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Oligosaccharide synthesis on a soluble, hyperbranched polymer support via thioglycoside activation

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Abstract—The synthesis of linear and branched di-, tri- and tetramannosides on a commercially available hyperbranched polyester as a soluble, high loading support is described. Glycosylation products were isolated in 26–63% yield as mixtures of anomers after total hydrolytic degradation of the polymer. All polymer-bound intermediates were purified through simple extraction or precipitation. Solution-phase NMR and MALDI-TOF were used to monitor the progress of the reaction directly on the hyperbranched polymer support.

1. Introduction

The development of insoluble polymeric supports[1] to facilitate the purification of synthetic intermediates has dramatically streamlined the synthesis of natural and unnatural molecules.[2] However, the lower kinetic reactivity of polymer-supported substrates, which depends greatly on the swelling characteristics of the polymer–substrate conjugate, impedes the routine application of solution-phase synthetic procedures to the solid phase. The use of highly-crosslinked, macroporous supports that do not require swelling for reactivity,[3] and insoluble resins grafted with soluble linear[4] or dendritic polymers,[5] partially circumvents these kinetic impediments. Similarly, the solubility of non-crosslinked polymers, such as linear polystyrene (PS) and poly(ethylene glycol) (PEG),[6] permits supported reactions to be performed in solution. However, the practical utility of many of these linear supports is limited by low loading capacities. Dendrimers[7] and hyperbranched polymers[8] possess an $(x-1)n+1$ ($n=$ degree of polymerization) number of terminal groups for an AB_x-type repeat unit. Accordingly, polyamidoamine (PAMAM),[9] carbosilane,[10] polyglycerol,[11] and dye-conjugated[12] dendrimers have been employed as high-loading, soluble supports for organic synthesis.[13] The utility of dendrimer-based supports is severely limited by the high cost of their synthesis. However, a perfectly branched dendrimer architecture is not absolutely required for efficacy as a high loading support. For example, hyperbranched polymers are highly branched polymers that are prepared in a single synthetic step with degrees of branching that are typically less than 50%. Therefore, these imperfect analogs may serve as practical, low cost alternatives to dendrimers (Fig. 1). Despite the tremendous potential of these readily accessible materials to serve as inexpensive, high-loading dendritic supports, they have received only limited attention as synthetic[14] or catalyst[15] supports.

Although the preparation of peptides[16] and nucleotides[17] on solid phase has become routine, oligosaccharide synthesis on polymer supports[18] remains problematic due to the structural complexity of oligosaccharides and difficulties associated with glycosyl bond formation. However, recent success in the automation of solid-phase oligosaccharide

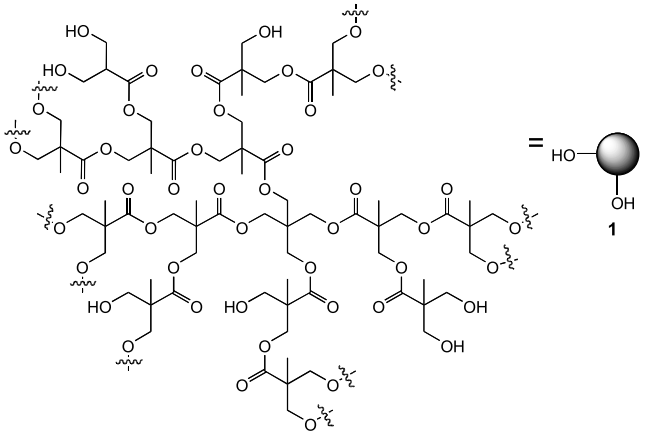

Figure 1. The hyperbranched polymer (Boltorn™).

Keywords: Carbohydrates; Glycosylations; Polymers; Dendrimers; Solid-phase.
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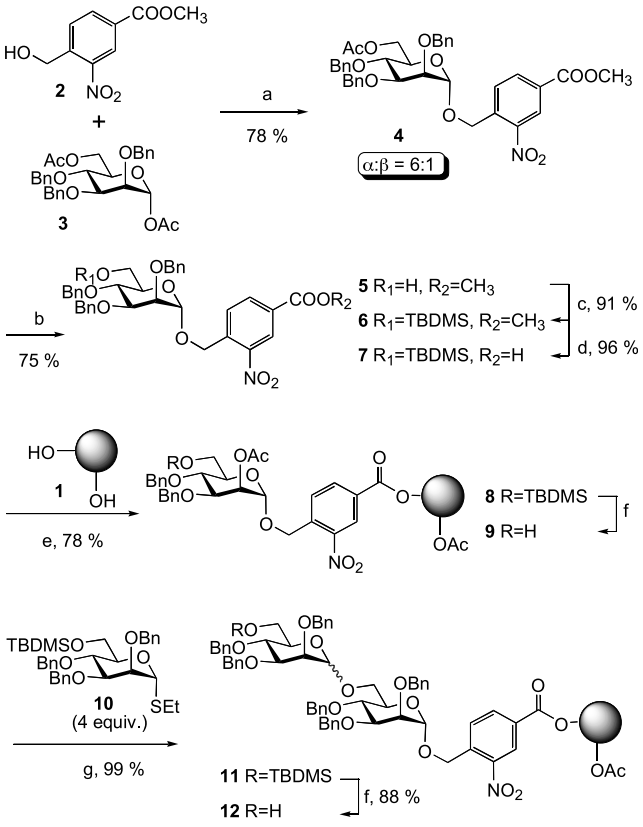
synthesis bodes well for the development of a routine solid-phase approach to these molecules.[19] In a preliminary communication,[20] we demonstrated that the synthesis of disaccharides via thioglycoside activation[21] could be performed using this hyperbranched polymer as a support. We report herein the extension of this approach to the preparation of linear and branched di-, tri- and tetrasaccharides. This paper focuses on hyperbranched polymer supported assembly of oligomannosides[22,23] because of their important role as a structural constituent of *N*-glycans.[24]

2. Results and discussion

The hyperbranched polymer support employed in this study is constructed via an acid-catalyzed polymerization of dimethylolpropionic acid in the presence of pentaerythritol as a central core (Fig. 1, commercially available as Boltorn™ H-50).[25] This support exhibits several properties that facilitate purification and analysis of polymer-bound intermediates: (1) the polymer-bound intermediates tend to exhibit high solubility in most aprotic solvents but very low solubility in methanol, from which they can be quantitatively precipitated. Although purification by size exclusion chromatography (SEC) remains as a potential purification method, we found that precipitation was much more expedient, especially on preparative scale. (2) Direct mass spectral analysis of the polymer-bound disaccharides can be achieved by photolytic release of the disaccharide from the support with the MALDI-TOF laser. (3) The support undergoes rapid hydrolytic degradation to water-soluble materials thereby permitting product purification by extraction. This method typically provides more efficient cleavage from the support than photolysis of the 2-nitrobenzyl linkage. (4) The high intrinsic loading capacity of Boltorn™ H-50 polymer (8.8 mmol/g OH-groups; nominal M_w = 14,500, pdi = 2.0) permits relatively large amounts of substrates per gram of polymer support to be immobilized.

