Synthesis of a αMan(1→3)αMan(1→2)αMan Glycocluster Presented on a β-Cyclodextrin Scaffold

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Application of 6-thio-α- and β-cyclodextrins as the core component for the construction of multivalent carbohydrate structures is described. The method employed for the attachment of monomeric glycosides to a cyclodextrin core is based on the efficient nucleophilic displacement of bromide from an *N*-bromoacetamido functionality. The *N*-bromoacetyl group was positioned at the end of a spacer arm of glycosides and reacted with thiol groups present on the primary face of thiolated cyclodextrins. This coupling reaction led to per-functionalised cyclodextrins which were thiother-linked to appended saccharides through a short spacer arm. Both components of the key conjugation step – 6-thiocyclodextrins and monomeric glycosides – were used without protecting groups, thus facilitating isolation and purification of target compounds. Glycoclusters incorporating six and seven α-D-mannopyranose or β-D-glucopyranose residues linked to cyclodexrin cores were synthesized in 57–75 % yield. Using the same technique, 3-(2-bromoacetamido)propyl trioside, incorporating a synthetic Man-α-(1→3)-Man-α-(1→2)-Man fragment of antigenic yeast mannan, was attached to per-6-thio-β-cyclodextrin to afford a heptavalent glycocluster.

Introduction

The efficiency of many biological processes depends on the multivalent character of interactions between participating ligands and oligomeric or oligovalent receptors.[1] This mode of interactions has a particular importance for biological events associated with recognition of glycoconjugates by carbohydrate-binding proteins (lectins) since monovalent carbohydrate–protein interactions are characterized by low binding affinities. Multivalent presentation of carbohydrate ligands frequently compensates for low affinity by simultaneous formation of multiple carbohydrate-protein complexes that result in a high observed affinity.[2] The fact that this enhancement of carbohydrate binding affinity has potential biomedical applications stimulates further studies of multivalent interactions and a search for efficient synthetic inhibitors and activators based on multivalent carbohydrate ligands.[3] Following pioneering work by Lee,[4] a great variety of scaffolds have been examined for creating molecules presenting carbohydrates in multivalent displays.[5] These multivalent architectures include monosaccharide-centered oligovalent clusters,[6] linear glycopolymers,[7] glycodendrimers,[8] sugar-bearing liposomes,[9] glyconanoparticles,[10] supramolecular assemblies with terminal sugar residues,[11] glycopeptides[12] and glycosylated macrocycles such as cyclodextrins (CDs),[13] calixarenes[13a,14] and cyclic peptides.[15] If the spatial arrangement of carbohydrate-binding sites in an oligovalent protein receptor is known then optimization of the architecture of multivalent carbohydrate ligands can lead to the design of highly efficient inhibitors.[16] However, if the identity of a natural carbohydrate ligand is not clearly established, then synthetic well-defined multivalent sugar ligands can serve as probes for elucidating the specificity of the carbohydrate-binding sites of a receptor.[7b] In addition, there are several different mechanisms by which multivalent ligands can interact with receptors resulting in several different topologies[17] of protein–carbohydrate complexes, e.g. multidimentional cross-linked carbohydrate-lectin complexes.[18] Therefore, a diverse range of synthetic multivalent carbohydrates is required to study a wide variety of lectins.

Naturally occurring glycoproteins often contain several copies of the same terminal mono- or oligosaccharide within their oligoantennary structures. Mannoproteins of yeast are particularly remarkable in that respect − their highly-branched polysaccharide outer chains are composed[19] of multiple side chains attached to O-2 of the mannopyranose (Man) residues of the backbone α-(1→6)-mannan (Figure 1). These side chains consist of mannopyranose, α-Man-(1→2)-α-Man and α-Man-(1→3)-α-Man-(1→2)-α-Man oligosaccharides. Oligomannosides from a structural repertoire of yeast mannans are capable of specific interactions with the host immune system.[20] Investigation of the high molecular weight mannan of the baker's

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yeast (*Saccharomyces cerevisiae*),[21] which has both allergenic and antigenic activities, showed[22] that these activities may be ascribed to a terminal α-Man-(1→3)-α-Man-(1→2)-α-Man trisaccharide fragment of the highly branched polysaccharide structure. A disturbed immune response to antigens of yeast antigenic mannans is specifically observed[23,24] in patients with Crohn's desease (inflammatory bowel disease) who develop *anti-S. cerevisiae* antibodies. It has also been noticed[24] that the level of mannose-binding lectins, a crucial component of the human innate immune system,[25] was reduced in the same group of patients. Well-defined synthetic models of the polysaccharide would be a useful tool for the investigation of allergenicity and antigenicity of yeast mannans. However, synthesis of large multi-branched oligomannan structures is a difficult task,[26] while the construction of multivalent mimics incorporating specific mannoligosaccharides as carbohydrate ligands and unnatural multivalent cores seems to present a reasonable alternative. A range of mannodendrimers containing only monosaccharide units on each of the arms has been synthesized and evaluated[27] as inhibitors of the binding of the yeast mannan antigens to Crohn's patients *anti-S.cerevisiae* antibodies. However, no activity was observed and it was concluded that epitopes containing more than one mannopyranose residue are required for recognition by these antibodies.

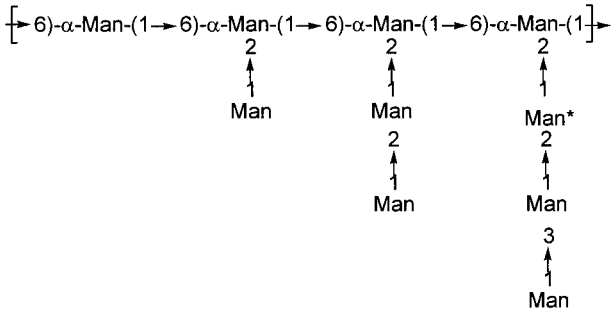


Figure 1. Schematic representation for the polysaccharide structure of the mannan from *S.cerevisiae*.[19b] The sequential arrangement of the different side chains is arbitrary. The asterisk * indicates the presence of the following substituents at O-6: 30% H, 20% PO_3^{2-}, 20% α-Man-OPO_2^{2-}, and 30% α-Man-(1→3)-α-Man-OPO_2^{2-}.

α-D-Mannopyranose-containing glycoconjugates and lectins capable of binding mannopyranose are abundant in biological systems and often involved in critical recognition events at the cellular level. Thus, serum mannose-binding lectins[25] and mannose receptors of macrophages and dendritic cells[28] are pivotal in defence against pathogens. The defence mechanism relies on interaction of the lectins with multiple sugars present on the pathogen surface. The same type of interaction can be utilized by some infectious agents, such as the virus HIV-1, which choose dendritic cell receptors as their targets in order to escape immune surveillance.[29] On the other hand, the adhesion of pathogens to human cells can be mediated by the attachment of bacterial lectins to mannopyranose residues of glycoproteins expressed on human cells.[30] The diversity of biological interactions in which mannopyranose participates as a ligand calls for mechanims that allow different mannopyranose-bearing entities to be distinguished. Clearly, elongation of a saccharide chain can increase specificity of the mannose ligand binding, as in the case of interaction of high-mannose oligosaccharides of envelope glycoprotein gp120 of HIV virus with human antibody 2G12[31] and protein cyanovarin-N.[32] In other cases, arrangement of mannopyranose ligands into clusters can be a key factor in the observed specificity.[33] Therefore, design of efficient probes for studying mannopyranose binding by natural receptors may be based on multivalent structures and can include mannooligosaccharides as ligands. In recent years, syntheses of cluster glycosides incorporating multiple mannopyranoside,[6b,15b,34] mannobioside[35] and mannononaoside[31c] ligands and a series of mannodendrimers[27,36] has been described.

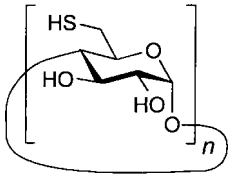
In the present work we studied conjugation of unprotected per-6-thiolated-α- and β-cyclodextrins with unprotected monosaccharide ligands equipped with glycosidically-linked spacer arms incorporating the bromoacetamido group as the reactive moiety. This method was then applied for the synthesis of a thioether-linked glycocluster based on β-cyclodextrin core which bears seven copies of the trisaccharide unit α-Man-(1→3)-α-Man-(1→2)-α-Man. It was envisaged that this glycocluster would provide an effective mimic of the outer chain of yeast mannan.

Results and Discussion

General Strategy: Conjugation of saccharides to a cyclodextrin core through the formation of the thioether linkage has been one of the approaches used for the construction of cyclodextrin-based glycoclusters.[13] In these approaches, thioether linkages were created by reaction of per-6-deoxy-6-iodo- or per-6-deoxy-6-chloroacetamido β-cyclodextrin with thiol-containing sugars[34f–34h,37] or by radical addition of 1-thio-sugars to cyclodextrins per-*O*-allylated in primary, secondary or both primary and secondary faces.[38] In most cases, both carbohydrate monomers and cyclodextrins were in fully protected form and synthesis of target conjugates required removal of a large number of protecting groups. In our strategy we apply unprotected polythiolated cyclodextrins as a core and unprotected bromoacetylated spacer-armed sugars as reactive electrophiles for making thioether linkages using efficient coupling technique.[39] To prove the concept, we first investigated the practicalities of such conjugation using known per-6-thio-cyclodextrins (TCDs) as cores and 3-bromoacetamidopropyl glycosides of mannopyranose and glucopyranose as ligands. Then the multiple attachment of a more complex saccharide motif was studied. Trisaccharide α-Man-(1→3)-α-Man-(1→2)-α-Man-$OCH_2CH_2CH_2NHCOCH_2Br$ was used as this motif, for which a practical synthesis based on the trichloroacetimidate glycosylation method[40] was developed.

Synthesis of Cyclodextrin-Based α-D-Mannopyranoside and β-D-Glucopyranoside Clusters Using Thiolated Cyclodextrins as Core Units: Core per-6-thio-α-CD (**1a**) and per-

6-thio-β-CD (**1b**) were synthesized in high yields using a well-established procedure[41] involving treatment of per-6-(deoxyhalo)-cyclodextrins with thiourea at 70 °C in the absence of oxygen, followed by decomposition of thiuronium salt with aq. NaOH. Starting per-6-deoxy-6-iodo-β-CD was prepared by iodination[42] of β-CD with Ph_3P/I_2, whereas per-6-bromo-6-deoxy-α-CD is easier prepared[43] by bromination of α-CD with NBS/Ph_3P in DMF. Both per-6-thio-β-CD[41] and per-6-thio-α-CD[44] are solid compounds that are practically insoluble in water, a property which facilitates their isolation and increases their stability against air oxidation (Figure 2).

