**Synthesis of part structures of *Cryptococcus neoformans* serotype C capsular polysaccharide**

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**Abstract** – *Cryptococcus neoformans* is a fungal pathogen that can cause life-threatening infections in immunocompromised patients. The development of a vaccine based on the capsular polysaccharide of *C. neoformans* is still an open challenge due to the heterogeneity of the capsular polysaccharide and the difficulty of identifying protective epitopes. Therefore, construction of structurally defined part structures of the C. neoformans GXM capsule is in great demand. Herein is presented the synthesis of a 3-*O*-naphthalenylmethyl protected trisaccharide thioglycoside building block which is present in *C. neoformans* serotype C polysaccharide. Its property as a donor in a glycosylation reaction with a model acceptor has been evaluated together with its behavior as an acceptor following removal of the temporary protecting group. The heavily branched hexasaccharide was obtained in good yields and excellent -selectivity. The frame shifted octasaccharide structural triad motif for serotype C was also prepared following the same building block strategy. For the first time this structural motif, which is the most substituted amongst the four *C. neoformans* serotypes, was prepared. Three synthesized *C. neoformans* serotype C fragments of varying size, from penta- up to octasaccharide, were deprotected and will be included in unique glycoarrays to further investigate the possibility to develop a synthetic vaccine against this pathogen.

*Keywords:* *Vaccine; Cryptococcus neoformans; Thioglycoside; Building Block*

**1. Introduction**

Cryptococcus neoformans is an opportunistic pathogen that causes severe diseases, e.g. cryptococcal meningoencephalitis (cryptococcosis) and death in immunocompromised individuals, including AIDS patients[1](#_ENREF_34),2 and organ transplant recipients[3](#_ENREF_34) or other patients receiving immunosuppressive drugs.

A thick layer of polysaccharides envelops the fungal cells and represents an important virulence factor. The basic structural motif of the major polysaccharide, which comprises 90-95% of the whole capsule, consists of a trimer of -d-(1→3)-mannoses which is substituted at OH-2 with a -d-gluco­pyranosyl­uronic acid (GlcA) residue. Further substitutions of the mannan backbone with -d-xylo­pyranosyl residues at OH-2, and/or at OH-4 give rise to four different serotypes A-D (Figure 1).4-7

Although the relative abundance of the triads reported in Figure 1 allowed for serotyping, the glucurono-xylo-mannan (GXM) polysaccharide is highly heterogeneous and minor amounts of cross-serotype substituted mannoses are found in each serotype. Also, the capsular polysaccharide contains acetyl groups at the 6 positions of the mannan backbone8 and these are believed to be immunologically relevant at least for serotype A and serotype D.9 The degree of O-acetylation varies between the serotypes with an average of two acetates per triad for serotype A and serotype D, and lower degree of acetylation for serotype B and C.

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Figure 1. Suggested structures of *C. neoformans* GXM serotype triads.

Xylose substitution

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serotype** | **2** | **2”** | **4’** | **4”** |
| **A** | **X** | **X** |  |  |
| **B** | **X** | **X** | **X** |  |
| **C** | **X** | **X** | **X** | **X** |
| **D** |  | **X** |  |  |

To investigate in depth the immunological determinants of the *C. neoformans* capsular polysaccharide for the development of glycoconjugate vaccines, well-defined synthetic oligosaccharide structures are crucial.

Most approaches explored to target the synthesis of part structures of the GXM polysaccharide have required late modification on rather large structures, e.g. introduction of GlcA residues,10-15 introduction of acetyl groups,16 and oxidation of glucose 6-OH.17 An attempt based on this type of strategy failed in the preparation of the octasaccharide structural motif of serotype C.15

A complementary approach entails the preparation and consecutive assembly of already modified building blocks.18-25 In theory, only six building blocks, four disaccharides and two trisaccharides (Figure 2), are required for the construction of any *C. neoformans* mannan variant.



In previous publications,18-21 we reported the preparation of 3-*O*-allyl protected thioglycoside building blocks and their use in the manufacture of GXM fragments. However, in light of recent findings, showing the incompatibility of the double bond and the thioethyl group in subsequent transformations,23 we are currently re-investigating the synthesis of the desired building blocks replacing the allyl group with the naphthalenylmethyl (NAP) group.23-25 Herein we report the preparation of a new building block **V**, and the study of its behaviour as a donor and as an acceptor in glycosylation reactions, targeting serotype C triad structural motifs.

**2. Results and discussion**

Concurrent introduction of the two xylose moieties appeared to be the most efficient approach for the synthesis of disubstituted thioglycoside **5**. The benzylidene ring on **1**24 was opened regioselectively with NaCNBH3/HCl,26 providing 2,4-OH acceptor **2** in a 73% yield. The coupling of **2** and **3**27 was carried out using TMSOTf in the presence of (commercial) acid-washed molecular sieves to prevent orthoester formation, affording compound **4** in a 68% yield (Scheme 1).



The benzoate groups, which were required to ensure the -selectivity in the glycosylation reaction, were then exchanged with benzyl groups giving building block **5** in an 80% yield.

For the purpose of preparing nonacetylated serotype C structures, this last step was unnecessary and **4** could have been used directly. However, owing to the large heterogeneity of the GXM capsular polysaccharide, minor quantities of this trisaccharide are also present in serotype A and D. Therefore, a 2,4-di-xylose trisaccharide building block, that only presents protecting groups compatible with acetyl groups and could be used in the synthesis of any GXM fragment, was selected.

The benzyl protected building block **5** was tested as a donor in a dimethyl(methylthio)sulfonium [DMTST]-promoted glycosylation reaction with a model mannose acceptor **6**.19 The reaction proceeded smoothly and afforded the spacer equipped tetrasaccharide **7** in an 84% yield with complete -selectivity (Scheme 2). This is in agreement with results previously obtained with the analogous 3-*O*-allyl thioglycoside derivative.19

The removal of the NAP protecting group by reaction with DDQ in a mixture of CH2Cl2/t-BuOH proceeded in a 70% yield giving acceptor **8** allowing the preparation of 2,3,4-tri-*O*-glycosylated structures. Thus, acceptor **8** was reacted with disaccharide thioglycoside **9**,24 again using DMTST as a promoter. Hexasaccharide **10** was obtained with complete -selectivity in an excellent 93% yield. The anomeric configurations were unambiguously confirmed by the one bond 1H–13C coupling constant values (only the mannose backbone coupling constants are reported for clarity: 1*J*C-H 173 Hz, 1*J*C-H 174 Hz, 1*J*C-H 170 Hz).

We then turned our attention to the preparation of the octasaccharide structural motif for serotype C. This is the largest and most branched structural motif of the four serotypes (A-D), having two disubstituted mannoses in the triad (Figure 1). As mentioned earlier, the synthesis of this heavily branched structure is still an open challenge. A previous synthetic attempt by Zao and Kong15 based on the introduction of the GlcA moiety on a heptasaccharide acceptor at the end of the synthetic pathway, a strategy proven successful for formation of a serotype B heptasaccharide,14 failed for type C.

First the spacer equipped trisaccharide **14** was prepared following the same sequence of reactions described above for building block **5** (Scheme 3). Regioselective benzylidene ring opening on **11**25 (→**12**), followed by coupling with the trichloroacetimidate donor **3** (→**13**), and final exchange of benzoates with benzyl groups gave the desired derivative **14** in a 46% overall yield.



The standardised sequence of reactions for the structure elongation, NAP removal-DMTST promoted glycosylation, was applied to **14** affording first acceptor **15** in an 82% yield and then -linked pentasaccharide **16** in an 82% yield after coupling with donor **9**.24 (Scheme 4)

Consecutive DDQ-promoted cleavage of the orthogonal protecting group gave the new acceptor **17** (68%) ready for the final glycosylation with trisaccharide thioglycoside **18**25. This glycosylation, for the first time, afforded the serotype C octasaccharide **19** together with its  anomer **20** in a 3:1 ratio and a 74% yield.