2.1. O-6 Glycoside bond

2.1.1. Preparative scale synthesis of a supported disaccharide. The 2-nitrobenzyl alcohol photolabile linker (**2**)[26] was glycosylated with 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-mannose (**3**)[27] affording the mannoside **4** (α:β = 6:1) in 78% yield (Scheme 1). The anomers could not be separated and were carried through the rest of the synthesis as a mixture. Deprotection of the 6-*O*-acetyl group with potassium carbonate in methanol–THF followed by silylation with TBDMSCl/Et_3N afforded the methyl ester **6**. Subsequent hydrolysis in a biphasic mixture of THF and aqueous KOH provided the carboxylic acid **7**, which was coupled to the Boltorn H-50 polyester (**1**) using EDCI in THF–pyridine. After an extractive aqueous work-up, the polymer-mannoside conjugate **8** was desilylated with excess HF·pyridine and glycosylated with the thioethyl donor **10**[28] affording polymer-supported disaccharide **11** (99% for both steps, loading level: 0.59 mmol/g).[29] Desilylation with HF–pyridine provided multigram quantities of the polymer-acceptor conjugate **12** in 88% yield (loading level: 0.64 mmol/g).[29b]

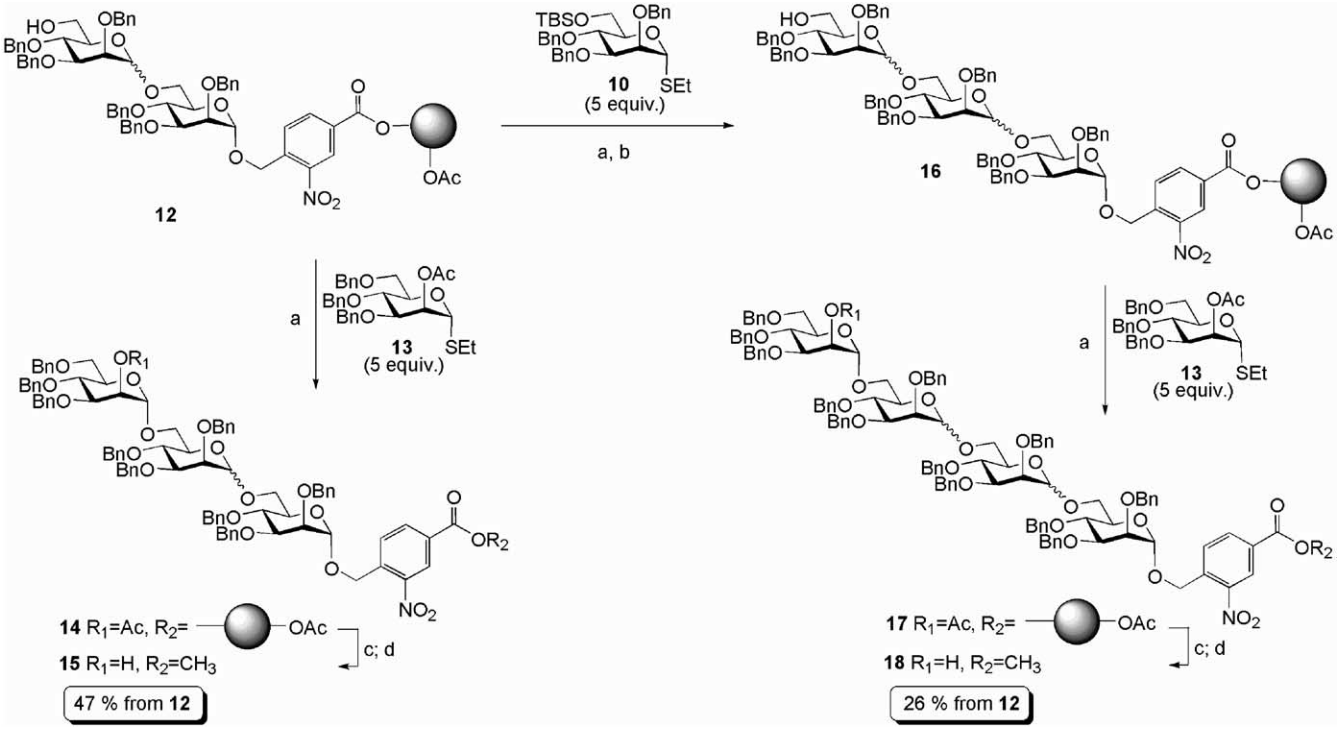


Scheme 1. Reagents and conditions: (a) $BF_3 \cdot OEt_2$, CH_2Cl_2, 0 °C; (b) K_2CO_3, MeOH–THF; (c) TBDMSCl, cat. DMAP, Et_3N, CH_2Cl_2; (d) NaOH, THF–H_2O, then dil H_2SO_4; (e) EDCI, cat. DMAP, THF–pyridine, then CH_3COCl; (f) HF–pyridine, THF; (g) NIS, cat. TfOH, CH_2Cl_2, −40 °C.

2.1.2. Synthesis of linear tri- and tetrasaccharides. Linear tri-and tetrasaccharides were prepared from **12** by an iterative glycosylation–deprotection sequence (Scheme 2). Glycosylation of **12** with 5 equiv of the thioethyl glycoside donor **13**[30] using *N*-iodosuccinimide (NIS) and cat. trifluoromethanesulfonic acid (TfOH) in acetonitrile at −40 °C followed by precipitation into methanol afforded polymer-bound trisaccharide **14**. Hydrolytic degradation of the support with NaOH in H_2O–THF at 65 °C and treatment with diazomethane[31] afforded the trisaccharide **15** as a mixture of anomers in 47% yield after chromatographic purification. Similarly, treatment of **12** with the activated thioglycoside **10**, silyl deprotection with HF–pyridine, and further glycosylation of trisaccharide **16** with **13** afforded the tetrasaccharide **18** in 26% isolated yield as a mixture of anomers following hydrolytic liberation of the product from the polymer support. The complexity of the NMR spectra of the oligosaccharide products **15** and **18** did not allow the ratios of the individual anomers to be determined. However, in the case of **15** small amounts of pure trisaccharides were obtained and their gate-decoupled ^{13}C NMR spectra were consistent with α,α,α (δ 98.4 ($^1J_{C-H}$ = 168.5 Hz), 100.7 ($^1J_{C-H}$ = 171.7 Hz), 102.4 ($^1J_{C-H}$ = 164.5 Hz)) and α,β,α (δ 98.4 ($^1J_{C-H}$ = 173.4 Hz), 98.5 ($^1J_{C-H}$ = 156.8 Hz), 100.2 ($^1J_{C-H}$ = 173.4 Hz)) configurations.[32]

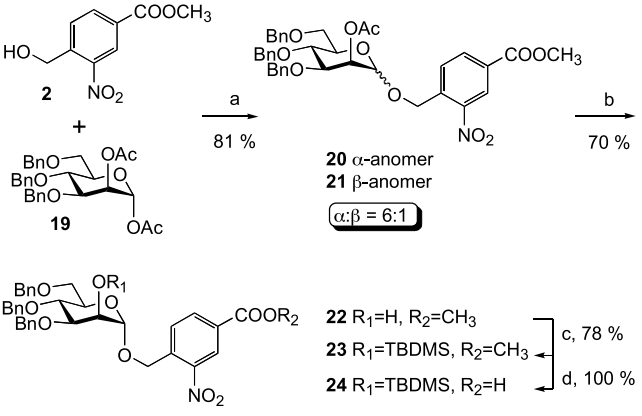
2.2. O-2 Glycoside bond: disaccharide synthesis

Mannose–mannose α-glycosidic bonds in *N*-glycans occur

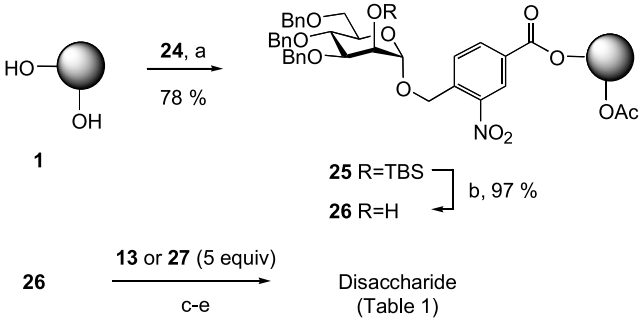


Scheme 2. Reagents and conditions: (a) NIS-cat. TfOH, CH_3CN, −40 °C; (b) HF·pyridine, THF; (c) NaOH, THF–H_2O, 65 °C, then dil H_2SO_4; (d) CH_2N_2, ether.

