Novel Solutions for Vaccines and Diagnostics to Combat Brucellosis

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ADDITIONAL GLYCOCONJUGATES USED IN ELISA SCREENING

Oligosaccharides available from other studies were activated and conjugated to BSA according to methods reported elsewhere to provide glycoconjugates S28-S37.\(^1\)\(^2\) The number of hapten groups per BSA was 10-15.

Figure S1
Figure S2  Pentasaccharide methyl glycosides were synthesized as previously described$^{3,4}$ and used as inhibitors of sera raised to vaccine 2.
III. GENERAL INFORMATION

Materials and Methods
Analytical TLC was performed on Silica Gel 60-F254 (Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 10% sulfuric acid in ethanol. All commercial reagents were used as supplied. Column chromatography was performed on Silica Gel 230-400 mesh, 60 Å (Silicycle, Ontario) with HPLC quality solvents. Bovine Serum Albumin (purchased from Sigma Aldrich) was used. Molecular sieves (3 Å or 4 Å), were crushed and stored in an oven at 150 °C after activation at 500 °C for 48 h and dried under vacuum before use. Organic solutions were dried with anhydrous MgSO4 prior to concentration under vacuum at <40 °C (bath). All final compounds were purified by reverse phase chromatography performed on a Waters 600 HPLC system, using a Beckmann semi preparative C-18 column (10 x 250 mm, 5 μ) with a combination of acetonitrile and water as eluents. Products were detected with a Waters 2487 UV detector. Optical rotations were measured with a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at 22 ± 2 °C. [α]D values are given in units of 10^-1 deg cm^2 g^-1. 1H NMR spectra were recorded on 500, 600 or 700 MHz spectrometers. First order proton chemical shifts δH are referenced to either residual CHCl3 (δH 7.26, CDCl3) or CD2HOD (δH 3.30, CD3OD), or external acetone (δH 2.225, D2O). The assignment of resonances for all compounds was made by two dimensional homonuclear shift correlation and for a limited subset also by heteronuclear chemical shift correlation experiments. Specifically for mono- to trisaccharides: peak assignments were based on 2D-{1H}1H-gCOSY experiments. Peak assignments for tetra- to hexasaccharides were based on 2D-{1H}1H-gCOSY, selective 1D-{1H}-CSSF-TOCSY (Chemical Shift Selective Filter - TOCSY) experiments and selective 1D-ROESY experiments. Mass analysis was performed by positive-mode electrospray ionization on a hybrid sector-TOF mass spectrometer and for protein glycoconjugates by MALDI mass analysis, employing sinapinic acid as matrix.

The numbering used for resonance assignments was as follows:
IV. SYNTHESIS OF D RHAMNOSE AND 4-AZIDO-4,6-DIDEOXY-α-D-MANNOPYRANOSE SYNTONS

Synthesis of Methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (S8).
The key precursor S8 was prepared according to Scheme S1 and analytical data for the title compound was essentially the same as previously described.\(^5\)\(^6\)

Scheme S1. Conditions: a) AcCl, MeOH, 70 °C, 6 h; b) DMP, PTSA, rt, H\(_2\)O, 4 h; c) Ph\(_3\)P, Imid, I\(_2\), PhMe, 10 min; d) Pd/C, H\(_2\), rt, EtOH/Et\(_3\)N, 16 h; e) i) OxCl, DMSO, DIPEA, CH\(_2\)Cl\(_2\), -78 °C-rt, 16 h; ii) NaBH\(_4\), EtOH, rt, 2 h; f) i) MsCl, Py, 0 °C-rt, 2 h; ii) TFA/H\(_2\)O (9:1), CH\(_2\)Cl\(_2\), rt, 10 min. g) NaN\(_3\), 15-crown-5, DMF, 100 °C, 6 h.

V. SYNTHESIS OF GLYCOSYL DONORS
A. Synthesis of glycosyl donor S9 & S10:

Ethyl 4-azido-3-\(O\)-benzyl-4,6-dIDEOXY-1-thio-\(α\)-D-mannopyranoside (S9).

Analytical data for the title compound was essentially the same as previously described.\(^7\)\(^9\)

4-Azido-2,3-di-\(O\)-benzoyl-4,6-dideoxy-\(α\)-D-mannopyranosyl trichloroacetimidate (S10).

