Glycerol and Glycerol Glycol Glycodendrimers

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Non-covalent interactions between structural parts of complex oligosaccharides and saccharide-recognising proteins are of crucial importance for many cell communication phenomena. Specificity of such interactions and stability of these ligand-receptor complexes are achieved through multivalent interactions between multiple copies of a saccharide ligand and a corresponding number of protein receptors. Substances presenting multiple copies of the saccharide ligand on easily accessible scaffold molecules therefore appear to be promising tools for study of multivalent interactions and their possible inhibition. Such multivalent glycomimetics can be prepared by attachment of saccharide residues to the surface functional groups of dendrimers. In the course of our work, we have prepared novel glycodendrimers with glycerol and glycerol glycol polyether scaffolds. Isopropylidene-protected hydroxyethyl mannoside was chosen as the carbohydrate component, with the construction of the dendritic structures proceeding by a convergent approach featuring iterative Williamson etherification and ozonolysis/hydride reduction steps. Deprotected representatives of such structures are potential inhibitors of mannose-binding lectins of *E. coli*. Three representative compounds were deprotected and their anti-adhesive properties were examined. The route to these glycodendrimers was also evaluated in terms of synthetic chemistry.

Introduction

Carbohydrate-protein interactions are of fundamental importance in many biological processes.[1,2] Surfaces of eukaryotic cells are covered in an array of glycoproteins and glycolipids, called glycocalyx. According to state of the art knowledge in glycobiology biological information is encoded within the structures of the glycoconjugates.[3−5] The composition of the glycocalyx is dependent on the type and stage of development of a cell and can change during the course of pathological events.[1] It is assumed that information is decoded by the formation of non-covalent complexes between component parts of the saccharide assembly and special protein receptors known as lectins and selectins.[6] Ligand−receptor interactions between single saccharide moieties and their protein receptors are weak, dissociation constants of the formed complexes typically being in the millimolar range.[7] Specificity of the interactions and stability of the ligand-receptor complexes are achieved through multivalent interactions between multiple copies of the saccharide ligand and a corresponding number of protein receptors.[8] Multivalent carbohydrate-protein interactions are of crucial importance in, for example, fertilisation, cell-cell adhesion[9] and immune responses,[10] but also in pathological events such as inflammatory processes,[11,12] cell degeneration[3] and infections[2] by bacteria, viruses and parasites. In many cases, carbohydrate-mediated adhesion of the pathogen to the carbohydrates of its host cell through its lectins is an absolutely necessary prerequisite for infection.[13,14]

The search for structurally simple − and hence easily accessible − compounds capable of successfully mimicking the naturally occurring complex saccharide structures has thus become an important scientific task.[15,16] Such multivalent glycomimetics are on one hand valuable tools with which to obtain better insights into carbohydrate-mediated processes, while on the other hand they have considerable potential as therapeutics against, for example, bacterial infection, by inhibition of the adhesion process. A common approach to artificial glyco ligands is the attachment of those saccharide moieties necessary for recognition to structurally simple, branched scaffold molecules. Dendrimers,[17−20] monodisperse macromolecules grown through iterative repetition of two reaction sequences, are − thanks to their highly branched molecular architectures and their multiple and uniform functional surface groups − especially well suited for the construction of multivalent glycomimics.[21,22] Our group has introduced several examples of glycodendrimers with PAMAM (polyamido amine),[23,24] carbohydrate-peptide,[25] and carbosilane[26,27] architectures. In this paper we report a new class of glycodendrimers with an aliphatic polyether scaffold. We selected this architecture because polyethers appear superior to PAMAM or carbosilane structures in several regards. Apart

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from being chemically inert, they can be expected, unlike the hydrophobic carbosilanes, to exhibit good solubility properties in aqueous media. PAMAM dendrimers contain basic tertiary amine centres that are extensively protonated under physiological conditions and can thus potentially enter into unspecific ionic interactions with proteins, which do not have to be feared when polyether structures are employed.

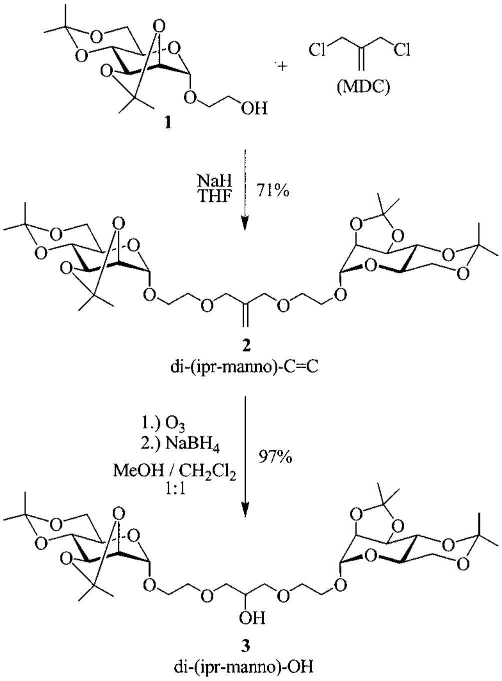
Aromatic polyether dendrimers, introduced by Fréchet and co-workers,[28,29] have become widely used scaffold structures for various applications.[30] Aliphatic polyether dendrimers on the other hand, were rarely encountered[31,32] in the literature until recently, due to reactivity problems during synthesis. An efficient pathway to these structures was developed, again by Fréchet et al.,[33−35] by the use of a sequence of iterative Williamson etherification steps with the activated alkenyl halide MDC (methallyl dichloride, 3-chloro-2-chloromethyl-1-propene), possessing two leaving groups in the allyl position, and hydroboration-oxidation of the double bond with 9-BBN in a convergent dendrimer synthesis. We have utilised a modified version of this synthetic strategy to construct multivalent glycomimetics with an aliphatic polyether scaffold.

Results and Discussion

Our desired target compounds were mannose-coated polyether glycodendrimers of different generations, which are potential inhibitors of mannose-binding lectins of *E. coli*. For the convergent dendrimer syntheses a protected ω-hydroxy-functionalised spacer mannoside was needed. This then could be treated with MDC to provide an ether-linked mannose dimer with a double bond at the focal point. Transformation of the double bond into a hydroxy function could be achieved by hydroboration with 9-BBN, followed by oxidation with aqueous sodium hydroxide/hydrogen peroxide, as described by Fréchet, to afford a primary hydroxy group. An alternative transformation, proceeding with the loss of one carbon atom, was the ozonolysis of the double bond and reduction of the carbonyl compound with sodium borohydride,[36] to afford a secondary alcohol. While hydroboration-oxidation by the reagent 9-BBN produces 1,4-cyclooctanediol as a by-product that has to be removed by chromatography, ozonolysis-hydride reduction in most cases proceeded smoothly, providing pure products without chromatography. This method was therefore selected for the construction of the desired glycodendrimers, thus giving them a polyglycerol ether scaffold.

The IUPAC names of the compounds described here are long, complicated and give no opportunity to conclude the actual structure from the name in a fast and easy manner. We have therefore designed a simple system to name the compounds. The name of each compound consists of a prefix, a middle part and a suffix. The prefixes *di*-, *tetra*- and *octa*- give the number of saccharide residues in the molecule, an additional *g*- in front identifying a structure with glycol spacers between glycerol branching units. The middle part shows whether the saccharide moieties are protected *(ipr-manno)* or deprotected *(manno)*. The suffix indicates the functionality at the focal point of the dendritic wedge. A *−C=C* suffix stands for a double bond, *-OH* for a hydroxy function, *-O-allyl* for an allyl ether and *−O-ethylene-OH* for a mono glycol ether residue.

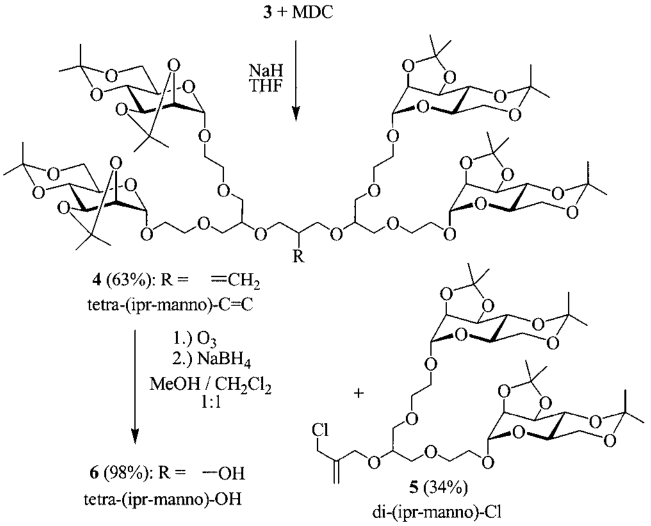
Isopropylidene-protected hydroxy ethyl mannoside (**1**) served as the carbohydrate component. An excess of **1** was treated with MDC and sodium hydride suspension in dry THF, giving unsaturated mannose dimer di-(ipr-manno)−C=C (**2**) in good yield, while unchanged starting mannoside (**1**) could be recovered during chromatographic purification. Compound **2** was in turn ozonised and reduced with sodium borohydride to yield alcohol di-(ipr-manno)−OH (**3**) without chromatography in excellent yield and in pure form (Scheme 1).



Scheme 1. Reaction sequence for the construction of glycerol glycodendrimers

For the synthesis of the next dendritic generation, di-(ipr-manno)−OH (**3**) was again treated with MDC. Along with the desired product tetra-(ipr-manno)−C=C (**4**) − obtained in lower yield − and recovered starting material **3**, the monosubstitution product of MDC, di-(ipr-manno)−Cl (**5**), was formed in substantial amount. Transformation of the unsaturated tetramer **4** into the alcohol tetra-(ipr-manno)−OH (**6**) proceeded smoothly (Scheme 2).