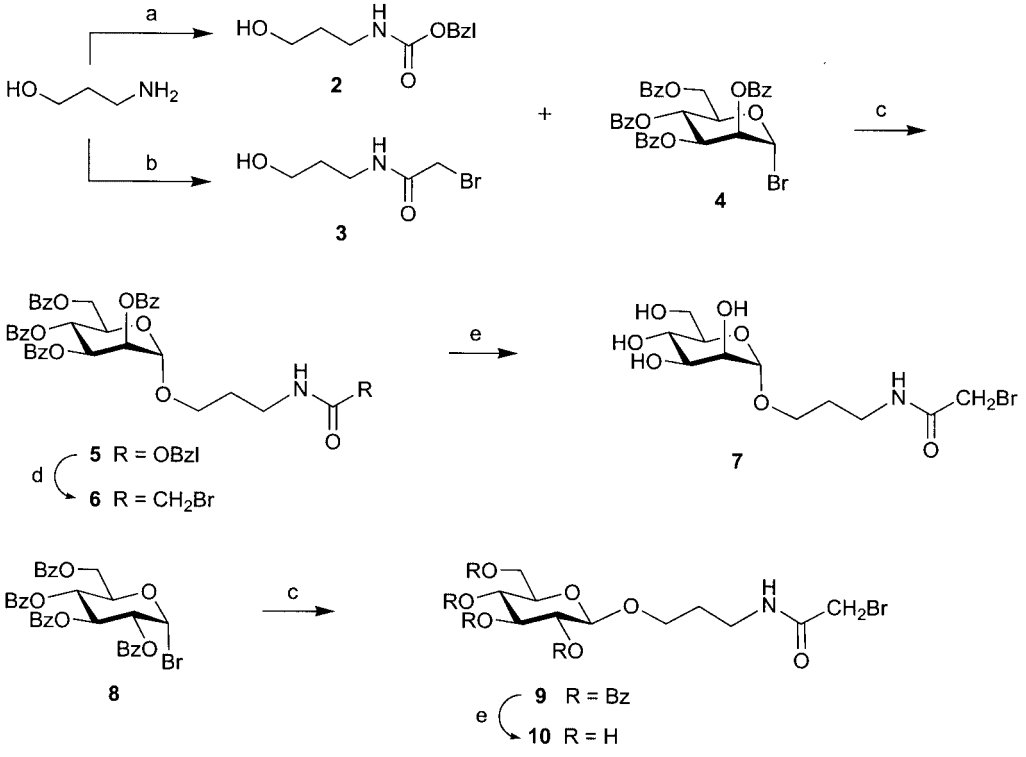


1a $n = 6$
1b $n = 7$

Figure 2. Per-6-thio-α-cyclodextrin (**1a**) and per-6-thio-β-cyclodextrin (**1b**).

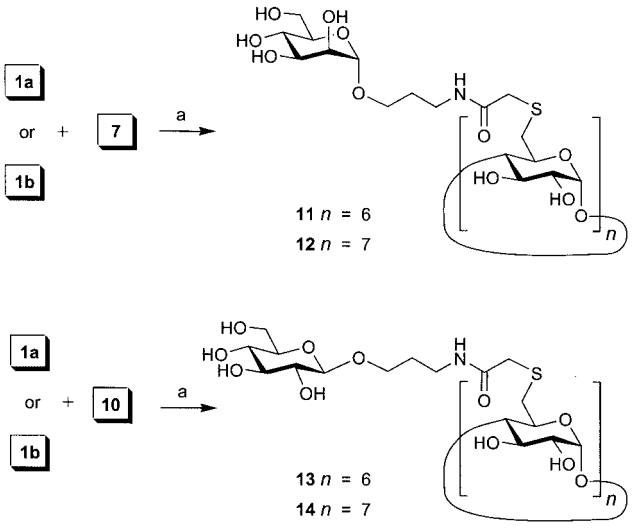
The possibility of conjugation of multiple sugar residues with thiolated cyclodextrins was examined initially with mannopyranoside **7** (Scheme 1), incorporating a short linker between monosaccharide and bromoacetamide. To synthesize glycoside **7**, alcohol **2**[45] was reacted with mannopyranosyl bromide **4** in the presence of iodine[46] in MeCN to give *N*-benzyloxycarbonyl derivative **5** in 72% yield. Selective deprotection of the amino group by hydrogenolysis followed by bromoacetylation with $BrCH_2COBr$ in the presence of Et_3N led to bromoacetamide **6** in overall 72% yield. De-*O*-benzoylation of **6** was carefully carried out with NaOMe in MeOH to afford water-soluble target glycoside **7** in 90% yield. Alternatively, mannopyranoside **7** was synthesized by glycosylation of 3-*N*-bromoacetyl 3-aminopropanol (**3**). Glysosyl acceptor **3** was prepared through *N*-bromoacetylation of aminopropanol with $BrCH_2COBr/Et_3N$ at –50 °C. Under these conditions partial *O*-bromoacetylation also occurred and treatment with Et_3N in MeOH was required to deprotect OH groups. The *N*-bromoacetyl group proved to be sufficiently stable to glycosylation conditions[46] involving I_2 as a promoter, and glycoside **6** was obtained in 65% yield by this approach. Though application of *N*-Cbz-protected alcohol **2** in synthesis of **7** required additional deprotection–derivatization steps, use of this glycosyl acceptor[47] is preferable compared to that of potentially labile bromoacetamide **3**.

Thiolated cyclodextrins can be solubilized at high pH in water,[41] and water was initially considered as a solvent for carrying out conjugation of unprotected glycosides **7** and **10** with TCDs **1a** and **1b**. However, the bromoacetamido group was not sufficiently stable to highly basic aqueous



Scheme 1. Synthesis of spacer-armed glycoside derivatives **7** and **10**. a) BzlOCOCl, Na_2CO_3, H_2O, 90%; b) 1. $BrCH_2COBr$, CH_2Cl_2, Et_3N, –50 °C; 2. Et_3N, MeOH, 44%; c) I_2, CH_2Cl_2, 72% for **5**, 65% for **6**, 63% for **9**; d) 1. H_2-Pd/C, EtOH, EtOAc; 2. $BrCH_2COBr$, CH_2Cl_2, Et_3N, –50 °C, 72%; e) NaOMe, MeOH, 90% for **7**, 94% for **10**.

media and attempted reactions of **7** with **1b** using such conditions gave very poor results. Thus, conjugation of TCDs **1a** and **1b** was accomplished with excess of bromoacetylated spacer-armed mannopyranoside **7** using conditions of thioether linkage formation[48] involving reaction in a degassed 9:1 DMF-water mixture in the presence of K_2CO_3 (Scheme 2). The reaction mixtures were acidified after stirring for 18 h at room temperature, and high molecular weight products **11** and **12**, which were freely soluble in water, were separated from low molecular weight substances using size exclusion chromatography with 0.1% aq. CF_3CO_2H as a mobile phase (Figure 3).



Scheme 2. a) DMF/H_2O, 2:1, K_2CO_3, 64% for **11**, 69% for **12**, 57% for **13** and 75% for **14**.

As a result of the increased molecular sizes of compounds **11** and **12** in comparison to starting TCDs, the elution time for **11** and **12** was less than the elution time for a sample of the β-cyclodextrin standard. Presence of mannopyranoside residues in molecules of **11** and **12** was evident from their ^{13}C NMR spectroscopic data recorded in D_2O. Although not all resonances can be assigned in these spectra, signals belonging to mannopyranoside and linker portions of the molecules can be identified by comparison with precursor **7** and alcohol **3** (Figure 4).

The cyclodextrin parts of **11** and **12** showed relatively week resonances in ^{13}C NMR spectra (cf. ref.[37a]), apparently as a result of restricted mobility of the core region of these molecules. ^1H NMR spectra of **11** and **12** showed clearly only the signal corresponding to methylene protons of the $CH_2CH_2CH_2$ group ($\delta \approx 1.6$ ppm), while all other resonances were considerably broadened and overlapping. The most important information about the structure of synthesized cluster glycosides was obtained using MALDI-TOF mass spectrometry which confirmed successful per-substitution of TCDs with spacer-armed mannopyranosyl residues. Mass spectra of cluster glycoside **11** revealed the presence of only one molecular ion, observed as a sodium

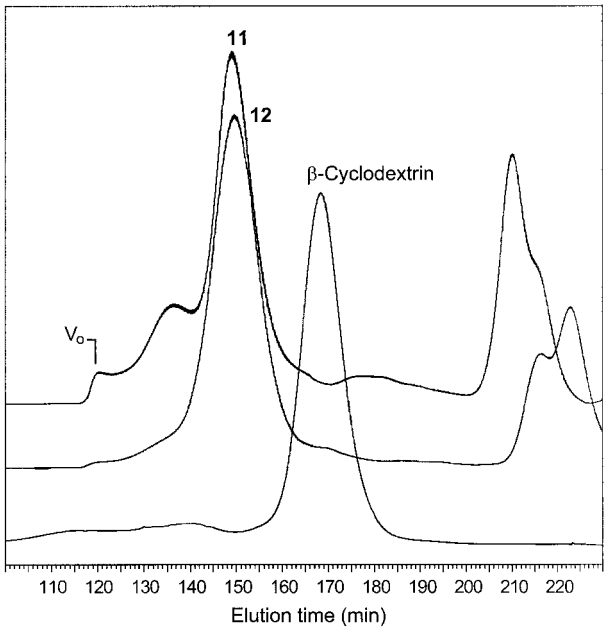


Figure 3. Elution profiles of products from conjugation reactions between TCD **1a** and **7** (top), **1b** and **7** (middle) obtained on Toyopearl HW-40S column (1.5 × 85 cm) using a differential refractometer as a detector. Products **11** and **12** were collected between 140 and 160 min. Low molecular weight products were eluted after 200 min, elution time of the void volume was 120 min. For comparison, the elution profile of β-cyclodextrin obtained in the same column (peak maximum at 170 min) is shown.

