Oligomannoside mimetics by glycosylation of 'octopus glycosides' and their investigation as inhibitors of type 1 fimbriae-mediated adhesion of *Escherichia coli*

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The glycocalyx of eukaryotic cells is composed of glycoconjugates, which carry highly complex oligosaccharide portions. To elucidate the biological role and function of the glycocalyx in cell–cell communication and cellular adhesion processes, glycomimetics have become targets of glycosciences, which resemble the composition and structural complexity of the glycocalyx constituents. Here, we report about the synthesis of a class of oligosaccharide mimetics of a high-mannose type, which were obtained by mannosylation of spacered mono- and oligosaccharide cores. These carbohydrate-centered cluster mannosides have been targeted as inhibitors of mannose-specific bacterial adhesion, which is mediated by so-called type 1 fimbriae. Their inhibitory potencies were measured by ELISA and compared to methyl mannoside as well as to a series of mannobiosides, and finally to the polysaccharide mannan. The obtained results suggest a new interpretation of the mechanisms of bacterial adhesion according to a macromolecular rather than a multivalency effect.

Introduction

Eukaryotic cells are covered by a nano-dimensioned carbohydrate layer, which is called the glycocalyx. The glycocalyx consists of a large number of structurally highly diverse glycoconjugates, which are partly embedded in the cell membrane with their non-carbohydrate moiety or stick to the cell surface by other non-covalent interactions.[1]

Research in the field of glycobiology has revealed that the glycocalyx is essential for cellular communication[2] however, the principles and mechanisms underlying its biological function are not well understood and are difficult to investigate.

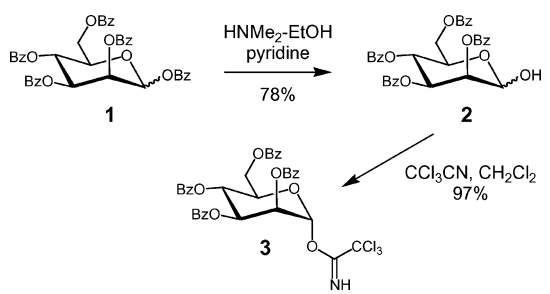
Particularly, this is due to the enormous complexity of the glycocalyx, which can be regarded as a molecular super-system. One useful approach in coping with the problems of experimental carbohydrate biology is the synthesis and investigation of molecules, which mimic the highly complex and hyperbranched character of the glycoconjugates found in the glycocalyx. Many examples of such glycomimetics have been published and reviewed.[3] Among the different architectures which have been employed for the conjugation of glycoligands, the use of carbohydrates as core molecules has led to the synthesis of the so-called carbohydrate-centered glycoclusters.[4]

These often large molecules have been built up using monosaccharide cores, which were uniformly spacer-modified at their hydroxyl groups to prevent steric hindrance upon ligation with peripheral carbohydrates. For such spacered core molecules the name 'octopus glycosides' has been coined alluding to their multi-arm like shape.[5]

Many different chemistries have been used for ligation of glycoligands to the oligofunctional carbohydrate cores,[4] among which only glycosylation leads to products which closely resemble the composition of structures found in nature. This approach is outlined in Fig. 1. As octopus-type core molecules, uniformly *O*-(ω-hydroxy-spacer)-modified glycosides are employed, which are turned into carbohydrate-centered cluster glycosides by classic glycosylation. Degradation of such oligosaccharide mimetics should lead to compounds found in the natural environment as the only degradation products, namely the sugar components and ethylene or propylene glycol.

The degree of difficulty of this synthetic pathway is mainly defined by the glycosylation step, which is complicated by steric hindrance occurring in the formed products. This demanding oligo-glycosylation reaction can be regarded as a multi-step synthesis as the reactivity of partially glycosylated intermediates decreases with increasing degree of glycosylation, the last glycosylation step becoming the most difficult one.

We have recently tackled this problem in the preparation of anomerically functionalized carbohydrate-centered glycoclusters and have achieved the exhaustive mannosylation of four hydroxyl groups using the mannosyl donor **3** (Scheme 1).[6] Then it has become our goal to establish this chemistry for the exhaustive glycosylation of octopus glycosides based on monosaccharide as



Scheme 1

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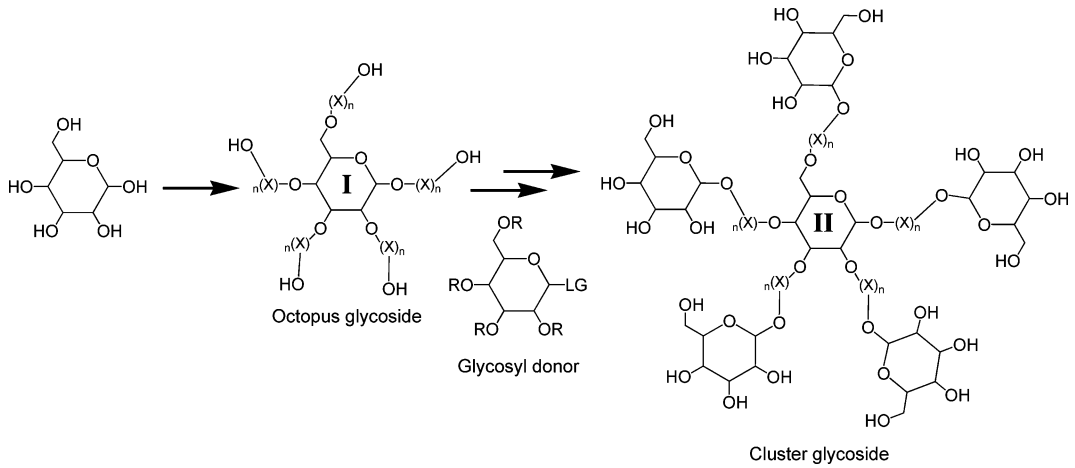


Fig. 1 This cartoon exemplifies the structure of *O*-(ω-hydroxy-spacer)-modified glycosides (**I**), which can be used as oligofunctional scaffold molecules of an "octopus-type". They can be turned into carbohydrate-centered cluster glycosides (**II**) by classic glycosylation.

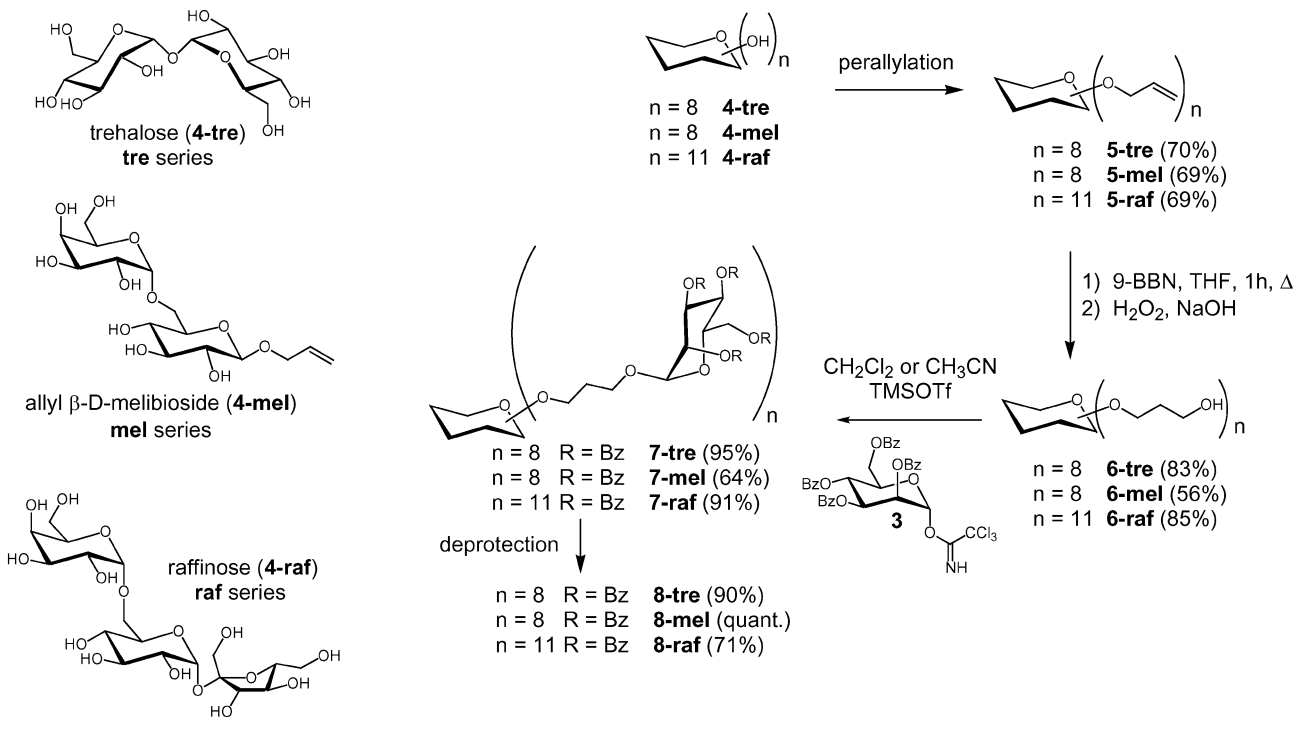
well as oligosaccharide cores. This would allow us to access a class of high-molecular weight oligosaccharide mimetics, which in the case of hydrolysis, deliver propylene oxide as the only byproduct in addition to the sugar constituents.

The first carbohydrate core we used in these experiments was D-glucose, and for further studies we selected two disaccharides and one trisaccharide (*cf.* Scheme 2a). For glycosylation of the carbohydrate-derived core molecules we chose α-mannosylation for two reasons: (i) high mannose-type glycoclusters are of considerable biological interest[7] and (ii) α-mannosylation is afflicted with the problem of glycosyl orthoester formation[8] which we wished to solve.

Results and discussion

Synthesis of the mannosyl donor

For mannosylation of the diverse spacer-modified carbohydrate cores, the trichloroacetimidate method was selected.[9] To reduce the probability of glycosyl orthoester formation during the mannosylation step benzoyl protective groups were used instead of acetyl groups.[10] As we anticipated that we would need a considerable excess of glycosyl donor to achieve high yields, we first optimized the synthesis of the mannosyl trichloroacetimidate **3** (Scheme 1). The synthesis of the reducing sugar **2** from the pentabenzoate **1**

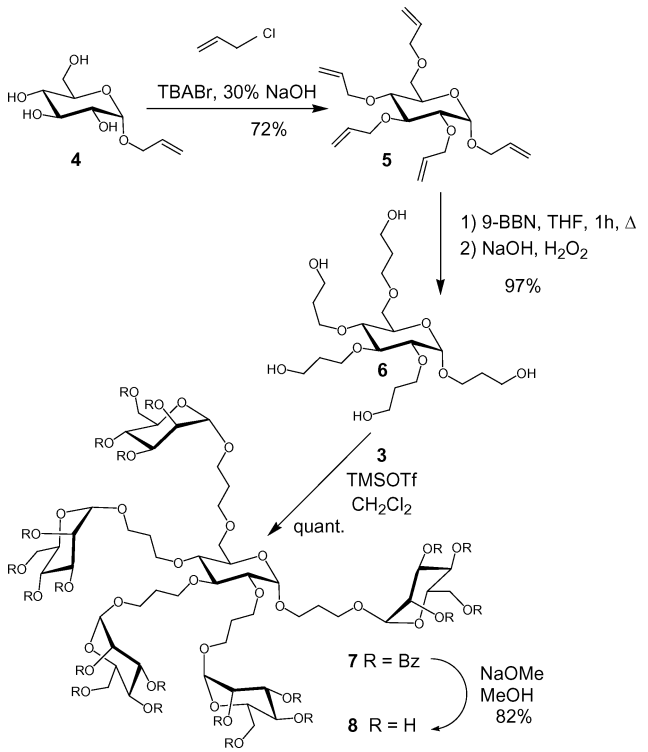


Scheme 2

could be significantly improved. While anomeric deacetylation is easily achieved in good yield by different procedures,[11] regioselective 1-*O*-debenzoylation is less common, especially in the *manno* series. We found that ethanolic dimethylamine[12] can be used to effect anomeric debenzoylation of **1** in pyridine solution, yielding **2** in nearly 80% after 1.5 h.[13] In addition, the synthesis of the imidate **3** was optimized by a prolonged reaction time of 12 h, which increased the yield in this step from the reported 75% (3 h reaction time)[14] to 97%.