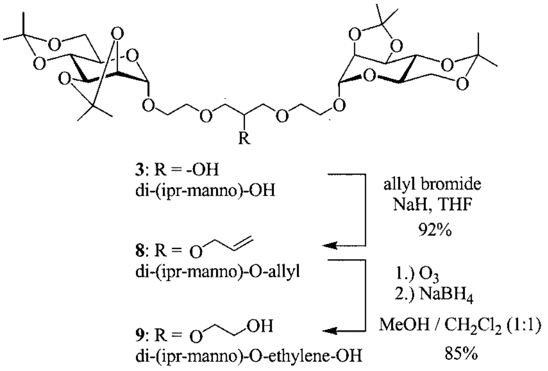
The intended coupling of two molecules of **6** to MDC under the previously employed conditions failed to give the desired octamer, only minor amounts of monosubstitution product of MDC with **6** being observed. Prolonged reaction times did not improve matters, with the reaction remaining incomplete, probably due to steric hindrance after the initial attack of the first molecule of **6** onto MDC.



Scheme 2. Preparation of tetra-(ipr-manno)−C=C (**4**) also produced the by-product di-(ipr-manno)−Cl (**5**); conversion of **4** into the alcohol tetra-(ipr-manno)-OH (**6**) was effected in very good yield

In order to enhance the efficiency of the reaction, the solvent/base system was changed from THF/sodium hydride to DMSO/sodium hydride. In the former system the base is insoluble in the solvent, so deprotonation of the alcohol can only take place on the interface of the solid base and the liquid phase. When sodium hydride is added to DMSO, the solvent itself is first deprotonated to form its anion. The sodium salt of the solvent's anion is soluble in DMSO, thus transforming the subsequent deprotonation of the alcohol component into a homogeneous process and so enhancing the reactivity of the base. By this approach, octa-(ipr-manno)−C=C (**7**) could be prepared, albeit in low yield (Figure 1).

To build up structures larger than mannose tetramers in more satisfactory yields, a more spacious design of the dendritic polyether structure seemed most promising, to mitigate the steric hindrance problems during synthesis. A strategy to achieve a more spacious architecture was the introduction of aliphatic alkyl ether spacers into the dendritic scaffold, separating the glycerol branching units and thus making the structure more flexible. A suitable compound for a start on this approach was prepared by etherification of alcohol di-(ipr-manno)−OH (**3**) with allyl bromide. The reaction product di-(ipr-manno)−O-allyl (**8**) was then converted into di-(ipr-manno)−O-ethylene−OH (**9**). Both steps proceeded smoothly and with good yields and without need for purification of the reaction products (Scheme 3).



Scheme 3. Preparation of the starting material for the construction of more spacious aliphatic polyether scaffolds

Primary alcohol **9** could now be introduced into the reaction cycle for the construction of polyether dendrimers, providing a glycerol glycol scaffold in which the glycerol branching units are separated by ethylene glycol spacers. Etherification of di-(ipr-manno)−O-ethylene−OH (**9**) with MDC yielded g-tetra-(ipr-manno)−C=C (**10**) along with a

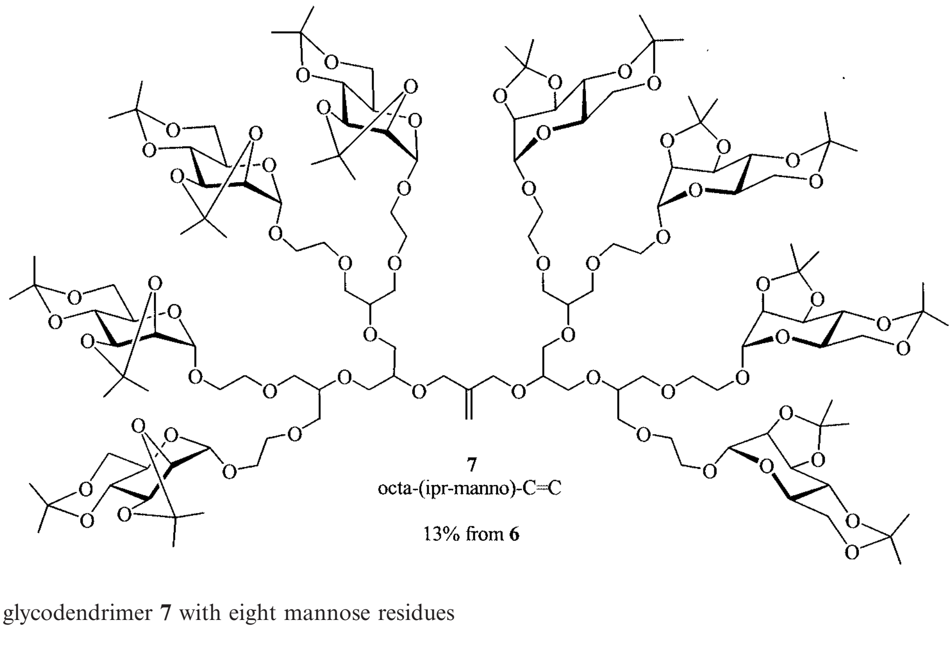
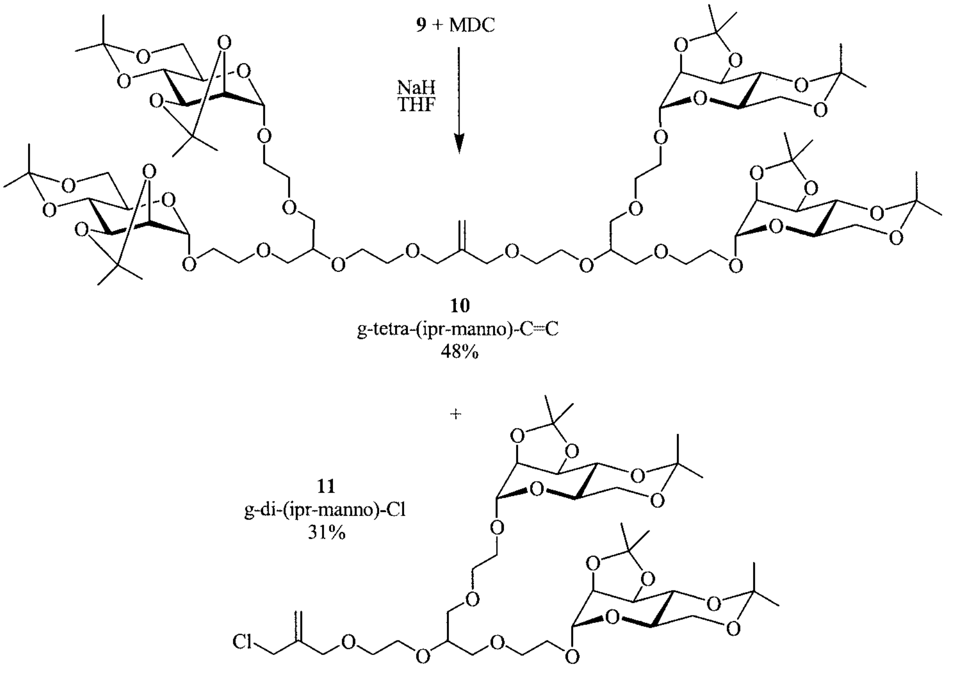


Figure 1. Glycerol glycodendrimer **7** with eight mannose residues



Scheme 4. Preparation of glycerol glycol glycodendrimer **10**

substantial amount of the monosubstitution product of MDC g-di-(ipr-manno)−Cl (**11**) and recovered starting material **9** (Scheme 4).

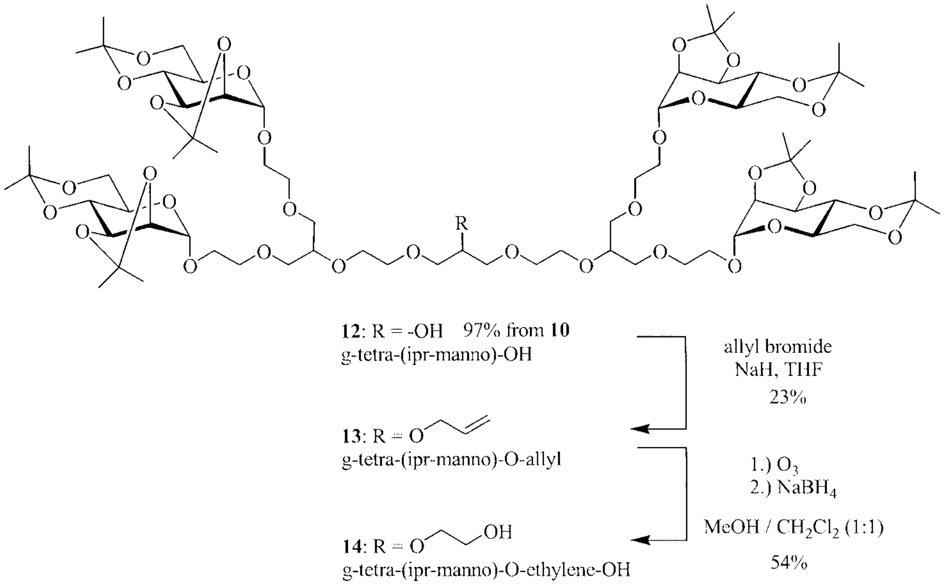
While ozonisation and hydride reduction of g-tetra-(ipr-manno)−C=C (**10**) to alcohol g-tetra-(ipr-manno)−OH (**12**) was effected with the usual ease, etherification of **12** with allyl bromide proved to be unexpectedly difficult. Under both standard (NaH, THF) and modified (NaH, DMSO) conditions, the reaction proceeded only sluggishly and remained incomplete even after prolonged reaction times. Furthermore, decomposition of the starting material was observed. The desired compound g-tetra-(ipr-manno)−O-allyl (**13**) was thus obtained in only 23% yield after chromatography. Ozonisation and reduction of **13** to alcohol g-tetra-(ipr-manno)−O-ethylene−OH (**14**) also did not work as expected. Compound **14** was isolated only in moderate yield, and chromatographic purification also became necessary. These results are summarised in Scheme 5.

Alcohol g-tetra-(ipr-manno)−O-ethylene−OH (**14**) was then coupled to MDC by use of sodium hydride in DMSO. The protected mannose octamer g-octa-(ipr-manno)−C=C (**15**) was isolated in 28% yield (Figure 2).

