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Synthesis of three trisaccharide congeners to investigate frame shifting of β1,2-mannan homo-oligomers in an antibody binding site

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D.R.B. wishes to dedicate this manuscript to Dr. Malcom Perry on the occasion of his retirement from the National Research Council of Canada

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Synthesis of terminal mono-deoxy and mono-*O*-methyl congeners

ABSTRACT

Homopolysaccharides such as the protective β1,2-mannan present in the cell wall of *Candida albicans* have the capability to bind to antibody in numerous frame shifted modes. A protective monoclonal antibody C3.1 binds this antigen and exhibits a unique binding profile where di and trisaccharides are the most potent inhibitors, while the intrinsic affinities of tetrasaccharide and larger oligomers dramatically decrease with increasing chain length. The design, synthesis and inhibitory activity of three β1,2-linked trisaccharide congeners is reported. Selective functional group modification was introduced at the terminal reducing or non-reducing mannose residues so that each trisaccharide would be capable of binding to antibody in only one of the possible frame shifted modes. Inhibition data show that C3.1 has the highest affinity for internally situated disaccharide epitopes but can bind the terminal non-reducing disaccharide of a trisaccharide epitope.

1. Introduction

Candida albicans is a fungal commensal that can cause serious infections in individuals with a compromised immune system or for those in a deliberately immunosuppressed condition such as individuals undergoing solid organ transplant or certain cancer therapies.[1,2] The incidence of hospital acquired infection continues to rise in North America and passive and active immunizations are treatment options that are beginning to be considered.[2–5] Two cell wall carbohydrate antigens have been the focus of vaccine research. One is the β-glucan[4,5] and the other is the β-mannan which is a minor component of the cell wall phosphomannan.[6–10] Monoclonal antibodies that recognize the β-mannan confer protection in passive transfer experiments.[11,12]

One of these antibodies C3.1, an IgG3κ has been the subject of detailed chemical mapping and conformational studies.[13,14] It shows a unique dependence on oligosaccharide size. Di and tri-saccharides of β1,2 linked mannohexosides are optimal inhibitors while larger oligosaccharides exhibit increasingly diminished activity.[13] We have attributed this observation to a relatively well organized solution conformation for the β1,2-oligomannan which precludes binding of larger structures.[13] We imagine that oligosaccharide segments of larger oligomers must protrude from the binding site and those parts adjacent to the site likely make unfavourable contacts with the antibody surface. Since the oligosaccharide is somewhat rigid an energetic penalty will be associated with altered conformations that avoid these contacts and consequently will result in lower intrinsic affinity. To explore these unique binding characteristics, in the absence of a solved crystal structure of the antibody and bound ligand, we have mapped the fine specificity of the binding site of C3.1 with functional group replacement[14] and NMR methods.[15] Surprisingly the primary polar contacts between a disaccharide epitope and antibody involve O-3 and O-4 of the terminal reducing residue and only one hydroxyl group at C-4′ of the non-reducing residue makes an important polar contact at the periphery of the binding site.[14]

The structure of the glycan component of *C. albicans* phosphomannan has been elucidated by Suzuki's group[16–20] and reveals an α1,6-mannan backbone with α1,2-linked side chains that incorporate two types of β-mannan chains. One is linked glycosidically to the α1,2-linked side chains. The second is attached to an

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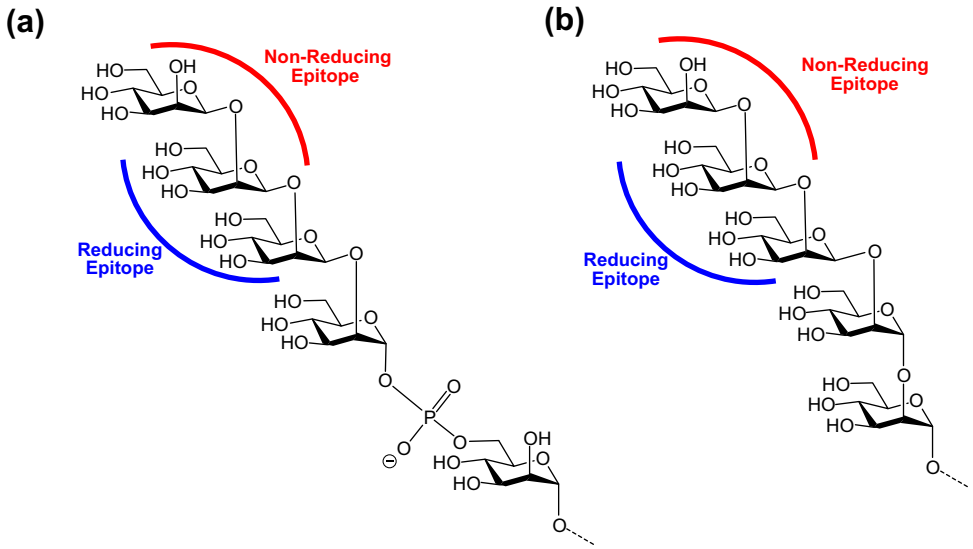


Figure 1. Two forms of β1,2-mannan trisaccharide epitopes found in the cell wall of *Candida albicans*. If an antibody recognizing a β1,2-mannan disaccharide is capable of frame shifting along the homo-oligomeric antigen chain both forms, an internal disaccharide epitope (blue) and external disaccharide (red) can be bound by antibodies. The β1,2-mannan trisaccharide may be attached (a) to the phosphomannan side chain residues via a phosphodiester or (b) the same trisaccharide can be attached to side chain residues via a glycosidic bond.

α-mannose residue through a phosphodiester. The precise lengths of the β1,2-linked mannose oligosaccharides are dependent on growth conditions and have been variously reported to range between 1 and 4 residues to as high as 14.[21]

Our mapping data are decisive with respect to the involvement of the terminal reducing residue of the disaccharide as the primary recognition element.[14] This is surprising since many protective antibodies bind to the most exposed residues at the distal end of cell surface antigens.[22,23] These results pose an important question. When the β-mannan of the cell wall antigen is larger than a disaccharide, which disaccharide element is bound by the antibody? Does the antibody always bind to the terminal reducing disaccharide or can it frame shift and bind a non-reducing disaccharide? The two binding modes are illustrated for the two types of *C. albicans* oligosaccharide epitopes present in the cell wall phosphomannan as acid labile phosphodiester linked β-mannan or the β-mannan linked glycosidically to the α-(1→2) mannose residues (Fig. 1).

2. Results and discussion

In order to identify whether frame shifted disaccharide epitopes are bound by C3.1 we utilized the findings of chemical mapping studies.[13,14] The binding site was mapped by monodeoxy and mono-*O*-methyl congeners. The C-3 and C-4 monodeoxy or mono-*O*-methyl ether analogues were all inactive in inhibition of C3.1 binding to synthetic β-mannan glycoconjugate coated EIA plates.[14] The C-4′ monodeoxy disaccharide congener was also inactive, while the C-4′ mono-*O*-methyl ether had an inhibitory potency of 5% relative to the native β-mannan disaccharide. Therefore if we constructed trisaccharides with a C-4″ monodeoxy residue (Fig. 2, compound **1**) and C-4 monodeoxy or C-4 mono-*O*-methyl groups (Fig. 2, compounds **2** and **3**), we anticipated that these trisaccharides would be forced into a single binding motif. Thus **1** should be bound only via its internal disaccharide, while **2** and **3** prevent binding of the reducing disaccharide and require the non-reducing disaccharide to be recognized.

Chemical synthesis of trisaccharides **1–3** (Fig. 2) incorporating these functional group replacements that abrogate binding to internal or external disaccharide elements was undertaken.

2.1. Synthesis of 4″-deoxy trisaccharide analogue 1

Disaccharide **4** prepared by a published procedure[24] was glycosylated by the 4-deoxy thioglycoside **5**[14] to give trisaccharide **6** in 69% yield. Debenzoylation gave the 4-deoxy derivative **7**. Epimerization at C-2″ to give the 4-deoxy derivative **8** was accomplished by two reactions involving oxidation to give the C-2″ uloside (not isolated) which was immediately reduced with good stereoselectivity by L-selectride®.[24] Hydrogenolysis of **8** using 10% Pd/C as a catalyst afforded the globally deprotected 4″-deoxy-trisaccharide analogue **1** in 80% yield (Scheme 1). Purifica-

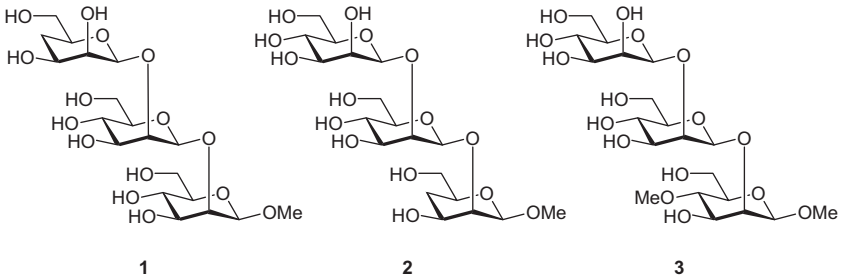


Figure 2. Trisaccharide congener **1** lacking a crucial hydroxyl group in the terminal non-reducing residue is designed to force antibody recognition via the internal disaccharide epitope. Trisaccharides **2** and **3** lack a crucial hydroxyl group in the terminal reducing residue and should force recognition of the external disaccharide epitope.

tion of the final compound was accomplished by HPLC with 70% CH_3CN—30% H_2O as eluent on a TSK-GEL Amide-80 column.

