Multivalent ligands for the mannose-specific lectin on type 1 fimbriae of *Escherichia coli*: syntheses and testing of trivalent α-D-mannoside clusters

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The syntheses of the triantennary cluster α-D-mannosides 16, 19, 23 and 24 and their capacities to inhibit mannose-dependent binding of *E. coli* HB 101 (pPKl4) are described. The cluster glycosides are formed by glycosylation of tris-(3-hydroxypropyl)nitromethane 13, and by linking of suitable mannoside derivatives *via* amide and thiourea bonds to tris-(2-carboxyethyl)nitromethane 17 and tris-(2-aminoethyl)amine 20. Functionalized mannosides are attached to the core molecules at the 6-position of the sugar ring to allow variation of the introduced aglycone moieties in order to compare their effects on the inhibitory potencies of the derived mannoside clusters. The 6-peptide-bridged cluster mannoside 19 displays the highest binding potency towards the type 1 fimbrial lectin of *E. coli* as tested by inhibition agglutination tests and ELISA.

Introduction

Carbohydrate protein interactions play an important role in cell adhesion[1,2] such as leukocyte trafficking[3,4] or microbial adhesion.[5] Carbohydrate derivatives have been designed to interfere in both processes, for example to control leukocyte recruitment in case of chronic or acute inflammatory diseases[6–8] or to inhibit the attachment of pathogens to their host cells. As firm adhesion of the microbe to the host cell surface often is a prerequisite for infection, a carbohydrate-based anti-adhesion therapy is a promising approach to the prevention of microbial infection.[9] Development of bacterial resistance as in the case of antibiotic treatment may be less likely to occur in the case of such treatment.

Multivalent glycomimetics have been shown to be potent inhibitors of carbohydrate–protein interactions in many different adhesion systems *in vitro*, such as in the adhesion of influenza virus[10] or *Streptococcus suis*[11] with IC_{50}-values as low as the nM range.[11] In our own studies we have used multivalent mannose derivatives to inhibit adhesion of *Escherichia coli* HB 101 (pPKl4) to erythrocytes and yeast mannan.[12,13] *E. coli* are important pathogens in urogenital and gastrointestinal infections. Like many other bacterial strains, they carry carbohydrate-recognition domains on long proteinogenous filaments on their surfaces, called fimbriae or pili. *E. coli* possesses different fimbriae, classified according to their carbohydrate specificity. The α-mannoside-specific type 1 fimbriae have been shown to be one of the important factors for *E. coli* virulence,[14,15] enabling the bacteria to adhere to the cell surface carbohydrates of their host cells.

Our recent work on testing multivalent mannose derivatives as inhibitors of haemagglutination with *E. coli* revealed trivalent mannosyl clusters as the best inhibitors among a number of mannose clusters with different carbohydrate valencies.[12,13] This was in keeping with earlier work, mainly from Ofek and Sharon[16–20] who have suggested a trisected carbohydrate binding pocket as a model for the type 1 fimbriae agglutinin of *E. coli*. Capitalizing on these findings, we have concentrated our efforts on the synthesis of trivalent cluster mannosides.

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Results and discussion

To allow for a broad variation in the structures of the glycoclusters, including the spatial arrangement of the mannose residues, we have considered different attachment sites at the sugar ring, as well as different linkage types. Mannosylation of a trivalent core was our first approach; however, to allow for variation of the aglycone moiety of the target mannosides, clustering *via* the 6-position of the α-mannoside ring was the basis for the design of the other synthesized cluster mannosides. Thus, the effect of different aglycone moieties on the resulting binding capacities can be explored, while the overall structure of the glycoclusters is maintained. This is especially attractive, as *p*-nitrophenyl α-D-mannoside binds approx. 80-fold better to the type 1 fimbrial lectin than does the analogous methyl glycoside.[16–20] Furthermore, a decrease in binding potency upon modification of the 6-position of the carbohydrate ring is not to be expected as it is known from various other lectins that the 2-, 3- and 4-hydroxy groups of the carbohydrate moiety are usually essential for binding to the lectin, whereas the 6-OH group often plays a less important role in the recognition process.[21]

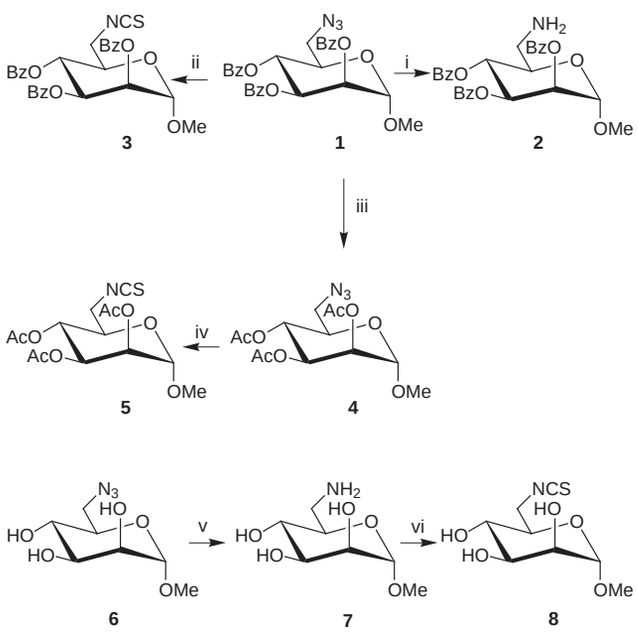
Syntheses of precursor molecules for cluster glycoside synthesis

Trifunctional core molecules were chosen for the synthesis of the target mannoside clusters, which were equipped with an additional group, suitable to be turned into a tether later in the synthesis, for both further enlargement of the glycoclusters and attachment of biolabels such as biotin or fluorescent markers. Tris-(hydroxymethyl)aminomethane (Tris) has been used as a core molecule for glycocluster synthesis in glycosylation after protection of the amino function.[22–24] The triol tris-(3-hydroxypropyl)nitromethane **13** appeared to be advantagous compared with Tris for its good solubility in organic solvents, greater sterical availability of the hydroxy groups, and its nitro group, which could serve as a masked amino function. Thus, compound **13**

was selected as an acceptor molecule for the mannosylation. Its synthesis started from nitromethane and acrylic acid *tert*-butyl ester to give the triester in a Michael addition.[25] The triester was saponified to the corresponding triacid **17**, which was a suitable core molecule in peptide-coupling reactions. The triacid **17** could be chemoselectivity reduced to the nitro-trialcohol **13** by using BH_3·THF as described.[25] Thus, compound **13** was obtained in 68% overall yield starting from nitromethane. Furthermore, tris-(2-aminoethyl)amine **20** was also used as core molecule.

The benzyl-protected mannosyl trichloroacetimidate **14**[26] was used as glycosyl donor for the complete glycosylation of the triol **13** in order to control orthoester formation, which regularly predominated when the analogous acetylated mannosyl donor was used.

To gain 6-functionalized mannosides for the synthesis of 6-linked glycoclusters, the primary hydroxy groups of methyl α-D-mannopyranoside and *p*-nitrophenyl α-D-mannopyranoside **9** were modified. Starting from the known benzoylated 6-azido-6-deoxy mannoside **1**,[27] the corresponding 6-amine **2** could be obtained by catalytic hydrogenation without migration of a benzoyl group occurring (Scheme 1). The

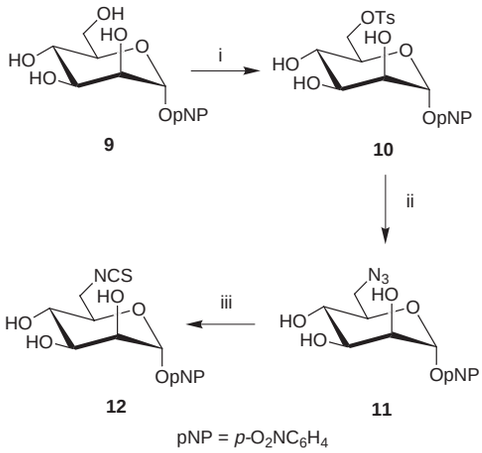


Scheme 1 *Reagents*, *conditions and yields*: i, H_2, Pd/C (10%), 1:1 EtOAc–EtOH, rt, 89%; ii, CS_2, $(EtO)_3P$, toluene, 80 °C, 4 h, 92%; iii (a) NaOMe, MeOH; (b) Ac_2O, pyridine, rt, 90%; iv, CS_2, $(EtO)_3P$, toluene, 80 °C, 6 h, 87%; v, H_2, Pd/C (10%), 1:1 EtOAc–EtOH, 84%; vi, $Cl_2C(S)$, 4:1 EtOH–water, diisopropylethylamine, rt, 96%

amino-functionalized mannoside **2** later served as 'mannoside donor' in the peptide-coupling reaction with the triacid **17**. The 6-azido group in compound **1** was also efficiently converted into an isothiocyanato function by the reaction with CS_2 and triethyl phosphite[28] to form mannoside **3**. Furthermore, mannoside **1** was converted into triacetate **4** following a deprotection–acetylation sequence. The acetylated azide **4** was then elaborated to give the 6-deoxy-6-isothiocyanato mannoside **5** by use of CS_2 and triethyl phosphite in excellent yield. Its unprotected analogue **8**[29] was obtained by hydrogenation of the unprotected azide **6** to form the amine **7**,[30] followed by treatment of amine **7** with thiophosgene.[29]

The formation of the isothiocyanato function could easily be monitored by IR [$\nu(N_3)$ at 2104 cm^{-1}, ν(NCS) at 2114 cm^{-1}] and by ^{13}C NMR spectroscopy which displayed the NCS carbon at δ_C 134.4. The 6-deoxy-6-isothiocyanato glycosides **3**, **5** and **8** were used for the synthesis of thiourea-bridged glycoclusters.

