Trivalent α-D-mannoside clusters as inhibitors of type-1 fimbriae-mediated adhesion of *Escherichia coli*: structural variation and biotinylation

Thisbe K. Lindhorst,[a]* Sven Kötter,[a] Ulrike Krallmann-Wenzel[b] and Stefan Ehlers[b]

[a] *Institute of Organic Chemistry, Christiana Albertina University of Kiel, Otto-Hahn-Platz 4, D-24098 Kiel, Germany. E-mail: tklind@oc.uni-kiel.de; Fax +49 431 880 7410*
[b] *Division of Molecular Infection Biology, Research Center Borstel, Parkallee 22, D-23845 Borstel, Germany. E-mail: sehlers@fz-borstel.de; Fax +49 4537 188 481*

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Structural modifications of trivalent cluster mannosides are presented to further elucidate the ligand preferences of the type-1 fimbrial lectin of *Escherichia coli*. Two types of variations are performed, either regarding the aglycone part of cluster mannosides of type **2**, leading to **27**, or altering the spacer lengths of mannosyl clusters of type **1**, leading to clusters **20**–**22**. Biotinylation of the cluster mannoside with the highest affinity to the type-1 fimbrial lectin is also shown (**33**). Testing of the inhibitory potencies of the synthesised cluster glycosides as inhibitors of mannose-specific (type-1 fimbriae-mediated) binding of *E. coli* to mannan in an enzyme-linked immunosorbent assay (ELISA) suggests that a structural preorganisation as given in cluster **2a** can be favourably combined with greater spacer flexibility as in cluster **22**.

Introduction

Bacteria often use their own specialised proteins, so-called lectins, to interact with carbohydrate structures on the surface of potential host cells.[1] By establishing many of such carbohydrate–protein contacts, bacteria accomplish adhesion to the host cell surface, an event which is of crucial importance for cell infection. Synthetic carbohydrate derivatives have been designed to interfere in the microbial adhesion process by competing for the carbohydrate-recognition domains of the involved lectins.[2] This has been demonstrated to work *in vitro*,[3] to allow the evaluation of such an approach towards an anti-adhesion therapy to be used for certain *in vivo* applications.[4]

As mannose-specific adhesion is among the most widely distributed types of carbohydrate-specific bacterial adhesion,[5] our interest has been directed towards the development of oligomannoside mimetics to serve as high-affinity inhibitors in this system. Mannose-specific adhesion of bacteria is mediated by so-called type-1 fimbriae, long proteinogenous appendages on the bacterial surface, which contain several copies of the relevant carbohydrate-recognition domains. The adhesin is called the FimH protein, which has received much attention recently.[6] It is encoded by the *fimH* gene within the gene cluster responsible for type-1 fimbriae assembly.[7]

As part of our former work we have synthesised a series of trisaccharide mimetics to serve as ligands for the type-1 fimbrial adhesin.[8] These trivalent glycoclusters either were obtained by mannosylation of a branched oligo-alcohol to yield cluster mannosides such as compound **1** (Scheme 1) or were the result of a peptide-coupling reaction of a triacid and a 6-amino-6-deoxymannoside, which can be regarded as a 'glycoside donor' (Scheme 1). This approach led to cluster glycosides such as **2a** and allows us to vary the aglycone part to provide an array of clusters such as **2b**. Clusters **1** and **2b** were shown to serve as particularly successful inhibitors of type-1 fimbriae-mediated adhesion of *Escherichia coli* to high mannose-type surfaces,[9] having up to 10^3-fold better inhibitory potencies than monovalent methyl α-D-mannoside (MeMan). As the conformational preorganisation as well as conformational flexibility of the sugar epitopes have to be regarded as important parameters for ligand binding, we decided to vary the structures of cluster mannosides **1** and **2** in order to obtain more information about the investigated ligand–receptor system from additional data on structure–activity relationships.

By combination of the synthetic strategies which led to **1** and **2** we planned to vary (i) the length of spacers and (ii) the aglycone moieties of related glycoclusters. Furthermore, it was of interest to utilise the architecture of the cluster mannosides prepared, to introduce a bio-label, *e.g.* by biotinylation.

Results and discussion

Synthetic part

To provide a collection of spacer-modified cluster mannosides, we set out to synthesise a set of 'mannoside donors' with ω-amino-functionalised aglycone moieties. Three different ω-halogen-functionalised alkyl and ethoxyalkyl alcohols (**3**, **4**, and **5**, respectively) were used as glycosyl acceptors in the glycosylation reactions which eventually led to the desired 'glycoside donors' **16**, **17**, and **18** (Scheme 2). The mannosyl trichloroactimidate **6**[10] served as glycosyl donor in the Lewis acid-catalysed reaction leading to the acetyl-protected glycosides **7**, **8**, and **9** in excellent yields. In the next synthetic step the halogen substituents in the aglycone moieties of these glycosides could be substituted by azide in almost quantitative reactions to give mannosides **10**, **11**, and **12**, respectively, which were in turn de-acetylated under Zemplén conditions[11] and subsequently reduced to the unprotected ω-amino-functionalised mannosides **16**, **17**, and **18**. These could be taken through an 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)-assisted peptide-coupling reaction with the triacid **19**[12] (Scheme 3) without further protection and deprotection steps to provide the trivalent cluster mannosides **20**, **21**, and **22**, which were tested as inhibitors of type-1 fimbriae-mediated bacterial adhesion (*vide supra*).

In the second synthetic part, it was our goal to replace the methyl aglycone groups in cluster mannoside **2a** by *p*-nitrophenyl moieties. This is of interest as aromatic moieties can increase the affinity of a given carbohydrate ligand to its

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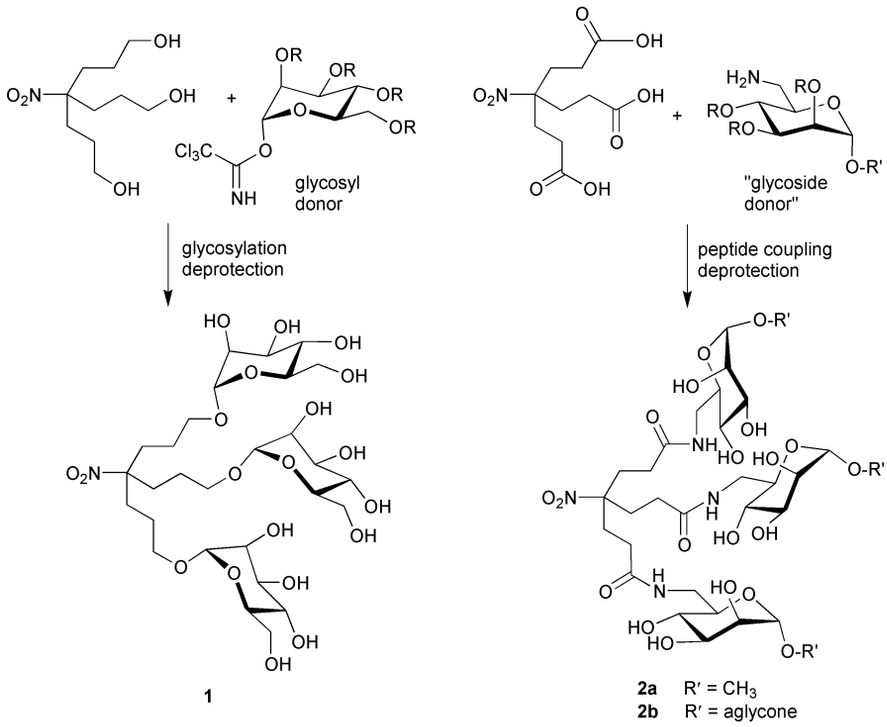
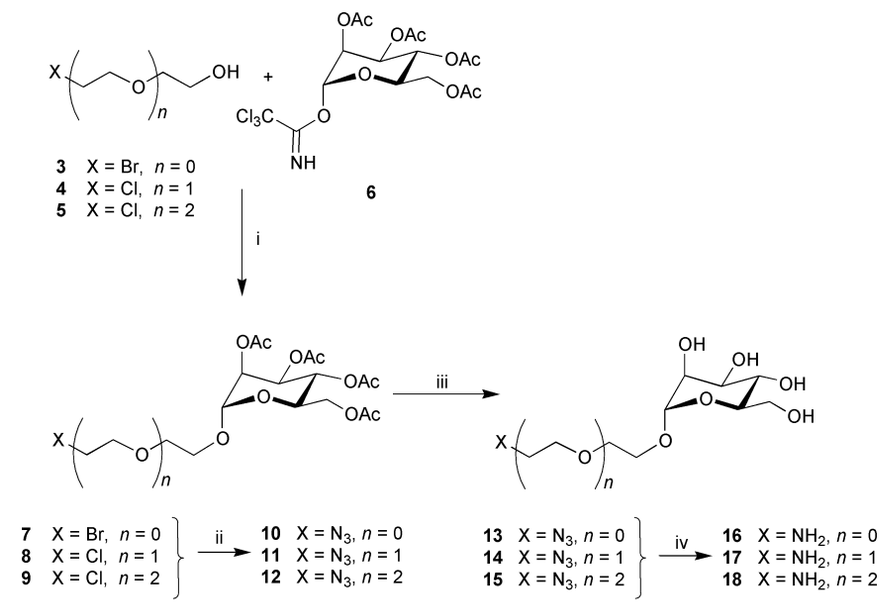


Scheme 1 Cluster glycosides can be obtained by glycosylation of branched oligo-alcohols with classical glycosyl donors (route to cluster **1**) or by (peptide) coupling of functionalised glycosides, which can be regarded as 'glycoside donors' to suitable core molecules (route to clusters **2**). The latter route allows variation of the aglycone moiety R′.



