Synthesis of Carbohydrate-Centered Oligosaccharide Mimetics Equipped with a Functionalized Tether

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Synthetic glycoclusters have gained substantial attention as mimetics of multivalent glycoconjugates. For their proposed glycobiological applications, it is advantageous to incorporate a functionalized tether into the clusters, which allows coupling to solid supports and other molecules such as reporter groups or even bioactive molecules. We herein report the use of carbohydrates as oligofunctional scaffolds for the synthesis of tethered cluster mannosides. Glycocluster **11** was prepared following two different pathways, starting either from glucose or the nonreducing disaccharide trehalose. The oligo alcohols **5** and **14** served as acceptors in the subsequent oligo-mannosylation reaction, in which three main problems were overcome: (i) incomplete glycosylation, (ii) cleavage of the core-glycoside, and (iii) ortho ester formation. Optimum conditions for the glycosylation were identified utilizing an advanced MALDI-TOF protocol.

Introduction

One of the main objectives in glycobiology is the investigation of carbohydrate-protein interactions, which play essential roles in cell−cell communication.[1,2] A major benefit of a more detailed understanding of these molecular recognition processes would be effective intervention in the associated pathological phenomena, along with the development of carbohydrate-based drugs such as carbohydrate-based antiadhesives to prevent microbial infections.[3] To simplify the synthesis of the necessary saccharides as well as to improve their biological functions, a large number of neoglycoconjugates[4] and glycomimetics[5] have been synthesized including multivalent glycoconjugates of various architectures.[6] The latter have been designed as to mimic oligosaccharides or oligoantennary glycoconjugates.[7]

For all potential glycobiological applications of (multivalent) glycomimetics, it is advantageous to have the respective molecules equipped with a handle to allow the attachment of reporter groups or labels such as various dyes or biotin, thus facilitating the necessary glycobiological assays. Furthermore, a functionalized tether might be used for drug targeting or for the attachment to solid phases,[8] improving the perspectives of synthesis, purification, and molecular diversity as well as isolation of lectins.

It was recently shown that carbohydrate derivatives can serve as multivalent scaffolds for the synthesis of oligosaccharide mimetics.[9−12] In this paper, an important extension of this concept is described, in which carbohydrate-centered glycoclusters carrying a functionalized tether are synthesized. Incorporation of a spacer arm to allow further conjugation of the oligosaccharide mimetic has just recently been recognized as a potential advantage of the *n*-pentenyl glycoside methodology.[13]

In this paper two approaches for the synthesis of tethered glycoclusters were followed: (i) the monosaccharide pathway using D-glucose as the core molecule, and (ii) the trehalose pathway, in which the nonreducing disaccharide was appropriately modified to serve as scaffold for oligoglycosylation, followed by cleavage of the interglycosidic bond. A mannosyl donor was used for the glycosylation reactions, as cluster mannosides are under special investigation in our laboratory as inhibitors of mannose-specific adhesion of *Escherichia coli*.[7,14,15]

Results and Discussion

Monosaccharide Pathway. To transform D-glucose into an oligo-hydroxylated core molecule equipped with a functionalized tether at the anomeric center, the most simple and shortest glycosidation method available could be utilized as the first step. Starting with glucose

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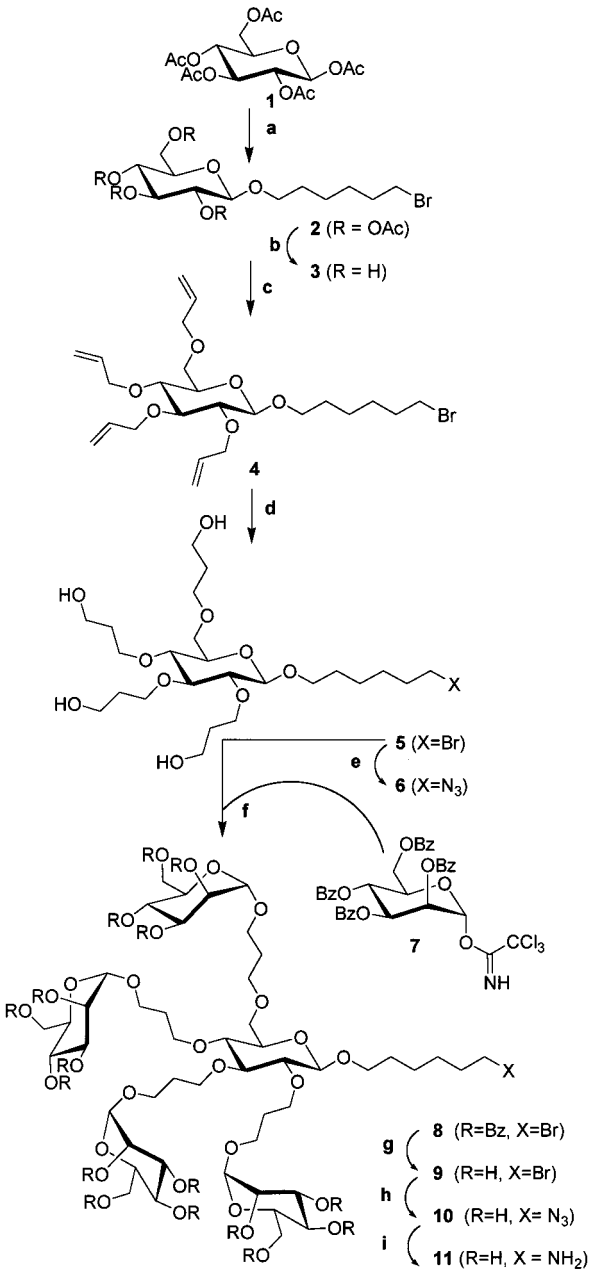
Scheme 1. Monosaccharide Pathway[a]



[a] Key: (a) $BF_3{\cdot}Et_2O$, 6-bromohexanol, CH_2Cl_2; (b) NaOMe, MeOH; (c) allyl bromide, NaOH, tetrabutylammonium bromide, rt; (d) 9-BBN-H, THF; (e) NaN_3, DMF; (f) TMS-OTf, CH_2Cl_2; (g) NaOMe, MeOH−THF; (h) NaN_3, DMF; (i) H_2, Pd−C.