In order to change the methyl aglycone of mannoside **8** into a *p*-nitrophenyl moiety, *p*-nitrophenyl mannoside **9** was selectively tosylated at the primary hydroxy group to form compound **10**, which was converted into the 6-azido-6-deoxy mannoside **11** by nucleophilic displacement reaction (Scheme 2). Subsequent



Scheme 2 *Reagents*, *conditions and yields*: i, TsCl, pyridine, 0 °C to rt, 57%; ii, NaN_3, DMF, 70 °C, 8 h, 91%; iii, CS_2, $(EtO)_3P$, DME, 80 °C, 6 h, 76%

reaction of the unprotected triol **11** with triethyl phosphite and CS_2 afforded compound **12** in high yield.

Compounds **14**, **2**, **3**, **5**, **8** and **12** served as building blocks in the subsequent synthesis of mannoside clusters.

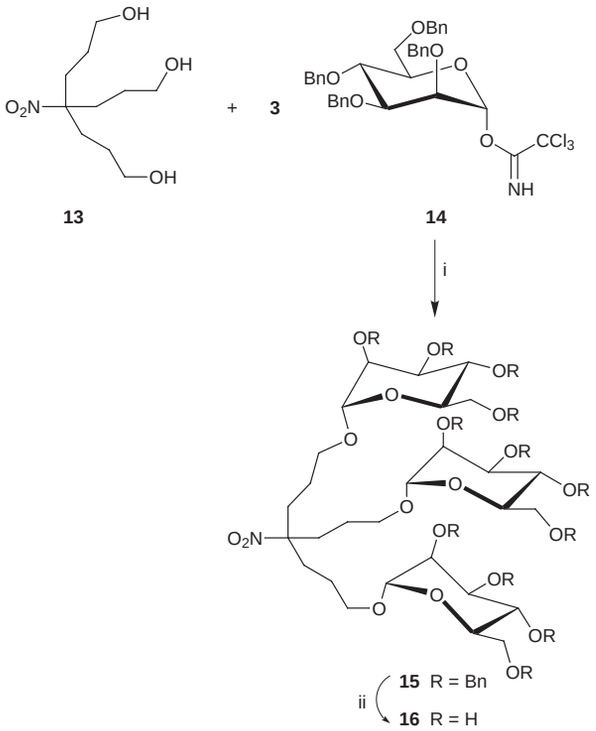
Syntheses of mannoside clusters

Glycosylation of the trialcohol **13** was carried out according to the method established by Schmidt *et al.*[31] using 3.9 mol equiv. of the mannosyl trichloroacetimidate **14** and TMSOTf (trimethylsilyl trifluoromethanesulfonate) as Lewis acid catalyst. The right choice of solvent and reaction conditions was essential for the success of this reaction. Freshly distilled, dry THF was used and the amount of added promotor TMSOTf was carefully controlled. Glycosylation yields were strongly dependent on the reaction temperature. Performing the reaction at −30 °C gave 25% product, reaction at −45 °C yielded 39%, glycosylation at −50 °C led to 73%, and reaction at −65 °C finally afforded the benzylated trivalent cluster mannoside **15** in 74% yield. Using such optimized conditions, the cluster mannoside **15** could also be obtained on a multigram scale. Complete debenzylation of compound **15** was achieved by hydrogenation at 60 °C and 60 bar† H_2 with Pd on charcoal as catalyst and led to the unprotected triantennary *O*-glycosidic α-D-mannoside cluster **16** in 77% yield (Scheme 3). The structures of triantennary products **15** and **16** were easily confirmed by NMR spectroscopy, which revealed the correct integration ratios of the core methylene protons and the multiplets for the sugar rings in the ^1H NMR spectrum.

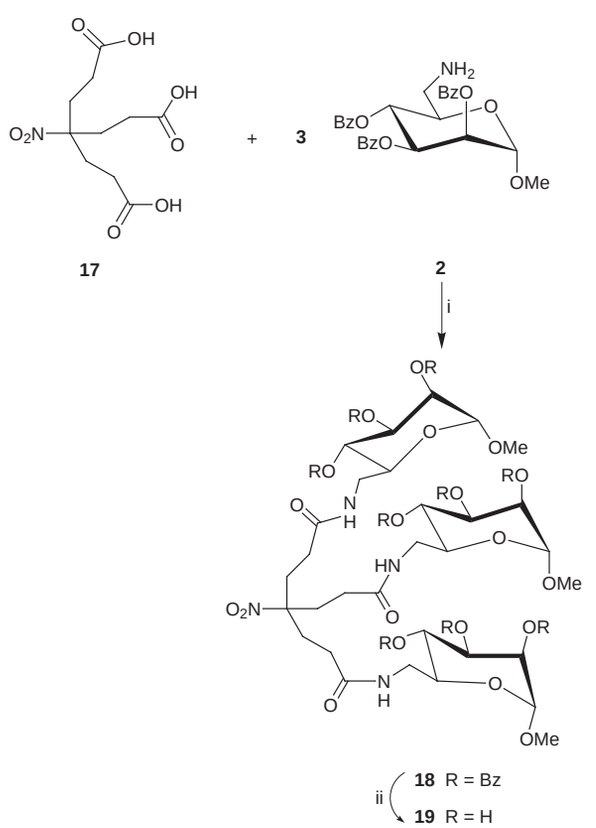
Furthermore, the 6-modified benzoylated methyl mannoside **2** was coupled to the nitroacid **17** under standard peptide-coupling conditions[32,33] to give the 6-linked cluster **18** in good yield (Scheme 4). This was debenzoylated by the Zemplén method to yield the unprotected 6-peptide-bridged trimannoside **19** in a quantitative reaction.

The 6-deoxy-6-isothiocyanato mannosides **3**, **5**, **8** and **12** were treated with tris-(2-aminoethyl)amine **20** to form thiourea-bridged trivalent mannoside clusters, based on the thiourea-bridging strategy which has recently been introduced (Scheme 5).[34] A slight excess of the benzolyated isothiocyanate **3** and tris-(2-aminoethyl)amine **20** led to compound **21** in over 90% yield. However, removal of the benzoyl protecting groups was difficult and the unprotected cluster **23** was obtained in yields

† 1 bar = 10^5 Pa.

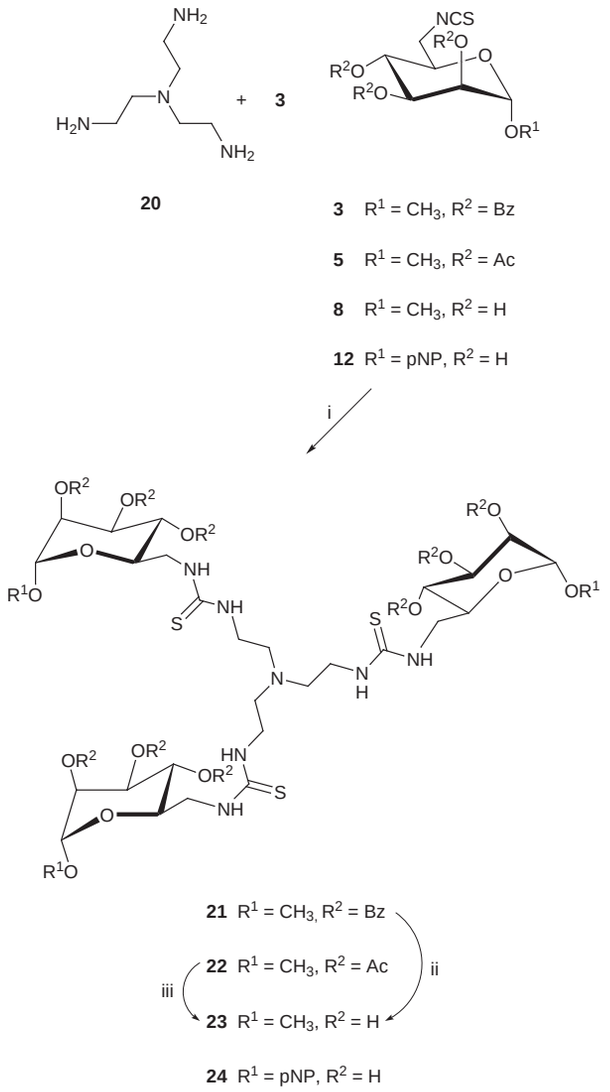


Scheme 3 *Reagents*, *conditions and yields*: i, 0.02 M TMSOTf in CH_2Cl_2, THF, 4 Å, N_2, −65 °C to rt, 74%; ii, H_2, Pd/C, 3:1 EtOH–THF, 77%



Scheme 4 *Reagents*, *conditions and yields*: i, DCC, HOBT, DMF, 0 °C to rt, 73%; ii, NaOMe, MeOH, 98%

not higher than 27%. Also, the analogous coupling reaction using the OH-unprotected isothiocyanate **8** gave the glycocluster **23** in only poor yields of approximately 20%, due to solubility problems. However, when the acetylated 6-isothiocyanate **5** was treated with tris-(2-aminoethyl)amine, the trivalent cluster **22** was formed in excellent yields and, also,



Scheme 5 *Reagents*, *conditions and yields*: i, CH_2Cl_2 50 °C, 30 min (for **3**, **5** or **12**), 92% ($R^1 = CH_3$, $R^2 = Bz$), 91% ($R^1 = CH_3$, $R^2 = Ac$), 89% ($R^1 = pNP$, $R^2 = H$); 4:1 EtOH–water, 50 °C, 12 h, (for **8**), 23%; ii, 1 M NaOMe, 5:1 MeOH–DMF, 50 °C, 3 days, 27%; iii, 1 M NaOMe, MeOH, rt, 82%

was readily deprotected under Zemplén conditions to form the thiourea-bridged target cluster **23**, which was easily purified by size-exclusion chromatography.