The incomplete -selectivity of the glycosylation reaction is in contrast to our previous findings when the same thioglycoside donor **18** was used for preparing serotype B heptasaccharide structural motif. The difference between the two acceptors used lies in the extra 4-*O*-linked xylopyranosyl residue present in compound **17**. This addition obviously has an impact on the stereoselectivity of the glycosylation. Also, NMR analysis of compound **20** at 25 °C in CDCl3 gave unexpected results, which included missing anomeric cross peaks in the 1H-13C HSQC NMR spectrum. By carrying out the NMR experiments at 50 °C the expected peaks were observed. This behaviour is in agreement with hindered rotation28 for compound **20** and was observed also during the preparation of building block **18**.25

Derivatives **10**, **17**, and **19** were deprotected by means of hydrogenolysis affording compounds **21**, **22**, and **23** in approximately 50% yields (Scheme 5). Longer reaction times were required for the reaction to go to completion in comparison with what was observed before in the deprotection of similar structures.24,25 Also, yields of isolated compounds were slightly lower. All the deprotected compounds contain the pentasaccharide frame, characteristic of *Cryptococcus neoformans* serotype C structures, but in different settings. These structures are now added to our library of synthetic GXM capsular polysaccharide fragments and will be used to prepare extended glycoarrays to further investigate the possibility to identify protective epitopes against *Cryptococcus neoformans*.



**3. Conclusion**

The orthogonally 2-naphtalenylmethyl (NAP) protected trisaccharide thioglycoside building block corresponding to *Cryptococcus neoformans* GXM serotype C structures has been prepared. Its ability as a donor in DMTST-promoted glycosylation reactions with a model monosaccharide acceptor has been proved. Subsequent removal of the temporary protecting group converted the tetrasaccharide into a new acceptor, which was shown to work well in the subsequent glycosylation reaction, allowing effective construction of the heavily branched 2,3,4-subtituted motif.

Also, and for the first time, the presented modular approach made possible the preparation of the spacer equipped octasaccharide **19**, corresponding to the structural motif of serotype C.

The protected octasaccharide presents a temporary protecting group on the non-reducing end mannose residue allowing access to even larger structures following the same sequence of reactions (NAP removal-DMTST glycosylation) shown above.

Three unprotected spacer equipped structures, all containing the distinctive *Cryptococcus neoformans* GXM serotype C pentasaccharide, were synthesized and will be used to prepare unique glycoarrays with the aim of identifying protective epitopes against this pathogen.

**4. Experimental Section**

**4.1. General methods**

TLC was carried out on precoated 60 F254 silica gel alumina plates (Merck) using UV-light and/or 8% H2SO4 and/or AMC-solution (ammonium molybdate, cerium (IV) sulphate, 10% H2SO4 [5:0.1:100, w/w/v] for visualization. Flash column chromatography was performed on silica gel (Merck, pore size 60 Å, particle size 40-63m). NMR spectra were recorded in CDCl3 (internal Me4Si *d* = 0.00 ppm) at 25 °C on a Varian instrument (500 MHz for 1H and 125 MHz for 13C or 600 MHz for 1H and 150 MHz for 13C). Coupling constants are given in Hertz (Hz). HRMS spectra were recorded on a micromass LCT instrument using electrospray ionisation (ESI) in either the positive or negative modes. Optical rotations were measured with a Perkin-Elmer 343 polarimeter at the sodium D-line (589 nm) at 20 °C using a 1 dm cell. All reactions containing air- and moisture-sensitive reagents were carried out under an argon atmosphere. Organic phases were dried over MgSO4 before evaporation, which was performed under reduced pressure at temperatures not exceeding 40 °C.

**4.2. Ethyl 6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)-1-thio--d-mannopyranoside (2)**

Sodium cyanoborohydride (1.98 g, 31.51 mmol) was added to a solution of **1** (2.01 g, 4.45 mmol) in dry THF (40 mL) containing crushed molecular sieves (3 Å, 200 mg). A 1 M solution of HCl in Et2O (25.0 mL, 25.0 mmol) was added dropwise at 20 °C (until pH 1-2). The reaction mixture was stirred until no starting material was detected by TLC (toluene-EtOAc, 7:3). After 3 h, Et3N (6 mL) was added, followed by dropwise addition of MeOH (20 mL). CH2Cl2 (30 mL) was added and the solids were removed by filtration through a short pad of Celite®. The filtrate was concentrated *in vacuo*, and then re-dissolved and co-evaporated with MeOH (3 × 20 mL). Purification by flash column chromatography (cyclohexane-EtOAc 8:2→4:6) gave **2** as a white foam (1.46 g, 3.23 mmol, 73%); *R*f 0.31 (7:3 toluene-EtOAc); []D20 +126.9 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.85 – 7.74 (m, 4H), 7.50 – 7.43 (m, 3H), 7.33 – 7.22 (m, 5H), 5.35 (d, *J* 1.3 Hz, 1H), 4.83 (d, *J* 11.7 Hz, 1H), 4.77 (d, *J* 11.7 Hz, 1H), 4.60 (d, *J* 12.0 Hz, 1H), 4.54 (d, *J* 12.0 Hz, 1H), 4.16 – 4.11 (m, 1H), 4.12– 4.09 (m, 1H), 3.98 (td, *J* 2.6 Hz, *J* 9.5 Hz, 1H), 3.79 – 3.66 (m, 3H), 2.78 (d, *J* 2.8 Hz, 1H), 2.71 (d, *J* 2.6 Hz, 1H), 2.67 – 2.50 (m, 2H), 1.26 (t, *J* 7.4 Hz, 3H); 13C NMR (126 MHz, CDCl3)  138.0, 135.1, 133.4, 133.2, 128.6, 128.5, 128.1, 127.8, 127.8, 127.7, 127.0, 126.4, 126.2, 125.8, 83.7, 79.9, 73.7, 72.2, 70.9, 70.3, 69.5, 68.4, 25.0, 14.9; HRMS (ESI): [M+Na]+ *m/z* Calcd for C26H30O5SNa, 477.1712; found, 477.1712.

**4.3. Ethyl 2,3,4-tri-*O*-benzoyl--d-xylopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)-1-thio--d-mannopyranoside (4)**