Oligomannosylation of a spacered glucose core

The mannosyl trichloroacetimidate **3** was then employed for the glycosylation of all five hydroxyl groups of the octopus glucoside **6** (Scheme 3). As previously reported,[5] this uniformly *O*-(3-hydroxypropyl)-modified pentanol can be readily prepared starting from the fully *O*-allylated glucose derivative **5**, which is derived from allyl α-D-glucoside (**4**). A hydroboration–oxidation sequence leads to **6** in high yield. The published procedure for the synthesis of **6**[5] was modified in that the reaction mixture was kept at reflux temperature for 1 h during hydroboration.



Scheme 3

The first attempt of mannosylation of the core molecule **6** using the glycosyl donor **3** to achieve **7** was hampered by the formation of glycosyl orthoester byproducts. This finding was unexpected because it has been reported that the benzoyl-protected mannosyl trichloroacetimidate **3** does not to lead to orthoester formation in other difficult glycosylation reactions such as in case of the mannosylation of a tether-functionalized carbohydrate-centered tetraol[6] or in the mannosylation of sterically hindered polyols.[15] Unfortunately, formation of glycosyl orthoester byproducts during the mannosylation of spacered carbohydrate cores remained a severe problem throughout all the work reported here.

It turned out that orthoester formation during the attempted synthesis of **7** could be avoided when a large excess of the imidate **3** and elevated TMSOTf concentrations were used in the glycosylation step. Exhaustive mannosylation of all hydroxyl groups was accomplished in very concentrated solution, using 10 mg acceptor in 1 ml CH_2Cl_2. This optimized mannosylation protocol delivered the target cluster mannoside **7** in quantitative yield. The ^{1}H NMR spectrum of this cluster is complex however, it can be fully assigned. The chiral core glucose influences its periphery, resulting in different signal sets for the protons of each individual mannosyl residue.

Subsequent deacylation of **7** under Zemplén conditions[16] finally furnished the unprotected target glycocluster **8** after 2 days, in high overall yield.

When this synthetic pathway had been elaborated with glucose as the core, we set out to apply this successful approach to di- and oligosaccharides. Three sugars were selected as starting material, the disaccharide allyl melibioside (**mel** series) and the non-reducing glycosides trehalose (**tre** series) and raffinose (**raf** series) (Scheme 2a). Whereas melibiose and trehalose are eight-functional scaffolds, suited to the assembly of eight mannosyl residues, even eleven sugar rings can be attached to a raffinose-derived scaffold molecule.

Firstly, we anticipated that the reaction conditions established for the synthesis of the glucose-centered derivatives **5**–**8** (Scheme 3) would also be suited to perform the analogous chemistry starting from allyl melibioside (**4-mel**), trehalose (**4-tre**) and raffinose (**4-raf**), respectively. However, it turned out that some reaction conditions had to be optimized for every individual sugar which was used as the carbohydrate core.

While for the allylation of **4**, phase transfer catalysis with TBABr, 33% NaOH and allyl chloride was successful (Scheme 3), perallylation of the higher polar trehalose **4-tre** under the same conditions gave only poor results. Perallylation of **4-tre** to form **5-tre** was achieved with NaH and allyl bromide in DMF. Hydroboration of perallylated trehalose **5-tre** to form **6-tre**,[17] as well as of the melibiose- and the raffinose-centered analogs **5-mel** and **5-raf**, proceeded as with the glucose derivative **5** however, instead of using $MgSO_4$ as a drying reagent, Sephadex LH-20 had to be employed with dry methanol as the eluent.

For the critical mannosylation step, which leads to the desired carbohydrate-centered oligomannoside mimetics (Scheme 2b), the solubility of the carbohydrate-centered oligo-alcohols was an important parameter. Because **6-tre**, **6-mel**, and **6-raf** were of higher polarity than the glucose-centered pentaol **6**, which is even soluble in CH_2Cl_2, glycosylation had to be performed in a more polar solvent than CH_2Cl_2. Complete mannosylation of the trehalose-centered octaol **6-tre**[17] with **3** as the donor was possible in acetonitrile. In contrast to glycosylation of **7**, mannosylation of **6-tre** in acetonitrile had to be carried out in very dilute solution with less that 0.1 mg acceptor per 1 mL acetonitrile.

Our search for optimized mannosylation conditions was doomed to navigate between three undesired complications (i) incomplete mannosylation, thus leading to structural defects in the respective product, (ii) orthoester formation and (iii) cleavage of an interglycosidic bond. The reaction conditions had to be carefully fine-tuned for each case in order to obtain perfect products. Optimization of the various glycosylation parameters was possible by close monitoring the glycosylation reaction and the subsequent

deprotection step by MALDI-TOF MS. The optimized reaction conditions reported here (*cf.* Experimental section), resulted from 25 glycosylation experiments.

To achieve complete mannosylation of **6-tre**, **6-mel**, as well as **6-raf**, higher temperatures at the beginning of the glycosylation reaction turned out to be beneficial. However, the interglycosidic bonds in trehalose, melibiose, and raffinose, respectively, are of different stability and are sensitive to elevated reaction temperatures. When the raffinose-centered polyol **6-raf** (Scheme 2b) and the melibiose-centered polyol **6-mel** were glycosylated for 2 h at 80 °C, the α(1 → 6)-interglycosidic bond in melibiose remained unaffected, whereas the α(1 → 1)-interglycosidic linkage between the melibiose and fructose subunits of raffinose was completely cleaved. Finally we found out that for the mannosylation of the melibiose-centered oligo-alcohol **6-mel** the reaction mixture was best kept at 75 °C for 2 h and then stirred at room temperature, whereas in the case of the trehalose analog, **6-tre** stirring for 2 h at 60 °C gave the best results. The raffinose analog **6-raf** was kept at 60 °C for only 10 minutes before the glycosylation mixture was further stirred at room temperature.

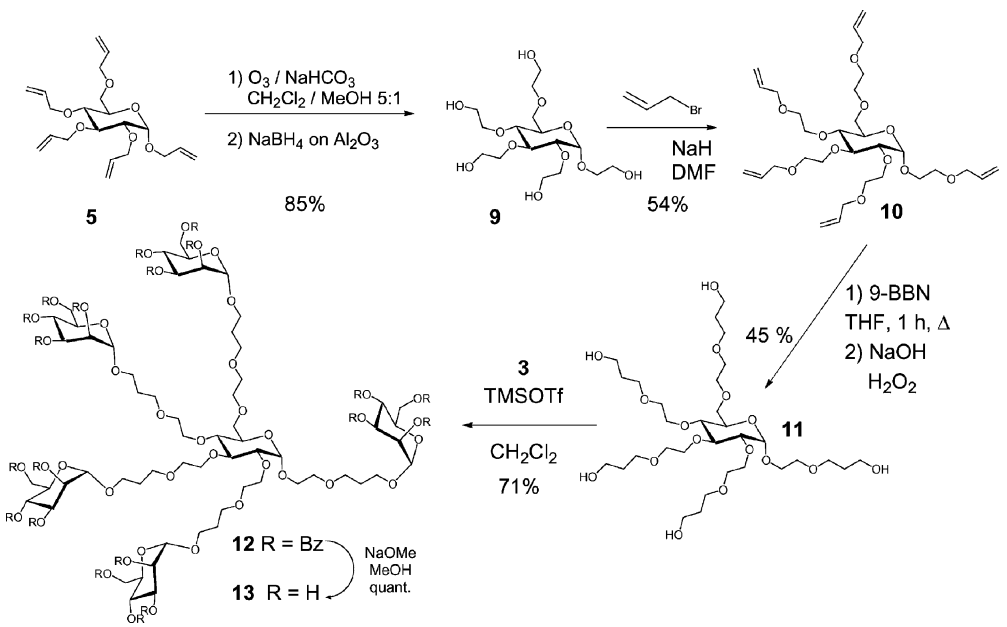
The excess of mannosyl donor **3**, which was used, as well as the amount of Lewis acid employed, could be tuned to an optimum, which was common for all three oligosaccharide series. The reaction time was of minor importance for the result of the glycosylation reaction.

Spectroscopic characterization of the carbohydrate-centered cluster mannosides **7-mel**, **7-tre** and **7-raf**, respectively, was possible by means of 2D-NMR and mass spectrometry. As the formation of β-mannosides during mannosylation reactions using trichloroacetimidates in acetonitrile was reported in the literature,[18] the anomeric configuration of the prepared cluster mannosides was confirmed by means of their $J_{C\text{-}1,H\text{-}1}$ hetero coupling constants, which are significantly lower for α-mannosides than for the respective β-anomers.[19]

Unfortunately, deprotection of the *O*-benzoylated glycoclusters **7-tre**, **7-mel** and **7-raf** (Scheme 2b) was not trivial. While the benzoylated glucose-centered cluster glycoside **7** could be deprotected under standard Zemplén conditions,[16] a stepwise deprotection protocol was necessary for the oligosaccharide-centered cluster mannosides. In this procedure the benzoylated sugar was first dissolved in THF and treated with methanolic sodium methoxide solution. After 1–4 h the partially deprotected glycocluster precipitated from the THF solution, which was concentrated and subsequently subjected to a standard Zemplén procedure in methanol, leading to fast and complete debenzoylation in nearly quantitative yields. The acidic ion exchange resin Amberlite IR 120, which was used after deprotection for neutralization also effected cleavage of orthoesters, when these had been formed during the glycosylation step.

Then we wished to further extend the elaborated synthetic protocol to spacered carbohydrate scaffolds with varied spacer lengths. This was of interest as the spacer length of cluster mannosides has been shown to be an important parameter when ligand–receptor interactions are to be optimized.[20] Indeed, the strategy reported herein for the synthesis of carbohydrate-centered glycoclusters could be adapted to the hydroxyethyleneglycol-modified glucoside **11** (Scheme 4). The latter was obtained from **5** by ozonisation and reductive work-up leading to **9**,[5] followed by perallylation to deliver **10**, which was finally submitted to hydroboration–oxidation. Complete glycosylation of the 6-hydroxy-3-oxa-hexyl-spacered octopus glycoside **11** employing **3** as the mannosyl donor led to **12** in yields around 70%. Deprotection to the OH-free cluster mannoside **13** with extended spacers was possible according to Zemplén in quantitative yield.

At this point it can be concluded that the methodology reported here allows the synthesis of multivalent glucose-, trehalose-, melibiose-, and raffinose-centered cluster mannosides. Optimized reaction conditions for each sugar series and each synthetic step have been elaborated. The carbohydrate-centered glycoclusters **8**, **8-tre**, **8-mel**, **8-raf**, and **13** closely resemble the elemental composition of the natural example structures and thus are prime candidates as oligosaccharide mimetics of a high-mannose type.



Scheme 4

Inhibition of type 1 fimbriae-mediated bacterial adhesion

Many biological processes occurring at cell surfaces are dependent on interactions with high-mannose glycoconjugates.[21] Bacterial adhesion is also often mediated through the interactions of bacterial fimbriae with mannose-containing conjugates of the host cell glycocalyx. Fimbriae are adhesive proteinogenous organells on the bacterial surface, which carry lectin domains.[22] *Escherichia coli* possess so-called type 1 fimbriae, which have been attributed a specificity for terminal α-mannosyl residues. The lectin portion of type 1 fimbriae has been identified as the protein FimH.[23]

The oligomannoside mimetics prepared herein have been targeted to investigate the inhibition of mannose-specific bacterial adhesion to the glycocalyx of their host cells. They were tested for their potency as inhibitors of type 1 fimbriae-mediated bacterial adhesion and were expected to perform much better than methyl α-D-mannoside (MeMan), a known inhibitor of type 1 fimbriae-mediated bacterial adhesion,[24] owing to their complex multivalent structure. We anticipated varying inhibitory potencies depending on the number of α-mannosyl residues exposed on the carbohydrate core as well as depending on the spatial circumstances of their orientation.

Table 1 summarizes the results which were obtained when the unprotected oligomannoside mimetics **8**, **8-tre**, **8-mel**, **8-raf**, and **13** were tested for their potency as inhibitors of type 1 fimbriae-mediated bacterial adhesion using an enzyme-linked immunosorbent assay (ELISA). The microtiter plates used were coated with mannan from *Saccharomyces cerevisiae* constituting a highly branched polysaccharide with an α-1,6-linked polymannoside backbone from which α-D-mannosyl residues branch out from the 2- and the 3-position.[25] Recombinant *Escherichia coli* bacteria (*E. coli* HB101 pPKl4[26]) were employed, carrying type 1 fimbriae as the only fimbriae which are expressed on the bacterial surface. IC_{50} values were determined for each cluster mannoside according to standard methods and reflect the concentration at which 50% of bacterial adhesion to the mannan surface is inhibited by an investigated inhibitor. Results in triplicate were used for plotting of the inhibition curves for each individual ELISA experiment (Fig. 2). Typically, the IC_{50} values obtained from several independently performed tests were in the range of ±15% for example, IC_{50} values determined for the standard MeMan varied between 1.2 and 6.9 millimolar. However, the relative inhibitory potencies calculated from independent series of data were highly reproducible.