In the case of the OH-unprotected 6-NCS-modified pNP mannoside **12**, the thiourea-bridging reaction with tris-(2-aminoethyl)amine leading to product **24** (Scheme 5) was successfully performed using aprotic solvents such as CH_2Cl_2 (89%) or DME (86%).

Testing of the inhibition properties of the mannoside clusters 16, 19, 23 and 24

Initially, the cluster mannosides were tested as inhibitors of the haemagglutination of guinea pig erythrocytes by *E. coli* HB 101 (pPKl4) in classical inhibition haemagglutination tests. In these assays, the inhibition titre (IT) is the lowest concentration of the inhibitor at which no agglutination occurs by macroscopic inspection. These results are highly reproducible but, however, have to be considered semi-quantitative due to the experimental set-up. A newly developed enzyme-linked immunosorbent assay (ELISA) has allowed us to measure IC_{50}-values for the inhibition of *E. coli* adhesion to yeast mannan. IC_{50}-Values reflect the inhibitor concentration which causes 50% inhibition of bacterial binding to yeast mannan. Duplicate results were used for the construction of the inhibition curves for each individual ELISA experiment. Typically, the intra-assay variation of an

Table 1 Inhibition titres (IT) obtained from inhibition haemagglutination tests and IC_{50}-values measured by ELISA are listed together with the relative values based on methyl α-D-mannopyranoside (RIT and $RIC_{50} \equiv 1$) as standard. IC_{50}-Values are average values from at least three independent ELISA experiments and the resulting standard deviations are indicated. The relative inhibition potencies based on moles of mannose moieties present in the tested compounds ('valency-corrected' values) are also listed for both tests.

	Methyl α-D-mannopyranoside	**9**	**16**	**19**	**23**	**24**
IT [μM][a]	3900	42	18	1.1	6900	
RIT	1	93	217	3545	0.57	
RIT based on moles of mannose	1	93	72	1182	0.19	
IC_{50} [μM][b]	3500	49	39	11	5333	429
S[c]	800	17	5	5	1892	140
RIC_{50}	1	71	90	318	0.66	8.2
RIC_{50} based on moles of mannose	1	71	30	106	0.22	2.7

[a] Obtained from inhibition haemagglutination tests. [b] Obtained by ELISA. [c] Standard deviation.

individual ELISA is very small, whereas the IC_{50}-values obtained from several independently performed ELISAs differ significantly. However, when relative inhibition potencies (RIPs) are calculated from independently obtained data, the results are highly reproducible. The results obtained from both tests are listed in Table 1. IC_{50}-Values are average values from at least three independent ELISA experiments and are depicted together with the resulting standard deviations.

The mannoside clusters **16**, **19**, **23** and **24** were compared with the known low-affinity inhibitor methyl α-D-mannopyranoside and the potent aromatic inhibitor **9**. Interestingly, the mannoside clusters greatly differed in their inhibitory potencies. The *O*-glycosidic cluster **16** and the peptide-linked cluster **19** were the best inhibitors of *E. coli* adhesion, clearly surpassing the inhibitory capacity of compound **9**. On the other hand, thiourea-linked cluster **23** had a very high IT and IC_{50}, and therefore was a worse inhibitor than methyl α-D-mannopyranoside. The differences in the inhibition potencies of compound **23** on the one hand and compounds **16** and **19** on the other may be due to conformational differences or may be related to the nature of the linkage. Introduction of hydrophobic phenyl moieties in the case of cluster **24**, instead of the methyl aglycones in cluster **23**, decreased IC_{50}-values over 100-fold, as was expected; however, the inhibitory potency of the aromatic mannoside **9** was not reached. This result was obtained by ELISA only, as limited water solubility of cluster **24** did not allow an inhibition haemagglutination test to be carried out in this case.

Depending on the assay system used, the tested compounds differed in terms of the absolute concentrations at which inhibition of adhesion occurred. In the ELISA, IC_{50}-values were higher than titres obtained in the inhibition haemagglutination test in the case of glycoclusters **16** and **19**. This effect was extreme for the best glycocluster **19**, which showed a 10-fold higher inhibitory concentration when determined by ELISA. However, when relative inhibitory potencies (RIT and RIC_{50}) were calculated to compare different compounds, the values are similar regardless of the assay system used. The discrepances obtained by both methods are not surprising, as the tests reflect very different aspects of *E. coli* adhesion. While in the ELISA *E. coli* adhesion to yeast mannan is inhibited, in the case of the inhibition agglutination test the bacteria bind to erythrocytes. Erythrocytes are likely to possess a greater variety of oligosaccharide structures on their surface than is reflected by yeast mannan. Furthermore, the sandwich ELISA rather has the characteristics of a two-dimensional system in contrast to the more complex haemagglutination assay. It is important to keep such differences in mind when *in vitro* results form the basis for predicting the usefulness of synthetic compounds in *in vivo* situations.

In order to estimate the net effect of clustering, the inhibitory potencies of the glycoclusters **16** and **19** were compared based on the number of mannose moieties they contained (RIT and RIC_{50} in Table 1). Whereas the RIT of cluster **16** is in the range of that shown by compound **9**, the valency-corrected RIT of the peptide-linked mannoside cluster **19** remains approximately 10-times higher than that of compound **9**. This effect was not observed in the ELISA.

As the carbohydrate-recognition domains (CRDs) are placed at the tips and along the shafts of the fimbriae[35] multivalent carbohydrate derivatives may display enhanced inhibitory capacities in the assays used due to simultaneous binding to multiple CRDs. This type of enhancement effect evident with clustering of carbohydrate substructures has been termed the 'glycoside clustering effect'.[36] Owing to the size of the tested cluster mannosides **16**, **19**, **23** and **24** we can exclude the possibility that their mannose moieties are simultaneously recognized by more than one CRD per pilus. It is, however, possible that they bind to adjacent CRDs on adjacent fimbriae at the same time. On the other hand, binding could also occur to a single CRD only, and thus different inhibitory potencies would result from the different fit of the glycoclusters into the CRD. This model is supported by the findings of Ofek and Sharon who described trisaccharides as particularly suitable ligands for the type 1 fimbrial lectin.[16,20] We are currently addressing these questions in further studies. For the design of even more potent inhibitors of the binding of type 1 fimbriated *E. coli*, the peptide-linked mannoside cluster **19** should serve as an excellent prototype structure and we expect the introduction of more hydrophobic aglycone moieties to further improve its inhibitory capacity.

Conclusions

A set of trivalent α-D-mannoside clusters was synthesized which displayed widely different binding potencies towards the type 1 fimbrial lectin of *E. coli* HB 101 (pPKL4). Our studies using variable cluster mannoside structures provide further insight into the putative three-dimensional structure of the carbohydrate recognition domain. In addition, the potent cluster **19** will be extremely useful as a lead structure for the synthesis of even more potent inhibitors of *E. coli* binding with inhibitory potencies expected in the nanomolar range.

Experimental

Materials

Methyl α-D-mannoside was purchased from Merck, *p*-nitrophenyl α-D-mannoside was from SENN Chemicals. TMSOTf (Fluka) and tris-(2-aminoethyl)amine (Aldrich) were used without further purification. F-shaped 96-well microtiter plates from Sarstedt were used for ELISA and V-microtiter plates from Nunc for inhibition haemagglutination tests. Mannan from *Saccharomyces cerevisiae* was purchased from Sigma and was used in 50 mM aq. Na_2CO_3 (1 mg ml^{-1}; pH 9.6). The polyclonal anti-fimA antibody was a kind gift from Prof. Dr J. Hacker (Würzburg) and peroxidase-conjugated goat anti-rabbit antibody (IgG, H+L) was purchased from Dianova. Skimmed milk was from Glücksklee, Tween 20 from Roth,

ABTS [2,2′-azidobis-(3-ethylbenzothiazoline-6-sulfonic acid)] from Sigma and thimerosal {2-[(ethylmercurio)thio]benzoic acid, sodium salt} was from Merck. A recombinant type 1 fimbriated *E. coli* strain, *E. coli* HB101 (pPKl4),[37] was used and cultured as described.[12] Light petroleum refers to the fraction with distillation range 50–70 °C.