TMSOTf (0.28 mL, 1.54 mmol) was added to a solution of donor **3** (3.51 g, 5.8 mmol) and acceptor **2** (907 mg, 2.00 mmol) in dry CH2Cl2 (60 mL) containing crushed molecular sieves (AW-300, 200 mg) kept at −78 °C in an atmosphere of nitrogen. The temperature was then allowed to rise to 0 °C (TLC, toluene-EtOAc, 9:1). After 2 h, the reaction mixture was neutralised with Et3N (0.4 mL), the solids were removed by filtration, and the filtrate was concentrated *in vacuo* to a yellowish foam. Purification by flash column chromatography (toluene-EtOAc, 98:2→7:3) gave **4** (1.83 g, 68%) as a colourless foam; *R*f 0.57 (9:1 toluene-EtOAc); []D20 -8.7 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3) δ 8.06 – 8.03 (m, 2H), 8.02 – 7.99 (m, 2H), 7.98 – 7.89 (m, 9H), 7.84 (d, *J* 8.4 Hz, 1H), 7.82 – 7.78 (m, 2H), 7.59 (dd, *J* 1.7 Hz, *J* 8.4 Hz, 1H), 7.56 – 7.19 (m, 23H), 7.15 – 7.11 (m, 2H), 5.72 (t, *J* 6.5 Hz, 1H), 5.61 (t, *J* 7.7 Hz, 1H), 5.45 (dd, *J* 4.8 Hz, *J* 6.5 Hz, 1H), 5.32 (dd, *J* 5.9 Hz, *J* 7.8 Hz, 1H), 5.29 – 5.23 (m, 2H), 5.19 (td, *J* 4.6 Hz, *J* 7.5 Hz, 1H), 5.02 (d, *J* 4.8 Hz, 1H), 4.98 (d, *J* 11.9 Hz, 1H), 4.89 (d, *J* 11.9 Hz, 1H), 4.82 (d, *J* 5.9 Hz, 1H), 4.57 (dd, *J* 3.9 Hz, 12.3 Hz, 1H), 4.30 – 4.17 (m, 3H), 4.18 – 4.06 (m, 2H), 4.00 – 3.94 (m, 1H), 3.62 (dd, *J* 6.3 Hz, *J* 12.3 Hz, 1H), 3.45 – 3.35 (m, 2H), 3.20 (dd, *J* 7.5 Hz, *J* 12.2 Hz, 1H), 2.53 – 2.35 (m, 1H), 1.12 (t, *J* 7.4 Hz, 1H); 13C NMR (126 MHz, CDCl3)  165.6, 165.5, 165.5, 165.5, 165.2, 165.1, 138.5, 135.6, 133.4, 133.4, 133.4, 133.4, 133.2, 133.1, 130.1, 130.0, 130.0, 130.0, 129.9, 129.6, 129.4, 129.4, 129.4, 129.3, 129.3, 128.5, 128.5, 128.3, 128.3, 128.0, 127.8, 127.6, 127.4, 127.1, 126.4, 126.3, 126.0, 100.4, 98.8, 81.5, 76.5, 73.0, 72.5, 71.7, 71.1, 70.9, 70.2, 69.9, 69.4, 69.1, 68.9, 61.6, 61.1, 25.3, 14.9; HRMS (ESI): [M+Na]+ *m/z* Calcd for C78H70O19SNa, 1365.4130; found, 1365.4177.

**4.4. Ethyl 2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)-1-thio--d-mannopyranoside (5)**

Sodium methoxide (72 mg, 1.33 mmol) was added to a solution of **4** (580 mg, 0.43 mmol) in dry methanol (21 mL). The mixture was stirred at 20 °C overnight (TLC, CH2Cl2-MeOH, 9:1). After complete conversion, Dowex® (H+) acidic ion exchange resin was added for neutralisation, the resin was filtered off, washed with methanol, and the filtrate was concentrated *in vacuo*. The crude was dissolved in dry DMF (36 mL) and sodium hydride (400 mg, 10 mmol, 60% oil dispersion) was added to the solution kept at 0 °C in an atmosphere of nitrogen. After 15 min, benzyl bromide (0.6 mL, 5 mmol) was added at 0 °C under vigorous stirring. The temperature was then allowed to rise to 20 °C overnight (TLC, toluene-EtOAc, 8:2). After complete consumption of the starting material (16h), residual sodium hydride was quenched with MeOH (2 mL), and then with H2O (30 mL). The resulting mixture was extracted with Et2O (3 × 30 mL), the layers were separated, and the organic layer was dried over MgSO4. The solids were filtered off, and the filtrate was concentrated *in vacuo*. Purification by flash column chromatography (toluene-EtOAc, 98:2→7:3) gave **5** (435 mg, 80%) as a colourless syrup; *R*f 0.36 (9:1 toluene-EtOAc); []D20 +37.9 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.87 (d, *J* 1.5 Hz, 1H), 7.84 – 7.80 (m, 1H), 7.78 – 7.74 (m, 2H), 7.55 (dd, *J* 1.7 Hz, *J* 8.5 Hz, 1H), 7.47 – 7.43 (m, 2H), 7.39 – 7.14 (m, 33H), 7.08 – 7.02 (m, 2H), 5.38 (d, *J* 2.1 Hz, 1H), 5.06 (d, *J* 10.3 Hz, 1H), 4.92 – 4.67 (m, 9H), 4.64 (d, *J* 11.7 Hz, 1H), 4.60 – 4.53 (m, 2H), 4.49 (d, *J* 10.2 Hz, 1H), 4.42 – 4.36 (m, 2H), 4.30 – 4.21 (m, 3H), 4.16 (dd, *J* 2.2 Hz, *J* 3.4 Hz, 1H), 4.04 (ddd, *J* 1.8 Hz, *J* 4.4 Hz, *J* 9.5 Hz, 1H), 3.92 (dd, *J* 5.2 Hz, *J* 11.5 Hz, 1H), 3.86 – 3.75 (m, 3H), 3.65 – 3.40 (m, 6H), 3.31 (dd, *J* 6.7 Hz, *J* 9.2 Hz, 1H), 3.17 (dd, *J* 9.8 Hz, *J* 11.6 Hz, 1H), 2.93 (dd, *J* 10.0 Hz, *J* 11.8 Hz, 1H), 2.65 – 2.51 (m, 2H), 1.24 (t, *J* 7.4 Hz, 3H); 13C NMR (126 MHz, CDCl3)  139.0, 138.8, 138.7 (2C), 138.4, 138.4, 138.3, 136.5, 133.4, 133.0, 129.1, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 126.6, 126.3, 126.0, 125.7, 103.6, 103.3, 84.2, 84.1, 82.5, 82.1, 81.2, 78.4, 77.7, 77.0, 77.0, 75.6 (2C), 75.1, 75.0, 74.8, 73.5, 73.1, 73.0, 72.3, 72.1, 69.0, 64.1, 63.7, 25.6, 15.1; HRMS (ESI): [M+Na]+ *m/z* Calcd for C78H82O13SNa, 1281.5374; found, 1281.5388.

**4.5. 2-Azidoethyl (2,3,4-tri-*O*-benzyl--d-xylopyranosyl)-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-(6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)--d-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl--d-mannopyranoside (7)**

A mixture of **6** (30 mg, 57.7 mol), **5** (109 mg, 86.5 mol) and crushed molecular sieves (4 Å, 40 mg) in dry Et2O (5 mL) was stirred at 20 °C for 30 min. The reaction mixture was cooled to 0 °C, DMTST (67 mg, 0.26 mmol) was added. The cooling bath was removed after the addition and stirring was continued at 20 °C for 1h. The reaction was quenched with Et3N (0.2 mL) and the solids were removed by filtration through a pad of Celite®. The filtrate was concentrated *in vacuo* and the crude was purified by flash column chromatography (SiO2, cyclohexane-EtOAc, 95:5→70:30) to give **7** (83 mg, 84%) as a colourless syrup; *R*f 0.48 (9:1 toluene-EtOAc); []D20 +9.3 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.83 (s, 1H), 7.73 (d, *J* 7.9 Hz, 1H), 7.67 (t, *J* 9.6 Hz, 2H), 7.53 (dd, *J* 8.4, 1H), 7.42 – 7.01 (m, 52H), 5.19 (s, 1H), 5.04 (d, *J* 10.6 Hz, 1H), 4.88 – 4.78 (m, 5H), 4.76 – 4.72 (m, 2H), 4.70 – 4.36 (m, 13H), 4.30 – 4.19 (m, 4H), 4.11 – 4.04 (m, 2H), 4.02 – 3.92 (m, 3H), 3.91 – 3.37 (m, 15H), 3.36 – 3.20 (m, 4H), 2.98 (t, *J* 10.8 Hz, 1H), 2.78 (t, *J* 10.6 Hz, 1H); 13C NMR (126 MHz, CDCl3)  139.1, 139.0, 138.8, 138.6, 138.6 (2C), 138.5, 138.4, 138.4, 138.3, 136.6, 133.4, 133.0, 129.0, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.2, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 127.2, 127.1, 126.7, 126.5, 126.0, 125.7, 103.8 (*J*C-H 162 Hz), 103.3 (*J*C-H 159.5 Hz), 100.0 (*J*C-H 171 Hz), 97.9 (*J*C-H 170.5 Hz), 84.2, 83.6, 82.6, 81.1, 79.3, 78.5, 78.4, 77.5, 76.2, 75.8, 75.6, 75.4, 74.9, 74.9, 74.8, 74.5, 74.3, 73.5, 73.2, 73.2, 73.0, 72.9, 72.8, 72.7, 72.3, 69.2, 69.1, 66.7, 63.9, 63.7, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C105H109N3O19Na, 1738.7553; found, 1738.7620.