pentaacetate (**1**) a BF_3-etherate catalyzed reaction[16] with an equimolar amount of 6-bromohexanol led to formation of glucoside **2**. Subsequent deprotection under Zemplén conditions gave the unprotected glycoside **3** (Scheme 1). The next step necessitated allylation of the primary and secondary hydroxyl groups in **3**, excluding inter- and intramolecular side reactions arising from the participation of the bromide function of the aglycon moiety. This was accomplished by modification of a literature-known procedure[17] using PTC conditions and by reducing the reaction temperature from 40 to 20 °C. The yield of 42% obtained in this step is significantly lower than the reported yields when the same procedure was used for per-allylation of nonfunctionalized carbohydrates,[18] and this reflects the difficulties connected with modification of the tethered derivatives described herein. The subsequent hydroboration step with 9-BBN-H was accompanied with an unexpectedly large number of side reactions which could, however, be reduced by modification of the conditions previously described [18] using a reaction time of 1 h at room temperature. During the oxidation step employing $NaOH/H_2O_2$, an intramolecular ring closure with elimination of 9-BBN-Br was observed, as well as several other side reactions. This observation is analogous to work published by H. C. Brown[19] and could correspondingly be avoided by replacing aqueous NaOH with aqueous NaOAc. Thus, the glucose-based tetraol equipped with a bromo-functionalized tether (**5**) was obtained, which is the acceptor molecule for the following glycosylation reaction. Glycosylation of **5**, however, is a nontrivial task even in view of the advantages of relatively well-advanced modern glycosidation methodology. Multiple glycosylations of relatively small core molecules are difficult, because the core becomes increasingly sterically hindered during proceeding glycosylation and the last glycosylation step consequently becomes the slowest and most critical of all. This problem was frequently monitored by MALDI-TOF measurements during the glycosylation reaction. An additional problem is that the separation of the target molecules **8** and **15** (vide supra) from a mixture of structurally imperfect, however very similar products is hardly possible. Therefore, formation of side products arising from incomplete glycosylation of the core polyols had to be avoided by using a large excess of the glycosyl donor. Glycosyl imidates[20] proved to be suited for this reaction. A problem connected with the special case of mannosylations is the formation of glycosyl ortho esters, which is especially favored in the *manno*-series due to the relatively high stability of the intermediate acyloxonium ion.[21] While the utilization of an ether-protected mannosyl trichloroacetimidate would exclude the possibility of ortho ester formation, the ether-protected mannosyl donor was not suitable for the preparation of **8**. Finally, the benzoylated mannosyl imidate **7**[22] proved to be a suitable donor in this reaction, being superior to acetylated as well as benzylated analogues. However, a relatively high concentration of Lewis acid was necessary to avoid ortho ester formation completely. This implied a restriction concerning the reaction time, which must be limited to exclude anomerization at the anomeric center of the glucoside core. Whereas with the ω-azido-functionalized glycoside **6** no time frame could be found in which ortho ester rearrangement was complete while the anomeric configuration remained unchanged, the bromide **5** allowed the preparation of the ortho ester-free fully mannosylated glycocluster **8** when the reaction was performed in concentrated solution in CH_2Cl_2. When the imidate excess was reduced by 50%, NMR analysis of the products revealed no difference, however a minor additional peak occurring in the MALDI-TOF mass spectrum indicated the formation of clusters missing one mannosyl residue.

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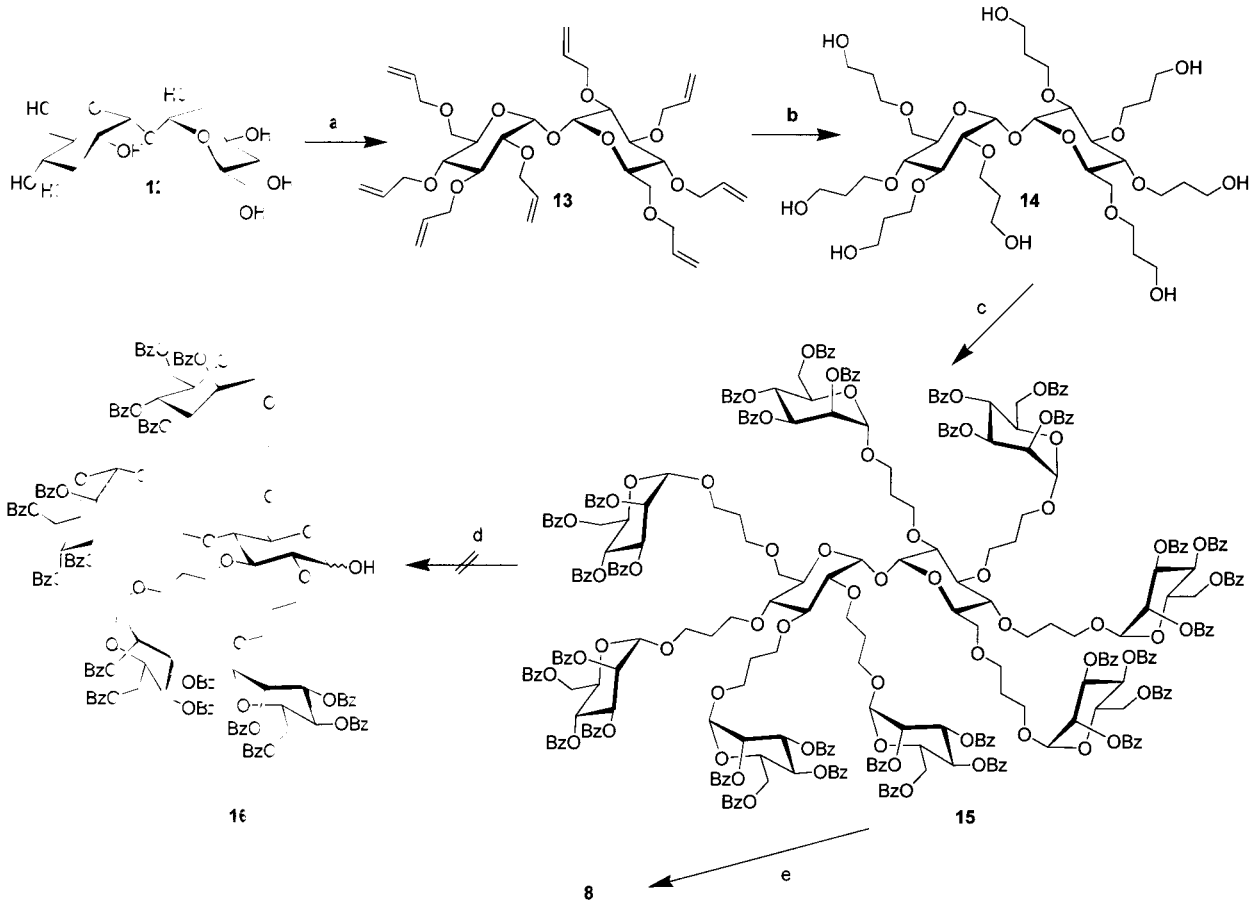
Scheme 2. Trehalose Pathway[a]