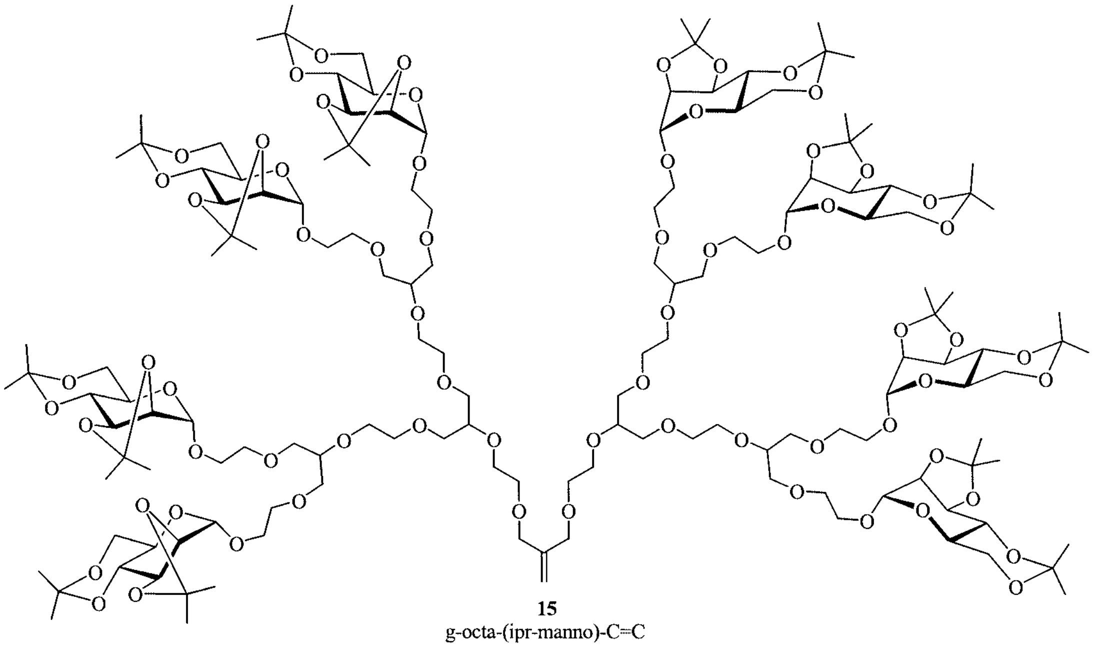
This result may be regarded as a success, however, in comparison with the 13% yield obtained in the synthesis of glycerol glycodendrimer octa-(ipr-manno)−C=C (**7**). The increase in yield may be attributable to the increased reactivity of the primary OH group of alcohol g-tetra-(ipr-manno)−O-ethylene−OH (**14**) in the Williamson etherification step with MDC and the more spacious design of the dendritic wedge, as well as better accessibility of the focal OH. However, the low yield in the etherification of g-tetra-(ipr-manno)−OH (**12**) with allyl bromide once again highlights the difficulty even for relatively small reagents to reach the reactive site at the focal point once four saccharide moieties are attached to the polyether scaffold. Here it has to be borne in mind that each of the saccharide units constitutes a tricyclic ring system with considerable steric demand, so that, unsurprisingly, four of them might well be able to reduce accessibility − and therefore reactivity − at the focal point considerably.

To access free OH compounds that could be evaluated in terms of their anti-adhesive potency towards lectins of *E. coli*, the acid-labile isopropylidene protective groups had to be removed without the acid-sensitive glycosidic linkages being affected. The literature describes the removal of ketal protective groups with many different acids in various solvents. Several deprotection methods, such as HCl/methanol,[37] TFA/water,[38] acidic ion-exchange resin/methanol/THF,[39] aqueous acetic acid[40] and *p*-toluenesulfonic acid in the presence of dimercaptopropane in THF,[41] were tried out. Whereas milder acid catalysts such as acetic acid and ion-exchange resin were far from cleaving all protective groups even after several days, use of *p*-toluenesulfonic acid/dimercaptopropane caused extensive decomposition. The best results were obtained by use of TFA/water, so this method was employed for deprotection. The method still failed to effect the quantitative deprotection reported in the literature, though this is not surprising, since only one ketal protective group was to be removed in the original example, while multiple isopropylidene groups had to be cleaved off for complete deprotection of the glycodendrimers, which obviously is no longer a trivial problem.

Di-(ipr-manno)−C=C (**2**), tetra-(ipr-manno)−C=C (**4**) and g-tetra-(ipr-manno)−OH (**12**) were selected as representative members of the glycerol and glycerol glycol glycodendrimer families for deprotection. In each case, TFA/water (9:1) was added to the starting material, followed by the immediate removal of the acid in vacuo, for prolonged



Scheme 5. Compounds from the glycerol glycol glycodendrimer family



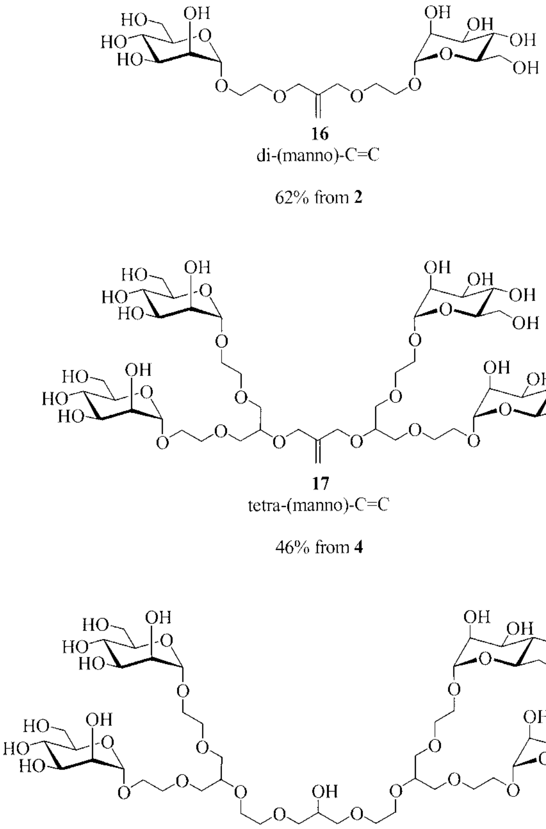
15
g-octa-(ipr-manno)-C=C

28% from **14**

Figure 2. Glycerol glycol glycodendrimer **15** with eight mannose residues

exposure of the compounds to the reagent caused cleavage of one or more glycosidic bonds. The crude products, still containing minor amounts of species with a single isopropylidene group, were purified by MPLC on reversed-phase silica gel. The three deprotected compounds di-(manno)−C=C (**16**), tetra-(manno)−C=C (**17**) and g-tetra-(manno)−OH (**18**) were thus obtained; the results are summarised in Figure 3. No deprotection of the octavalent compounds octa-(ipr-manno)−C=C (**7**) and g-octa-(ipr-manno)−C=C (**15**) was attempted, due to the low molar amounts of material available.

Finally, di-(manno)−C=C (**16**), tetra-(manno)−C=C (**17**) and g-tetra-(manno)−OH (**18**) were tested for their capacity to inhibit mannose-specific adhesion of *E. coli*, with use of a recombinant strain, *E. coli* HB 101 (pPK14), expressing only type 1 fimbriae on its surface.[42] A sandwich-type ELISA (enzyme-linked immunosorbent assay) was employed with microtiter plates, coated with yeast mannan.[43] *E. coli* readily binds to the mannan surface on the microtiter plates through mannose-specific lectins. Binding of bacteria can be inhibited by compounds containing α-mannosyl residues at different concentrations reflecting their in-



16
di-(manno)-C=C

62% from **2**

17
tetra-(manno)-C=C

46% from **4**

18
g-tetra-(manno)-C=C

66% from **12**

Figure 3. Deprotection of representative compounds of the glycerol and glycerol glycol glycodendrimers

hibitory potency. As adhesion of bacteria can be quantified by a peroxidase-based colour reaction, an ELISA allows IC_{50} values to be measured for each compound capable of inhibiting *E. coli* adhesion in a concentration-dependent manner. The IC_{50} values obtained in the ELISA employed reflect the inhibitor concentration causing 50% inhibition of bacterial binding to yeast mannan. Average IC_{50} values from three independently performed ELISAs are collected in Table 1. As was to be expected, methyl α-D-mannoside (MeMan) inhibited the adhesion of *E. coli* HB 101 (pPK14) at millimolar concentrations, whereas *p*-nitrophenyl α-D-mannoside (*p*NPMan), due to its aromatic aglycon,[44] has an inhibitory potency better by approximately two orders of magnitude.[45,46]

None of the tested glycodendrons **16**, **17**, and **18** is a better inhibitor than *p*NPMan. Due to their spacer characteristics the measurered IC_{50} values have been compared to methyl α-D-mannoside (MeMan) to deduce relative inhibition potencies (RIC_{50}), which are typically highly reproducible.

From the available knowledge about the adhesive protein of type 1 fimbriae, FimH,[47] it is likely that the tested glycodendrons bind to single FimH molecules and so the measured IC_{50} values may be regarded as affinities. Nevertheless, it can be assumed that the multiple α-mannosyl moieties present interact with several sites of the adhesin, giving slightly improved binding activities of the glycodendrimers **16**, **17** and **18** in relation to MeMan. Thanks to their multivalent structures, however, the tested glycodendrimers may even effect receptor clustering,[48] a possibility that should also be considered as it has important biological consequences.[49]

Table 1. Anti-adhesive properties of the three deprotected glycodendrimers

IC_{50} [μML^{-1}]	MeMan	*p*NPMan	**16**	**17**	**18**
IC_{50} [a] (assay 1)	748	24	162	190	94
IC_{50} [a] (assay 2)	897	25	76	183	63
IC_{50} [a] (assay 3)	1510	19	115	187	90
Average IC_{50}	1052	23	118	187	82
Standard deviation	400	4	43	3	17
RIC_{50} [b]	1	50	6	10	13

[a] IC_{50}: 50% of binding inhibited. [b] RIC_{50} Relative IC_{50} based on MeMan (=1)

Conclusion

Two novel families of glycodendrimers with two new aliphatic polyether structures have successfully been introduced. For structures with up to four mannose residues, the glycerol glycodendrimers are accessible on multigram scales, which is an important distinction between this method and other glycodendrimer syntheses. Unfortunately, the octavalent compound in the glycerol series, octa-(ipr-manno)−C=C (**7**) could be obtained only in poor yield, probably due to steric problems. An approach to reduction of steric hindrance through the use of a more flexible and spacious dendritic scaffold resulted in the preparation of the glycerol glycol glycodendrimers, the synthesis of which again proceeded smoothly up to the tetravalent compounds. Etherification of the tetramer g-tetra-(ipr-manno)−OH (**12**) with allyl bromide proved to be difficult. The final construction of the target compound g-octa-(ipr-manno)−C=C (**15**) proceeded with significantly increased yield relative to that of the corresponding glycerol glycodendrimer **7**.