2.2. Synthesis of 4-deoxy and 4-*O*-methyl trisaccharide analogues 2 and 3

Two different monosaccharides **9** and **10** were required as the starting point for the synthesis of trisaccharide analogues **2** and **3**. Starting from methyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene β-D-glucopyranoside both glycosyl acceptors the 4-deoxy **9** and the **4**-*O*-methyl **10** were synthesized via the selectively protected methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl β-D-glucopyranoside according to a previous publication from our group.[24]

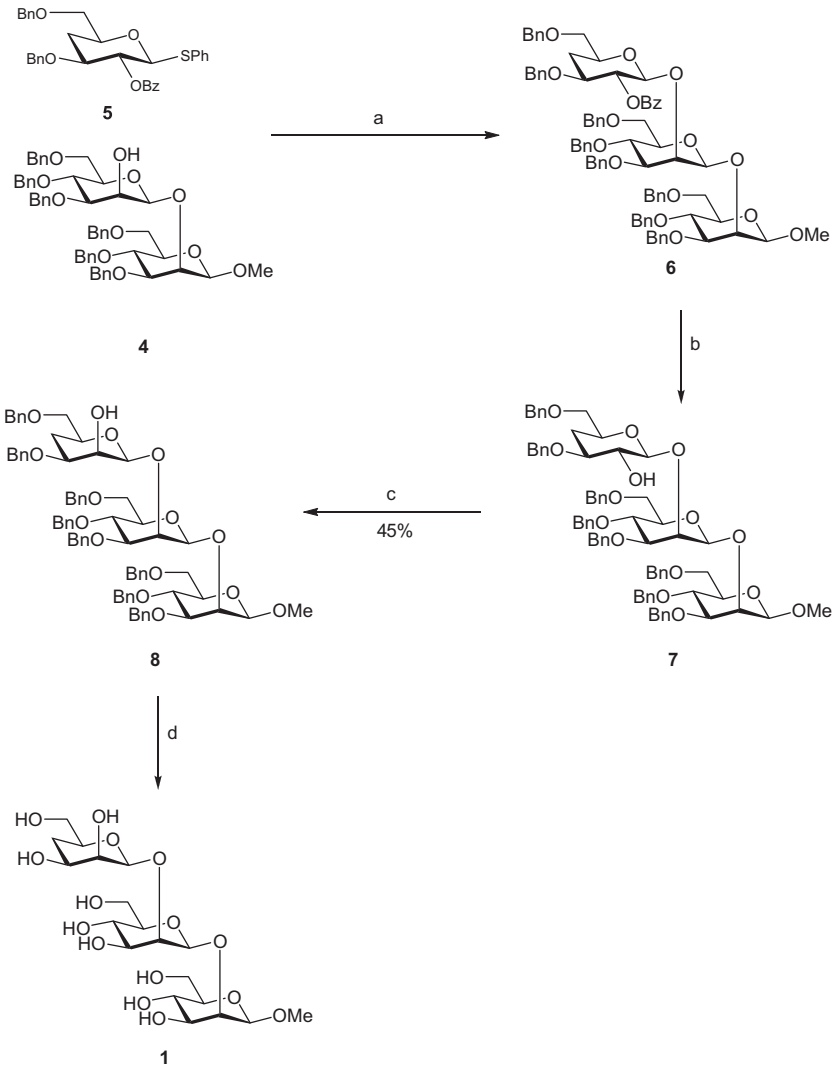
The acceptors **9** and **10** were glycosylated by trichloroacetimidate donor **11**[25] to give disaccharides **12** and **13** (Scheme 2). Deacetylation provided disaccharides **14** and **15** and as described previously epimerization at C-2′ was achieved by an oxidation and reduction sequence using Ac_2O/DMSO and then L-selectride® to give the disaccharide acceptors **16** and **17**, the synthesis of which had been performed as part of our disaccharide mapping studies.[24]

Disaccharide acceptors **16** and **17** were glycosylated by trichloroacetimidate **11**[25] to provide trisaccharides **18** and **19** (Scheme 3). Employing a similar set of transformations, transesterification afforded **20** and **21**. Then the C-2″ hydroxyl groups of these two trisaccharides were oxidized and the keto derivatives reduced to afford the manno derivatives **22** and **23**. Catalytic hydrogenation of **22** and **23** afforded the desired 4-deoxy and 4-*O*-methyl trisaccharide analogues **2** and **3**. Purification of **2** and **3** was accomplished by HPLC, using the same conditions as described for **1**.

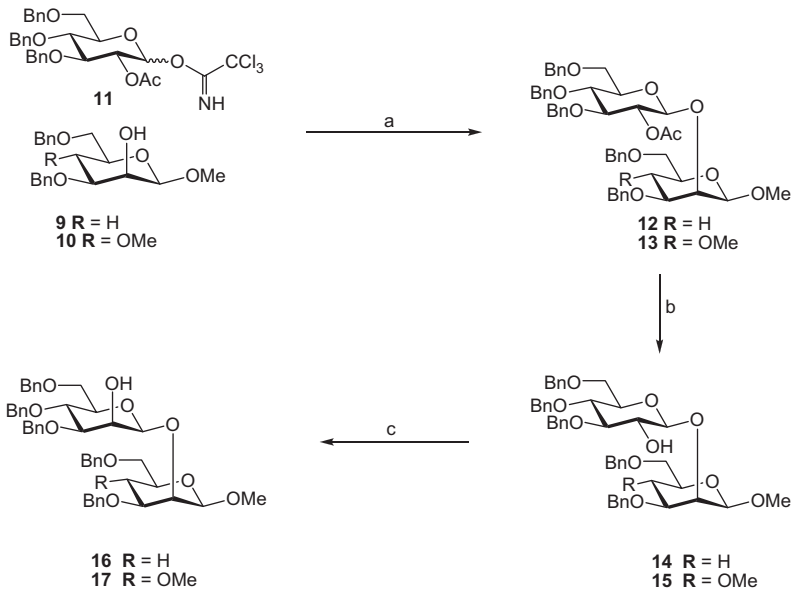
2.3. Enzyme immunoassay evaluation of the inhibitory power of trisaccharides 1–3

Competitive ELISA was used to compare the inhibitory activity of trisaccharides **1**–**3** and the native trisaccharide, methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside (Fig. 3).

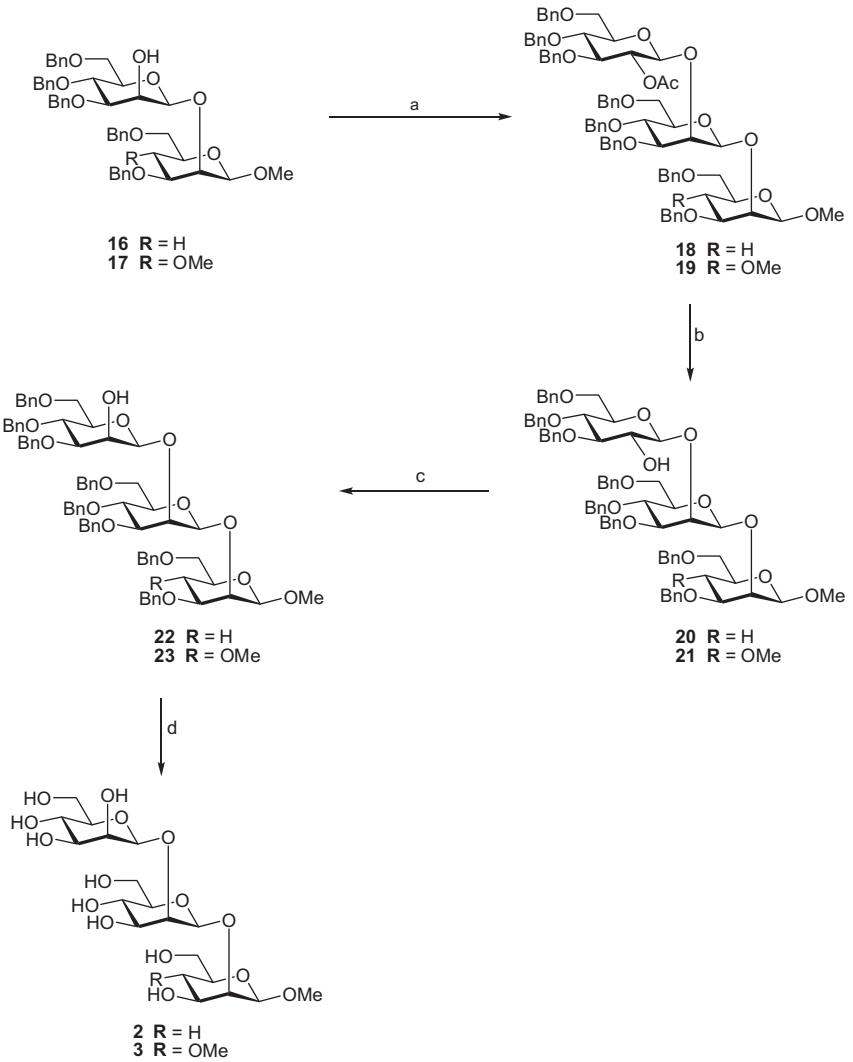
Native (1→2)-β-D-mannopyranose trisaccharide conjugated to BSA in PBS was used to coat 96-well ELISA plates.[14,25] Solutions of mAb IgG C3.1 mixed with serial dilutions of inhibitor were assayed in triplicate. Bound C3.1 monoclonal antibody was detected by a goat anti-mouse IgG antibody conjugated to the enzyme horseradish peroxidase. Percent inhibition was plotted against inhibitor concentration (Fig. 3) and IC_{50} values



Scheme 1. Reagents and conditions: (a) NIS, AgOTf, CH_2Cl_2, 4 Å MS, −30 °C, 69%; (b) NaOMe, MeOH, CH_2Cl_2, 85%; (c) (i) Ac_2O, DMSO, (ii) L-Selectride, THF, −78 °C, 45%; (d) H_2, Pd/C, MeOH, CH_2Cl_2, 80%.