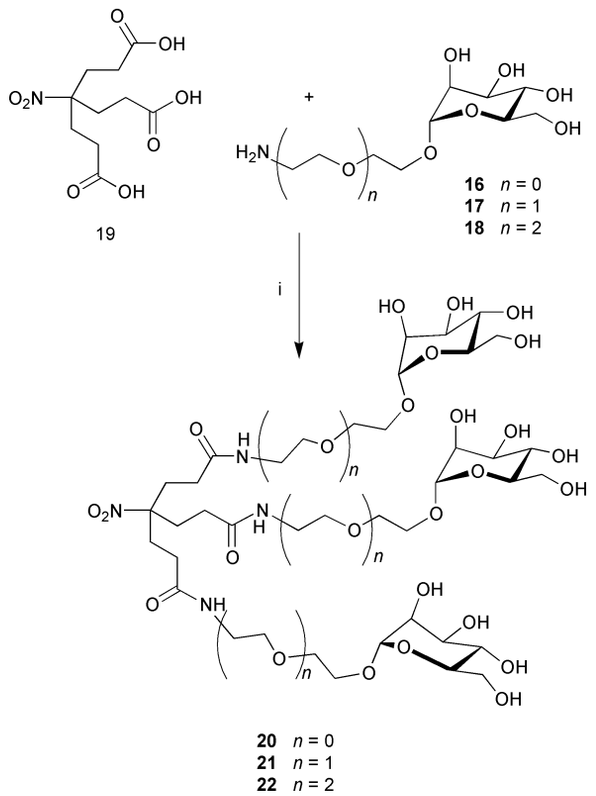
Scheme 2 *Reagents, conditions, and yields*: i, $BF_3{\cdot}Et_2O$ (for **3**), TMSOTf (for **4** and **5**), CH_2Cl_2, rt, 96% (**7**), 92% (**8**), 89% (**9**); ii, NaN_3, DMF, 50–60 °C, 98% (**10**), 99% (**11**), 97% (**12**); iii, NaOMe, MeOH, rt, 99% (**13**), 99% (**14**), 97% (**15**); iv, H_2, Pd–C (10%), 1 : 1 ethyl acetate–EtOH, 98% (**16**), 98% (**17**), 98% (**18**).

lectin receptor by hydrophobic interactions. The inhibitory potency of *p*-nitrophenyl α-D-mannoside (*p*NPMan), for example, is approximately 100 times higher than that of the respective methyl mannoside.[3a,5] As cluster glycoside **2a** was shown to be an excellent ligand for the type-1 fimbrial lectin earlier by our group[9] it could be expected that its *p*NP analogue **27** would perform even better.

For the preparation of **27** a 6-amino-6-deoxy-functionalised *p*NP mannoside was required, which was obtained starting from *p*NPMan **23**. Regioselective activation of the 6-position followed by nucleophilic displacement of the sulfonate from **24** led to the 6-azido-6-deoxymannoside **25** (Scheme 4).[9] For the reduction of the azide function in **25** catalytic hydrogenation could not be used as the *p*-nitro group had to be kept intact. Finally, Staudinger reduction[13] was the method of choice after it was found that the intermediate triphenylphosphine which was formed in the reaction of **25** with triphenylphosphine could be readily hydrolysed using silica gel. Peptide coupling of the unprotected amine **26** with the triacid **19** gave the cluster mannoside **27** in excellent yield, carrying three *p*-nitrophenyl α-D-mannosyl units.

In order to facilitate biological testing, the third part of this study was dedicated to biotinylation of trivalent cluster mannosides. This was exemplified using cluster **2a**, as it is the most potent inhibitor of mannose-specific bacterial adhesion in a series of synthetic trivalent cluster mannosides.[8a] To allow biotinylation, we tried to reduce the nitro group in **2a** to generate an amino function; however, all our attempts led to

unsatisfactory results. Therefore, the desired amino group was introduced at an earlier stage of the synthesis. The tris(*tert*-butyl ester) **28**[12] was reduced to the γ-amino triacid **29** (Scheme 5), followed by introduction of the biotin label to yield **30** in excellent yield. The *tert*-butyl esters were cleaved under acidic conditions, followed by peptide coupling with methyl 6-amino-6-deoxy-α-D-mannoside **32**[14] to yield the desired biotinylated trivalent cluster mannoside **33** in a clean reaction. The structures of all prepared cluster mannosides could unequivocally be confirmed by means of NMR spectroscopy. The obtained data were supported by matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry in all cases.

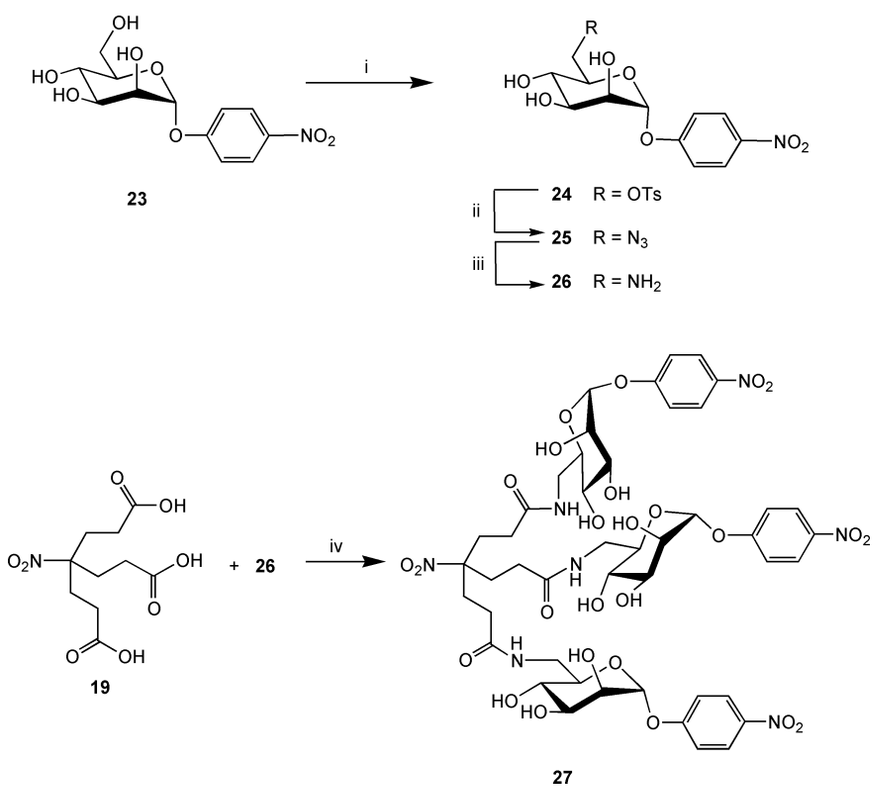


Scheme 3 *Reagents, conditions, and yields*: i, 3.3 equiv. amine (**16**, **17**, or **18**, respectively), EEDQ, DMA, 60 °C, 4 d, 74% (**20**), 69% (**21**), 59% (**22**).

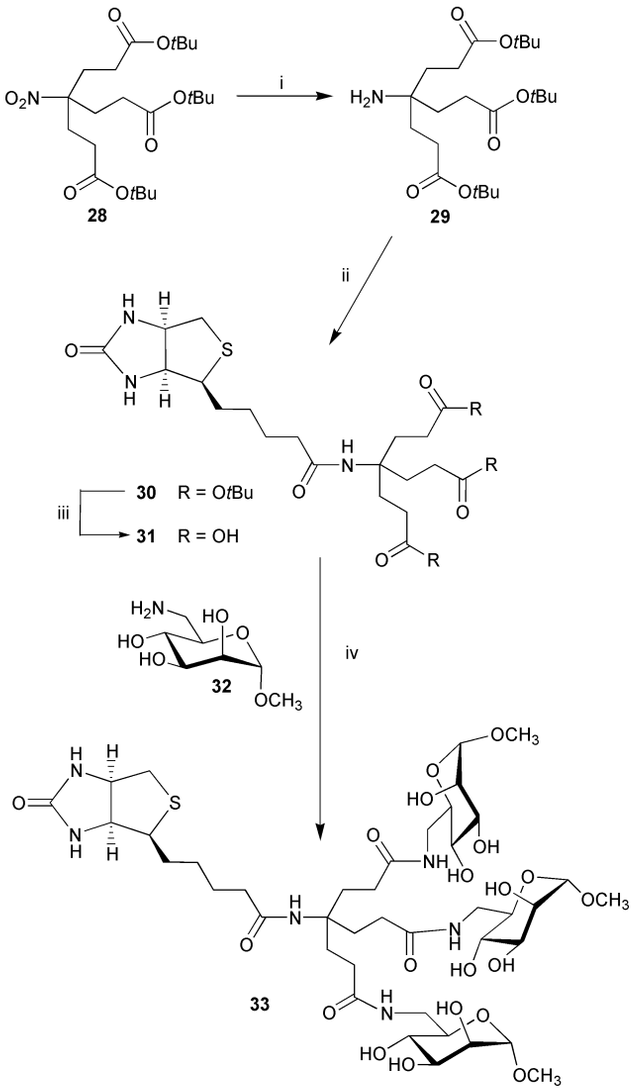
Testing part

The prepared cluster mannosides were tested for their capacity to inhibit mannose-specific adhesion of *E. coli*, using a bacterial strain, *E. coli* HB 101 (pPKl4), which expresses only type-1 fimbriae on its surface.[15] As assay a sandwich-type enzyme-linked immunosorbent assay (ELISA) was used in which the microtitre plate was coated with yeast mannan.[9] Adhesion of bacteria and inhibition of adhesion were determined *via* an antibody–enzyme conjugate-based colour reaction. These ELISAs allowed us to measure IC_{50}-values for the inhibition of *E. coli* adhesion which 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 individual ELISA is very small, whereas the IC_{50}-values obtained from several independently performed ELISAs differ significantly. However, when relative inhibition potencies are calculated from independently obtained data, the results are highly reproducible. The results obtained are listed in Table 1. IC_{50}-Values are average values from five independent ELISA experiments and are depicted together with the resulting standard deviations.

The new clusters were compared with the known trivalent cluster mannosides **1** and **2a** (Scheme 1), which were shown earlier to surpass the inhibitory potency of *p*NPMan without containing an aromatic moiety. Unfortunately, the water solubility of **27**, the *p*NP analogue of **2a**, was not good enough to provide reliable data. The spacer-modified cluster mannosides **20**, **21**, and **22**, however, revealed interesting results. None of these compounds reached the inhibitory potency of **1** or **2a**.



Scheme 4 *Reagents, conditions, and yields*: i, TsCl, pyridine, 0 °C ⟶ rt, 57%; ii, NaN_3, DMF, 80 °C, 91%; iii, Ph_3P, silica gel, 4 : 1 THF–water, 45 °C, 98%; iv, EEDQ, DMA, 5 d, 40 °C, 91%.



Scheme 5 *Reagents, conditions, and yields*: i, H_2, T1 Raney-nickel, EtOH, 3 bar, 2 d, 60 °C, 99%; ii, biotin, EDC–1-HOBT (1 : 2), DIPEA, DMF, 0 °C ⟶ rt, 97%; iii, HCO_2H, 99%; iv, EDC–1-HOBT (1 : 2), DIPEA, DMF, 0 °C ⟶ rt, 91%.