Table 1 Potencies of the prepared carbohydrate-centered cluster mannosides, mannobio- and triosides and mannan as inhibitors of mannose-specific adhesion of *E. coli*, compared to MeMan, as determined by ELISA. IC_{50} values are average values of at least three independent assays and are listed together with their standard deviations (s.d.). So-called relative inhibitory potencies (RIP) are relative to the IC_{50} value measured for methyl α-D-mannoside (**1**); thus the inhibitory potency of **1** has been defined as RIP ≡ 1. All RIP values are average values of at least three independently determined RIPs and listed together with their standard deviations

Mannose-containing molecules	IC_{50}[a]	s.d.	RIP	s.d.
MeMan	3.6	1.2	1	—
1 → 2Dis	1.1[b]	0.5	2.9	1.0
1 → 3Dis	0.4[c]	0.3	11	7.0
1 → 4Dis	2.8	0.5	0.8	0.35
1 → 6Dis	2.2[d]	1.1	1.4	0.5
1 → 3,6Tris	0.15[e]	0.03	20	0.6
8	0.048	0.028	180	32
8-Tre	0.054	0.038	170	46
8-Mel	0.016	0.00058	230	74
8-Raf	0.031	0.0003	250	74
13	0.027	0.0032	130	38
8-Mel(def)	0.041	0.0018	190	18
Mannan	0.0086	0.0038	190	51

[a] [mmolar]. [b] Reported: 1.3.[28] [c] Reported: 1.2.[28] [d] Reported: 0.5.[28] [e] Reported: 10.5.[28]

The IC_{50}s determined for an individual cluster mannoside were related to the IC_{50} value of the standard inhibitor MeMan, which was measured on the same microtiter plate affording a relative ranking of the tested compounds regarding their inhibitory potency. Relative inhibitory potencies (RIP values) were thus based on the inhibitory potency of MeMan in the same test with RIP (MeMan) ≡ 1 (Table 1). Rather unnatural synthetic mannosides such as methylumbelliferyl α-D-mannoside were not considered in this study (*cf.* the subsequent paper[27]).

Unexpectedly, the inhibitory potencies of all carbohydrate-centered cluster mannosides tested, **8**, **8-tre**, **8-mel**, **8-raf**, and **13**, were almost the same when compared to MeMan with RIP values around 200 (Fig. 3). Their inhibitory potency was increased by two orders of magnitude compared to the simple methylmannoside. To allow a better assessment of this finding, the clusters were compared to a series of mannobiosides, allyl 2-*O*-α-D-mannosyl-α-D-mannoside (**1 → 2Dis**), allyl 3-*O*-α-D-mannosyl-α-D-mannoside (**1 → 3Dis**), allyl 4-*O*-α-D-mannosyl-α-D-mannoside (**1 → 4Dis**), allyl 6-*O*-α-D-mannosyl-α-D-mannoside (**1 → 6Dis**), and the branched mannotrioside allyl 3,6-di-*O*-(α-D-mannosyl)-α-D-mannoside(**1 → 3,6Tris**), which were prepared according to reported procedures.[28] Whereas, the carbohydrate-centered glycoclusters prepared herein form a group of inhibitors which perform around two orders of magnitude better than MeMan regardless of their valency or spatial arrangement, the tested mannobiosides hardly exceed the inhibitory potency of MeMan or are up to one order of magnitude more potent (**1 → 3Dis** and **1 → 3,6Tris**), respectively (Table 1).

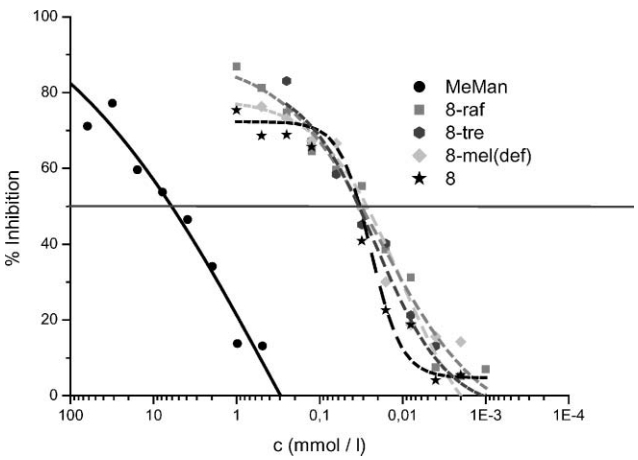


Fig. 2 Example for the sigmoidal fitting of the data obtained by ELISA, from which IC_{50} values were obtained. On each ELISA plate, the standard methyl α-D-mannoside (MeMan) was included.

Our results with the known di- and trisaccharides are in rough accordance with the literature[29] and can be rationalized according to ligand–receptor interactions, in other words, on the basis of their

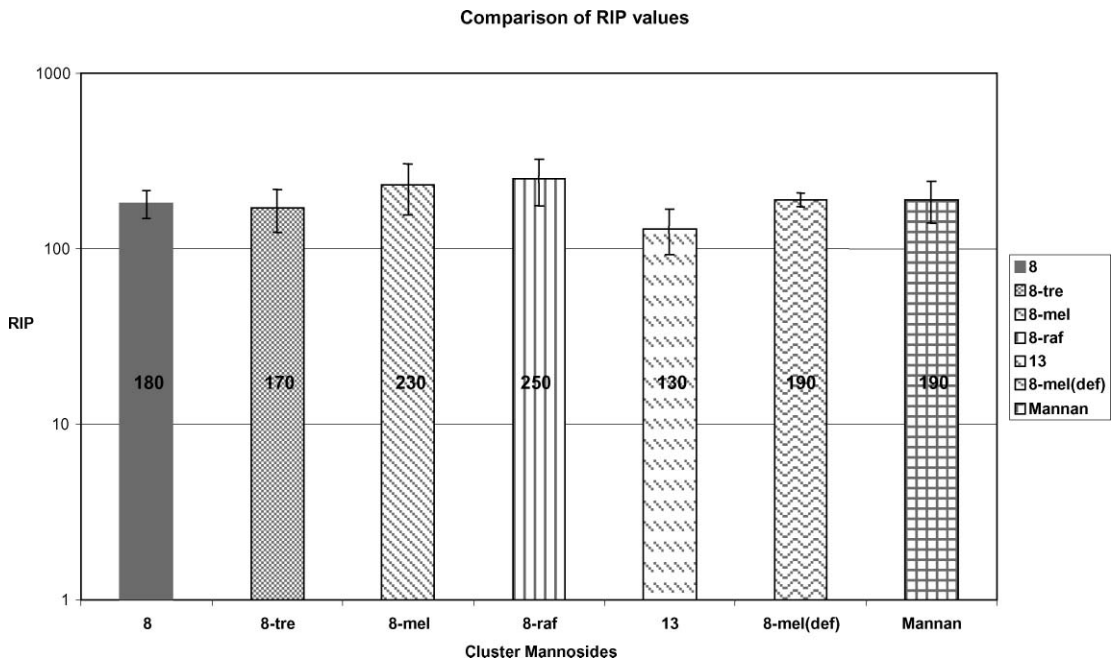


Fig. 3 Comparison of the relative inhibitory potencies of the prepared carbohydrate-centered cluster mannosides as well as mannan, based on MeMan (RIP ≡ 1, not shown). Standard deviations are indicated.

molecular interactions with the carbohydrate-recognition domain (CRD) of the fimbrial lectin FimH. However, the findings collected for the various cluster mannosides cannot be rationalized this way. Referring to reported multivalency effects in the inhibition of type 1 fimbriae-mediated bacterial adhesion, we had expected much better values (lower IC_{50}s) for the novel hyperbranched oligomannoside mimetics.

From molecular dynamics simulations, which we have performed prior to our synthetic work,[30] we have an idea of the conformational behaviour of our target cluster mannosides **8**, **8-tre**, **8-mel**, and **8-raf**. Modeling had revealed that the carbohydrate-centered cluster mannosides enclose a spherical conformational space. The average distance of the cluster center to an exposed mannosyl residue is in rough accordance with the radius of the respective conformational sphere and this is increasing from 7.8 Å for **8** to 9.2 for **8-tre**, 9.4 for **8-mel**, and finally 9.7 for **8-raf**. These differences, however, were not reflected in the measured inhibitory potencies and even a much more spaced cluster mannoside such as **13** received a similar ranking as all other cluster mannosides tested. Moreover, a structurally defective analogue of **8-mel**, termed as **8-mel(def)**, hardly showed any difference when compared to the perfect cluster **8-mel** (Table 1).

While we had expected to measure different inhibitory potencies depending on the valency of a specific cluster and its three-dimensional characteristics, the findings summarized in Table 1 prompted us to consider an alternative mechanism for the involved molecular interactions. Our considerations led us to test mannan itself as an inhibitor of type 1 fimbriae-mediated bacterial adhesion to the mannan-coated surface of the poylstyrene plate used in the ELISA. The concentration of mannan was calculated on the basis of its average molecular weight and this delivered a RIP value of 190. Thus, mannan is ranking in the range of all carbohydrate-centered cluster mannosides which we have tested before.

This is a very interesting result, suggesting that mannan, as well as the class of carbohydrate-centered cluster mannosides prepared and investigated herein, other than the di- and trisaccharides tested, share a structural feature which is responsible for inhibition of the adhesion of *E. coli* in the employed ELISA. This inhibitory effect can neither be interpreted according to standard structure–activity relationships nor can it be understood on the basis of solitary or multivalent interactions of ligands and their receptors.[31] Multivalency effects, which are important in biology and especially in glycobiology have been reasoned according to different principles, such as a chelate or a statistical effect;[32] our findings suggest the addition of a new mechanistic principle to the knowledge reported so far. The observed inhibitory potencies may owe to a feature which is typical for macromolecules and the interactions they form, rather than for distinct molecular epitopes. We will consider such a macromolecular effect in our future experiments and we will attempt to elucidate the underlying mechanistic principles.

On the other hand, a second part of our work on the mechanisms of carbohydrate-based bacterial adhesion must focus on the detailed inspection of the interactions between the CRD of FimH and mannoside ligands, and this is discussed in the subsequent paper.[27]

Conclusions

We have to conclude that understanding of fimbriae-mediated bacterial adhesion might require at least two different points of view. One perspective deals with the interpretation of results obtained from hemagglutination inhibition assays or ELISA. Inhibition of bacterial adhesion as observed in this testing system cannot be rationalized on the basis of the known crystal structure of FimH[33] however, it can neither be interpreted in the sense of a classical

"multivalency effect". Rather than that, a macromolecular effect should be considered as rational for the inhibition of bacterial adhesion to the gylcocalyx or a glycocalyx mimetic.

Nevertheless, in addition to more such supramolecular considerations, the molecular details of interactions of the FimH CRD and mannoside ligands have to be investigated, utilizing the known crystal structure of FimH. This second aspect of our research is highlighted in the successive contribution.[27]

Experimental

General remarks

Optical rotations were determined with a Perkin Elmer 241 polarimeter (10 cm cells, Na-D-line: 589 nm). NMR spectra were recorded at 400 or 500 MHz on Bruker AMX-400 and Bruker DRX-500 instruments with Me_4Si (δ 0) as the internal standard. All reactions were monitored by TLC on silica gel FG_{254} (Merck) with detection by UV light and/or by charring with 10% ethanolic sulfuric acid. Flash column chromatography was performed on silica gel 60 (200–400 mesh, Macherey Nagel & Co). Gel permeation chromatography was carried out on Sephadex G-15 (Pharmacia) if not otherwise stated. Elemental analyses were determined by the Microanalytical Laboratory of the Department of Organic Chemistry at the University of Hamburg. Optical densities (ODs) were measured on an Asys DigiScan 400 ELISA reader at 405 nm with the reference read to 492 nm. ELISA plates were incubated at 37 °C.