Scheme 2. Reagents and conditions: (a) **11**, TMSOTf, CH_2Cl_2, 4 Å MS, −40 °C, **9** + **11**→**12**, 81%, **10** + **11**→**12**, 83%; (b) NaOMe, MeOH, CH_2Cl_2 **12**→**14**, 98%, **13**→**15**, 92%; (c) (i) Ac_2O, DMSO, (ii) L-Selectride, THF, −78 °C, **14**→**16**, 58%, **15**→**17**, 79%.



Scheme 3. Reagents and conditions: (a) **11**, TMSOTf, CH_2Cl_2, 4 Å MS, −30 °C **16** + **11**→**18**, 61%, **17** + **11**→**19**, 80%, (b) NaOMe, MeOH, CH_2Cl_2 **18**→**20**, 85%, **19**→**21**, 93%; (c) (i) Ac_2O, DMSO, (ii) L-Selectride, THF, −78 °C, **20**→**22**, 82%, **21**→**23**, 66%, (d) H_2, Pd/C, MeOH, CH_2Cl_2, **22**→**2**, 42%, **23**→**3**, 47%.

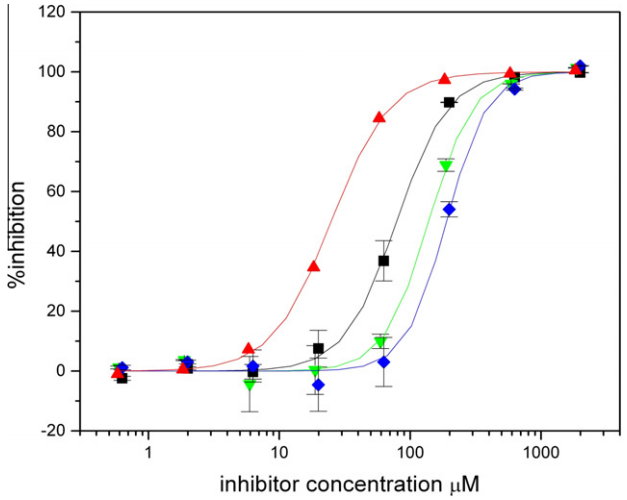


Figure 3. Competitive ELISA. Inhibition of Mab C3.1 binding to antigen coat plates by ▲ methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside and trisaccharides congeners ■ **1**, ♦ **2**, ▼ **3**.

Table 1
IC_{50} and relative free energy for native trisaccharide and trisaccharide congeners **1**–**3**

Trisaccharide	IC_{50} (μM)	Relative potency	Δ(ΔG)[a] (kcal mol^{-1})
Native	25 ± 1	100	0
1	79 ± 4	32	0.68
2	189 ± 11	13	1.2
3	139 ± 9	18	1.0

[a] The calculated standard error on all three values of Δ(ΔG) was less than 0.04 kcal mol^{-1}.

were used to determine the potency of inhibition and the relative change in free energy of binding (Table 1).

2.4. Conclusion

Inhibition data demonstrate that antibody C3.1 has a preference for binding the reducing disaccharide element of a trisaccharide, since analogue **1** is bound approximately twofold more tightly than either **2** or **3**. If the reducing end was exclusively preferred over the non-reducing end, a modification of the C-4 hydroxyl group (compounds **2** and **3**) would result in a complete loss of inhibition. This was not observed, as compounds **2** and **3** were both active. This clearly establishes that the non-reducing disaccharide element can also bind in the C3.1 binding site. The possibility of a frame shift to bind the non-reducing end disaccharide element at least for a trisaccharide epitope is thus confirmed. This is important since Cutler has reported that di and trisaccharide sequences are heavily expressed on growing *C. albicans* cultures[26] and C3.1 would clearly be capable of binding to either of these structures.

It is interesting to note that although compound **1** exhibits the preferred recognition element, there was a difference in binding energy between the native trisaccharide and **1**. The potency of **1** is only 32% of that of the native trisaccharide, a difference in free energy of 0.68 kcal mol^{-1}. It is possible that the replacement of the hydroxyl group by a hydrogen at C-4″ leads to unfavourable interactions with the antibody, at the periphery of the binding site. We favour the interpretation that 4″-OH is likely involved in a hydrogen bonding network with water at the periphery of the binding site. A 4″-deoxy trisaccharide analogue would have reduced affinity for binding, due to the loss of this hydrogen bonding capability. Relatively small changes in binding activity of this type have been seen in other detailed studies of congener binding to antibodies and lectins[27] when the functional group modifications similar to those we have described were employed. If we consider the opposite end of the binding site, which would accommodate the reducing mannose residue of compounds **2** and **3** we see that these compounds are approximately sixfold weaker inhibitors than **1**. Our NMR and homology model of the antibody binding site suggest β-mannose residues at this end of the groove type binding site encounter increasingly unfavourable interactions with the antibody surface.[15] We therefore attribute the weaker inhibitory power of compounds **2** and **3** to unfavourable antibody–trisaccharide contacts.

3. Experimental

3.1. General methods

All chemical reagents were of analytical grade and used as obtained from commercial sources unless otherwise indicated. Solvents used in water-sensitive reactions were purified by successive passage through columns of alumina and copper under nitrogen, except for DMSO, which was distilled under vacuum and collected over 4 Å molecular sieves. Unless otherwise noted, reactions were carried out at room temperature, and water-sensitive reactions were performed under an atmosphere of argon. Molecular sieves were flame dried and then allowed to cool to room temperature under argon before use. Reactions were monitored by analytical thin-layer chromatography (TLC) performed on Silica Gel 60-F254 (E. Merck). Plates were visualized under UV light, and/or by treatment with 5% sulfuric acid in ethanol followed by heating. Organic solvents were removed under vacuum at <40 °C. Medium-pressure chromatography was conducted using silica gel (230–400 mesh, Silicycle, Montreal) at flow rates between 5 and 10 mL min^{-1}. Following deprotection, final compounds were purified by HPLC (flow rate = 3.0 mL/min, diameter = 7.8 mm) with 70% CH_3CN—30% H_2O as eluent on a TSK-GEL Amide-80 column, concentrated under reduced pressure to remove acetonitrile, and then lyophilized. ^{1}H NMR spectra were recorded at 500 or 600 MHz, and chemical shifts, reported in δ (ppm), were referenced to internal residual protonated solvent signals or to external acetone (0.1% ext. acetone @ δ 2.225 ppm) in the case of D_2O. ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts are referenced to internal CDCl3 (δ 77.23) or external acetone (δ 31.07). First order chemical shifts of ^{1}H and ^{13}C are reported to the second and first decimal place, respectively. High resolution mass spectra were obtained on a Micromass Zabspec TOF-mass spectrometer by analytical services in the department. Optical rotations were determined with a Perkin-Elmer, model 241, polarimeter at room temperature using the sodium D-line and are reported in units of deg mL g^{-1} dm^{-1}. Elemental analysis was performed by analytical services of this department.

3.2. Enzyme linked immunosorbent inhibition assay

The mAb C3.1 was produced as a cell culture harvest supernatant (8.9 mg/mL) and was diluted 1:5000 for ELISA. A solution of synthetic *C. albicans* (1→2)-β-D-mannopyranose trisaccharide conjugated to BSA in PBS (5 μg/mL) was used to coat the 96-well ELISA plate (100 μL) overnight at 4 °C. Plates were washed five times with PBST buffer. Solutions of mAb IgG C3.1 mixed with inhibitor in PBST at concentrations ranging from 0.316 μg/mL up to of 1 mg/mL were added to the microtitre plates in triplicate and incubated at room temperature for 2 h. Goat anti-mouse IgG antibody conjugated to the horseradish peroxidase (diluted 1:5000, Kirkegaard & Perry Laboratories) in PBST was added and incubated at room temperature for 30 min. The plates were washed five times

with PBST buffer. The enzyme substrate 3,3′,5,5′-tetramethylbenzidine (100 μL, Kirkegaard & Perry Laboratories) was added and incubated at room temperature. After 15 min, the colour reaction was stopped by the addition of 1 M H_3PO_4 (100 μL). The absorbance of the solution was measured at 450 nm. Percent inhibition was calculated from the absorbance readings of the sample in relative to those of wells containing antibody without inhibitor.