most often at C-6, C-2 and more rarely at C-3 hydroxyl groups.[23] Accordingly, disaccharides were elaborated from C-2 on the hyperbranched polymer support. The 2-nitrobenzyl linker-mannose conjugate (**24**) was prepared from 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-mannose[33] (**19**) via a $BF_3 \cdot OEt_2$-promoted glycosylation with linker alcohol **2** as shown in Scheme 3. The acetyl protected mannoside was formed as a mixture of two anomers (**20**, **21**) in a 6:1 ratio. It is noteworthy that hydrolysis of the acetoxy group in β-anomer **21** proceeded much slower than for α-anomer **20**, presumably a consequence of the increased steric bulk of the β-substituent. Therefore, selective hydrolysis of the α-anomer **20** provided **22** in 70% isolated yield as a single anomer, along with 7% of recovered **21**. The β-configuration of **21** was confirmed by gate-decoupled ^{13}C NMR (δ 99.4, $^1J_{C-H} = 155.3$ Hz).[32] Silylation of the 2-hydroxyl group in **22** with TBDMSOTf and lutidine[34] followed by saponification of the methyl ester provided the carboxylic acid **24**, which was coupled with EDCI to the Boltorn™ H-50 (**1**) polymer support in 86% yield (loading level: 0.64 mmol/g).[29b] Deprotection of the axial 2-OTBDMS group in the product (**25**) proved to be significantly more difficult in comparison with the 6-OTBDMS analog **11**, most likely for steric reasons (Scheme 4). Accordingly, exposure to a large excess of HF–pyridine in THF at reflux was required to achieve 95% deprotection of the TBDMS groups. Fortuitously, the factors responsible for hindering C-2 desilylation did not impede glycosylation of the C-2 hydroxyl group in the polymer-bound acceptor **26**. Accordingly, glycosylation of **26** with donors **13** and **27**[35] provided disaccharides **28** in 41% and **29** in 63% isolated yield as pure α-anomers (Scheme 4, Table 1).

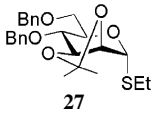
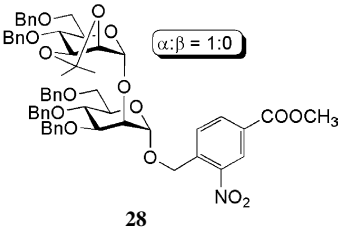
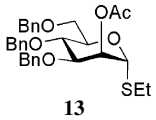
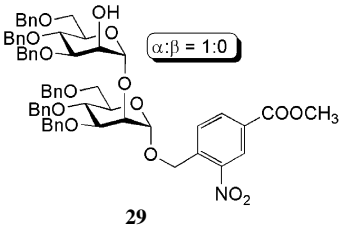


Scheme 3. Reagents and conditions: (a) $BF_3 \cdot OEt_2$, CH_2Cl_2, 0 °C; (b) K_2CO_3, MeOH–THF (from **20**; **21** was recovered in 7% isolated yield); (c) TBDMSOTf, 2,6-lutidine, CH_2Cl_2; (d) NaOH, THF–H_2O, then dil H_2SO_4.



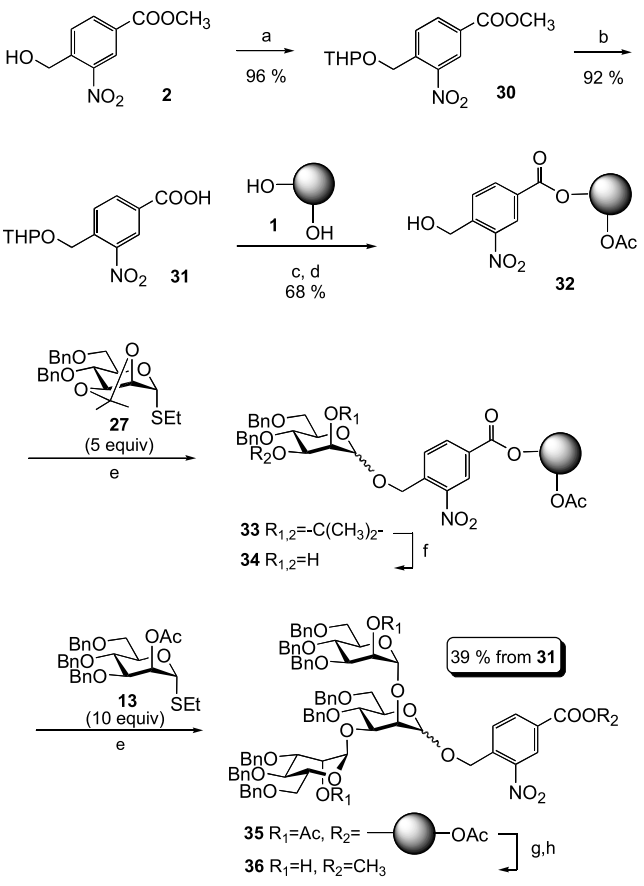
Scheme 4. Reagents and conditions: (a) EDCI, cat. DMAP, THF–pyridine, then excess CH_3COCl; (b) HF·pyridine, THF, 65 °C; (c) NIS-cat. TfOH, CH_3CN, −40 °C; (d) KOH, THF–H_2O, 65 °C, then dil H_2SO_4; (e) CH_2N_2, ether.

Table 1. Disaccharides extended from C-2 (Scheme 4)

Donor	Disaccharide	Yield (%)
 **27**	 **28**	63
 **13**	 **29**	41

2.3. Synthesis of 2,3-branched trisaccharides

The synthesis of 2,3-branched oligosaccharides was addressed using 2,3-isopropylidene thioethyl donor **27** as a protected branching unit (Scheme 5). Accordingly, polymer-supported linker **32**, prepared by EDCI mediated coupling of 3-nitro-4-(tetrahydropyran-2-yloxymethyl)benzoic acid, **31**, to the Boltorn™ H-50 polymer (loading 1.36 mmol/g)[29b] and acid-catalyzed THP deprotection, was glycosylated with the glycosyl donor **27**. Cleavage of the 2,3-isopropylidene protecting group in **33** with 1,3-propanedithiol–trifluoroacetic acid (TFA) followed by simultaneous glycosylation at O-2 and O-3 with donor provided supported trisaccharide **35** after precipitation with methanol. Release of the product by treatment with aqueous KOH and methylation with diazomethane afforded branched trisaccharide **36** as a mixture of anomers in 39% overall yield from **32**, after chromatographic purification. The ratios of the individual anomers could not be determined due to the complexity of the ^{1}H NMR spectrum of **36**. However, inspection of the gate-decoupled ^{13}C NMR spectrum[32] revealed that α,α,α-stereoisomer was the major constituent of the mixture.



Scheme 5. Reagents and conditions: (a) DHP, cat. TsOH, CH_2Cl_2; (b) KOH, H_2O–THF, then dil H_2SO_4; (c) EDCI, cat. DMAP, THF–pyridine, then excess CH_3COCl; (d) cat. HCl, MeOH–CH_2Cl_2; (e) NIS-cat. TfOH, CH_3CN, −40 °C; (f) $HS(CH_2)_3SH$, TFA, CH_2Cl_2; (g) KOH, THF–H_2O, 65 °C, then dil H_2SO_4; (h) CH_2N_2, ether.

3. Conclusion

This work demonstrates the potential for hyperbranched polymers to serve as high loading, soluble supports for multistep synthesis of large, complex molecules. We prepared linear and branched di-, tri-, and tetrasaccharides on the hyperbranched polyester Boltorn™ H-50 as a soluble support in good yields on a preparative scale. Boltorn polyester shares numerous advantageous characteristics with linear polymers previously used as soluble supports. For example, separation from soluble by-products and excess of reagents can be accomplished by simple precipitation from a poor solvent (methanol).[36] The high solubility of all protected intermediates in most organic solvents ensured that synthetic protocols and analytical techniques used for conventional chemistry (solution ^{1}H and ^{13}C NMR and MALDI-TOF MS) could be applied to the supported reactions and intermediates with minimal changes, and specialized equipment was not required. In contrast to the traditional linear soluble supports, the high loading capacity (theoretically 8.8 mmol/g) and low cost (commercially available at US$ 5/kg) allows economical, large scale preparations to be possible. As a potential drawback, the base-labile nature of the polyester backbone limits the range of reactions and conditions applicable to Boltorn polymer supports. However, the preparation of new, more chemically robust hyperbranched polymers from readily available starting materials should remedy this problem.