Methyl α-D-mannoside was purchased from Fluka, F-shaped 96-well microtiter plates from Sarstedt. Mannan from *Saccharomyces cerevisiae* was purchased from Sigma and was used in 50 mM aq. Na_2CO_3 (1 mg ml^{-1}; pH 9.6). The polyclonal anti-fimA antibody was a kind gift from Prof. Dr J. Hacker (Würzburg) and peroxidase-conjugated goat anti-rabbit antibody (IgG, H + L) was purchased from Dianova. Skimmed milk was from Ulzena, Tween 20 from Roth, ABTS [2,2-azidobis-(3-ethylbenzothiazoline-6-sulfonic acid)] from Fluka and thimerosal {2-(ethylmercurio)thiobenzoic acid, sodium salt} was from Merck. A recombinant type 1 fimbriated *E. coli* strain, *E. coli* HB101 (pPKl4),[26] was used and cultured as described earlier.[34]

Buffers. PBS (phosphate-buffered saline) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4{\cdot}2H_2O$ and 0.2 g KH_2PO_4 in 1000 ml of bidest. water (pH 7.2). PBSE was PBS buffer + 100 mg l^{-1} thimerosal, PBSET was PBSE buffer + 200 µl l^{-1} tween 20. Substrate buffer was 0.1 M sodium citrate dihydrate, adjusted to pH 4.5 with citric acid. For preparation of the ABTS solution, ABTS (1 mg per ml) was dissolved in substrate buffer and 0.1% H_2O_2 (25 µl per ml) was added.

ELISA

To determine the potencies of the various cluster mannosides tested as inhibitors of type 1 fimbriae-mediated adhesion of *E. coli*, an ELISA was used as published earlier.[34] Polystyrene microtiter plates were coated with mannan solution (100 µl per well) and dried overnight at 37 °C. The plates were blocked once with 5% skimmed milk in PBSE for 30 min at 37 °C. The wells were washed with PBSE (150 µl) and then PBSE (50 µl) and inhibitor solutions (50 µl) were added. Inhibitor solutions were diluted serially two-fold in PBSE. Bacterial suspension (50 µl per well) was added and the plate was left at 37 °C for 1 h to allow sedimentation of the bacteria. Then each well was washed four times with PBSE (150 µl) and 50 µl of the first antibody (anti-fimA antibody, solution as optimized prior to the experiments) in 2% skimmed milk was added. The plates were incubated for 30 min and then washed twice with PBSET and the second antibody was added (50 µl). The plates were incubated for 30 min and then washed three times with PBSET and once with PBSE and substrate buffer. ABTS solution (50 µl) was added, incubated for 60 min at 37 °C. For ELISA controls, bacterial adhesion to blocked, uncoated microtiter plates was checked, and the reaction of the employed antibodies with yeast mannan was tested and found to be negligible. The percentage inhibition was calculated as $[OD(nI) - OD(I) \times 100 \times [OD(nI)]^{-1}$ (nI: no inhibitor, I: with inhibitor).

IC_{50} values are average values from at least three independent assays and are listed together with their standard deviations. Relative inhibitory potencies (RIPs) are based on the IC_{50} value of methyl α-D-mannopyranoside (MeMan), with RIP (MeMan) ≡ 1.

Tetra-*O*-2,3,4,6-benzoyl-mannopyranose (2). To a solution of 1,2,3,4,6-penta-*O*-benzoyl-mannose (**1**, 5.00 g, 7.13 mmol) in pyridine (50 mL), an ethanolic solution of dimethylamine (5.6 M, 35 mL) was added and the reaction mixture was stirred for 1.5 h at rt. Then the reaction was quenched by the addition of toluene (100 mL), the organic phase was washed three times with brine, dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (toluene–ethyl acetate, 5 : 1) to yield the product (3.31 g, 78%) as a white solid. Anal. Calcd. for $C_{34}H_{28}O_{10}$: C, 68.45; H 4.73. Found: C, 68.60; H, 4.73%.

[3-*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propyl] 2,3,4,6-tetra-*O*-[3-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propyl]-α-D-glucopyranoside (7). To a solution of the pentaol **6**[5] (22 mg, 0.047 mmol) and the mannosyl donor **3** (1.59 g, 2.14 mmol) in dry CH_2Cl_2 (4 mL) TMS-OTf (5% in CH_2Cl_2, 0.2 mL,) was added under N_2 and the solution was stirred at rt overnight. Then $NaHCO_3$ (1 g) and CH_2Cl_2 (50 mL) were added, the solution was filtered, concentrated and the residue purified by flash chromatography (light petroleum ether–ethyl acetate, 2 : 1 → 1 : 1) to yield the title cluster mannoside (157 mg, quant.) as a white amorphous solid. $[\alpha]_D^{20}$ −21.9 (*c* 1.89, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11–7.91 (m, 30H, aryl-H), 7.87–7.77 (m, 10H, aryl-H), 7.56–7.48 (m, 10H, aryl-H), 7.42–7.14 (m, 50H, aryl-H), 6.17, 6.15, 6.14, 6.14, and 6.14 (each dd ≈ t, 5H, $J_{3,4man}$ $J_{4,5man}$ 9.1 Hz, H-4_{man}), 6.11, 5.98, 5.91, 5.91, and 5.91 (each dd, 5H, $J_{2,3man}$ 3.0 Hz, H-3_{man}), 5.77, 5.72, 5.72, 5.72, 5.70 (each dd, 5H, H-2_{man}), 5.17, 5.14, 5.14, 5.12, 5.11 (d, 5H, $J_{1,2man}$ 1.5 Hz, H-1_{man}), 5.05 (d, 1H, $J_{1,2glc}$ 3.6 Hz, H-1_{glc}), 4.74–4.66 (m, 5H, H-6_{man}), 4.56 (m, 1H, H-5_{man}), 4.53–4.41 (m, 10H, 5 H-6_{man}, 4 H-5_{man}), 4.08–3.62 (m, 24H, H-5_{glc}, 2 H-6_{glc}, 10 OC*H*HCH$_2$CH$_2$O, 10 OCH$_2$CH$_2$C*H*HO, H-3_{glc}), 3.50 (dd ~ t, 1H, $J_{3,4glc}$ 9.1 Hz, H-4_{glc}), 3.40 (dd, 1H, $J_{2,3glc}$ 9.1 Hz, H-2_{glc}), 2.15–2.04 (m, 10H, OCH$_2$C*H*HCH$_2$O) ppm. ^{1}H-^{1}H COSY δ = 3.70 (H-3) ppm. ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 166.2–166.0, 165.5–165.2 (20 *C*OOR), 133.4–132.9, 130.0–128.2 (100 aryl-C), 97.8, 97.8, 97.7, 97.7, 97.5 (C-1_{man}), 97.0 (C-1_{glc}), 81.9 (C-3_{glc}), 80.6 (C-2_{glc}), 77.8 (C-4_{glc}), 70.6, 70.6, 70.5, 70.5, 70.5 (C-2_{man}), 70.5 (C-5_{glc}), 70.4, 70.3, 70.3, 70.2, 70.2 (C-3_{man}), 70.2 (CH$_2$*C*H$_2$OC-3_{glc}), 69.4

(C-6_{glc}), 69.1, 67.9, 67.9 (3 CH_2CH_2O), 68.9, 68.9, 68.8, 68.8, 68.7 (C-5_{man}), 67.0, 67.0, 66.9, 66.9, 66.8 (C-4_{man}), 66.2, 65.7, 65.5, 65.3, 65.0, 64.9 (5 CH_2CH_2OC-1_{man}, CH_2CH_2OC-1_{glc}), 62.9, 62.8, 62.8, 62.8, 62.8 (C-6_{man}), 30.7, 30.4, 30.3, 29.8, 29.7 ($OCH_2CH_2CH_2O$) ppm. Anal. Calcd. for $C_{191}H_{172}O_{56}$: C, 68.21; H 5.15. Found: C, 68.12; H, 5.25%. MALDI-TOF MS: 3383.46 $(M + Na)^+$ ion.