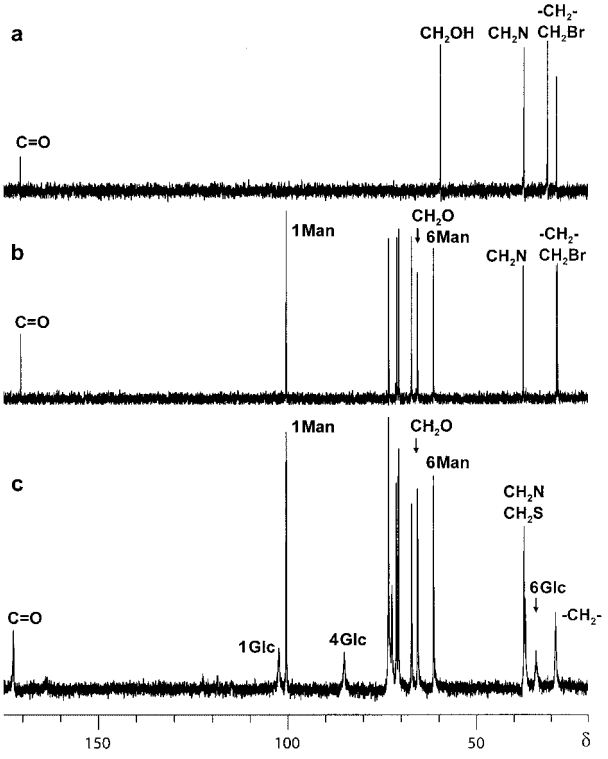
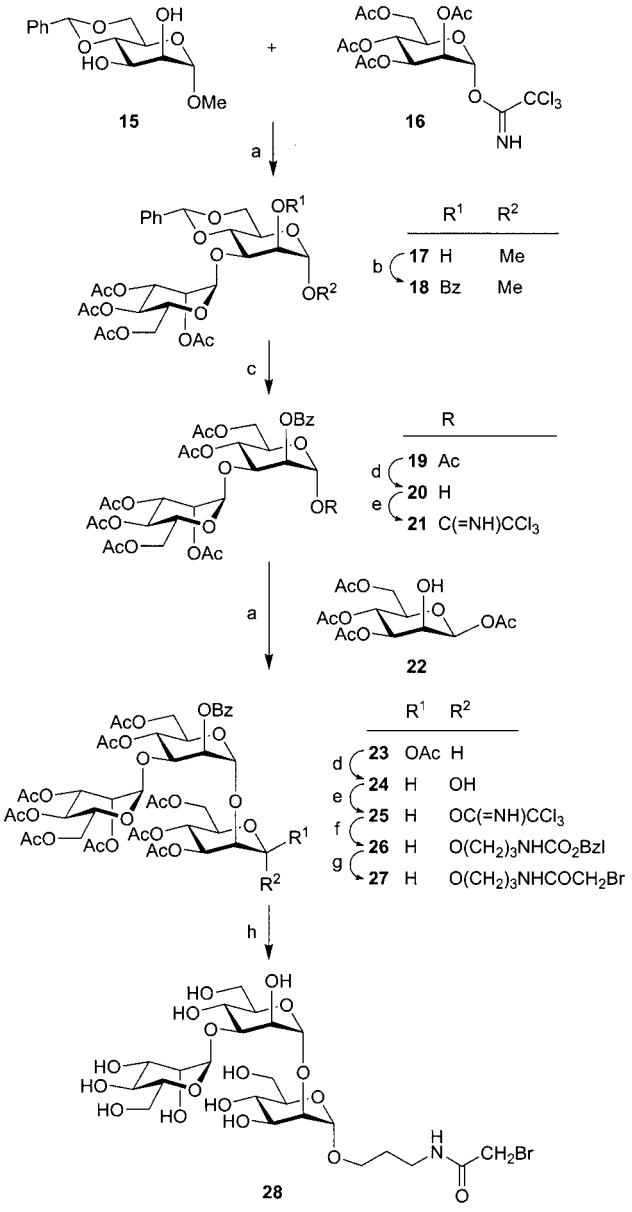


Figure 4. ^{13}C NMR spectra of 2-bromo-*N*-(3-hydroxypropyl)acetamide (**3**) (**a**), mannopyranoside **7** (**b**), and cluster mannopyranoside **12** (**c**) in D_2O.

adduct $[M + Na]^+$ (major peak m/z = 2754.9). Similarly, heptavalent cluster glycoside **12** gave a potassium adduct of the molecular ion $[M + K]^+$ (major peak m/z = 3227.1). In both cases, calculated isotope distributions for these peaks were a perfect match with recorded spectra. Conjugation of the bromoacetamido derivative of glucopyranose **10** with TCDs **1a** and **1b** gave results similar to those obtained for mannopyranose ligand **9**. Hexa- and heptavalent glucopyranoside–CD glycoclusters **12** and **13** (Scheme 2) were isolated by size exclusion chromatography in 57 and 75% yield, respectively, and their structures were in agreement with NMR spectroscopic data and results of MALDI-TOF mass spectrometry analyses. Thus, nucleophilic displacement can serve as a suitable procedure for the formation of thioether linkages between C-6 of TCDs and multiple unprotected carbohydrates equipped with a bromoacetylated spacer-arm.

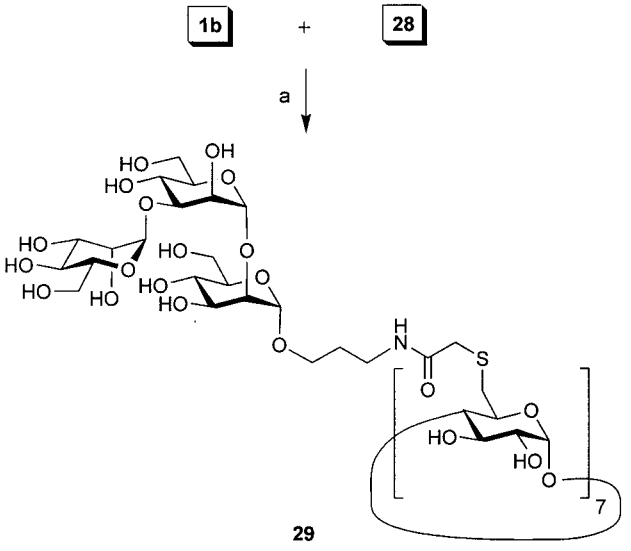
Practical Synthesis of a Spacer-Armed αMan(1→3)αMan-(1→2)αMan Trisaccharide: To create three α-mannopyranosidic bonds required for the construction of a mannotrioside bearing a spacer-arm, we applied the same trichloroacetimidate glycosylation strategy[40] repetitively starting from the "non-reducing end" of the oligosaccharide (Scheme 3). Thus, readily available diol **15**[49] was regioselectively 3-*O*-α-mannosylated with trichloroacetimidate **16** in the presence of $BF_3{\cdot}OEt_2$ to give 2-OH derivative **17** in 88% yield. High regioselectivity had also been observed earlier[50] in the reaction of the acceptor **15** with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide, though the reported efficiency of that glycosylation was much lower (18% yield of the disaccharide). Benzoylation of **17** produced 2-*O*-benzoyl derivative **18** (95%) which served as a starting material in a three-step preparation of the glycosyl donor **21**. The reaction steps included acetolysis, selective deprotection of the anomeric acetate and treatment with Cl_3CCN/DBU. Acetolysis of **18** using 1% H_2SO_4 in Ac_2O/AcOH mixture led to the replacement of both glycosidic methyl group and 4,6-*O*-benzylidene group with acetyl groups, affording derivative **19** in 78% yield. Upon treatment with $NH_2NH_2{\cdot}HOAc$ in DMF, 1-*O*-acetyl derivative **19** was transformed into the hemiacetal **20** in 93% yield. Reaction of **20** with Cl_3CCN/DBU gave trichloroacetimidate **21** (79%) which was used in $BF_3{\cdot}OEt_2$-catalysed coupling with known[51] tetraacetate **22**. The latter reaction furnished the desired trisaccharide **23** in 55% yield. Replacement of the 1-*O*-acetyl group in **23** with 1-*O*-trichloroacetimidoyl group was performed using the same reagents as in the transformation of 1-*O*-acetyl derivative **19** into trichloroacetimidate **21** to afford, via hemiacetal **24**, the trisaccharide donor **25** in 68% overall yield. Glycosylation of *N*-benzyloxycarbonyl-protected aglycon **2** with **25** in the presence of TMSOTf gave the mannotrioside **26** in 84% yield. Hydrogenolytic cleavage of the *N*-benzyloxycarbonyl group in **26** led to the liberation of an amino group which was immediately bromoacetylated with $BrCH_2COBr/Et_3N$ at –35 °C to afford fully protected trisaccharide **27** in 75% yield. Deesterification of **27** with 0.01 M NaOMe in MeOH furnished the target trisaccharide **28** in 87% yield.



Scheme 3. Synthesis of spacer-armed manotrioside derivative **28**. a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 83% for **17**, 55% for **23**; b) BzCl, C_5H_5N, CH_2Cl_2, 88% over two steps (a and b); c) 1% H_2SO_4, Ac_2O/AcOH, 78%; d) $NH_2NH_2{\cdot}HOAc$, DMF, 93% for **20**, 85% for **24**; e) Cl_3CCN, DBU, CH_2Cl_2, 79% for **21**, 89% for **25**; f) **2**, TMSOTf, CH_2Cl_2, 91%; g) 1. H_2-Pd/C, EtOH/EtOAc; 2. $BrCH_2COBr$, CH_2Cl_2, Et_3N, –50 °C, 75%; h) 0.01 M NaOMe, MeOH, 87%.

Conjugation of mannotrioside **28** with TCD **1b** (Scheme 4) was carried out under conditions developed for the conjugation of simpler saccharides **7** and **10** with TCDs using slightly less molar excess of the bromoacetamide **28** (1.1 equiv.) compared to bromoacetamides **7** and **10** (1.4 equiv.). The product was freely soluble in water at neutral pH and was purified using size-exclusion chromatography to give cyclodextrin glycocluster **29** (Figure 4). The ^{13}C NMR spectrum of **29** clearly showed resonances of the appended spacer-armed mannotrioside residues which practically coincided with those in the non-conjugated derivative **28**, though the signals in the spectrum of **29** are

noticeably broadened and vary greatly in intensities. The central TCD core can be identified by the presence of clearly visible, but weak, signals at δ = 85.1 and δ = 34.1 ppm which correspond to C-1 and C-6 of glucopyranosyl residues, respectively. Other TCD resonances are hidden by overlapping signals of mannopyranosyl residues. Heptavalency of the synthetic glycocluster followed unequivocally from the presence of a strong peak at *m*/*z* = 5496.6, corresponding to the potassium adduct of the molecular ion of compound **29** in its MALDI-TOF mass spectrum obtained with KOAc as an additive.