4. Experimental

4.1. General

THF was distilled from sodium/benzophenone ketyl; CH_3CN, CH_2Cl_2 and pyridine were distilled from calcium hydride; methanol was dried over 3 Å molecular sieves. Chromatographic separations were performed on silica gel 60 (230–400 mesh, 60 Å) using the flash technique in the indicated solvents as mobile phase. TLC was performed on silica gel 60-F_{254} plates. Visualization of the compounds was accomplished by UV-detection (254 nm) or staining with 10% H_2SO_4. The solvents used for extraction and

column chromatography were removed on a rotary evaporator (40 mm Hg). All glycosylation reactions were performed under anhydrous conditions under an argon atmosphere. The precipitated polymers were centrifuged in an Eppendorf centrifuge at 5000 rpm for time sufficient to achieve complete precipitation (5–60 min). NMR spectra were recorded at Bruker DPX-250, AC-300, DPX-400 and DRX-500 spectrometers, referenced to the residual deuterated solvent peaks and the chemical shifts expressed with respect to tetramethylsilane (TMS). MALDI-TOF mass spectra were recorded using 2,3-dihydrobenzoic acid as matrix in THF. Elemental analyzes were obtained at Atlantic Microlabs, Norcross, GA. All samples for elemental analysis were dried for 16 h in vacuum over P_2O_5 at 56 °C (refluxing acetone).

4.1.1. 4-Methoxycarbonyl-2-nitrobenzyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranoside (4). Methyl 4-hydroxymethyl-2-nitrobenzoate **2** (3.51 g, 6.6 mmol, 1 equiv) and **3** (1.52 g, 7.2 mmol, 1.09 equiv) and were dissolved in dry CH_2Cl_2 (12 mL). The solution was cooled in an ice bath and $BF_3 \cdot OEt_2$ (4.1 mL, 4.68 g, 33 mmol, 5 equiv) were added. After stirring at 0 °C for 6 h, the solution was diluted with CH_2Cl_2 (50 mL), washed with water (50 mL), saturated $NaHCO_3$ solution (2×100 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and the solvent was evaporated. The acetate **4** (3.54 g, 5.1 mmol, 78%) was obtained as a pale yellow foam after column chromatography (toluene/ether, 6:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (s, 3H), 3.78–3.85 (m, 2H), 3.92–4.05 (m, 5H), 4.14–4.39 (m, 2H), 4.60–5.19 (m, 9H), 7.54 (d, J=8.1 Hz, 1H), 8.24 (dd, J=8.1, 1.7 Hz, 1H), 8.70 (d, J=1.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7, 52.6, 63.3, 65.6, 70.7, 71.8, 72.2, 74.3, 74.3, 75.1, 79.5, 97.9, 125.8, 127.5–129.2 (multiple carbons), 130.5, 133.9, 137.8, 138.0, 138.2, 147.1, 164.6, 170.7. Anal. Calcd for $C_{38}H_{39}NO_{11}$: C, 66.56; H, 5.69; N, 2.04. Found: C, 66.82; H, 5.69; N, 2.10.

4.1.2. 4-Methoxycarbonyl-2-nitrobenzyl 2,3,4-tri-*O*-benzyl-α/β-D-mannopyranoside (5). The acetate **4** (7.17 g, 10.5 mmol, 1 equiv) was dissolved in a mixture of THF (15 mL) and methanol (45 mL) and cooled in an ice bath. Finely powdered K_2CO_3 (2.77 g, 20 mmol, 1.90 equiv) was added and the mixture stirred for 1 h 15 min. The solution was acidified with 10% aqueous H_2SO_4 (5 mL), the organic solvents were removed and the organic material was extracted in ethyl acetate (100 mL). The organic layer was washed with saturated $NaHCO_3$ solution (2×50 mL), brine (30 mL), dried ($MgSO_4$) and evaporated. The alcohol **5** (5.06 g, 7.88 mmol, 75%) was obtained as pale yellow foam after column chromatography (hexane/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (broad s, 1H), 3.75–3.81 (m, 2H), 3.85–4.08 (m, 8H), 4.61–5.09 (m, 8H), 7.08–7.43 (m, 15H), 7.30–7.48 (m, 15H), 7.61 (d, J=8.1 Hz, 1H), 8.23 (dd, J=8.1, 1.5 Hz, 1H), 8.69 (d, J=1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.7, 62.1, 65.8, 72.3, 72.9, 72.9, 74.5, 74.5, 75.2, 79.5, 98.4, 125.8, 127.5, 127.5–129.2 (multiple carbons), 130.5, 134.0, 137.9, 138.1, 138.2, 138.2, 147.1, 164.6. Anal. Calcd for $C_{36}H_{37}NO_{10}$: C, 67.17; H, 5.79; N, 2.18. Found: C, 67.44; H, 5.91; N, 1.93.

4.1.3. 4-Methoxycarbonyl-2-nitrobenzyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α/β-D-mannopyranoside (6). To a solution of **5** (2.46 g, 3.8 mmol, 1 equiv) in dry CH_2Cl_2 (10 mL) and Et_3N (2.8 mL), DMAP (50 mg, 0.4 mmol, 0.11 equiv) and TBDMSCl (0.60 g, 4.0 mmol, 1.05 equiv) were added in succession and the solution stirred for 4 h. The mixture was diluted with ethyl acetate (50 mL), washed successively with water (30 mL), 5% H_2SO_4 (3×30 mL), saturated $NaHCO_3$ solution (2×30 mL), brine (20 mL), dried ($MgSO_4$) and evaporated. After column chromatography (hexane/ethyl acetate, 7:1), **6** (2.63 g, 3.5 mmol, 91%) was obtained as a yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.13 (s, 6H), 0.94 (s, 9H), 3.63–3.67 (m, 1H), 3.91–4.01 (m, 3H), 4.02–4.06 (m, 5H), 4.70–5.16 (m, 8H), 7.24–7.36 (m, 15H), 7.69 (d, J=8.1 Hz, 1H), 8.29 (dd, J=8.1, 1.3 Hz, 1H), 8.75 (d, J=1.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.77, −4.73, 18.2, 26.3, 53.2, 63.8, 65.9, 72.8, 72.9, 73.1, 74.6, 75.1, 75.4, 80.0, 98.2, 126.3, 128.1–128.8 (multiple carbons), 129.1, 130.9, 134.5, 138.7, 138.8, 139.0, 139.2, 147.6, 165.2. Anal. Calcd for $C_{42}H_{51}NO_{10}Si$: C, 66.56; H, 6.78; N, 1.85. Found: C, 66.60; H, 6.63; N, 1.74.

4.1.4. 4-Oxycarbonyl-2-nitrobenzyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α/β-D-mannopyranoside (7). Solution of **6** (2.63 g, 3.47 mmol, 1 equiv) in THF (30 mL) was mixed with freshly prepared 2 M aqueous KOH solution (30 mL). The mixture was stirred for 6 h, then acidified with 10% H_2SO_4 (pH=3) and extracted with ethyl acetate (3×30 mL), washed with water (5×30 mL), dried ($MgSO_4$) and evaporated. The carboxylic acid **7** (2.48 g, 3.33 mmol, 96%) was obtained as pale yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.13 (s, 6H), 0.94 (s, 9H), 3.63–3.67 (m, 1H), 3.91–4.01 (m, 3H), 4.02–4.06 (m, 5H), 4.70–5.16 (m, 8H), 7.24–7.36 (m, 15H), 7.69 (d, J=8.1 Hz, 1H), 7.80 (broad s, 1H), 8.29 (dd, J=8.1, 1.3 Hz, 1H), 8.75 (d, J=1.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.83, −4.73, 18.2, 26.3, 53.2, 63.8, 65.9, 72.8, 74.7, 73.1, 75.2, 75.3, 75.7, 89.9, 98.1, 126.3, 128.1–128.8 (multiple carbons), 129.1, 130.9, 134.5, 138.7, 138.8, 139.0, 139.2, 147.5, 168.9. Anal. Calcd for $C_{41}H_{49}NO_{10}Si$: C, 66.20; H, 6.64; N, 1.88. Found: C, 66.47; H, 6.64; N, 1.71.