[3-*O*-(α-D-Mannopyranosyloxy)propyl] 2,3,4,6-tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-α-D-glucopyranoside (8). To a solution of **7** (329 mg, 0.098 mmol) in dry MeOH (50 mL), NaOMe (10 mg Na in 20 mL MeOH) was added and the solution was stirred for 2 d at rt. Then it was neutralized with Amberlite IR 120, filtered and purified on Sephadex LH-20 (with MeOH as the eluent) to yield the unprotected title compound (102 mg, 82%) as a colorless amorphous solid. $[\alpha]_D^{20}$ +13.5 (*c* 1.85, MeOH). ^{1}H NMR (400 MHz, D_4-MeOH): δ = 4.95 (d, 1H, $J_{1,2glc}$ 3.56 Hz, H-1_{glc}), 4.82, 4.81 (3×), 4.80 (d, 5H, H-1_{man}), 3.97–3.51 (m, 54H, H-3_{glc}, H-5_{glc}, 2 H-6_{glc}, 5 H-2_{man}, 5 H-3_{man}, 5 H-4_{man}, 5 H-5_{man}, 10 H-6_{man}, 20 $OCHCH_2CH_2O$), 3.31 (dd ≈ t, 1H, *J* 9.1 Hz, H-4_{glc}), 3.30 (dd ≈ t, 1H, *J* 9.1 Hz, H-2_{glc}), 2.00–1.83 (m, 10H, OCH_2CHCH_2O) ppm. ^{1}H-^{1}H COSY: δ = 3.56 (H-3_{glc}), 3.65 (H-5_{glc}) ppm. ^{1}H-^{13}C HMQC: δ = 3.88 (H-6_{man}), 3.85 (H-2_{man}), 3.77 (H-6′$_{man}$), 3.74 (H-3_{man}), 3.67 (H-4_{man}), 3.56 (H-5_{man}) ppm. ^{13}C NMR (100.67 MHz, D_4-MeOH): δ = 102.8 (5 C-1_{man}), 99.2 (C-1_{glc}), 84.2 (C-3_{glc}), 83.0 (C-2_{glc}), 80.5 (C-4_{glc}), 75.8, 75.8 (3×), 75.7 (5 C-5_{man}), 73.9, 73.8 (4×) (5 C-3_{man}), 73.3 (5×) (5 C-2_{man}), 72.9 (C-5_{glc}), 72.7, 72.0, 71.8, 70.4, 70.1 (C-6_{glc}, CH_2CH_2O), 69.8, 69.8, 69.8, 69.7, 69.7 (5 C-4_{man}), 67.1 (CH_2CH_2OC-1_{glc}), 66.7, 66.6, 66.6, 66.5, 66.4 (CH_2CH_2OC-1_{man}), 64.1 (5×) (5 C-6_{man}), 32.9, 32.7, 32.5, 32.1, 31.8 (5 $OCH_2CH_2CH_2O$) ppm. ^{1}H-^{13}C HMBC: δ = 72.0 or 71.8 (C-6_{glc}) ppm. MALDI-TOF MS: 1303.78 $(M + Na)^+$ ion.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-(3-hydroxypropyl)-D-trehalose (6-tre). Perallylated trehalose **5-tre**[17] (370 mg, 0.56 mmol) was dissolved in dry THF (20 mL), treated with 9-BBN (20 mL, 10 mmol) and the reaction mixture was heated under reflux for 1 h. An excess of hydride was hydrolyzed with water at 0 °C and then aqueous NaOH (3 M, 10 mL) was added, followed by dropwise addition of aqueous H_2O_2 (30%, 10 mL) at 0 °C. The reaction mixture was stirred overnight at rt and saturated with K_2CO_3. The phases were separated and the aqueous phase extracted with THF (40 mL) three times. The combined organic phases was concentrated, the residue dissolved in a minimum amount of methanol and passed over a Sephadex LH-20 column (eluent MeOH). Then purification was accomplished on silica gel (CH_2Cl_2–MeOH, 3 : 1) to obtain the pure title compound (372 mg, 0.46 mmol, 83%) as a colorless syrup. $[\alpha]_D^{20}$ +70.1 (*c* 2.19, MeOH). ^{1}H NMR (500 MHz, D_4-MeOH): δ = 5.20 (d, 2H, $J_{1,2}$ = 3.1 Hz, 2 H-1), 3.98–3.84, 3.79–3.58 (m, 8H und 32H, 8 $OCH_2CH_2CH_2OH$, 8 $OCH_2CH_2CH_2OH$, 2 H-5, 2 H-6, 2 H-6′, 2 H-3), 3.35–3.28 (m, 4H, 2 H-2, 2 H-4), 1.92–1.77 (m, 16H, 8 $OCH_2CH_2CH_2OH$) ppm. ^{1}H–^{1}H-COSY: δ = 3.95 (H-5), 3.64 (H-3) ppm. ^{13}C NMR (100.67 MHz, D_4-MeOH): δ = 94.9 (2 C-1), 83.8 (2 C-3), 82.7 (2 C-2), 80.5 (2 C-4), 73.3 (2 C-5), 72.6, 72.0, 71.9, 70.7, 70.5 (8 $OCH_2CH_2CH_2OH$, 2 C-6), 61.5, 61.3, 61.2, 61.2 (8 $OCH_2CH_2CH_2OH$), 35.7, 35.5, 35.4, 34.8 (8 $OCH_2CH_2CH_2OH$) ppm. ^{1}H–^{13}C-HMBC: δ = 72.0 or 71.9 (C-6) ppm. MALDI-TOF MS: *m*/*z* = 829.38 $[M + Na]^+$ (806.45 calcd. for $C_{36}H_{70}O_{19}$). Anal. Calcd. for $C_{36}H_{70}O_{19} \times 2\ H_2O$ (842.97): C, 51.29; H, 8.85. Found: C, 51.69; H, 8.80%.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-[3-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propyl]-D-trehalose (7-tre). The octaol **6-tre** (50 mg, 0.062 mmol) and the mannosyl donor **3** (5.0 g, 6.7 mmol) were dissolved in dry acetonitrile (400 mL) under an argon atmosphere and the solution was heated to 65 °C. Then TMS-OTf (0.05 ml) was added and it was stirred for 1 h at 65 °C. Then, it was cooled to rt, more mannosylimidate **3** (1.2 g, 1.6 mmol) was added and the reaction mixture was stirred at rt overnight. The solution was neutralized with solid $NaHCO_3$, it was filtered, neutralized and concentrated. The residue was purified by three subsequent purification steps. First GPC on Sephadex LH-20 was performed (eluent CH_2Cl_2–MeOH, 1 : 1), followed by flash chromatography on silica gel (hexane–ethyl acetate, 45 : 55) and finally another GPC on Sephadex LH-20 (eluent CH_2Cl_2–MeOH, 1 : 1) gave the pure title cluster mannoside (318 mg, 0.059, 95%) as an amorphous white solid. $[\alpha]_D^{20}$ −20.3 (*c* 0.64, CH_2Cl_2). ^{1}H NMR (500 MHz, D_6-DMSO, 403 K): δ = 8.02–7.92, 7.87–7.82, 7.72–7.65, 7.64–7.56, 7.52–7.20 (each m, 32 H, 16 H, 16 H, 16 H 80 H, aryl-H), 6.03–5.95 (m, 8H, 8 H-4_{man}), 5.88, 5.85, 5.85, 5.84 (each dd, each 2H, $J_{2,3man}$ 3.0 Hz, $J_{3,4man}$ 9.9 Hz, 8 H-3_{man}), 5.71, 5.68, 5.68, 5.65 (each dd, each 2H, $J_{1,2man}$ 1.7 Hz, 8 H-2_{man}), 5.24 (d, 2H, $J_{1,2glc}$ 3.6 Hz, 2 H-1_{glc}), 5.23, 5.18, 5.16, 5.13 (each d, each 2H, 8 H-1_{man}), 4.67–4.47 (m, 24H, 8 H-5_{man}, 8 H-6_{man}, 8 H-6′$_{man}$), 4.05–3.64 (m, 40H, 2 H-3_{glc}, 2 H-5_{glc}, 2 H-6_{glc}, 2 H-6′$_{glc}$, 8 glc-OCH_2, 8 man-OCH_2), 3.40 (dd ≈ t, 2H, *J* 9.1 Hz, 2 H-4_{glc}), 3.39 (dd, 2H, $J_{2,3glc}$ 9.1 Hz, 2 H-2_{glc}), 2.11–1.95 (m, 16H, 8 $CH_2CH_2CH_2$) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ = 167.5–166.5 (32 CO_2Ph), 134.5–129.5 (160 aryl-C), 99.3, 99.0, 89.9, 89.9 (8 C-1_{man}), 95.1 (2 C-1_{glc}), 83.0 (2 C-3_{glc}), 81.7 (2 C-2_{glc}), 79.1 (2 C-4_{glc}), 72.3 (2 C-5_{glc}), 72.0, 71.9, 71.9, 71.8 (8 C-2_{man}), 71.7, 71.6, 71.5, 71.5 (8 C-3_{man}), 70.2, 70.1, 70.1, 70.0 (8 C-5_{man}), 70.7, 70.5, 69.6, 69.3 (8 (glc)OCH_2, 2 C-6_{glc}*), 68.3 (3×), 68.1 (8 C-4_{man}), 67.5, 67.2, 66.6 (2×) (8 man-OCH_2), 64.2, 64.2, 64.1 (2×) (8 C-6_{man}), 32.0, 31.8, 31.7, 31.3 (8 $OCH_2CH_2CH_2O$) ppm. * The signal for one CH_2 moiety is superimposed by the signal for C-3_{man}. MALDI-TOF MS: *m*/*z* = 5454.33 $[M + Na]^+$ (5431.71 calcd. for $C_{308}H_{278}O_{91}$). Anal. Calcd. for $C_{308}H_{278}O_{91}$ (5435.54): C, 68.08; H 5.16. Found: C, 67.77; H, 5.06%.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-[3-(α-D-mannopyranosyloxy)propyl]-D-trehalose (8-tre). To a solution of **7-tre** (110 mg, 0.020 mmol) in dry THF (50 mL), NaOMe (10 mg Na in 20 mL MeOH) was added and it was stirred for 4 h at rt. Then, it was concentrated, the residue dissolved in MeOH (50 mL) and NaOMe (10 mg Na in 20 mL MeOH) was added. This reaction mixture was stirred overnight at rt, then neutralized with Amberlite IR 120, filtered, concentrated and purified on Sephadex LH-20 (with MeOH as the eluent) to yield the title glycocluster (38 mg, 90%) as a colorless amorphous solid. $[\alpha]_D^{20}$ +72.9 (*c* 0.17, MeOH). ^{1}H NMR (400 MHz, D_4-MeOH): δ = 5.22 (d, 2H, $J_{1,2glc}$ 3.5 Hz, 2 H-1_{glc}), 4.82 (6×), 4.79 (2×) (each d ≈ s, 8H, 8 H-1_{man}), 4.04–3.49 (m, 88H, 32 $OCHHCH_2$, 2 H-3_{glc}, 2 H-5_{glc}, 2 H-6_{glc}, 2-H-6′$_{glc}$, 8 H-2_{man}, 8 H-3_{man}, 8 H-4_{man}, 8 H-5_{man}, 8 H-6_{man}, 8 H-6′$_{man}$), 3.37–3.29 (m, 4H + MeOH, 2 H-2_{glc}, 2 H-4_{glc}), 2.00–1.77 (m, 16H, OCH_2CHCH_2O) ppm. ^{13}C NMR (100.67 MHz, D_4-MeOH): δ = 102.8 (2×), 102.7 (6×) (8 C-1_{man}), 95.1 (2×) (2 C-1_{glc}), 84.0 (2×) (2 C-3_{glc}), 82.7 (2×) (2 C-2_{glc}), 80.5 (2×) (2 C-4_{glc}), 75.9 (2×), 75.8 (6×) (8 C-5_{man}), 73.8 (8×) (8 C-3_{man}), 73.4 (4×), 73.3 (4×) (8 C-2_{man}), 72.7 (2×), 71.8 (2×), 71.7 (2×), 70.6 (2×), 70.5 (2×) (2 C-6_{glc}, (glc)OCH_2), 69.8 (6×), 69.7 (2×) (8 C-4_{man}), 67.0 (2×), 66.7 (2×), 66.5 (2×), 66.4 (2×)

(CH_2CH_2OC-1_{man}), 64.1 (8×) (8 C-6_{man}), 33.0 (2×), 32.7 (2×), 32.6 (2×), 32.1 (2×) (8 $OCH_2CH_2CH_2O$) ppm. MALDI-TOF MS: 2125.89 $(M + Na)^+$ ion.

Allyl 2,3,4-tri-*O*-allyl-6-(2,3,4,6-tetra-*O*-allyl-α-D-galactopyranosyloxy)-β-D-glucopyranoside (5-mel). To a suspension of the allyl melibioside (**4-mel**) (1.2 g, 3.1 mmol) in dry DMF (100 mL), NaH (2.5 g, 65 mmol) was added at rt. 1 h later, allyl bromide (3.1 mL, 37 mmol) was added and the reaction mixture was stirred overnight at rt. Then the reaction was quenched with ice water at 0 °C and diluted with toluene (100 mL). The organic phase was separated, consecutively washed with aqueous NaCl solution (2 ×) and water (6 ×), dried over $MgSO_4$, filtered, concentrated and the residue was purified by flash chromatography (toluene–ethyl acetate, 7 : 1) to yield the title compound (1.4 g, 69%) as a colorless syrup. $[\alpha]_D^{20}$ +48.0 (*c* 3.42, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.01–5.84 (dddd ≈ m, 8H, 8 OCH_2CHCH_2), 5.33–5.20 (m, 8H, 8 OCH_2CHCHH), 5.20–5.11 (m, 8H, 8 OCH_2CHCHH), 5.05 (d, 1H, $J_{1,2gal}$ 3.6 Hz, H-1_{gal}), 4.41–3.94 (m, 18H, H-1_{glc}, H-5_{gal}, 16 $OCHHCHCH_2$), 3.85–3.74 (m, 4H, H-6_{glc}, H-$6'_{glc}$, H-2_{gal}, H-4_{gal}), 3.71 (dd, 1H, $J_{2,3gal}$ 10.2 Hz, $J_{3,4gal}$ 2.5 Hz, H-3_{gal}), 3.59 (dd, 1H, $J_{6,5gal}$ 7.6 Hz, $J_{6,6'gal}$ 9.2 Hz, H-6_{gal}), 3.52 (dd, 1H, $J_{6',5gal}$ 6.1 Hz, H-$6'_{gal}$), 3.40 (m, 1H, H-5_{glc}), 3.38–3.30 (m, 2H, H-3_{glc}, H-4_{glc}), 3.19 (dd ≈ t, 1H, $J_{1,2glc}$ and $J_{2,3glc}$ 8.4 Hz, H-2_{glc}) ppm. ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 135.9, 135.7, 135.7, 135.6, 135.5, 135.3, 135.0, 134.6 (8 OCH_2CHCH_2), 117.4, 117.2, 117.2, 117.1, 117.1, 117.0, 116.8, 116.6 (8 OCH_2CHCH_2), 102.7 (C-1_{glc}), 98.2 (C-1_{gal}), 84.6 (C-3_{glc}), 82.1 (C-2_{glc}), 78.2 (C-3_{gal}), 78.1 (C-4_{glc}), 76.7 (C-2_{gal}), 75.3 (C-4_{gal}), 75.0 (C-5_{glc}), 74.7, 74.4, 74.1, 74.0, 72.6, 72.2, 72.1, 70.2 (OCH_2CHCH_2), 69.3 (C-5_{gal}), 69.0 (C-6_{gal}), 66.9 (C-6_{glc}) ppm. Anal. Calcd. for $C_{36}H_{54}O_{11}$: C, 65.24; H 8.21. Found: C, 65.30; H, 8.27%.