The divalent and tetravalent glycerol dendrimers **2** and **4** and the tetravalent glycerol glycol dendrimer **12** were deprotected as representative compounds of the polyether family. With the resulting polyether glycodendrimers **16**, **17** and **18**, three compounds could be biologically evaluated.

Experimental Section

General Remarks: Reactions requiring dry or oxygen-free conditions were carried out under argon (Schlenk conditions). THF and *n*-hexane were dried by distillation from sodium/potassium ketyl, methanol by distillation from magnesium turnings, and dichloromethane by distillation from calcium hydride, each under argon. Dry DMSO was purchased from Fluka. Sodium hydride (Merck) was used as a suspension in paraffin oil (content 55−65%). Pure sodium hydride was obtained from the suspension by washing with dry *n*-hexane under argon and drying in vacuo. **Caution, the resulting white powder is extremely inflammable, especially on contact with water!** TLC was performed on GF_{254} silica gel plates (Merck), detection was effected by charring with 10% sulfuric acid in ethanol followed by heat treatment. Flash chromatography was performed on silica gel 60 (230−400 mesh, particle size 0.040−0.063 mm, Merck). RP MPLC was performed with a Büchi 688 chromatography pump on a pre packed Lobar column, size B (310−25) LiChroprep RP 18, 40−63 μm (Merck). Optical rotations were measured with Perkin−Elmer polarimeters 241 and 243 (sodium D-line: 589 nm, length of cell 1 dm) in the solvents given. NMR spectra were recorded with Bruker DRX 500 (500 MHz for ^{1}H, 125.47 MHz for ^{13}C) and ARX 300 instruments (300 MHz for ^{1}H. The spectra were calibrated on the solvent peak ($CDCl_3$: 7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C; [D$_4$]methanol: 3.35 ppm for ^{1}H and 49.30 ppm for ^{13}C). Assignment of the peaks was achieved with aid of 2D NMR techniques (^{1}H-^{1}H-COSY and HSQC). Peak values that could not be unequivocally assigned to one atom, and may therefore be interchangeable, are marked with an asterisk. In the reported data the abbreviation "su" denotes a sugar moiety. The hydrogen and carbon atoms within the scaffold are named as follows: starting from the mannosyl residue, which is abbreviated as su, the hydrogens of the ethylene glycol spacer are named *H-a*, *H-b*, *H-c* and *H-d*, while the carbon atoms are called *C-ab* and *C-cd*. The dendritic scaffold is now viewed along its branches from outside to the inside. The hydrogen atoms of the methylene group are named $H\text{-}e_1$ and $H\text{-}f_1$, the carbon is named $C\text{-}e_1f_1$. If the next position is a tetrahedral branching point or a tetrahedral centre of the molecule, the hydrogen atom is named $H\text{-}x_1$, the carbon atom $C\text{-}x_1$. According to this system, naming is continued until the centre of the molecule is reached. If the structure contains glycol spacer units between the glycerol branching units, they are not named specially, but are indicated by $O-CH_2-CH_2-O$. The system is shown in Figure 4.

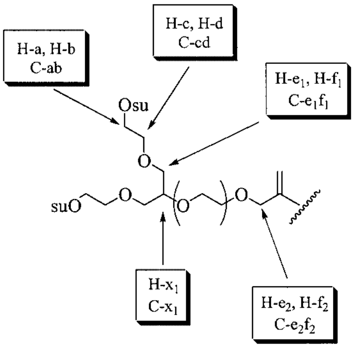


Figure 4. Assignation of the hydrogen and carbon atoms of the dendritic scaffold

MALDI TOF mass spectra were recorded with a Bruker Biflex III 19 kV instrument, DHB (2,5-dihydroxybenzoic acid) in acetonitrile/water (1:2) with 0.1% TFA was used as matrix. For sample preparation, a drop of matrix solution was first placed on the target and left to evaporate. Afterwards, a solution of the sample in THF (protected compounds) or in methanol (deprotected derivatives) was placed on the pre-crystallised matrix. Elemental analyses were performed in the Institute of Inorganic Chemistry, Christiana-Albertina-University Kiel. Even though NMR studies showed no contamination within the samples (with the exception of compound **7**), correct analyses could not be obtained for all substances. Even samples that contained no impurities according to analytical HPLC failed to give correct values. A recombinant type 1 fimbriated *E. coli* strain, *E coli* HB101 (pPK14)[42] was used in ELISA (enzyme linked immunosorbent assay), which was carried out as described earlier.[43] Sarstedt F-shaped 96-well microtiter plates were used. Mannan from *Saccharomyces cerevisae* was purchased from Sigma and was used in 50 mM aq. Na_2CO_3 (1 mg/mL; pH 9.6). The polyclonal anti-fimA antibody was a kind gift from Prof. Dr. S. Ehlers (Research Center Borstel, Germany) and peroxidase-conjugated goat anti-rabbit antibody (IgG, H+L) was purchased from Dianova. Skimmed milk was from Ulzena, Tween 20 from Roth, ABTS [2,2′-azidobis(3-ethylbenzothiazoline-6-sulfonic acid)] from Sigma, and thimerosal {2-[(ethylmercurio)thio]benzoic acid, sodium salt} from Merck.

Procedure A − Etherification of Alcohols with MDC: Under Schlenk conditions the alcohol component (3 equiv.) was dissolved in dry THF (c = 0.50−1.00 mol/L). Sodium hydride suspension (corresponding to 3.13 equiv. pure sodium hydride) and MDC (1 equiv.) was added, and the resulting mixture was heated at reflux for 12−15 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature, quenched with water and subsequently saturated with potassium carbonate. The phases were separated, and the aqueous layer was extracted with three portions of ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate and filtered, and the solvent was removed under vacuum. The resulting crude product was purified by flash chromatography on silica gel with the solvent combinations given for the particular compound.

Procedure B − Ozonolysis and Reduction of Olefinic Double Bonds to Hydroxy Functions: The unsaturated compound (1 equiv.) was dissolved in dry methanol/dichloromethane 1:1 (c = 0.13 mol/L) and cooled to −78 °C. Ozone was bubbled through the solution until it turned blue, and the complete consumption of the olefin was then verified by TLC. After the removal of excess ozone by use of a vigorous stream of nitrogen, sodium borohydride (10 equiv.) was added. While stirring for 12−15 h the mixture was allowed to slowly warm to room temperature. Completion of the reduction was checked by TLC. The reaction mixture was quenched by addition of saturated ammonium chloride solution followed by stirring for 1 h. The phases were separated and the aqueous layer was extracted with three portions of dichloromethane. The combined organic phases were washed with water, dried over anhydrous magnesium sulfate and filtered. Evaporation of the solvent yielded the target compound in pure form.

Procedure C − Allylation of Hydroxy Functions: Under Schlenk conditions the alcohol component (1 equiv.) was dissolved in dry THF (c = 0.17−0.31 mol/L). Sodium hydride suspension (corresponding to 1.11 equiv. pure sodium hydride) and allyl bromide (3 equiv.) were added and the resulting mixture was heated at reflux for 12−15 h. Progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature, quenched with water and subsequently saturated with potassium carbonate. The phases were separated and the aqueous layer was extracted with three portions of ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate and filtered. Removal of the solvent in vacuo yielded the target compound in pure form.

Procedure D − Deprotection of Diisopropylidene-Protected Mannopyranosyl Derivatives with TFA/H_2O: TFA/H_2O (9:1, 10 mL) was added to the isopropylidene-protected compound and the solvent mixture was immediately removed on a rotary evaporator connected to an oil pump. The residue was co-evaporated with toluene and subsequently purified by MPLC on RP-18 silica gel (methanol/H_2O, 1:4).

Di-(ipr-manno)−C=C (2): Reaction conditions and workup were as described in Procedure A, with hydroxyethyl mannoside **1** (12.66 g, 41.59 mmol), 60% sodium hydride suspension (1.74 g), MDC (1.17 mL, 13.86 mmol), dry THF (40 mL), TLC (cyclohexane/ethyl acetate, 1:1). Purification was by flash chromatography on silica gel (cyclohexane/ethyl acetate, 2:1). Two fractions were collected, the first consisted of title compound **2** and the second contained unconsumed starting mannoside **1**. Recovered hydroxyethyl mannoside **1** (4.04 g, 13.28 mmol). Title compound **2** (4.61 g, 6.97 mmol, 71%) was obtained as a colourless oil. $[\alpha]_D^{20} = +3.9$ ($c = 0.8$ in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 1.32, 1.40, 1.49, 1.52$ (each s, 24 H, $-CH_3$ isopropylidene), 3.54−3.64 (m, 8 H, H-5, H-b, H-c, H-d), 3.69−3.76 (m, 4 H, H-4, H-6′), 3.76−3.81 (m, 2 H, H-a), 3.84 (dd, $^3J_{5,6} = 5.7$, $^2J_{6,6'} = 10.8$ Hz, 2 H, H-6), 4.01 (m_c, 4 H, H-e_1, H-f_1), 4.13 (dd, $^3J_{2,3} = 5.7$, $^3J_{3,4} = 7.9$ Hz, 2 H, H-3), 4.19 (dd ≈ d, $^3J_{1,2} \approx 0$, $^3J_{2,3} = 5.7$ Hz, 2 H, H-2), 5.04 (d ≈ s, $^3J_{1,2} \approx 0$ Hz, 2 H, H-1), 5.20 (m_c, 2 H, C=CH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 18.8, 26.2, 28.2, 29.1$ (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.1 (CH_2, C-6), 66.7 (CH_2, C-ab), 69.1 (CH_2, C-cd), 71.8 (CH_2, C-e_1f_1), 72.7 (CH, C-4), 74.9 (CH, C-3), 76.0 (CH, C-2), 97.9 (CH, C-1), 99.7, 109.4 (C, Me−C−Me isopropylidene), 114.5 (CH_2, C=CH_2), 142.2 (C, C=CH_2) ppm. MALDI TOF MS: $m/z = 683.4$ $[M + Na]^+$, 699.3 $[M + K]^+$ found for $C_{32}H_{52}O_{14}$ (calcd. 660.34). $C_{32}H_{52}O_{14}$ (660.76): calcd. C 58.2, H 7.9; found C 58.4, H 7.9.