4.1.5. 4-Methoxycarbonyl-2-nitrobenzyl 6-(6-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranoside (15). A. To a solution of **7** (2.30 g, 3.09 mmol, 1 equiv), Boltorn H-50 polymer **1** (1.41 g, 12.4 mmol OH-groups, 4 equiv) and DMAP (42 mg, 0.34 mmol, 0.11 equiv) in dry pyridine (3.7 mL) and dry THF (4.8 mL), EDCI (0.65 g, 3.40 mmol, 1.10 equiv) was added and the mixture stirred for 16 h. It was then cooled in an ice bath and acetyl chloride (0.83 mL, 0.92 g, 11.7 mmol, 3.78 equiv) was added dropwise. The mixture was warmed up to room temperature and stirred for additional 8 h, poured in water (100 mL) and extracted with ethyl acetate (2×30 mL). The combined organic extracts were washed with 10% H_2SO_4 (2×30 mL), saturated $NaHCO_3$ solution (2×30 mL), brine (30 mL), dried ($MgSO_4$) and evaporated, affording **8** (4.20 g, 2.60 mmol, 84%, loading level: 0.62 mmol/g) as pale yellow foam.

B. A solution of **8** from the previous step in THF (25 mL) was then treated with HF·pyridine (0.83 g, 8.33 mmol,

3.19 equiv) and the solution stirred for 16 h. The solution was diluted with ethyl acetate (50 mL) and washed with water (50 mL), 10% H_2SO_4 (2×20 mL) and saturated $NaHCO_3$ solution (2×20 mL). After drying ($MgSO_4$) and evaporation, **9** (3.80 g) was obtained as a pale yellow foam. This material was used directly for the next step.

C. The polymer-immobilized acceptor **9** (3.80 g, 2.61 mmol, 1 equiv) and the thioethyl glycoside **10** (6.09 g, 10.40 mmol, 4 equiv) were dissolved in dry CH_2Cl_2 (10 mL). After a clear solution had formed, the flask was cooled to −40 °C. NIS (2.56 g, 11.40 mmol, 4.40 equiv) was added, followed by TfOH (90 μL, 156 mg, 1.04 mmol, 0.40 equiv). After stirring for 30 min at −40 °C, the mixture was diluted with CH_2Cl_2 (50 mL), washed with 10% $NaHSO_3$ solution (100 mL), saturated $NaHCO_3$ solution (50 mL), brine (10 mL), dried ($MgSO_4$) and concentrated in vacuum. The crude polymer was dissolved in CH_2Cl_2 (10 mL) and precipitated out of methanol (250 mL). After decanting of the supernatant, rinsing with methanol (3×10 mL) and drying in vacuum, the disaccharide **11** (4.36 g, mmol, 99%, loading level: 0.59 mmol/g) was obtained as a pale yellow foam.

D. From **11** (4.22 g, 2.57 mmol, 1 equiv), dissolved in THF (20 mL) and HF·pyridine (0.57 g, 5.66 mmol, 2.20 equiv), **12** (3.52 g, mmol, 88%, loading level: 0.64 mmol/g) was obtained as a yellow foam following the procedure (Part B) for preparation of **9**.

E. The glycosyl acceptor **12** (156 mg, 0.1 mmol, 1 equiv) and the glycosyl donor **13** (0.27 g, 0.5 mmol, 5 equiv) were dissolved in dry CH_3CN (0.6 mL). After stirring at room temperature for 15 min, the mixture was cooled to −40 °C. NIS (135 mg, 0.6 mmol, 6 equiv) and TfOH (2 μL, 3.4 mg, 0.022 mmol, 0.20 equiv) were added in succession. After stirring for 20 min at −40 °C, the reaction mixture was diluted with ethyl acetate (5 mL) and washed with 10% $NaHSO_3$ solution (5 mL), saturated $NaHCO_3$ solution (5 mL) and brine (2 mL). After drying ($MgSO_4$) and concentrating in vacuum, the polymer-immobilized trisaccharide **14** (194 mg) was purified by dissolving in CH_2Cl_2 (0.5 mL) and precipitating out of methanol (15 mL), decanting the supernatant, rinsing with methanol (3× 3 mL) and drying under high vacuum.

F. The immobilized trisaccharide **14** was heated at reflux in a mixture of THF (2 mL) and 2 M aqueous NaOH solution (2 mL) for 16 h. H_2SO_4 (10%) was added (pH=3) and the product was extracted into ethyl acetate (3×5 mL). The combined organic extracts were washed with water (5× 10 mL), brine (5 mL), dried ($MgSO_4$) and evaporated. The crude product was dissolved in diethyl ether (2 mL) and an excess of freshly prepared diazomethane solution in diethyl ether was added dropwise until the evolution of N_2 had ceased. The excess of diazomethane was destroyed by dropwise addition of glacial acetic acid. The ether solution was washed with saturated $NaHCO_3$ solution (2×5 mL), brine (5 mL), dried ($MgSO_4$) and evaporated. The trisaccharide **15** (73 mg, 0.047 mmol, 47%) was obtained as a pale yellow foam after chromatography on silica gel (hexane/ethyl acetate, 2:1). MALDI-TOF MS: *m/z* calcd for $C_{90}H_{93}NNaO_{20}$ $(M+Na)^+$: 1530.62, found: 1525.8. Anal. Calcd for $C_{90}H_{93}NO_{20}$: C, 71.65; H, 6.21; N, 0.93. Found: C, 71.25; H, 6.27; N, 0.97.

A small portion of the product was chromatographically separated into two fractions. The (α,α,α)-trisaccharide: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.89 (broad s, 1H), 3.63–4.03 (m, 22H), 4.12–4.17 (m, 1H), 4.51–4.96 (m, 22H), 5.03–5. 15 (m, 3H), 7.25–7.49 (m, 45H), 7.58 (d, J=8.1 Hz, 1H), 8. 23 (dd, J=8.1, 1.5 Hz, 1H), 8.66 (d, J=1.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.2, 66.2, 67.0, 68.1, 68.9, 69. 4, 71.5, 71.6, 71.8, 72.7, 72.9, 73.3, 73.8, 74.3, 74.5, 74.7, 74.7, 74.9, 75.1, 75.3, 75.5, 75.5, 75.7, 79.7, 80.2, 82.7, 98. 4 ($^1J_{C-H}$=168.5 Hz), 100.7 ($^1J_{C-H}$=171.7 Hz), 102.4 ($^1J_{C-H}$=164.5 Hz), 126.4, 128.0–128.9 (multiple carbons), 130.9, 134.5, 138.4–139.2 (multiple carbons), 147.4, 165.2.