3-Hydroxypropyl 2,3,4-tri-*O*-(3-hydroxypropyl)-6-[2,3,4,6-tetra-*O*-(3-hydroxypropyl)-α-D-galactopyranosyloxy]-β-D-glucopyranoside (6-mel). To a solution of 5-mel (240 mg, 0.36 mmol) in dry THF (20 mL), 9-BBN (0.5 M solution in THF, 12 mL) was added under an N_2 atmosphere and the solution was stirred at 60 °C for 1 h. Then the excess of 9-BBN was destroyed by dropwise addition of water at 0 °C. The hydroboration mixture was oxidized by the addition of aqueous NaOH (3 M, 12 mL) and aqueous H_2O_2 (30%, 12 mL) at 0 °C, followed by stirring at rt overnight. The aqueous phase was saturated with K_2CO_3 and the THF phase was separated. The aqueous phase was extracted twice with THF (40 mL). The combined organic phases were concentrated and purified on Sephadex LH-20 (eluent MeOH) and by flash chromatography (CH_2Cl_2–MeOH, 3 : 1) to afford the title compound (162 mg, 56%) as a colorless oil. $[\alpha]_D^{20}$ +53.1 (*c* 0.52, MeOH). ^{1}H NMR (500 MHz, D_4-MeOH): δ = 5.14 (d ≈ s, 1H, H-1_{gal}), 4.31 (d, 1H, $J_{1,2glc}$ 7.6 Hz, H-1_{glc}), 4.01 (ddd ≈ t, 1H, $J_{5,6gal}$ and $J_{5,6'gal}$ 6.6 Hz, H-5_{gal}), 3.99–3.60 (m, 38H, 16 $OCHHCH_2CH_2OH$, 16 OCH_2CH_2CHHOH, H-2_{gal}, H-3_{gal}, H-4_{gal}, H-6_{gal}), 3.58 (dd, 1H, $J_{6,6'gal}$ 9.2 Hz, H-$6'_{gal}$), 3.42–3.26 (m, 3H + MeOH, H-3_{glc}, H-4_{glc}, H-5_{glc}), 3.05 (dd ≈ t, 1H, $J_{2,3glc}$ 8.1 Hz, H-2_{glc}), 1.92–1.77 (m, 16H, OCH_2CHHCH_2OH) ppm. ^{13}C NMR (100.67 MHz, D_4-MeOH): δ = 105.7 (C-1_{glc}), 99.7 (C-1_{gal}), 87.2 (C-3_{glc}), 84.9 (C-2_{glc}), 80.9, 80.5, 79.3, 78.1 (C-2_{gal}, C-3_{gal}, C-4_{gal}, C-4_{glc}), 77.0 (C-5_{glc}), 72.7, 72.7, 72.1, 72.0, 72.0 (2×), 70.5, 70.1, 70.0, 68.8, 68.3 (8 $OCH_2CH_2CH_2OH$, C-6_{glc}, C-6_{gal}), 71.6 (C-5_{gal}), 61.6, 61.6, 61.5, 61.5, 61.4, 61.3, 61.2, 61.1 (8 $OCH_2CH_2CH_2OH$), 35.7, 35.5, 35.3, 35.2, 35.2, 35.1, 35.0 (8 $OCH_2CH_2CH_2OH$) ppm. MALDI-TOF MS: 828.59 $(M + Na)^+$ ion. Anal. Calcd. for $C_{36}H_{70}O_{19}\cdot H_2O$: C, 52.41; H 8.80. Found: C, 52.37; H, 8.66%.

[3-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propyl] 2,3,4-tri-*O*-[3-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-propyl]-6-{2,3,4,6-tetra-*O*-[3-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propyl]-α-D-galactopyranosyloxy}-β-D-glucopyranoside (7-mel). A solution of the octaol **6-mel** (0.034 g, 0.042 mmol) and the mannosyl donor 3 (5.0 g, 6.7 mmol) in dry acetonitrile (400 mL) was heated to 75 °C, TMS-OTf (0.05 mL) was added and the reaction mixture was stirred at this temperature for 2 h. Then, additional donor **3** (1.2 g, 1.6 mmol) was added and the mixture was stirred overnight at rt. Then it was neutralized with $NaHCO_3$ (10 g), filtered, concentrated and the residue was purified on Sephadex LH-20 (CH_2Cl_2–MeOH, 1 : 1), followed by flash chromatography (hexane–ethyl acetate, 9 : 11) to yield the title cluster mannoside (145 mg, 64%) as a white amorphous solid. $[\alpha]_D^{20}$ −36.0 (*c* 0.30, CH_2Cl_2). ^{1}H NMR (500 MHz, D_6-DMSO, 353 K): δ = 7.98–7.87, 7.84–7.79, 7.66–7.55, 7.48–7.18 (each m, 32 H, 16 H, 32H, 80H, aryl-H), 6.01–5.93 (m, 8 H, 8 H-4_{man}), 5.83–5.75 (m, 8 H, 8 H-3_{man}), 5.66–5.60 (m, 8H, 8 H-2_{man}), 5.18–5.10 (m, 9H, H-1_{gal}, 8 H-1_{man}), 4.64–4.55, 4.53–4.43 (each m, 8H, 16H, 8 H-5_{man}, 8 H-6_{man}, 8 H-$6'_{man}$), 4.37 (d, 1H, $J_{1,2glc}$ 7.6 Hz, H-1_{glc}), 4.00–3.32 (m, 44H, H-3_{glc}, H-4_{glc}, H-5_{glc}, H-6_{glc}, H-$6'_{glc}$, H-2_{gal}, H-3_{gal}, H-4_{gal}, H-5_{gal}, H-6_{gal}, H-$6'_{gal}$, 16 $OCHHCH_2CH_2OH$, 16 OCH_2CH_2CHHO, H-2_{glc}), 2.05–1.85 (m, 16H, OCH_2CHHCH_2O) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ = 166.0–165.1 (32 aryl-C), 133.3–128.2 (160 aryl-C), 103.5 (C-1_{glc}), 97.8–97.5 (C-1_{gal}, 8 C-1_{man}), 84.9 (C-3_{glc}), 82.6 (C-2_{glc}), 78.5, 78.2, 77.1, 75.9 (C-2_{gal}, C-3_{gal}, C-4_{gal}, C-4_{glc}), 75.0 (C-5_{glc}), 70.6–70.4 (8 C-2_{man}), 70.3–70.1 (8 C-3_{man}), 69.1 (C-5_{gal},), 68.9–68.6 (8 C-5_{man}), 66.9–66.8 (8 C-4_{man}), 70 (2×), 69.6, 69.5, 69.4, 68.2, 67.8 (2×), 66.2–65.7 (C-6_{glc}, C-6_{gal}, 8 man-OCH_2, 4 glc-OCH_2, 4 gal-OCH_2), 62.7 (8 C-6_{man}), 30.6–29.7 (11 $OCH_2CH_2CH_2O$) ppm. MALDI-TOF MS: 5454.1 $(M + Na)^+$ ion.

[3-(α-D-Mannopyranosyloxy)propyl] 2,3,4-tri-*O*-[3-(α-D-mannopyranosyloxy)propyl]-6-{2,3,4,6-tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-α-D-galactopyranosyloxy}-β-D-glucopyranoside (8-mel). To a solution of the protected cluster **7-mel** (81 mg, 0.015 mmol) in dry THF (50 mL), NaOMe (10 mg Na in 20 mL MeOH) was added and the solution was stirred for 4 h at rt. Then it was concentrated and the residue dissolved in MeOH (50 mL). Then again NaOMe (10 mg Na in 20 mL MeOH) was added and the reaction mixture was stirred overnight at rt, neutralized with Amberlite IR 120, filtered and the residue was purified on Sephadex LH-20 (eluent MeOH) to yield the unprotected title compound (32 mg, quant.) as a colorless amorphous solid. $[\alpha]_D^{20}$ +66.0 (*c* 0.59, MeOH). ^{1}H NMR (300 MHz, D_4-MeOH): δ = 5.14 (d ≈ s, 1H, H-1_{gal}), 4.85–4.78 (m, 8H, 8 H-1_{man}), 4.31 (d, 1H, $J_{1,2glc}$ 7.3 Hz, H-1_{glc}), 4.06–3.51 (m, 86H, H-5_{gal}, 16 $OCHHCH_2CH_2O$, 16 OCH_2CH_2CHHO, H-2_{gal}, H-3_{gal}, H-4_{gal}, H-6_{gal}, H-$6'_{gal}$, 8 H-4_{man}, 8 H-3_{man}, 8 H-2_{man}, 8 H-5_{man}, 8 H-6_{man}, 8 H-$6'_{man}$), 3.46–3.25 (m, 3H + MeOH, H-3_{glc}, H-4_{glc}, H-5_{glc}), 3.03 (dd ≈ t, 1H, $J_{2,3glc}$ 8.9 Hz, H-2_{glc}), 2.03–1.80 (m, 16H, OCH_2CHHCH_2O) ppm. ^{13}C NMR (125.77 MHz, D_4-MeOH): δ = 104.9 (C-1_{glc}), 102.1–101.9 (8×) (8 C-1_{man}), 98.9 (C-1_{gal}),. 86.3 (C-3_{glc}), 84.2 (C-2_{glc}), 80.0, 79.3, 78.8, 77.5 (C-2_{gal}, C-3_{gal}, C-4_{gal}, C-4_{glc}), 76.4 (C-5_{glc}), 74.9 (8×) (8 C-5_{man}), 72.9 (8×) (8 C-3_{man}), 72.5 (8×) (8 C-2_{man}), 71.0

(C-5$_{gal}$), 71.9, 71.7, 71.3, 71.2, 70.9, 69.6, 69.1, 67.8, 67.0, 64.6 (C-6$_{glc}$, C-6$_{gal}$, 8 glc-O*C*H$_2$),68.9 (8×) (8 C-4$_{man}$), 66.0–65.7 (8×) (8 man-O*C*H$_2$), 63.2 (8×) (8 C-6$_{man}$), 32.0, 31.8 (5×), 31.4, 31.2 (8 OCH$_2$*C*H$_2$CH$_2$O) ppm. MALDI-TOF MS: 2126.1 (M + Na)$^+$ ion.

Undeca-*O*-allyl-D-raffinose (5-raf). To a suspension of raffinose (**4-raf**) (3.0 g, 5.0 mmol) in dry DMF (100 mL), NaH (3.5 g, 80 mmol) and 1 h later, allyl bromide (6.1 mL, 72.1 mmol) were added and the reaction mixture was stirred overnight at rt. Then the reaction was quenched with ice water at 0 °C and toluene (100 mL) was added. The organic phase was separated, consecutively washed with aqueous NaCl (2 ×) and water (6 ×), dried over $MgSO_4$, filtered, concentrated and the residue was purified by flash chromatography (toluene–ethyl acetate, 7 : 1) to yield the title compound (1.4 g, 69%) as a colorless syrup. $[\alpha]_D^{20}$ +81.4 (*c* 0.91, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.02–5.82 (dddd ≈ m, 11H, 11 OCH$_2$C*H*CH$_2$), 5.51 (d, 1H, $J_{1,2glc}$ 3.6 Hz, H-1$_{glc}$), 5.34–5.20 (ddd, 11H, 11 OCH$_2$CHC*H*H), 5.20–5.07 (ddd, 11H, 11 OCH$_2$CHCH*H*), 5.06 (d, 1H, $J_{1,2gal}$ 3.6 Hz, H-1$_{gal}$), 4.40–4.29 (m, 3H, OC*H*HCHCH$_2$), 4.26–3.89 (m, 24H, H-3$_{frc}$, H-4$_{frc}$, H-5$_{frc}$, H-5$_{gal}$, H-5$_{glc}$, 19 OC*H*HCHCH$_2$), 3.84 (dd, 1H, H-6$_{glc}$), 3.83 (dd, 1H, H-4$_{gal}$), 3.80 (dd, 1H, $J_{2,3gal}$ 10.2 Hz, H-2$_{gal}$), 3.70 (dd, 1H, $J_{3,4gal}$ 3.1 Hz, H-3$_{gal}$), 3.68–3.57 (m, 6H, H-1$_{frc}$, H-6$_{frc}$, H-6′$_{frc}$, H-3$_{glc}$, H-6$_{glc}$, H-6$_{gal}$), 3.54 (dd ≈ t, 1H, $J_{3,4glc}$ and $J_{3,5glc}$ 9.2 Hz, H-4$_{glc}$), 3.49 (dd, 1H, $J_{6',5gal}$ 5.6 Hz, $J_{6,6'gal}$ 9.2 Hz, H-6′$_{gal}$), 3.43 (d, 1H, $J_{1,1'frc}$ 11.2 Hz, H-1′$_{frc}$), 3.26 (dd, 1H, $J_{2,3glc}$ 9.2 Hz, H-2$_{glc}$) ppm. ^{1}H-^{13}C HMQC: δ = 4.24 (H-3$_{frc}$), 4.01 (H-4$_{frc}$), 3.97 (H-5$_{frc}$) ppm. ^{1}H-^{13}C HMBC: δ = 3.92 (H-5$_{gal}$) ppm. ^{1}H-^{1}H COSY: δ = 3.94 (H-5$_{glc}$) ppm. ^{13}C NMR (100.67 MHz, $CDCl_3$): δ = 136.1, 135.9, 135.8, 135.8, 135.7, 135.5, 135.2, 135.2, 135.1, 135.0, 135.0 (11 OCH$_2$*C*HCH$_2$), 117.4, 117.3, 117.3 (2×), 117.3, 117.2, 116.8, 116.7, 116.6 (2×), 116.4 (11 OCH$_2$CH*C*H$_2$), 104.8 (C-1$_{frc}$), 98.6 (C-1$_{gal}$), 90.3 (C-1$_{glc}$), 83.8 (C-3$_{frc}$), 82.4 (C-4$_{frc}$), 81.7 (C-3$_{glc}$), 79.9, 79.8 (C-2$_{glc}$, C-5$_{frc}$), 78.2 (C-3$_{gal}$), 77.6 (C-4$_{glc}$), 76.8 (C-2$_{gal}$), 75.2 (C-4$_{gal}$), 74.4 (2×), 74.1, 72.9, 72.7, 72.6, 72.1 (2×), 71.9, 71.9, 71.8, 71.6 (C-6$_{frc}$, OCH$_2$CHCH$_2$), 71.5 (C-5$_{glc}$), 69.5 (C-5$_{gal}$), 69.1 (C-6$_{gal}$), 66.4 (C-6$_{glc}$) ppm. MALDI-TOF MS: 967.94 (M + Na)$^+$ ion. Anal. Calcd. for $C_{51}H_{76}O_{16}$: C, 64.81; H, 8.10. Found: C, 64.65; H, 8.11%.