Di-(ipr-manno)−OH (3): Reaction conditions and workup were as described in Procedure B, with di-(ipr-manno)−C=C (**2**, 4.20 g, 6.35 mmol), sodium borohydride (2.40 g, 63.50 mmol), dry methanol/dichloromethane (1:1, 50 mL), TLC (cyclohexane/ethyl acetate, 1:1). Title compound **3** (4.11 g, 6.18 mmol, 97%) was obtained as a colourless foam without further purification. $[\alpha]_D^{20} = +3.2$ ($c = 0.9$ in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 1.32, 1.40, 1.49, 1.52$ (each s, 24 H, $-CH_3$ isopropylidene), 2.70 (d, $^3J_{x1,OH} = 2.2$ Hz, 1 H, −OH), 3.47−3.67 (m, 12 H, H-5, H-b, H-c, H-d, H-e_1, H-f_1), 3.69−3.76 (m, 4 H, H-4, H-6′), 3.82−3.88 (2 dd, $^3J_{5,6} = 5.5$, $^2J_{6,6'} = 10.8$ Hz, 2 H, H-6), 3.76−3.82 (m, 2 H, H-a), 3.89−4.00 (m, 1 H, H-x_1), 4.12 (dd, $^3J_{2,3} = 6.1$, $^3J_{3,4} = 7.7$ Hz, 2 H, H-3), 4.18 (dd ≈ d, $^3J_{1,2} \approx 0$, $^3J_{2,3} = 6.1$ Hz, 2 H, H-2), 5.03 (d ≈ s, $^3J_{1,2} \approx 0$ Hz, 2 H, H-1) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 18.8, 26.1, 28.2, 29.0$, ($CH_3$, $-CH_3$ isopropylidene), 61.4 (CH, C-5), 62.0 (CH_2, C-6), 66.6 (CH_2, C-ab), 69.4 (CH, C-x_1), 70.4 (CH_2, C-e_1f_1), 72.4 (CH_2, C-cd), 72.7 (CH, C-4), 74.8 (CH, C-3), 76.0 (CH, C-2), 97.9 (CH, C-1), 99.7, 109.4 (C, Me−C−Me isopropylidene) ppm. MALDI TOF MS: $m/z = 687.4$ $[M + Na]^+$ and 703.4 $[M + K]^+$ found for $C_{31}H_{52}O_{15}$ (calcd. 664.33). $C_{31}H_{52}O_{15}$ (664.74): calcd. C 56.0, H 7.9; found C 55.6, H 8.0.

Tetra-(ipr-manno)−C=C (4): Reaction conditions and workup were as described in Procedure A, with di-(ipr-manno)−OH (**3**, 3.76 g, 5.65 mmol), 60% sodium hydride suspension (235 mg), MDC (0.16 mL, 1.88 mmol), dry THF (10 mL), TLC (cyclohexane/ethyl acetate, 1:2). Purification was by flash chromatography on silica gel (cyclohexane/ethyl acetate, 1:1). Three fractions were collected; the first contained monosubstitution product **5**, the second title compound **4** and the third consisted of unconsumed di-(ipr-manno)−OH (**3**). Recovered di-(ipr-manno)−OH (**3**, 875 mg 1.32 mmol). Title compound **4** (1.64 g, 1.19 mmol, 63%) was obtained as a colourless foam. $[\alpha]_D^{20} = +2.4$ ($c = 1.1$ in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 1.32, 1.39, 1.49, 1.52$ (each s, 48 H, $-CH_3$ isopropylidene), 3.51−3.66 (m, 26 H, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, H-5), 3.68−3.79 (m, 12 H, H-a, H-4, H-6′), 3.81−3.87 (2 dd, $^3J_{5,6} = 5.7$, $^2J_{6,6'} = 10.8$ Hz, 4 H, H-6), 4.10−4.14 (m, 8 H, H-3, H-e_2, H-f_2), 4.17 (dd ≈ d, $^3J_{1,2} \approx 0$, $^3J_{2,3} = 5.7$ Hz, 4 H, H-2), 5.03 (d ≈ s, $^3J_{1,2} \approx 0$ Hz, 4 H, H-1), 5.19 (m_c, 2 H, C=CH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 18.8, 26.2, 28.2, 29.1$ (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.6 (CH_2, C-ab), 70.4 (CH_2, C-e_1f_1), 70.7 (CH_2, C-e_2f_2), 71.0, 71.1 (CH_2, C-cd), 72.7 (CH, C-4), 74.8 (CH, C-3), 76.0 (CH, C-2), 77.0 (CH, C-x_1), 97.8, 97.9 (CH, C-1), 99.7, 109.4 (C, Me−C−Me isopropylidene), 114.3 (CH_2, C=CH_2), 143.1 (C, C=CH_2) ppm. MALDI TOF MS: $m/z = 1403.6$ $[M + Na]^+$ and 1419.6 $[M + K]^+$ found for $C_{66}H_{108}O_{30}$ (calcd. 1380.69). $C_{66}H_{108}O_{30}$ (1381.57): calcd. C 57.4, H 7.9; found C 57.0, H 8.0.

Di-(ipr-manno)−Cl (5): **5** (479 mg, 640 μmol, 34%) was isolated as a greenish oil. $[\alpha]_D^{20} = +3.1$ ($c = 1.0$ in $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 1.32, 1.39, 1.49, 1.52$ (each s, 24 H, $-CH_3$ isopropylidene) 3.49−3.80 (m, 19 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, H-5), 3.81−3.90 (2 dd, $^3J_{5,6} = 5.6$, $^2J_{6,6'} = 10.5$ Hz, 2 H, H-6), 4.05−4.26 (m, 8 H, H-2, H-3, H-e_2, H-f_2), 5.03 (d ≈ s, $^3J_{1,2} \approx 0$ Hz, 2 H, H-1), 5.19−5.31 (m, 2 H, C=CH_2) ppm.

Tetra-(ipr-manno)−OH (6): Reaction conditions and workup were as described in Procedure B, with tetra-(ipr-manno)−C=C (**4**, 4.06 g, 2.65 mmol), sodium borohydride (1.01 g, 26.50 mmol), dry methanol/dichloromethane (1:1, 40 mL), TLC (cyclohexane/ethyl acetate, 1:2). Title compound **6** (3.95 g, 2.85 mmol, 98%) was obtained as a colourless foam without further purification. $[\alpha]_D^{20} = +1.8$ ($c = 0.85$ in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 1.32, 1.39, 1.49, 1.52$ (each s, 48 H, $-CH_3$ isopropylidene), 3.49−3.80 (m, 43 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, H-e_2, H-f_2, H-5, -OH), 3.81−3.87 (2 dd, 4 H, H-6), 3.87−3.39 (m, 1 H, H-x_2), 4.12 (m, 4 H, H-3), 4.17−4.21 (2 dd ≈ 2 d, 4 H, H-2), 5.03 (d ≈ s, $^3J_{1,2} \approx 0$ Hz, 4 H, H-1) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 18.8, 26.2, 28.2, 29.0$, ($CH_3$, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.5 (CH_2, C-ab), 69.7 (CH, C-x_2), 70.3, 70.4, 71.1, 71.2, (CH_2, C-e_1f_1, C-e_2f_2), 72.0, 72.1 (CH_2, C-cd), 72.7 (CH, C-4), 74.8 (CH, C-3), 75.9 (CH, C-2), 78.6 (CH, C-x_1), 97.8 (CH, C-1), 99.7, 109.4 (C, Me−C−Me isopropylidene) ppm. MALDI TOF MS: $m/z = 1407.9$ $[M + Na]^+$ and 1423.9 $[M + K]^+$ found for $C_{65}H_{108}O_{31}$ (calcd. 1384.69).

Octa-(ipr-manno)−C=C (7): Under Schlenk conditions, tetra-(ipr-manno)−OH (**6**, 300 mg, 217 μmol) was dissolved in dry DMSO (2 mL). Pure sodium hydride (5 mg, 225 μmol) and MDC (6 μL, 72 μmol) were added and the resulting mixture was stirred at 50 °C for 15 h. TLC (cyclohexane/ethyl acetate, 1:4) revealed a very slow conversion into the product. To accelerate the reaction, a small amount of tetra-*n*-butylammonium iodide was added and the mixture was kept at 50 °C for another 24 h and then cooled to room temperature. Quenching was effected with water (2 mL) and the mixture was extracted with three portions of diethyl ether. The combined organic phases were washed with water, dried over anhydrous magnesium sulfate and filtered, and the solvent was removed under vacuum. Purification by flash chromatography on silica gel (cyclohexane/ethyl acetate, 1:3) yielded two fractions; the first contained title compound **7** and the second consisted of unconsumed tetra-(ipr-manno)−OH (**6**). Recovered tetra-(ipr-manno)−OH (**7**, 150 mg 108 μmol). Title compound **7** (25 mg, 9 μmol, 13%) was obtained as a colourless oil that according to the NMR spectra still contained minor impurities. $[\alpha]_D^{20}$ = +2.0 (*c* = 0.7 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.32, 1.39, 1.49, 1.52 (each s, 96 H, $-CH_3$ isopropylidene), 3.49−3.79 (m, 86 H, H-a, H-4, H-6′,H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, H-e_2, H-f_2, H-x_2, H-5), 3.83 (dd, $^3J_{5,6}$ = 5.7, $^2J_{6,6'}$ = 10.8 Hz, 8 H, H-6), 4.05−4.14 (m, 12 H, H-3, H-e_3, H-f_3), 4.17 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.5 Hz, 8 H, H-2), 5.02 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 8 H, H-1), 5.19 (m_c, 2 H, $C=CH_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.7, 26.2, 28.2, 29.0 (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.5 (CH_2, C-ab), 70.0, 70.3, 70.4, 70.6, 70.8, 70.9 (CH_2, C-cd, C-e_1f_1, C-e_2f_2, C-e_3f_3), 72.7 (CH, C-4), 74.7 (CH, C-3), 75.9 (CH, C-2), 77.4 (CH, C-x_2), 78.5 (CH, C-x_1), 97.7, 97.8(CH, C-1), 99.6, 109.4 (C, Me−*C*−Me isopropylidene), 113.3 (CH_2, $C=CH_2$), 143.2 (C, $C=CH_2$) ppm. MALDI TOF MS: *m*/*z* = 2844.6 [M + Na]$^+$ and 2860.5 [M + K]$^+$ found for $C_{134}H_{220}O_{62}$ (calcd. 2821.41).