[a] Key: (a) allyl bromide, NaH, DMF; (b) 9-BBN-H, THF; (c) **7**, TMS-OTf, acetonitrile; (d) aqueous TFA, THF, or acetonitrile; (e) 6-bromohexanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2.

Thus, no further attempts were made to optimize the amount of imidate necessary for the glycosylation reaction, especially in light of the fact that **7** is an inexpensive, easily obtained and relatively stable derivative of mannose.[23]

After **8** had been obtained in pure form, deprotection was achieved under modified Zemplén conditions. As the benzoyl-protected cluster is hardly soluble in methanol and deprotection in a THF−MeOH mixture did not go to completion, a stepwise protocol was introduced. Thus, the majority of the benzoate groups was first removed with sodium methoxide in THF−MeOH, and then this solvent mixture was replaced by pure MeOH to achieve complete deprotection. The unprotected cluster **9** was then subjected to an S_N2 substitution reaction using sodium azide, followed by catalytic reduction of the intermediate **10** to obtain cluster **11** carrying the amino-functionalized tether.

Trehalose Pathway. Considering the difficulties connected with the monosaccharide pathway which was used first for the preparation of **11**, an alternative route was sought in which the symmetrical, nonreducing disaccharide trehalose (**12**) was used as the starting material, which can be considered an anomerically protected glucose. Cleavage of the interglycosidic bond at an intermediate stage of the synthesis would lead to two equivalents of the reducing glucose-centered glycocluster **16**.

This reaction sequence was started with the perallylation of trehalose using allyl bromide and NaH in DMF to yield **13**, followed by the hydroboration−oxidation sequence leading to the octaol **14** (Scheme 2). No difficulties were observed in this step which gave the product in 83% isolated yield, in contrast to the case of the analogous synthesis of the monosaccharide derivative **5**, which gave low yields.

Mannosylation of **14**, as with **5**, was complicated by the aforementioned problems of incomplete glycosylation, ortho ester-formation and undesired cleavage of the interglycosidic bond accompanied by subsequent glycosylation of the anomeric center. Fortunately, intermolecular benzoyl group migration leading to benzoylation of the spacer hydroxyl groups, as it has been reported for polymer-supported oligosaccharide synthesis,[24] was never observed.

To find optimum conditions allowing the synthesis of structurally perfect **15** from **14**, means for monitoring the reaction and the formation of the various side products had to be developed. When the course of the glycosylation reaction was periodically monitored by MALDI-TOF-MS, incomplete glycosylation as well as cleavage and destruction of the core could be seen, but no ortho ester formation could be observed. However, when the product of the glycosylation reaction was subjected to silica gel filtration followed by basic deprotection and neutralization with

(23) See the Supporting Information.

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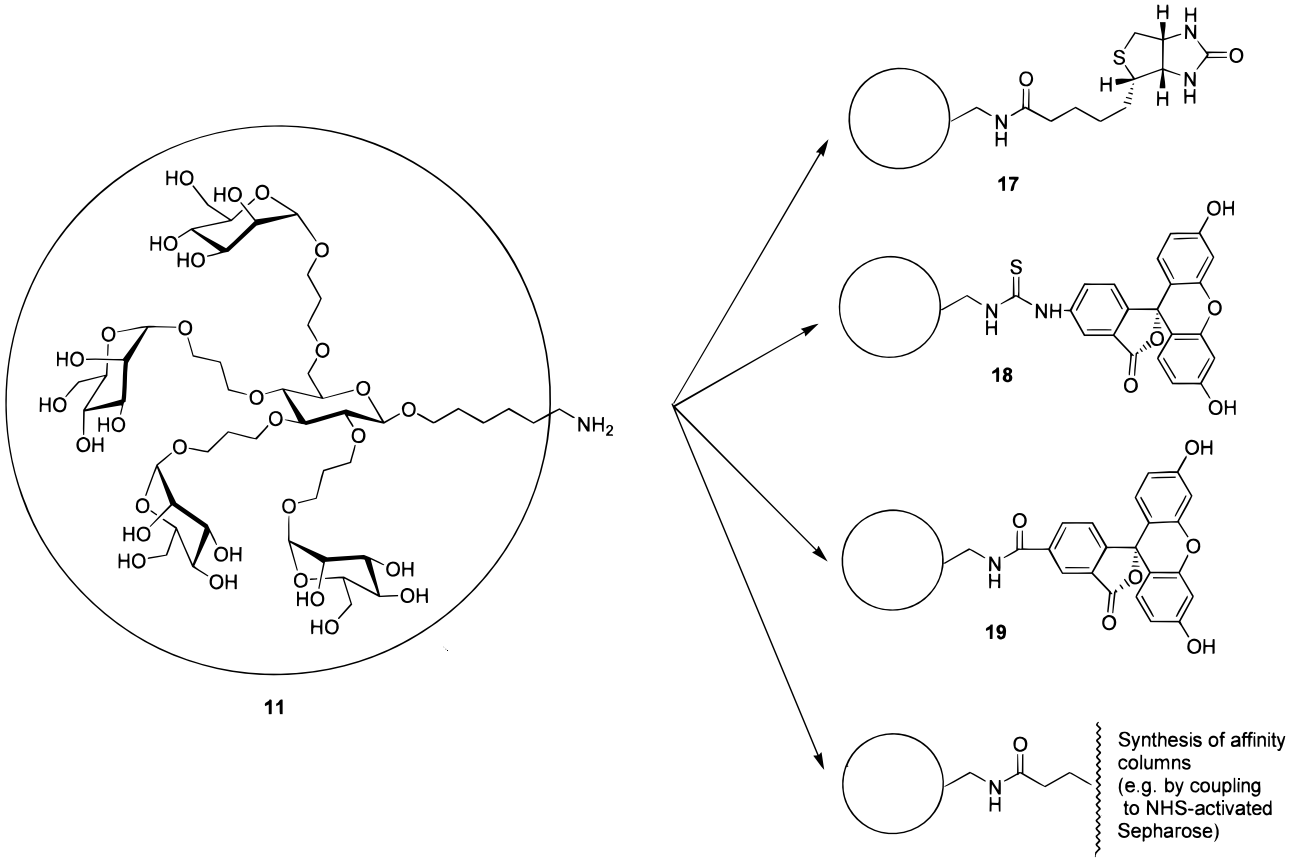
Scheme 3