3.2.1. Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-deoxy-β-D-*xylo*-hexopyranosyl-(1→2) 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (6)

Disaccharide acceptor **4**[24] (0.050 g, 0.056 mmol) and thioglycoside donor **5**[14] (0.040 g, 0.074 mmol) were dried together in a pear shaped flask (50 mL) under vacuum overnight. The contents of the flask were dissolved in CH_2Cl_2 (1.5 mL) and activated 4 Å molecular sieves were added. The solution was cooled to −30 °C (dry ice–acetone bath). NIS (0.017 g, 76 mmol) was added. After 20 min, AgOTf (0.011 g, 43 mmol) was added. After 1 h, the reaction mixture was neutralized with Et_3N and filtered through celite. The filtrate was washed with saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, distilled water and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography (19:1, toluene–EtOAc) gave **6** (0.051 g, 69%) as a clear and colourless syrup. R_f 0.26 (1:2, hexanes–EtOAc); $[\alpha]_D$ −34.5 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.22–8.20 (m, 2H, ArH), 7.42–7.00 (m, 43H, ArH), 5.52 (d, 1H, $J_{1'',2''}$ = 8.1 Hz, H-1″), 5.35 (dd, 1H, $J_{1'',2''}$ = 8.1 Hz, $J_{2'',3''}$ = 9.2 Hz, H-2″), 4.95–4.88 (m, 3H, PhCH_2O), 4.70–4.66 (m, 2H, H-2′, PhCH_2O), 4.63–4.58 (m, 2H, PhCH_2O), 4.58–4.52 (m, 4H, H-1′, PhCH_2O), 4.51–4.41 (m, 4H, PhCH_2O), 4.34 (d, 1H, J_{gem} = 10.9 Hz, PhCH_2O), 4.22 (s, 1H, H-1), 4.18 (d, 1H, J_{gem} = 12.1 Hz, PhCH_2O), 4.08–4.04 (m, 2H, H-2, PhCH_2O), 3.85 (m, 1H, H-5″), 3.74–3.68 (m, 4H, H-4, H-6a, H-3″, H6a″), 3.60–3.49 (m, 4H, H-3, H-6b, H-4′, H6a′, H6b″), 3.46 (dd, 1H, $J_{2',3'}$ = 2.9 Hz, $J_{3',4'}$ = 9.3 Hz, H-3′), 3.43 (m, 1H, H-5), 3.41–3.38 (m, 4H, H-5, CH_3O), 3.12 (dd, 1H, $J_{5',6'}$ = 6.9 Hz, J_{gem} = 10.7 Hz, H-6b′), 2.12 (ddd, 1H, $J_{3'',4eq''}$ = 5.3 Hz, J_{gem} = 12.7 Hz, $J_{4eq'',5''}$ = 1.8 Hz, H-4$_{eq}$″), 1.67 (q, 1H, J_{gem} = 11.7 Hz, H-4$_{ax}$″). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.5 (*C*=O), 138.7 (Ar), 138.6 (Ar), 138.5 (Ar), 138.4(09) (Ar), 138.4(05) (Ar), 138.3(9) (Ar), 138.3 (Ar), 138.2 (Ar), 134.5 (Ar), 133.5 (Ar), 134.5 (Ar), 132.2 (Ar), 131.3 (Ar), 130.3 (Ar), 128.5 (Ar), 128.3(5) (Ar), 128.3(4) (Ar), 128.3(3) (Ar), 128.2(4) (Ar), 128.2(0) (Ar), 128.1(5) (Ar), 128.1(4) (Ar), 128.1(3) (Ar), 128.1(2) (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6(3) (Ar), 127.6(1) (Ar), 127.5(8) (Ar), 127.5(5) (Ar), 127.5(3) (Ar), 127.4(9) (Ar), 127.4(2) (Ar), 127.3(4) (Ar), 127.3(0) (Ar), 127.2 (Ar), 103.3 (C-1′), 101.8 (C-1), 99.7 (C-1″), 80.1, 80.0 (C-3′, C-3), 76.4 (C-3″), 76.0, 75.7, 75.5 (C-5′, C-2, C-5), 75.2, 75.1 (PhCH$_2$O, C-6), 75.1, 75.0 (C-2″, C-4′), 74.5 (C-2′), 73.4 (PhCH$_2$O), 73.3 (PhCH$_2$O), 73.1 (PhCH$_2$O), 72.8 (C-6″), 70.8 (C-5″), 70.7, 70.5 (C-6′, PhCH$_2$O), 70.3 (C-4), 70.0 (PhCH$_2$O), 69.8 (PhCH$_2$O), 69.7 (PhCH$_2$O), 56.6 (CH$_3$O), 33.8 (C-4″). HRESIMS: calcd for $C_{82}H_{86}O_{16}Na$ (M+Na): 1349.5808, found 1349.5795. Anal. Calcd for $C_{82}H_{86}O_{16}$: C, 74.19; H, 6.53. Found: C, 73.75; H, 6.75.

3.2.2. Methyl 3,6-di-*O*-benzyl-4-deoxy-β-D-*xylo*-hexopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (7)

Trisaccharide **6** (0.204 g, 0.154 mmol) was dissolved in a mixture of MeOH (4 mL) and CH_2Cl_2 (2 mL). A 1 M solution of NaOMe/MeOH (1 mL, 1 mmol) was added. The reaction was stirred for 24 h. The reaction mixture was neutralized with Amberlite IR 120 and filtered to remove the resin. The filtrate was concentrated under reduced pressure. Purification by silica gel chromatography (4:1, toluene–EtOAc) gave **7** (0.159 g, 85%) as a white film. R_f 0.54 (1:1, hexanes–EtOAc); $[\alpha]_D$ −54.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42–7.38 (m, 2H, ArH), 7.38–7.33 (m, 4H, ArH), 7.33–7.20 (m, 25H, ArH), 7.20–7.14 (m, 9 H, ArH), 5.10 (d, 1H, J_{gem} = 11.1 Hz, PhCH_2O), 5.04 (d, 1H, J_{gem} = 12.1 Hz, PhCH_2O), 5.00 (d, 1H, J_{gem} = 11.1 Hz, PhCH_2O), 4.97 (d, 1H, J_{gem} = 12.6 Hz, PhCH_2O), 4.85 (s, 1H, H-1′), 4.78 (d, 1H, J_{gem} = 11.9 Hz, PhCH_2O), 4.63–4.59 (m, 2H, H-1″, PhCH_2O), 4.56–4.51 (m, 3H, PhCH_2O), 4.51–4.43 (m, 5H, H-2, H-2′, PhCH_2O), 4.43–4.35 (m, 3H, PhCH_2O), 4.26 (s, 1H, H-1), 4.18 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.87 (dd, 1H, $J_{5,6a}$ = 4.5 Hz, J_{gem} = 10.6 Hz, H-6a), 3.82–3.72 (m, 3H, H-6b, H-4′, H-6a″), 3.66–3.61 (m, 2H, H-2″, H-6b″), 3.56–3.52 (m, 2H, H-3′, H-6a′), 3.52 (s, 3H, OCH_3), 3.50–3.46 (m, 2H, H-3, H-5″), 3.42–3.34 (m, 4H, H-5, H-5′, H-6b′, H-3″), 1.84 (dd, 1H, $J_{3'',4eq''}$ = $J_{4eq'',5''}$ = 5.2 Hz, J_{gem} = 12.3 Hz, H-4$_{eq}$″), 1.49 (q, 1H, J_{gem} = 12.0 Hz, H-4$_{ax}$″). ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.2 (Ar), 138.9 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1(4) (Ar), 138.1(0) (Ar), 138.0 (Ar), 128.3(4) (Ar), 128.3(1)(Ar), 128.2(4)(Ar), 128.2(3)(Ar), 128.1(7)(Ar), 128.1(4)(Ar), 128.0(8) (Ar), 128.0(4) (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(3) (Ar), 127.7(2) (Ar), 127.6 (Ar), 127.5(7) (Ar), 127.5(2) (Ar), 127.4(9) (Ar), 127.4(3)(Ar), 127.4(0)(Ar), 127.3(4)(Ar), 127.3(0)(Ar), 127.2(9)(Ar), 127.1 (Ar), 105.5 (C-1″), 102.3 (C-1), 99.9 (C-1′), 80.1 (C-3′), 79.8 (C-3), 79.6 (C-3″), 75.7, 75.5 (C-5, C-2″), 75.4 (PhCH$_2$O), 75.1 (C-5″), 75.1 (PhCH$_2$O), 74.7 (C-4′), 74.3 (C-2′), 73.8 (PhCH$_2$O), 73.4 (PhCH$_2$O), 73.3 (PhCH$_2$O), 72.7, 72.6 (C-6′, PhCH$_2$O), 70.9, 70.8 (C-2, C-5′), 70.3 (C-6″), 69.9 (C-6), 69.8 (PhCH$_2$O), 57.5 (OCH$_3$), 33.5 (C-4″). HRESIMS: calcd for $C_{75}H_{82}O_{15}Na$ (M+Na): 1245.5546, found 1245.5532. Anal. Calcd for $C_{75}H_{82}O_{15}$: C, 73.63; H, 6.76. Found: C, 73.61; H, 6.77.