Table 1 IC_{50}-Values measured by ELISA are listed together with the relative values based on MeMan. IC_{50}-Values are average values from five independent ELISA experiments and the resulting standard deviations are indicated

	MeMan	*p*NPMan	**1**	**2a**	**20**	**21**	**22**
IC_{50} (μM)[a]	4250	43	17	4	238	222	134
S^b	1173	14	5	0.7	90	65	59
RIC_{50}[c]	1	99	250	1063	18	19	32

[a] Obtained by ELISA. [b] Standard deviation. [c] Relative IC_{50}-values based on methyl α-D-mannoside (MeMan) as standard.

This suggests that the structural positioning of the α-mannosyl units especially in **2a** is advantageous over the other tested analogues for receptor binding and was disrupted by increased length of the spacers. While **20** and **21** performed almost equally well, the most flexible cluster glycoside prepared so far, **22**, showed a significant increase in inhibitory potency compared with its less flexible counterparts **20** and **21**.

While it seems that more flexibility of the α-mannosyl moieties, as presented in glycoclusters **20**–**22**, is appreciated in the molecular recognition process, the structural preorganisation which is provided in the structurally very different cluster mannosides **1** and **2a** is of greater importance for lectin binding in this case. These data suggested that we combine a structural assembly of mannosyl units as in **2a** with a flexible spacer architecture as given in **22**. Thus, our future work will be directed towards the multiple, flexible assembly of glycocluster **2a** in a multivalent neoglycoconjugate, which can be provided by the principal architecture as chosen in **22**.

In summary, we showed the synthesis of spacer-modified cluster mannosides **20**, **21**, and **22** in excellent yields and demonstrated the utilisation of the contained nitro group for biotinylation. Biotinylation of the cluster mannoside adds an option for assaying biological functions. An aglycone-modified analogue of cluster mannoside **2a** could also be prepared containing three *p*-nitrophenyl moieties, which, unfortunately, showed limited water solubility and could, therefore, not be tested for its affinity to the type-1 fimbrial lectin. The inhibitory potencies of clusters **20**, **21**, and **22** did not surpass that of 'lead' cluster **2a**. However, the most flexible cluster **22** showed almost double the affinity of its counterparts **20** and **21**. These results suggest attempts should be made to combine the structural characteristics of clusters **2a** and **22**, which will be subject of our future work.

Experimental

Materials

Methyl α-D-mannoside was purchased from Merck, *p*-nitrophenyl α-D-mannoside was from SENN Chemicals. The ω-halogen-substituted alcohols **3**, **4**, and **5** were purchased from Aldrich. F-Shaped 96-well microtitre plates from Sarstedt were used for ELISAs. Mannan from *Saccharomyces cerevisiae* was purchased from Sigma and was used in 50 mM aq. Na_2CO_3 (1 mg cm^{-3}; pH 9.6). A polyclonal anti-fimA antibody was used for ELISAs, and was produced at Borstel Research Centre. All the other material needed for ELISA as well as the required buffers was used as reported earlier. The recombinant type-1 fimbriated *E. coli* strain, *E. coli* HB101 (pPKl4),[15] used was cultured as reported in the literature.[3a,9] Light petroleum refers to the fraction with boiling range 40–60 °C.

General methods

Optical absorbance (*A*) was measured on a Dynatech 5000 ELISA reader at 405 nm with the reference red at 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 Macherey 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-2-ol, 1 : 1) with subsequent heating. NMR spectra were measured on a Bruker AMX 400 (400 MHz for ^1H and 100.67 MHz for ^{13}C NMR) or a DRX 500 (500 MHz for ^1H and 125.84 MHz for ^{13}C NMR). Chemical shifts are in ppm, relative to internal $SiMe_4$ (TMS) (0.00 ppm for ^1H and ^{13}C NMR) or solvent peaks which were calibrated as follows: $CDCl_3$ (δ 7.26 for ^1H and 77.00 for ^{13}C NMR), D_2O [δ 4.65 for ^1H; for ^{13}C NMR CD_3CN (δ 1.30) or D_6-acetone (δ 30.60) was added], D_4-methanol (δ 3.35 for ^1H and 49.30 for ^{13}C NMR). *J*-Values are in Hz. Where necessary, two-dimensional ^1H–^1H or ^1H–^{13}C homonuclear/two-dimensional correlation spectroscopy (COSY) experiments were performed for complete signal assignments. Optical-rotation values were obtained using a Perkin-Elmer polarimeter 341 or 243 (Na-D line, 589 nm, cell length 10 cm). $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra were measured on a VG Analytical 70-250S (FAB MS) or a Finnigan MAT 95 (MALDI-TOF MS) instrument. ELISAs were performed as described earlier. For ELISA controls, bacterial adhesion to blocked, uncoated microtitre 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 $[A(\mathrm{nI}) - A(\mathrm{I})] \times 100 \times [A(\mathrm{nI})]^{-1}$ (nI: no inhibitor, I: with inhibitor).

2-Bromoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 7

2-Bromoethanol **3** (345 mg, 2.76 mmol) and the trichloroacetimidate **6** (1.63 g, 3.31 mmol) were dissolved in dry CH_2Cl_2 (50 cm^3) and the glycosylation reaction was started by the addition of $BF_3{\cdot}Et_2O$ (0.01 cm^3) at rt. After stirring of the mixture at rt for 15 h, triethylamine (0.2 cm^3) was added, the reaction mixture was concentrated under reduced pressure and the residual syrup was purified by flash chromatography (toluene–ethyl acetate, 4 : 1) to give the desired *mannopyranoside* **7** (1.21 g, 96%) as an amorphous white solid (Found: C, 42.24; H, 5.12; Br, 17.45. $C_{16}H_{23}BrO_{10}$ requires C, 42.21; H, 5.09; Br, 17.55%); $[\alpha]_D^{20}$ +37.3 (*c* 0.75 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.32 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 9.7, 3-H), 5.25 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 5.25 (1 H, dd, 2-H), 4.85 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.24 (1 H, dd, $J_{5,6}$ 6.1, 6-H), 4.11 (1 H, dd, $J_{5,6'}$ 2.04, $J_{6,6'}$ 12.7, 6-H), 4.11 (1 H, m_c, 5-H), 3.91 (2 H, m_c, OCH_2-CH_2Br), 3.49 (2 H, t, *J* 6.1, OCH_2CH_2Br), 2.13, 2.08, 2.03, 1.97 (each 3 H, 4 s, 4 × $COCH_3$); δ_C (100.67 MHz; $CDCl_3$; Me_4Si) 170.65, 170.04, 169.89, 169.78 (4 × $COCH_3$), 97.73 (C-1), 69.42, 69.00, 68.95, 68.48 (C-2, -3, -4, -5), 66.00 (OCH_2CH_2Br), 62.41 (C-6), 29.60 (OCH_2CH_2Br), 20.84, 20.73, 20.68, 20.65 (4 × $COCH_3$); *m/z* (MALDI-TOF) 455.25 (M^+ + H. $C_{16}H_{24}$-BrO_{10} requires *m/z* 455.06), *m/z* (MALDI-TOF) 477.23 (M^+ + Na. $C_{16}H_{23}BrNaO_{10}$ requires *m/z* 477.04), *m/z* (MALDI-TOF) 493.18 (M^+ + K. $C_{16}H_{23}BrKO_{10}$ requires *m/z* 493.01).

2-(2-Chloroethoxy)ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 8

The alcohol **4** (698 mg, 5.60 mmol) and the trichloroacetimidate **6** (3.31 g, 6.72 mmol) were dissolved in dry CH_2Cl_2 (60 cm^3) and the glycosylation reaction was started by the addition of a solution of trifluoromethyl trifluoromethanesulfonate (TMSOTf) in dry CH_2Cl_2 (0.02 M; 0.2 cm^3) at 0 °C. After stirring of the mixture at rt for 4 h, additional TMSOTf solution (0.4 cm^3) was added and then the reaction mixture was stirred for 40 h at rt. Triethylamine (0.2 cm^3) was added, the mixture was concentrated under high vacuum and the residue was purified by flash chromatography (ethyl acetate–toluene, 3 : 1) to yield the *title glycoside* (2.35 g, 92%) as a colourless syrup (Found: C, 47.57; H, 6.04; Cl, 7.72. $C_{18}H_{27}ClO_{11}$ requires C, 47.53; H, 5.98; Cl, 7.79%); $[\alpha]_D^{20}$ +120.5 (*c* 0.75 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.37 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.8, 3-H), 5.29 (1 H, dd ≈ t, $J_{4,5}$ 9.8, 4-H), 5.27 (1 H, dd, 2-H), 4.89 (1 H, d, $J_{1,2}$ 1.6, 1-H), 4.29 (1 H, dd, $J_{5,6}$ 6.6, 6-H), 4.11 (1 H, dd, $J_{5,6'}$ 2.2, $J_{6,6'}$ 12.6, 6-H′), 4.13–4.10 (1 H, m, 5-H), 3.86–3.81 (1 H, m, $OCHCH_2OCH_2CH_2Cl$), 3.77–3.75 (2 H, m, OCH_2-$CH_2OCH_2CH_2Cl$), 3.71–3.69 (3 H, m, $OCHCH_2OCH_2$-CH_2Cl), 3.63 (2 H, t, *J* 5.7, $OCH_2CH_2OCH_2CH_2Cl$), 2.16, 2.11, 2.04, 1.99 (each 3 H, 4 s, 4 × $COCH_3$); δ_C (100.67 MHz; $CDCl_3$; Me_4Si) 170.64, 170.02, 169.89, 169.73 (4 × $COCH_3$), 97.61 (C-1), 71.37 ($OCH_2CH_2OCH_2CH_2Cl$), 70.05 (OCH_2CH_2-OCH_2CH_2Cl), 69.52 (C-2), 69.06 (C-3), 68.39 (C-5), 67.13 ($OCH_2CH_2OCH_2CH_2Cl$), 66.13 (C-4), 62.43 (C-6), 42.80 (OCH_2CH_2Cl), 20.85, 20.72, 20.65, 20.64 (4 × $COCH_3$); *m/z* (MALDI-TOF) 477.04 (M^+ + Na. $C_{18}H_{27}ClNaO_{11}$ requires *m/z* 477.11), *m/z* (MALDI-TOF) 493.02 (M^+ + K. $C_{18}H_{27}$-$ClKO_{11}$ requires *m/z* 493.09).