**4.6. 2-Azidoethyl (2,3,4-tri-*O*-benzyl--d-xylopyranosyl)-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-(6-*O*-benzyl--d-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl--d-mannopyranoside (8)**

DDQ (15 mg, 66 mol) was added to a vigorously stirred solution of **7** (56 mg, 32.6 mol) in CH2Cl2/*t*-BuOH (5.5 mL, 10:1) at 20 °C. After 1.5h, the reaction was quenched by adding sat. NaHCO3 solution (20 mL). The resulting mixture was stirred for 20 min and then was extracted once with CH2Cl2 (20 mL), the layers were separated, and the organic layer was washed with 10% aq. Na2S2O3 solution (20 mL), dried over MgSO4 and concentrated *in vacuo* to a yellow oil. Purification by flash column chromatography on silica gel (toluene-EtOAc, 95:5→70:30) gave **8** (36 mg, 70%) as a colourless syrup; *R*f 0.29 (9:1 toluene-EtOAc); []D20 +22.5 (*c* 0.7, CHCl3); 1H NMR (500 MHz, CDCl3)  7.42 – 6.99 (m, 50H), 5.22 (s, 1H), 5.03 (d, *J* 10.5 Hz, 1H), 4.87 – 4.41 (m, 18H), 4.23 (d, *J* 7.7 Hz, 1H), 4.19 – 3.92 (m, 9H), 3.91 – 3.44 (m, 14H), 3.37 – 3.22 (m, 5H), 3.12 (t, *J* 11.1 Hz, 1H), 2.71 (t, *J* 11.0 Hz, 1H); 13C NMR (126 MHz, CDCl3)  139.0, 138.8, 138.6, 138.6, 138.5, 138.4, 138.4, 138.3 (2C), 138.2, 129.0, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.6, 127.5, 127.4, 127.1, 127.1, 104.1, 103.4, 100.3, 97.7, 84.1, 83.7, 81.9, 81.2, 80.0, 78.6, 78.3, 77.8, 77.4, 77.2, 75.8, 75.6, 75.2, 75.0, 74.7, 74.6, 73.6, 73.5, 73.3, 73.1, 72.4 (2C), 71.9, 69.2, 69.1, 68.4, 66.7, 64.3, 64.1, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C94H101N3O19Na, 1598.6927; found, 1598.6926.

**4.7. 2-Azidoethyl (2,3,4-tri-*O*-benzyl--d-xylopyranosyl)-(1→2)-(4,6-di-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)--d-mannopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)]-[2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-(6-*O*-benzyl--d-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl--d-mannopyranoside (10)**

A mixture of **8** (36 mg, 22.8 mol), **9** (33 mg, 34.8 mol) and crushed molecular sieves (4 Å, 20 mg) in dry Et2O (5 mL) was stirred at 20 °C for 30 min. The reaction mixture was cooled to 0 °C and DMTST (26 mg, 0.1 mmol) was added. The cooling bath was removed after the addition and stirring was continued at 20 °C for 1h. The reaction was quenched with Et3N (50 L) and the solids were removed by filtration through a pad of Celite®. The filtrate was concentrated *in vacuo* and the crude was purified by flash column chromatography (SiO2, toluene-EtOAc, 95:5→9:1) to give **10** (52 mg, 93%) as a colourless syrup; *R*f 0.54 (9:1 toluene-EtOAc); []D20 -9.7 (*c* 1.0, CHCl3); 1H NMR (600 MHz, CDCl3)  7.89 (d, *J* 3.8 Hz, 1H), 7.77 – 7.66 (m, 3H), 7.51 (dd, *J* 9.1, 3.4 Hz, 1H), 7.46 – 7.36 (m, 5H), 7.36 – 7.04 (m, 72H), 5.46 (d, *J* 3.9 Hz, 1H), 5.20 (d, *J* 3.8 Hz, 1H), 5.13 (dd, *J* 11.2, 3.9 Hz, 1H), 5.06 – 5.00 (m, 2H), 4.90 (dd, *J* 3.8 Hz, *J* 11.1 Hz,1H), 4.87 – 4.83 (m, 2H), 4.83 – 4.72 (m, 6H), 4.71 – 4.66 (m, 3H), 4.65 – 4.46 (m, 12H), 4.44 – 4.24 (m, 10H), 4.19 – 4.06 (m, 5H), 4.06 – 3.92 (m, 5H), 3.90 – 3.72 (m, 7H), 3.71 – 3.51 (m, 9H), 3.45 (td, *J* 3.8 Hz, *J* 8.9 Hz,1H), 3.39 (ddt, *J* 3.4 Hz, *J* 6.8 Hz, *J* 13.6 Hz, 1H), 3.33 – 3.15 (m, 6H), 2.93 (td, *J* 4.0 Hz, *J* 11.2 Hz,1H), 2.72 – 2.65 (m, 1H); 13C NMR (151 MHz, CDCl3)  139.5, 139.1, 139.0, 138.9, 138.9, 138.7, 138.6, 138.5, 138.5, 138.4, 138.4, 138.3, 136.2, 133.4, 133.0, 129.0, 129.0, 128.8, 128.8, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 127.1, 127.1, 127.0, 126.8, 126.0, 125.9, 104.7 (*J*C-H 163 Hz), 103.9 (*J*C-H 160 Hz), 103.3 (*J*C-H 162 Hz), 101.0 (*J*C-H 173 Hz), 99.2 (*J*C-H 174 Hz), 97.8 (*J*C-H 170 Hz), 84.3, 83.6, 83.4, 82.6, 81.0, 80.9, 80.3, 79.1, 78.7, 78.2, 77.9, 77.7, 76.9, 75.6, 75.4, 75.3, 75.2, 75.0, 75.0, 74.6, 74.5, 74.4, 74.2, 73.6, 73.5, 73.4, 73.4, 73.0, 72.9, 72.6, 72.5, 72.4, 72.4, 72.2, 72.2, 69.9, 69.3, 68.6, 66.7, 64.2, 63.9, 63.2, 50.5; [M+Na]+ *m/z* Calcd for C151H157N3O28Na, 2483.0851; found, 2483.0886.