acidic ion-exchange resin, MALDI-TOF analysis of the deprotected cluster glycoside revealed ortho ester portions formed during the glycosylation step. To use MALDI-TOF-MS as a reliable tool in this context, the reproducibility of MALDI-TOF spectra had to be ensured. Thus, sample preparation for MALDI-TOF-MS in which dihydroxybenzoic acid is commonly used with carbohydrates,[25] leading to relatively low reproducibility of the obtained spectra,[26] was improved by evaluation of several other matrixes. Finally the premix technique with norHarmane[27] was modified, using acetone and sodium iodide as cationizer to avoid $[M + \text{matrix}]^+$ peaks. With this technique in hand, optimum conditions for the glycosylation could be established.

Because of the high polarity of the polyol-type scaffold **14**, acetonitrile had to be used as the solvent in the glycosylation reaction. The first glycosylation experiments, as evaluated for the synthesis of **8**, led to incomplete glycosylation with approximately five instead of eight mannosyl residues attached to the core. Therefore a two-step glycosylation procedure was attempted, in which the second glycosylation step was carried out in CH_2Cl_2. This approach resulted in clusters carrying up to seven mannosyl moieties. To further enhance the glycosylation reaction, more concentrated solutions in acetonitrile were used. This approach, which was successful with **8**, failed completely in this case, yielding products in which only two of eight spacer arms of the core were mannosylated. Interestingly, extreme dilution of the reaction partners opened the way to complete glycosylation of **14**. While for the glycosylation of **5** in CH_2Cl_2 concentrations of >10 mg acceptor polyol/mL were optimal, the same result was obtained with **14** in acetonitrile at concentrations of < 0.1 mg acceptor polyol/mL.

The remaining ortho ester problem could not be solved by rearrangement reaction using elevated concentrations of Lewis acid as shown in the synthesis of **8**, however, it could be supressed by using elevated reaction temperatures during the initial phase of the glycoslyation reaction.

Thus the "octopus" glycocluster **15** was ultimately obtained in pure form. As cleavage of the interglycosidic bond was shown to be a constant problem during its synthesis, it was anticipated that this interglycosidic cleavage would be easy to utilize in order to obtain two equivalents of the reducing glycocluster **16** after the mannosylation step. However, this approach was not successful as **15** was stable even under the conditions of up to 20% of 1 M aqueous TFA used in organic cosolvents such as THF or acetonitrile at temperatures up to 80 °C. Fortunately, it was possible to split **15** with $BF_3 \cdot Et_2O$ and a trace of water in CH_2Cl_2, however further degradation also occurred during this reaction. Adding an alcohol component instead of water decreased the extent of degradation significantly. This led to the appealing possibility to synthesize **8** from **15** using 6-bromoethanol in the presence of a large excess $BF_3 \cdot Et_2O$, which unhappily produced an anomeric mixture.

Labeling of 11. The amino function of the tethered cluster mannoside **11** can serve as a handle for a large

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number of further modifications (Scheme 3) which are advantageous for many glycobiological applications.[28] Thus the amino group allows attachment of labels such as biotin, (fluorescent) dyes[29,30] or coupling to solid supports in order to form matrixes for affinity chromatography.

The most popular methodologies used for linking molecules via their amino groups are peptide coupling utilizing succinimidyl-activated esters, or thiourea-bridging using isothiocyanates as reaction partners. Thus, glycocluster **11** could be turned into the biotin-labeled derivative **17** using a tethered succinimidyl ester of biotin. The fluorescein-labeled cluster **18** proved to be too instable for definitive characterization, while for the amide-linked analogue **19** sufficient ^{13}C NMR spectral data were obtained. Interestingly the NMR spectra of the biotinylated cluster **17** changed when the NMR sample in D_2O was stored over several days. In the ^{13}C NMR spectrum a peak at 55.8 ppm for the central carbon of the biotin bicycle was decreased while a corresponding resonance started to show at 62.4 ppm. Correspondingly, the multiplet for the attached proton showing in the ^1H NMR shifted from 3.91 to 4.31 ppm. To date this phenomenon remains unexplained.

Summary

In conclusion, we have introduced two synthetic pathways leading to selectively functionalized cluster glycosides such as **11**, in which the amino-functionalized aglycone can serve as a tether to allow various useful modifications. This was demonstrated by the synthesis of biotinylated cluster **17**. The key problems connected with the synthesis and analysis of oligomannosylation and oligomannosylated clusters were solved so that this type of molecule is now available for glycobiological applications such as for the investigation of carbohydrate-protein interactions and affinity chromatogaphy.