Scheme 4. a) K_2CO_3, DMF/H_2O, 9:1, 54%.

Conclusions

We have successfully demonstrated that readily available per-6-thio-cyclodextrins (TCDs) can be used as convenient scaffolds for attachment of spacer-armed glycosides and creating multivalent saccharide structures. An electrophilic *N*-bromoacetyl group, which was employed in the formation of thioether linkages, was introduced into glycoside ligands at a late stage of the glycoside construction or as an *N*-protecting functionality for aglycon in the process of glycoside preparation. Conjugation of spacer-armed glycosides to TCD scaffolds was carried out in aqueous DMF using highly efficient nucleophilic displacement of bromide with thiolated cyclodextrins. Both TCD scaffolds and saccharide ligands were used unprotected thus facilitatating isolation of target cyclodextrin glycoclusters by application of size exclusion chromatography directly to the mixtures of reaction products. The methodology was successfully applied for the synthesis of a heptavalent cyclodextrin cluster bearing mannotrioside ligands representing side chains of antigenic yeast mannans.

Experimental Section

General Methods: Reactions were carried out in dry solvents using septa and syringes for addition of reagents. Anhydrous DMF was purchased from Aldrich, CH_2Cl_2 and MeCN were distilled from CaH_2, MeOH was distilled from $Mg(OMe)_2$, pyridine was distilled from P_2O_5 and stored over 4 Å molecular sieves. Cation-exchange resins were washed with water and dry MeOH before use. TLC was performed on precoated aluminium plates (Silica Gel 60 F_{254}, Merck). Spots were visualized by exposure to UV light or by immersion into 5% ethanolic H_2SO_4 followed by heating to 150 °C. Solutions of reaction products were dried with $MgSO_4$ and solvents were evaporated under reduced pressure at 25–40 °C. Column chromatography was performed on silica gel (40–70 μm, BDH-Merck). Optical rotations were measured at 25 °C using a Perkin–Elmer 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 24 °C with a Varian Gemini 2000 spectrometer at 300 and 75 MHz, respectively, or with a Varian Unity Plus spectrometer at 400 and 100 MHz, respectively, using TMS (for solution in $CDCl_3$) or MeOH (δ = 49.9 ppm, for solutions in D_2O) as internal standards. Resonance assignments were made with the aid of gCOSY and gHSQC experiments. In cases where spectral dispersion was poor, only selected diagnostic NMR spectroscopic data are given; other spectral features were in agreement with the proposed structures. Accurate electrospray ionisation mass spectra (HR ESI-MS) were obtained using positive ionization mode on a Finnigan MAT 900 XLT mass spectrometer. For compounds with molecular masses over 1000, low resolution ESI-MS or LSIMS were obtained with the same instrument or low resolution MALDI-TOF MS were obtained with an Applied Biosystems Voyager mass spectrometer and experimental data were matched to theoretical isotope patterns.

2-Bromo-*N*-(3-hydroxypropyl)acetamide (3): To a stirred solution of 3-aminopropan-1-ol (3.8 mL, 50 mmol) in CH_2Cl_2 (40 mL) at –50 °C a solution of bromoacetyl bromide (8.7 mL, 100 mmol) in CH_2Cl_2 (40 mL) and a solution of Et_3N (13.9 mL, 100 mmol) in CH_2Cl_2 (40 mL) were slowly and simultaneously added from two separate syringes. After completion of the addition, the reaction mixture was poured onto iced water, the organic phase was separated, washed with diluted aq. $NaHCO_3$ and concentrated to give a brown syrup. This residue was treated with Et_3N (5 mL) in MeOH (40 mL) for 1 h, concentrated and purified by chromatography (toluene/EtOAc, 1:9) to give bromoacetamide **3** (6.6 g, 67%) as a colourless oil. R_f = 0.22 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.74 (m, 2 H, $CH_2CH_2CH_2$), 2.85 (broad s, 1 H, OH), 3.44 (m, 2 H, CH_2N), 3.66 (m, 2 H CH_2OH), 3.87 (m, 2 H, CH_2Br), 3.66 (dd, *J* = 6.0 Hz, 1 H, OH), 7.11 (broad s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.8 (CH_2Br), 31.4 ($CH_2CH_2CH_2$), 37.4 (CH_2N), 59.7 (CH_2OH), 167.3 (C=O) ppm.

3-[(Benzyloxycarbonyl)amino]propyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranoside (5): A mixture of the mannopyranosyl bromide **4** (2.17 g, 3.30 mmol) and the alcohol **2**[45] (690 mg, 3.30 mmol) in toluene was concentrated and dried in vacuo. The residue was dissolved in MeCN (30 mL) and iodine (1.15 g, 4.50 mmol) was added. After 3 h at room temperature the reaction mixture was quenched with 10% aq. $Na_2S_2O_3$, diluted with CH_2Cl_2 (60 mL) and washed with saturated aq. $NaHCO_3$. The organic layer was concentrated and the residue was purified by chromatography (toluene/EtOAc, 95:5) to give the title compound **5** (1.87 g, 72%). R_f = 0.56 (toluene/EtOAc, 9:1). $[\alpha]_D$ = –44.5 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.94 (m, 2 H, $CH_2CH_2CH_2$), 3.49 (m, 2 H, CH_2N), 3.63 (m, 1 H, OC*H*aHbCH$_2$), 3.92 (m, 1 H, OCHa*H*bCH$_2$), 4.68 (dd, *J* = 2.5, *J* = 12.0, 1 H, H-6b), 4.99 (dd, *J* = 4.4, *J* = 12.0, 1 H, H-6a), 4.95 (m, 1 H, H-5), 5.03 (broad t, 1 H, NH), 5.08 (s, 1 H, CH_2Bn), 5.12 (s, 1 H, H-1), 5.70, (m, 1 H, H-2), 5.89 (dd, *J* = 3.3, *J* = 9.9 Hz, 1 H, H-3), 6.10 (t, *J* = 9.9 Hz, 1 H, H-4) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 29.7 ($CH_2CH_2CH_2$), 38.3 (CH_2N), 63.0 (C-6), 66.2 (OCH_2CH_2), 66.7

(CH_2Ph), 67.0 (C-4), 68.9 (C-5), 70.1 (C-3), 70.5 (C-2), 97.9 (C-1), 156.7 (NHC=O), 165.6, 165.7, 166.4 (C=O) ppm. HR ESI-MS: calcd. for $C_{39}H_{40}BrN_2O_{11}$ ($[M + H]^+$): m/z = 788.2702, found: m/z = 788.2704.

3-(2-Bromoacetamido)propyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranoside (6). Method A: A mixture of *N*-benzyloxycarbonyl derivative **5** (1.15 g, 1.46 mmol) and 10% Pd/C in EtOAc/AcOH mixture (50 mL, 9:1) was hydrogenated for 18 h, the catalyst was filtered off and the solution was concentrated. The residue showed a single spot on TLC with R_f = 0.72 (toluene/MeOH, 9:1) visualised by ninhydrin spray. The product was dissolved in CH_2Cl_2 (10 mL), Et_3N (0.23 mL, 1.70 mmol) was added, the solution was cooled to –35 °C and a solution of $BrCH_2COBr$ (0.15 mL, 1.70 mmol) in CH_2Cl_2 (10 mL) was slowly added at this temperature. The mixture was diluted with CH_2Cl_2 (30 mL), washed with saturated aq. $NaHCO_3$ (1 × 20 mL), concentrated and the residue was purified by chromatography (toluene/EtOAc, 4:1) to give the bromoacetamide **6** (815 mg, 72%). R_f = 0.47 (toluene/EtOAc, 3:2). $[\alpha]_D$ = –46 (c = 0.9, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.97 (m, 2 H, $CH_2CH_2CH_2$), 3.51 (m, 2 H, CH_2N), 3.66 (m, 1 H, OCHaHbCH$_2$), 3.92 (s, 2 H, CH_2Br), 3.98 (m, 2 H, OCHaHbCH$_2$), 4.46–4.58 (m, 2 H, H-5, H-6a), 4.77 (broad d, $J_{6A,6B}$ = 12 Hz, 1 H, H-6b), 5.13 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H-1), 5.79 (dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 3.2 Hz, 1 H, H-2), 5.94 ($J_{2,3}$ = 3.2, $J_{3,4}$ = 10.1 Hz, 1 H, H-3), 6.16 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10 Hz, 1 H, H-4), 6.96 (m, 1 H, NH), 7.2–8.1 (m, 20 H, Bz) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 28.9 ($CH_2CH_2CH_2$), 29.2 (CH_2Br), 38.0 (CH_2N), 62.9 (C-6), 66.8 (OCH_2CH_2), 66.9 (C-4), 69.0 (C-5), 70.2 (C-3), 70.45 (C-2), 97.9 (C-1), 165.6, 165.7, 165.8, 166.2, 166.3 (C=O) ppm. HR ESI-MS: calcd. for $C_{39}H_{40}BrN_2O_{11}$ ($[M + NH_4]^+$): m/z = 791.1810, found: m/z = 791.1809.

Method B: A mixture of mannopyranosyl bromide **4** (530 mg, 0.80 mmol) and alcohol **3** (340 mg, 1.75 mmol) in toluene was concentrated and dried in vacuo, re-dissolved in MeCN (6 mL) and then iodine (0.54 g, 2.12 mmol) was added. After 3 h at room temperature the reaction was quenched with 10% aq. $Na_2S_2O_3$, diluted with CH_2Cl_2 (20 mL) and washed with saturated aq. $NaHCO_3$. The organic layer was dried and concentrated and the residue was purified by flash chromatography (toluene/EtOAc, 95:5) to give compound **6** (320 mg, 52%).