The (α,β,α)-trisaccharide: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1. 89 (broad s, 1H), 3.63–4.03 (m, 22H), 4.12–4.17 (m, 1H), 4. 51–4.96 (m, 22H), 5.03–5.15 (m, 3H), 7.25–7.49 (m, 45H), 7.59 (d, J=8.0 Hz, 1H), 8.22 (dd, J=8.0, 1.3 Hz, 1H), 8.70 (d, J=1.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.2, 60. 8, 66.1, 67.4, 67.5, 68.4, 69.3, 71.6, 71.8, 71.9, 72.0, 72.8, 73.1, 73.5, 73.8, 74.7, 74.8, 74.9, 75.1, 75.4, 75.4, 75.6, 75. 6, 80.0, 80.0, 80.2, 98.4 ($^1J_{C-H}$=173.4 Hz), 98.5 ($^1J_{C-H}$= 156.8 Hz), 100.2 ($^1J_{C-H}$=173.4 Hz), 126.3, 127.9–128.9 (multiple carbons), 130.1, 134.5, 138.4–138.8 (multiple carbons), 147.8, 165.2.

4.1.6. 4-Methoxycarbonyl-2-nitrobenzyl 6-(6-(6-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranoside (18). From the glycosyl acceptor **12** (321 mg, 0.2 mmol, 1 equiv) and the glycosyl donor **10** (0.68 g, 1 mmol, 5 equiv) in dry CH_3CN (1 mL), following the procedure for preparation of compound **14**, polymer-supported, 6-*O*-TBDMS-protected trisaccharide (432 mg) was obtained as a pale yellow foam after precipitation from methanol (30 mL, containing five drops Et_3N), decanting of the supernatant, washing with methanol (3×2 mL) and drying in vacuum. The polymer was treated with HF·pyridine (0.5 mL, 0.5 g, 5.0 mmol, 2.50 equiv) in THF (3 mL), following the procedure for compound **12**. From the glycosyl acceptor **16** (obtained as described above, 0.1 mmol, 1 equiv) and the glycosyl donor **13** (0.54 g, 1 mmol, 5 equiv) in dry CH_3CN (1 mL), following the procedure for preparation of **14**, the polymer-supported tetrasaccharide **17** (534 mg) was obtained as a pale yellow foam after precipitation into methanol (30 mL), decanting of the supernatant, rinsing with methanol (3×2 mL) and drying in vacuum. From **17** (536 mg), following the general procedure for compound **15**, the tetrasaccharide (mixture of anomers) **18** (100 mg, 0.52 mmol, 26%) was obtained as a pale yellow foam after chromatography on silicagel (hexane/ethyl acetate, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (broad s, 1H), 3.05–4.05 (m, 33H), 4.22–5.13 (m, 33H), 6.94–7.32 (m, 60H), 7.42 (m, 1H), 8.11 (m, 1H), 8.68 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.2, 66.8–82.0 (multiple carbons), 98.4, 98.5, 98.9, 102.2, 126.4, 127.8–128.8 (multiple carbons), 129.3, 134.4, 138.7–139.0 (multiple carbons), 147.8, 165.2. MALDI-TOF MS: *m/z* calcd for $C_{117}H_{121}NNaO_{25}$ $(M+Na)^+$: 1962.81, found

1957.6. Anal. Calcd for $C_{117}H_{121}NO_{25}$: C, 72.39; H, 6.28; N, 0.72. Found: C, 72.55; H, 6.19; N, 0.82.

4.1.7. 4-Methoxycarbonyl-2-nitrobenzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranoside (20, 21). From **19** (4.74 g, 22.4 mmol, 1.20 equiv) and **2** (10.0 g, 18.7 mmol, 1 equiv), activated with $BF_3 \cdot OEt_2$ (6.0 mL, 6.92 g, 48.8 mmol, 2.61 equiv) in dry CH_2Cl_2 (30 mL), **20** and **21** (10.4 g, 15.1 mmol, 81%, α/β 6:1) were obtained as pale yellow foam after column chromatography (toluene/ether, 5:1) as described for compound **4**. The major anomer (**20**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.22 (s, 3H), 3.77 (m, 1H), 3.82–3.85 (m, 3H), 3.97–4.03 (m, 5H), 4.08 (m, 1H), 4.55–4.82 (m, 8H), 4.92 (d, $J=10.7$ Hz, 1H), 5.00–5.06 (m, 2H), 5.19 (m, 1H), 5.34 (s, 1H), 5.49 (m, 1H), 7.23–7.40 (m, 15H), 7.79 (d, $J=8.1$ Hz, 1H), 8.32 (dd, $J=8.1$, 1.7 Hz, 1H), 8.77 (d, $J=1.7$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.5, 53.2, 66.3, 67.0, 69.1, 72.4, 72.7, 74.0, 74.6, 75.7, 78.2, 98.2, 126.4, 128.1–128.9 (multiple carbons), 129.2, 131.1, 134.7, 138.2, 138.6, 138.7, 147.5, 165.2, 170.9. Anal. Calcd for $C_{38}H_{39}NO_{11}$: C, 66.56; H, 5.73; N, 2.04. Found: C, 6.47; H, 5.47; N, 1.97.

4.1.8. 4-Methoxycarbonyl-2-nitrobenzyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (22) and 4-methoxycarbonyl-2-nitrobenzyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (21 recovered). Following the procedure for compound **5**, acetate deprotection of the mixture of **20** and **21** (10.4 g, 15.2 mmol, 1 equiv) with K_2CO_3 (2.10 g, 15 mmol, 1 equiv) in dry THF (15 mL) and dry methanol (50 mL) after 1 h 45 min afforded **22** (6.83 g, 10.6 mmol, 70%) as a pale yellow foam after column chromatography (hexane/ethyl acetate, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (broad s, 1H), 3.60–3.72 (m, 2H), 3.85 (m, 2H), 3.92 (s, 3H), 4.04 (s, 1H), 4.46 (d, $J=9.4$ Hz, 1H), 4.47 (d, $J=12.2$ Hz, 1H), 4.58 (d, $J=12.1$ Hz, 1H), 4.68 (s, 2H), 4.77 (d, $J=10.7$ Hz, 1H), 4.77 (d, $J=10.7$ Hz, 1H), 4.90–5.06 (m, 3H), 7.13–7.32 (m, 15H), 7.65 (d, $J=8.1$ Hz, 1H), 8.19 (dd, $J=8.1$, 1.7 Hz, 1H), 8.63 (d, $J=1.7$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.2, 66.3, 68.7, 69.1, 72.2, 72.6, 74.0, 74.5, 75.7, 80.3, 99.8, 126.4, 128.0–129.0 (multiple carbons), 129.3, 131.1, 134.5, 138.2, 138.5, 138.8, 147.7, 165.2. Anal. Calcd for $C_{36}H_{37}NO_{10}$: C, 67.17; H, 5.79; N, 2.18. Found: C, 67.08; H, 5.93; N, 2.11.

Unreacted **21** (0.70 g, 1.1 mmol, 7%) was recovered as a pale yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.19 (s, 3H), 3.46 (m, 1H), 3.64–3.74 (m, 3H), 3.84–3.92 (m, 5H), 4.43–4.85 (m, 9H), 4.92 (m, 1H), 5.06 (d, $J=19.2$ Hz, 1H), 5.28 (d, $J=19.2$ Hz, 1H), 5.69 (d, $J=3.4$ Hz, 1H), 7.14–7.30 (m, 15H), 7.84 (d, $J=10.2$ Hz, 1H), 8.22 (dd, $J=10.2$, 2.0 Hz, 1H), 8.65 (d, $J=2.0$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1, 53.2, 68.3, 68.5, 69.3, 72.1, 74.0, 74.7, 75.7, 76.2, 80.6, 99.4, 126.2, 128.1–128.9 (multiple carbons), 129.3, 130.8, 134.7, 138.0, 138.6, 139.6, 147.1, 165.3, 170.1.