Experimental Section

MALDI-TOF Techniques. For MALDI-TOF measurements, acetone-soluble samples were prepared using the premix technique with nor-Harmane[27] and NaI in acetone as matrix. Highly polar compounds were prepared using the layer technique with dihydroxybenzoic acid (DHB) in water−acetonitrile (2:1) with 0.1% TFA as matrix.[25]

6-Bromohexyl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranoside (2). To a solution of **1** (8 g, 21 mmol) and 1-bromohexanol (3 mL, 22 mmol) in dry CH_2Cl_2 (20 mL) boron trifluoride-etherate (10 mL, 81 mmol) was added. The reaction mixture was stirred for 1 h in the dark, CH_2Cl_2 (100 mL) was added and the solution was neutralized with saturated aqueous $NaHCO_3$ solution before being washed twice with water. The organic phase was dried ($MgSO_4$), filtered, concentrated, purified by column chromatography (light petroleum-EtOAc 3:2) and recrystallized from diethyl ether to yield **2** (3.8 g, 36%) as a white solid: mp 103 °C; $[\alpha]_D$ −21 (*c* 1.2, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) 5.39 (t, 1H, J = 9.2 Hz), 5.27 (t, 1H, J = 9.2 Hz), 5.17 (dd, 1H, J = 9.2, 8.1 Hz), 4.68 (d, 1H, J = 8.1 Hz), 4.45 (dd, 1H, J = 4.6, 12.2 Hz), 4.32 (dd, 1H, J = 2.0, 12.2 Hz), 4.06 (m, 1H), 3.87 (dddd∼m, 1H), 3.67 (m, 1H), 3.58 (t, 1H), 2.27, 2.23, 2.21, 2.19 (4 s, 12H), 2.03 (m, 2H), 1.77 (m, 2H), 1.62 (m, 2H), 1.62 (m, 2H) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$) 171.1, 170.7, 169.8, 169.7, 101.2, 73.3, 72.2, 71.8, 70.3, 68.9, 62.4, 34.2, 33.0, 29.6, 28.2, 25.4, 21.2, 21.1, 21.0, 21.0 ppm. Anal. Calcd. for $C_{20}H_{31}BrO_{10}$: C, 46.98; H, 6.11; Br, 15.63. Found: C, 46.91;.H, 6.11; Br, 15.48.

6-Bromohexyl *β*-D-Glucopyranoside (3). To a solution of **2** (1.3 g, 2.6 mmol) in dry MeOH (20 mL), a solution of NaOMe in MeOH (0.1 M, 2 mL) was added. The reaction mixture was stirred overnight, neutralized with Amberlite IR 120, filtered and concentrated to yield **3** (0.91 g, quant) as a colorless syrup: $[\alpha]_D$ −15.4 (*c* 0.95, MeOH); ^1H NMR (500 MHz, MeOH-d_4) 4.29 (d, 1H, J = 7.9 Hz), 3.95 (m, 1H), 3.90 (dd, 1H, J = 4.7, 12.0 Hz), 3.70 (dd, 1H, J = 1.9, 12.0 Hz), 3.60 (m, 1H), 3.49 (t, 2H, J = 6.9 Hz), 3.39 (t, 1H + MeOH, J = 8.5 Hz), 3.32 (t, 1H, J = 8.5 Hz), 3.29 (m, 1H), 3.21 (t, 1H, J = 8.5 Hz), 1.90 (m, 2H), 1.68 (m, 2H), 1.63−1.41 (m, 4H) ppm; ^{13}C NMR (100.6 MHz, MeOH-d_4) 100.6, 79.3, 79.1, 76.3, 72.9, 71.9, 64.0, 35.5, 35.2, 31.8, 30.2, 27.4 ppm. Anal. Calcd. for $C_{12}H_{23}BrO_6$: C, 41.99; H, 6.75; Br, 23.28. Found: C, 41.79; H, 6.84; Br, 23.25.

6-Bromohexyl 2,3,4,6-Tetra-*O*-allyl-*β*-D-glucopyranoside (4). To a solution of **3** (1.27 g, 3.70 mmol) in water (5 mL), aqueous sodium hydroxide (40 g NaOH in 60 mL H_2O) and tetrabutylammonium bromide (2 g) were added. While the reaction mixture was vigorously stirred at 20 °C, allyl bromide (3 mL, 36 mmol) was added over 1 h. After an additional 2.5 h of vigorous stirring, the reaction was quenched by addition of toluene (100 mL). The phases were separated and the organic phase was washed with water (6×), dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography (toluene−EtOAc 9:1) to yield **4** (0.78 g, 42%) as a colorless syrup: $[\alpha]_D$ −4.6 (*c* 1.74, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) 6.06−5.90 (m, 4H), 5.35−5.26 (m, 4H), 5.24−5.17 (m, 1H), 4.42−4.26 (m, 5H) 4.24−4.03 (m, 1H), 3.95 (m, 1H), 3.75 (dd, 1H, J = 1.5, 11.2 Hz), 3.65 (dd, 1H, dd, J = 2.5, 11.2 Hz), 3.52 (m, 1H), 3.45 (t, 2H, J = 7.1 Hz), 3.43−3.34 (m, 3H), 3.23 (t, 1H, J = 8.1 Hz), 1.90 (m, 2H), 1.66 (m, 2H), 1.54−1.39 (m, 4H) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$) 136.0, 135.8, 135.5, 135.4, 117.6, 117.6, 117.4, 117.3, 104.1, 84.8, 82.3, 78.3, 75.5, 75.1, 74.5, 74.3, 73.1, 70.4, 69.7, 34.4, 33.4, 30.1, 28.6, 25.9 ppm. Anal. Calcd for $C_{24}H_{39}BrO_6$: C, 57.25; H, 7.81; Br, 15.87. Found: C, 57.46; H, 7.85; Br, 15.90.

6-Bromohexyl 2,3,4,6-Tetra-*O*-(3-hydroxypropyl)-*β*-D-glucopyranoside (5). To a solution of **4** (0.78 mg, 1.55 mmol) in dry THF (30 mL), 0.5 M 9-BBN−H solution in THF (28 mL, 14 mmol) was added and the reaction mixture was stirred at room temperature for 1.5 h. The excess reagent was destroyed by adding ice water. Oxidation was subsequently achieved by the simultaneous dropwise addition of 3M aqueous NaOAc (14 mL) and 30% hydrogen peroxide (14 mL) followed by stirring overnight at room temperature. The mixture was saturated with K_2CO_3 and the phases were separated. The aqueous phase was washed with THF (2x) and the combined organic phases were concentrated and purified by flash chromatography (EtOAc−MeOH 9:1) to yield **5** (0.48 g, 54%) as a colorless syrup: $[\alpha]_D$ −10.2 (*c* 0.96, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) 4.35 (d, 1H, J = 7.6 Hz), 4.14 (m, 1H), 4.08−3.97 (m, 4H), 3.95−3.75 (m, 14H), 3.60 (m, 1H), 3.53 (t, 2H, J = 7.1 Hz), 3.43−3.34 (m, 3H), 3.21 (t, 1H, J = 8.1 Hz), 2.88 (s, 4H), 2.03−1.88 (m, 10H), 1.75 (m, 2H), 1.58 (m, 2H), 1.51 (m, 2H) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$) 103.7, 84.6, 82.3, 78.9, 74.9, 71.8, 71.7, 71.0, 70.6, 70.2, 70.1, 61.5, 61.4, 61.0, 60.6, 34.2, 33.3, 33.2, 33.0, 32.9, 32.4, 29.8, 28.2, 25.6 ppm. Anal. Calcd for $C_{24}H_{47}BrO_{10}$: C, 50.09; H, 8.23; Br, 13.88. Found: C, 49.95; H, 8.39; Br, 13.75.

