testing. The relative inhibitory potencies (RIP) are referenced to the IC_{50} value obtained for an individual MeMan standard on the same test plate, with RIP(MeMan) ≡ 1 (see Exp. Sect.). RIP_{vc} = valence corrected RIP; SD = standard deviation.

To interpret the obtained testing results, two important features of the inhibitor molecules must be considered, namely their mannose valency and the properties of the aglycon moieties that link a mannosidic moiety to the cysteine scaffold. The monovalent mannoside **22** gave an IC_{50} value of 70 μM, corresponding to an inhibitory potency, which is 38 times higher than that of the reference compound MeMan. The elevated inhibitory potency can be attributed to π–π stacking interactions of the phenyl aglycon with two tyrosine residues flanking the entrance of the bacterial adhesin FimH, an effect that is known and expected.[15,16] When two phenyl mannoside moieties were scaffolded in one molecule such as in the case of **16** and **24**, different effects were seen. Whereas in the case of **16** the RIP value went up to ca. 180, the potency of the tetravalent cluster mannoside **24** as inhbitor of type 1 fimbriae mediated bacterial adhesion was weaker, only reaching about one third of that of **16** [RIP(**24**) = 63]. It can be assumed that, in addition to the effect of scaffolding, also the carbohydrate environment of a glycosidic moiety effects its ligand properties. This observation might be of considerable importance in a natural adhesion system and needs further investigation.

The divalent cluster mannoside **21**, lacking any aromatic aglycon moiety, showed only a slightly improved inhibitory potency when compared to MeMan. This finding can be assigned to a statistical multivalency effect, which is, however, not very pronounced in this case. Overall, the aromatic nature of the linker moieties in the tested mannosides seem to exert a dominant effect on receptor binding, much more than mannoside valency. Thus, for judgment of the valency-corrected RIP values (RIP_{vc}) the ratio of aromatic to aliphatic aglycon residues in the respective cluster mannoside should be taken into account. The trivalent cluster mannoside **16**, which performed best in the testing system, can profit from a combination of ligand multivalency and a high contribution of two phenyl moieties to π–π stacking stabilisation of the lectin–ligand complex. In the higher-valent cluster **24**, on the other hand, only 50% of all aglycon linkers are aromatic, thus its RIP_{vc} is significantly lowered.

Conclusions

It has been especially difficult to design high-affinity carbohydrate ligands for lectin proteins.[18] This research field is complicated by a number of effects, which are affiliated with weak binding and multivalency. In addition to the configuration of the glycone part of a glycosidic ligand, the aglycon moiety can contribute to binding and, moreover, the aglycon can also affect the conformational properties of a given ligand such as its overall flexibility and its minimum-energy conformation. A multitude of published studies[16,19] as well as the herein described work on carbohydrate binding of type 1-fimbriated *E. coli* have shown, that also in this testing system, which seems to be ruled by the monovalent α-D-mannoside-specific fimbrial lectin FimH, multivalency makes a difference, and even more the nature of the mannosidic aglycon moieties and mannoside scaffolding are important. With respect to the investigation of these effects and their meaning in type 1 fimbriae mediated bacterial adhesion the current study paves the way to a future project by highlighting some effects of structural variation of cysteine-scaffolded cluster mannosides on the inhibitory potency of the respective glycoclusters.

Moreover, a synthetic concept was introduced, which is suited to easily vary carbohydrate valency and aglycon properties of cluster glycosides, which are of interest for biological testing. Carbohydrate clustering on a cysteine scaffold was shown to even work with unprotected mannosides, and in addition, this project can be easily extended towards the synthesis of glycoclusters of a "mixed" type[20] and to solid-phase synthesis. It is obvious that the exemplified approach can be used as versatile tool box for a relatively rapid access to variable synthetic lectin ligands.

Experimental Section

General: All solvents were distilled prior to use. Commercially available starting materials, reagents and anhydrous DMF were used without further purification unless otherwise noted. Air- and/or moisture-sensitive reactions were carried out under 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 with an apparatus of BÜCHI Labortechnik GmbH by using a LiChroprep RP18 (40–60 μm, Merck) column for reversed-phase silica gel chromatography. ^{1}H and ^{13}C NMR spectra were recorded with 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 the following shifts: $CHCl_3$ (internal tetramethylsilane: ^{1}H: δ = 0.00 ppm; ^{13}C: δ = 77.00 ppm), MeOH (^{1}H: δ = 3.31 ppm; ^{13}C: δ = 49.05 ppm) or H_2O (δ = 4.65 ppm). For signal assignment, nuclei of the sugar ring, which are coupled to the cysteine amino group are primed, those of the glycoside ligated to the thiol function are double-primed. ESI-MS measurements were recorded with a Mariner ESI-ToF 5280 (Applied Biosystems) instrument and MALDI-MS measurements with a MALDI-ToF-MS Biflex III (Bruker) instrument. Optical rotations were measured with a Perkin–Elmer 241 polarimeter (Na_D line: 589 nm; length of cell: 1 dm).

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

GFP-Based Bacterial Adhesion Assay:[17] Black (Thermo Fisher Scientific, Nunc Maxisorp) 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) at 37 °C for 2 h and then washed with PBST (3 × 150 μL/well). Serial dilutions of the examined inhibitor (**14**, **21**, **22**, or **24**) 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 at 37 °C for 45 min. 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. The percentage inhibition was calculated as $\{[F(nI) - F(I)] \times 100 \times [F(nI)]^{-1}\}$ (F: fluorescence; nI: no inhibitor, I: with inhibitor). All assays were performed by using triplicate samples of each concentration. Relative inhibitory potencies (RIPs) are based on the average IC_{50} value of duplicate MeMan samples on the same plate [RIP(MeMan) ≡ 1], and the RIP values reported in Figure 2 are average values of three RIPs. LB: lysogeny broth; rpm: revolutions per minute; PBS: phosphate-buffered saline; PBST: PBS + 0.05% Tween 20.