Buffers. PBS (phosphate-bufferred saline) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4 \cdot 2H_2O$ and 0.2 g KH_2PO_4 in 1000 ml of distilled, deionized water (pH 7.2). PBSE (PBS buffer used for ELISA) was used as 150 mM solution [pH 7.2; NaCl (136.90 g l^{-1}), KCl (2.68 g l^{-1}), Na_2HPO_4 (8.0 g l^{-1}), KH_2PO_4 (2.4 g l^{-1}), 0.01% thimerosal]. PBSET was PBSE buffer + 0.05% Tween 20. Substrate buffer was 0.1 M sodium citrate dihydrate, adjusted to pH 4.5 with citric acid. For preparation of the ABTS solution, ABTS (1 mg) was dissolved in substrate buffer (1 ml) by sonification and 0.1% H_2O_2 (25 µl per ml) was added.

General methods

Optical densities (ODs) were measured on a Dynatech 5000 ELISA reader at 405 nm with the reference read to 490 nm. ELISA plates were incubated at 37 °C. For flash chromatography Merck silica gel 60 (0.040–0.063 mm, 230–400 mesh) was used. TLC was performed on Alugram® Sil G/UV_{254} plates, purchased from Machery&Nagel, or on Kieselgel 60 F_{254} plates from Merck. Detection was carried out under UV light or by spraying with 20% ethanolic sulfuric acid or ninhydrin solution (2% in water–propan-1-ol, 1:1) with subsequent heating. Size-exclusion chromatography was performed on Sephadex G-15 (60–181 µm) and 15 mM aq. NH_4HCO_3 as the eluent. NMR spectra were measured on a Bruker AMX 400 (400 MHz for ^{1}H and 100.67 MHz for ^{13}C NMR) or a DRX 500 (500 MHz for ^{1}H and 125.84 MHz for ^{13}C NMR) spectrometer. Chemical shifts are in ppm, relative to internal $SiMe_4$ (0.00 ppm for ^{1}H and ^{13}C NMR) or solvent peaks which were calibrated as follows: $CDCl_3$ (δ 7.26 for ^{1}H and δ_C 77.00 for ^{13}C NMR), D_2O [δ 4.65 for ^{1}H; for ^{13}C NMR CD_3CN (δ_C 1.30) or [$^2H_6$]acetone (δ_C 30.60) was added], [$^2H_4$]methanol (δ 3.35 for ^{1}H and δ_C 49.30 for ^{13}C NMR), [$^2H_6$]DMSO (δ 2.49 for ^{1}H and δ_C 39.70 for ^{13}C NMR). *J* Values are in Hz. Where necessary, two-dimensional ^{1}H–^{1}H or ^{1}H–^{13}C chemical-shift-correlation spectroscopy (COSY) experiments were performed for complete signal assignments. Mps were determined on a Leitz heating table microscope and are uncorrected. Optical rotation values were obtained using a Perkin-Elmer polarimeter 341 or 243 (Na-D line, 589 nm; 20 °C; cell length 10 cm), and are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on an FT-IR ATI Mattson Instruments (USA) spectrometer. Mass spectra were measured on a VG Analytical 70-250S (FAB-MS) or a Finnigan MAT 95 (MALDI-TOF MS) instrument.

Inhibition haemagglutination tests

For inhibition of haemagglutination (= erythrocyte agglutination) of guinea pig erythrocytes on microtiter plates serially two-fold diluted carbohydrate solutions were used as described.[12] The lowest concentration that inhibited haemagglutination was determined visually as haemagglutination inhibition titre (IT).

ELISA

The ELISA protocol used to determine the inhibitory potencies of the cluster mannosides towards type 1 fimbriated *E. coli* was established on the basis of published procedures.[38–40] In preliminary experiments, the optimal concentrations for mannan, bacteria and antibody solutions were determined (data not shown). Polystyrene microtiter plates were coated with mannan solution (100 µl per well) and dried overnight at 37 °C. The plates were washed once with PBS (150 µl per well) and then blocked with 5% skimmed milk in PBSE for 30 min at 37 °C. The wells were washed with PBSE (150 µl) and then PBSE (50 µl) and inhibitor solution (50 µl) were added. Inhibitor solutions were diluted serially two-fold in PBSE. Bacterial suspension (50 µl per well) was added and the plate was left at 37 °C for 1 h to allow sedimentation of the bacteria. Then each well was washed four times with PBSE (150 µl) and 50 µl of the first antibody (anti-fimA antibody, solution as optimized prior to the experiments) in 2% skimmed milk was added. The plates were incubated for 30 min and then washed twice with PBSET and the second antibody was added (50 µl). The plates were incubated for 30 min and then subsequently washed three times with PBSET, PBSE and substrate buffer. ABTS solution (50 µl) was added, incubated for 30 min at 37 °C, and the reaction was stopped by adding of 2% oxalic acid (50 µl).

For ELISA controls, bacterial adhesion to blocked, uncoated microtiter plates was checked, and the reaction of antibodies with carbohydrate derivatives as well as the reaction of the employed antibodies with yeast mannan was tested and found to be negligible. The low background obtained from these control experiments was subtracted when calculating the IC_{50} values. The percentage inhibition was calculated as [OD(nl) − OD(I)] × 100 × [OD(nl)]$^{-1}$ (nl: no inhibitor, I: with inhibitor).

Methyl 6-amino-2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranoside 2

Methyl 6-azido-2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranoside **1**[27] (1.40 g, 2.64 mmol) was dissolved in ethyl acetate–EtOH (1:1; 20 ml) and hydrogenated after the addition of Pd catalyst (10% on charcoal; 200 mg) at atmospheric pressure. After the reaction was complete (*ca.* 3.5 h), the mixture was filtered over Celite and the filtrate was concentrated *in vacuo* and purified by flash chromatography (toluene–acetone, 5:1) to yield the *title compound* **2** (1.19 g, 89%) as a slightly yellow syrup; $[\alpha]_D^{20}$ −51.7 (*c* 0.89, $CHCl_3$); R_f (toluene–acetone, 5:1) 0.12 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 8.13–7.23 (15 H, m, ArH), 5.92 (1 H, dd, $J_{3,4}$ 10.2, 3-H), 5.83 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 5.67 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 4.97 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.02 (1 H, ddd, $J_{5,6}$ 3.6, 5-H), 3.52 (3 H, s, OCH_3), 3.00–2.90 (2 H, m, $J_{5,6'}$ 5.6, 6-H_2) and 1.54 (1 H, br s, NH); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 165.96, 165.48, 165.42 (C=O), 133.48, 133.43, 133.07, (aryl-CH), 129.93–128.19 (m, aryl-C), 98.58 (C-1), 71.36, 70.64, 69.84, 67.63 (C-2, -3, -4, -5), 55.35 (OCH_3) and 42.54 (C-6); *m/z* (FAB MS) 506.6 (M^+ + H. $C_{28}H_{28}NO_8$ requires *m/z*, 506.18).

Methyl 2,3,4-tri-*O*-benzoyl-6-deoxy-6-isothiocyanato-α-D-mannopyranoside 3

A mixture of methyl 6-azido-2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranoside **1**[27] (0.50 g, 0.94 mmol), CS_2 (3.3 ml, 4.6 mmol) and triethyl phosphite (0.8 ml, 0.055 mmol) in dry toluene (10 ml) was stirred at 80 °C for 10 h. Water (10 ml) was added and the mixture was left at room temperature (rt) overnight. Then the aq. phase was extracted three times with CH_2Cl_2 (50, 50 and 30 ml) and the combined organic phases were subsequently washed successively with 1 M H_2SO_4 and saturated aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (light petroleum–ethyl acetate, 4:1) yielded the *title compound* **3** (474 mg, 92%) as a solid, mp 227 °C (Found: C, 63.6; H, 4.6; N, 2.55; S, 5.8. $C_{29}H_{25}NO_8S$ requires C, 63.61; H, 4.60; N, 2.56; S, 5.85%); $[\alpha]_D^{20}$ −29.6 (*c* 1.0, $CHCl_3$); ν_{NCS} 2114 cm^{-1}; R_f (light petroleum–ethyl acetate, 4:1) 0.17 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 8.15–7.24 (15 H, m, ArH), 5.90 (1 H, dd, $J_{3,4}$ 10.2, 3-H), 5.82 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 5.70 (1 H, dd, $J_{2,3}$ 3.1, 2-H), 5.02 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.25 (1 H, ddd, $J_{5,6}$ 5.6, 5-H), 3.81 (1 H, dd, $J_{6,6'}$ 14.8, 6-H), 3.76 (1 H, dd, $J_{5,6'}$ 3.6, 6-H′) and 3.58 (3 H, s, OCH_3); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 165.55, 165.45, 165.36 (C=O), 133.71–128.28 (m, aryl-C), 98.85 (C-1), 70.10 (C-2), 69.45 (C-3), 69.29 (C-5), 67.73 (C-4), 56.00 (OCH_3) and 46.18 (C-6); *m/z* (EIMS) 548 (M^+ + H. $C_{29}H_{26}NO_8S$ requires *m/z*, 548.14).

Methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside 4

To a solution of methyl 6-azido-2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranoside **1**[27] (1.591 g, 2.99 mmol) in dry MeOH (45 ml) was added a catalytic amount of NaOMe solution (1M in MeOH; 1.5 ml) and the mixture was stirred at rt. After the reaction was complete (TLC toluene–ethyl acetate, 25:1), the solution was neutralized with ion-exchange resin (Levatit SP 1080 H^+), then filtered, and the filtrate was concentrated *in vacuo*. The residual unprotected mannoside **6** was dissolved in dry pyridine (40 ml), acetic anhydride (4.3 ml, 45 mmol) was added, and the reaction mixture was stirred at rt until the reaction was complete (TLC toluene–ethyl acetate, 25:1). Pyridine was removed *in vacuo* and the residue was purified by flash chromatography (toluene–ethyl acetate, 25:1) to give the *title compound* **4** (930 mg, 90%) as a syrup; $[\alpha]_D^{20}$ +80.8 (*c* 1.50, $CHCl_3$) {lit.,[41] $[\alpha]_D$ +63° (*c* 1, $CHCl_3$)}; R_f (toluene–ethyl acetate, 5:1) 0.30 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 5.33 (1 H, dd, $J_{3,4}$ 9.7, 3-H), 5.31 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 5.24 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 4.73 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.94 (1 H, ddd, $J_{5,6}$ 7.1, 5-H), 3.45 (3 H, s, OCH_3), 3.38 (1 H, dd, $J_{6,6'}$ 13.2, 6-H), 3.29 (1 H, dd, $J_{5,6'}$ 2.6, 6-H′) and 2.16, 2.05 and 2.00 (each 3 H, each s, 3 × Ac); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 169.97, 169.80, 169.77 (C=O), 98.36 (C-1), 69.87, 69.40, 68.75, 67.12 (C-2, -3, -4, -5), 55.37 (OCH_3), 51.02 (C-6), 20.81, 20.62 and 20.59 (3 × $CO\mathit{C}H_3$).

Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-isothiocyanato-α-D-mannopyranoside 5

A mixture of compound **4** (898 mg, 2.60 mmol), CS_2 (7.6 g, 6 ml, 0.10 mol) and triethyl phosphite (1.3 g, 1.4 ml, 8.06 mmol) in dry toluene (14 ml) was heated at 80 °C for 6 h under nitrogen. Then water (50 ml) was added and the aq. phase was extracted with CH_2Cl_2 three times. The combined organic phases were subsequently washed successively with 1 M H_2SO_4 and saturated aq. NaCl, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (toluene–ethyl acetate, 5:1) afforded the *title compound* **5** (820 mg, 87%) as a solid; mp 120–121.5 °C (lit.,[29] 114–115 °C); $[\alpha]_D^{20}$ +70.1 (*c* 0.95, $CHCl_3$) [lit.,[29] $[\alpha]_D$ +62 (*c* 1.1, $CHCl_3$)]; R_f (toluene–ethyl acetate, 2:1) 0.47 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 5.35 (1 H, dd, $J_{3,4}$ 10.2, 3-H), 5.23 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 5.21, dd ≈ t, $J_{4,5}$ 9.7, 4-H), 4.76 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.95 (1 H, ddd, $J_{4,5}$ 9.7, 5-H), 3.62 (2 H, m, 6-H_2), 3.46 (3 H, s, OCH_3) and 2.17, 2.07 and 2.00 (each 3 H, each s, 3 × Ac); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 169.97, 169.79, 169.74 (C=O), 134.36 (NCS), 98.51 (C-1), 69.28 (C-2), 69.01 (C-5), 68.57 (C-3), 67.21 (C-4), 55.72 (OCH_3), 46.12 (C-6) and 20.82, 20.60 and 20.58 (3 × $CO\mathit{C}H_3$).

***p*-Nitrophenyl 6-*O*-tosyl-α-D-mannopyranoside 10**

To a solution of *p*-nitrophenyl α-D-mannopyranoside **9** (3.64 g, 12.08 mmol) in dry pyridine (60 ml) was added dropwise a solution of tosyl chloride (2.76 g, 14.5 mmol) in dry pyridine (60 ml) within 45 min at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then 1 h at rt. After completion of the reaction (TLC ethyl acetate–toluene, 9:1), saturated aq. NaCl (150 ml) was added and the aq. phase was extracted three times with ethyl acetate (70 ml each). The combined organic phases were dried over $MgSO_4$, filtered and concentrated. Flash chromatography (ethyl acetate–toluene, 9:1) afforded the *title glycoside* **10** (3.16 g, 57%) as an amorphous solid (Found: C, 50.2; H, 4.7; N, 3.1. $C_{19}H_{21}NO_{10}S$ requires C, 50.11; H, 4.65; N, 3.08%); $[\alpha]_D^{20}$ +102.7 (*c* 1.30, MeOH); R_f (ethyl acetate–toluene, 9:1) 0.33 (UV; H_2SO_4); δ_H(400 MHz; D_3COD) 8.19 (2 H, d, ^3J 9.16, ArH), 7.73 (2 H, d, ^3J 8.65, tosyl H), 7.41 (2 H, d, tosyl H), 7.19 (2 H, d, ArH), 5.58 (1 H, d, $J_{1,2}$ 1.15, 1-H), 4.36 (1 H, dd, $J_{5,6'}$ 1.5, 6′-H), 4.24 (1 H, dd, $J_{6,6'}$ 11.2, 6-H), 4.05 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 3.86 (1 H, dd, $J_{3,4}$ 8.7, 3-H), 3.69 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 3.64 (1 H, ddd ≈ m, $J_{5,6}$ 6.6, 5-H) and 2.48 (3 H, s, tosyl CH_3); δ_C(100.67 MHz; D_3COD) 162.73, 146.78, 144.22, 134.72 (aryl-C_q), 131.22, 118.06 (aryl-CH), 129.30, 126.90 (tosyl-CH), 100.22 (C-1), 73.64, 72.43, 71.64, 68.09 (C-2, -3, -4, -5), 70.65 (C-6) and 21.87 (Ar$\mathit{C}H_3$).

***p*-Nitrophenyl 6-azido-6-deoxy-α-D-mannopyranoside 11**

A mixture of the mannoside **10** (1.67 g, 3.67 mmol) and sodium azide (1.20 g, 18.3 mmol) in dry DMF (40 ml) was stirred at 70 °C for 8 h. After the reaction was complete (TLC ethyl acetate–toluene, 19:1) the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate. The excess of sodium azide and formed sodium tosylate were removed by filtration and the filtrate was evaporated and purified by flash chromatography (ethyl acetate–toluene, 19:1) to afford the *title mannoside* **11** (1.09 g, 91%) as an amorphous solid (Found: C, 44.3; H, 4.3; N, 17.1. $C_{12}H_{14}N_4O_7$ requires C, 44.18; H, 4.33; N, 17.17%); $[\alpha]_D^{20}$ +63.7 (*c* 0.87, MeOH); R_f (ethyl acetate–toluene, 19:1) 0.21 (UV; H_2SO_4); δ_H(400 MHz; D_3COD) 8.26, 7.32 (each 2 H, each d, ^3J 9.15, ArH), 5.73 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.11 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 3.92 (1 H, dd, $J_{3,4}$ 9.2, 3-H), 3.75 (1 H, dd ≈ t, $J_{4,5}$ 9.2, 4-H), 3.67 (1 H, ddd, $J_{5,6}$ 5.1, 5-H) and 3.47–3.46 (2 H, m, $J_{5,6'}$ 4.1, $J_{6,6'}$ 13.7, 6-H_2); δ_C(100.67 MHz; D_3COD) 162.74 (*i*-aryl-C_q), 144.24 (*p*-aryl-C_q), 126.97, 117.93 (aryl-CH), 100.06 (C-1), 75.49 (C-5), 72.34 (C-3), 71.72 (C-2), 69.33 (C-4) and 53.02 (C-6); *m*/*z* (FAB MS) 327 (M^+ + H. $C_{12}H_{15}N_4O_7$ requires 327.09).