3-(2-Bromoacetamido)propyl α-D-Mannopyranoside (7): A solution of the tetrabenzoate **6** (1.16 g mg, 1.50 mmol) in MeOH (30 mL) was treated with 1 M NaOMe in MeOH (1 mL) and the solution was kept for 2 h at 20 °C. The mixture was neutralised with Amberlite IRA-120 (H^+), the resin was filtered off and washed with MeOH and the filtrate was concentrated to afford the tetraol **7** (510 mg, 95%). R_f = 0.49 (CH_2Cl_2/MeOH, 4:1). $[\alpha]_D$ = +42 (c = 1.05, H_2O). ^1H NMR (400 MHz, $CDCl_3$): 1.66 (m, 2 H, $CH_2CH_2CH_2$), 3.10–3.20 (m, 2 H, CH_2N), 3.32–3.40 (m, 1 H, OCHaHbCH$_2$), 3.40–3.82 (7 H, H Man and OCHaHbCH$_2$), 4.66 (broad s, 1 H, H-1 Man) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.2 ($CH_2CH_2CH_2$), 28.5 (CH_2Br), 37.5 (CH_2N), 61.3 (C-6), 65.5 (OCH_2CH_2), 67.2, 70.5, 71.0, 73.1 (C-2–C-5), 100.3 (C-1), 170.4 (C=O) ppm. HR ESI-MS: calcd. for $C_{11}H_{24}BrN_2O_7$ ($[M + NH_4]^+$): m/z = 375.0761, found: m/z = 375.0760.

3-(2-Bromoacetamido)propyl 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranoside (9): A solution of the glucopyranosyl bromide **8** (2.07 g, 3.15 mmol) and the alcohol **3** (1.46 g, 7.49 mmol) in toluene was concentrated and dried in vacuo, re-dissolved in MeCN (25 mL) and iodine (2.36 g, 9.29 mmol) was added. After 3 h at room temperature the reaction was quenched with $Na_2S_2O_3$, diluted with CH_2Cl_2 (20 mL) and washed with saturated aq. $NaHCO_3$. The organic layer was dried and concentrated and the residue was purified by flash chromatography (toluene/EtOAc, 9:1→7:3) to give compound **9** (1.54 g, 63%). R_f = 0.39 (toluene/EtOAc, 7:3). $[\alpha]_D$ = +6 (c = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.78 (m, 2 H, $CH_2CH_2CH_2$), 3.30 (m, 2 H, CH_2N), 3.64 (m, 1 H, s, OCHaHbCH$_2$), 3.92 (AB system, 2 H, CH_2Br), 4.02 (m, 2 H, OCHaHbCH$_2$), 4.18 (m, 1 H, H-5), 4.49 (dd, $J_{5,6a}$ = 3, $J_{6a,6b}$ = 12.0 Hz, 1 H, H-6a), 4.74 (dd, $J_{5,6b}$ = 1.5, $J_{6a,6b}$ = 12.0 Hz, 1 H, H-6b), 4.84 (d, $J_{1,2}$ = 9.0 Hz, 1 H, H-1), 5.51 (dd, $J_{1,2} \approx J_{2,3} \approx$ 10 Hz, 1 H, H-2), 5.71 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10 Hz, 1 H, H-4), 5.96 (dd, $J_{2,3} \approx J_{3,4}$ = 10 Hz, 1 H, H-2), 6.91 (m, 1 H, NH), 7.26–8.10 (m, 20 H, Bz) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 28.9 ($CH_2CH_2CH_2$), 29.2 (CH_2Br), 38.2 (CH_2N), 63.0 (C-6), 68.8 (OCH_2CH_2), 69.7 (C-4), 72.2 (C-2), 72.7, 72.9 (C-3, C-5), 101.5 (C-1), 165.4, 165.7, 166.0, 166.2, 166.4 (C=O) ppm. HR ESI-MS: calcd. for $C_{39}H_{40}BrN_2O_{11}$ ($[M + NH_4]^+$): m/z = 774.1545, found: m/z = 774.1538.

3-(2-Bromoacetamido)propyl β-D-Glucopyranoside (10): A solution of the tetrabenzoate **9** (1.06 g, 1.37 mmol) in MeOH (20 mL) was treated with 1 M NaOMe in MeOH (2.2 mL) and kept for 2 h at 20 °C. The mixture was neutralized with Amberlite IRA-120 (H^+), the resin was filtered off and washed with MeOH and the filtrate was concentrated to afford the tetraol **10** (460 mg, 94%). R_f = 0.47 (CH_2Cl_2/MeOH, 4:1). $[\alpha]_D$ = –19 (c = 1.1, H_2O). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.65 (m, 2 H, $CH_2CH_2CH_2$), 3.07 (dd, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.4 Hz, 1 H, H-2), 3.12–3.22 (m, 3 H, H-4, CH_2N), 3.22–3.29 (m, 1 H, H-5) 3.29 (dd, $J_{2,3} \approx J_{3,4} \approx$ 9 Hz, 1 H, H-3), 3.49–3.56 (m, 2 H, H-6a, OCHaHbCH$_2$), 3.70 (s, 2 H, CH_2Br), 3.74–3.81 (m, 2 H, H-6b, OCHaHbCH$_2$), 4.26 (d, 1 H, H-1) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.2 ($CH_2CH_2CH_2$), 28.5 (CH_2Br), 37.35 (CH_2N), 61.2 (C-6), 68.2 (OCH_2CH_2), 70.2 (C-4), 73.6 (C-2), 76.2, 76.4 (C-3, C-5), 102.8 (C-1), 170.6 (C=O) ppm. HR ESI-MS: calcd. for $C_{11}H_{24}BrN_2O_7$ ($[M + NH_4]^+$): m/z = 375.0761, found: m/z = 375.0764.

General Procedure for the Synthesis of Cyclodextrin Glycoclusters 11–14: A degassed aq. 1 M K_2CO_3 solution (0.3 mL) was added to a degassed solution of per-6-thio-cyclodextrin (21.4 mg of **1a** or 25.0 mg of **1b**, 20 μmol) and compound **7** or compound **10** (71.4 mg, 200 μmol) in DMF (2.7 mL) and the mixture was stirred for 20 h at room temperature under N_2. Aqueous 1 M AcOH was added to adjust pH of the reaction mixture to 7 (paper indicator) and solvents were evaporated in vacuo. Purification of the residue using gel permeation chromatography on Toyopearl HW-40S column (1.5 × 85 cm) with 0.1% aq. CF_3CO_2H as a mobile phase furnished target glycoclusters.

Compound 11: Yield 35 mg, 64%. $[\alpha]_D$ = +42 (c = 1.0, H_2O). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 28.8 ($CH_2CH_2CH_2$), 33.8 (C-6), 37.1, 37.4 (CH_2N, CH_2S), 61.4 (C-6′), 65.7 (OCH_2CH_2), 67.0, 70.6, 71.2, 73.3 (C-2′–C-5′), 85.3 (C-4), 100.4 (C-1′), 102.4 (C-1), 172.6 (C=O) ppm. MALDI-TOF MS: m/z = 2754.9 $[M + Na]^+$. $C_{102}H_{174}N_6O_{66}S_6Na$ (2754.9).

Compound 12: Yield 44 mg, 69%. $[\alpha]_D$ = +57 (c = 1.3, H_2O). ^{13}C NMR (400 MHz, $CDCl_3$): δ = ^{13}C NMR (400 MHz, $CDCl_3$): δ = 28.8 ($CH_2CH_2CH_2$), 34.1 (C-6), 36.9, 37.3 (CH_2N, CH_2S), 61.3 (C-6′), 65.6 (OCH_2CH_2), 67.2, 70.6, 72,4, 73.2 (C-2′–C-5′), 84.9 (C-4), 100.4 (C-1′), 102.4 (C-1), 172.3 (C=O) ppm. MALDI-TOF MS: m/z = 3227.1 $[M + K]^+$. $C_{119}H_{203}N_7O_{77}S_7K$ (3227.0).

Compound 13: Yield 31 mg, 57%. $[\alpha]_D$ = +11 (c = 1.2, H_2O). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 28.9 ($CH_2CH_2CH_2$), 33.8 (C-6), 37.2 (× 2, CH_2N, CH_2S), 61.2 (C-6′), 68.2 (OCH_2CH_2), 70.1 (C-4′), 73.5 (C-2′), 76.2, 76.4 (C-3′, C-5′), 85.1 (C-4), 102.8 (C-1′), 172.6 (C=O) ppm. MALDI-TOF MS: m/z = 2754.7 $[M + Na]^+$. $C_{102}H_{174}N_6O_{66}S_6Na$ (2754.9).

Compound 14: Yield 48 mg, 75%. $[\alpha]_D$ = +17 (*c* = 1.0, H_2O). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 29.1 ($CH_2CH_2CH_2$), 33.96 (C-6), 36.8, 37.2 (CH_2N, CH_2S), 61.2 (C-6′), 68.2 (OCH_2CH_2), 70.1 (C-4′), 72.3, 72.7, 73,2 (C-2, C-3, C-5), 73.6 (C-2′), 76.2, 76.4 (C-3′, C-5′), 84.9 (C-4), 102.1 (C-1), 102.8 (C-1′), 172.6 (C=O) ppm. MALDI-TOF MS: *m*/*z* = 3226.7 $[M + K]^+$. $C_{119}H_{203}N_7O_{77}S_7K$ (3227.0).

Methyl 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-4,6-*O*-benzylidene-α-D-mannopyranoside (17): A mixture of the diol **15** (250 mg, 0.50 mmol), trichloroacetimidate **16** (140 mg, 0.50 mmol) and mol. sieves (4 Å, 0.5 g) in CH_2Cl_2 (5 mL) was stirred for 30 min and cooled to –10 °C. To this mixture a solution of $BF_3{\cdot}OEt_2$ (0.012 mL, 0.1 mmol) in CH_2Cl_2 (5 mL) was slowly added, stirring continued for 30 min at 0 °C and then Et_3N (0.1 mL) was added. The mixture was diluted with CH_2Cl_2, washed with saturated aq. $NaHCO_3$, dried and concentrated. Chromatography of the residue (toluene/EtOAc, 1:1→1:2) afforded the disaccharide **17** (300 mg,83%). R_f = 0.6 (toluene/EtOAc, 1:3). $[\alpha]_D$ = +61 (*c* = 1.15, $CHCl_3$). Ref.[50] $[\alpha]_D$ = +67. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.01, 2.08, 2.11, 2.12 (4 s, 12 H, Ac), 2.69 (broad s, 1 H, OH), 3.42 (s, 3 H, Me), 3.85 (m, 1 H, H-5), 3.90 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10 Hz, 1 H, H-6a), 4.07–4.13 (m, 2 H, H-2, H-5′), 4.13–4.23 (m, 3 H, H-3, H-4, H-6′a,), 4.27 (dd, $J_{5,6b}$ = 5.8, $J_{6a,6b}$ = 12.0 Hz, 1 H, H-6′b), 4.32 (dd, $J_{5,6b}$ = 4, $J_{6a,6b}$ = 9.5 Hz, 1 H, H-6b), 4.80 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1), 5.25 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10 Hz, 1 H, H-4′), 5.30 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1′), 5.37 (dd, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.8 Hz, 1 H, H-3′), 5.42 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.5 Hz, 1 H, H-2′), 5.62 (s, 1 H, PhC*H*), 7.33–7.38 (m, 3 H, Ph), 7.44–6.47 (m, 2 H, Ph) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 21.0, 21.1 (×2), 21.2 (*C*H_3CO), 55.3 (OMe), 63.0 (C-6′), 63.9 (C-5), 66.7 (C-4′), 68.7 (C-6), 69.3, 69.4, 69.6 (C-2, C-2′, C-3′), 70.8 (C-5′), 73.9, 75.9 (C-3, C-4), 98.7 (C-1′), 101.7 (×2, C-1, *C*HPh), 126.4, 128.5, 129.2 (Ph), 170.1, 170.6 (×2), 171.2 (CH_3CO) ppm. HR ESI-MS: calcd. for $C_{28}H_{36}O_{15}$ $([M + H]^+)$: *m*/*z* = 613.2127, found: *m*/*z* = 613.2132.