Di-(ipr-manno)−O-allyl (8): Reaction conditions and workup were as described in Procedure C, with di-(ipr-manno)−OH (**3**, 5.16 g, 7.77 mmol), 60% sodium hydride suspension (340 mg), allyl bromide (1.97 mL, 23.30 mmol), dry THF (25 mL), TLC (cyclohexane/ethyl acetate, 1:1). Title compound **8** (5.03 g, 7.14 mmol, 92%) was obtained as a colourless foam without further purification. $[\alpha]_D^{20}$ = +2.4 (*c* = 1.0 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.32, 1.39, 1.48, 1.52 (each s, 24 H, $-CH_3$ isopropylidene), 3.51−3.79 (m, 19 H, H-a, H-4, H-6′, H-x_1, H-b, H-c, H-d, H-e_1, H-f_1, H-5), 3.81−3.88 (2 dd, $^3J_{5,6}$ = 5.7, $^2J_{6,6'}$ = 10.8 Hz, 2 H, H-6), 4.08−4.15 (m, 4 H, $CH_2=CH-CH_2-O$, H-3), 4.17 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.7 Hz, 2 H, H-2), 5.03 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 2 H, H-1), 5.14 (m_c, 1 H, $CHH=CH-CH_2-O$), 5.27 (m_c, 1 H, $CHH=CH-CH_2-O$), 5.90 (m_c, 1 H, $CH_2=CH-CH_2-O$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.8, 26.1, 28.2, 29.0 (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.6 (CH_2, C-ab), 70.4 (CH_2, C-e_1f_1), 71.3, 71.4 (CH_2, $CH_2=CH-CH_2-O$, C-cd), 72.7 (CH, C-4), 74.8 (CH, C-3), 76.0 (CH, C-2), 77.0 (CH, C-x_1), 97.8 (CH, C-1), 99.6, 109.4 (C, Me−*C*−Me isopropylidene), 116.9 (CH_2, $CH_2=CH-CH_2-O$), 135.3 (CH, $CH_2=CH-CH_2-O$) ppm. MALDI TOF MS: *m*/*z* = 727.2 [M + Na]$^+$ and 743.2 [M + K]$^+$ found for $C_{34}H_{56}O_{15}$ (calcd. 704.36). $C_{34}H_{56}O_{15}$ (704.81): calcd. C 57.9, H 8.0; found C 57.9, H 8.1.

Di-(ipr-manno)−O-ethylene-OH (9): Reaction conditions and workup were as described in Procedure B, with di-(ipr-manno)-O-allyl (**8**, 5.03 g, 7.14 mmol), sodium borohydride (2.70 g, 71.40 mmol), dry methanol/dichloromethane 1:1 (60 mL), TLC (cyclohexane/ethyl acetate, 1:1). Title compound **9** (4.29 g, 6.05 mmol, 85%) was obtained as a colourless foam without further purification. $[\alpha]_D^{20}$ = +2.6 (*c* = 0.9 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.32, 1.39, 1.49, 1.52 (each s, 24 H, $-CH_3$ isopropylidene), 3.15 (br. s, 1 H, -OH), 3.45−3.89 (m, 25 H, H-6, H-a, H-4, H-6′, H-x_1, H-b, H-c, H-d, $O-CH_2-CH_2-OH$, H-e_1, H-f_1, H-5), 4.08−4.23 (m, 4 H, H-2, H-3), 5.03, 5.04 (each d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 2 H, H-1) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.8, 26.1, 28.2, 29.0 (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 61.9 (CH_2, $O-CH_2-CH_2-OH$), 62.0 (CH_2, C-6), 66.5 (CH_2, C-ab), 70.4 (CH_2, C-e_1f_1), 71.5, 71.6 (CH_2, C-cd), 72.3 (CH_2, $O-CH_2-CH_2-OH$), 72.7 (CH, C-4), 74.8 (CH, C-3), 75.9, 76.0 (CH, C-2), 78.2 (CH, C-x_1), 97.8, 97.9 (CH, C-1), 99.7, 109.4 (C, Me−*C*−Me isopropylidene) ppm. MALDI TOF MS: *m*/*z* = 731.5 [M + Na]$^+$ and 747.5 [M + K]$^+$ found for $C_{33}H_{56}O_{16}$ (calcd. 708.36).

g-Tetra-(ipr-manno)−C=C (10): Reaction conditions and workup were as described in Procedure A, with di-(ipr-manno)-O-ethylene-OH (**9**, 4.29 g, 6.05 mmol), 60% sodium hydride suspension (274 mg), MDC (0.17 mL, 2.01 mmol), dry THF (12 mL), TLC (cyclohexane/ethyl acetate, 1:1). Purification was by flash chromatography on silica gel (cyclohexane/ethyl acetate, 2:1). Three fractions were collected; the first contained monosubstitution product g-di-(ipr-manno)−Cl (**11**), the second title compound **10**, and the third consisted of unconsumed di-(ipr-manno)-O-ethylene-OH (**9**). Recovered di-(ipr-manno)-O-ethylene-OH (**9**, 875 mg, 1.32 mmol). Title compound **10** (1.42 g, 0.96 mmol, 48%) was obtained as a colourless foam. $[\alpha]_D^{20}$ = +2.5 (*c* = 1.1 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.32, 1.39, 1.49, 1.52 (each s, 48 H, $-CH_3$ isopropylidene), 3.50−3.80 (m, 46 H, H-a, H-4,H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, $O-CH_2-CH_2-O$, H-5), 3.80−3.87 (2 dd, $^3J_{5,6}$ = 5.5, $^2J_{6,6'}$ = 10.6 Hz, 4 H, H-6), 3.98 (m_c, 4 H, H-e_2, H-f_2), 4.12 (dd, $^3J_{2,3}$ = 5.7, $^3J_{3,4}$ = 7.9 Hz, 4 H, H-3), 4.18 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.7 Hz, 4 H, H-2), 5.03 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 4 H, H-1), 5.16 (m_c, 2 H, $C=CH_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.8, 26.2, 28.2, 29.1 (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.6 (CH_2, C-ab), 69.8, 69.9 ($O-CH_2-CH_2-O$), 70.4 (CH_2, C-e_1f_1), 71.2 (CH_2, C-cd), 71.8 (CH_2, C-e_2f_2), 72.7 (CH, C-4), 74.8 (CH, C-3), 76.0 (CH, C-2), 78.4 (CH, C-x_1), 97.8 (CH, C-1), 99.7, 109.4 (C, Me−*C*−Me isopropylidene), 113.6 (CH_2, $C=CH_2$), 142.6 (C, $C=CH_2$) ppm. MALDI TOF MS: *m*/*z* = 1492.0 [M + Na]$^+$ and 1507.9 [M + K]$^+$ found for $C_{70}H_{116}O_{32}$ (calcd. 1468.74).

g-Di-(ipr-manno)−Cl (11): **11** (500 mg, 620 µmol, 31%) was obtained as a greenish oil. $[\alpha]_D^{20}$ = +2.7 (*c* = 1.2 in $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.32, 1.39, 1.49, 1.52 (each s, 24 H, $-CH_3$ isopropylidene), 3.50−3.79 (m, 23 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, H-5), 3.81−3.87 (2 dd, $^3J_{5,6}$ = 5.6, $^2J_{6,6'}$ = 10.5 Hz, 2 H, H-6), 4.09 (m_c, 4 H, H-e_2, H-f_2), 4.12 (dd, $^3J_{2,3}$ = 5.5, $^3J_{3,4}$ = 7.9 Hz, 2 H, H-3), 4.18 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.5 Hz, 2 H, H-2), 5.03 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 2 H, H-1), 5.19−5.29 (m, 2 H, $C=CH_2$).

g-Tetra-(ipr-manno)-OH (12): Reaction conditions and workup were as described in Procedure B, with g-tetra-(ipr-manno)−C=C (**10**, 1.42 g, 966 µmol), sodium borohydride (361 mg, 9.66 mmol), dry methanol/dichloromethane (1:1, 40 mL), TLC (cyclohexane/ethyl acetate, 1:2). The title compound **12** (1.38 g, 936 µmol, 97%) was obtained as a colourless foam without further purification. $[\alpha]_D^{20}$ = +3.1 (*c* = 0.8 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.32, 1.39, 1.49, 1.52 (each s, 48 H, $-CH_3$ isopropylidene), 3.43−3.80 (m, 51 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, $O-CH_2-CH_2-O$, H-e_2, H-f_2, H-5, -OH), 3.84 (dd, $^3J_{5,6}$ = 5.5, $^2J_{6,6'}$ = 10.6 Hz, 4 H, H-6), 3.89 (m, 1 H, H-x_2), 4.12 (dd, $^3J_{2,3}$ = 5.7, $^3J_{3,4}$ = 7.7 Hz, 4 H, H-3), 4.18 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.7 Hz, 4 H, H-2), 5.03 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 4 H, H-1) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.8, 26.2, 28.2, 29.1 (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.6 (CH_2, C-ab), 69.3 (CH, C-x_2), 70.4 (CH_2, C-e_1f_1), 69.7*, 71.0* (CH_2, $O-CH_2-CH_2-O$), 71.2, 71.3 (CH_2, C-cd), 72.6* (CH_2, C-e_2f_2), 72.7 (CH, C-4), 74.8 (CH, C-3), 76.0 (CH, C-2), 78.3 (CH, C-x_1), 97.8 (CH, C-1), 99.7, 109.4 (C, Me−*C*−Me isopropylidene) ppm. MALDI TOF MS: *m*/*z* = 1495.9 [M + Na]$^+$ and 1511.9 [M + K]$^+$ found for $C_{69}H_{116}O_{33}$ (calcd. 1472.74).