General Procedure for Peptide Coupling: The carboxy-functionalised mannoside (1–1.2 equiv.) and HATU (1.1–1.2 equiv.) were dissolved in dry DMF under nitrogen and cooled to 0 °C. To this solution DIPEA (1.1–1.2 equiv.) was added, and the mixture was shaken for 2 min. Then the amine (1.4–2 equiv.), dissolved in dry DMF (4–10 mL), was added dropwise. After 4 h, the cooling was removed and the reaction mixture stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product purified by silica gel chromatography.

General Procedure for Disulfide Formation: The protected thiol (1 equiv.) was dissolved in dry MeOH (2 mL), and a solution of iodine (1.5 equiv. in 5 mL dry MeOH) was added. The reaction mixture was stirred at room temperature for 15 min and then quenched by addition of sodium thiosulfate solution (3 mL, 0.01 N in MeOH), filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography.

General Procedure for Deacetylation: The acetylated compound was dissolved in dry methanol (10 mL/g), and sodium methoxide (100 μL, 1 M in MeOH) was added under nitrogen. The reaction mixture was stirred at room temperature until the reaction was complete (minimum 3 h, maximum overnight), then it was neutralised by the addition of Amberlite IR 120 (H^+), filtered, and the solution was concentrated under reduced pressure. The crude product was purified by chromatography on normal-phase or RP silica gel.

General Procedure for Fmoc Deprotection: The Fmoc-protected amine (1 equiv.) and morpholine (6 equiv.) were dissolved in DMF (10 mL) and stirred at room temperature for 4 h. Then, the solvent was removed under reduced pressure and the crude product dissolved in MeOH. The white precipitate formed was filtered off and the solvent removed under reduced pressure to give the desired deprotected amine.

***N*-[2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl]-*S*-(triphenylmethyl)-L-cysteinamide (4):** According to the general procedure for Fmoc deprotection, mannoside **3** (3.93 g, 4.10 mmol) and morpholine (2.63 mL, 30.1 mmol) were allowed to react in dry DMF (10 mL) for 1.5 h. The product **4** (2.40 g, 83%) was obtained after silica gel chromatography (EtOAc/toluene, 1:1) as a pale yellow syrup. ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ = 7.47–7.44 (m, 6 H, Ar-H), 7.34 (t, ^3J = 7.4 Hz, 6 H, Ar-H), 7.27 (t, ^3J = 7.3 Hz, 3 H, Ar-H), 5.33 (dd, ^3J = 3.4, ^3J = 10.0 Hz, 1 H, 3-H), 5.30 (dd, ^3J = 3.4, ^3J = 1.7 Hz, 1 H, 2-H), 5.29 (dd ≈ t, ^3J = 10.0 Hz, 1 H, 4-H), 4.92 (d, ^3J = 1.7 Hz, 1 H, 1-H), 4.27 (dd, ^3J = 4.9, ^2J = 12.3 Hz, 1 H, 6a-H), 4.11 (dd, ^3J = 2.3, ^2J = 12.3 Hz, 1 H, 6b-H), 4.07 (ddd, ^3J = 2.3, ^3J = 4.9, ^3J = 10.0 Hz, 1 H, 5-H), 3.81 (m$_c$, 1 H, OC*H*HCH$_2$), 3.73–3.54 (m, 3 H, OCH*H*CH$_2$, CH$_2$C*H*$_2$N), 3.20–3.15 (m, 1 H, α-H), 2.61 (dd, ^3J = 6.5, ^2J = 12.2 Hz, 1 H, βa-H), 2.45 (dd, ^3J = 6.5, ^2J = 12.2 Hz, 1 H, βb-H), 2.13, 2.06, 2.04, 2.00 (each s, each 3 H, 4 OAc) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH): δ = 171.3 (*C*OC-α), 170.1, 169.9, 169.4, 169.2 (4 *C*OCH$_3$), 96.7 (C-1), 68.6 (C-3), 68.3 (C-2), 67.9 (C-5), 67.2 (O*C*H$_2$CH$_2$), 65.6 (C-4), 62.2 (C-6), 53.6 (C-α), 39.8 (CH$_2$*C*H$_2$N), 32.9 (C-β), 20.6, 20.5, 20.5, 20.4 (4 CO*C*H$_3$) ppm. HRESI-MS: calcd. for [$C_{38}H_{44}N_2O_{11}S$ + H]$^+$ 737.2744; found 737.2739.

Allyl [4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenyl]-acetate (8): The allyl ester **6**[8] (4.06 g, 21.1 mmol) and mannose pentaacetate **7** (4.13 g, 10.8 mmol) were combined in a Schlenk flask and dried in vacuo for more than 70 min. This mixture was dissolved in dry CH_2Cl_2 (7 mL), and BF_3–Et_2O (159 μL, 23.5 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 30 min and then stirred at room temperature overnight. Ice-cold water (100 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were subsequently washed with aq. $NaHCO_3$ and water (40 mL each), dried with $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane/EtOAc, 3:2) to yield **8** (3.50 g, 62%) as a colourless syrup. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.22 (d, ^3J = 6.6 Hz, 2 H, Ar-H), 7.05 (^3J = 6.6 Hz, 2 H, Ar-H), 5.90 (dddd ≈ ddt, ^3J = 5.7, ^3J = 10.4, ^3J = 17.2 Hz, 1 H, CH$_2$C*H*CH$_2$), 5.56 (dd, ^3J = 3.5, ^3J = 10.0 Hz, 1 H, 3-H), 5.50 (d, ^3J = 1.9 Hz, 1 H, 1-H),