Methyl 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (18): A solution of $BF_3{\cdot}OEt_2$ (0.6 mL, 4.8 mmol) in CH_2Cl_2 (10 mL) was added to a mixture of the diol **15** (5.60 g, 20.3 mmol), trichloroacetimidate **16** (10,0 g mg, 20.3 mmol) and mol. sieves 4 Å (19 g) in CH_2Cl_2 (75 mL) at –15 °C and the reaction mixture was stirred for 30 min at 0 °C before being treated with Et_3N (2.0 mL). The reaction mixture was diluted with CH_2Cl_2 (100 mL), filtered, and the filtrate was washed with saturated aq. $NaHCO_3$, dried and concentrated. After drying in vacuo the residue was dissolved in pyridine (100 mL), DMAP (100 mg) and BzCl (2.32 mL, 20.0 mmol) were added and the mixture was kept for 18 h at 20 °C. After careful treatment with MeOH (1.0 mL) the solution was diluted with CH_2Cl_2 (200 mL), washed successively with saturated aq. $NaHCO_3$ (2 × 100 mL) and water (100 mL), dried and concentrated. The residue was purified by column chromatography (toluene/EtOAc, 20:1→4:1) to give **18** (12.8 g, 88% over two steps). R_f = 0.28 (toluene/EtOAc, 3:1). $[\alpha]_D$ = –7 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.83, 1.93, 1.98, 2.04 (4 s, 12 H, Ac), 3.34 (s, 3 H, Me), 3.78–3.87 (m, 2 H, H-5, H-6a), 4.02 (dd, $J_{5,6a}$ = 2, $J_{6a,6b}$ = 11.8 Hz, 1 H, H-6′a), 4.05–4.13 (m, 2 H, H-4, H-5′), 4.18 (dd, $J_{5,6b}$ = 5.8, $J_{6a,6b}$ = 11.8 Hz, 1 H, H-6′b), 4.24 (m, 1 H, H-6b), 4.33 (dd, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 4.78 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1), 5.08–5.17 (m, 3 H, H-1′, H-3′, H-4′), 5.23 (dd, $J_{1,2}$ = 2, $J_{2,3}$ = 2.5 Hz 1 H, H-2′), 5.40 (dd, $J_{1,2}$ = 1.5, $J_{2,3}$ = 3.5 Hz, 1 H, H-2), 5.57 (s, 1 H, PhC*H*), 7.00–7.60 (m, 8 H, Ph), 8.05 (m, 2 H, Ph) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 21.0 (×2), 21.1 (×2) (CH_3CO), 55.45 (OMe), 62.9 (C-6′), 63.9 (C-5), 66.4 (C-4′), 69.1, 69.2, (C-6, C-3′), 69.6 (C-2′, C-5′), 72.2, 72.3 (C-2, C-3), 79.4 (C-4), 99.1 (C-1′), 100.0 (C-1), 101.8 (*C*HPh), 125.7–129.2 (Ph), 166.4, 169.9, 170.1, 170.2, 171.0 (C=O) ppm. HR ESI-MS: calcd. for $C_{35}H_{40}O_{16}$ $([M + NH_4]^+)$: *m*/*z* = 734.2655, found: *m*/*z* = 734.2653.

1,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-α-D-mannopyranoside (19): A solution of H_2SO_4 in Ac_2O (2% v/v, 40 mL) was slowly added to a stirred solution of mannobioside **18** (10.0 g, 14.0 mmol) in Ac_2O (40 mL) at –10 °C and stirring was continued for 4 h at room temperature. The mixture was poured into crushed ice (300 g), stirred for 6 h, extracted with CH_2Cl_2 (3 × 100 mL) and extracts were dried and concentrated. The product obtained after the residual Ac_2O was evaporated with toluene under reduced pressure was purified by flash chromatography (toluene/EtOAc, 1:1), to afford compound **19** (10.9 g, 78%). R_f = 0.26 (toluene/EtOAc, 1:1). $[\alpha]_D$ = +2.5 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.96, 1.97, 2.09, 2.12, 2.15, 2.19, 2.20 (7 s, 21 H, Ac), 3.78–3.87 (m, 2 H, H-5, H-6a), 3.98 (dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 12.5 Hz, 1 H, H-6′a), 4.01–4.08 (m, 2 H, H-5, H-5′), 4.10–4.18 (m, 2 H, H-6′b, H-6a), 4.24 (m, 1 H, H-6b), 4.26–4.31 (m, 2 H, H-3, H-6b), 5.07 (m, 2 H, H-1′, H-2′), 5.16 (dd, $J_{2,3}$ = 2.2, $J_{3,4}$ = 10.0 Hz, 1 H, H-3′), 5.25 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10.0 Hz, 1 H, H-4′), 5.45 (dd, $J_{1,2}$ = 1.5, $J_{2,3}$ = 3.3 Hz, 1 H, H-2), 5.58 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10.0 Hz, 1 H, H-4), 6.28 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1), 7.50 (m, 1 H, Ph), 7.64 (m, 2 H, Ph), 8.12 (m, 2 H, Ph) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 21.0 (×4), 21.1, 21.2, 21.3 (*C*H_3CO), 62.5 (C-6, C-6′), 66.0 (C-4′), 67.0 (C-4), 68.8, (C-3′), 69.9, 70.1, 70.7, 71.1 (C-2, C-5, C-2′, C-5′), 76.5 (C-3), 90.8 (C-1), 99.6 (C-1′), 129.1, 129.4, 130.4, 134.3 (Ph), 165.8–171.1 (8 C, C=O) ppm. LSIMS: *m*/*z* = 681 $[M–OAc]^+$. $C_{33}H_{40}O_{19}$ (740.22): calcd. C 53.51; H 5.44; found C 53.77, H 5.48.

4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-α-D-mannopyranoside (20): A solution of the 1-*O*-acetyl derivative **19** (7.40 g, 10.0 mmol) and $NH_2NH_2{\cdot}HOAc$ (1.10 g, 11.0 mmol) in DMF (40 mL) was stirred for 4 h at room temperature. The solution was diluted with EtOAc (250 mL), washed with water (3 × 100 mL) and the organic layer was dried and concentrated. Column chromatography (toluene/EtOAc, 1:1) afforded hemiacetal **20** (6.50 g, 93%). R_f = 0.61 (toluene/EtOAc, 3:1). $[\alpha]_D$ = –21 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.95 (× 2), 2.09, 2.11, 2.14, 2.16, (6 s, 18 H, Ac), 3.93 (dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 11.9 Hz, 1 H, H-6′a), 4.02–4.27 (m, 4 H, H-5, H-6a, 6b, H-5′, H-6′b), 4.39 (dd, $J_{2,3}$ = 2.2, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 4.56 (broad s, 1 H, OH), 5.01–5.07 (m, 2 H, H-1′, H-2′), 5.17 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10.0 Hz, 1 H, H-3′), 5.20 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10.0 Hz, 1 H, H-4′), 5.41 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1), 5.45 (m, 1 H, H-2), 5.52 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H-4), 7.50 (m, 1 H, Ph), 7.64 (m, 2 H, Ph), 8.12 (m, 2 H, Ph) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 21.0 (×4), 21.15, 21.2 (*C*H_3CO), 62.6, 63.0 (C-6, C-6′), 66.2 (C-4′), 67.7 (C-4), 68.85, 69.9 (C-5, C-3′), 69.6 (C-5′), 70.2 (C-2), 75.8 (C-3), 92.3 (C-1), 99.3 (C-1′), 129.0, 129.6, 130.3, 134.0 (Ph), 166.2–171.1 (×7, C=O) ppm. HR ESI-MS: calcd. for $C_{31}H_{38}O_{18}$ $([M + H]^+)$: *m*/*z* = 716.2396, found: *m*/*z* = 716.2390.

4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-mannopyranoside (21): A solution of DBU (0.42 mL, 2.8 mmol) in CH_2Cl_2 (10 mL) was added to a solution of hemiacetal derivative **20** (6.50 g, 9.3 mmol) and Cl_3CCN (14.1 mL, 141 mmol) in CH_2Cl_2 (140 mL) at –5 °C and stirred for 1 h. The solution was concentrated at 25 °C to 20 mL volume and a crude product was immediately purified by flash chromatography (toluene/EtOAc, 9:1→7:3) to furnish imidate **21** (6.2 g, 79%). R_f = 0.49 (toluene/EtOAc, 1:1). $[\alpha]_D$ = +3 (*c* = 1.2, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.88, 189, 1.99, 2.02, 2.06, 2.10 (6 s, 18 H, Ac), 3.83 (dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 11.9 Hz, 1

H, H-6′a), 3.96 (m, 1 H, H-5′), 4.03–4.17 (m, 3 H, H-5, H-6a, H-6′b), 4.20 (dd, $J_{5,6a}$ = 3.5, $J_{6a,6b}$ = 12.0 Hz, 1 H, H-6b), 4.30 (dd, $J_{2,3}$ = 2.2, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 4.99–5.03 (m, 2 H, H-1′, H-2′), 5.08 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10.0 Hz, 1 H, H-3′), 5.18 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10.0 Hz, 1 H, H-4′), 5.41 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H-1), 5.53–5.59 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H-4), 7.50 (m, 1 H, Ph), 7.64 (m, 2 H, Ph), 8.12 (m, 2 H, Ph), 8.78 (s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 20.8, 20.9, 21.1 (×6, *C*H_3CO), 62.25 (C-6, C-6′), 65.8 (C-4′), 66.7 (C-4), 68.7, (C-3′), 69.8, 69.9 (C-2′, C-5′), 70.3 (C-2), 71.6 (C-5), 76.1 (C-3), 90.9 (C-1), 99.6 (C-1′), 129.0, 129.3, 130.3, 134.2 (Ph), 159.7–170.7 (×8, C=O) ppm. LSIMS: *m*/*z* = 684 [M + Na]$^+$, 681 [M–OC(NH)CCl_3]$^+$. $C_{33}H_{38}Cl_3NO_{18}$ (841.12).