3.2.3. Methyl 3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (8)

Acetic anhydride (2.5 mL, 26 mmol) was added to a solution of trisaccharide **7** (0.159 g, 0.130 mmol) in DMSO (5.0 mL, 70 mmol). The reaction was stirred overnight. The mixture was concentrated under reduced pressure and the residue was redissolved in THF (5 mL). This solution was cooled to −78 °C (dry ice–acetone bath). A 1.0 M solution of L-Selectride® in THF (0.52 mL, 0.52 mmol) was added dropwise. The reaction was stirred at −78 °C for 3 h and then warmed to room temperature over 1 h. The reaction was quenched with MeOH and diluted with CH_2Cl_2. It was washed with 10% aqueous H_2O_2, 1 M aqueous NaOH, distilled water and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography (2:1, hexanes–EtOAc) gave **8** (0.071 g, 45%) as a clear and colourless syrup. R_f 0.57 (1:1, hexanes–EtOAc); $[\alpha]_D$ −76.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.51–7.48 (m, 2H, ArH), 7.41–7.38 (m, 2H, ArH), 7.36–7.20 (m, 29 H, ArH), 7.15–7.12 (m, 3H, ArH), 7.08–7.04 (m, 4H, ArH), 5.02 (s, 1H, H-1′), 5.01–4.94 (m, 3H, PhCH_2O), 4.92 (s, 1H, H-1″), 4.86 (s, 1H, J_{gem} = 10.4 Hz, PhCH_2O), 4.67–4.62 (m, 2H, H-2′, PhCH_2O), 4.59 (d, 1H, $J_{2,3}$ = 3.4 Hz, H-2), 4.56–4.34 (m, 9 H, PhCH_2O), 3.87 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, H-4′), 3.82 (dd, 1H, J_{gem} = 10.6 Hz, $J_{5,6a}$ = 4.4 Hz, H-6a), 3.77–3.80 (m, 2H, H-6b, H-6a′), 3.72–3.66 (m, 2H, H-6b′, H-4), 3.61–3.56 (m, 3H, H-3, H-3′, H-6a″), 3.56–3.50 (m, 5H, H-5′, H-6b″, CH_3O), 3.46–3.40 (m, 2H, H-5, H-5″), 3.35 (ddd, 1H, $J_{2'',3''}$ = 2.8 Hz, $J_{3'',4ax''}$ = 11.6 Hz, $J_{3'',4eq''}$ = 4.8 Hz, H-3″), 1.78 (q, 1H, J_{gem} = 12.0 Hz, H-4$_{ax}$″), 1.64 (dd, 1H, J_{gem} = 11.0 Hz, $J_{3'',4eq''}$ = 4.8 Hz H-4$_{eq}$″). ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.6 (Ar), 138.4 (Ar), 138.2(7) (Ar), 138.2(2) (Ar), 138.1(94) (Ar), 138.1(66) (Ar), 138.1(45) (Ar), 138.1(27) (Ar), 129.0 (Ar), 128.4 (Ar), 128.3(5) (Ar), 128.3(4) (Ar), 128.3(2) (Ar), 128.2(7) (Ar), 128.2(2) (Ar), 128.1(6) (Ar), 128.1(2) (Ar), 128.0(8) (Ar), 128.0(6) (Ar), 127.8 (Ar), 127.7(9) (Ar), 127.7(8) (Ar), 127.7(4) (Ar), 127.7(2) (Ar), 127.6(7) (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 127.2 (Ar), 102.8 (C-1), 100.8 (C-1″), 100.0 (C-1′), 80.3(2), 80.2(7) (C-3, C-3′), 76.6 (C-3″), 75.4 (PhCH$_2$O), 75.3, 75.2 (C-5, C-5′), 75.1 (PhCH$_2$O), 74.7, 74.5 (C-4, C-4′), 73.6 (PhCH$_2$O), 73.4(5) (PhCH$_2$O), 73.4(3) (PhCH$_2$O), 73.0 (PhCH$_2$O), 71.8 (C-5″), 71.2 (C-2′), 70.3 (C-6″), 69.9 (C-6′), 69.6 (PhCH$_2$O), 69.4(4) (C-6), 69.4(1) (C-2), 69.3 (PhCH$_2$O), 66.4 (C-2″), 57.4

(CH_3O), 28.6 (C-4″). HRESIMS: calcd for $C_{75}H_{82}O_{15}Na$ (M+Na): 1245.5546, found 1245.5545. Anal. Calcd for $C_{75}H_{82}O_{15}$: C, 73.63; H, 6.76. Found: C, 73.62; H, 6.72.

3.2.4. Methyl 4-deoxy-β-D-*lyxo*-hexopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranoside (1)

Trisaccharide **8** (0.073 g, 0.060 mmol) was dissolved in a mixture of CH_2Cl_2 (5 mL) and MeOH (5 mL). 10% Pd/C (0.050 g) was added and the reaction was evacuated and purged with H_2(g). The mixture was stirred overnight under a hydrogen atmosphere. The catalyst was separated by filtration through celite and the filtrate was concentrated under reduced pressure. Purification of the product by HPLC gave **1** (0.024 g, 80%) as a white solid. R_f 0.02 (9:1, CH_2Cl_2–MeOH); $[\alpha]_D$ −60.0 (*c* 0.9, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 4.86 (s, 1H, H-1′), 4.75 (s, 1H, H-1″), 4.61 (s, 1H, H-1), 4.31 (d, 1H, $J_{2',3'}$ = 3.1 Hz, H-2′), 4.20 (d, 1H, $J_{2,3}$ = 3.2 Hz, H-2), 4.01 (d, 1H, $J_{2'',3''}$ = 2.9 Hz, H-2″), 3.91 (dd, 1H, J_{gem} = 4.7 Hz, $J_{5,6a}$ = 2.2 Hz, H-6a), 3.89 (dd, 1H, J_{gem} = 4.7 Hz, $J_{5',6a'}$ = 2.2 Hz, H-6a′), 3.86 (ddd, 1H, $J_{2'',3''}$ = 3.1 Hz, $J_{3'',4ax''}$ = 11.9 Hz, $J_{3'',4eq''}$ = 5.1 Hz, H-3″), 3.74 (t, 1H, J_{gem} = $J_{5',6b'}$ = 6.2 Hz, H-6b′), 3.71 (t, 1H, J_{gem} = $J_{5,6b}$ = 5.9 Hz, H-6b), 3.67 (m, 1H, H-3), 3.66–3.58 (m, 4H, H-3′, H-5″, H-6a″, H-6b″), 3.57 (m, 1H, H-4′), 3.52 (s, 3H, CH_3O), 3.47 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 3.37–3.33 (m, 2H, H-5, H-5′), 1.64 (dd, 1H, $J_{3'',4eq''}$ = 5.0 Hz, J_{gem} = 11.3 Hz, H-4_{eq}″), 1.50 (q, 1H, J_{gem} = 11.9 Hz, H-4_{ax}″). ^{13}C NMR (125 MHz, D_2O): δ 102.1(8) (C-1), 102.1(7) (C-1″), 101.5 (C-1′), 79.0(6) (C-2′), 79.0(1) (C-2), 77.2, 77.1 (C-5, C-5′), 73.7, 73.1 (C-3′, C-5″), 72.9 (C-3), 69.9 (C-2″), 68.8 (C-3″), 68.2 (C-4), 67.9 (C-4′), 64.8 (C-6″), 63.3 (C-6′), 61.6 (C-6), 57.8 (CH_3O), 29.4 (C-4″). HRESIMS: calcd for $C_{19}H_{34}O_{15}Na$ (M+Na): 525.179, found 525.1786.

3.2.5. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-glucopyranosyl trichloro acetimidate (11)[25]

DBU (1.1 mL, 7.4 mmol) was added dropwise to a solution of 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-glucopyranose (13.90 g, 28.22 mmol) and Cl_3CCN (20.0 mL, 199 mmol) in CH_2Cl_2 (100 mL). The reaction was stirred for 3 h. The solution was washed with saturated aqueous NH_4Cl. The organic phase was dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel chromatography (2:1, hexanes–EtOAc with 1% Et_3N) gave **11** (17.91 g, 97%) as a yellow syrup.

3.2.6. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (18)

Disaccharide acceptor **16**[24] (0.097 g, 0.12 mmol) and monosaccharide donor **11**[25] (0.117 g, 0.184 mmol) were dried together in a pear shaped flask (50 mL) under vacuum overnight. The contents of the flask were dissolved in CH_2Cl_2 (2 mL) and activated 4 Å molecular sieves were added. The solution was cooled to −30 °C (dry ice–acetone bath). TMSOTf (6 μL, 0.03 mmol) was added and the reaction was stirred for 1 h. The mixture was neutralized with Et_3N, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (19:1, toluene–EtOAc) gave **18** (94 mg, 61%) as a white syrup. R_f 0.41 (2:1, hexanes–EtOAc); $[\alpha]_D$ −55.5 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42–7.16 (m, 40 H, ArH), 5.22 (d, 1H, $J_{1'',2''}$ = 8.1 Hz, H-1″), 5.18 (t, 1H, $J_{1'',2''}$ = $J_{2'',3''}$ = 8.5 Hz, H-2″), 4.98 (d, 1H, J_{gem} = 10.9 Hz, PhCH_2O), 4.95 (d, 1H, J_{gem} = 12.1 Hz, PhCH_2O), 4.86 (d, 1H, J_{gem} = 11.0 Hz, PhCH_2O), 4.80 (d, 1H, J_{gem} = 11.9 Hz, PhCH_2O), 4.75–4.73 (m, 2H, PhCH_2O), 4.71 (s, 1H, H-1′), 4.63 (d, 1H, J_{gem} = 12.3 Hz, PhCH_2O), 4.61–4.58 (m, 2H, PhCH_2O), 4.58–4.55 (m, 2H, PhCH_2O), 4.55–4.46 (m, 6H, PhCH_2O), 4.17 (s, 1H, H-1), 4.14 (d, 1H, $J_{2,3}$ = 2.8 Hz, H-2), 3.80–3.76 (m, 3H, H-6a, H-6a′, H-3″), 3.75–3.69 (m, 2H, H-6b, H-5″), 3.67–3.60 (m, 5H, H-4′, H-6b′, H-4″, H-6a″, H-6b″), 3.57 (m, 1H, H-5), 3.53–3.46 (m, 3H, H-3, H-3′, H-5′), 3.45 (s, 3H, CH_3O), 1.97 (s, 3H, C(O)CH_3), 1.92 (m, 1H, H-4_{ax}), 1.75 (dd, 1H, $J_{3,4eq}$ = $J_{4eq,5}$ = 4.5 Hz, J_{gem} = 12.5 Hz, H-4_{eq}). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5 (*C*=O), 138.7 (Ar), 138.5 (Ar), 138.4(4) (Ar), 138.3(8) (Ar), 138.2(5) (Ar), 138.2(3) (Ar), 138.1(9) (Ar), 128.4 (Ar), 128.3 (Ar), 128.2(9) (Ar), 128.2(7) (Ar), 128.2(4) (Ar), 128.1(8) (Ar), 128.1(3) (Ar), 128.0(9) (Ar), 128.0(0) (Ar), 127.8(6) (Ar), 127.8(4) (Ar), 127.6(9) (Ar), 127.6(4) (Ar), 127.6(1) (Ar), 127.5(6) (Ar), 127.5(2) (Ar), 127.4(9) (Ar), 127.4(5) (Ar), 127.4(4) (Ar), 127.4(3) (Ar), 127.3(9) (Ar), 127.3(1) (Ar), 102.6 (C-1), 101.9 (C-1′), 100.9 (C-1″), 83.0 (C-3″), 80.0 (C-3′), 78.3 (C-5″), 75.5 (C-3), 75.1 (PhCH_2O), 74.9 (PhCH_2O), 74.8 (C-4′), 74.6 (C-2′), 74.4 (PhCH_2O), 74.2 (C-5′), 73.7 (C-2), 73.4 (PhCH_2O), 73.3(4) (PhCH_2O), 73.2(7) (C-2″), 73.2(0) (PhCH_2O), 73.1 (PhCH_2O), 72.0 (C-5), 71.9 (C-4″), 70.7 (C-6′), 69.8 (C-6″), 69.5 (C-6), 68.8 (PhCH_2O), 56.8 (OCH_3), 29.6 (C-4), 21.2 (C(O)CH_3). HRESIMS: calcd for $C_{77}H_{84}O_{16}Na$ (M+Na): 1287.5652, found 1287.5639. Anal. Calcd for $C_{77}H_{84}O_{16}$: C, 73.08; H, 6.69. Found: C, 72.83; H, 6.66.