5.44 (dd, ^3J = 1.9, ^3J = 3.5 Hz, 1 H 2-H), 5.35 (dd ≈ t, ^3J = 10.0 Hz, 1 H, 4-H), 5.28 (dddd ≈ dq, ^4J = 1.4, ^3J = 10.4, ^3J = 17.2 Hz, 1 H, CH_2CHCHH), 5.22 (dddd ≈ dq, ^4J = 1.4, ^3J = 10.4 Hz, 1 H, CH_2CHCHH), 4.65–4.53 (m, 2 H, CH_2CHCH_2), 4.28 (dd, ^3J = 5.2, ^2J = 12.2 Hz, 1 H, 6a-H), 4.15–4.10 (m, 1 H, 5-H), 4.07 (dd, ^3J = 2.4, ^2J = 12.2 Hz, 1 H, 6b-H), 3.60 (s, 2 H, OCH_2C-Ar), 2.05, 2.04, 2.03, 2.03 (each s, each 3 H, 4 O*Ac*) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.1, 170.5, 169.9, 169.9, 169.7 (4 *C*OCH$_3$, *C*OOallyl), 154.8 (C-Ar), 132.0 (CH_2CHCH_2), 130.5, 128.6 (C-Ar), 118.3 (CH_2CHCH_2), 116.6 (C-Ar), 95.9 (C-1), 69.4 (C-2), 69.2 (C-5), 68.9 (C-3), 66.0 (C-4), 65.4 (CH_2CHCH_2), 62.1 (C-6), 40.4 (CH_2COO), 21.0, 20.8, 20.6, 20.6 (4 CO*C*H$_3$) ppm. HRESI-MS: calcd. for $[C_{25}H_{30}O_{12} + Na]^+$ 545.1635; found 545.1629.

***N*-[4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenyl]acetyl-*S*-(triphenylmethyl)-L-cysteine [2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl]amide (10):** The allyl ester **8** (3.20 g, 6.12 mmol) and $Pd(PPh_3)_4$ (50 mg, 43 μmol) were dissolved in dry THF (20 mL) under nitrogen. Morpholine (2.2 mL, 25.1 mmol) was added, and the mixture was stirred at room temperature for 3 h. The solvent was evaporated, and the crude product was filtered through a short silica gel column and washed (cyclohexane/EtOAc, 1:1) to give the carboxylic acid **9** (2.03 g, 69%). A portion of this product (680 mg, 1.41 mmol) was treated with the amine **4** (865 mg, 1.17 mmol) by addition of HATU (535 mg, 1.40 mmol) and DIPEA (244 μL, 1.40 mmol) in DMF (4 mL) according to the general coupling procedure. Silica gel column chromatography (CH_2Cl_2/EtOAc, 1:1) gave the title compound **10** (857 mg, 60%) as a colourless foam. $[\alpha]_D^{20}$ = +44.2 (*c* = 1.1 in MeOH). ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ = 7.38–7.35 (m, 6 H, Ar-H), 7.34–7.28 (m, 9 H, Ar-H), 7.26 (d, ^3J = 7.3 Hz, 2 H, Ar-H), 7.08 (d, ^3J = 8.5 Hz, 2 H, Ar-H), 5.57 (d, ^3J = 1.8 Hz, 1 H, 1′-H), 5.50 (dd, ^3J = 3.4, ^3J = 10.0 Hz, 1 H, 3-H), 5.47 (dd, ^3J = 1.8, ^3J = 3.4 Hz, 1 H, 2′-H), 5.35 (dd ≈ t, ^3J = 10.3 Hz, 1 H, 4′-H), 5.33 (dd, ^3J = 3.4, ^3J = 10.3 Hz, 1 H, 3′-H), 5.29 (dd, ^3J = 1.6, ^3J = 3.2 Hz, 1 H, 2-H), 5.25 (dd ≈ t, ^3J = 10.0 Hz, 1 H, 4-H), 4.86 (br. s, 1 H, 1-H), 4.26–4.22 (m, 2 H, 6a-H, α-H), 4.18 (dd, ^3J = 4.2, ^2J = 12.3 Hz, 1 H, 6′a-H), 4.09–4.02 (m, 3 H, 6b-H, 5-H, 5′-H), 3.97 (dd, ^3J = 2.5, ^2J = 12.3 Hz, 1 H, 6′b-H), 3.78–3.73 (m, 2 H, OCH_2CH_2) 3.56–3.51 (m, 2 H, CH_2C-Ar), 3.42–3.30 (m, 2 H, OCH_2CH_2), 2.59 (dd, ^3J = 6.0, ^2J = 12.5 Hz, 1 H, βa-H), 2.52–2.46 (m, 1 H, βb-H), 2.13, 2.12, 2.05, 2.04, 1.99, 1.97 (each s, 24 H, 8 O*Ac*) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH): δ = 173.6 (CH_2*C*ONH), 173.0 (α-CONH), 172.2, 171.6, 171.5, 171.0 (8 *C*OCH$_3$), 156.0, 145.9, 131.5, 131.4, 130.7, 129.1, 127.9, 117.9 (C-Ar), 98.9 (C-1), 97.1 (C-1′), 70.8 (C-2), 70.6 (C-2′), 70.6 (C-5′), 70.5 (C-3), 70.5 (C-5), 70.4 (C-3′), 69.9 (C-4′), 69.8 (C-4), 67.9 (*C*Ph$_3$), 67.7 (OCH_2CH_2), 63.5 (C-6′), 63.2 (C-6), 53.8 (C-α), 42.8 (CH_2CH_2N), 40.3 (CH_2C-Ar), 35.2 (C-β), 20.8, 20.7, 20.6, 20.5 (8 CO*C*H$_3$) ppm. HRESI-MS: calcd. for $[C_{38}H_{44}N_2O_{11}S + Na]^+$ 1223.3882; found 1223.3877.