2-[2-(2-Chloroethoxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 9

The alcohol **5** (673 mg, 3.99 mmol) and the trichloroacetimidate **6** (2.36 g, 4.79 mmol) were dissolved in dry CH_2Cl_2 (50 cm^3) and the glycosylation reaction was started by the addition of a solution of TMSOTf in dry CH_2Cl_2 (0.02 M; 0.05 cm^3) at 0 °C. After stirring of the mixture at rt for 1 h, additional TMSOTf solution (0.3 cm^3) was added and then the reaction mixture was stirred for 12 h at rt. Triethylamine (0.2 cm^3) was added, the mixture was concentrated under high vacuum and the residue was purified by flash chromatography (ethyl acetate–light petroleum, 1 : 1) to yield the *title glycoside* (1.78 g, 89%) as a colourless syrup (Found: C, 48.13; H, 6.28; Cl, 7.05. $C_{20}H_{31}ClO_{12}$ requires C, 48.15; H, 6.26; Cl, 7.11%); $[\alpha]_D^{20}$ +119.1 (*c* 0.30 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.36 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 10.2, 3-H), 5.29 (1 H, dd ≈ t, 4-H), 5.27 (1 H, dd, 2-H), 4.88 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.30 (1 H, dd, $J_{5,6}$ 5.1, 6-H), 4.11 (1 H, dd, $J_{5,6'}$ 2.3, $J_{6,6'}$ 12.2, 6-H′), 4.07 (1 H, ddd, $J_{4,5}$ 10.2, 5-H), 3.86–3.76 (3 H, m, $OCHCH_2OCH_2CH_2$-OCH_2CH_2Cl), 3.70–3.63 (9 H, m, $OCHCH_2OCH_2CH_2$-OCH_2CH_2Cl), 2.16, 2.11, 2.06, 1.99 (each 3 H, 4 s, 4 × $COCH_3$); δ_C (100.67 MHz; $CDCl_3$; Me_4Si) 170.65, 170.01, 169.87, 169.70 (4 × $COCH_3$), 97.69 (C-1), 71.36 ($OCH_2CH_2OCH_2CH_2OCH_2$-CH_2Cl), 70.70, 70.63, 70.01 ($OCH_2CH_2OCH_2CH_2OCH_2CH_2$-Cl), 69.56 (C-2), 69.04 (C-3), 68.39 (C-5), 67.36 (OCH_2CH_2-$OCH_2CH_2OCH_2CH_2Cl$), 66.13 (C-4), 62.39 (C-6), 42.73 (OCH_2CH_2Cl), 20.87, 20.72, 20.67, 20.65 (4 × $COCH_3$); *m/z* (MALDI-TOF) 521.82 (M^+ + Na. $C_{20}H_{31}ClNaO_{12}$ requires *m/z* 521.14), *m/z* (MALDI-TOF) 537.63 (M^+ + K. $C_{20}H_{31}$-$ClKO_{12}$ requires *m/z* 537.11).

2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 10

The bromo-substituted mannoside **7** (1.2 g, 2.64 mmol) and sodium azide (1.37 g, 21 mmol) were dissolved in dry DMF (50 cm^3) and the reaction mixture was stirred at 60 °C for 15 h. The solvent was removed under high vacuum and the residue was dissolved in ethyl acetate (50 cm^3) and filtered. The filtrate was evaporated under reduced pressure and purified by flash chromatography (light petroleum–ethyl acetate, 1 : 1) to yield the *title azide* (1.08 g, 98%) as an amorphous solid (Found: C, 46.09; H, 5.58; N, 10.02. $C_{16}H_{23}N_3O_{10}$ requires C, 46.04; H, 5.55; N, 10.07%); $[\alpha]_D^{20}$ +50.2 (*c* 0.75 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.37 (1 H, dd, $J_{2,3}$ 3.1, $J_{3,4}$ 10.2, 3-H), 5.30 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 5.28 (1 H, dd, 2-H), 4.88 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.30 (1 H, dd, $J_{5,6}$ 5.6, 6-H), 4.13 (1 H, dd, $J_{5,6'}$ 2.5, $J_{6,6'}$ 12.2, 6-H′), 4.05 (1 H, ddd, 5-H), 3.88 (1 H, m_c, $OCHH$), 3.68 (1 H, m_c, $OCHH$), 3.48 (2 H, m_c, CH_2N_3), 2.22, 2.11, 2.06, 2.00 (each 3 H, 4 s, 4 × $COCH_3$); δ_C (100.67 MHz; $CDCl_3$) 170.59, 169.98, 169.78, 169.73 (4 × $COCH_3$), 97.74 (C-1), 69.38 (C-2), 68.85 (C-3, C-5), 67.02 (OCH_2CH_2), 66.01 (C-4), 62.45 (C-6), 50.34 (CH_2N_3), 20.83, 20.70, 20.67, 20.62 (4 × $COCH_3$); *m/z* (MALDI-TOF) 418.36 (M^+ + H. $C_{16}H_{24}N_3O_{10}$ requires *m/z* 418.15), *m/z* (MALDI-TOF) 440.37 (M^++Na. $C_{16}H_{23}N_3$-NaO_{10} requires *m/z* 440.13), *m/z* (MALDI-TOF) 456.31 (M^+ + K. $C_{16}H_{23}KN_3O_{10}$ requires *m/z* 456.10).

2-(2-Azidoethoxy)ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 11

The chloro-substituted mannoside **8** (1.60 g, 3.52 mmol) and sodium azide (1.83 g, 28.15 mmol) were dissolved in dry DMF (60 cm^3) and the reaction mixture was stirred at 60 °C for 16 h. The solvent was removed under high vacuum, the residue was dissolved in ethyl acetate (50 cm^3) and the solution was filtered to remove inorganic salts. The filtrate was evaporated under reduced pressure and purified by flash chromatography (light petroleum–ethyl acetate, 1 : 1) to yield the *title azide* (1.62 g, 99%) as a colourless syrup (Found: C, 46.87; H, 5.96; N, 9.10. $C_{18}H_{27}N_3O_{11}$ requires C, 46.85; H, 5.90; N, 9.11%); $[\alpha]_D^{20}$ +105.7 (*c* 0.60 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.38 (1H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 10.2, 3-H), 5.29 (1 H, dd ≈ t, $J_{4,5}$ 10.2, 4-H), 5.28 (1 H, dd, 2-H), 4.89 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.29 (1 H, dd, $J_{5,6}$ 5.1, 6-H), 4.11 (1 H, dd, $J_{5,6'}$ 2.6, $J_{6,6'}$ 12.2, 6-H′), 4.08 (1 H, ddd, 5-H), 3.88–3.81 (1 H, m, $OCHCH_2OCH_2CH_2Cl$),

3.69–3.67 (5 H, m, OCH*HC*H_2O*C*$H_2$$CH_2$Cl), 3.40 (2 H, t, *J* 5.1, $OCH_2CH_2OCH_2CH_2$Cl), 2.16, 2.11, 2.04, 2.00 (each 3 H, 4 s, 4 × COCH_3); δ_C (100.67 MHz; $CDCl_3$; Me_4Si) 170.64, 170.02, 169.89, 169.74 (4 × *C*OCH_3), 97.71 (C-1), 70.17 (OCH_2CH_2-OCH_2CH_2Cl), 70.04 ($OCH_2CH_2OCH_2CH_2$Cl), 69.52 (C-2), 69.06 (C-3), 68.42 (C-5), 67.28 ($OCH_2CH_2OCH_2CH_2$Cl), 66.13 (C-4), 62.43 (C-6), 50.71 (OCH_2CH_2Cl), 20.85, 20.70, 20.65, 20.64 (4 × CO*C*H_3); *m*/*z* (MALDI-TOF) 484.05 (M^+ + Na. $C_{18}H_{27}N_3NaO_{11}$ requires *m*/*z* 484.15), *m*/*z* (MALDI-TOF) 500.02 (M^+ + K. $C_{18}H_{27}N_3KO_{11}$ requires *m*/*z* 500.13).

2-[2-(2-Azidoethoxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 12

Mannoside **9** (1.02 g, 2.05 mmol) was dissolved in dry DMF (70 cm^3), sodium azide (1.06 g, 16.36 mmol) was added, and the reaction mixture was stirred at 50 °C for 48 h. Then, the solvent was removed under high vacuum and the residue was passed through a silica gel column (light petroleum–ethyl acetate, 1 : 1) to yield the *title compound* (1.02 g, 2.02 mmol, 97%) as a colourless syrup (Found: C, 47.55; H, 6.21; N, 8.29. $C_{20}H_{31}N_3O_{12}$ requires C, 47.52; H, 6.18; N, 8.31%); $[\alpha]_D^{20}$ +61.5 (*c* 0.22 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.37 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 10.2, 3-H), 5.29 (1 H, dd ≈ t, $J_{4,5}$ 9.7, 4-H), 5.27 (1 H, dd, 2-H), 4.88 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.30 (1 H, dd, $J_{5,6}$ 4.6, 6-H), 4.10 (1 H, dd, $J_{5,6'}$ 2.6, $J_{6,6'}$ 12.2, 6-H′), 4.07 (1 H, ddd, 5-H), 3.86–3.76 (1 H, m, OC*H*$HCH_2OCH_2CH_2OCH_2CH_2N_3$), 3.71–3.65 (9 H, m, OCH*HC*H_2O*C*H_2*C*H_2O*C*$H_2$$CH_2N_3$), 3.40 (2 H, t, *J* 5.1, $OCH_2CH_2N_3$), 2.16, 2.11, 2.05, 1.99 (each 3 H, 4 s, 4 × COCH_3); δ_C (100.67 MHz; $CDCl_3$) 170.61, 169.97, 169.82, 169.67 (4 × *C*OCH_3), 97.65 (C-1), 70.72 ($OCH_2CH_2OCH_2$-$CH_2OCH_2CH_2N_3$), 70.62, 70.02, 69.99 ($OCH_2CH_2OCH_2$-$CH_2OCH_2CH_2N_3$), 69.498 (C-2), 69.00 (C-3), 68.34 (C-5), 67.33 ($OCH_2CH_2OCH_2CH_2OCH_2CH_2N_3$), 66.08 (C-4), 62.339 (C-6), 50.59 ($OCH_2CH_2OCH_2CH_2OCH_2CH_2N_3$), 20.82, 20.67, 20.62, 20.61 (4 × CO*C*H_3); *m*/*z* (MALDI-TOF) 528.18 (M^+ + Na. $C_{20}H_{31}N_3NaO_{12}$ requires *m*/*z* 528.18), *m*/*z* (MALDI-TOF) 544.11 (M^+ + K. $C_{20}H_{31}KN_3O_{12}$ requires *m*/*z* 544.15).