3.2.7. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (19)

Disaccharide acceptor **17**[24] (0.6755 g, 0.8228 mmol) and monosaccharide donor **11**[24] (0.786 g, 1.23 mmol) were dried together in a pear shaped flask (50 mL) under vacuum overnight. The contents of the flask were dissolved in CH_2Cl_2 (5 mL) and activated 4 Å molecular sieves were added. The solution was cooled to −35 °C (dry ice–acetone bath). TMSOTf (37 μL, 0.20 mmol) was added and the reaction was stirred from −35 °C to −25 °C over 2 h. The mixture was neutralized with Et_3N, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (4:1, hexanes–EtOAc) gave **19** (0.856 g, 80%) as a white solid. R_f 0.32 (2:1, hexanes–EtOAc); $[\alpha]_D$ −40.0 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.34 (m, 4H, ArH), 7.31–7.16 (m, 34H, ArH), 7.10–7.07 (m, 2H, ArH), 5.20–5.14 (m, 2H, H-1″, H-2″), 4.95 (d, 1H, J_{gem} = 11.0 Hz, PhCH_2O), 4.92 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.80 (d, 2H, J_{gem} = 11.5 Hz, PhCH_2O), 4.74–4.68 (m, 3H, PhCH_2O), 4.62 (d, 1H, J_{gem} = 12.9 Hz, PhCH_2O), 4.59 (s, 1H, H-1′), 4.59–4.49 (m, 5H, PhCH_2O), 4.49–4.40 (m, 4H, H-2′, PhCH_2O), 4.20 (s, 1H, H-1), 4.10 (d, 1H, $J_{2,3}$ = 2.8 Hz, H-2), 3.84 (m, 1H, H-3″), 3.78–3.68 (m, 6H, H-6a, H-6b, H-4″, H-5″, H-6a″, H-6b″), 3.68–3.56 (m, 4H, H-4, H-4′, H-6a′, H-6b′), 3.52 (s, 3H, 4-OCH_3), 3.48–3.42 (m, 2H, H-3′, H-5′), 3.41 (s, 3H, 1-OCH_3), 3.39 (dd, 1H, $J_{2,3}$ = 2.8 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.26 (m, 1H, H-5), 1.95 (s, 3H, C(O)CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5 (*C*=O), 138.7(32) (Ar), 138.7(28) (Ar), 138.5 (Ar), 138.4(09) (Ar), 138.4(03) (Ar), 138.3 (Ar), 138.2 (Ar), 128.2(6) (Ar), 128.2(5) (Ar), 128.2(3) (Ar), 128.2(1) (Ar), 128.1(8) (Ar), 128.1(6) (Ar), 128.1(5) (Ar), 128.0(3) (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5(5) (Ar), 127.5(3) (Ar), 127.5(1) (Ar), 127.4(6) (Ar), 127.4(1) (Ar), 127.4(0) (Ar), 127.3(8) (Ar), 127.3(7) (Ar), 127.3(5) (Ar), 127.3(0) (Ar), 102.1 (C-1′), 102.0 (C-1), 101.0 (C-1″), 83.4 (C-3″), 80.3 (C-3′), 79.9 (C-3), 78.4 (C-4), 76.0 (C-2), 75.4(0), 75.3(6), 75.0 (C-5, C-5′, C-5″), 75.1 (PhCH_2O), 74.8 (PhCH_2O), 74.6, 74.5 (C-4′, C-4″), 74.6 (PhCH_2O), 73.3 (C-2″), 73.2(9) (PhCH_2O), 73.2 (PhCH_2O), 73.0 (PhCH_2O), 71.9 (C-2′), 70.8 (C-6″), 69.8 (PhCH_2O), 69.7 (C-6′), 69.5 (PhCH_2O), 69.3 (C-6), 61.0 (4-CH_3O), 56.9 (1-CH_3O), 22.6 (C(O)CH_3). HRESIMS: calcd for $C_{78}H_{86}O_{17}Na$ (M+Na): 1317.5757, found 1317.5758. Anal. Calcd for $C_{78}H_{86}O_{17}$: C, 72.31; H, 6.69. Found: C, 72.26; H, 6.67.

3.2.8. Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (20)

Trisaccharide **18** (0.085 g, 0.067 mmol) was dissolved in a mixture of MeOH (4 mL) and CH_2Cl_2 (2 mL). A 1 M solution of NaOMe/MeOH (1 mL, 1 mmol) was added. The reaction was stirred for 24 h. The reaction mixture was neutralized with Amberlite IR 120 and filtered to remove the resin. The filtrate was concentrated.

Purification by silica gel chromatography (4:1, toluene–EtOAc) gave **20** (0.070 g, 85%) as a clear and colourless syrup. R_f 0.41 (2:1, hexanes–EtOAc); $[\alpha]_D$ −65.0 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.42–7.22 (m, 36H, ArH), 7.21–7.15 (m, 4H, ArH), 5.07 (d, 1H, J_{gem} = 11.4 Hz, PhCH_2O), 4.99 (d, 1H, J_{gem} = 10.8 Hz, PhCH_2O), 4.98 (d, 1H, J_{gem} = 12.6 Hz, PhCH_2O), 4.91 (s, 1H, H-1′), 4.89 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.87 (d, 1H, J_{gem} = 10.9 Hz, PhCH_2O), 4.79 (d, 1H, J_{gem} = 11.2 Hz, PhCH_2O), 4.77 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.62 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.56 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.55–4.44 (m, 6H, H-2′, PhCH_2O), 4.42 (d, 1H, J_{gem} = 12.1 Hz, PhCH_2O), 4.41 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.37 (d, 1H, $J_{2,3}$ = 2.8 Hz, H-2), 4.18 (s, 1H, H-1), 3.81 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, H-4′), 3.77 (t, 1H, $J_{1'',2''} = J_{2'',3''}$ = 8.5 Hz, H-2″), 3.76–3.67 (m 3H, H-4′, H-5′, H-6a′), 3.67–3.61 (m, 4H, H-6a, H-6b, H-6b′, H-3″), 3.61–3.52 (m, 4H, H-5, H-3′, H-6a′, H-6b′), 3.50 (s 3H, OCH_3), 3.49–3.44 (m, 2H, H-3, H-5′), 1.96 (q, 1H, J_{gem} = 12.1 Hz, H-4_{ax}), 1.74 (ddd, 1H, $J_{3,4eq}$ = 4.6 Hz, J_{gem} = 12.5 Hz, $J_{4eq,5}$ = 1.8 Hz, H-4_{eq}). ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.2 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3(4) (Ar), 138.2(7) (Ar), 138.1(4) (Ar), 138.1(3) (Ar), 128.4 (Ar), 128.3(1) (Ar), 128.2(9) (Ar), 128.2(7) (Ar), 128.2(5) (Ar), 128.2(0) (Ar), 128.1(6) (Ar), 128.1(2) (Ar), 128.1(0) (Ar), 127.9(9) (Ar), 127.9(7) (Ar), 127.8 (Ar), 127.7(5) (Ar), 127.7(2) (Ar), 127.6 (Ar), 127.5 (Ar), 127.4(9) (Ar), 127.4(8) (Ar), 127.3(4) (Ar), 127.3(2) (Ar), 104.6 (C-1″), 102.7 (C-1), 99.9 (C-1′), 85.8 (C-3″), 80.1 (C-3′), 77.2 (C-5′), 75.3(5), 75.2(9) (C-3, C-2′), 75.1 (PhCH_2O), 75.0 (PhCH_2O), 74.8, 74.6 (C-4′, C-2″), 74.5 (PhCH_2O), 74.0, 73.8 (C-4″, C-5″), 73.6 (PhCH_2O), 73.4 (PhCH_2O), 73.3 (PhCH_2O), 73.0 (C-6″), 72.1 (C-5), 70.1 (C-6′), 70.0 (C-2), 69.8 (C-6), 69.7 (PhCH_2O), 69.2 (PhCH_2O), 57.3 (OCH_3), 29.7 (C-4). HRESIMS: calcd for $C_{75}H_{82}O_{15}Na$ (M+Na): 1245.5546, found 1245.5539. Anal. Calcd for $C_{75}H_{82}O_{15}$: C, 73.63; H, 6.76. Found: C, 73.35; H, 6.69.