4-Methylphenyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (13): The trichloroacetimidate **12** (1.00 g, 2.03 mmol) and *p*-cresol (264 mg, 2.44 mmol) were coevaporated with toluene (5 mL). This mixture was dissolved in dry CH_2Cl_2 (20 mL) and cooled to 0 °C. TMSOTf (10 mL, 0.01 mmol in dry CH_2Cl_2) was added dropwise over 30 min. The solution was stirred at room temperature overnight and then washed with satd. $NaHCO_3$ solution (3× 5 mL). The organic phase was dried with $MgSO_4$ and filtered. The solvent was evaporated, and the crude product was purified by column chromatography (cyclohexane/EtOAc, 1:1) to obtain the title compound **13** (750 mg, 84%) as a colourless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.09 (d, ^3J = 8.6 Hz, 2 H, Ar-H), 6.97 (d, ^3J = 8.6 Hz, 2 H, Ar-H), 5.56 (dd, ^3J = 3.5, ^3J = 10.0 Hz, 1 H, 3-H), 5.47 (d, ^3J = 1.7 Hz, 1 H, 1-H), 5.44 (dd, ^3J = 1.9, ^3J = 3.5 Hz, 1 H, 2-H), 5.36 (dd ≈ t, ^3J = 10.1 Hz, 1 H, 4-H), 4.28 (dd, ^3J = 5.2, ^2J = 12.1 Hz, 1 H, 6a-H), 4.11 (ddd, ^3J = 2.3, ^3J = 5.2, ^3J = 10.1 Hz, 1 H, 5-H), 4.07 (dd, ^3J = 2.3, ^2J = 12.1 Hz, 1 H, 6b-H), 2.30, 2.19, 2.05, 2.04, 2.03 (each s, each 3 H, 4 O*Ac*, CH_3-Ar) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.6, 170.0, 170.0, 169.8 (4 *C*OCH$_3$), 153.5, 132.5, 130.0, 116.4 (C-Ar), 96.0 (C-1), 65.3, 69.0, 68.9, 60.0 (C-2, C-3, C-4, C-5), 62.2 (C-6), 20.9, 20.7, 20.7, 20.6 (4 CO*C*H$_3$, Ar-*C*H$_3$) ppm. HRESI-MS: calcd. for $[C_{21}H_{26}O_{10} + Na]^+$ 461.1424; found 461.1439.

4-(Bromomethyl)phenyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (14): Mannoside **13** (206 mg, 470 μmol), 1,3-dibromo-5,5-dimethylhydantoine (DBDMH) (60 mg, 210 μmol) and AIBN (3 mg, 20 μmol) were dissolved in dry benzene (10 mL) under nitrogen. The reaction mixture was refluxed for 90 min and simultaneously irradiated with a 150 W tungsten lamp. The solvent was evaporated, and the crude product was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 3:1). The title compound was obtained as a colourless solid (194 mg, 80%). $[\alpha]_D^{20}$ = +56.8 (*c* = 1.25 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34 (d, ^3J = 8.7 Hz, 2 H, Ar-H), 7.06 (d, ^3J = 8.7 Hz, 2 H, Ar-H), 5.55 (dd, ^3J = 3.6, ^3J = 10.0 Hz, 1 H, 3-H), 5.53 (d, ^3J = 1.8 Hz, 1 H, 1-H), 5.43 (dd, ^3J = 1.8, ^3J = 3.6 Hz, 1 H, 2-H), 5.36 (dd ≈ t, ^3J = 10.0 Hz, 1 H, 4-H), 4.48 (s, 2 H, CH_2Br), 4.27 (dd, ^3J = 5.8, ^2J = 12.7 Hz, 1 H, 6a-H), 4.04–4.09 (m, 2 H, 5-H, 6b-H), 2.20, 2.05, 2.04, 2.03 (each s, each 3 H, 4 O*Ac*) ppm. ^{13}C NMR (500 MHz, $CDCl_3$): δ = 170.6, 170.0, 170.0, 169.7 (4 *C*OCH$_3$), 155.5, 132.5, 130.5, 116.7 (C-Ar), 95.6 (C-1), 69.3, 69.2, 68.8, 65.9 (C-2, C-3, C-4, C-5), 62.1 (C-6), 33.1 (CH_2Br), 20.9, 20.7, 20.7, 20.7 (4 CO*C*H$_3$) ppm. HRESI-MS: calcd. for $[C_{21}H_{25}BrO_{10} + Na]^+$ 539.0523, 541.0506; found 539.0468, 541.0482.

***N*-[4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)phenyl]acetyl-*S*-[4-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)benzyl]-L-cysteine [2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl]amide (15):** The trityl-protected sulfide **10** (650 mg, 541 μmol) was dissolved in dry CH_2Cl_2 (5 mL) under nitrogen. Then triethylsilane (0.41 mL, 2.5 mmol) and trifluoroacetic acid (190 μL, 2.5 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated, and the crude product was purified on a short silica gel column (CH_2Cl_2/EtOAc, 1:1) to give the desired product **11** (512 mg, 99%). A portion of this thiol **11** (100 mg, 104 μmol) and sodium hydride (2.5 mg, 0.1 mmol) were suspended in dry DMF (5 mL) and treated with ultrasound for 10 min. Then, the benzyl bromide **14** (49 mg, 95 μmol) was added, and the reaction mixture was stirred with ultrasound for further 3 h. The mixture was concentrated under reduced pressure, and the crude product was purified by GPC on Sephadex LH-20 (eluent: MeOH) to yield the glycocluster **15** (45 mg, 34%) as a colourless solid. ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 7.31–7.28 (m, 4 H, Ar′′-H, Ar′-H), 7.12–7.08 (m, 4 H, Ar′′-H, Ar′-H), 5.64 (br. s, 1 H, 1′-H), 5.54 (br. s, 1 H, 1′′-H), 5.53–5.45 (m, 4 H, 3′-H, 3′′-H, 3-H, 4-H), 5.42 (dd, ^3J = 1.8, ^3J = 3.5 Hz, 1 H, 2′-H), 5.38–5.30 (m, 3 H, 2-H, 2′′-H, 4′′-H), 5.26 (dd ≈ t, ^3J = 10.2 Hz, 1 H, 4′-H), 4.88 (br. s, 1 H, 1-H), 4.56–4.50 (m, 1 H, α-H), 4.31–4.21 (m, 4 H, 5′′-H, 6a-H, 6a′-H, 6a′′-H), 4.16–4.05 (m, 5 H, 5-H, 5′-H, 6b-H, 6b′-H, 6b′′-H), 3.84–3.80 (m, 1 H, OC*H*HCH$_2$), 3.74–3.71 (m, 2 H, SCH_2C-Ar), 3.62–3.50 (m, 4 H, OCH*H*CH$_2$, CH_2N, C_{Ar}C*H*HCO), 3.46–3.42 (m, 1 H, Ar-CH*H*CO), 2.90–2.85 (m, 1 H, βa-H), 2.72–2.66 (m, 1 H, βb-H), 2.22, 2.17, 2.10, 2.09, 2.05, 2.05, 2.04, 2.01, 2.00, 2.00, 1.99, 1.99 (each s, each 3 H, 12 O*Ac*) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH): δ = 173.7 (CH_2*C*ONH), 173.1 (α-*C*O), 172.9, 172.8, 172.5, 172.4, 172.2, 171.6, 171.5, 171.5 (12 *C*OCH$_3$), 156.0, 155.0, 134.1, 131.6, 131.5, 131.4, 118.0, 117.9 (C-Ar), 98.9