Undeca-*O*-(3-hydroxypropyl)-D-raffinose (6-raf). To a solution of **5-raf** (535 mg, 0.57 mmol) in dry THF (20 mL), 9-BBN (0.5 M solution in THF, 26 mL) was added under an N_2 atmosphere and the solution was stirred at reflux temperature for 1 h. Then the excess of 9-BBN was destroyed by dropwise addition of water at 0 °C. The hydroboration mixture was oxidized by the addition of aqueous NaOH (3 M, 13 mL) and aqueous H_2O_2 (30%, 13 mL) at 0 °C. Then it was stirred at rt overnight and the aqueous phase was saturated with K_2CO_3. The phases were separated and the aqueous phase was extracted twice with THF (40 mL). The combined organic phases was concentrated and the residue was purified by two subsequent procedures, first by GPC on Sephadex LH-20 (eluent MeOH), followed by flash chromatography (CH_2Cl_2–MeOH, 3 : 1) to afford the pure title compound (538 mg, 85%) as a colorless oil. $[\alpha]_D^{20}$ +66.1 (*c* 0.36, MeOH). ^{1}H NMR (500 MHz, D_4-MeOH): δ = 5.63 (d, 1H, $J_{1,2glc}$ 3.6 Hz, H-1$_{glc}$), 5.18 (d, 1H, H-1$_{gal}$), 4.15 (d, 1H, H-3$_{frc}$), 4.05–3.47 (m, 61H, H-3$_{glc}$, H-4$_{glc}$, H-5$_{glc}$, H-6$_{glc}$, H-6′$_{glc}$, H-1$_{frc}$, H-1′$_{frc}$, H-4$_{frc}$, H-5$_{frc}$, H-6$_{frc}$, H-6′$_{frc}$, H-2$_{gal}$, H-3$_{gal}$, H-4$_{gal}$, H-5$_{gal}$, H-6$_{gal}$, H-6′$_{gal}$, 22 OC*H*HCH$_2$CH$_2$OH, 22 OCH$_2$CH$_2$C*H*HOH), 3.26 (dd, 1H, $J_{2,3glc}$ 9.1 Hz, H-2$_{glc}$), 1.92–1.77 (m, 22H, 22 OCH$_2$C*H*HCH$_2$OH) ppm. ^{1}H-^{1}H COSY : δ = 3.57 (H-3$_{glc}$), 3.68 (H-2$_{gal}$). ^{1}H-^{13}H HSQC : δ = 3.51 (H-4$_{glc}$), 3.99 (H-5$_{glc}$), 3.90 (H-6$_{glc}$), 3.77 (H-6′$_{glc}$), 3.69 (H-1$_{frc}$), 3.49 (H-1′$_{frc}$), 4.02 (H-4$_{frc}$), 3.89 (H-5$_{frc}$), 3.71 (H-6$_{frc}$), 3.71 (H-6′$_{frc}$), 3.70 (H-3$_{gal}$), 3.86 (H-4$_{gal}$), 3.97 (H-5$_{gal}$) ppm. ^{13}C NMR (100.67 MHz, D_4-MeOH): δ = 106.7 (C-2$_{frc}$), 99.9 (C-1$_{gal}$), 92.0 (C-1$_{glc}$), 86.5 (C-3$_{frc}$), 84.4 (C-4$_{frc}$), 84.1 (C-3$_{glc}$), 82.9 (C-2$_{glc}$), 81.9 (C-5$_{frc}$), 80.8 (C-3$_{gal}$), 79.9 (C-4$_{glc}$), 79.3 (C-2$_{gal}$), 78.0 (C-4$_{gal}$), 74.4 (C-1$_{frc}$), 74.1 (C-6$_{frc}$), 73.8 (C-5$_{glc}$), 71.6 (C-5$_{gal}$), 72.7, 72.5, 71.8 (2×), 70.8, 70.6, 70.5 (2×), 70.1, 70.0, and 69.9 (2× (C-6$_{gal}$, 11 OC*H*$_2$CH$_2$CH$_2$OH), 67.8 (C-6$_{glc}$), 61.6, 61.6, 61.6, 61.5, 61.4, 61.3 61.2 (3× 61.2, 61.0 (11 OCH$_2$CH$_2$*C*H$_2$OH), 35.7, 35.5, 35.3 (3×), 35.2, 35.2 (2×), and 34.9 (3× (11 OCH$_2$*C*H$_2$CH$_2$OH) ppm. MALDI-TOF MS: 1165.87 (M + Na)$^+$ ion. Anal. Calcd. for $C_{51}H_{98}O_{27}\cdot 2H_2O$: C, 51.94; H 8.72. Found: C, 51.99; H, 8.64%.

Undeca-*O*-[3-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-propyl]-D-raffinose (7-raf). To a solution of **6-raf** (39 mg, 0.034 mmol) and the mannosyl donor **3** (5.0 g, 6.7 mmol) in dry acetonitrile (400 mL), TMS-OTf (0.05 mL) was added under N_2 at 60 °C and the solution was stirred at 60 °C for 10 min. Then the heating was removed and stirring was continued for 2 h at rt. Additional donor **3** (2.0 g, 2.7 mmol) was added and the reaction mixture was stirred overnight at rt. Then $NaHCO_3$ (10 g) was added, it was filtered and purified by subsequent procedures, first on Sephadex LH-20 (CH_2Cl_2–MeOH, 1 : 1) and then by flash chromatography (hexane–ethyl acetate, 4 : 6) to yield the title copound (231 mg, 91%) as a white amorphous solid. $[\alpha]_D^{20}$ −30.0 (*c* 0.17, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.12–7.70 (m, 88H, aryl-H), 7.55–7.00 (m, 132H, aryl-H), 6.25–6.05 (m, 11 H, 11 H-4$_{man}$), 5.98–5.86 (m, 11 H, 11 H-3$_{man}$), 5.78–5.60 (m, 12 H, 11 H-2$_{man}$, H-1$_{glc}$), 5.23–4.92 (m, 12H, H-1$_{gal}$, 11 H-1$_{man}$), 4.80–4.56, 4.53–4.30 (each m, 11H, 22H, 11 H-5$_{man}$, 11 H-6$_{man}$, 11 H-6′$_{man}$), 4.16–3.26 (m, 64H, H-3$_{frc}$, H-3$_{glc}$, H-4$_{glc}$, H-5$_{glc}$, H-6$_{glc}$, H-6′$_{glc}$, H-1$_{frc}$, H-1′$_{frc}$, H-4$_{frc}$, H-5$_{frc}$, H-6$_{frc}$, H-6′$_{frc}$, H-2$_{gal}$, H-3$_{gal}$, H-4$_{gal}$, H-5$_{gal}$, H-6$_{gal}$, H-6′$_{gal}$, 22 OC*H*HCH$_2$CH$_2$OH, 22 OCH$_2$CH$_2$C*H*HOH, H-2$_{glc}$), 2.24–1.90 (m, 22H, OCH$_2$C*H*HCH$_2$O) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ = 165.9–165.1 (44 aryl-C), 133.2–128.2 (220 aryl-C), 104.5 (C-2$_{frc}$), 97.9–97.5 (C-1$_{gal}$, 11 C-1$_{man}$), 90.3 (C-1$_{glc}$), 83.8, 83.3, 82.2, 80.8, 79.6, 78.5, 77.2, 75.7, and 75.5 (C-3$_{frc}$, C-4$_{frc}$, C-3$_{glc}$, C-2$_{glc}$, C-5$_{frc}$, C-3$_{gal}$, C-4$_{glc}$, C-2$_{gal}$, C-4$_{gal}$), 73.4–71.5 (C-1$_{frc}$, C-6$_{frc}$, C-5$_{glc}$), 70.5–70.1 (11 C-2$_{man}$, 11 C-3$_{man}$, C-5$_{gal}$), 68.7 (11 C-5$_{man}$), 70.0, 69.2, 69.1, 68.4–67.3 (11 (gal-, glc-, frc-OC*H*$_2$, C-6$_{gal}$), 66.7 (11 C-4$_{man}$), 66.0–65.4 (C-6$_{glc}$, 11 man-OC*H*$_2$), 62.6 (11 C-6$_{man}$), 30.6–29.7 (11 OCH$_2$*C*H$_2$CH$_2$O) ppm. MALDI-TOF MS: 7525.7 (M + Na)$^+$ ion.

Undeca-*O*-[3-(α-D-mannopyranosyloxy)propyl]-D-raffinose (8-raf). The protected cluster mannoside **7-raf** (231 mg, 0.031 mmol) was suspended in dry THF (50 mL), NaOMe (10 mg Na in 20 mL MeOH) was added and the reaction mixture was stirred at rt for 4 h. Then, it was concentrated, the residue was dissolved in MeOH (50 mL) and NaOMe (10 mg Na in 20 mL MeOH) was added. This reaction mixture was stirred overnight at rt, then it was neutralized with Amberlite IR 120, filtered and the residue was purified on Sephadex LH-20 (eluent MeOH) to yield the unprotected title compound (64 mg, 71%) as a colorless amorphous solid. $[\alpha]_D^{20}$ +71.5 (*c* 0.13, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.62 (d, 1H, $J_{1.2glc}$ 3.5 Hz, H-1$_{glc}$), 5.17 (s, 1H, H-1$_{gal}$), 4.86–4.79 (m, 11H, 11

H-1$_{man}$), 4.15 (d, 1H, H-3$_{frc}$), 4.07–3.47, 3.40–3.22 (m, 125H and 3H + MeOH, H-3$_{glc}$, H-5$_{glc}$, H-6$_{glc}$, H-6′$_{glc}$, H-1$_{frc}$, H-1′$_{frc}$, H-4$_{frc}$, H-5$_{frc}$, H-6$_{frc}$, H-6′$_{frc}$, H-2$_{gal}$, H-3$_{gal}$, H-4$_{gal}$, H-5$_{gal}$, H-6$_{gal}$, H-6′$_{gal}$, 22 OC*H*HCH$_2$CH$_2$O, 22 OCH$_2$CH$_2$C*H*HO, 11 H-4$_{man}$, 11 H-3$_{man}$, 11 H-2$_{man}$, 11 H-5$_{man}$, 11 H-6$_{man}$, 11 H-6′$_{man}$, H-2$_{glc}$, H-4$_{glc}$), 2.02–1.83 (m, 22H, 22 OCH$_2$C*H*HCH$_2$OH) ppm. ^{13}C NMR (100.67 MHz, D$_4$-MeOH): δ = 101.9 (11×) (11 C-1$_{man}$), 99.3 (C-1$_{gal}$), 91.3 (C-1$_{glc}$), 85.6 (C-3$_{frc}$), 83.5 (2×) (C-4$_{frc}$, C-3$_{glc}$), 82.1 (C-2$_{glc}$), 81.0 (C-5$_{frc}$), 80.1 (C-3$_{gal}$), 79.1 (C-4$_{glc}$), 78.8 (C-2$_{gal}$), 77.4 (C-4$_{gal}$), 74.9 (11×) (11 C-5$_{man}$), 73.3 (2×) (C-1$_{frc}$, C-6$_{frc}$), 73.0 (12×) (C-5$_{glc}$, 11 C-3$_{man}$), 77.5 (11×) (11 C-2$_{man}$), 71.7 (2×), 71.1 (2×), 69.7 (4×), and 69.1 (2×) (CH$_2$, C-6$_{gal}$, 11 gal-, glc-, frc-OCH$_2$), 70.9 (C-5$_{gal}$), 68.9 (11×) (11 C-4$_{man}$), 66.0 (6×), 65.7 (6×) (C-6$_{glc}$, 11 CH$_2$CH$_2$O(C-1$_{man}$)), 63.2 (11×) (11 C-6$_{man}$), 32.0–31.2 (11×) (11 OCH$_2$CH$_2$CH$_2$O) ppm. MALDI-TOF MS: 2948.6 (M + Na)$^+$ ion.