3.2.9. Methyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (21)

Trisaccharide **19** (0.856 g, 0.661 mmol) was dissolved in a mixture of MeOH (6 mL) and CH_2Cl_2 (3 mL). A 1 M solution of NaOMe/MeOH (1 mL, 1 mmol) was added. The reaction was stirred for 24 h. The reaction mixture was neutralized with Amberlite IR 120 and filtered to remove the resin. The filtrate was concentrated. Purification by silica gel chromatography (4:1, toluene–EtOAc) gave **21** (0.7666 g, 93%) as a white solid. R_f 0.64 (1:1, hexanes–EtOAc); $[\alpha]_D$ −58.0 (*c* 1.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.45–7.42 (m, 2H, ArH), 7.39–7.35 (m, 4H, ArH), 7.35–7.21 (m, 29 H, ArH), 7.21–7.14 (m, 5H, ArH), 5.02–4.97 (m, 4H, PhCH_2O), 4.88–4.84 (m, 2H, H-1′, PhCH_2O), 4.72 (d, 1H, $J_{1'',2''}$ = 7.8 Hz, H-1″), 4.70–4.65 (m, 2H, PhCH_2O), 4.61 (d, 1H, J_{gem} = 12.2 Hz, PhCH_2O), 4.54–4.46 (m, 4H, H-2′, PhCH_2O), 4.46–4.41 (m, 4H, H-2, PhCH_2O), 4.38 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.37 (d, 1H, J_{gem} = 12.1 Hz, PhCH_2O), 4.25 (s, 1H, H-1), 3.82–3.70 (m, 6H, H-4, H-6a, H-6b, H-6a′, H-2″, H-5″), 3.68–3.60 (m, 4H, H-4′, H-6b′, H-3″, H-6a″), 3.55–3.52 (m, 3H, H-3′, H-4″, H-6b″), 3.50 (s, 3H, 4-OCH_3), 3.49 (s, 3H, 1-OCH_3), 3.48 (m, 1H, H-5′), 3.40 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.29 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 5.0 Hz, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.2 (Ar), 138.6 (Ar) 138.4(1) (Ar), 138.3(9) (Ar), 138.2(4) (Ar), 138.2(0), 138.1(4) (Ar), 138.0(8) (Ar), 129.0 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2(64) (Ar), 128.2(56) (Ar), 128.2(4) (Ar), 128.2(2) (Ar), 128.1(9) (Ar), 128.0(8) (Ar), 127.9(7) (Ar), 127.9(4) (Ar), 127.8(7) (Ar), 127.8(1) (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4(8) (Ar), 127.4(7) (Ar), 127.4(3) (Ar), 127.3(7) (Ar), 127.3 (Ar), 127.0 (Ar), 125.3 (Ar), 105.1 (C-1″), 102.1 (C-1), 99.7 (C-1′), 86.5 (C-4′), 80.1, 80.0 (C-3, C-3′), 77.2 (C-2″), 75.6 (C-5), 75.3 (C-5′), 75.1(9), 75.1(6) (C-2′, C-5″), 75.1(2) (PhCH_2O), 74.9, 74.8 (C-6′, PhCH_2O), 74.7, 74.6, 74.5 (C-2, C-4, C-4″), 73.6 (PhCH_2O), 73.4 (PhCH_2O), 73.2 (PhCH_2O), 70.8 (C-3″), 70.2, 70.0 (C-6, PhCH_2O), 69.6(9), 69.6(6) (C-6″, PhCH_2O), 61.0 (4-CH_3O), 57.4 (1-CH_3O). HRESIMS: calcd for $C_{76}H_{84}O_{16}Na$ (M+Na): 1275.5652, found 1275.5648. Anal. Calcd for $C_{76}H_{84}O_{16}$: C, 72.82; H, 6.75. Found: C, 72.72; H, 6.69.

3.2.10. Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-deoxy-β-D-*lyxo*-hexopyranoside (22)

Acetic anhydride (1.0 mL, 11 mmol) was added to a solution of trisaccharide **20** (0.045 g, 0.037 mmol) in DMSO (2.0 mL, 28 mmol). The reaction was stirred overnight. The mixture was concentrated under reduced pressure and the residue was redissolved in THF (2 mL). This solution was cooled to −78 °C. A solution of L-Selectride® in THF (0.15 mL, 0.15 mmol) was added dropwise. The reaction was stirred at −78 °C for 2.5 h and was then warmed to room temperature over 2 h. The reaction was quenched with MeOH and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, where it was washed with 10% aqueous H_2O_2, 1 M aqueous NaOH, distilled water, and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography (2:1, hexanes–EtOAc) gave **20** (0.037 g, 82%) as a clear and colourless syrup. R_f 0.06 (4:1, toluene–EtOAc); $[\alpha]_D$ −35.5 (*c* 1.4, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.48–7.46 (m, 2H, ArH), 7.40–7.37 (m, 2H, ArH), 7.31–7.16 (m, 36H, ArH), 5.05 (s, 1H, H-1′), 5.01–4.95 (m, 4H, H-1″, PhCH_2O), 4.90 (d, 1H, J_{gem} = 10.7 Hz, PhCH_2O), 4.61 (d, 1H, $J_{2',3'}$ = 3.2 Hz, H-2′), 4.57–4.54 (m, 2H, H-1, PhCH_2O), 4.54–4.51 (m, 2H, PhCH_2O), 4.50–4.47 (m, 2H, PhCH_2O), 4.48–4.45 (m, 2H, PhCH_2O), 4.45–4.43 (m, 2H, H-2″, PhCH_2O), 4.40 (d, 1H, J_{gem} = 12.0 Hz, PhCH_2O), 4.31 (d, 1H, J_{gem} = 12.1 Hz, PhCH_2O), 4.27 (d, 1H, J_{gem} = 9.9 Hz, PhCH_2O), 4.23 (s, 1H, H-1), 4.04 (d, 1H, J_{gem} = 12.2 Hz, PhCH_2O), 3.89 (d, 1H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 3.82 (d, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.5 Hz, H-4″), 3.78 (dd, 1H, $J_{5',6a'}$ = 1.7 Hz, J_{gem} = 10.5 Hz, H-6a′), 3.73 (dd, 1H, $J_{5'',6a''}$ = 1.9 Hz, J_{gem} = 10.4 Hz, H-6a″), 3.71–3.68 (m, 2H, H-6b′, H-6b″), 3.62–3.59 (m, 2H, H-5, H-3′), 3.55–3.50 (m, 3H, H-3, H-6a, H-5′), 3.49 (s, 3H, OCH_3), 3.45–3.37 (m, 3H, H-6b, H-3″, H-5″), 1.64 (ddd, 1H, $J_{3,4eq}$ = 4.5 Hz, J_{gem} = 12.4 Hz, $J_{4eq,5}$ = 1.8 Hz, H-4_{eq}), 1.46 (q, 1H, J_{gem} = 12.2 Hz, H-4_{ax}). ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.6 (Ar), 138.5 (Ar), 138.3(7) (Ar), 138.3(2) (Ar), 138.2 (Ar), 138.1(6) (Ar), 138.1(3) (Ar), 138.0 (Ar), 129.2 (Ar), 128.3(7) (Ar), 128.3(1) (Ar), 128.2(7) (Ar), 128.2(4) (Ar), 128.2(2) (Ar), 128.2(1) (Ar), 128.2(0) (Ar), 128.0(6) (Ar), 128.0(4) (Ar), 127.9(8) (Ar), 127.8(7) (Ar), 127.7(9) (Ar), 127.7(1) (Ar), 127.6(6) (Ar), 127.6(2) (Ar), 127.5(9) (Ar), 127.5(1) (Ar), 127.4(9) (Ar), 127.3(6) (Ar), 127.3(5) (Ar), 127.2(6) (Ar), 103.2 (C-1), 100.7 (C-1″), 99.6 (C-1′), 81.4 (C-3″), 80.1 (C-3′), 75.3 (PhCH_2O), 75.2 (PhCH_2O), 75.0(7), 75.0(6) (C-5′, C-5″), 74.6 (C-3), 74.2(7) (C-4′), 74.2(0) (C-4″), 73.5 (PhCH_2O), 73.4 (PhCH_2O), 73.3 (PhCH_2O), 72.7(0), 72.6(8) (C-6, C-2′), 71.9 (C-5), 70.2 (C-6′), 69.9 (C-6), 69.5 (PhCH_2O), 69.4 (PhCH_2O), 69.2 (PhCH_2O), 67.6 (C-2), 66.8 (C-2″), 57.2 (OCH_3), 30.2 (C-4). HRESIMS: calcd for $C_{75}H_{82}O_{15}Na$ (M+Na): 1245.5546, found 1245.5539.