(C-1), 97.2 (C-1′), 97.2 (C-1′′), 70.8 (C-2), 70.7 (C-2′), 70.6 (C-5′), 70.5 (C-2′′), 70.4 (C-3′), 70.0 (C-5), 69.9 (C-3), 69.9 (C-5′′), 69.8 (C-3′′), 67.5 (O*C*H$_2$CH$_2$), 67.2 (C-4′′), 67.2 (C-4′), 67.1 (C-4), 63.5 (C-6′), 63.5 (C-6′′), 63.3 (C-6), 54.0 (C-α), 42.7 (CH$_2$*C*H$_2$N), 40.3 (*C*H$_2$C-Ar), 36.3 (Ar-*C*H$_2$S), 34.2 (C-β), 20.8, 20.7, 20.6, 20.5 (12 CO*C*H$_3$) ppm. HRESI-MS: calcd. for [$C_{62}H_{78}N_2O_{32}S$ + Na]$^+$ 1417.4156; found 1417.4151.

***N*-[4-(α-D-Mannopyranosyloxy)phenyl]acetyl-*S*-[4-(α-D-mannopyranosyl)benzyl]-L-cysteine [2-(α-D-Mannopyranosyloxy)ethyl]-amide (16):** The protected glycocluster **15** (45 mg, 32 μmol) was treated according to the general procedure for deacetylation. The crude product was purified by HPLC (RP-8) to yield **16** (28 mg, 98%) as a colourless lyophilisate. HPLC: t_R = 11.3 min (A = water, B = acetonitrile + 1% TFA, 20% B → 80% B, 100 min, 10 mL/min]. $[\alpha]_D^{20}$ = +41.9 (*c* = 1.1 in MeOH). ^{1}H NMR (500 MHz, D_2O): δ = 7.29 (d, ^3J = 8.8 Hz, 2 H, Ar′′-H), 7.23 (d, ^3J = 8.3 Hz, 2 H, Ar′-H), 7.13 (d, ^3J = 8.8 Hz, 2 H, Ar′′-H), 7.06 (d, ^3J = 8.3 Hz, 2 H, Ar′-H), 5.59 (d, ^3J = 1.5 Hz, 1 H, 1′′-H), 5.58 (br. s, 1 H, 1′-H), 4.83 (d, ^3J = 1.4 Hz, 1 H, 1-H), 4.32 (dd, ^3J = 1.5, ^3J = 8.3 Hz, 1 H, 2-H), 4.18–4.15 (m, 2 H, 2′-H, 2′′-H), 4.08–4.05 (m, 2 H, 3′-H, 3′′-H), 4.05–4.00 (m, 1 H, OC*H*HCH$_2$), 3.93–3.88 (m, 2 H, α-H, OCH*H*CH$_2$), 3.86–3.83 (m, 2 H, 6a-H, 6b-H), 3.79–3.70 (m, 7 H, 6′a-H, 6′′a-H, 6′b-H, 6′′b-H, 3-H, 4′-H, 4′′-H), 3.69–3.66 (m, 4 H, $C_{Ar}CH_2S$, 5′-H, 5′′-H), 3.63 (dd ≈ t, ^3J = 9.2 Hz, 1 H, 4-H), 3.59–3.56 (m, 3 H, Ar-C*H*$_2$CO, 5-H), 3.45–3.42 (m, 1 H, C*H*HN), 3.39–3.36 (m, 1 H, CH*H*N), 2.92 (dd, ^3J = 5.5, ^2J = 14.1 Hz, 1 H, βa-H), 2.74 (dd, ^3J = 8.3, ^2J = 14.1 Hz, 1 H, βb-H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 176.4 (CH$_2$*C*ON), 174.2 (α-*C*ON), 156.6, 156.5, 134.3, 132.4, 132.1, 131.1, 119.2 (C-Ar), 101.9 (C-1), 100.1 (C-1′, C-1′′), 75.3 (C-5′, C-5′′), 74.7 (C-5), 72.5 (C-3′, C-3′′), 72.4 (C-3), 72.0 (C-2), 71.9 (C-2′, C-2′′), 68.7 (C-4), 68.5 (C-4′′), 68.4 (C-4′), 68.1 (O*C*H$_2$CH$_2$), 62.9 (C-6′, C-6′′), 62.6 (C-6), 55.1 (C-α), 42.9 (*C*H$_2$C-Ar), 40.8 (*C*H$_2$N), 36.9 (Ar-*C*H$_2$S), 34.5 (C-β) ppm. MALDI-ToF-MS: calcd. for [$C_{38}H_{54}N_2O_{20}S$ + Na]$^+$ 914.03; found 913.30.