***p*-Nitrophenyl 6-deoxy-6-isothiocyanato-α-D-mannopyranoside 12**

A mixture of the mannoside **11** (800 mg, 2.45 mmol), CS_2 (7.1 ml, 0.12 mol) and triethyl phosphite (1.65 ml, 8.51 mmol) in dry DME (25 ml) was stirred at 80 °C for 5 h. Then water (100 ml) was added and the mixture was kept at rt for 12 h. Then the aq. phase was extracted with CH_2Cl_2 (100, 50 and 50 ml) and the combined organic phases were subsequently washed with 1 M H_2SO_4 and saturated aq. NaCl, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (toluene–ethyl acetate, 1:1) afforded the *title compound* **12** (395 mg, 47%, 76% based on converted starting material) as an amorphous solid and starting material **21** (230 mg, 0.705 mmol) was recovered (Found: C, 45.7; H, 4.1; N, 8.2; S, 9.35. $C_{13}H_{14}N_2O_7S$ requires C, 45.61; H, 4.12; N, 8.18; S, 9.37%); $[\alpha]_D^{20}$ +85.2 (*c* 0.86, MeOH); R_f (ethyl acetate–toluene, 2:1) 0.74 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 8.24 (2 H, d, ^3J 9.16, ArH), 7.20 (2 H, d, ArH), 5.74 (1 H, d, $J_{1,2}$ 1.0, 1-H), 4.56 (1 H, dd, $J_{3,4}$ 9.7, 3-H), 4.24 (1 H, dd, $J_{2,3}$ 3.1, 2-H), 4.21 (1 H, ddd, $J_{5,6}$ 7.1, 5-H), 4.13 (2 H, dd, $J_{5,6'}$ 2.5, $J_{6,6'}$ 10.2, 6-H_2) and 3.85 (1 H, dd ≈ t, $J_{4,5}$ 9.7, 4-H); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 160.32 (*i*-aryl-C_q), 143.05 (*p*-aryl-C_q), 134.09 (C_q, NCS), 126.11 (aryl-CH), 116.36 (aryl-CH), 97.08 (C-1), 75.16 (C-3), 70.12 (C-2), 69.85 (C-4), 65.89 (C-5) and 65.75 (C-6); *m*/*z* (FAB MS) 365 (M^+ + Na. $C_{13}H_{14}SO_7N_2Na$ requires *m*/*z*, 365.04).

Nitrotris-[3-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyloxy)-propyl]methane 15

The mannosyl donor **14**[26] (7.48 g, 10.92 mmol) and the triol **13**[25] (660 mg, 2.81 mmol) were dissolved in dry THF (100 ml) under nitrogen and the solution was stirred with molecular sieves (4 Å, 4 g) at −65 °C for 1 h. The reaction was started by the addition of TMSOTf (0.02 M solution in CH_2Cl_2; 100 μl) during 10 min and the mixture was stirred for 2 h at −65 °C and for 3 h at −30 °C. Additional TMSOTf (0.02 M solution in CH_2Cl_2; 150 μl) was added and the mixture was stirred at rt overnight. Then saturated aq. $NaHCO_3$ (100 cm^3) was added and the aq. phase was extracted with CH_2Cl_2 (150, 100 and 50 cm^3). The combined organic phases were concentrated, and purified by flash chromatography (light petroleum–ethyl acetate, 3:1) to afford the *title mannoside cluster* **15** (3.75 g, 74%) as a syrup (Found: C, 74.6; H, 6.9; N, 0.8. $C_{112}H_{123}NO_{20}$ requires C, 74.60; H, 6.88; N, 0.78%); $[\alpha]_D^{20}$ +16.3 (*c* 1.24, in $CHCl_3$); R_f (light petroleum–ethyl acetate, 1:1) 0.72 (UV; H_2SO_4); δ_H(400 MHz;

$CDCl_3$; Me_4Si) 7.36–7.23 (60 H, m, ArH), 4.84 (3 H, d, ^2J 11.2, benzyl CH_2), 4.82 (3 H, d, $J_{1,2}$ 2.0, 1-H), 4.74–4.47 (21 H, m, benzyl CH_2), 3.96 (3 H, dd ≈ t, $J_{4,5}$ 9.2, 4-H), 3.84 (3 H, dd, $J_{3,4}$ 9.7, 3-H), 3.76–3.68 (12 H, m, $J_{2,3}$ 3.1, 2-, 5H and 6-H_2), 3.62 (3 H, m_c, $CH_2CH_2CH_2O$), 3.31 (3 H, m_c, $CH_2CH_2CH_2O$), 1.88–1.82 (6 H, m, $CH_2CH_2CH_2O$) and 1.42–1.35 (6 H, m, $CH_2CH_2CH_2O$); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 138.35, 138.28, 138.22, 138.19 (aryl C_q), 128.29–127.50 (aryl CH), 97.90 (C-1), 93.65 (CNO_2), 80.04 (C-3), 75.12 (benzyl CH_2), 74.83 (C-4), 74.75 (C-2), 73.31, 72.65 and 72.13 (benzyl CH_2), 71.89 (C-5), 69.07 (C-6), 66.79 ($CH_2CH_2CH_2O$), 32.08 ($CH_2CH_2CH_2O$) and 23.73 ($CH_2CH_2CH_2O$); *m/z* (FAB-MS) 1827.8 (M^+ + Na. $C_{112}H_{123}NNaO_{20}$ requires *m/z*, 1824.85).

Nitrotris-[3-(α-D-mannopyranosyloxy)propyl]methane 16

The benzylated cluster **15** (750 mg, 0.416 mmol) and Pd catalyst (0.80 g; 10% on charcoal) were suspended in EtOH–THF (50 ml; 3:1) and the mixture was stirred at 60 °C under H_2 60 bar for 6 h. Then the catalyst was removed by filtration over a bead of Celite, and the filtrate was concentrated, and purified by size-exclusion chromatography on Sephadex G-15 (with 15 mM aq. NH_4HCO_3 as eluent). After lyophilization the *unprotected cluster* **16** (231 mg, 77%) was obtained as a lyophilizate; $[\alpha]_D^{20}$ +44.2 (*c* 0.97, water); R_f (propan-2-ol–water, 7:3, 1% NH_3) 0.38 (UV; H_2SO_4); δ_H(400 MHz; D_2O) 4.69 (3 H, d, $J_{1,2}$ 1.5, 1-H), 3.78 (3 H, dd, $J_{2,3}$ 3.6, 2-H), 3.72 (3 H, dd, $J_{3,4}$ 11.7, 3-H), 3.65–3.55 (9 H, m, 6-, 5- and 4-H), 3.51–3.46 (6 H, m, 6-H′, CH_2O), 3.42–3.37 (3 H, m, CH_2O), 1.90 (6 H, m_c, $CH_2CH_2CH_2O$) and 1.40 (6 H, m_c, $CH_2CH_2CH_2O$); δ_C(100.67 MHz; D_2O) 100.06 (C-1), 95.52 (CNO_2), 73.17, 71.01, 70.45 and 67.12 (C-2, -3, -4 and C-5), 67.43 (C-6), 61.29 ($CH_2CH_2CH_2O$), 32.02 ($CH_2CH_2CH_2O$) and 23.51 ($CH_2CH_2CH_2O$); *m/z* (FAB-MS) 722.5 (M^+ + H. $C_{28}H_{52}NO_{20}$ requires *m/z*, 722.31).

Nitrotris-{2-[(2′,3′,4′-tri-*O*-benzoyl-6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)carbamoyl]ethyl}methane 18

A mixture of the mannoside **2** (1.12 g, 2.22 mmol), the triacid **17**[25] (0.20 g, 0.72 mmol) and HOBT (0.60 g, 4.44 mmol) in dry DMF (10 ml) was stirred at 0 °C for 30 min. Then DCC (0.46 g, 2.23 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min and at rt overnight. The mixture was filtered and the filtrate was concentrated. The resulting crude product was diluted in ethyl acetate (80 ml) and subsequently washed successively with hydrochloric acid (5%; twice), saturated aq. $NaHCO_3$ and water. The organic phase was concentrated, and purified by flash chromatography (light petroleum–ethyl acetate, 1:3) to afford the *title cluster* **18** (0.92 g, 73%) as a solid (Found: C, 64.9; H, 5.2; N, 3.2. $C_{94}H_{90}N_4O_{29}$ requires C, 64.90; H, 5.21; N, 3.22%); $[\alpha]_D^{20}$ −99.6 (*c* 0.70, $CHCl_3$); R_f (ethyl acetate–light petroleum, 3:1) 0.21 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 8.07–7.24 (45 H, m, ArH), 6.25 (3 H, t, $J_{NH,6'}$ 5.6, $J_{NH,6''}$ 6.6, NH), 5.85 (3 H, dd, $J_{3',4'}$ 10.2, 3′-H), 5.70 (3 H, dd ≈ t, $J_{4',5'}$ 10.2, 4′-H), 5.63 (3 H, dd, $J_{2',3'}$ 3.6, 2′-H), 4.92 (3 H, d, $J_{1',2'}$ 1.5, 1′-H), 4.12 (3 H, ddd, $J_{5',6'}$ 5.6, 5′-H), 3.80 (3 H, ddd, $J_{5',6''}$ 2.5, 6′-H′), 3.47 (9 H, s, OCH_3), 3.30 (3 H, ddd, $J_{6',6''}$ 14.2, 6′-H) and 2.30–2.11 (12 H, m, CH_2CH_2CO); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 170.94 (NHCO), 165.98, 165.39 and 165.35 (3 × C=O), 133.69–128.27 (m, aryl-C), 98.53 (C-1′), 93.01 (C_qNO_2), 70.51 (C-2′), 69.62 (C-3′), 69.00 (C-5′), 67.93 (C-4′), 55.58 (OCH_3), 39.81 (C-6′), 30.69 (CH_2CH_2CO) and 30.56 (CH_2CH_2CO); *m/z* (FAB-MS) 1739.9 (M^+ + H. $C_{94}H_{91}N_4O_{29}$ requires *m/z*, 1739.58).