1,3,4,6-Tetra-*O*-acetyl-2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-α-D-mannopyranosyl]-β-D-mannopyranose (23): A mixture of imidate **21** (5.38 g, 6.38 mmol), alcohol **22**[51] (2.44 g, 7.00 mmol) and powdered mol. sieves (4 Å, 6.5 g) in CH_2Cl_2 (60 mL) was stirred for 30 min under N_2 and cooled to 0 °C. To this mixture a solution of BF_3·OEt_2 (0.19 mL, 1.60 mmol) in CH_2Cl_2 (5 mL) was added via a syringe and, after stirring for 30 min at 0–10 °C, TLC indicated no presence of starting material. The reaction mixture was treated with Et_3N (0.5 mL) and filtered, the filtrate was diluted with CH_2Cl_2 (100 mL) and washed successively with saturated aq. $NaHCO_3$ and water. After removal of solvents in vacuo the residue was purified by column chromatography (toluene/EtOAc, 4:1→1:1) to afford the trisaccharide **23** (3.61 g, 55%). R_f = 0.45 (toluene/EtOAc, 2:3). $[\alpha]_D$ = –16 (*c* = 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.93, 1.98, 2.08, 2.09, 2.11, 2.12, 2.17, 2.19, 2.21, 2.22 (10 s, 30 H, CH_3CO), 3.84 (m, 1 H, H-5), 3.89 (dd, $J_{5,6a}$ = 2.3, $J_{6a,6b}$ = 12.5 Hz, 1 H, H-6″a), 3.97 (m, 1 H, H-5″), 4.07–4.19 (m, 3 H, H-6a, H-6′a, H-6″b), 4.22 (broad d, 1 H, H-2), 4.30 (dd, $J_{5,6a}$ = 5.5, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6b), 4.34 (dd, $J_{5,6a}$ = 4.2, $J_{6a,6b}$ = 11.3 Hz, 1 H, H-6″b), 4.41 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 10.0 Hz, 1 H, H-3′), 4.47 (m, 1 H, H-5′), 5.10 (broad s, 1 H, H-1″), 5.14–5.22 (m, 3 H, H-3, H-2″, H-3″), 5.24 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1′), 5.28 (dd, $J_{3,4} \approx J_{4,5} \approx$ 10.0 Hz, 1 H, H4″), 5.32 (dd, $J_{1,2} \approx J_{2,3} \approx$ 9.8 Hz, 1 H, H-4), 5.52 (broad d, 1 H, H-2′), 5.65 (dd, $J_{3,4} \approx J_{4,5}$ = 9.8 Hz, 1 H, H-4′), 5.82 (broad s, 1 H, H-1), 7.53 (m, 2 H, Ph), 7.65 (m, 1 H, Ph), 8.13 (m, 1 H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8–21.3 (*C*H_3CO), 60.7 (C-6″), 62.1 (C-6), 62.5 (C-6′), 65.7, 66.2, 66.8 (C-4, C-4′, C-4″), 69.0, 69.4, 69.7, 69.9 (C5′, C-2″, C-3″, C-5″), 71.7 (C-5), 72.5 (C-2′), 73.5 (C-3), 75 (C-2), 76.9 (C-3′), 91.4 (C-1), 98.4 (C-1′), 99.6 (C-1″), 129.0, 129.4, 130.2, 134.1 (Ph), 165.7–170.9 (×10, C=O) ppm. HR ESI-MS: calcd. for $C_{45}H_{56}O_{27}$ ([M + NH_4]$^+$): *m*/*z* = 1046.3347, found: *m*/*z* = 1046.3326.

3,4,6-Tetra-*O*-acetyl-2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-2-*O*-benzoyl-α-D-mannopyranose (24): A solution of the trisaccharide **23** (3.44 g, 3.34 mmol) and NH_2NH_2·HOAc (305 mg, 3.34 mmol) in DMF (30 mL) was stirred for 2 h at room temperature, diluted with EtOAc (200 mL), washed with water (3×75 mL) and the organic layer was dried and concentrated. Column chromatography (toluene/EtOAc, 1:1) afforded the hemiacetal **24** (2.80 g, 85%). R_f = 0.43 (toluene/EtOAc, 3:7). $[\alpha]_D$ = –10 (*c* = 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.93, 1.98, 2.08, 2.09, 2.11, 2.14, 2.17 (×2), 2.21, (9 s, 27 H, CH_3CO), 3.40 (broad s, 1 H, OH), 3.86 (dd, $J_{5,6a}$ = 2.3, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6″a), 3.96 (m, 1 H, H-5″), 4.07 (dd, $J_{5,6a}$ = 4.0, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6″b), 4.13 (m, 1 H, H-2), 4.14–4.27 (m, 6 H, H-5, H-6a, H-6b, H-5′, H-6′a, H-6′b), 4.32 (dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.8 Hz, 1 H, H-3′), 5.10 (d, $J_{1,2}$ = 2.0 Hz, 1 H, H-1″), 5.14–5.20 (m, 3 H, H-1′, H-2″, H-3″), 5.28 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H4″), 5.32 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.6 Hz, 1 H, H-4), 5.39 (dd, $J_{1,2}$ = 2.0, $J_{1,OH}$ = 4.3 Hz, 1 H, H-1), 5.43 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10.0 Hz, 1 H, H-3), 5.48 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.2 Hz, 1 H, H-2′), 5.55 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H-4′), 7.55 (m, 2 H, Ph), 7.65 (m, 1 H, Ph), 8.12 (m, 1 H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.0–21.2 (*C*H_3CO), 62.1 (C-6″), 62.8, 63.1 (C-6, C-6′), 65.8 (C-4″), 66.8 (C-4), 67.2 (C-4′), 68.7, 69.1, 69.7, 69.9 (×2) (C-5, C-5′, C-2″, C-3″, C5″), 70.3 (C-3), 71.8 (C-2′), 76.9 (C-3′), 78.0 (C-2), 93.2 (C-1), 99.2 (C-1′), 99.6 (C-1″), 129.1, 129.4, 130.3, 134.2 (Ph), 165.9–171.2 (C=O) ppm. HR ESI-MS: calcd. for $C_{43}H_{54}O_{26}$ ([M + NH]$^+$): *m*/*z* = 1004.3242, found: *m*/*z* = 1004.3247.

3,4,6-Tri-*O*-acetyl-2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-α-D-mannopyranosyl]-1-*O*-trichloroacetimidoyl-α-D-mannopyranose (25): A mixture of hemiacetal **24** (2.60 g, 2.63 mmol), Cl_3CCN (1.18 mL, 11.8 mmol) and powdered K_2CO_3 (1.18 g) in CH_2Cl_2 (25 mL) was stirred at room temperature for 2 h, diluted with CH_2Cl_2 (75 mL) and K_2CO_3 was then removed by filtration. The filtrate was concentrated and the residue was purified by short-column chromatography (toluene/EtOAc/Et_3N, 50:50:1) to give imidate **25** (2.66 g, 89%). R_f = 0.39 (toluene/EtOAc, 1:1). $[\alpha]_D$ = +5 (*c* = 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.93, 1.98, 2.07, 2.09 (×2), 2.10, 2.13, 2.17, 2.18, 2.21, (9 s, 27 H, CH_3CO), 3.82 (dd, $J_{5,6a}$ = 2.5, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6″a), 3.96 (m, 1 H, H-5″), 4.06 (dd, $J_{5,6a}$ = 4.0, $J_{6a,6b}$ = 12.3 Hz, 1 H, H-6″b), 4.17–4.30 (m, 6 H, H-5, H-6a, H-6b, H-5′, H-6′a, H-6′b), 4.30–4.35 (m, 2 H, H-2, H-3′), 5.12 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H-1″), 5.14–5.20 (m, 2 H, H-2″, H-3″), 5.22 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H-1′), 5.28 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H4″), 5.40 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 5.45 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H-4), 5.49 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.2 Hz, 1 H, H-2′), 5.56 (dd, $J_{3,4} \approx J_{4,5} \approx$ 9.8 Hz, 1 H, H-4′), 6.45 (d, $J_{1,2}$ = 2.0 Hz, 1 H, H-1), 7.52 (m, 2 H, Ph), 7.65 (m, 1 H, Ph), 8.12 (m, 1 H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.0- 21.3 (*C*H_3CO), 62.1(C-6″), 62.5, 62.7 (C-6, C-6′), 65.8, 65.9 (C-4, C-4″), 67.0 (C-4′), 69.1, 69.9 (×2), 70.2 (×2) (C-3, C-2″, C-3″, C-5″, C-5 or C-5′), 71.6, 71.7 (C-3′, C-5 or C-5′), 75.5 (C-2), 76.9 (C-3), 95.9 (C-1), 99.4, 99.6 (C-1′, C-1″), 129.2, 129.4, 130.3, 134.3 (Ph), 160.4–170.9 (C=O) ppm. HR ESI-MS: calcd. for $C_{45}H_{58}Cl_3N_2O_{26}$ ([M + NH_4]$^+$): *m*/*z* = 1147.2338, found: *m*/*z* = 1147.2322.