g-Tetra-(ipr-manno)-O-allyl (13): Reaction conditions and workup were as described in Procedure C, with g-tetra-(ipr-manno)-OH (**12**, 1.22 g, 825 µmol), 60% sodium hydride suspension (50 mg), allyl bromide (0.10 mL, 1.24 mmol), dry THF (5 mL), TLC (cyclohexane/ethyl acetate, 1:1). TLC revealed the presence of impurities in the product, so purification by flash chromatography on silica gel was performed (cyclohexane/acetone 2:1). Title compound **13** (291 mg, 192 µmol, 23%) was obtained as a colourless foam. $[\alpha]_D^{20}$ = +1.9 (*c* = 0.8 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ =

1.32, 1.39, 1.48, 1.52 (each s, 48 H, $-CH_3$, isopropylidene), 3.47−3.79 (m, 51 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, $O-CH_2-CH_2-O$, H-e_2, H-f_2, H-x_2, H-5), 3.81−3.87 (2 dd, $^3J_{5,6}$ = 5.7, $^2J_{6,6'}$ = 10.8 Hz, 4 H, H-6), 4.08−4.15 (m, 6 H, $CH_2=CH-CH_2-O$, H-3), 4.17 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.7 Hz, 4 H, H-2), 5.03 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 4 H, H-1), 5.12 (m_c, 1 H, $CHH=CH-CH_2-O$), 5.25 (m_c, 1 H, $CHH=CH-CH_2-O$), 5.89 (m_c, 1 H, $CH_2=CH-CH_2-O$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.8, 26.2, 28.2, 29.1 (CH_3, $-CH_3$ isopropylidene), 61.3 (CH, C-5), 62.0 (CH_2, C-6), 66.6 (CH_2, C-ab), 70.4 (CH_2, C-e_1f_1), 69.8*, 71.0* (CH_2, $O-CH_2-CH_2-O$), 71.2 (CH_2, C-cd), 71.3* (CH_2, $CH_2=CH-CH_2-O$), 71.6* (CH_2, C-e_2f_2), 72.8 (CH, C-4), 74.8 (CH, C-3), 76.0 (CH, C-2), 77.2, 77.1 (CH, C-x_2), 78.3 (CH, C-x_1), 97.8 (CH, C-1), 99.7, 109.4 (C, $Me-C-Me$ isopropylidene), 116.7 (CH_2, $CH_2=CH-CH_2-O$), 135.3 (CH, $CH_2=CH-CH_2-O$) ppm. MALDI TOF MS: *m*/*z* = 1535.9 $[M + Na]^+$ and 1551.8 $[M + K]^+$ found for $C_{72}H_{120}O_{33}$ (calcd. 1512.77).

g-Tetra-(ipr-manno)-O-ethylene-OH (14): Reaction conditions and workup were as described in Procedure B, with g-tetra-(ipr-manno)-O-allyl (**13**, 277 mg, 183 µmol), sodium borohydride (69 mg, 1.83 mmol), dry methanol/dichloromethane 1:1 (10 mL), TLC (cyclohexane/ethyl acetate, 1:3). After workup some minor impurities showed up on TLC, so the crude product was purified by flash chromatography on silica gel (ethyl acetate/methanol 49:1). The title compound **14** (150 mg, 99 µmol, 54%) was obtained as a colourless oil. $[\alpha]_D^{20}$ = +1.7 (*c* = 0.8 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.29, 1.36, 1.46, 1.49 (each s, 48 H, $-CH_3$ isopropylidene), 2.25 (br. s, 1 H, -OH), 3.40−3.76 (m, 55 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, $O-CH_2-CH_2-O$, H-e_2, H-f_2, H-x_2, $O-CH_2-CH_2-OH$, H-5), 3.78−3.84 (2 dd, $^3J_{5,6}$ = 5.7, $^2J_{6,6'}$ = 10.8 Hz, 4 H, H-6), 4.09 (dd, $^3J_{2,3}$ = 5.5, $^3J_{3,4}$ = 7.9 Hz, 4 H, H-3), 4.15 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.5 Hz, 4 H, H-2), 5.00 (each d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 2 H, H-1) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.7, 26.1, 28.1, 29.0 (CH_3, $-CH_3$ isopropylidene), 61.1 (CH, C-5), 61.7 (CH_2, $O-CH_2-CH_2-OH$), 61.9 (CH_2, C-6), 66.5 (CH_2, C-ab), 69.6* (CH_2, $O-CH_2-CH_2-O$), 70.3 (CH_2, C-e_1f_1), 70.9* (CH_2, $O-CH_2-CH_2-O$), 71.1 (C-cd), 71.6* (CH_2, C-e_2f_2), 72.3 (CH_2, $O-CH_2-CH_2-OH$), 72.6 (CH, C-4), 74.7 (CH, C-3), 75.9 (CH, C-2), 78.1 (CH, C-x_2), 78.2 (CH, C-x_1), 97.7 (CH, C-1), 99.6, 109.3 (C, $Me-C-Me$ isopropylidene) ppm. MALDI TOF MS: *m*/*z* = 1540.0 $[M + Na]^+$ and 1555.9 $[M + K]^+$ found for $C_{71}H_{120}O_{34}$ (calcd. 1516.77).

g-Octa-(ipr-manno)-C=C (15): Under Schlenk conditions, g-tetra-(ipr-manno)-O-ethylene-OH (**14**, 150 mg, 99 µmol) was dissolved in dry DMSO (1 mL). Pure sodium hydride (3 mg, 103 µmol) and MDC (3 µL, 33 µmol) were added and the resulting mixture was stirred at 50 °C for 48 h. The reaction was monitored by TLC (ethyl acetate/methanol, 10:1). After the mixture had cooled to room temperature, quenching was effected with water (2 mL) and the mixture was extracted with three portions of diethyl ether. The combined organic phases were washed with water, dried over anhydrous magnesium sulfate and filtered, and the solvent was removed under vacuum. Purification by flash chromatography on silica gel (ethyl acetate/methanol, 49:1) yielded two fractions; the first contained title compound **15** and the second consisted of unconsumed g-tetra-(ipr-manno)-O-ethylene-OH (**14**). Recovered g-tetra-(ipr-manno)-O-ethylene-OH (**14**, 25 mg, 16 µmol). Title compound **15** (85 mg, 28 µmol, 28%) was obtained as a colourless foam. $[\alpha]_D^{20}$ = +1.4 (*c* = 1.1 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.31, 1.37, 1.47, 1.51 (each s, 96 H, $-CH_3$ isopropylidene), 3.45−3.78 (m, 110 H, H-a, H-4, H-6′, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, $O-CH_2-CH_2-O$, H-e_2, H-f_2, H-x_2, $O-CH_2-CH_2-O$, H-5), 3.79−3.86 (2 dd, $^3J_{5,6}$ = 5.7, $^2J_{6,6'}$ = 10.8 Hz, 8 H, H-6), 3.96 (m_c, 4 H, H-e_3, H-f_3), 4.02 (dd, $^3J_{2,3}$ = 5.5, $^3J_{3,4}$ = 7.9 Hz, 8 H, H-3), 4.16 (dd ≈ d, $^3J_{1,2}$ ≈ 0, $^3J_{2,3}$ = 5.5 Hz, 8 H, H-2), 5.01 (d ≈ s, $^3J_{1,2}$ ≈ 0 Hz, 8 H, H-1), 5.13 (m_c, 2 H, $C=CH_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.7, 26.1, 28.2, 29.0 (CH_3, $-CH_3$ isopropylidene), 61.2 (CH, C-5), 62.0 (CH_2, C-6), 66.5 (CH_2, C-ab), 69.7, 70.3, 70.9, 71.0, 71.1, 71.3, 71.7 (CH_2, C-cd, C-e_1f_1, $O-CH_2-CH_2-O$, C-e_2f_2, $O-CH_2-CH_2-O$, C-e_3f_3), 72.7 (CH, C-4), 74.7 (CH, C-3), 75.9 (CH, C-2), 78.2 (CH, C-x_1), 78.3 (CH, C-x_2), 97.7 (CH, C-1), 99.6, 109.3 (C, $Me-C-Me$ isopropylidene), 113.5 (CH_2, $C=CH_2$), 142.4 (C, $C=CH_2$) ppm. MALDI TOF MS: *m*/*z* = 3108.8 $[M + Na]^+$ and 3124.7 $[M + K]^+$ found for $C_{146}H_{244}O_{68}$ (calcd. 3085.56).

Di-(manno)-C=C (16): Reaction conditions and workup were as described in Procedure D, with di-(ipr-manno)−C=C (**2**, 189 mg, 286 µmol). Title compound **16** (88 mg, 176 µmol, 62%) was obtained as a colourless foam. $[\alpha]_D^{20}$ = +61.1 (*c* = 5.4 in methanol). ^{1}H NMR (500 MHz, [D_4]methanol, 25 °C): δ = 3.61 (ddd, 2 H, H-5), 3.64−3.70 (m, 8 H, H-4, H-b, H-c, H-d), 3.72−3.79 (m, 4 H, H-6, H-3), 3.84−3.92 (m, 6 H, H-2, H-6′, H-a), 4.09 (m_c, 4 H, H-e_1, H-f_1), 4.85 (d, $^3J_{1,2}$ = 1.7 Hz, 2 H, H-1), 5.24 (m_c, 2 H, $C=CH_2$) ppm. ^{13}C NMR (125 MHz, [D_4]methanol, 25 °C): δ = 63.1 (CH_2, C-6), 68.0 (CH_2, C-ab), 68.8 (CH, C-4), 70.7 (CH_2, C-cd), 72.4 (CH, C-2), 72.8 (CH, CH_2, C3, C-e_1f_1), 74.8 (CH, C-5), 101.9 (CH, C-1), 115.1 (CH_2, $C=CH_2$), 144.6 (C, $C=CH_2$) ppm. MALDI TOF MS: *m*/*z* = 523.2 $[M + Na]^+$ and 539.2, $[M + K]^+$ found for $C_{20}H_{36}O_{14}$ (M = 500.21).