4.1.9. 4-Oxycarbonyl-2-nitrobenzyl 3,4,6-tri-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (24). A. 4-Methoxycarbonyl-2-nitrobenzyl 3,4,6-tri-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (**23**)

To a solution of **22** (6.0 g, 9.3 mmol, 1.00 equiv) in dry CH_2Cl_2 (45 mL), 2,6-lutidine (2.1 mL, 1.93 g, 19 mmol, 2.04 equiv) were added, followed by dropwise addition of TBSOTf (2.6 mL, 2.99 g, 11.3 mmol, 1.22 equiv). After 5 h, the solution was diluted with CH_2Cl_2 (150 mL), washed with 10% aqueous H_2SO_4 (100 mL), saturated $NaHCO_3$ solution (2×150 mL), brine (50 mL), dried ($MgSO_4$) and evaporated. After column chromatography (hexane/ethyl acetate, 6:1), **23** (5.5 g, 7.25 mmol, 78%) was obtained as a yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ −0.49 (s, 3H), −0.43 (s, 3H), 0.77 (s, 9H), 3.45–4.00 (m, 10H), 4.38–4.84 (m, 9H), 4.99 (d, $J=9.3$ Hz, 1H), 7.03–7.58 (m, 15H), 7.57 (d, $J=8.1$ Hz, 1H), 8.07 (dd, $J=8.1$, 1.6 Hz, 1H), 8.54 (d, $J=1.6$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.88, −1.28, 18.6, 26.2, 53.1, 66.3, 69.7, 70.2, 72.8, 73.5, 73.7, 74.9, 75.5, 80.2, 101.2, 126.3, 127.8–128.8 (multiple carbons), 129.3, 131.0, 134.5, 138.8, 139.0, 139.0, 147.7, 165.2.

B. 4-Oxycarbonyl-2-nitrobenzyl 3,4,6-tri-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (**24**)

Following the procedure for compound **7**, from **23** (3.60 g, 4.75 mmol, 1.00 equiv) dissolved in THF (23 mL) and 2 M aqueous KOH solution (23 mL) after stirring for 4 h, **24** (3.53 g, 4.75 mmol, 100%) was obtained as a pale yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14 (s, 3H), 0.19 (s, 3H), 0.97 (s, 9H), 3.80–4.20 (m, 10H), 4.57–5.02 (m, 9H), 5.18 (d, $J=9.3$ Hz, 1H), 7.23–7.47 (m, 15H), 7.77 (d, $J=8.2$ Hz, 1H), 8.26 (dd, $J=8.2$, 1.5 Hz, 1H), 8.69 (d, $J=1.5$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.25, −3.92, 18.7, 26.3, 66.5, 69.7, 70.2, 72.9, 73.5, 73.7, 75.0, 75.6, 80.1, 101.1, 126.8, 128.0–128.9 (multiple carbons), 129.3, 130.5, 134.9, 138.5, 138.7, 138.9, 139.9, 147.7, 168.5. Anal. Calcd for $C_{41}H_{49}NO_{10}Si$: C, 66.20; H, 6.64; N, 1.88. Found: C, 66.00; H, 6.72; N, 1.87.

4.1.10. 4-Methoxycarbonyl-2-nitrobenzyl 2-(4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (28). Following the procedure for compound **8**, the carboxylic acid **24** (1.33 g, 1.79 mmol, 1.00 equiv) and Boltorn H-50 polymer (0.82 g, 7.16 mmol, 4.00 equiv) were coupled in dry pyridine (2 mL) and dry THF (7 mL) using EDCI (0.38 g, 1.97 mmol, 1.10 equiv) and DMAP (0.02 g, 0.18 mmol, 0.10 equiv). The polymer was then capped with acetyl chloride (0.51 mL, 0.71 g, 7.16 mmol, 400 mol%). The polymer-immobilized product **25** (2.30 g, 1.54 mmol, 86%) was obtained as a pale yellow foam after the usual work-up and precipitation from methanol (50 mL), decanting of the supernatant, rinsing with methanol (3×5 mL) and drying in high vacuum. To a solution of **25** (1.83 g, 1.24 mmol, 1.00 equiv) in THF (7 mL), HF·pyridine (0.76 g, 7.7 mmol, 6.21 equiv) was added and the solution stirred for 12 h at reflux. After cooling to room temperature, the mixture was diluted with ethyl acetate (75 mL) and washed with water (50 mL), 10% aqueous H_2SO_4 (2×50 mL), saturated $NaHCO_3$ solution (2×50 mL) and brine (50 mL). After drying ($MgSO_4$) and evaporation, **26** was obtained as a pale yellow foam (1.46 g, 1.15 mmol, 94%, loading level: 0.79 mmol/g). The glycosylation-cleavage procedure used compounds **14** and **15** was applied using 2 M aqueous KOH solution. From glycosyl acceptor **26** (126 mg, 0.1 mmol, 1.00 equiv) and glycosyl donor **27** (222 mg, 0.5 mmol,

5.00 equiv), **28** (63 mg, 0.063 mmol, 63%) was isolated after column chromatography (hexanes/ethyl acetate, 3:1) as a pale yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (s, 3H), 1.53 (s, 3H) 3.49–3.80 (m, 7H), 3.90–3.98 (m, 6H), 4.18 (s, 1H), 4.30–4.38 (m, 2H), 4.49–4.55 (m, 5H), 4.65–5.04 (m, 6H), 5.14 (d, J=1.8 Hz), 5.40 (s, 1H), 7.15–7.35 (m, 25H), 7.60 (d, J=8.1 Hz, 1H), 8.17 (dd, J=8.1, 1.7 Hz, 1H), 8.68 (d, J=1.7 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.9, 28.5, 53.1, 66.1, 69.2, 69.5, 70.1, 72.7, 73.0, 73.1, 73.8, 73.8, 74.4, 74.4, 75.0, 75.6, 76.3, 76.3, 79.0, 79.6, 99.4 ($^1J_{C-H}$=173.1 Hz), 99.4 ($^1J_{C-H}$=173.1 Hz), 109.7, 126.2, 127.7–128.7 (multiple carbons), 128.9, 134.5, 138.6–138.7 (multiple carbons), 147.3, 165.3. Anal. Calcd for $C_{59}H_{63}NO_{15}$: C, 69.06; H, 6.19; N, 1.37. Found: C, 68.66; H, 6.31; N, 1.36.

4.1.11. 4-Methoxycarbonyl-2-nitrobenzyl 2-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (29). The glycosylation-cleavage procedure for compounds **14** and **15** was applied using 2 M aqueous KOH. From glycosyl acceptor **26** (126 mg, 0.1 mmol, 1.00 equiv) and glycosyl donor **13** (268 mg, 0.5 mmol, 5.00 equiv), **29** (44 mg, 0.041 mmol, 41%) was isolated after column chromatography (hexanes/ethyl acetate, 2:1) as a pale yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (broad s, 1H), 3.65–3.95 (m, 15H), 4.03–4.14 (m, 2H), 4.65–4.80 (m, 14H), 5.06–5.13 (m, 3H), 7.12–7.33 (m, 30H), 7.58 (d, J=8.1 Hz, 1H), 8.17 (dd, J=8.1, 1.7 Hz, 1H), 8.65 (d, J=1.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.1, 66.2, 68.9, 69.5, 69.6, 72.1, 72.6, 72.9, 73.1, 73.8, 73.8, 74.8, 75.0, 75.3, 75.4, 75.6, 79.6, 80.4, 99.4 ($^1J_{C-H}$=172.4 Hz), 101.6 ($^1J_{C-H}$=172.0 Hz), 126.3, 127.8–128.8 (multiple carbons), 128.9, 128.9, 134.5, 138.4–138.7 (multiple carbons), 147.7, 165.3. Anal. Calcd for $C_{63}H_{65}NO_{15}$: C, 70.31; H, 6.09; N, 1.30. Found: C, 70.26; H, 6.23; N, 1.31.