3-[(Benzyloxycarbonyl)amino]propyl 3,4,6-Tri-*O*-acetyl-2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-α-D-mannopyranosyl]-α-D-mannopyranoside (26): A mixture of the imidate **25** (1.65 g, 1.46 mmol), alcohol **2** (600 mg, 2.87 mmol) and powdered mol. sieves (4 Å, 0.5 g) in CH_2Cl_2 (30 mL) was stirred for 30 min under N_2 and then cooled to 0 °C. To this mixture a solution of TMSOTf (30 μL, 0.15 mmol) in CH_2Cl_2 (5 mL) was added via a syringe and the mixture was stirred for 1.5 h to allow the temperature to rise gradually to 10 °C before addition of Et_3N (0.1 mL). The mixture was filtered through Celite, the filtrate was diluted with CH_2Cl_2 (100 mL) and washed successively with saturated aq. $NaHCO_3$ and water. Solvents were evaporated in vacuo to give crude product which was purified by column chromatography (toluene/Me_2CO, 4:1) to afford the trisaccharide **26** (1.56 g, 91%). R_f = 0.32 (toluene/EtOAc, 1:1). $[\alpha]_D$ = –1 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.83 (m, 2 H, $CH_2CH_2CH_2$), 1.94, 1.95, 2.04, 2.06 (×2), 2.09, 2.14 (×2), 2.18 (9 s, 27 H, CH_3CO), 3.30 (m, 2 H, CH_2N), 3.51 (m, 1 H, OC*H*aHbCH_2), 3.79 (m, 1 H, OCHa*H*bCH_2), 3.82 (dd, $J_{5,6a}$ = 2.4, $J_{6a,6b}$ = 12.4 Hz, 1 H, H-6″a), 3.91–3.99 (m, 2 H, H-5, H-5″), 4.04 (dd, $J_{5,6b}$ = 4.0, $J_{6a,6b}$ = 12.4 Hz, 1 H, H-6″b), 4.07 (broad d, *J* = 3 Hz, 1 H, H-2), 4.10–4.18 (m, 2 H, H-6a, H-5′), 4.18–4.26 (m, 2 H, H-6b, H-6′a, H-6′b), 4.29 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.7 Hz, 1 H, H-3′), 4.95 (broad s, 1 H, H-1), 5.08 (broad s, 3 H, H-1″, C*H*$_2$Ph), 5.10–

5.19 (m, 3 H, H-1′, H-2′′, H-3′′), 5.20–5.34 (m, 3 H, H-3, H-4, H-4′′), 5.45 (dd, $J_{3,4} \approx J_{4,5} \approx 9.8$ Hz, 1 H, H-4′′), 5.40 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 5.45 (m, 1 H, H-2′), 5.52 (dd, $J_{3,4} \approx J_{4,5} \approx 10$ Hz, 1 H, H-4′), 7.52 (m, 2 H, Ph), 7.65 (m, 1 H, Ph), 8.12 (m, 1 H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8–21.6 (CH_3CO), 32.7 ($CH_2CH_2CH_2$), 38.0 (CH_2N), 61.9 (C-6′′), 62.9 (×2, C-6, C-6′), 65.5 (C-4′′), 66.0 (OCH_2CH_2), 66.6, 66.8 (C-4, C-4′′), 67.2 (C-4′), 68.7, 68.9, 69.4, 69.7 (×2) (C-2, C-5′, C-2′′, C-3′′, C-5′′), 70.4 (C-3), 71.5 (C-2′), 76.6 (C-3′), 77.1 (C-2), 98.7 (C-1), 99.0 (C-1′′), 99.4 (C-1′), 128.9, 129.2, 130.1, 134.0 (Ph), 165.6–170.8 (×9, C=O) ppm. MALDI-TOF MS: *m/z* = 1184.4 [M + Li]$^+$. $C_{54}H_{67}NO_{28}$ (1177.4).

3-(2-Bromoacetamido)propyl 3,4,6-Tri-*O*-acetyl-2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-*O*-benzoyl-α-D-mannopyranosyl]-α-D-mannopyranoside (27): Compound **26** (250 mg, 0.212 mmol) was dissolved in a mixture of EtOH (20 mL), EtOAc (5 mL) and AcOH (0.2 mL) and hydrogenated (H_2, 10% Pd/C, 1 atm) at room temperature until TLC showed no more starting material present in the mixture (3 h). The mixture was filtered through Celite, concentrated and dried in vacuo. The residue was dissolved in CH_2Cl_2 (5.0 mL) and Et_3N (0.04 mL) and a solution of $BrCH_2COBr$ (0.026 mL, 0.3 mmol) in CH_2Cl_2 (2.0 mL) was carefully added at –50 °C under N_2. The mixture was stirred for 1 h, diluted with CH_2Cl_2 (20 mL), washed with water (1 × 10 mL) and concentrated. The residue was purified by flash chromatography (toluene/Me_2CO, 7:3) to give the bromoacetamide **27** (186 mg, 75%). R_f = 0.44 (toluene/EtOAc, 3:2). $[\alpha]_D$ = +1 (c = 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.83 (m, 2 H, $CH_2CH_2CH_2$), 1.94, 1.95, 2.04, 2.06 (×2), 2.09, 2.14 (×2), 2.18 (9 s, 27 H, CH_3CO), 3.30 (m, 2 H, CH_2N), 3.51 (m, 1 H, OCHaHbCH$_2$), 3.79 (m, 1 H, OCHaHbCH$_2$), 3.82 (dd, $J_{5,6a}$ = 2.4, $J_{6a,6b}$ = 12.4 Hz, 1 H, H-6′′a), 3.91–3.99 (m, 1 H, H-5, H-5′′), 4.04 (dd, $J_{5,6b}$ = 4.0, $J_{6a,6b}$ = 12.4 Hz, 1 H, H-6′′b), 4.07 (broad d, J = 3 Hz, 1 H, H-2), 4.10–4.18 (m, 2 H, H-6a, H-5′), 4.18–4.26 (m, 2 H, H-6b, H-6′a, H-6′b), 4.29 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 9.7 Hz, 1 H, H-3′), 4.95 (broad s, 1 H, H-1), 5.08 (broad s, 3 H, H-1′′, CH_2Ph), 5.10–5.19 (m, 3 H, H-1′, H-2′′, H-3′′), 5.20–5.34 (m, 3 H, H-3, H-4, H-4′′), 5.45 (dd, $J_{3,4} \approx J_{4,5} \approx 9.8$ Hz, 1 H, H4′′), 5.40 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.8 Hz, 1 H, H-3), 5.45 (m, 1 H, H-2′), 5.52 (dd, $J_{3,4} \approx J_{4,5} \approx 10$ Hz, 1 H, H-4′), 7.52 (m, 2 H, Ph), 7.65 (m, 1 H, Ph), 8.12 (m, 1 H, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8–21.0 (CH_3CO), 28.9, 29.2 ($CH_2CH_2CH_2$, CH_2Br), 38.1 (CH_2N), 61.8 (C-6′), 62.7, 62.9 (C-6, C-6′′), 65.5 (C-4′′), 66.6 (×2, CH_2 aglycon, C-4), 67.0 (C-4′), 68.8 (C-5 or C-5′′), 68.9 (C-3′′), 69.5 (C-5′′), 69.7 (×2, C-2′′, C-5′′ or C-5), 70.3 (C-3), 71.5 (C-2′), 76.6 (C-3′), 77.2 (C-2), 98.7 (C-1), 99.1 (C-1′), 99.3 (C-1′′), 128.9, 129.2, 130.1, 134.0 (Ph), 165.6–170.8 (×9, C=O) ppm. ES MS: *m/z* = 1183.3 [M + NH_4]$^+$, 1188.6 [M + Na]$^+$. $C_{48}H_{62}NO_{27}$ (1165.3).

3-(2-Bromoacetamido)propyl 2-*O*-[3-*O*-(α-D-Mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (28): Methanolic 1 M NaOMe (0.02 mL) was added to a solution of the trisaccharide **27** (180 mg, 0.154 mmol) in MeOH (2 mL) and the mixture was stirred for 1 h at room temperature. The reaction mixture was neutralized with Amberlite IRA-120 (H^+), the resin was filtered off and washed with MeOH. The solutions were combined and concentrated and the residue was dried in vacuo to afford compound **28** (92 mg, 87%). $[\alpha]_D$ = +68 (c = 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.65 (m, 2 H, $CH_2CH_2CH_2$), 3.13 (m, 2 H, CH_2N), 3.31–3.77 (m, 19 H, H-3–H-6 Man, OCH_2CH_2, CH_2Br), 3.77, 3.87, 4.03 (three broad s, 3 H, H-2 Man), 4.82, 4.90, 4.95 (three broad s, 3 H, H-1 Man) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 28.2 ($CH_2CH_2CH_2$), 28.5 (C-6 Glc), 37.6 (CH_2N), 61.4 and 61.5 (C-6 Man), 65.7 (OCH_2CH_2), 66.7, 67.3, 67.4, 70.0, 70.5, 70.7, 70.8, 73.2, 73.75, 73.8, 78.3, 79.2, (C-2–C-5 Man), 98.8 and 102.7 (1 C and 2 C, C-1 Man), 170.4 (C=O) ppm. HR ESI-MS: calcd. for $C_{23}H_{44}BrN_2O_{17}$ ([M + NH_4]$^+$): *m/z* = 699.1818, found: *m/z* = 699.1812.

Mannotrioside-β-cyclodextrin Glycocluster 29: A degassed 0.5 M aq. K_2CO_3 solution was added to a degassed solution of per-6-thio-β-cyclodextrin (**1b**, 5.5 mg, 4.4 μmol) and compound **28** (30 mg, 44 mmol) in DMF (0.8 mL) to make pH of the reaction mixture 8–8.5 (paper indicator). The mixture was stirred for 24 h at room temperature under N_2, 1 M aq. AcOH was added to adjust pH to 6 and then solvents were evaporated in vacuo. Purification of the residue using gel permeation chromatography on Toyopearl HW-40S column (1.5 × 85 cm) with 0.1% aq. CF_3CO_2H as a mobile phase furnished the target glycocluster **29** (13.0 mg, 54%). $[\alpha]_D$ = +74 (c = 0.8, H_2O). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 28.8 ($CH_2CH_2CH_2$), 34.1 (C-6 Glc), 37.1, 37.4 (CH_2N, CH_2S), 61.5 (C-6 Man), 65.8, 66.7, 67.3, 67.4, 70.0, 70.5, 70.8, 73.3, 73.7, 73.9, 78.3, 79.2 (C-2–C-5 Man), 85.1 (C-4 Glc), 98.8 (C-1 Man), 102.7 (C-1 Man), 102.8 (C-1 Man), 172.4 (C=O) ppm. MALDI-TOF MS: *m/z* = 5496.6 [M + K]$^+$. $C_{203}H_{343}N_7O_{147}S_7K$ (5496.7).

Supporting Information (see footnote on the first page of this article) for this article contains original ^{1}H and ^{13}C NMR spectra for compounds described in the Experimental Section and MALDI TOF mass spectra for glycoclusters **11**–**14** and **29**.

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