Tetra-(manno)-C=C (17): Reaction conditions and workup were as described in Procedure D, with tetra-(ipr-manno)−C=C (**4**, 100 mg, 72 µmol). Title compound **17** (35 mg, 33 µmol, 46%) was obtained as a colourless foam. $[\alpha]_D^{20}$ = +59.8 (*c* = 0.9 in methanol). ^{1}H NMR (500 MHz, [D_4]methanol, 25 °C): δ = 3.58−3.80 (m, 38 H, H-3, H-4, H-5, H-6, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1), 3.84−3.94 (m, 12 H, H-2, H-6′, H-a), 4.24 (m_c, 4 H, H-e_2, H-f_2), 4.85 (d, $^3J_{1,2}$ = 1.7 Hz, 4 H, H-1), 5.28 (m_c, 2 H, $C=CH_2$) ppm. ^{13}C NMR (125 MHz, [D_4]methanol, 25 °C): δ = 63.2 (CH_2, C-6), 68.0 (CH_2, C-ab), 68.9 (CH, C-4), 71.9 (CH_2, C-e_2f_2), 72.0, 72.3 (CH_2, C-cd, C-e_1f_1), 72.4 (CH, C-2), 72.8 (CH, C-3), 74.9 (CH, C-5), 78.7 (CH, H-x_1), 102.0 (CH, C-1), 115.5 (CH_2, $C=CH_2$), 145.3 (C, $C=CH_2$) ppm. MALDI TOF MS: *m*/*z* = 1083.7 $[M + Na]^+$ and 1099.7 $[M + K]^+$ found for $C_{42}H_{76}O_{30}$ (calcd. 1060.44).

g-Tetra-(manno)-OH (18): Reaction conditions and workup were as described in Procedure D, with g-tetra-(ipr-manno)-OH (**12**, 94 mg, 64 µmol). Purification was effected by MPLC on RP 18 silica gel (methanol/H_2O 1:3). Title compound **18** (49 mg, 42 µmol, 66%) was obtained as a colourless foam. $[\alpha]_D^{20}$ = +61.6 (*c* = 1.2 in methanol). ^{1}H NMR (500 MHz, [D_4]methanol, 25 °C): δ = 3.50−3.92 (m, 60 H, H-2, H-3, H-4, H-5, H-6, H-6′, H-a, H-b, H-c, H-d, H-e_1, H-f_1, H-x_1, H-e_2, H-f_2, $O-CH_2-CH_2-O$), 3.93−3.3.99 (m, 1 H, H-x_2), 4.86 (d ≈ br. s, $^3J_{1,2}$ ≈ 0 Hz, 4 H, H-1) ppm. ^{13}C NMR (125 MHz, [D_4]methanol, 25 °C): δ = 63.2 (CH_2, C-6), 68.0 (CH_2, C-ab), 68.9 (CH, C-4), 70.9 (CH, C-x_2), 71.1, 72.0, 72.3, 72.4 (CH_2, C-cd, C-e_1f_1, $O-CH_2-CH_2-O$), 72.4 (CH, C-2), 72.8 (CH, C-3), 74.0 (CH_2, C-e_2f_2), 74.9 (CH, C-5), 79.9 (CH, C-x_1), 101.9 (CH, C-1) ppm. MALDI TOF MS: *m*/*z* = 1175.9 $[M + Na]^+$ and 1191.9, $[M + K]^+$ found for $C_{45}H_{84}O_{33}$ (calcd. 1152.49).

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[1] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683–720.

[2] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794.

[3] A. Varki, *Glycobiology* **1993**, *3*, 97–130.

[4] A. Kobata, *Glycoconjugate J.* **2000**, *17*, 443–464.

[5] C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357–2364.

[6] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674.

[7] E. J. Toone, *Curr. Opin. Struct. Biol.* **1994**, *4*, 719–728.

[8] Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321–327.

[9] K. Drickamer, *J. Biol. Chem.* **1988**, *263*, 9557–9560.

[10] P. M. Rudd, T. Elliott, P. Cresswell, I. A. Wilson, R. A. Dwek, *Science* **2001**, *291*, 2370–2376.

[11] W. I. Weis, M. E. Taylor, K. Drickamer, *Immunol. Rev.* **1998**, *163*, 19–34.

[12] T. A. Springer, *Cell* **1994**, *76*, 301–314.

[13] E. H. Beachey, *J. Infect. Dis.* **1981**, *143*, 325–345.

[14] K. A. Krogfelt, *Rev. Infect. Dis.* **1991**, *13*, 721–735.

[15] R. Roy, *Curr. Opin. Struct. Biol.* **1996**, *6*, 692–702.

[16] Th. K. Lindhorst, *Top. Curr. Chem.* **2002**, *218*, 201–235.

[17] A. W. Bosman, H. M. Janssen, E. W. Meijer, *Chem. Rev.* **1999**, *99*, 1665–1688.

[18] M. Fischer, F. Vögtle, *Angew. Chem.* **1999**, *111*, 934–955; *Angew. Chem. Int. Ed.* **1999**, *38*, 884–905.

[19] O. A. Matthews, A. N. Shipway, J. F. Stoddart, *Prog. Polym. Sci.* **1998**, *23*, 1–56.

[20] D. A. Tomalia, H. Dupont Durst, *Top. Curr. Chem.* **1993**, *165*, 193–313.

[21] N. Röckendorf, Th. K. Lindhorst, *Top. Curr. Chem.* **2001**, *217*, 201–238.

[22] J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555–578.

[23] Th. K. Lindhorst, C. Kieburg, *Angew. Chem.* **1996**, *108*, 2083–2086; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953–1956.

[24] C. Kieburg, Th. K. Lindhorst, *Tetrahedron Lett.* **1997**, *38*, 3885–3888.

[25] K. Sadalapure, Th. K. Lindhorst, *Angew. Chem.* **2000**, *112*, 2066–2069; *Angew. Chem. Int. Ed.* **2000**, *39*, 2010–2013.

[26] M. M. K. Boysen, Th. K. Lindhorst, *Org. Lett.* **1999**, *1*, 1925–1927.

[27] M. M. K. Boysen, Th. K. Lindhorst, *Tetrahedron* **2003**, *59*, 3895–3898.

[28] C. J. Hawker, J. M. J. Fréchet, *J. Chem. Soc., Chem. Commun.* **1990**, 1010–1013.

[29] C. J. Hawker, J. M. J. Fréchet, *J. Am. Chem. Soc.* **1990**, *112*, 7638–7647.

[30] S. M. Grayson, J. M. J. Fréchet, *Chem. Rev.* **2001**, *101*, 3819–3867.

[31] A. B. Padias, H. K. Hall Jr., D. A. Tomalia, J. R. McConnell, *J. Org. Chem.* **1987**, *52*, 5305–5312.

[32] H. Nemoto, J. G. Wilson, H. Nakamura, Y. Yamamoto, *J. Org. Chem.* **1992**, *57*, 435.

[33] M. Jayaraman, J. M. J. Fréchet, *J. Am. Chem. Soc.* **1998**, *120*, 12996–12997.

[34] S. M. Grayson, M. Jayaraman, J. M. J. Fréchet, *Chem. Commun.* **1999**, 1329–1330.

[35] S. M. Grayson, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2000**, *122*, 10335–10344.

[36] J. A. Sousa, A. A. L. Bluhm, *J. Org. Chem.* **1960**, *25*, 108–111.

[37] T. P. Kogan, B. Dupré, K. M. Keller, I. L. Scott, H. Bui, R. V. Market, P. J. Beck, J. A. Voytus, B. M. Revelle, D. Scott, *J. Med. Chem.* **1995**, *38*, 4976–4984.

[38] D. K. Baeschlin, A. R. Chaperon, L. G. Green, M. G. Hahn, S. J. Ince, S. V. Ley, *Chem. Eur. J.* **2000**, *6*, 172–186.

[39] S. M. Grayson, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2000**, *122*, 10335–10344.

[40] M. W. Lewbart, J. J. Schneider, *J. Org. Chem.* **1969**, *34*, 3505–3512.

[41] D. R. Williams, S.-Y. Sit, *J. Am. Chem. Soc.* **1984**, *106*, 2949–2954.

[42] P. Klemm, B. J. Jørgensen, I. van Die, H. de Ree, H. Bergmans, *Mol. Gen. Genet.* **1985**, *199*, 410–414.

[43] S. Kötter, U. Krallmann-Wenzel, S. Ehlers, Th. K. Lindhorst, *J. Chem. Soc., Perkin Trans. 1* **1998**, 2193–2200.

[44] N. Nagahori, R. T. Lee, S.-I. Nishimura, D. Pagé, R. Roy, Y. C. Lee, *ChemBioChem* **2002**, *3*, 836–844.

[45] N. Sharon, *FEBS Lett.* **1987**, *217*, 145–157.

[46] Th. K. Lindhorst, S. Kötter, J. Kubisch, U. Krallmann-Wenzel, S. Ehlers, V. Kren, *Eur. J. Org. Chem.* **1998**, 1669–1674.

[47] C. S. Hung, J. Bouckaert, D. Hung, J. Pinkner, C. Widberg, A. DeFusco, C. G. Auguste, R. Strouse, S. Langermann, G. Waksman, S. J. Hultgren, *Mol. Microbiol.* **2002**, *44*, 903–915.

[48] A. Siriwardena, M. R. Jørgensen, M. A. Wolfert, M. L. Vandenplas, J. N. Moore, G.-J. Boons, *J. Am. Chem. Soc.* **2001**, *123*, 8145–8146.

[49] J. D. Klemm, S. L. Schreiber, G. R. Crabtree, *Ann. Rev. Immunol.* **1998**, *16*, 569–592.

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