2-Azidoethyl α-D-mannopyranoside 13

The protected mannoside **10** (810 mg, 1.94 mmol) was dissolved in dry MeOH (80 cm^3) and sodium methanolate solution (1 M in MeOH) was added until pH 9 was reached. Then the reaction mixture was stirred at rt until the deprotection reaction was complete (TLC *n*-PrOH–water, 7 : 3, +1% NH_3; R_f 0.61, H_2SO_4). Then it was neutralised with ion-exchange resin (Levatit 1080 H^+), filtered, and the solvent was removed *in vacuo*. The deprotected title compound (481.3 mg, 99%) was obtained as a white amorphous solid and used without further purification; $[\alpha]_D^{20}$ +139.2 (*c* 0.50 in $CHCl_3$); δ_H (400 MHz; CD_3OD) 4.82 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.88 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 10.7, 3-H), 3.86 (1 H, dd ≈ t, $J_{4,5}$ 9.2, 4-H), 3.84 (1 H, dd, 2-H), 3.74 (1 H, dd, $J_{5,6'}$ 2.1, $J_{6,6'}$ 12.2, 6-H′), 3.71 (1 H, dd, $J_{5,6}$ 5.6, 6-H), 3.62–3.57 (3 H, m, O*C*H_2CH_2, 5-H), 3.40–3.36 (2 H, m, OCH_2N_3); δ_C (100.67 MHz; CD_3OD) 102.03 (C-1), 74.88, 72.86, 72.39, 68.88 (C-2, -3, -4, -5), 68.07 (O*C*H_2CH_2), 63.21 (C-6), 52.03 (CH_2N_3).

2-(2-Azidoethoxy)ethyl α-D-mannopyranoside 14

The protected mannoside **11** (1.42 g, 3.077 mmol) was dissolved in dry MeOH (80 cm^3) and sodium methanolate solution (1 M in MeOH) was added until pH 9 was reached. Then the reaction mixture was stirred at rt until the deprotection reaction was complete (TLC *n*-PrOH–water, 7 : 3, +1% NH_3; R_f 0.59, H_2SO_4). It was neutralised with ion-exchange resin (Levatit 1080 H^+), filtered, and the solvent was removed *in vacuo*. The deprotected title compound (891.3 mg, 99%) was obtained as a colourless syrup and used without further purification; $[\alpha]_D^{20}$ +62.9 (*c* 1.05 in MeOH); δ_H (400 MHz, CD_3OD) 4.84 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.91 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 9.2, 3-H), 3.88 (1 H, dd ≈ t, $J_{4,5}$ 9.2, 4-H), 3.87 (dd, 1 H, 2-H), 3.75 (1 H, dd, $J_{5,6'}$ 2.5, $J_{6,6'}$ 12.2, 6-H′), 3.73 (1 H, dd, $J_{5,6}$ 5.1, 6-H), 3.74–3.58 (6 H, m, 3 × OCH_2), 3.61 (1H, ddd, 5-H), 3.43–3.39 (2 H, m, CH_2N_3); δ_C (100.67 MHz; CD_3OD) 102.11 (C-1), 74.90, 72.85, 72.39, 68.86 (C-2, -3, -4, -5), 71.55, 71.47 (2 × OCH_2), 68.16 (O*C*$H_2CH_2N_3$), 63.20 (C-6), 52.03 (*C*H_2N_3); *m*/*z* (MALDI-TOF) 316.18 (M^+ + Na. $C_{10}H_{19}N_3NaO_7$ requires *m*/*z* 316.11), *m*/*z* (MALDI-TOF) 332.14 (M^+ + K. $C_{10}H_{19}N_3NaO_7$ requires *m*/*z* 332.09).

2-[2-(2-Azidoethoxy)ethoxy]ethyl α-D-mannopyranoside 15

The protected mannoside **12** (650 mg, 1.29 mmol) was dissolved in dry MeOH (80 cm^3), and sodium methanolate solution (1 M in MeOH) was added until pH 9 was reached. Then the reaction mixture was stirred at rt until the deprotection reaction was complete (TLC *n*-PrOH–water, 7 : 3, +1% NH_3; R_f 0.60, H_2SO_4). It was neutralised with ion-exchange resin (Levatit 1080 H^+), filtered, and the solvent removed *in vacuo*. After flash chromatography (*i*-PrOH–water, 7 : 3, +1% NH_3) the deprotected *title compound* (423 mg, 97%) was obtained as colourless syrup (Found: C, 42.75; H, 6.90; N, 12.42. $C_{12}H_{23}N_3O_8$ requires C, 42.73; H, 6.87; N, 12.46%); δ_H (400 MHz; CD_3OD) 4.84 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.89 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 10.7, 3-H), 3.89 (1 H, dd ≈ t, $J_{4,5}$ 9.2, 4-H), 3.85 (1 H, dd, 2-H), 3.79–3.63 (12 H, m, $J_{6,6'}$ 11.2, 6-H, 6-H′, 5 × CH_2), 3.60 (1 H, ddd, $J_{5,6}$ 5.6, $J_{5,6'}$ 2.0, 5-H), 3.43–3.39 (2 H, m, CH_2N_3); δ_C (100.67 MHz; CD_3OD) 102.05 (C-1), 74.90 (C-5), 72.87 (C-2), 72.41 (C-3), 72.01, 71.85, 71.74, 71.49 (4 × OCH_2), 68.90 (C-4), 68.09 ($COCH_2$), 63.23 (C-6), 52.06 (CH_2N_3); *m*/*z* (MALDI-TOF) 338.34 (M^+ + H. $C_{12}H_{24}N_3O_8$ requires *m*/*z* 338.16), *m*/*z* (MALDI-TOF) 360.32 (M^+ + Na. $C_{12}H_{23}N_3NaO_8$ requires *m*/*z* 360.14), *m*/*z* (MALDI-TOF) 376.40 (M^+ + K. $C_{12}H_{23}$-KN_3O_8 requires *m*/*z* 376.11).

2-Aminoethyl α-D-mannopyranoside 16

The azide **13** (420 mg, 1.69 mmol) was dissolved in ethyl acetate–ethanol (10 cm^3; 1 : 1), palladium catalyst (10% Pd-C, 50 mg) was added and the reaction mixture was hydrogenated at 1 bar until the reduction was complete (TLC *i*-PrOH–water, 7 : 3, +1% NH_3; R_f 0.04, H_2SO_4). Then, it was filtered through a Celite bed, washed with ethyl acetate–ethanol (1 : 1) and the filtrate was concentrated under low pressure. Purification by flash chromatography (*i*-PrOH–water, 6 : 2) gave the title amine (370.2 mg, 98%) as a colourless syrup; $[\alpha]_D^{20}$ +84.5 (*c* 1.20 in MeOH); δ_H (400 MHz; D_2O) 4.72 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.82 (1 H, dd, 2-H), 3.74 (1 H, dd, $J_{5,6'}$ 1.5, 6-H′), 3.68 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 9.7, 3-H), 3.61 (1 H, dd ≈ t, $J_{4,5}$ 10.7, 4-H), 3.61 (1 H, dd, $J_{5,6}$ 6.1, $J_{6,6'}$ 12.2, 6-H), 3.50 (2 H, m_c, O*C*$H_2CH_2NH_2$), 3.38 (1 H, ddd, 5-H), 2.74–2.63 (2 H, m, $OCH_2CH_2NH_2$); δ_C (100.67 MHz; D_2O) 100.23 (C-1), 73.08, 70.90, 70.38, 67.16 (C-2, -3, -4, -5), 67.16 (O*C*$H_2CH_2NH_2$), 61.31 (C-6), 40.29 (OCH_2-*C*H_2NH_2); *m*/*z* (MALDI-TOF) 224.33 (M^+ + H. $C_8H_{18}NO_6$ requires *m*/*z* 224.11), *m*/*z* (MALDI-TOF) 246.25 (M^+ + Na. $C_8H_{17}NNaO_6$ requires *m*/*z* 246.10), *m*/*z* (MALDI-TOF) 262.18 (M^+ + K. $C_8H_{17}KNO_6N$ requires *m*/*z* 262.07).

2-(2-Aminoethoxy)ethyl α-D-mannopyranoside 17

The azide **14** (521 mg, 1.78 mmol) was dissolved in ethyl acetate–ethanol (10 cm^3; 1 : 1), palladium catalyst (10% Pd-C, 50 mg) was added and the reaction mixture was hydrogenated at 1 bar until the reduction was complete (TLC *n*-PrOH–water, 7 : 3, +1% NH_3; R_f 0.07, H_2SO_4). Then, it was filtered through a Celite bed, washed with ethyl acetate–ethanol (1 : 1), and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (*i*-PrOH–water, 8 : 2) gave the title amine (465 mg, 98%) as a colourless syrup; $[\alpha]_D^{20}$ +57.2 (*c* 0.75 in H_2O); δ_H (400 MHz; D_2O) 4.74 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.81 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 3.75–3.54 (6 H, m, 3-, 4-, 5-H, 6-H_2,

OC*H*HCH$_2$NH$_2$), 3.52–3.49 (3 H, m, OCH*H*CH$_2$O), 3.47–3.43 (2 H, m, OCH$_2$), 2.66 (2 H, t, *J* 5.6, OCH$_2$C*H*$_2$NH$_2$); δ_C (100.67 MHz; D$_2$O) 100.31 (C-1), 73.15, 70.88, 70.34, 67.12 (C-2, -3, -4, -5), 72.23 (OCH$_2$*C*H$_2$O), 69.69 (O*C*H$_2$CH$_2$NH$_2$), 66.81 (O*C*H$_2$CH$_2$O), 61.30 (C-6), 40.16 (OCH$_2$*C*H$_2$NH$_2$); *m*/*z* (MALDI-TOF) 268.29 (M^+ + H. $C_{10}H_{22}NO_7$ requires *m*/*z* 268.14), *m*/*z* (MALDI-TOF) 290.25 (M^+ + Na. $C_{10}H_{21}NNaO_7$ requires *m*/*z* 290.12), *m*/*z* (MALDI-TOF) 306.20 (M^+ + K. $C_{10}H_{21}KNO_7$ requires 306.10).