6-Bromohexyl 2,3,4,6-Tetra-*O*-[3-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)propyl]-*β*-D-glucopyranoside (8). A mixture of **5** (0.055 g, 0.096 mmol) and **7** (2.8 g, 3.8 mmol) in dry CH_2Cl_2 was concentrated and dried under high vacuum. The resulting solid was dissolved in dry CH_2Cl_2 (3 mL). TMS-OTf solution (0.1 mL, 0.5 mL TMS-Tf 0.5 mL in 10 mL CH_2Cl_2) was added and the reaction mixture was stirred for 6 h at room temperature before being neutralized with $NaHCO_3$ (10 g), filtered, concentrated and purified by flash chromatography (light petroleum−EtOAc 3:2 → 2:3). Subsequent gel filtration on Sephadex LH-20 (CH_2Cl_2−MeOH 1:1)

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yielded **8** (262 mg, 95%) as an amorphous white solid: $[\alpha]_D$ −45.9 (*c* 0.23, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) 8.10−8.06, 8.04−7.97, 7.94−7.86, 7.83−7.78, 7.57−7.50 (m, each 8H), 7.48−7.16 (m, 40H + $CDCl_3$), 6.18−6.09 (m, 4H), 5.98−5.89 (m, 4H), 5.73, 5.72, 5.70, 5.69 (each dd, 4H, *J* = 1.6 and 3.1 Hz), 5.15, 5.13, 5.11, 5.06 (each d, 4H, *J* = 1.6 Hz), 4.73−4.64 (m, 4H), 4.52−4.41 (m, 8H), 4.25 (d, 1H, *J* = 7.6 Hz), 4.05−3.65 (m, 19H), 3.49 (m, 1H), 3.37−3.31 (m, 3H), 3.28 (t, 2H, *J* = 6.9 Hz), 3.12 (t, 1H, *J* = 8.1 Hz), 2.12−1.96 (m, 8H), 1.80−1.73 (m, 2H), 1.64−1.56 (m, 2H), 1.42−1.30 (m, 4H) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$) 166.1, 165.-165.2, 133.3−133.0, 129.9−128.2, 103.4, 97.7 (3×), 97.6, 84.9, 82.4, 78.1, 74.8, 70.5 (2×), 70.5 (2×), 70.3, 70.2, 70.2 (2×), 70.2, 69.8, 69.7, 69.5, 69.1, 68.8 (3×), 68.7, 68.0, 66.1, 66.0, 65.4 (2×), 66.9, 66.9 (2×), 66.9, 62.8 (4×), 33.9, 32.7, 30.6, 30.4 (2×), 29.7, 29.5, 27.9, 25.2 ppm. Anal. Calcd for $C_{160}H_{151}BrO_{46}$: C, 66.50; H, 5.27; Br, 2.77. Found: C, 66.48; H, 5.22; Br, 2.80.

6-Bromohexyl 2,3,4,6-Tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-*β*-D-glucopyranoside (9). To a solution of **8** (0.418 g, 0.145 mmol) in THF (20 mL) a solution of NaOMe in MeOH (0.1 M, 10 mL) was added. The reaction mixture was stirred for 1 h at room temperature and then concentrated. The residue was dissolved in MeOH (50 mL), then a solution of NaOMe in MeOH (0.1 M, 10 mL) was added and the reaction mixture was stirred overnight at room temperature before being neutralized with Amberlite IR 120, filtered, concentrated and purified by gel permeation chromatography on Sephadex LH-20 (MeOH) to yield **9** (167 mg, 94%) as a colorless syrup: $[\alpha]_D$ + 47.1 (*c* 1.9, MeOH); ^{1}H NMR (500 MHz, MeOH-d_4) 4.81, 4.80, 4.80, 4.79 (each d, 4H, *J* = 1.6 Hz), 4.30 (d, 1H, *J* = 7.6 Hz), 3.96−3.82 (m, 17H), 3.79−3.54 (m, 27H), 3.50 (t, 2H, *J* = 6.8 Hz), 3.34 (m, 1H + MeOH), 3.31−3.26 (m, 2H), 3.03 (t, 1H, *J* = 8.3 Hz), 1.98−1.85 (m, 10H), 1.67 (m, 2H), 1.58−1.43 (m, 4H) ppm; ^{13}C NMR (100.6 MHz, MeOH-d_4) 105.9, 102.8 (4×), 87.2, 84.5, 80.5, 77.0, 75.8 (3×), 75.7, 73.8 (4×), 73.4 (4×), 72.8, 71.8 (4×), 70.5, 69.8 (2×), 69.7 (2×), 66.9, 66.8, 66.5 (2×), 64.1 (4×), 35.7, 35.1, 32.9, 32.8, 32.7, 32.1, 31.8, 30.1, 27.6 ppm; MALDI-TOF-MS *m*/*z* 1245.2 $[M + Na]^+$, calcd for $C_{48}H_{87}BrO_{30}$ 1222.4.