Analytical data for the title compound was essentially the same as previously described.\(^1\)
B. Synthesis of thioglycoside donors 11:

Scheme S2. Conditions: a) Ac₂O, AcOH, H₂SO₄, rt, 6 h; b) BF₃·Et₂O, p-Toluenethiol, CH₂Cl₂, 0°C to rt, 12 h.

Methyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (S11).

A solution of S11 (5 g, 17.05 mmol) in acetic anhydride/acetic acid/sulfuric acid (50:20:0.5, 50 mL) was stirred at 21 °C for 3 h, and then poured into ice-cold 1M K₂CO₃ solution (80 mL). The mixture was then diluted with CH₂Cl₂ (~100 mL) and washed with water (2 x 30 mL), sat. aq. NaHCO₃ (35 mL), and brine (15 mL). The organic phase was separated, dried over MgSO₄, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound S12 (5.6 g, 91%) as a sticky liquid. Analytical data for the title compound was essentially the same as previously described.⁴

1,2-di-O-acetyl-4-azido-4,6-dideoxy-α-D-mannopyranose (S12).

p-Tolyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside (11).
To the stirred solution of S12 (0.78 g, 2.15 mmol) and p-toluenethiol (0.4 g, 3.22 mmol) in anhydrous CH₂Cl₂ (15 mL) at 0 °C, BF₃·Et₂O (0.32 mL, 2.57 mmol) was added dropwise. When TLC showed the reaction was completed, the mixture was then diluted with CH₂Cl₂ (~50 mL) and washed with water (2 x 10 mL), sat. aq. NaHCO₃ (15 mL), and brine (10 mL). The organic phase was separated, dried over MgSO₄, and concentrated in vacuo. The residue was purified by flash column chromatography (Ethyl acetate – hexane gradient elution) to give 11 as a sticky liquid (0.854 g, 92.9%). Analytical data for 11: Rf = 0.7 (Ethyl acetate /hexane, 1/3, v/v); [α]D²¹ = +135.5 (c = 2.25, CHCl₃); ¹H NMR (700 MHz, CDCl₃): δ: 7.36 – 7.43 (m, 4 H, ArH), 7.30 – 7.35 (m, 3 H, ArH), 7.12 (d, J=8.1 Hz, 2 H, ArH), 5.59 (dd, J=3.1, 1.7 Hz, 1 H, H-2), 5.36 (d, J=1.5 Hz, 1 H, H-1), 4.72 (d, J=11.1 Hz, 1 H, -CHPh), 4.57 (d, J=11.1 Hz, 1 H, -CHPh), 4.02 – 4.08 (m, 1 H, H-5), 3.80 (dd, J=10.0, 3.2 Hz, 1 H, H-3), 3.51 (t, J=10.0 Hz, 1 H, H-3), 2.33 (s, 3 H, -CH₃), 2.12 (s, 3 H, -COCH₃), 1.36 (d, J=6.3 Hz, 3 H, H-6); ¹³C NMR (176 MHz, CDCl₃): δ: 170.0, 138.1, 137.0, 132.4, 132.3, 129.9, 129.8, 129.6, 128.5, 128.4, 128.1, 86.4, 76.4, 71.7, 69.1, 68.2, 64.2, 21.1, 21.0, 18.4 ppm; HRMS (ESI): m/z calcd for C₂₂H₂₅N₃O₄SNa [M+Na]+: 450.1458, found: 450.1465.

VI SYNTHESIS OF LINKER AND ATTACHMENT TO TERMINAL RHAMNOSE

C. Synthesis of Linker bromoalkane 12:

Scheme S3. Conditions: a) i) BzCl, DMAP, Et₃N, CH₂Cl₂, 0°C to rt, 16 h; ii) BnBr, NaH, DMF, 0°C to rt, 12 h; b) i) NaOMe, CH₃OH, rt, 6 h; ii) CBr₄, TPP, CH₂Cl₂, 0°C to rt, 2 h.

5-(N-benzyl((benzyloxy)carbonyl)amino)pentanol benzoate (S13).

Benzoyl chloride (0.88 mL, 7.59 mmol) was added dropwise to a stirred solution of benzyl (5-hydroxypentyl) carbamate (commercially available) (1.5 g, 6.32 mmol) in anhydrous CH₂Cl₂ (15 mL) containing Et₃N (1.76 mL, 1.26 mmol) at 0 °C. After 1 minute DMAP (1.7 g, 13.9 mmol) in anhydrous CH₂Cl₂ (10 mL) was added dropwise to the reaction mixture and stirred at rt overnight. The resulting mixture was diluted with CH₂Cl₂ (~30 mL) and washed with aq. HCl (1M, 1 x 10 mL), water (60 mL), sat. aq. NaHCO₃ (30 mL), and brine (30 mL). The organic phase was separated, dried over MgSO₄, and concentrated in vacuo. The residue was quickly filtered off on silica gel (ethyl acetate – hexane gradient elution) to afford the almost pure compound as oil. This crude material was directly used for benzylolation.