3.2.11. Methyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-methyl-β-D-mannopyranoside (23)

Acetic anhydride (2.5 mL, 26 mmol) was added to a solution of trisaccharide **21** (0.2829 g, 0.2257 mmol) in DMSO (5.0 mL, 70 mmol). The reaction was stirred overnight. The mixture was concentrated under reduced pressure and the residue was redissolved in THF (5 mL). This solution was cooled to −78 °C. A solution of L-Selectride® in THF (0.90 mL, 0.90 mmol) was added dropwise. The reaction was stirred at −78 °C for 2 h and was then warmed to room temperature overnight. The reaction was quenched with MeOH and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, where it was washed with 10% aqueous H_2O_2, 1 M aqueous NaOH, distilled

water, and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography (2:1, hexanes–EtOAc) gave **23** (0.188 g, 66%) as a clear and colourless syrup. R_f 0.20 (4:1, toluene–EtOAc); $[\alpha]_D$ −53.0 (*c* 2.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.52–7.49 (m, 2H, ArH), 7.42–7.39 (m, 2H, ArH), 7.32–7.20 (m, 31H, ArH), 7.19–7.15 (m, 5H, ArH), 5.06 (s, 1H, H-1″), 5.05 (s, 1H, H-1′), 4.99 (d, 1H, J_{gem} = 11.8 Hz, PhC*H*$_2$O), 4.98 (d, 1H, J_{gem} = 10.9 Hz, PhC*H*$_2$O), 4.94–4.90 (m, 2H, PhC*H*$_2$O), 4.67 (d, 1H, $J_{2',3'}$ = 3.1 Hz, H-2′), 4.63 (d, 1H, J_{gem} = 12.2 Hz, PhC*H*$_2$O), 4.58 (d, 1H, $J_{2,3}$ = 3.4 Hz, H-2), 4.57–4.52 (m, 2H, PhC*H*$_2$O), 4.52–4.47 (m, 4H, PhC*H*$_2$O), 4.45–4.40 (m, 2H, PhC*H*$_2$O), 4.32 (d, 1H, J_{gem} = 9.9 Hz, PhC*H*$_2$O), 4.29 (s, 1H, H-1), 4.28–4.25 (m, 2H, H-2″, PhC*H*$_2$O), 4.05 (d, 1H, J_{gem} = 12.2 Hz, PhC*H*$_2$O), 3.89 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 3.87 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.4 Hz, H-4″), 3.79 (dd, 1H, $J_{5',6a'}$ = 1.6 Hz, J_{gem} = 10.5 Hz, H-6a′), 3.73–3.70 (m, 2H, H-6a, H-6b′), 3.70–3.66 (m, 2H, H-6a″, H-6b″), 3.64 (dd, 1H, $J_{5,6b}$ = 5.0 Hz, J_{gem} = 10.5 Hz, H-6b), 3.61 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 9.3 Hz, H-3′), 3.53 (ddd, 1H, $J_{4',5'}$ = 9.6 Hz, $J_{5',6a'}$ = 1.7 Hz, $J_{5',6b'}$ = 6.0 Hz, H-5′), 3.48 (s, 3H, 1-OC*H*$_3$), 3.47 (m, 1H, H-5″), 3.46–3.42 (m, 2H, H-3, H-3″), 3.31 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 1.6 Hz, $J_{5,6b}$ = 5.0 Hz, H-5), 3.25 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.21 (s, 3H, 4-OC*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.5(9) (Ar), 138.5(6) (Ar), 138.4(0) (Ar), 138.3(7) (Ar), 138.3(0) (Ar), 138.2(3) (Ar), 138.1(7) (Ar), 138.0 (Ar), 129.1 (Ar), 138.3(4) (Ar), 128.3(2) (Ar), 128.2(6) (Ar), 128.2(5) (Ar), 128.2(1) (Ar), 128.2(0) (Ar), 128.1 (Ar), 128.0(8) (Ar), 128.0(2) (Ar), 127.9(8) (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.6(7) (Ar), 127.5(4) (Ar), 127.5(3) (Ar), 127.5(1) (Ar), 127.4(9) (Ar), 127.3(6) (Ar), 127.2 (Ar), 127.1(5) (Ar), 102.6 (C-1), 100.0 (C-1″), 99.8 (C-1′). 82.5 (C-3″), 80.4 (C-3), 80.2 (C-3′), 76.1 (C-4), 75.2(7), 75.2(6) (C-5, C-5′), 75.2(1) (PhC*H*$_2$O), 75.0 (PhC*H*$_2$O), 74.9(7) (C-5″), 74.4(5), 74.4(2) (C-4′, C-4″), 73.5 (PhC*H*$_2$O), 73.4 (PhC*H*$_2$O), 73.3 (PhC*H*$_2$O), 71.4 (C-2′), 70.2 (C-6′), 69.8 (C-6″), 69.8 (PhC*H*$_2$O), 69.5(2) (PhC*H*$_2$O), 69.4(8) (PhC*H*$_2$O), 69.3 (C-6), 68.9 (C-2), 67.1 (C-2″), 60.8 (4-OC*H*$_3$), 57.3 (4-OC*H*$_3$). HRESIMS: calcd for $C_{76}H_{84}O_{16}Na$ (M+Na): 1275.5652, found 1275.5641. Anal. Calcd for $C_{76}H_{84}O_{16}$: C, 72.82; H, 6.75. Found: C, 72.83; H, 6.77.

3.2.12. Methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-4-deoxy-β-D-*lyxo*-hexopyranoside (2)

Trisaccharide **22** (0.037 g, 0.030 mmol) was dissolved in a mixture of CH_2Cl_2 (2.5 mL) and MeOH (2.5 mL). 10% Pd/C (50 mg) was added and the reaction vessel was evacuated and purged with H_2(g). The mixture was stirred overnight under a hydrogen atmosphere. The catalyst was separated by filtration through celite and the filtrate was concentrated under reduced pressure. The crude product was dissolved in water and lyophilized. Purification of the product by HPLC gave **2** (0.0064 g, 42%) as a white solid. $[\alpha]_D$ −41.0 (*c* 0.6, H_2O); ^1H NMR (600 MHz, D_2O): δ 4.95 (s, 1H, H-1′), 4.90 (s, 1H, H-1″), 4.48 (s, 1H, H-1), 4.35 (d, 1H, $J_{2'',3''}$ = 3.4 Hz, H-2″), 4.15 (d, 1H, $J_{2',3'}$ = 3.0 Hz, H-2′), 4.09 (d, 1H, $J_{2,3}$ = 2.7 Hz, H-2), 3.93–3.88 (m, 3H, H-3, H-6a′, H-6a″), 3.75–3.67 (m, 3H, H-6a, H-6b′, H-6b″), 3.66–3.59 (m, 3H, H-5, H-6b, H-3″), 3.59–3.54 (m, 3H, H-3′, H-4′, H-4″), 3.53 (s, 3H, OC*H*$_3$), 3.40–3.34 (m, 2H, H-5′, H-5″), 1.73 (dd, 1H, $J_{3,4eq}$ = 4.3 Hz, J_{gem} = 11.8 Hz, H-4$_{eq}$), 1.41 (q, 1H, J_{gem} = 12.0 Hz, H-4$_{ax}$). ^{13}C NMR (125 MHz, D_2O): δ 102.7 (C-1), 101.7 (C-1′), 101.6 (C-1″), 79.1 (C-2″), 77.9 (C-2), 77.2(3), 77.2(1) (C-5′, C-5″), 73.9, 73.7 (C-5, C-3′), 73.1 (C-3″), 71.3 (C-2′), 68.1 (C-3), 68.0, 67.7 (C-4′, C-4″), 64.8 (C-6), 62.0, 61.7 (C-6′, C-6″), 57.9 (OC*H*$_3$), 30.9 (C-4). HRESIMS: calcd for $C_{19}H_{34}O_{15}Na$ (M+Na): 525.179, found 525.1789.

3.2.13. Methyl β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-4-*O*-methyl-β-D-mannopyranoside (3)

Trisaccharide **23** (0.089 g, 0.071 mmol) was dissolved in a mixture of CH_2Cl_2 (5 mL) and MeOH (5 mL). 10% Pd/C (50 mg) was added and the reaction vessel was evacuated and purged with H_2(g). The mixture was stirred overnight under a hydrogen atmosphere. The catalyst was separated by filtration through celite and the filtrate was concentrated under reduced pressure. The crude product was dissolved in water and lyophilized. Purification of the product by HPLC gave **3** (0.018 g, 47%) as a white solid. $[\alpha]_D$ −53.0 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, D_2O): δ 4.93 (s, 1H, H-1″), 4.89 (s, 1H, H-1′), 4.57 (s, 1H, H-1), 4.34 (d, 1H, $J_{2',3'}$ = 3.1 Hz, H-2′), 4.20 (d, 1H, $J_{2,3}$ = 3.0 Hz, H-2), 4.18 (d, 1H, $J_{2'',3''}$ = 3.2 Hz, H-2″), 3.93–3.88 (m, 3H, H-6a, H-6a″, H-6b″), 3.69 (m, 4H, H-3, H-6b, H-6a′, H-6b′), 3.65–3.60 (m, 2H, H-3′, H-3″), 3.60–3.55 (m, 2H, H-4′, H-4″), 3.53 (s, 3H, 4-OC*H*$_3$), 3.51 (s, 3H, 1-OC*H*$_3$), 3.40–3.33 (m, 3H, H-5, H-5′, H-5″), 3.26 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz). ^{13}C NMR (125 MHz, D_2O): δ 102.1 (C-1), 101.7 (C-1″), 101.5 (C-1′), 79.1 (C-2), 78.9 (C-2′), 78.2 (C-4), 77.1(4), 77.1(2) (C-5′, C-5″), 76.0 (C-5), 73.8 (C-3″), 73.0 (C-3′), 72.7 (C-3), 71.4 (C-2″), 67.8, 67.7 (C-4′, C-4″), 62.0 (C-6), 61.6 (C-6′, C-6″), 61.2 (4-OC*H*$_3$), 58.0 (1-OC*H*$_3$). HRESIMS: calcd for $C_{20}H_{36}O_{16}Na$ (M+Na): 555.1896, found 555.1895.

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Supplementary data

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