***N*-(Fluoren-9-ylmethoxycarbonyl)-*S*-(triphenylmethyl)-L-cysteine [2-(α-D-Mannopyranosyloxy)ethyl]amide (18):** According to the general coupling procedure, Fmoc-Cys(Trt)-OH (**2**) (1.28 g, 2.18 mmol), the mannoside **17** (970 mg, 4.34 mmol), HATU (913 mg, 2.40 mmol) and DIPEA (418 μL, 2.40 mmol) were allowed to react in DMF (4 mL). Silica gel column chromatography (cyclohexane/EtOAc/MeOH, 4:7:1) gave the title compound (326 mg, 81%) as a colourless foam. ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 7.84–7.79 (m, 2 H, Ar-H), 7.70 (t, ^3J = 6.5 Hz, 2 H, Ar-H), 7.44–7.41 (m, 8 H, Ar-H), 7.33–7.23 (m, 11 H, Ar-H), 4.77 (d, ^3J = 1.6 Hz, 1 H, 1-H), 4.48 (dd, ^3J = 7.2, ^3J = 10.1 Hz, 1 H, C*H*H-Fmoc), 4.33 (dd, ^3J = 7.0, ^3J = 10.1 Hz, 1 H, CH*H*-Fmoc), 4.27 (dd, ^3J = 7.1, ^3J = 6.7 Hz, 1 H, C*H*-Fmoc), 3.96 (dd, ^3J = 5.7, ^3J = 8.4 Hz, 1 H, α-H), 3.86–3.81 (m, 2 H, 6a-H, 2-H), 3.76–3.69 (m, 3 H, OC*H*HCH$_2$, 3-H, 6b-H), 3.63 (dd ≈ t, *J* = 9.6 Hz, 1 H, 4-H), 3.55–3.48 (m, 2 H, 5-H, OCH*H*CH$_2$), 3.44–3.36 (m, 2 H, OCH$_2$C*H*$_2$), 2.63 (dd, ^3J = 5.2, ^2J = 12.5 Hz, 1 H, βa-H), 2.52 (dd, ^3J = 8.6, ^2J = 12.5 Hz, 1 H, βb-H) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH): δ = 172.8 (*C*OC-α), 158.1 (*C*OO-Fmoc), 145.9, 145.1, 142.6, 130.7, 128.9, 128.7, 128.2, 127.9, 126.2, 120.9 (C-Ar), 101.6 (C-1), 74.7 (C-5), 72.6 (C-3), 72.0 (C-2), 68.7 (C-4), 68.1 (*C*H$_2$-Fmoc), 67.0 (O*C*H$_2$CH$_2$), 62.9 (C-6), 55.6 (C-α), 48.4 (*C*H-Fmoc), 40.3 (OCH$_2$*C*H$_2$), 35.1 (C-β) ppm. HRESI-MS: calcd. for [$C_{45}H_{46}N_2O_9S$ + Na]$^+$ 813.2822; found 813.2382.

***N*-[2-(α-D-Mannopyranosyloxy)ethyl]-*S*-(triphenylmethyl)-L-cysteine Amide (19):** According to the general procedure for Fmoc deprotection, glycoamino acid **18** (1.77 g, 2.24 mmol) and morpholine (1.17 mL, 13.4 mmol) were allowed to react in DMF (10 mL). The title compound (1.27 g, quant.) was used in the next step without further purification. ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 7.45 (d, ^3J = 8.0 Hz, 6 H, Ar-H), 7.34 (dd, ^3J = 7.6, ^3J = 7.6 Hz, 6 H, Ar-H), 7.26 (t, *J* = 7.3 Hz, 3 H, Ar-H), 4.79 (d, ^3J = 1.5 Hz, 1 H, 1-H), 3.87–3.82 (m, 2 H, 6a-H, 2-H), 3.79 (ddd, ^3J = 4.5, ^3J = 6.4, ^2J = 10.1 Hz, 1 H, OC*H*HCH$_2$), 3.74–3.68 (m, 2 H, 3-H, 6b-H), 3.63 (dd ≈ t, *J* = 9.6 Hz, 1 H, 4-H), 3.59–3.43 (m, 2 H, OCH*H*CH$_2$, 5-H), 3.46–3.39 (m, 2 H, OCH$_2$C*H*$_2$), 3.17 (dd ≈ t, ^3J = 6.5 Hz, 1 H, α-H), 2.59 (dd, ^3J = 6.0, ^2J = 12.1 Hz, 1 H, βa-H), 2.44 (dd, ^3J = 7.0, ^2J = 12.1 Hz, 1 H, βb-H) ppm. ^{13}C NMR (150 MHz, [D$_4$]-MeOH): δ = 164.8 (*C*OC-α), 146.1, 130.7, 128.9, 127.8 (C-Ar), 101.6 (C-1), 74.8 (C-5), 72.6 (C-3), 72.0 (C-2), 68.7 (C-4), 67.1 (O*C*H$_2$CH$_2$), 62.9 (C-6), 55.3 (C-α), 40.2 (OCH$_2$*C*H$_2$), 38.2 (C-β) ppm. HRESI-MS: calcd. for [$C_{30}H_{36}N_2O_7S$ + H]$^+$ 569.2321; found 569.2402.