Tris-{2-[6′deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)carbamoyl]ethyl}nitromethane 19

A solution of the protected cluster mannoside **18** (270 mg, 0.155 mmol) in diethyl ether–MeOH (1:2.5; 10 ml) was treated with NaOMe solution (1 M in MeOH; 1.5 ml) and stirred at rt for 2 days. Then the reaction mixture was neutralized with ion-exchange resin (Levatit SP 1080 H^+), the resin was filtered off, and the filtrate was concentrated. The resulting crude material was purified by size-exclusion chromatography on Sephadex G-15 (with 15 mM aq. NH_4HCO_3 as eluent) to yield the *unprotected cluster* **19** (122 mg, 98%) as a amorphous solid; $[\alpha]_D^{20}$ +64.9 (*c* 1.02, water); R_f (propan-1-ol–water, 7:3, 1% NH_3) 0.38 (UV; H_2SO_4); δ_H(400 MHz; D_2O) 4.59 (3 H, d, $J_{1,2}$ 1.5, 1′-H), 3.78 (3 H, dd, $J_{2',3'}$ 3.6, 2′-H), 3.60 (3 H, dd, $J_{3',4'}$ 9.2, 3′-H), 3.50–3.45 (6 H, m, $J_{5',6'}$ 7.6, 5′-H, 6′-H′), 3.41 (3 H, dd ≈ t, $J_{4',5'}$ 9.2, 4′-H), 3.29 (3 H, dd, $J_{6',6''}$ 14.8, 6′-H) and 3.24 (9 H, s, OCH_3); δ_C(100.67 MHz; D_2O) 174.97 (NHCO), 101.23 (C-1′), 93.69 (C_qNO_2), 70.99 (C-5′), 70.72 (C-3′), 70.23 (C-2′), 68.40 (C-4′), 55.01 (OCH_3), 40.42 (C-6′), 30.98 (CH_2CH_2CO) and 30.35 (CH_2CH_2CO); *m/z* (FAB-MS) 803.7 (M^+ + H. $C_{31}H_{55}N_4O_{20}$ requires *m/z*, 803.34).

Tris-{2-[3-(2′,3′,4′-tri-*O*-benzoyl-6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)thioureido]ethyl}amine 21

To a solution of the mannoside **3** (394 mg, 0.72 mmol) in dry CH_2Cl_2 (40 ml) was added tris-(2-aminoethyl)amine **20** (31.9 mg, 0.218 mmol) slowly at 50 °C. The mixture was stirred at 50 °C until the reaction was complete (TLC ethyl acetate–light petroleum, 2:1). The solvent was removed *in vacuo* and the remaining syrup was purified by flash chromatography (ethyl acetate–light petroleum, 2:1) to yield the *title cluster* **21** (359 mg, 92%) as a slightly yellow amorphous solid (Found: C, 62.4; H, 5.25; N, 5.5. $C_{93}H_{93}N_7O_{24}S_3$ requires C, 62.44; H, 5.24; N, 5.48%); $[\alpha]_D^{20}$ −92.6 (*c* 1.79, $CHCl_3$); R_f (ethyl acetate–light petroleum, 2:1) 0.20 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 8.08, 7.93 and 7.80 (each 6 H, each d, ArH), 7.54–7.23 (27 H, m, ArH), 7.08 (6 H, br s, NH), 5.83 (3 H, dd, $J_{3',4'}$ 9.7, 3′-H), 5.70 (3 H, dd ≈ t, $J_{4',5'}$ 9.7, 4′-H), 5.61 (3 H, dd, $J_{2',3'}$ 3.1, 2′-H), 4.85 (3 H, d, $J_{1',2'}$ 2.0, 1′-H), 4.20 (3 H, br s, 5′-H), 4.11–3.94 (3 H, br s, 6′-H), 3.77–3.67 (3 H, br s, 6′-H), 3.58 [6 H, br s, $CH_2NHC(S)$], 3.42 (9 H, s, OCH_3) and 2.50 (6 H, s, NCH_2); δ_C(62.89 MHz; $CDCl_3$) 171.11, 165.89 and 165.40 (C=O), 133.46, 133.37 and 133.08 (aryl-C_q), 129.93–128.18 (aryl-CH), 98.51 (C-1′), 70.26 (C-2′), 69.76 (C-3′), 69.51 (C-5′), 68.02 (C-4′), 60.35 (C-6′), 55.60 (OCH_3), 53.43 (NCH_2) and 45.16 [$CH_2NHC(S)$]; *m/z* (FAB-MS) 1788.9 (M^+ + H. $C_{93}H_{94}N_7O_{24}S_3$ requires *m/z*, 1788.55).

Tris-{2-[3-(2′,3′,4′-tri-*O*-acetyl-6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)thioureido]ethyl}amine 22

To a solution of the mannoside **5** (317 mg, 0.877 mmol) in dry CH_2Cl_2 (30 ml) was added tris-(2-aminoethyl)amine **20** (39 mg, 0.266 mmol) slowly at 50 °C. The reaction mixture was stirred at 50 °C until the reaction was complete (TLC ethyl acetate–light petroleum, 2:1). The solvent was removed *in vacuo* and the remaining syrup was purified by flash chromatography (ethyl acetate) to yield the *title cluster* **22** (298 mg, 91%) as an amorphous solid (Found: C, 46.9; H, 6.15; N, 7.95; S, 7.8. $C_{48}H_{75}N_7O_{24}S_3$ requires C, 46.86; H, 6.14; N, 7.97; S, 7.82%); $[\alpha]_D^{20}$ +43.6 (*c* 0.84, $CHCl_3$); R_f (ethyl acetate–EtOH, 9:1) 0.39 (UV; H_2SO_4); δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.25 and 7.05 (3 H, br s, NH), 5.32 (3 H, dd, $J_{3',4'}$ 9.7, 3′-H), 5.23 (3 H, dd, $J_{2',3'}$ 3.6, 2′-H), 5.12 (3 H, dd ≈ t, $J_{4',5'}$ 10.2, 4′-H), 4.71 (3 H, d, $J_{1',2'}$ 1.5, 1′-H), 3.90 (3 H, m_c, 5′-H), 3.84–3.50 [12 H, br m, 6′-H_2, C(S)NHCH_2], 3.41 (9 H, s, OCH_3), 2.67 (6 H, br s, NCH_2) and 2.17, 2.09 and 1.99 (each 9 H, each s, 3 × Ac); δ_C(100.67 MHz; $CDCl_3$; Me_4Si) 182.82 (N*C*S), 170.07, 169.82 and 169.67 (C=O), 98.25 (C-1′), 69.36 (C-2′), 69.25 (C-5′), 68.77 (C-3′), 67.04 (C-4′), 55.28 (OCH_3), 52.62 (NCH_2), 45.05 (C-6′), 42.43 [C(S)NHCH_2] and 20.74, 20.73 and 20.49 [C(O)CH_3].

Tris-{2-[3-(6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)thioureido]ethyl}amine 23

(a) To a solution of the unprotected mannoside **8** (136 mg, 0.578 mmol) in EtOH–ethyl acetate (4:1; 10 ml) was added tris-(2-aminoethyl)amine **20** (25.6 mg, 0.175 mmol) slowly at 50 °C. The reaction mixture was stirred at 50 °C until the reaction was

complete (TLC ethyl acetate–light petroleum, 2:1). The solvent was removed *in vacuo* and the remaining syrup was purified by gel-permeation chromatography on Sephadex G-15 with 15 mM aq. NH_4HCO_3 as eluent to yield the *title cluster* **23** (35 mg, 23%) as a lyophilizate after repeated lyophilization with distilled deionized water until mass stability.

(b) A solution of the benzoylated cluster **21** (236 mg, 0.132 mmol) in dry 5:1 MeOH–DMF (60 ml) was treated with NaOMe solution (1 M in MeOH; 1.5 ml) and the reaction mixture was stirred at rt for 1 week. It was neutralized with ion-exchange resin (Levatit SP 1080 H^+), the resin was filtered off, and the filtrate was concentrated, and purified by size-exclusion chromatography on Sephadex G-15 (15 mM aq. NH_4HCO_3 as eluent). The *unprotected title cluster* **23** (30 mg, 27%) was obtained as a lyophilizate after repeated lyophilization with distilled, deionized water until mass stability.