**4.8. 2-Azidoethyl 6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)--d-mannopyranoside (12)**

Sodium cyanoborohydride (1.86 g, 29.60 mmol) was added to a solution of **11** (2.1 g, 4.40 mmol) in dry THF (40 mL) containing crushed molecular sieves (3 Å, 200 mg). A 1 M solution of HCl in Et2O (25.0 mL, 25.0 mmol) was added dropwise at 20 °C (until pH 1-2). The reaction mixture was stirred until no starting material was detected by TLC (toluene-EtOAc, 7:3). After 3 h, Et3N (6 mL) was added, followed by dropwise addition of MeOH (20 mL). CH2Cl2 (30 mL) was added and the solids were removed by filtration through a short pad of Celite®. The filtrate was concentrated *in vacuo*, and then re-dissolved and co-evaporated with MeOH (3 × 20 mL). Purification by flash column chromatography (toluene-EtOAc 98:2→1:1) gave **12** as a white foam (1.70 g, 3.56 mmol, 81%); *R*f 0.36 (7:3 toluene-EtOAc); []D20 +16.2 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.86 – 7.76 (m, 4H), 7.50 – 7.44 (m, 3H), 7.38 – 7.22 (m, 5H), 4.91 (d, *J* 1.6 Hz, 1H), 4.85 (d, *J* 11.8 Hz, 1H), 4.79 (d, *J* 11.8 Hz, 1H), 4.60 (d, *J* 12.1 Hz, 1H), 4.56 (d, *J* 12.0 Hz, 1H), 4.05 (td, *J* 1.7 Hz, *J* 3.0 Hz, 1H), 3.94 (td, *J* 2.5 Hz, *J* 9.2 Hz, 1H), 3.87 (ddd, *J* 3.7 Hz, *J* 5.9 Hz, *J* 10.7 Hz, 1H), 3.80 – 3.72 (m, 4H), 3.58 (ddd, *J* 3.9 Hz, *J* 6.3 Hz*, J* 10.5 Hz, 1H), 3.39 – 3.29 (m, 2H), 2.59 (d, *J* 2.5 Hz, 1H), 2.54 (d, *J* 2.6 Hz, 1H); 13C NMR (126 MHz, CDCl3)  138.1, 135.3, 133.4, 133.2, 128.7, 128.5, 128.1, 127.8, 127.8, 127.8, 127.1, 126.4, 126.3, 125.8, 99.7, 79.4, 73.7, 72.3, 71.0, 70.4, 67.9, 66.8, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C26H29N3O6Na, 502.1954; found, 502.1940.

**4.9. 2-Azidoethyl 2,3,4-tri-*O*-benzoyl--d-xylopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)--d-mannopyranoside (13)**

TMSOTf (0.15 mL, 0.83 mmol) was added dropwise to a solution of donor **3** (1.68 g, 2.77 mmol) and acceptor **12** (509 mg, 1.06 mmol) in dry CH2Cl2 (28 mL) containing crushed molecular sieves (AW-300, 150 mg) kept at −78 °C in an atmosphere of nitrogen. The temperature was then allowed to rise to 0 °C. After 1.5 h, the reaction mixture was neutralised with Et3N (0.4 mL), the solids were removed by filtration through a pad of Celite®, and the filtrate was concentrated *in vacuo* to a yellowish foam. Purification by flash column chromatography (toluene-EtOAc, 98:2→7:3) gave **13** (1.07 g, 74%) as a colourless foam; *R*f 0.57 (9:1 toluene-EtOAc); []D20 -39.0 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  8.05 – 8.00 (m, 4H), 7.98 – 7.88 (m, 9H), 7.85 (d, *J* 8.5 Hz, 1H), 7.82 – 7.79 (m, 2H), 7.59 (dd, *J* 8.6, 1.4 Hz, 1H), 7.57 – 7.29 (m, 18H), 7.29 – 7.20 (m, 5H), 7.15 – 7.12 (m, 2H), 5.70 (t, *J* 6.4 Hz, 1H), 5.60 (t, *J* 7.7 Hz, 1H), 5.47 – 5.38 (m, 1H), 5.31 (dd, *J* 5.9 Hz, *J* 7.9 Hz, 1H), 5.26 (td, *J* 3.8 Hz, *J* 6.2 Hz, 1H), 5.17 (td, *J* 4.5 Hz, *J* 7.5 Hz, 1H), 5.03 (d, *J* 4.7 Hz, 1H), 4.98 (d, *J* 11.8 Hz, 1H), 4.87 (d, *J* 11.9 Hz, 1H), 4.83 (d, *J* 2.7 Hz, 1H), 4.78 (d, *J* 5.9 Hz, 1H), 4.59 (dd, *J* 3.8 Hz, *J* 12.4 Hz, 1H), 4.25 (d, *J* 12.2 Hz, 1H), 4.23 – 4.15 (m, 2H), 4.10 (d, *J* 12.2 Hz, 1H), 4.08 – 4.02 (m, 2H), 3.71 (ddd, *J* 3.5 Hz, *J* 5.9 Hz, *J* 10.1 Hz, 1H), 3.68 – 3.60 (m, 2H), 3.46 – 3.38 (m, 2H), 3.33 (dd, *J* 5.7 Hz, *J* 11.0 Hz, 1H), 3.27 (ddd, *J* 3.5 Hz, *J* 7.1 Hz, *J* 13.2 Hz, 1H), 3.23 – 3.12 (m, 2H); 13C NMR (126 MHz, CDCl3)  165.6, 165.5, 165.5, 165.4, 165.2, 165.1, 138.4, 135.8, 133.4 (3C), 133.4, 133.4, 133.4, 133.3, 133.1, 130.1, 130.0, 130.0, 129.9, 129.9, 129.5, 129.5, 129.4, 129.3, 129.3, 129.2, 128.5, 128.5, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.0, 127.8, 127.6, 127.5, 127.0, 126.4, 126.2, 126.0, 100.5, 99.2, 98.2, 76.8, 75.9, 75.2, 73.1, 72.5, 71.8, 71.1, 70.9, 70.2, 69.8, 69.4, 69.1, 68.9, 66.7, 61.6, 61.0, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C78H69N3O20Na, 1390.4372; found, 1390.4353.

**4.10. 2-Azidoethyl 2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)--d-mannopyranoside (14)**

Sodium methoxide (100 mg, 1.85 mmol) was added to a solution of **13** (812 mg, 0.59 mmol) in dry methanol (30 mL). The mixture was stirred at 20 °C overnight. After complete conversion, Dowex® (H+) acidic ion exchange resin was added for neutralisation, the resin was filtered off, washed with methanol, and the filtrate was concentrated *in vacuo*. The crude was dissolved in dry DMF (50 mL) and sodium hydride (560 mg, 14 mmol, 60% oil dispersion) was added to the solution kept at 0 °C in an atmosphere of nitrogen. After 15 min, benzyl bromide (0.83 mL, 6.98 mmol) was added at 0 °C under vigorous stirring. The temperature was then allowed to rise to 20 °C overnight (TLC, toluene-EtOAc, 8:2). After 16h, residual sodium hydride was quenched with MeOH (2 mL), and then with H2O (50 mL). The resulting mixture was extracted with Et2O (3 × 500 mL), the layers were separated, and the organic layer was dried over MgSO4. The solids were filtered off, and the filtrate was concentrated *in vacuo*. Purification by flash column chromatography (toluene-EtOAc, 99:1→7:3) gave **14** (590 mg, 77%) as a colourless syrup; *R*f 0.46 (9:1 toluene-EtOAc) []D20 +15.0 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.84 – 7.80 (m, 1H), 7.78 – 7.73 (m, 2H), 7.56 (dd, *J* 1.6 Hz, *J* 8.4 Hz, 1H), 7.48 – 7.41 (m, 2H), 7.38 – 7.02 (m, 32H), 5.03 (d, *J* 10.4 Hz, 1H), 4.93 (d, *J* 2.4 Hz, 1H), 4.91 (d, *J* 12.8 Hz, 1H), 4.89 – 4.77 (m, 5H), 4.76 – 4.67 (m, 3H), 4.65 – 4.50 (m, 4H), 4.42 (d, *J* 7.3 Hz, 1H, H-1xyl), 4.37 (d, *J* 7.6 Hz, 1H, H-1xyl), 4.34 – 4.25 (m, 2H), 4.22 (t, *J* 8.5 Hz, 1H), 4.11 (dd, *J* 2.4 Hz, *J* 3.4 Hz, 1H), 3.93 (dd, *J* 5.3 Hz, *J* 11.6 Hz, 1H), 3.90 (dd, *J* 3.4 Hz, *J* 8.4 Hz, 1H), 3.87 – 3.73 (m, 4H), 3.66 – 3.46 (m, 6H), 3.43 (t, *J* 8.9 Hz, 1H), 3.33 – 3.27 (m, 3H), 3.19 (dd, *J* 9.9 Hz, *J* 11.6 Hz, 1H), 2.92 (dd, *J* 10.0 Hz, *J* 11.8 Hz, 1H); 13C NMR (126 MHz, CDCl3)  138.9, 138.8, 138.7, 138.7, 138.4, 138.3, 138.3, 136.6, 133.4, 133.0, 128.9, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 126.6, 126.4, 125.9, 125.7, 103.7 (2C), 98.4, 84.2, 84.0, 82.4, 81.3, 78.3, 77.7, 76.5, 75.6, 75.6, 75.5, 75.0, 75.0, 74.7, 73.5, 73.1 (2C), 72.4, 72.1, 69.0, 66.7, 64.1, 63.7, 50.6; HRMS (ESI): [M+Na]+ *m/z* Calcd for C78H81N3O14Na, 1306.5616; found, 1306.5642.