6-Azidohexyl 2,3,4,6-Tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-*β*-D-glucopyranoside (10). A solution of **9** (0.172 g, 0.141 mmol) in DMF (20 mL) was treated with sodium azide (0.056 g, 0.860 mmol) and the reaction mixture stirred at 60 °C for 1 h. The solution was concentrated and purified on Sephadex LH-20 (MeOH) yielding **10** (163 mg, 97%) as a colorless syrup: $[\alpha]_D$ + 40.1 (*c* 1.7, MeOH); ^{1}H NMR (500 MHz, MeOH-d_4) 4.82−4.78 (m, 4H), 4.30 (d, 1H, *J* = 7.6 Hz), 3.97−3.82 (m, 17H), 3.79−3.54 (m, 27H), 3.36−3.32 (m, 3H + MeOH), 3.30−3.26 (m, 2H), 3.03 (t, 1H, *J* = 8.0 Hz), 1.98−1.85 (m, 8H), 1.71−1.61 (m, 4H), 1.53−1.42 (m, 4H) ppm; ^{13}C NMR (100.6 MHz, MeOH-d_4) 105,9, 102.8 (3×), 102.7, 87.2, 84.9, 80.5, 77.0, 75.8 (3×), 75.7, 73.8 (4×), 73.4 (4×), 72.8, 71.9, 71.8 (3×), 70.5, 69.8 (2×), 69.8, 69.7, 66.9, 66.8, 66.5 (2×), 64.1 (4×), 53.6, 32.9, 32.7, 32.1, 31.9, 31.1, 28.7, 28.0 ppm; MALDI-TOF-MS *m*/*z* 1208.3 $[M + Na]^+$, calcd for $C_{48}H_{87}N_3O_{30}$ 1185.5.

6-Aminohexyl 2,3,4,6-Tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-*β*-D-glucopyranoside (11). A solution of **10** (0.159 g, 0.134 mmol) in MeOH (20 mL) was stirred with 5% Pd−C under 1 atm of hydrogen for 2 h. The mixture was filtered over a thin bed of Sephadex LH-20, which was washed with MeOH, and the filtrate was concentrated to give **11** (149 mg, 96%) as a colorless syrup: $[\alpha]_D$ + 55.1 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, MeOH-d_4) 4.82−4.78 (m, 4H), 4.30 (d, 1H, *J* = 7.6 Hz), 3.97−3.82 (m, 17H), 3.79−3.53 (m, 27H), 3.40−3.33 (m, 1H + MeOH), 3.30−3.25 (m, 2H), 3.03 (t, 1H, *J* = 7.8 Hz), 2.74 (t, 1H, *J* = 7.1 Hz), 1.98−1.85 (m, 8H), 1.71−1.63 (m, 2H), 1.62−1.52 (m, 2H), 1.51−1.39 (m, 4H) ppm; ^{13}C NMR (125.8 MHz, MeOH-d_4) 105,8, 102.8 (4×), 87.2, 84.9, 80.5, 77.0, 75.8 (4×), 73.8 (4×), 73.4 (4×), 72.8, 71.9, 71.9, 71.8 (2×), 70.5, 69.8, 69.8, 69.8, 69.7, 66.9, 66.8, 66.6 (2×), 64.1 (4×), 43.3, 33.9, 32.9, 32.7, 32.7, 32.1, 31.9, 28.8, 28.2 ppm; MALDI-TOF-MS *m*/*z* 1182.3 $[M + Na]^+$, calcd for $C_{48}H_{89}NO_{30}$ 1159.6.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-allyl-trehalose (13). To a suspension of **12** (3.1 g, 9.1 mmol) in dry DMF (100 mL), NaH (3.8 g, 99 mmol) and allyl bromide (8 mL, 95 mmol) were added and the reaction mixture was stirred overnight at room temperature. 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 sodium chloride solution (2×) and water (6×), dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography (toluene-EtOAc 7:1) to yield **13** (4.22 g, 70%) as a colorless syrup: $[\alpha]_D$ +134.8 (*c* 1.27, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) 6.03−5.77 (m, 8H), 5.34−5.20 (m, 8H), 5.20−5.07 (m, 10H), 4.36, 4.32, 4.25 (each m, each 2H), 4.16−4.01 (m, 10H), 3.96 (m, 2H), 3.70 (t, 2H, *J* = 9.2 Hz), 3.62 (dd, 2H, *J* = 10.7, 3.6 Hz), 3.54 (dd, 2H, *J* = 10.7, 2.0 Hz), 3.45 (t, 2H, *J* = 9.7 Hz), 3.37 (dd, 2H, *J* = 9.7, 4.1 Hz) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$) 135.9, 135.5, 135.2, 135.0, 117.5, 117.1, 117.1, 116.6, 94.6, 81.6, 79.6, 77.7, 74.6, 74.3, 72.9, 72.2, 70.8, 68.8 ppm. Anal. Calcd for $C_{36}H_{54}O_{11}$: C, 65.24; H, 8.21. Found: C, 65.23; H, 8.18.