2-[2-(2-Aminoethoxy)ethoxy]ethyl α-D-mannopyranoside 18

The azide **15** (380 mg, 1.13 mmol) was dissolved in ethyl acetate–ethanol (10 cm^3; 1 : 1), palladium catalyst (10% Pd-C, 60 mg) was added and the reaction mixture was hydrogenated at 1 bar until the reduction was completed (TLC *i*-PrOH–water, 7 : 3, +1% NH$_3$; R_f 0.05, H$_2$SO$_4$). Then, it was filtered through a Celite bed, washed with ethyl acetate–ethanol (1 : 1) and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (*i*-PrOH–water, 7 : 2) gave the title amine (345 mg, 98%) as a colourless syrup; $[\alpha]_D^{20}$ +32.6 (*c* 0.60 in MeOH); δ_H (400 MHz; CD$_3$OD) 4.87 (1 H, d, $J_{1,2}$ 1.5, 1-H), 3.89 (1 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 9.2, 3-H), 3.88 (1 H, dd ≈ t, 4-H), 3.85 (1 H, dd, 2-H), 3.79–3.62 (12 H, m, $J_{6,6'}$ 12.2, 6-H$_2$, 5 × CH$_2$), 3.58 (1 H, ddd, $J_{4,5}$ 10.2, $J_{5,6}$ 5.1, $J_{5,6'}$ 2.5, 5-H), 3.40 (2 H, m, OCH$_2$C*H*$_2$NH$_2$); δ_C (100.67 MHz; CD$_3$OD) 102.00 (C-1), 74.94 (C-5), 73.23 (OCH$_2$), 72.85 (C-2), 72.40 (C-3), 71.82, 71.61, 70.88 (3 × OCH$_2$), 68.93 (C-4), 67.85 (O*C*H$_2$CH$_2$), 63.23 (C-6), 41.68 (CH$_2$NH$_2$); *m*/*z* (MALDI-TOF) 312.23 (M^+ + H. $C_{12}H_{26}NO_8$ requires *m*/*z* 312.17), *m*/*z* (MALDI-TOF) 334.21 (M^+ + Na. $C_{12}H_{25}NNaO_8$ requires *m*/*z* 334.15), *m*/*z* (MALDI-TOF) 350.17 (M^+ + K. $C_{12}H_{25}KNO_8$ requires *m*/*z* 350.12).

Tris(2″-{[2′-(α-D-mannopyranosyloxy)ethyl]carbamoyl}ethyl)-nitromethane 20

A solution of the amino-functionalised mannoside **16** (29 mg, 0.130 mmol) and the triacid **19** (10.9 mg, 0.039 mmol) in dry dimethylacetamide (DMA) (10 cm^3) was treated with EEDQ (26.7 mg, 0.108 mmol) and stirred at 60 °C for 4 days. Then the solvent was removed by lyophilisation and the residue was taken up in water (50 cm^3). Impurities were extracted three times with Et$_2$O (each 40 cm^3), then the aqueous phase was set under slight vacuum to remove dissolved Et$_2$O, and was subsequently diluted to the three-fold volume with water. The resulting solution was freeze-dried and the obtained lyophilisate was subjected to gel-permeation chromatography (Biogel P2 extra fine; 15 mM aq. NH$_4$HCO$_3$ as the eluent) to yield the pure title cluster (26 mg, 74%) as a white lyophilisate; TLC *n*-PrOH–water, 7 : 3, + 1% NH$_3$ (R_f 0.11, H$_2$SO$_4$); $[\alpha]_D^{20}$ +43.6 (*c* 0.70 in MeOH); δ_H (400 MHz; CD$_3$OD) 4.86 (3 H, d, $J_{1,2}$ 1.5, 1-H), 3.92 (3 H, dd, 2-H), 3.89 (3 H, dd, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.2, 6-H^b), 3.88–3.77 (3 H, m, OC*H*H), 3.79 (3 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 9.2, 3-H), 3.77 (3 H, dd, $J_{5,6a}$ 5.6, 6-H^a), 3.67 (3 H, dd ≈ t, $J_{4,5}$ 9.7, 4-H), 3.62–3.58 (6 H, m, CH$_2$N), 3.52–3.37 (6 H, m, 5-H, OCH*H*), 2.30 (12 H, br s, O$_2$NC^{q}CH$_2$C*H*$_2$CO); δ_C (100.67 MHz; CD$_3$OD) 175.13 (C=O), 101.32 (C-1), 94.55 (C^{q}NO$_2$), 74.49 (C-5), 72.29 (C-3), 71.77 (C-2), 68.35 (C-4), 67.16 (O*C*H$_2$CH$_2$NH), 62.57 (C-6), 40.48 (OCH$_2$*C*H$_2$NH), 32.19 (*C*H$_2$CH$_2$CONH), 31.41 (CH$_2$*C*H$_2$CONH); *m*/*z* (MALDI-TOF) 915.48 (M^+ + Na. $C_{34}H_{60}N_4NaO_{23}$ requires *m*/*z* 915.35), *m*/*z* (MALDI-TOF) 931.46 (M^+ + K. $C_{34}H_{60}KN_4O_{23}$ requires *m*/*z* 931.33).

Tris[2‴-({2″-[2′-(α-D-mannopyranosyloxy)ethoxy]ethyl}-carbamoyl)ethyl]nitromethane 21

A solution of the amino-functionalised mannoside **17** (47.6 mg, 0.178 mmol) and the triacid **19** (14.96 mg, 0.054 mmol) in dry DMA (15 cm^3) was treated with EEDQ (52.84 mg, 0.214 mmol) and stirred at 60 °C for 4 days. Then the solvent was removed by lyophilisation and the residue was taken up in water (50 cm^3). Impurities were extracted three times with Et$_2$O (each 40 cm^3), then the aqueous phase was set under slight vacuum to remove traces of Et$_2$O. The residual solution was diluted to three-fold its volume with water and the resulting solution was freeze-dried. The obtained lyophilisate was subjected to gel-permeation chromatography (Biogel P2 extra fine; 15 mM aq. NH$_4$HCO$_3$ as the eluent) to yield the pure title cluster (38 mg, 69%) as a white lyophilisate; TLC *n*-PrOH–water, 7 : 3, + 1% NH$_3$ (R_f 0.14, H$_2$SO$_4$); $[\alpha]_D^{20}$ +20.0 (*c* 0.90 in MeOH); δ_H (400 MHz; CD$_3$OD) 4.85 (3 H, d, $J_{1,2}$ 1.9, 1-H), 3.88 (3 H, dd, 6-H^b), 3.86 (3 H, dd, 2-H), 3.75 (3 H, dd, $J_{6a,6b}$ 11.7, 6-H^a), 3.745 (3 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5, 3-H), 3.70–3.66 (12 H, m, CONHCH$_2$-C*H*$_2$OC*H*$_2$), 3.65 (3 H, dd ≈ t, $J_{4,5}$ 9.8, 4-H), 3.60 (3 H, ddd, $J_{5,6a}$ 5.7, $J_{5,6b}$ 2.2, 5-H), 3.60–3.58 (6 H, m, manOC*H*$_2$), 3.41 (6 H, t, *J* 5.4, CONHC*H*$_2$), 2.31–2.26 (12 H, m, O$_2$NC^{q}C*H*$_2$C*H*$_2$-CONH); δ_C (100.67 MHz; CD$_3$OD) 174.62 (C^q, C=O), 101.98 (C-1), 94.63 (C^{q}NO$_2$), 74.94 (C-5), 72.87 (C-3), 72.41 (C-2), 71.50 (CONHCH$_2$*C*H$_2$OCH$_2$), 70.81 (CONHCH$_2$CH$_2$-O*C*H$_2$), 69.00 (C-4), 68.05 (manO*C*H$_2$), 63.31 (C-6), 40.84 (CONH*C*H$_2$), 32.44 (O$_2$NC^q*C*H$_2$CH$_2$), 31.48 (O$_2$NC^{q}CH$_2$-*C*H$_2$); *m*/*z* (MALDI-TOF) 1047.55 (M^+ + Na. $C_{40}H_{72}N_4NaO_{26}$ requires *m*/*z* 1047.43), *m*/*z* (MALDI-TOF) 1063.55 (M^+ + K. $C_{40}H_{72}KN_4O_{26}$ requires *m*/*z* 1063.41).

Tris{2⁗-[(2‴-{2″-[2′-(α-D-mannopyranosyloxy)ethoxy]-ethoxy}ethyl)carbamoyl]ethyl}nitromethane 22

A solution of the amino-functionalised mannoside **18** (41.3 mg, 0.133 mmol) and the triacid **19** (10.2 mg, 0.037 mmol) in dry DMA (15 cm^3) was treated with EEDQ (39.4 mg, 0.214 mmol) and stirred at 60 °C for 4 days. Then the solvent was removed by lyophilisation and the residue was taken up in water (50 cm^3). Impurities were extracted three times with Et$_2$O (each 40 cm^3), then the aqueous phase was set under slight vacuum to remove traces of Et$_2$O. The residual solution was diluted to three times its volume with water and the resulting solution was freeze-dried. The obtained lyophilisate was subjected to gel-permeation chromatography (Biogel P2 extra fine; 15 mM aq. NH$_4$HCO$_3$ as the eluent) to yield the pure title cluster (25.3 mg, 59%) as a white lyophilisate; TLC *n*-PrOH–water, 7 : 3, + 1% NH$_3$ (R_f 0.13, H$_2$SO$_4$); $[\alpha]_D^{20}$ +23.4 (*c* 0.60 in H$_2$O); δ_H (400 MHz; D$_2$O) 4.74 (3 H, d, $J_{1,2}$ 1.5, 1-H), 3.81 (3 H, dd, 2-H), 3.73 (3 H, dd, $J_{5,6a}$ 6.6, 6-H^a), 3.72 (3 H, dd, $J_{2,3}$ 3.6, $J_{3,4}$ 9.1, 3-H), 3.66 (3 H, ddd, $J_{4,5}$ 9.1, 5-H), 3.62–3.56 (6 H, m, spacer-CH$_2$), 3.60 (3 H, dd, $J_{5,6b}$ 3.1, $J_{6a,6b}$ 11.2, 6-H^b), 3.55–3.53 (15 H, m, 12 spacer-CH$_2$, 4-H), 3.51–3.46 (12 H, m, spacer-CH$_2$), 3.24 (6 H, t, *J* 5.6, spacer-CH$_2$), 2.13 (12 H, br s, core-CH$_2$), 1.10 and 1.03 (together 3 H, t, *J* 7.1, NH); δ_C (100.67 MHz; D$_2$O) 174.81 (NHC=O), 100.30 (C-1), 94.43 (C^{q}NO$_2$), 73.13 (C-5), 70.891 (C-3), 70.35 (C-2), 70.02 (CONHCH$_2$*C*H$_2$OCH$_2$), 69.88 (manOCH$_2$*C*H$_2$OCH$_2$), 69.79 (manOCH$_2$CH$_2$O*C*H$_2$), 69.15 (CONHCH$_2$CH$_2$O*C*H$_2$), 67.12 (C-4), 66.71 (manO*C*H$_2$CH$_2$), 61.31 (C-6), 39.39 (CONH*C*H$_2$CH$_2$), 30.90 (O$_2$NC^q*C*H$_2$-CH$_2$CO), 30.38 (O$_2$NC^{q}CH$_2$*C*H$_2$CO); *m*/*z* (MALDI-TOF) 1179.54 (M^+ + Na. $C_{46}H_{84}N_4NaO_{29}$ requires *m*/*z* 1179.51), *m*/*z* (MALDI-TOF) 1195.54 (M^+ + K. $C_{46}H_{84}KN_4O_{29}$ requires *m*/*z* 1195.49).