(c) A solution of the acetylated cluster **22** (86 mg, 70 μmol) in dry MeOH (20 ml) was treated with NaOMe solution (1 M in MeOH; 1.5 ml) and the reaction mixture was stirred at rt until the deprotection was complete (TLC propan-1-ol–water, 7:3, 1% NH_3). The mixture was neutralized with ion-exchange resin (Levatit SP 1080 H^+), the resin was filtered off and the filtrate was concentrated, and purified by size-exclusion chromatography on Sephadex G-15 with (15 mM aq. NH_4HCO_3 as eluent). The *unprotected title cluster* **23** (49 mg, 82%) was obtained as a lyophilizate after repeated lyophilization with distilled, deionised water; $[\alpha]_D^{20}$ +14.0 (*c* 1.10, MeOH); R_f (propan-1-ol–water, 7:3, 1% NH_3) 0.37 (UV; H_2SO_4); δ_H(400 MHz; D_2O) 5.03 (3 H, d, $J_{1',2'}$ 1.5, 1′-H), 4.19 (3 H, dd, $J_{2',3'}$ 3.6, 2′-H), 4.01 (3 H, dd, $J_{3',4'}$ 9.7, 3′-H), 4.10–3.92 [15 H, m, C(S)NHCH_2, 5′-H and 6′-H_2], 3.86 (3 H, dd ≈ t, $J_{4',5'}$ 9.7, 4′-H), 3.67 (9 H, s, OCH_3) and 3.29 (6 H, br s, NCH_2); δ_C(100.67 MHz; D_2O, CD_3CN) 182.76 (NCS), 101.80 (C-1′), 71.76, 71.26, 70.81 and 68.80 (C-2′, -3′, -4′ and -5′), 55.50 (OCH_3), 53.83 (NCH_2), 45.54 (C-6′) and 42.00 [C(S)NHCH_2]; *m/z* (FAB-MS) 852 (M^+ + H. $C_{30}H_{58}N_7O_{15}S_3$ requires *m/z*, 852.32).

Tris-(2-{3-[6′-deoxy-1′-*O*-(*p*-nitrophenyl)-α-D-mannopyranos-6′-yl]thioureido}ethyl)amine 24

To a solution of the aromatic mannoside **12** (291 mg, 0.851 mmol) in dry CH_2Cl_2 (20 ml) was added tris-(2-aminoethyl)-amine **20** (34 mg, 0.234 mmol) slowly at 50 °C. The mixture was stirred at 50 °C until the reaction was complete (TLC ethyl acetate–MeOH–water, 7:2:1). The solvent was removed *in vacuo* and the remaining syrup was purified by flash chromatography (ethyl acetate–MeOH, 10:1, 1% methanolic NH_3; then ethyl acetate–MeOH–water, 7:2:1, 1% methanolic NH_3) to afford the *title cluster* **24** (244 mg, 89%) as a lyophilizate (Found: C, 46.2; H, 5.2; N, 11.9; S, 8.2. $C_{45}H_{60}N_{10}O_{21}S_3$ requires C, 46.07; H, 5.15; N, 11.94; S, 8.20%); $[\alpha]_D^{20}$ +89.9 (*c* 0.65, MeOH); R_f (ethyl acetate–MeOH–water, 7:2:1, 1% NH_3) 0.77 (UV; H_2SO_4); δ_H(400 MHz; D_3COD) 8.23 (6 H, d, ^3J 9.67, *m*-ArH), 7.30 (6 H, d, ^3J 9.16, *o*-ArH), 5.72 (3 H, d, $J_{1',2'}$ 1.5, 1′-H), 4.10 (3 H, dd, $J_{2',3'}$ 3.6, 2′-H), 3.96 (3 H, dd, $J_{3',4'}$ 9.2, 3′-H), 3.87 (3 H, ddd, $J_{5',6'}$ 5.1, 5′-H), 3 H, dd, $J_{6',6'}$ 10.2, 6′-H), 3.71 (3 H, dd ≈ t, $J_{4',5'}$ 9.7, 4′-H), 3.66 (3 H, dd, $J_{5',6''}$ 3.6, 6′-H), 3.47 [6 H, br s, C(S)NHCH_2] and 2.66 (6 H, br s, NCH_2); δ_C(100.67 MHz; D_3COD) 162.73 (*i*-aryl-C_q), 144.10 (*p*-aryl-C_q), 127.11 and 118.19 (aryl-CH), 100.16 (C-1′), 74.45, 72.03, 71.78 and 69.36 (C-2′), -3′, -4′ and -5′), 54.55 (NCH_2), 46.39 (C-6′) and 43.91 [C(S)NHCH_2]; *m/z* (MALDI-TOF) 1173.5 (M^+ + H. $C_{45}H_{61}N_{10}O_{21}S_3$ requires *m/z*, 1173.32).

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References

1 T. Feizi, *Curr. Opin. Struct. Biol.*, 1993, **3**, 701.
2 A. Varki, *Glycobiology*, 1993, **3**, 97.
3 T. A. Springer, *Cell*, 1994, **76**, 301.
4 L. A. Larsky, *Annu. Rev. Biochem.*, 1995, **64**, 113.
5 K.-A. Karlsson, *Curr. Opin. Struct. Biol.*, 1995, **5**, 622.
6 A. Giannis, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 178.
7 U. Sprengard, M. Schudok, W. Schmidt, G. Kretzschmar and H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 321.
8 S. A. D. Frees, W. Kosch, W. Way, J. C. Paulson, S. Sabesan, R. L. Halcomb and D.-H. Huang, *J. Am. Chem. Soc.*, 1995, **117**, 66.
9 *Toward anti-adhesion therapy for microbial diseases*, ed. I. Kahane and I. Ofek, *Adv. Exp. Med. Biol.*, 1996, **408**.
10 R. Roy, D. Zanini, S. J. Meunier and A. Romanowska, *J. Chem. Soc., Chem. Commun.*, 1993, 1869.
11 H. C. Hansen, S. Haataja, J. Finne and G. Magnusson, *J. Am. Chem. Soc.*, 1997, **119**, 6974.
12 Th. K. Lindhorst, C. Kieburg and U. Krallmann-Wenzel, *Glycoconjugate J.*, 1998, **15**, in the press.
13 U. Krallmann-Wenzel, D. Pagé, C. Kieburg, S. Ehlers, R. Roy and Th. K. Lindhorst, unpublished results.
14 H. Conell, W. Agace, P. Klemm, M. Schembri, S. Mårlid and C. Svanborg, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 9827.
15 E. V. Sokurenko, V. Chesuohova, R. J. Doyle and D. L. Hastky, *J. Biol. Chem.*, 1997, **272**, 17 880.
16 N. Firon, I. Ofek and N. Sharon, *Carbohydr. Res.*, 1983, **120**, 235.
17 N. Firon, I. Ofek and N. Sharon, *Infect. Immun.*, 1984, **43**, 1088.
18 J. R. Neeser, B. Koehlreutter and P. Wuersch, *Infect. Immun.*, 1986, **52**, 428.
19 N. Firon, S. Ashkenazi, D. Mirelman, I. Ofek and N. Sharon, *Infect. Immun.*, 1987, **55**, 472.
20 N. Sharon, *FEBS Lett.*, 1987, **217**, 145.
21 W. I. Weiss and K. Drickamer, *Annu. Rev. Biochem.*, 1996, **65**, 441.
22 Y. C. Lee, *Carbohydr. Res.*, 1978, **67**, 509.
23 E. A. L. Biessen, D. M. Beuting, H. C. P. F. Roelen, G. A. v. d. Marel, J. H. v. Boom and T. J. C. v. Berkel, *J. Med. Chem.*, 1995, **38**, 1538.
24 P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev and J. F. Stoddart, *Chem. Eur. J.*, 1996, **2**, 1115.
25 G. R. Newkome, C. N. Moorefield and K. J. Theriot, *J. Org. Chem.*, 1988, **53**, 5552.
26 W. Liao, D. Lu, A. Li and F. Kong, *J. Carbohydr. Chem.*, 1997, **16**, 877.
27 S. Jacobsen, *Acta Chem. Scand., Sect. B*, 1984, **38**, 157.
28 B. Schönecker and K. Ponsold, *J. Prakt. Chem.*, 1976, **318**, 483.
29 J. M. G. Fernández, C. O. Mellet and J. Fuentes, *J. Org. Chem.*, 1993, **58**, 5192.
30 P. Wang, G.-J. Shen, Y.-F. Wang, Y. Ichikawa and C.-H. Wong, *J. Org. Chem.*, 1993, **58**, 3985.
31 R. R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1984, 1343.
32 W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
33 Y. S. Klausner and M. Bodansky, *Synthesis*, 1972, 453.
34 Th. Lindhorst and C. Kieburg, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1953.
35 K. A. Krogfelt, H. Bergmans and P. Klemm, *Infect. Immun.*, 1990, **58**, 1995.
36 Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321.
37 P. Klemm, B. J. Jørgensen, I. v. Die, H. d. Ree and H. Bergmans, *Mol. Gen. Genet.*, 1985, **199**, 410.
38 E. V. Sokurenko, V. A. McMackin and D. L. Hasty, *Methods Enzymol.*, 1995, **253**, 519.
39 N. A. Nnalue, A. Weintraub, S. Oscarson and A. A. Lindberg, *Eur. J. Biochem.*, 1994, **220**, 973.
40 H.-M. Kuhn, L. Brade, B. J. Appelmelk, S. Kusumoto, E. T. Rietschel and H. Brade, *Infect. Immun.*, 1992, **60**, 2201.
41 S. Cottaz, J. S. Brimacombe and M. A. J. Ferguson, *Carbohydr. Res.*, 1993, **247**, 341.

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