***N*-Acetyl-*S*-(triphenylmethyl)-L-cysteine [2-(α-D-Mannopyranosyloxy)ethyl]amide (20):** Glycoamino acid **19** (420 mg, 739 μmol) was dissolved in DMF (5 mL), DIPEA (532 μL, 2.96 mmol) and acetic anhydride (280 μL, 2.96 mmol) were added, and the solution was stirred at room temperature for 4 h. The crude product was purified by silica gel chromatography (cyclohexane/EtOAc/MeOH, 1:4:2) to yield the title compound (452 mg, quant.) as a colourless foam. ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.09 (d, ^3J = 8.4 Hz, 1 H, N*H*Ac), 7.92 (t, ^3J = 5.6 Hz, 1 H, CH$_2$N*H*CO), 7.36–7.23 (m, 15 H, Ar-H), 4.59 (d, ^3J = 1.5 Hz, 1 H, 1-H), 4.31 (dd, ^3J = 6.2, ^3J = 8.0 Hz, 1 H, α-H), 3.66–3.59 (m, 2 H, 6a-H, 2-H), 3.54 (dd, ^3J = 5.9, ^2J = 10.1 Hz, 1 H, OC*H*HCH$_2$), 3.48–3.33 (m, 5 H, 3-H, 4-H, 6b-H, OCH*H*CH$_2$-H, 5-H), 3.22 (dd, ^3J = 6.9, ^2J = 12.6 Hz, 1 H, OCH$_2$CH*H*), 3.18 (dd, ^3J = 7.0, ^2J = 13.2 Hz, 1 H, OCH$_2$CH*H*) 2.35 (dd, ^3J = 6.1, ^2J = 11.7 Hz, 1 H, βa-H), 2.29 (dd, ^3J = 7.9, ^2J = 11.7 Hz, 1 H, βb-H), 1.83 (s, 3 H, NH*Ac*) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 169.7 (*C*OCH$_3$), 169.1 (*C*OC-α), 144.2, 129.0, 127.9, 126.6 (C-Ar), 99.9 (C-1), 73.8 (C-5), 70.8 (C-3), 70.1 (C-2), 66.9 (C-4), 65.8 (*C*Ph$_3$), 65.0 (O*C*H$_2$CH$_2$), 61.1 (C-6), 51.5 (C-α), 38.4 (OCH$_2$*C*H$_2$), 33.8 (C-β), 22.3 (CO*C*H$_3$) ppm. HRESI-MS: calcd. for [$C_{32}H_{38}N_2O_8S$ + Na]$^+$ 633.2247; found 633.2257.

***N*,*N*′-Diacetyl-L-cystine {Bis[2-(α-D-mannopyranosyloxy)ethyl]}-diamide (21):** According to the general procedure for disulfide formation, the tritylated thiol **20** (100 mg, 164 μmol) and iodine (63 mg, 250 μmol) were allowed to react in methanol (3 mL). The crude product was purified by silica gel chromatography (EtOAc/MeOH, 3:1 → EtOAc/MeOH, 1:1) to yield the disulfide **21** (36 mg, 60%) as a colourless foam. $[\alpha]_D^{20}$ = –10.5 (*c* = 1.1 in MeOH). ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ = 4.81 (d, ^3J = 1.8 Hz, 2 H, 1-H), 4.78 (dd, ^3J = 5.9, ^3J = 8.3 Hz, 1 H, α-H), 4.73 (dd, ^3J = 5.6, ^3J = 8.7 Hz, 1 H, α′-H), 3.88 (dd, ^3J = 2.4, ^2J = 11.9 Hz, 2 H, 6a-H), 3.86 (dd, ^3J = 1.7, ^3J = 3.3 Hz, 2 H, 2-H), 3.82 (ddd, ^3J = 4.4, ^3J = 6.7, ^2J = 10.9 Hz, 2 H, OC*H*HCH$_2$), 3.78–3.72 (m, 4 H, 3-H, 6b-H), 3.64 (dd ≈ t, *J* = 9.4 Hz, 2 H, 4-H), 3.60–3.55 (m, 4 H, OCH*H*CH$_2$, 5-H), 3.53–3.47 (m, 2 H, OCH$_2$CH*H*), 3.46–3.41 (m, 2 H, OCH$_2$CH*H*), 3.40–3.36 (m, 1 H, βa-H), 3.23 (dd, ^3J = 5.6, ^2J = 13.8 Hz, 1 H, β′a-H), 3.19 (dd, ^3J = 5.6, ^2J = 13.8 Hz, 1 H, βb-H), 2.98 (dd, ^3J = 8.7, ^2J = 13.8 Hz, 1 H, β′b-H), 2.06 (s, 6 H, NH*Ac*) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH): δ = 173.5, 173.4 (*C*OCH$_3$), 172.6, 172.3 (*C*OC-α), 101.6 (C-1), 74.7 (C-5), 72.5 (C-3), 72.0 (C-2), 68.8 (C-4), 66.9 (O*C*H$_2$CH$_2$), 63.0 (C-6), 54.1, 54.0 (C-α), 41.4, 41.0 (C-β), 40.5 (OCH$_2$*C*H$_2$), 22.6 (CO*C*H$_3$) ppm. HRESI-MS: calcd. for [$C_{26}H_{46}N_4O_{16}S_2$ + Na]$^+$ 757.2248; found 757.2207.

***N*-[4-(α-D-Mannopyranosyloxy)phenyl]acetyl-*S*-(triphenylmethyl)-L-cysteine [2-(α-D-Mannopyranosyloxy)ethyl]amide (23):** According