[2-(Allyloxy)ethyl] 2,3,4,6-tetra-*O*-[2-(allyloxy)ethyl]-α-D-glucopyranoside (10). The pentaol **9**[5] (165 mg, 0.41 mmol) was suspended in dry DMF (10 mL) and NaH (60% suspension in paraffin oil, 200 mg, 5.0 mmol) and after 0.5 h, allyl bromide (0.35 mL, 4.1 mmol), were added. The reaction mixture was stirred overnight at rt, was then cooled to 0 °C and water (100 mL) and toluene (100 mL) were added. The phases were separated and the organic phase was washed twice with satd. aqueous sodium chloride solution and twice with water. It was dried over MgSO$_4$, filtered and concentrated. Purification on silica gel (toluene–ethyl acetate, 1 : 1) delivered the title compound as a colorless syrup (134 mg, 54%). $[\alpha]_D^{20}$ +71.3 (*c* 0.38, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.97–5.81 (m, 5H, 5 OCH$_2$C*H*CH$_2$), 5.31–5.21 (m, 5H, 5 OCH$_2$CHC*H*H), 5.19–5.12 (m, 5H, 5 OCH$_2$CHCH*H*), 4.99 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.10–3.86, 3.83–3.49 (each m, 13H and 21H, H-3, H-5, H-6, H-6′, 5 OC*H*$_2$CH$_2$O, 5 OCH$_2$C*H*$_2$O, 5 OC*H*$_2$CHCH$_2$), 3.40 (dd ≈ t, 1H, *J* 9.5 Hz, H-4), 3.38 (dd, 1H, $J_{2,3}$ 9.6 Hz, H-2) ppm. ^{13}C NMR (75.47 MHz, CDCl$_3$): δ = 134.9, 134.9, 134.8 (2×), 134.7 (5 OCH$_2$*C*HCH$_2$), 116.9, 116.8 (2×), 116.7, 116.6 (5 OCH$_2$CH*C*H$_2$), 97.1 (C-1), 82.0 (C-3), 80.9 (C-2), 77.9 (C-4), 70.1 (C-5), 72.3, 72.2, 72.1, 72.1 (2×), 72.0, 72.0, 70.8, 70.6, 69.8, 69.7, 69.6 (2×), 69.4, 69.1, 66.8 (C-6, 5 O*C*H$_2$CH$_2$O, 5 OCH$_2$*C*H$_2$O, 5 O*C*H$_2$CHCH$_2$) ppm.

(6-Hydroxy-3-oxa-hexyl) 2,3,4,6-tetra-*O*-(6-hydroxy-3-oxa-hexyl)-α-D-glucopyranoside (11). The perallylated octopus glucoside **10** (136 mg, 0.23 mmol) was dissolved in dry THF (15 mL), treated with 9-BBN (5 mL, 2.5 mmol) and the reaction mixture was heated under reflux for 1 h. The excess hydride was destroyed by the addition of ice water and then aqueous NaOH (3 M, 2.5 mL) and aqueous H$_2$O$_2$ (30%, 2.5 mL) were added dropwise at 0 °C. The reaction mixture was stirred overnight at rt, then the solution was saturated with solid K$_2$CO$_3$, the phases were separated and the aqueous phase was washed with THF (2 × 40 mL). The combined organic phases was dried over MgSO$_4$ and the solvent was removed after filtration. Purification on silica gel (CH$_2$Cl$_2$–MeOH, 6 : 1 → 5 : 1) yielded the title compound as a colorless syrup (72 mg, 45%). $[\alpha]_D^{20}$ +53.7 (*c* 0.66, MeOH). ^{1}H NMR (300 MHz, D$_4$-MeOH): δ = 5.01 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.09–3.58 (m, 44H, H-3, H-5, H-6, H-6′, 5 OC*H*$_2$C*H*$_2$O, 5 OC*H*$_2$CH$_2$C*H*$_2$OH), 3.40 (d, 1H, $J_{2,3}$ 9.6 Hz, H-2), 3.35 (m, 1H + MeOH, H-4), 1.88–1.76 (m, 10H, 5 OCH$_2$C*H*$_2$CH$_2$OH) ppm. ^{13}C NMR (75.47 MHz, D$_4$-MeOH): δ = 99.4 (C-1), 84.5 (C-3), 83.2 (C-2), 80.4 (C-4), 72.7 (C-5), 74.6, 74.4, 73.1, 73.0, 72.9, 72.8, 72.7, 72.4, 72.2, 72.0, 69.3 (C-6, 5 OCH$_2$*C*H$_2$O), 70.3 (5 OCH$_2$CH$_2$CH$_2$OH), 61.3 (5 OCH$_2$CH$_2$*C*H$_2$OH), 34.9 (5 OCH$_2$*C*H$_2$CH$_2$OH) ppm. MALDI-TOF MS: *m*/*z* = 713.6 [M + Na]$^+$ (690.4 calcd. for C$_{31}$H$_{62}$O$_{16}$).

[6-*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-3-oxa-hexyl] 2,3,4,6-tetra-*O*-[6-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-3-oxa-hexyl]-α-D-glucopyranoside (12). A mixture of the pentaol **11** (45 mg, 0.065 mmol) and the mannosyl donor **3** (2.30 g, 3.1 mmol) was dried under high vacuum and then dissolved in dry CH$_2$Cl$_2$ (4 mL) under an argon atmosphere. A solution of TMS-OTf (5% in dry CH$_2$Cl$_2$, 0.2 mL) was added and the reaction mixture was stirred overnight at rt. Then it was neutralized with NaHCO$_3$ (1 g), filtered, concentrated and the residue was purified by flash chromatography on silica gel (cyclohexane–ethyl acetate, 3 : 2 → 1 : 1) to yield the title cluster mannoside as an amorphous colorless solid (164 mg, 71%). $[\alpha]_D^{20}$ −35.4 (*c* = 0.11, CH$_2$Cl$_2$). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.12–8.08, 8.06–8.02, 7.97–7.93, 7.85–7.81, 7.59–7.53, 7.44–7.48, 7.43–7.30, 7.26–7.20 (each m, 10H, 10H, 10H, 10H, 10H, 5H, 35H, 10H, aryl-H), 6.17–6.10 (m, 5H, 5 H-4$_{man}$), 5.91 (dd, 5H, $J_{2,3man}$ 3.3 Hz, $J_{3,4man}$ 10.3 Hz, 5 H-3$_{man}$), 5.70 (dd, 5H, $J_{1,2man}$ 1.6 Hz, 5 H-2$_{man}$), 5.10–5.08 (m, 5H, 5 H-1$_{man}$), 4.99 (d, 1H, $J_{1,2glc}$ 3.5 Hz, H-1$_{glc}$), 4.72–4.68 (m, 5H, 5 H-6$_{man}$), 4.51–4.46 (m, 5H, 5 H-6′$_{man}$), 4.45–4.40 (m, 5H, 5 H-5$_{man}$), 4.08–4.00, 3.96–3.89, 3.86–3.75, 3.74–3.58 (each m, 2H, 6H, 6H, 30H, 44H, H-3$_{glc}$, H-5$_{glc}$, H-6$_{glc}$, H-6′$_{glc}$, 5 OC*H*$_2$C*H*$_2$OC*H*$_2$CH$_2$C*H*$_2$O), 3.39–3.43 (m, 2H, H-4$_{glc}$, H-2$_{glc}$), 2.03–1.94 (m, 10H, 5 OCH$_2$C*H*$_2$CH$_2$O) ppm. ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 166.1–165.4 (20 *C*O$_2$Ph), 133.4–128.3 (100 aryl-C), 97.7 (5 C-1$_{man}$), 97.2 (C-1$_{glc}$), 82.1 (C-3$_{glc}$), 80.9 (C-2$_{glc}$), 78.1 (C-4$_{glc}$), 72.4, 72.2, 70.8–70.6, 70.1, 69.9, 69.7, 67.7, 66.8 (5 O*C*H$_2$*C*H$_2$O, C-6$_{glc}$) 70.6 (5 C-2$_{man}$), 70.3 (C-5$_{glc}$), 70.2 (5 C-3$_{man}$), 68.8 (5 C-5$_{man}$), 67.0 (5 C-4$_{man}$), 65.7–65.6 (5 (C-1$_{man}$)O*C*H$_2$), 62.9 (5 C-6$_{man}$), 29.8–29.7 (5 OCH$_2$*C*H$_2$CH$_2$O) ppm. MALDI-TOF MS: *m*/*z* = 3604.6 [M + Na]$^+$ (3581.2 calcd. for C$_{201}$H$_{192}$O$_{61}$).

[6-*O*-(α-D-Mannopyranosyloxy)-3-oxa-hexyl] 2,3,4,6-tetra-*O*-[6-*O*-(α-D-mannopyranosyloxy)-3-oxa-hexyl]-α-D-glucopyranoside (13). The protected cluster mannoside **12** (137 mg, 0.038 mmol) was dissolved in THF (20 mL) and treated with NaOMe (0.02 M in MeOH, 10 mL). The reaction mixture was stirred at rt for 0.5 h, then it was neutralized by the addition of ion exchange resin Amberlite IR 120, it was filtered, concentrated and then purified on Sephadex LH-20 with methanol as the eluent. The unprotected title compound was obtained as a colorless amorphous solid (57 mg, quant.). $[\alpha]_D^{20}$ +72.8 (*c* 0.53, MeOH). ^{1}H NMR (500 MHz, D$_4$-MeOH): δ = 5.02 (d, 1H, $J_{1,2glc}$ 3.7 Hz, H-1$_{glc}$), 4.81–4.79 (m, 5H, 5 H-1$_{man}$), 4.07–3.98, 3.94–3.53 (each m, 2H, 72H, H-3$_{glc}$, H-5$_{glc}$, 2 H-6$_{glc}$, 5 H-2$_{man}$, 5 H-3$_{man}$, 5 H-4$_{man}$, 5 H-5$_{man}$, 10 H-6$_{man}$, 5 OC*H*$_2$C*H*$_2$OC*H*$_2$CH$_2$C*H*$_2$O), 3.42 (dd, 1H, $J_{2,3}$ 9.7 Hz, H-2$_{glc}$), 3.35 (dd ≈ t, 1H + MeOH, *J* 9.4 Hz, H-4$_{glc}$), 1.95–1.87 (m, 10H, 5 OCH$_2$C*H*$_2$CH$_2$O) ppm. ^{13}C NMR (125.76 MHz, D$_4$-MeOH): δ = 102.7 (5 C-1$_{man}$), 99.4 (C-1$_{glc}$), 84.5 (C-3$_{glc}$), 83.2 (C-2$_{glc}$), 80.4 (C-4$_{glc}$), 75.7 (5 C-5$_{man}$), 74.6, 74.4 ((C-3$_{glc}$)O*C*H$_2$, (C-4$_{glc}$)O*C*H$_2$), 73.8 (5 C-3$_{man}$), 73.4 (5 C-2$_{man}$), 73.1, 72.9 (2x), 72.8, 72.7, 72.4, 72.2, 72.1 (C-6$_{glc}$, 2 (glc)O*C*H$_2$, 5 (glc)OCH$_2$*C*H$_2$), 72.8 (C-5$_{glc}$), 70.2 (man-OCH$_2$CH$_2$*C*H$_2$O), 69.8 (5 C-4$_{man}$), 69.4 (C-1$_{glc}$-O*C*H$_2$), 66.6 (5 man-O*C*H$_2$), 64.1 (5 C-6$_{man}$), 32.1 (5 OCH$_2$*C*H$_2$CH$_2$O) ppm. MALDI-TOF MS: *m*/*z* = 1523.8 [M + Na]$^+$ (1500.7 calcd.

for $C_{61}H_{112}O_{41}$). Anal. Calcd. for $C_{61}H_{112}O_{41}$: C, 48.79; H 7.52. Found: C, 49.09; H, 7.69%.

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