**4.11. 2-Azidoethyl 2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranoside (15)**

DDQ (267 mg, 1.20 mmol) was added to a vigorously stirred solution of **14** (428 mg, 0.33 mmol) in CH2Cl2/*t*-BuOH (44 mL, 10:1) at 20 °C. The progress of the reaction was monitored by TLC (toluene-EtOAc, 9:1). After 60 min, the reaction was quenched by adding sat. NaHCO3 solution (80 mL). The resulting mixture was extracted once with CH2Cl2 (50 mL), the layers were separated, and the organic layer was washed 10% aq. Na2S2O3 solution (20 mL), dried over MgSO4 and concentrated *in vacuo* to a yellow oil. Purification by flash column chromatography (SiO2, toluene-EtOAc, 95:5→70:30) gave **15** (313 mg, 82%) as a colourless syrup; *R*f 0.25 (9:1 toluene-EtOAc); []D20 +43.6 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.41 – 6.97 (m, 35H), 5.03 (d, *J* 10.3 Hz, 1H), 4.92 (s, 1H), 4.90 – 4.79 (m, 4H), 4.78 (s, 2H), 4.73 – 4.67 (m, 2H), 4.61 – 4.56 (m, 2H), 4.50 (d, *J* 10.3 Hz, 1H), 4.33 (d, *J* 7.7 Hz, 1H, H-1xyl), 4.25 (d, *J* 7.8 Hz, 1H, H-1xyl), 4.22 – 4.16 (m, 2H), 4.07 (bs, 1H), 4.00 – 3.82 (m, 5H), 3.78 – 3.74 (m, 2H), 3.70 (dd, *J* 4.3 Hz, *J* 10.5 Hz, 1H), 3.67 – 3.46 (m, 6H), 3.44 – 3.30 (m, 4H), 3.18 – 3.05 (m, 2H); 13C NMR (126 MHz, CDCl3)  138.9, 138.6, 138.6, 138.5, 138.4, 138.2, 138.2, 129.0, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 128.0, 128.0, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 103.9, 103.8, 98.2, 84.1, 84.1, 81.7, 81.3, 77.9, 77.8, 77.4, 77.3, 75.7 (2C), 75.3, 75.1, 73.5, 73.5, 73.0, 71.4, 68.7, 68.2, 66.8, 64.3, 64.2, 50.6; HRMS (ESI): [M+Na]+ *m/z* Calcd for C67H73N3O14Na, 1166.4990; found, 1166.4940.

**4.12. 2-Azidoethyl (2,3,4-tri-*O*-benzyl--d-xylopyranosyl)-(1→2)-(4,6-di-*O*-benzyl-3-*O*-(2-naphthalenylmethyl)--d-mannopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)][2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranoside (16)**

A mixture of **15** (53 mg, 46.5 mol), **9** (66 mg, 69.7 mol) and crushed molecular sieves (4 Å, 50 mg) in dry Et2O (5 mL) was stirred at 20 °C for 30 min. The reaction mixture was cooled to 0 °C and DMTST (36 mg, 139.5 mol) was added. The cooling bath was removed after the addition and stirring was continued at 20 °C for 2.5h. The reaction was quenched with Et3N (100 L) and the solids were removed by filtration through a pad of Celite®. The filtrate was concentrated *in vacuo* and the crude was purified by flash column chromatography on silica gel (toluene-EtOAc, 95:5→70:30) to give **16** (77 mg, 82%) as a colourless syrup; *R*f 0.43 (9:1 toluene-EtOAc); []D20 -3.0 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.86 (s, 1H), 7.70 (m, *3*H), 7.50 (d, *J* 9.8 Hz, 1H), 7.45 – 7.05 (m, 62H), 5.38 (s, 1H), 5.12 (d, *J* 10.7 Hz, 1H), 5.02 – 4.97 (m, 2H), 4.91 – 4.69 (m, 10H), 4.69 – 4.33 (m, 13H), 4.32 – 4.25 (m, 3H), 4.23 – 4.09 (m, 6H), 4.02 – 3.74 (m, 9H), 3.71 (dd, *J* 3.1 Hz, *J* 9.9 Hz, 1H), 3.67 – 3.39 (m, 8H), 3.35 – 3.17 (m, 6H), 3.04 (dd, *J* 9.5 Hz, *J* 11.9 Hz, 1H), 2.97 (t, *J* 11.3 Hz, 1H); 13C NMR (126 MHz, CDCl3)  139.5, 139.1, 139.0, 138.9, 138.8, 138.8, 138.7, 138.7, 138.5, 138.4, 138.4, 138.2, 136.2, 133.4, 133.0, 129.1, 129.0, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.2, 126.7, 126.1, 125.9, 104.4 (*J*C-H 160.5 Hz), 104.3 (*J*C-H 160.5 Hz), 103.6 (*J*C-H 161 Hz), 99.5 (*J*C-H 174.5 Hz), 98.4 (*J*C-H 171 Hz), 84.3, 83.8, 83.8, 82.5, 81.1 (2C), 79.1, 78.6, 78.1, 77.8, 77.8, 75.9, 75.5, 75.5, 75.4, 75.4, 75.0, 75.0, 74.9, 74.7, 74.4, 73.6, 73.4, 73.3, 73.2, 73.0, 72.4, 72.4, 72.0, 71.8, 69.9, 68.2, 66.7, 64.3, 64.0, 63.4, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C124H129N3O23Na, 2050.8915; found, 2050.8989.