4.1.12. Methyl 4-((tetrahydro-2[*H*]-pyran-2-yl)-oxymethyl)-3-nitrobenzoate (30). To a suspension of methyl 4-hydroxymethyl benzoate (**2**) (10.56 g, 50 mmol, 1.00 equiv) in dry dichloromethane (20 mL), 2,3-dihydro-4[*H*]-pyrane (9.12 mL, 8.42 g, 100 mmol, 2.00 equiv) was added, followed by *p*-toluenesulfonic acid monohydrate (0.19 g, 1 mmol, 0.02 equiv). After 15 min, the clear solution formed was diluted with dichloromethane (150 mL), washed with saturated $NaHCO_3$ solution (50 mL), dried ($MgSO_4$) and the solvent was evaporated in vacuum. The THP ether **30** (14.21 g, 96%) was obtained as a pale yellow oil after chromatography on silicagel (hexane/ethyl acetate, 5:1 containing 0.5% Et_3N). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.48–1.85 (m, 8H), 3.44–3.50 (m, 1H), 3.73–3.79 (m, 1H), 3.82 (s, 3H), 4.68–4.72 (m, 1H), 4.87 (d, J=16.1 Hz, 1H), 5.10 (d, J=16.1 Hz, 1H), 7.87 (d, J=8.1 Hz, 1H), 8.20 (dd, J=8.2, 1.5 Hz, 1H), 8.60 (d, J=1.5 Hz, 1H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 19.8, 25.7, 30.8, 53.0, 62.9, 66.1, 99.3, 126.1, 129.4, 130.6, 134.4, 140.4, 147.6, 165.3. HR-QTOF-MS: m/z calcd for $C_{14}H_{17}NNaO_6$ $(M+Na)^+$318.0954, found 318.0952.

4.1.13. 4-((Tetrahydro-[2*H*]-pyran-2yl)-oxymethyl)-3-nitrobenzoic acid (31). A solution of **30** (3.51 g, 11.9 mmol, 100 mol%) in THF (50 mL) was mixed with 2 M aqueous KOH solution (50 mL) and the two-phase mixture was stirred vigorously for 6 h at room temperature. The mixture was acidified with 10% aqueous H_2SO_4 (pH=4), extracted with ethyl acetate (3×30 mL), washed with water (5×50 mL), brine (30 mL), dried ($MgSO_4$) and the solvent was evaporated to afford **31** (3.08 g, 92%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.51–1.82 (m, 7H), 3.50–3.57 (m, 1H), 3.77–3.81 (m, 1H), 4.74–4.76 (m, 1H), 4.91 (d, J=16.4 Hz, 1H), 5.16 (d, J=16.4 Hz, 1H), 7.93 (d, J=8.2 Hz, 1H), 8.26 (dd, J=8.2, 1.7 Hz, 1H), 8.69 (d, J=1.7 Hz, 1H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 19.8, 25.7, 30.8, 53.0, 62.9, 66.1, 99.3, 126.1, 129.4, 130.6, 134.4, 140.4, 147.6, 165.3. HR-QTOF-MS: m/z calcd for $C_{13}H_{15}NNaO_6$ $(M+Na)^+$304.0797, found 304.0782.

4.1.14. 4-Methoxycarbonyl-2-nitrobenzyl 2-(3,4,6-tri-*O*-benzyl-D-mannopyranosyl)-3-(3,4,6-tri-*O*-benzyl-D-mannopyranosyl)-4,6-di-*O*-benzyl-D-mannopyranoside (36). The carboxylic acid **31** (2.97 g, 10.5 mmol, 1.00 equiv), Boltorn H-50 polymer (**1**) (4.72 g, 42 mmol, 4.00 equiv) and DMAP (130 mg, 1.1 mmol, 0.11 equiv) were dissolved in dry pyridine (15 mL) and dry THF (20 mL). EDCI (2.20 g, 11.5 mmol, 1.10 equiv) was added and the reaction mixture was stirred for 16 h. The mixture was cooled in an ice bath and acetyl chloride (2.85 mL, 3.14 g, 40 mmol, 3.81 equiv) was added dropwise. The reaction was warmed to room temperature with stirring for 8 h, poured into water (100 mL) and extracted with ethyl acetate (2×30 mL). The combined organic extracts were washed with 10% H_2SO_4 (2×30 mL), saturated $NaHCO_3$ solution (2×30 mL), brine (30 mL), dried ($MgSO_4$) and the solvent was evaporated to afford the THP-protected polymer-immobilized linker, which was used directly for the next step. THP-protected **32** was dissolved in CH_2Cl_2 and methanol (40 mL each) and concd HCl (2 mL) was added. After stirring for 4 h, saturated aqueous $NaHCO_3$ solution was added (pH=8). The organic solvents were removed on rotary evaporator and the product was extracted in ethyl acetate (3×30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The polymer-immobilized alcohol **32** (5.27 g, 68%, loading level: 1.36 mmol/g) was obtained as a pale yellow foam after dissolving the crude polymer in dichloromethane (10 mL) and precipitation into methanol (150 mL), decanting of the supernatant, rinsing with methanol (3×10 mL), and drying in high vacuum. From the polymer-supported linker **32** (73.5 mg, 0.1 mmol, 1.00 equiv) and the glycosyl donor **27** (0.23 g, 0.5 mmol, 5.00 equiv) in dry CH_3CN (0.6 mL), following the procedure for preparation of **14**, the monosaccharide **33** (110 mg) was obtained as a pale yellow foam after precipitation from methanol (15 mL, containing five drops of Et_3N), decanting of the supernatant, rinsing with methanol (3×2 mL), and drying in high vacuum. To **33** (110 mg, 0.1 mmol, 1.00 equiv) dissolved in dry CH_2Cl_2 (1 mL), 1,3-propanedithiol (100 μL, 108 mg, 0.5 mmol, 5.00 equiv) and TFA (30 μL, 44 mg, 0.39 mmol, 3.90 equiv) were added and the solution was stirred overnight. Excess Et_3N (60 μL) was used to quench the acid and the solution was concentrated. The diol **34** was obtained by precipitation into methanol (30 mL), decanting of the supernatant, rinsing with methanol (3×2 mL) and drying in high vacuum. The product was directly applied into the next step. Following the procedure for preparation of **14**, the glycosylation of the diol **34** and the glycosyl donor

13 (0.54 g, 1 mmol, 10.00 equiv) in dry CH_3CN (0.7 mL) was accomplished using NIS (0.27 g, 1.2 mmol, 12.00 equiv) and TfOH (10 μL, 17 mg, 0.1 mmol, 100 mol%). The polymer-immobilized trisaccharide **35** was obtained as a pale yellow foam after precipitation into methanol (15 mL), decanting of the supernatant, rinsing with methanol (3×2 mL) and drying in high vacuum. Following the procedure for compound **15**, the trisaccharide (mixture of anomers) **36** (56 mg, 0.039 mmol, 39%) was obtained as a pale yellow foam after chromatography on silicagel (hexane/ethyl acetate, 2:1). The major anomer (α,α,α): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (broad s, 2H), 3.75–5.26 (m, 58H), 7.28–7.37 (m, 40H), 7.75–8.00 (m, 1H), 8.07–8.23 (m, 1H), 8.72–8.78 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.2, 66.2, 66.4, 68.3, 69.1, 69.1, 69.3, 69.6, 69.7, 72.1, 72.1, 72.4, 72.5, 72.7, 73.8, 73.9, 74.0, 74.7, 74.7, 74.9, 75.2, 75.4, 75.5, 76.3, 80.3, 80.6, 99.1 ($^1J_{C-H}$=174.8 Hz), 99.2 ($^1J_{C-H}$=174.8 Hz), 99.9 ($^1J_{C-H}$=170.8 Hz), 126.3, 128.1–129.2 (multiple carbons), 130.7, 134.7, 137.9–139.3 (multiple carbons), 147.1, 165.3. MALDI-TOF MS: *m/z* calcd for $C_{80}H_{87}NNaO_{20}$ $(M+Na)^+$: 1440.57, found 1441.30; *m/z* calcd for $C_{80}H_{87}NKO_{20}$ $(M+K)^+$: 1456.68, found 1457.27. Anal. Calcd for $C_{80}H_{87}NO_{20}$: C, 70.27; H, 6.18; N, 0.99. Found: C, 70.04; H, 6.33; N, 0.89.

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