p-Nitrophenyl 6-amino-6-deoxy-α-D-mannopyranoside 26

To a solution of the 6-azido-6-deoxymannopyranoside **25**[9] (334.5 mg, 1.025 mmol) in tetrahydrofuran (THF)–water (4 : 1; 50 cm^3) was added PPh$_3$ (349.6 mg, 1.33 mmol) at rt and, after 30 min of stirring, silica gel (1 g) was added and the reaction mixture was stirred at 45 °C for 12 h. The silica gel was then filtered off, the solvents were removed *in vacuo*, and the residue was purified by flash chromatography (using two gradients: ethyl acetate–toluene, 19 : 1, + 3% pyridine; →ethyl acetate–MeOH, 2 : 1, + 3% pyridine; →ethyl acetate–MeOH, 4 : 1,

+ 3% pyridine) to give the title glycoside (302.4 mg, 98%) as a slightly yellow amorphous solid; $[\alpha]_D^{20}$ +82.8 (*c* 0.50 in MeOH); δ_H (400 MHz; CD_3OD) 8.27 (2 H, d, ^3J 9.2, *m*-ArH), 7.32 (2 H, d, ^3J 9.2, *o*-ArH), 5.71 (1 H, d, $J_{1,2}$ 1.5, 1-H), 4.09 (1 H, dd, $J_{2,3}$ 3.6, 2-H), 3.93 (1 H, dd, 3-H), 3.68 (1 H, dd ≈ t, $J_{3,4}$ 9.2, $J_{4,5}$ 9.7, 4-H), 3.49 (1 H, ddd, 5-H), 2.97 (1 H, dd, $J_{5,6'}$ 3.1, $J_{6,6'}$ 13.2, 6-H′), 2.82 (1 H, dd, $J_{5,6}$ 7.1, 6-H); δ_C (100.67 MHz; CD_3OD) 162.92 (*i*-aryl-C^q), 144.17 (*p*-aryl-C^q), 127.07 (*m*-aryl-CH), 117.93 (*o*-aryl-CH), 100.18 (C-1), 76.18 (C-5), 72.41 (C-3), 71.86 (C-2), 69.83 (C-4), 43.87 (C-6); *m*/*z* (MALDI-TOF) 301.14 (M^+ + H. $C_{12}H_{17}N_2O_7$ requires *m*/*z* 301.10), *m*/*z* (MALDI-TOF) 323.12 (M^+ + Na. $C_{12}H_{16}NaN_2O_7$ requires *m*/*z* 323.09), *m*/*z* (MALDI-TOF) 339.10 (M^+ + K. $C_{12}H_{16}KN_2O_7$ requires *m*/*z* 339.06).

Tris{2′-[(6-deoxy-1-*O*-*p*-nitrophenyl-α-D-mannopyranos-6-yl)carbamoyl]ethyl}nitromethane 27

Mannoside **26** (33.3 mg, 0.11 mmol) and triacid **19** (7.9 mg, 0.029 mmol) were dissolved in dry DMA (12 cm^3) and treated with EEDQ (32.9 mg, 0.133 mmol, 1.2 eq.) for 5 days. Then, the solvent was removed by lyophilisation and the resulting lyophilisate was dissolved in Et_2O–light petroleum–MeOH–water (2 : 1 : 1 : 1; 20 cm^3) to remove an excess of EEDQ and any formed quinoline. The aqueous phase was separated, again concentrated by lyophilisation, and the residue was purified on silica gel (ethyl acetate–methanol, 9 : 1) to yield the title cluster mannoside **27** (29.2 mg, 91%) as a white amorphous solid, $[\alpha]_D^{20}$ +78.6 (*c* 0.40 in MeOH); δ_H (400 MHz; CD_3OD) 8.26, 7.31 (each 6 H, 2 × d, ^3J 9.7, ArH), 5.69 (3 H, d, $J_{1,2}$ 1.5, 1-H), 4.09 (3 H, dd, $J_{2,3}$ 3.6, 2-H), 3.93 (3 H, dd, $J_{3,4}$ 9.2, 3-H), 3.65 (3 H, dd ≈ t, $J_{4,5}$ 9.2, 4-H), 3.62 (3 H, dd, $J_{5,6b}$ 3.6, $J_{6a,6b}$ 12.7, 6-H^b), 3.60 (3 H, dd, $J_{5,6a}$ 6.1, 6-H^a), 3.59–3.55 (3 H, m, 5-H), 2.12–1.98 (12 H, m, CH_2CH_2CO); δ_C (100.67 MHz; CD_3OD) 174.57 (NHCO), 162.84 (*i*-aryl-C^q), 144.21 (*p*-aryl-C^q), 127.09 (*m*-aryl-CH), 118.00 (*o*-aryl-CH), 100.16 (C-1), 94.49 (*C*^{q}-NO_2), 74.35 (C-5), 72.24 (C-3), 71.85 (C-2), 69.66 (C-4), 41.67 (C-6), 32.19 (CH_2CH_2CO), 31.39 (CH_2-CH_2CO); *m*/*z* (MALDI-TOF) 1146.34 (M^+ + Na. $C_{46}H_{57}N_7NaO_{26}$ requires *m*/*z* 1146.325), *m*/*z* (MALDI-TOF) 1162.33 (M^+ + K. $C_{46}H_{57}KN_7O_{26}$ requires *m*/*z* 1162.299).

Biotinamidotris[2-(*tert*-butoxycarbonyl)ethyl]methane 30

To a solution of (+)-biotin (307.6 mg, 1.26 mmol) and diisopropylethylamine (DIPEA) (0.3 cm^3) in dry dimethylformamide (DMF) (12 cm^3) was added a solution of the amine **29**[16] (561 mg, 1.26 mmol) in dry DMF (10 cm^3). Then the mixture was cooled to 0 °C and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) (241.3 mg, 1.26 mmol) and *N*-hydroxybenzotriazole (1-HOBT) (340.2 mg, 2.52 mmol) were added. The reaction mixture was stirred at 0 °C for 1 h followed by stirring at rt for 12 h. Then the solvent was removed *in vacuo* and the residue was purified by flash chromatography (ethyl acetate–MeOH, 4 : 1) to yield the biotinylated title compound (780 mg, 97%) as a white amorphous solid; δ_H (400 MHz; $CDCl_3$; Me_4Si) 6.69 (1 H, br s, N*H*CONH), 6.52 (1 H, br s, NHCON*H*), 6.21 (1 H, br s, CONHC^q), 4.45 (1 H, dd ≈ t, $^3J_{cis}$ 4.7, $^3J_{trans}$ 7.6, C*H*CH$_2$S), 4.30 (1 H, dd ≈ t, $^3J_{cis}$ 4.7, $^3J_{trans}$ 7.3, C*H*CHRS), 3.12 (1 H, m$_c$, CHC*H*RS), 2.88 (1 H, dd, ^3J 4.7, ^2J 12.6, CHC*H*HS), 2.73 (1 H, dd ≈ d, CHCH*H*S), 2.20 (6 H, m$_c$, CH_2COOBu^t), 2.15 (2 H, m$_c$, CH$_2$CH_2CH$_2$CH$_2$CONH), 1.97 (6 H, m$_c$, CH_2CH$_2$COOBu^t), 1.71 (1 H, m$_c$, C*H*HCH$_2$-CH$_2$CH$_2$CONH), 1.62 (3 H, m$_c$, CH*H*CH$_2$CH_2CH$_2$CONH), 1.42 (29 H, m$_c$, CH_2CONH, 3 × Bu^t); δ_C (100.67 MHz; $CDCl_3$) 172.96 (NHCO-biotinamide), 162.73 (NHCONH-biotin), 91.92 (C^q, CONH*C*), 82.07 (*C*^{q}Me$_3$), 61.86 (CONH*C*HCH$_2$S), 60.38 (CONH*C*HCHRS), 57.32 (CONHCH*C*HRS), 40.52 (CONHCH*C*H$_2$S), 36.76 (CH$_2$*C*H$_2$CH$_2$CH$_2$CONH), 32.08 (*C*H$_2$CH$_2$COO*t*Bu), 30.70 (*C*H$_2$CH$_2$CH$_2$CH$_2$CONH), 30.21 (CH$_2$*C*H$_2$COO*t*Bu), 29.82 (CH$_2$CH$_2$*C*H$_2$CH$_2$CONH), 28.68 (C*Me*$_3$), 27.94 (CH$_2$CH$_2$CH$_2$*C*H$_2$CONH); *m*/*z* (MALDI-TOF) 664.28 (M^+ + Na. $C_{32}H_{55}N_3NaO_8S$ requires *m*/*z* 664.36), *m*/*z* (MALDI-TOF) 680.17 (M^+ + K. $C_{32}H_{55}KN_3O_8S$ requires *m*/*z* 680.33).

Biotinamidotris(2-carboxyethyl)methane 31

The *tert*-butyl triester **30** (200 mg, 0.312 mmol) was stirred in formic acid (98%; 10 cm^3) for 5 h at rt. Then the solvent was removed *in vacuo* with repeated co-evaporation with toluene to yield the title triacid (146 mg, 99%) as a colourless syrup which was not further purified; TLC *n*-PrOH–water, 7 : 3, + 1% NH_3 (R_f 0.35, H_2SO_4); δ_H (500 MHz; CD_3OD) 8.63 (1 H, br s, NHCO), 4.53 (1 H, dd, $^3J_{cis}$ 4.4, $^3J_{trans}$ 8.2, C*H*CH$_2$S), 4.36 (1 H, dd, $^3J_{cis}$ 4.4, $^3J_{trans}$ 7.6, C*H*CHRS), 3.25 (1 H, ddd, CHC*H*RS), 2.97 (1 H, dd, ^3J 5.0, ^2J 12.6, CHC*H*HS), 2.74 (1 H, dd ≈ d, CHCH*H*S), 2.32 (6 H, m$_c$, CH$_2$CH_2COOH), 2.24 (2 H, m$_c$, CH$_2$CH_2CH$_2$CH$_2$CONH), 2.06 (6 H, m$_c$, CH_2CH$_2$COOH), 1.77 (1 H, m$_c$, C*H*HCH$_2$CH$_2$CH$_2$CONH), 1.68 (3 H, m$_c$, CH*H*CH$_2$CH_2CH$_2$CONH), 1.50 (2 H, m$_c$, CH$_2$CH$_2$CH$_2$CH_2-CONH); δ_C (125.77 MHz; CD_3OD) 177.45 (CH$_2$CH$_2$*C*OOH), 176.01 (CH$_2$*C*ONH), 166.47 (NH*C*ONH), 91.92 (*C*^{q}), 63.55 (*C*HCH$_2$S), 61.94 (*C*HCHRS), 58.89 (CH*C*HRS), 41.34 (CH*C*H$_2$S), 37.67 (CH$_2$*C*H$_2$CH$_2$CH$_2$CONH), 32.08 (*C*H$_2$-CH$_2$COOH), 30.78 (*C*H$_2$CH$_2$CH$_2$CH$_2$CONH), 30.03 (CH$_2$*C*H$_2$COOH), 29.57 (CH$_2$CH$_2$*C*H$_2$CH$_2$CONH), 27.26 (CH$_2$CH$_2$CH$_2$*C*H$_2$CONH) *m*/*z* (MALDI-TOF) 496.17 (M^+ + Na. $C_{20}H_{31}NaN_3O_8S$ requires *m*/*z* 496.17), *m*/*z* (MALDI-TOF) 512.13 (M^+ + K. $C_{20}H_{31}KN_3O_8S$ requires *m*/*z* 512.15).