**4.13. 2-Azidoethyl (2,3,4-tri-*O*-benzyl--d-xylopyranosyl)-(1→2)-(4,6-di-*O*-benzyl--d-mannopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)][2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranoside (17)**

DDQ (31 mg, 136 mol) was added to a vigorously stirred solution of **16** (77 mg, 38 mol) in CH2Cl2/*t*-BuOH (5 mL, 10:1) at 20 °C. After 1h, the reaction was quenched by adding sat. NaHCO3 solution (15 mL). The resulting mixture was stirred for 20 min and then extracted once with CH2Cl2 (20 mL). The layers were separated, and the organic layer was washed with 10% aq. Na2S2O3 solution (10 mL), and H2O (10 mL), dried over MgSO4 and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (toluene-EtOAc, 98:2→70:30) gave **18** (49 mg, 68%) as a colourless syrup; *R*f 0.48 (9:1 toluene-EtOAc); []D20 +21.6 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.53 – 6.98 (m, 60H), 5.39 (d, *J* 1.5 Hz, 1H), 5.08 (d, *J* 10.9 Hz, 1H), 5.03– 4.98 (m, 2H), 4.90 – 4.52 (m, 18H), 4.42 – 4.35 (m, 3H), 4.36 – 4.21 (m, 6H), 4.20 – 4.12 (m, 3H), 4.06 (dd, *J* 5.2 Hz, *J* 11.7 Hz, 1H), 4.03 – 3.93 (m, 3H), 3.88 (dd, *J* 3.7 Hz, *J* 10.8 Hz, 1H), 3.85 – 3.77 (m, 3H), 3.75 – 3.67 (m, 2H), 3.66 – 3.40 (m, 10H), 3.34 – 3.07 (m, 6H), 3.01 (d, *J* 9.0 Hz, 1H); 13C NMR (126 MHz, CDCl3)  139.4, 139.1, 138.9, 138.8, 139.0, 138.7 (2C), 138.5, 138.3, 138.3, 138.3, 138.2, 129.0, 128.9, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 104.5, 104.5, 104.3, 99.9, 98.4, 84.3, 83.9, 83.9, 82.6, 81.1, 80.9, 80.1, 79.1, 78.3, 77.9, 77.5, 77.3, 75.8, 75.8, 75.5, 75.5, 75.3, 75.0, 74.8, 74.6, 73.7, 73.5, 73.4, 73.2, 73.2, 72.8, 72.0, 71.8, 70.7, 69.8, 68.2, 66.7, 64.4, 64.1, 63.6, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C113H121N3O23Na, 1910.8289; found, 1910.8378.

**4.14. 2-Azidoethyl (benzyl 2,3,4-tri-*O*-benzyl--d-glucopyranosyluronate)-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)]-(4,6-di-*O*-benzyl--d-mannopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)][2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranoside (19)** and **2-Azidoethyl (benzyl 2,3,4-tri-*O*-benzyl--d-glucopyranosyluronate)-(1→2)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)]-(4,6-di-*O*-benzyl--d-mannopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl--d-xylopyranosyl-(1→2)][2,3,4-tri-*O*-benzyl--d-xylo­pyranosyl-(1→4)]-6-*O*-benzyl--d-mannopyranoside (20)**

A mixture of **17** (44 mg, 23.3 mol), 1**8** (48.7 mg, 35 mol) and crushed molecular sieves (4 Å, 50 mg) in dry Et2O (3.5 mL) was stirred at 20 °C for 30 min. The reaction mixture was cooled to 0 °C, freshly prepared DMTST (27 mg, 104 mol) was added. The cooling bath was removed and stirring was continued at 20 °C for 1.5h. The reaction was quenched by adding Et3N (80 L). The solids were removed by filtration through a pad of Celite®, and the filtrate was concentrated *in vacuo*. Purification by flash column chromatography (toluene-EtOAc, 98:2→70:30) gave **19** (44 mg, 57%) as a colourless syrup and **20** (13 mg, 17%) as a colourless syrup.

**Compound 19**

*R*f 0.61 (9:1 toluene-EtOAc); []D20 -11.6 (*c* 1.0, CHCl3); 1H NMR (500 MHz, CDCl3)  7.88 – 7.81 (m, 2H), 7.72 (d, *J* 8.2 Hz, 1H), 7.68 (d, *J* 8.5 Hz, 1H), 7.55 – 7.49 (m, 3H), 7.40 – 6.84 (m, 100H), 5.47 (s, 1H), 5.25 (s, 1H), 5.18 (d, *J* 10.6 Hz, 1H), 5.03 (d, *J* 12.2 Hz, 1H), 4.96 – 4.82 (m, 6H), 4.83 – 4.62 (m, 12H), 4.62 – 4.42 (m, 12H), 4.41 – 4.16 (m, 20H), 4.10 – 3.95 (m, 8H), 3.88 – 3.80 (m, 3H), 3.78 – 3.60 (m, 10H), 3.57 – 3.51 (m, 2H), 3.51 – 3.08 (m, 16H), 3.94 – 3.82 (m, 2H), 2.67 (t, *J* 11.4 Hz, 1H); 13C NMR (126 MHz, CDCl3)  168.2, 139.3, 139.3, 139.2, 139.0 (2C), 139.0 (2C), 138.8, 138.8 (2C), 138.7, 138.7, 138.4, 138.4, 138.4 (2C), 138.3, 138.3, 138.2, 135.9, 135.2, 133.3, 133.1, 129.2, 129.0, 128.9, 128.9, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.3, 128.2, 128.22, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 126.1, 125.9, 125.6, 104.9 (*J*C-H 161.0 Hz), 104.4 (2C, *J*C-H 162.0 Hz, *J*C-H 161.0 Hz), 103.6 (*J*C-H 162.0 Hz), 102.4 (*J*C-H 160.0 Hz), 99.9 (*J*C-H 175.5 Hz), 98.7 (*J*C-H 171.5 Hz), 98.7 (*J*C-H 171.5 Hz), 84.7, 84.2, 83.8 (2C), 83.5, 82.9, 82.6, 81.5 (2C), 80.9, 79.2, 79.0 (2C), 78.9, 78.7 (2C), 78.2, 76.7, 76.3, 76.2, 75.7 (2C), 75.3 (2C), 75.3, 75.2, 75.2, 75.1 (2C), 75.0, 74.9, 74.8, 74.8, 74.2 (2C), 73.7, 73.6 (2C), 73.5, 73.3 (2C), 73.0, 72.9, 72.6, 72.5, 72.0, 71.9, 70.9, 69.5, 68.2, 67.2, 66.7, 63.8, 63.8, 63.8, 63.7, 50.5; HRMS (ESI): [M+Na]+ *m/z* Calcd for C197H203N3O38Na, 3241.3942; found, 3241.3896.

**Compound 20**

*R*f 0.54 (9:1 toluene-EtOAc); []D20 -13.5 (*c* 1.0, CHCl3); 1H NMR (600 MHz, CDCl3)  7.90 (s, 1H), 7.77 – 7.73 (m, 2H), 7.70 (d, *J* 8.4 Hz, 1H), 7.56 (dd, *J* 1.6 Hz, *J* 8.5 Hz, 1H), 7.45 – 7.41 (m, 2H), 7.41 – 7.38 (m, 2H), 7.36 – 7.33 (m, 2H), 7.31 – 6.93 (m, 96H), 5.48 (d, *J* 1.5 Hz, 1H), 5.38 (d, *J* 7.3 Hz, 1H), 5.24 (d, *J* 11.7 Hz, 1H), 5.18 (d, *J* 10.5 Hz, 1H), 5.08 (d, *J* 11.6 Hz, 1H), 5.03 (d, *J* 12.0 Hz, 1H), 5.00 – 4.94 (m, 2H), 4.91 – 4.71 (m, 14H), 4.70 (d, *J* 4.3 Hz, 1H), 4.67 (d, *J* 6.2 Hz, 1H), 4.65 – 4.50 (m, 12H), 4.50 – 4.30 (m, 13H), 4.31 – 4.18 (m, 9H), 4.11 – 3.98 (m, 5H), 3.89 (dd, *J* 3.6 Hz, *J* 10.9 Hz, 1H), 3.83 – 3.78 (m, 4H), 3.76 – 3.58 (m, 10H), 3.58 – 3.54 (m, 1H), 3.53 – 3.48 (m, 4H), 3.46 – 3.39 (m, 3H), 3.38 – 3.35 (m, 2H), 3.32 – 3.23 (m, 3H), 3.21 – 3.17 (m, 2H), 3.11 – 3.07 (m, 2H), 2.84 (dd, *J* 9.7 Hz, *J* 11.9 Hz, 1H); anomeric peaks taken from the HSQC  104.6 (*J*C-H 159.6 Hz), 104.2 (*J*C-H 162.6 Hz), 103.5 (*J*C-H 160.2 Hz), 103.3 (*J*C-H 166.4 Hz), 102.9 (*J*C-H 164.4 Hz), 99.5 (*J*C-H 174.0 Hz), 98.5 (*J*C-H 154.2 Hz), 98.5 (*J*C-H 171.0 Hz);HRMS (ESI): [M+Na]+ *m/z* Calcd for C197H203N3O38Na, 3241.3942; found, 3241.4102.