to the general coupling procedure, the amine **19** (1.27 g, 2.24 mmol), the carboxylic acid **22** (503 mg, 1.60 mmol), HATU (669 mg, 1.76 mmol) and DIPEA (306 μL, 1.76 mmol) were allowed to react in DMF (4 mL). Silica gel column chromatography (cyclohexane/EtOAc/MeOH, 1:4:2) yielded the title compound (632 mg, 46%) as a colourless foam. $[\alpha]_D^{20}$ = +51.4 (*c* = 1.0 in MeOH). ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ = 7.39 (d, ^3J = 8.5 Hz, 6 H, Ar-H), 7.31 (dd, ^3J = 7.5, ^3J = 7.5 Hz, 6 H, Ar-H), 7.28–7.23 (m, 5 H, Ar-H), 7.07 (d, ^3J = 8.7 Hz, 2 H, Ar-H), 5.48 (d, ^3J = 1.9 Hz, 1 H, 1′-H), 4.77 (d, ^3J = 1.6 Hz, 1 H, 1-H), 4.20 (dd, ^3J = 6.1, ^3J = 8.1 Hz, 1 H, H-α), 4.03 (dd, ^3J = 1.9, ^3J = 3.4 Hz, 1 H, 2′-H), 3.94 (dd, ^3J = 3.4, ^3J = 9.5 Hz, 1 H, 3′-H), 3.85–3.82 (m, 2 H, 2-H, 6a-H), 3.79 (dd ≈ t, *J* = 9.7 Hz, 1 H, 4′-H), 3.75–3.70 (m, 5 H, 6a′-H, 6b′-H, 6b-H, 3-H, OC*H*HCH$_2$), 3.63 (dd ≈ t, *J* = 9.5 Hz, 1 H, 4-H), 3.63 (m$_c$, 1 H, 5′-H), 3.56–3.51 (m, 4 H, 5-H, COC*H*$_2$C-Ar, OCH*H*CH$_2$), 3.38–3.37 (m, 2 H, OCH$_2$C*H*$_2$), 2.60 (dd, ^3J = 6.0, ^2J = 12.4 Hz, 1 H, βa-H), 2.50 (dd, ^3J = 8.1 H, 12.4 Hz, 1 H, βb-H) ppm. ^{13}C NMR (150 MHz, [D$_4$]-MeOH): δ = 174.0 (NH*C*OCH$_2$), 172.4 (*C*OC-α), 157.0, 145.9, 131.3, 130.6, 130.4, 129.1, 127.9 (C-Ar), 117.8 (C-Ar), 101.6 (C-1), 100.2 (C-1′), 75.2 (C-5′), 74.6 (C-5), 72.5 (C-3), 72.4 (C-3′), 72.0 (C-2), 72.0 (C-2′), 68.7 (C-4), 68.2 (C-4′), 67.9 (*C*Ph$_3$), 66.8 (O*C*H$_2$CH$_2$), 62.9 (C-6), 62.5 (C-6′), 53.9 (C-α), 42.7 (CO*C*H$_2$C-Ar), 40.3 (OCH$_2$*C*H$_2$), 34.8 (C-β) ppm. MALDI-ToF-MS: calcd. for [C$_{44}$H$_{52}$N$_2$O$_{14}$S + Na]$^+$ 887.30; found 888.26; calcd. for [C$_{44}$H$_{52}$N$_2$O$_{14}$S + K]$^+$ 903.27; found 904.20. HRESI-MS: calcd. for [C$_{44}$H$_{52}$N$_2$O$_{14}$S + Na]$^+$ 887.3037; found 887.3081.

***N*,*N*′-Bis{[4-(α-D-mannopyranosyloxy)phenyl]acetyl}-L-cystine {Bis[2-(α-D-mannopyranosyloxy)ethyl]}diamide (24):** According to the general procedure for disulfide formation, the tritylated thiol **21** (112 mg, 129 μmol) and iodine (49 mg, 190 μmol) were allowed to react in methanol (3 mL). The crude product was purified by RP-MPLC (MeCN/H$_2$O, 1:1) followed by GPC on Sephadex LH-20 (MeOH) to yield pure **24** (31 mg, 39%) as a white lyophilisate. $[\alpha]_D^{20}$ = +36.1 (*c* = 0.6 in MeOH). ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ = 7.28 (d, ^3J = 8.8 Hz, 4 H, Ar-H), 7.10 (d, ^3J = 8.7 Hz, 4 H, Ar-H), 5.50 (d, ^3J = 1.8 Hz, 2 H, 1′-H), 4.80 (d, ^3J = 1.8 Hz, 2 H, 1-H), 4.76 (dd, ^3J = 5.2, ^3J = 9.1 Hz, 2 H, α-H), 4.04 (dd, ^3J = 1.8, ^3J = 3.4 Hz, 2 H, 2′-H), 3.94 (dd, ^3J = 3.4, ^3J = 9.5 Hz, 2 H, 3′-H), 3.90–3.74 (m, 8 H, 2-H, 4′-H, 6a-H, 6′a-H), 3.66–3.62 (m, 12 H, 3-H, 4-H, 5′-H, 6b-H, 6′b-H, OC*H*HCH$_2$), 3.58–3.51 (m, 8 H, 5-H, OC*H*$_2$C-Ar, OCH*H*CH$_2$), 3.45–3.40 (m, 4 H, OCH$_2$C*H*$_2$), 3.14 (dd, ^3J = 5.3, ^2J = 13.9 Hz, 2 H, βa-H), 2.96 (dd, ^3J = 9.1, ^2J = 13.9 Hz, 2 H, βb-H) ppm. ^{13}C NMR (150 MHz, [D$_4$]MeOH): δ = 174.4 (NH*C*OCH$_2$), 172.4 (NH*C*OC-α), 156.9, 131.4, 130.4, 118.0 (C-Ar), 101.6 (C-1), 100.3 (C-1′), 75.2 (C-5′), 74.7 (C-5), 72.5 (C-3), 72.4 (C-3′), 72.0 (C-2), 71.9 (C-2′), 68.8 (C-4), 68.4 (C-4′), 66.9 (O*C*H$_2$CH$_2$), 62.9 (C-6), 62.6 (C-6′), 54.2 (C-α), 42.7 (CO*C*H$_2$C-Ar), 41.4 (C-β), 40.4 (OCH$_2$*C*H$_2$) ppm. HRESI-MS: calcd. for [C$_{50}$H$_{74}$N$_4$O$_{28}$S$_2$ + Na]$^+$ 1265.3829; found 1265.3768; calcd. for [C$_{50}$H$_{74}$N$_4$O$_{28}$S$_2$ + K]$^+$ 1281.3568; found 1281.3164.

Supporting Information (see also the footnote on the first page of this article): Copies of ^{13}C NMR spectra of reported compounds and inhibition curves obtained in inhibition adhesion assays.

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