Biotinamidotris{2′-[(6-deoxy-1-*O*-methyl-α-D-mannopyranos-6-yl)carbamoyl]ethyl}methane 33

The biotinylated triacid **31** (67.29 mg, 0.1421 mmol) and the amino-functionalised glycoside **32** (87.85 mg, 0.455 mmol) were dissolved in dry DMF (50 cm^3). Then DIPEA (0.5 cm^3) was added, the mixture was cooled to 0 °C, and a solution of EDC (27.24 mg, 0.1421 mmol) and 1-HOBT (38.4 mg, 0.284 mmol) in dry DMF (10 cm^3) was added dropwise over a period of 20 min. The reaction mixture was stirred at 0 °C for 1 h and for 48 h at rt. Then, the solvent was removed under high vacuum and the residue was purified by gel chromatography (first on Sephadex LH-20 with MeOH as eluent, followed by Sephadex G-15 with 15 mM aq. NH_4HCO_3 as eluent) to yield the title compound (129 mg, 91%) as a slightly yellow amorphous solid after lyophilisation; TLC *n*-PrOH–water, 7 : 3, + 1% NH_3 (R_f 0.27, H_2SO_4); $[\alpha]_D^{20}$ +96.7 (*c* 0.45 in water); δ_H (400 MHz; $CDCl_3$; Me_4Si) 8.33 (6 H, br s, NHCO), 4.61 (3 H, d, 1-H), 4.47 (1 H, dd, $^3J_{cis}$ 4.6, $^3J_{trans}$ 7.6, C*H*CH$_2$S), 4.29 (1 H, dd, $^3J_{trans}$ 7.6, $^3J_{cis}$ 4.6, C*H*CHRS), 3.79 (3 H, dd, $J_{1,2}$ 1.5, $J_{2,3}$ 3.6, 2-H), 3.60 (3 H, dd, $J_{3,4}$ 9.7, 3-H), 3.51–3.46 (6 H, m, 5-H, 6-H^b), 3.42 (3 H, dd ≈ t, $J_{4,5}$ 9.7, 4-H), 3.30 (3 H, dd, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 12.7, 6-H^a), 3.24 (9 H, s, OCH_3), 3.23–3.05 (1 H, m, CHC*H*RS), 2.87 (1 H, dd, ^3J 4.6, ^2J 13.2, CHC*H*HS), 2.65 (1 H, dd ≈ d, CHCH*H*S), 2.22–2.05 (8 H, m, 3 × CH$_2$CH_2CONHCH$_2$, CH$_2$CH_2CH$_2$-CH$_2$CONH), 1.90–1.73 (8 H, m, CH_2CH$_2$CH$_2$CH$_2$CONH, 3 × CH_2CH$_2$CONHCH$_2$), 1.62–1.41 (2 H, m, CH$_2$CH$_2$CH_2-CH$_2$CONH), 1.31–1.24 (2 H, m, CH$_2$CH$_2$CH$_2$CH_2CONH); δ_C (100.67 MHz; $CDCl_3$; Me_4Si) 176.74 (*C*ONHCH$_2$man), 176.47 (NHCO-biotinamide), 165.73 (NHCONH-biotin), 101.17 (C-1), 92.92 (C^q, CONH*C*), 71.02 (C-5), 70.73 (C-3), 70.24 (C-2), 68.49 (C-4), 62.42 (CONH*C*HCHRS), 60.61 (CONH*C*HCH$_2$S), 55.69 (OCH_3), 54.99 (CH*C*HRS), 40.44 (C-6), 40.09 (SCH_2), 36.61 (CH$_2$*C*H$_2$CH$_2$CH$_2$CONH), 31.84 (*C*H$_2$CH$_2$CONHCH$_2$), 30.38 (*C*H$_2$CH$_2$CH$_2$CH$_2$CONH), 28.40 (CH$_2$*C*H$_2$CONHCH$_2$), 28.04 (CH$_2$CH$_2$*C*H$_2$CH$_2$CONH), 25.61 (CH$_2$CH$_2$CH$_2$*C*H$_2$CONH); *m*/*z* (MALDI-TOF) 999.41 (M^+ + H. $C_{41}H_{71}N_6O_{20}S$ requires *m*/*z* 999.44), *m*/*z* (MALDI-TOF) 1021.40 (M^+ + Na. $C_{41}H_{70}NaN_6O_{20}S$ requires *m*/*z* 1021.43), *m*/*z* (MALDI-TOF) 1037.52 (M^+ + K. $C_{41}H_{70}KN_6O_{20}S$ requires *m*/*z* 1037.40).

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References

1 (*a*) K.-A. Karlsson, *Curr. Opin. Struct. Biol.*, 1995, **5**, 622; (*b*) H. Lis and N. Sharon, *Chem. Rev.*, 1998, **98**, 637; (*c*) M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem.*, 1998, **110**, 2908; M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754.

2 Some leading references are: (*a*) C. A. Lingwood, *Curr. Opin. Chem. Biol.*, 1998, **2**, 695; (*b*) N. Firon, I. Ofek and N. Sharon, *Carbohydr. Res.*, 1983, **120**, 235; (*c*) N. Firon, I. Ofek and N. Sharon, *Infect. Immun.*, 1984, **43**, 1088; (*d*) W. J. Sanders, E. J. Gordon, O. Dwir, P. J. Beck, R. Alon and L. L. Kiessling, *J. Biol. Chem.*, 1999, **274**, 5271; (*e*) E. J. Gordon, J. E. Gestwicki, L. E. Strong and L. L. Kiessling, *Chem. Biol.*, 1999, **7**, 9; (*f*) J. E. Gestwicki, L. E. Strong and L. L. Kiessling, *Angew. Chem.*, 2000, **112**, 4741; J. E. Gestwicki, L. E. Strong and L. L. Kiessling, *Angew. Chem., Int. Ed.*, 2000, **39**, 4567; (*g*) W. B. Turnbull, A. R. Pease and J. F. Stoddart, *ChemBioChem*, 2000, **1**, 70; (*h*) C. Gege, J. Vogel, G. Bendas, U. Rothe and R. R. Schmidt, *Chem. Eur. J.*, 2000, **6**, 111; (*i*) V. Wittmann and S. Seeberger, *Angew. Chem.*, 2000, **112**, 4508; V. Wittmann and S. Seeberger, *Angew. Chem., Int. Ed.*, 2000, **39**, 4348; (*j*) S. D. Burke, Q. Zhao, M. C. Schuster and L. L. Kiessling, *J. Am. Chem. Soc.*, 2000, **122**, 4518; (*k*) Z. Gan and R. Roy, *Tetrahedron*, 2000, **56**, 1423.

3 (*a*) Th. K. Lindhorst, C. Kieburg and U. Krallmann-Wenzel, *Glycoconjugate J.*, 1998, **15**, 605; (*b*) H. C. Hansen, S. Haataja, J. Finne and G. Magnusson, *J. Am. Chem. Soc.*, 1997, **119**, 6974; (*c*) L. E. Strong and L. L. Kiessling, *J. Am. Chem. Soc.*, 1999, **121**, 6193; (*d*) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read and D. R. Bundle, *Nature*, 2000, **403**, 669; (*e*) D. Zanini and R. Roy, *J. Am. Chem. Soc.*, 1997, **119**, 2088.

4 (*a*) *Toward Anti-adhesion Therapy for Microbial Diseases*, *Adv. Exp. Med. Biol.*, 1996, **408**; (*b*) K. J. Yarema and C. R. Bertozzi, *Curr. Opin. Chem. Biol.*, 1998, **2**, 49.

5 N. Sharon, *FEBS Lett.*, 1987, **217**, 145.

6 D. Choudhury, A. Thompson, V. Stojanoff, S. Langermann, J. Pinkner, S. J. Hultgreen and S. D. Knight, *Science*, 1999, **285**, 1061.

7 (*a*) K. A. Krogfelt, H. Bergmany and P. Klemm, *Infect. Immun.*, 1990, **58**, 1995; (*b*) K. A. Krogfelt, *Rev. Infect. Dis.*, 1991, **13**, 721; (*c*) P. Klemm and K. A. Krogfelt, in *Fimbriae, Adhesion, Genetics, Biogenesis and Vaccines*, ed. P. Klemm, CRC Press, Boca Raton, 1994, p. 9.

8 (*a*) Th. K. Lindhorst, in *Bioorganic Chemistry*, ed. U. Diederichsen, Th. K. Lindhorst, B. Westermann and B. L. Wessjohann, Wiley-VCH, Weinheim, 1999, p. 133; (*b*) Th. K. Lindhorst, M. Dubber, U. Krallmann-Wenzel and S. Ehlers, *Eur. J. Org. Chem.*, 2000, 2027.

9 S. Kötter, U. Krallmann-Wenzel, S. Ehlers and Th. K. Lindhorst, *J. Chem. Soc., Perkin Trans. 1*, 1998, 2193.

10 R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21.

11 G. Zemplén and E. Pascu, *Ber. Dtsch. Chem. Ges.*, 1929, **62**, 1613.

12 G. R. Newkome, G. N. Moorefield and K. J. Theriot, *J. Org. Chem.*, 1988, **53**, 5552.

13 H. Staudinger and E. Hauser, *Helv. Chim. Acta*, 1921, **4**, 861.

14 P. Wang, G.-J. Shen, Y.-F. Wang, Y. Ichikawa and C. H. Wong, *J. Org. Chem.*, 1993, **58**, 3985.

15 P. Klemm, B. J. Jørgensen, I. v. Die, H. d. Ree and H. Bergmans, *Mol. Gen. Genet.*, 1985, **199**, 410.

16 G. R. Newkome, R. K. Behera, C. N. Moorefield and G. R. Baker, *J. Org. Chem.*, 1991, **56**, 7162.