**4.15. 2-Aminoethyl -d-xylopyranosyl-(1→2)--d-mannopyranosyl-(1→3)-[-d-xylopyranosyl-(1→2)]-[-d-xylo­pyranosyl-(1→4)]--d-mannopyranosyl-(1→3)--d-mannopyranoside (21)**

10% w Pd/C (35 mg, 32.0 mol) was added to a solution of **10** (51 mg, 20.7 mol) in AcOEt/H2O/AcOH (4:2:1, 1.75 mL). The mixture was hydrogenolysed in a high-pressure reactor (Berghof) at 20 °C (*p* = 25 bar). After 24 h, the solids were removed by filtration using a ‘sandwich filter’ (3 frits stacked on top of each other in the following order: 20 m, 10 m, 5 m), rinsed with H2O (3 × 2 mL) and EtOH (3 × 2 mL), and the filtrate was concentrated *in vacuo*. 10% w Pd/C (35 mg, 32.0 mol) was added to a solution of the crude in a 1:1 mixture of THF and H2O (3 mL). The reaction mixture was hydrogenolysed again in a high-pressure reactor (Berghof) at 20 °C (*p* = 25 bar). After 48 h, the solids were removed by filtration using a ‘sandwich filter’ (3 frits stacked on top of each other in the following order: 20 m, 10 m, 5 m), rinsed with H2O (3 × 2 mL) and EtOH (3 × 2 mL), and the filtrate was concentrated *in vacuo*. Purification by reversed-phase chromatography (C-18, H2O-MeOH, 9:1→8:2→7:3→6:4→2:8→0:10), followed by freeze-drying gave **21** (9.9 mg, 51%) as a colourless, amorphous solid; []D20 +20.0 (*c* 0.3, H2O); 1H NMR (500 MHz, D2O) δ 5.23 (s, 1H), 5.19 (s, 1H), 4.89 (s, 1H), 4.44 – 4.39 (m, 2H), 4.30 (d, *J* 7.5 Hz, 1H), 4.26 (bs, 2H), 4.14 (bs, 2H), 4.10 – 3.83 (m, 12H), 3.81 – 3.74 (m, 3H), 3.72 – 3.57 (m, 7H), 3.51 – 3.41 (m, 3H), 3.35 – 3.20 (m, 7H), 3.10 (t, *J* 7.8 Hz, 1H); anomeric peaks taken from the HSQC  103.8, 103.2, 102.8, 100.3, 99.8, 99.7;HRMS (ESI): [M+Na]+ *m/z* Calcd for C35H61NO28Na, 966.3278; found, 966.3296.

**4.16. 2-Aminoethyl -d-xylopyranosyl-(1→2)--d-mannopyranosyl-(1→3)-[-d-xylopyranosyl-(1→2)][-d-xylo­pyranosyl-(1→4)]--d-mannopyranoside (22)**

10% w Pd/C (45 mg, 42.4 mol) was added to a solution of compound **16** (45 mg, 22.2 mol) in AcOEt/H2O/AcOH (4:2:1, 3.5 mL). The mixture was hydrogenolysed in a high-pressure reactor (Berghof) at 20 °C (*p* = 30 bar). After 48 h, the solids were removed by filtration using a ‘sandwich filter’ (3 frits stacked on top of each other in the following order: 20 m, 10 m, 5 m), rinsed with H2O (3 × 2 mL) and EtOH (3 × 2 mL), and the filtrate was concentrated *in vacuo*. Purification by reversed-phase chromatography (C-18, H2O-MeOH, 9:1→8:2→7:3→6:4→2:8→0:10), followed by freeze-drying gave **22** (8.7 mg, 50%) as a colourless, amorphous solid; []D20 -15.0 (*c* 0.4, H2O); 1H NMR (600 MHz, CDCl3) 1H NMR (500 MHz, D2O)  5.21 (s, 1H), 4.99 (s, 1H), 4.48 – 4.40 (m, 2H), 4.30 (d, *J* 7.6 Hz, 1H), 4.23 – 4.10 (m, 3H), 4.08 – 3.73 (m, 13H), 3.72 – 3.58 (m, 4H), 3.52 – 3.40 (m, 3H), 3.39 – 3.14 (m, 8H); anomeric peaks taken from the HSQC  104.0, 103.3, 102.9, 100.1, 98.0;HRMS (ESI): [M+Na]+ *m/z* Calcd for C29H52NO23, 782.2930; found, 782.2962.

**4.17. 2-Aminoethyl -d-glucopyranosyluronate-(1→2)-[-d-xylopyranosyl-(1→4)]--d-mannopyranosyl-(1→3)-[-d-xylopyranosyl-(1→2)]--d-mannopyranosyl-(1→3)-[-d-xylopyranosyl-(1→2)][-d-xylo­pyranosyl-(1→4)]--d-mannopyranoside (23)**

10% w Pd/C (20 mg, 18.1 mol) was added to a solution of **19** (29 mg, 9.0 mol) in AcOEt/H2O/AcOH (4:2:1, 1.75 mL). The mixture was hydrogenolysed in a high-pressure reactor (Berghof) at 20 °C (*p* = 25 bar). After 24 h, the solids were removed by filtration using a ‘sandwich filter’ (3 frits stacked on top of each other in the following order: 20 m, 10 m, 5 m), rinsed with H2O (3 × 2 mL) and EtOH (3 × 2 mL), and the filtrate was concentrated *in vacuo*. 10% w Pd/C (20 mg, 18.1 mol) was added to a solution of the crude in a 1:1 mixture of THF and H2O (2 mL). The reaction mixture was hydrogenolysed again in a high-pressure reactor (Berghof) at 20 °C (*p* = 25 bar). After 48 h, the solids were removed by filtration using a ‘sandwich filter’ (3 frits stacked on top of each other in the following order: 20 m, 10 m, 5 m), rinsed with H2O (3 × 2 mL) and EtOH (3 × 2 mL), and the filtrate was concentrated *in vacuo*. Purification by reversed-phase chromatography (C-18, H2O-MeOH, 9:1→8:2→7:3→6:4→2:8→0:10), followed by freeze-drying gave **23** (5.7 mg, 51%) as a colourless, amorphous solid; []D20 +4.0 (*c* 0.3, H2O); 1H NMR (500 MHz, D2O)  5.26 (s, 1H), 5.21 (s, 1H), 4.98 (s, 1H), 4.52 – 4.38 (m, 4H), 4.29 – 4.24 (m, 1H), 4.24 – 4.16 (m, 3H), 4.14 – 3.96 (m, 9H), 3.96 – 3.84 (m, 5H), 3.84 – 3.74 (m, 4H), 3.74 – 3.57 (m, 9H), 3.56 – 3.23 (m, 14H), 3.20 – 3.09 (m, 2H); anomeric peaks taken from the HSQC  103.9, 103.8, 103.3, 102.8, 101.5, 100.1, 99.4, 98.8;HRMS (ESI): [M+H]+ *m/z* Calcd for C46H78NO38, 1252.4202; found, 1252.4203.

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