2,3,4,6,2′,3′,4′,6′-Octa-*O*-(3-hydroxypropyl)trehalose (14). To a solution of **13** (0.37 g, 0.56 mmol) in dry THF (20 mL), 9-BBN-H (0.5 M solution in THF, 20 mL, 10 mmol) was added and the reaction mixture was stirred for 1 h under reflux. The excess of reagent was destroyed with ice water and then oxidation was achieved by treatment with 3 M aqueous NaOH (10 mL) and dropwise addition of 30% hydrogen peroxide (10 mL). The reaction mixture was stirred overnight and saturated with K_2CO_3, and the phases were separated. The aqueous phase was washed with THF (2×) and the combined organic phases were concentrated and purified on Sephadex LH-20 (MeOH) followed by flash chromatography (CH_2Cl_2−MeOH 3:1) to yield **14** (372 mg, 83%) as a colorless syrup: $[\alpha]_D$ +70.1 (*c* 2.19, MeOH); ^{1}H NMR (400 MHz, MeOH-d_4) 5.20 (d, 2H, *J* = 3.1 Hz), 3.98−3.84 (m, 8H), 3.79−3.58 (m, 32H), 3.35−3.28 (m, 4H), 1.92−1.77 (m, 16H) ppm; ^{13}C NMR (100.6 MHz, MeOH-d_4) 94.9, 83.8, 82.7, 80.5, 73.3, 72.6, 72.0, 71.9, 70.7, 70.5, 61.5, 61.3, 61.2, 61.2, 35.7, 35.5, 35.4, 34.8 ppm; MALDI-TOF-MS *m*/*z* 829.4 $[M + Na]^+$, calcd for $C_{36}H_{70}O_{19}$ 806.5. Anal. Calcd for $C_{36}H_{70}O_{19}$ 2 H_2O: 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)]trehalose (15). A solution of **14** (0.050 g, 0.062 mmol) and **7** (5 g, 6.7 mmol) in dry acetonitrile (400 mL) was heated to 65 °C, TMS−OTf (0.5 mL) was added and the reaction mixture was stirred at this temperature for 1 h. Additional **7** (1.2 g, 1.6 mmol) was added and the solution was stirred overnight at room temperature. The reaction mixture was neutralized with $NaHCO_3$ (10 g), filtered, concentrated and purified on Sephadex LH-20 (CH_2Cl_2−MeOH 1:1) followed by flash chromatography (hexanes−EtOAc 9:11) to yield **15** (318 mg, 95%) as a white amorphous solid: $[\alpha]_D$ −20.3 (*c* 0.64, CH_2Cl_2); ^{1}H NMR (500 MHz, DMSO-d_6, 403 K) 8.02−7.92 (m, 32 H), 7.87−7.82 (m, 16 H), 7.72−7.65 (m, 16 H), 7.64−7.56 (m, 16 H), 7.52−7.20 (m, 80 H), 6.03−5.95 (m, 8 H), 5.88, 5.85, 5.85, 5.84 (each dd, each 2H, *J* = 2.8, 9.9 Hz), 5.71, 5.68, 5.68, 5.65 (each dd, each 2H, *J* = 1.7, 3.0 Hz), 5.24 (d, 2 H, *J* = 3.6 Hz), 5.23, 5.18, 5.16, 5.13 (each d, each 2H, *J* = 1.7 Hz), 4.67−4.47 (m, 24H), 4.05−3.64 (m, 40H), 3.40 (t, 2 H, *J* = 9.1 Hz), 3.39 (dd, 2 H), 2.11−1.95 (m, 16H) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$) 167.5−166.5, 134.5−134.3, 131.3−129.5, 99.3, 99.0, 89.9, 89.9, 95.1, 83.0, 81.7, 79.1, 72.3, 72.0, 71.9, 71.9, 71.8, 71.7, 71.6, 71.5, 71.5, 70.2, 70.1, 70.1, 70.0, 70.7, 70.5, 69.6, 69.3, 68.3 (3×), 68.1, 67.5, 67.2, 66.6 (2×), 64.2, 64.2, 64.1 (2×), 32.0, 31.8, 31.7, 31.3 ppm; MALDI-TOF-MS *m*/*z* 5454.3 $[M + Na]^+$, calcd for $C_{308}H_{278}O_{91}$ 5431.7. Anal. Calcd for $C_{308}H_{278}O_{91}$: C, 68.06; H, 5.16. Found: C, 67.77; H, 5.06.

6-d-Biotinamidohexyl 2,3,4,6-Tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-*β*-D-glucopyranoside (17). To a solution of **11** (0.026 g, 0.022 mmol) in aqueous $NaHCO_3$ (0.02M, 8 mL), sulfosuccinimidyl biotin (0.010 g, 0.023 mmol) was added. The solution was stirred for 5 h at room temperature, then poured onto Biogel-P2 and eluted with water to yield **17** (31 mg, quant.) as a white lyophilisate: ^{1}H NMR (500 MHz, D_2O) 4.80 (s, 4H), 4.55 (dd, 1H, *J* = 4.6, 8.1 Hz), 4.37 (m, 2H), 3.92−3.68 (m, 28H), 3.65−3.51 (m, 16H), 3.42 (m, 1H), 3.37−3.23 (m, 3H), 3.18−3.00 (m, 3H), 2.95 (dd, 1H, *J* = 5.1, 13.2 Hz), 2.73 (d, 1H, *J* = 12.7 Hz), 2.20 (t, 2H, *J* = 7.1 Hz), 1.94−

1.81 (m, 8H), 1.75−1.53 (m, 6H), 1.50−1.44 (m, 2H), 1.41−1.27 (m, 6H) ppm; ^{13}C NMR (100.6 MHz, D_2O, DEPT) 102.8, 100.1 (4×), 84.2, 81.9, 78.2, 74.0, 73.1 (4×), 71.4, 71.1, 71.0 (4×), 70.7, 70.6, 70.5 (4×), 69.2, 68.5, 67.1 (4×), 64.8 (2×), 64.7-(2×), 62.5, 61.3 (4×), 60.6, 56.5, 55.8, 40.1, 39.6, 35.9, 29.9, 29.8, 29.7, 29.1, 29.0, 28.7, 28.2, 28.1, 26.1, 25.6, 25.3 ppm; MALDI-TOF-MS *m*/*z* 1408.7 $[M + Na]^+$, calcd for $C_{58}H_{103}N_3O_{32}S$ 1385.6.

6-Carboxyfluoresceinylaminohexyl 2,3,4,6-Tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-*β*-D-glucopyranoside (19). To a solution of **11** (0.055 g, 0.047 mmol) and $NaHCO_3$ (100 mg) in MeOH (8 mL), 5(6)-carboxy-fluorescein-*N*-succinimidyl ester (25 mg) was added. The yellow solution was stirred for 5 h at room temperature in the dark, then poured onto Sephadex LH-20 and eluted with MeOH to yield **19** (21 mg, 29%) as a very instable light-sensitive, red solid: ^{13}C NMR (100.6 MHz, D_2O, DEPT) 131.7, 129.3, 128.6, 123.6, 102.8, 100.2 (4×), 84.2, 81.8, 78.1, 73.9, 73.1 (4×), 71.2, 71.0 (4×), 70.6, 70.5 (4×),70.3 (2×), 67.2 (2×), 67.1 (2×), 64.8−64.7 (4×), 61.3−61.2 (4×), 40.4, 29.9, 29.8 (2×), 29.0 (2×), 28.4, 25.9, 25.1 ppm; MALDI-TOF-MS *m*/*z* 1540.3 $[M + Na]^+$, calcd for $C_{69}H_{99}NO_{36}$ 1517.6.

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Supporting Information Available: Additional experimental data for **7**, **14**, **18**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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