To the solution of benzoyl protected compound (0.9 g, 2.63 mmol) dissolved in anhydrous DMF (10 mL) was added NaH (0.12 g, 2.89 mmol) at 0 °C. The mixture was stirred at 0 °C for 45 min, and then BnBr (0.37 mL, 3.16 mmol) were added. After stirring for another 12 h when TLC showed that the reaction was completed, it was quenched with H₂O at 0 °C, and the mixture was diluted with EtOAc. The aqueous layer was extracted with EtOAc (5 x 25 mL), and the organic
phases were combined and dried over Na$_2$SO$_4$. The desired product S13 (1.093 g, 96.1%) was obtained upon flash column chromatography (ethyl acetate – hexane gradient elution) of the condensed product. Analytical data for S13: R$^f$ = 0.6 (ethyl acetate/hexane, 1/3.5, v/v); $^1$H NMR (700 MHz, CDCl$_3$): $\delta$: 7.99 - 8.05 (m, 2 H, ArH), 7.54 (d, J=6.8 Hz, 1 H, ArH), 7.43 (t, J=7.6 Hz, 2 H, ArH), 7.24 - 7.38 (m, 9 H, ArH), 7.17 (br. s., 1 H, ArH), 5.17 (d, J=17.4 Hz, 2 H, -NCH$_2$Ph), 4.50 (d, J=16.6 Hz, 2 H, -OCH$_2$Ph), 4.18 - 4.33 (m, 2 H, -CH$_2$a), 3.17 - 3.34 (m, 2 H, -CH$_2$e), 1.65 - 1.80 (m, 2 H, -CH$_2$b), 1.52 - 1.64 (m, 2 H, -CH$_2$e), 1.30 - 1.45 (m, 2 H, -CH$_2$e); $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$: 166.6, 156.7, 156.2, 137.9, 136.8, 132.8, 130.4, 129.5, 128.5, 128.4, 128.3, 127.8, 127.3, 127.2, 67.2, 64.8, 64.7, 50.5, 50.2, 47.0, 46.0, 28.4, 27.8, 27.4, 23.3 ppm; HRMS (ESI): m/z calcd for C$_{27}$H$_{29}$NO$_4$Na [M+Na]+$^+$: 454.1989, found: 454.1986.

benzyl N-benzyl(5-bromopentanlyl)carbamate (12).

Sodium methoxide (~0.8 mL, 0.5 M solution) was added to a solution of S13 (1.0 g, 2.32 mmol) in CH$_3$OH (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 $^\circ$C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH$_3$OH. The combined filtrate was concentrated in vacuo and this crude material was directly used for bromination.

To the solution of deprotected compound (0.96 g, 2.92 mmol) dissolved in anhydrous CH$_2$Cl$_2$ (15 mL) were added CBr$_4$ (1.85 g, 5.55 mmol) and PPh$_3$ (1.54 g, 5.86 mmol) at 0 $^\circ$C. The reaction was allowed to warm up to room temperature and stirring for another 3 h. When TLC showed the reaction was completed, it was quenched with H$_2$O at 0 $^\circ$C, mixture was then diluted with CH$_2$Cl$_2$ (~50 mL) and washed with water (2 x 10 mL), sat. aq. NaHCO$_3$ (15 mL), and brine (15 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound 12 (1.085 g, 94.8%) as a liquid. Analytical data for 12: R$^f$ = 0.85 (ethyl acetate/hexane, 1/4, v/v); $^1$H NMR (700 MHz, CDCl$_3$): $\delta$: 7.22 - 7.43 (m, 9 H, ArH), 7.18 (br. s., 1 H, ArH), 5.18 (d, J=18.8 Hz, 2 H, -NCH$_2$Ph), 4.50 (d, J=12.9 Hz, 2 H, -OCH$_2$Ph), 3.15 - 3.39 (m, 4 H, -CH$_2$a, -CH$_2$e), 1.71 - 1.88 (m, 2 H, -CH$_2$b), 1.45 - 1.60 (m, 2 H, -CH$_2$e), 1.30 - 1.44 (m, 2 H, -CH$_2$e); $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$: 156.7, 156.2, 137.8, 136.7, 128.5(x2), 128.4, 128.0, 127.9, 127.4, 127.3, 127.2, 67.3, 67.2, 50.6, 50.3, 46.9, 46.0, 33.6, 33.4, 32.3(x2), 27.2, 26.8, 25.3 ppm; HRMS (ESI): m/z calcd for C$_{20}$H$_{24}$BrNO$_2$Na [M+Na]+$^+$: 412.0883, found: 412.0878.
D. Synthesis of the capping \( p \)-tolyl thioglycoside donor with attached tether 13:

![Scheme S4](image)

**Scheme S4.** Conditions: a) CbzBnN(CH\(_2\))\(_3\)Br, NaH, DMF, 0°C to rt, 18 h; b) TFA/H\(_2\)O (9:1), CH\(_2\)Cl\(_2\), rt, 10 min.; c) BzCl, DMAP, Et\(_3\)N, CH\(_2\)Cl\(_2\), 0°C to rt, 12 h; d) Ac\(_2\)O, AcOH, H\(_2\)SO\(_4\), rt, 4 h; e) BF\(_3\)•Et\(_2\)O, p-Toluenethiol, CH\(_2\)Cl\(_2\), 0°C to rt, 10 h.

**Methyl 2,3-O-isopropylidene-6-deoxy-\( \alpha \)-D-mannopyranoside (S5).**

![Methyl 2,3-O-isopropylidene-6-deoxy-\( \alpha \)-D-mannopyranoside (S5)](image)

Analytical data for the title compound was essentially the same as previously described.\(^5\)

**Methyl 4-O-(5'-N-benzyl-5'-N-carboxybenzyl-pentanyl) 2,3-O-isopropylidene-6-deoxy-\( \alpha \)-D-mannopyranoside (S14).**

![Methyl 4-O-(5'-N-benzyl-5'-N-carboxybenzyl-pentanyl) 2,3-O-isopropylidene-6-deoxy-\( \alpha \)-D-mannopyranoside (S14)](image)

To the solution of S5 (2.0 g, 9.17 mmol) dissolved in anhydrous DMF (15 mL) was added NaH (0.4 g, 10.08 mmol) at 0 °C. The mixture was stirred at 0 °C for 45 min, and then CbzBnN(CH\(_2\))\(_3\)Br (4.5 g, 11.01 mmol) were added. After stirring for another 12 h when TLC showed that the reaction was completed, it was quenched with H\(_2\)O at 0 °C, and the mixture was diluted with EtOAc. The aqueous layer was extracted with EtOAc (5 × 25 mL), and the organic phases were combined and dried over Na\(_2\)SO\(_4\). The desired product S14 (3.26 g, 73.2 %) along with eliminated alkene and small amount unreacted starting material S5 (0.16 g) were obtained upon flash column chromatography (ethyl acetate – hexane gradient elution) of the condensed product. Analytical data for S14: Rf = 0.6 (ethyl acetate/hexane, 1/4, v/v); [\( \alpha \)]D\(^{21}\) = +20.48 (c = 2.11, CHCl\(_3\)); \(^1\)H NMR (700 MHz, CDCl\(_3\)): \( \delta \): 7.15 - 7.45 (m, 10 H, ArH), 5.12 - 5.21 (m, 2 H, -NCH\(_2\)Ph), 4.86 (s, 1 H, H-1), 4.45 - 4.52 (m, 2 H, -OCH\(_2\)Ph), 4.10 - 4.14 (m, 2 H, H-2, H-3), 3.81 (br. s., 1 H, -CH\(_3\)), 3.56 - 3.64 (m, 1 H, H-5), 3.39 - 3.48 (m, 1 H, H-4), 3.38 (s, 3 H, -OCH\(_3\)), 3.19 - 3.34 (m, 2 H, -CH\(_3\)), 2.96 - 3.04 (m, 1 H, -CH\(_3\)), 1.49 - 1.61 (m, 4 H, -CH\(_3\), -CH\(_{2}\)), 1.51 (s, 3 H, -CH\(_3\)), 1.33 (s, 3 H, -CH\(_3\)), 1.21 - 1.31 (m, 5 H, H-6, -CH\(_{2}\)); \(^{13}\)C NMR (176...
10 MHz, CDCl$_3$): $\delta$: 156.7, 156.1, 137.9, 136.9, 136.8, 128.5, 128.4, 127.9, 127.8, 127.3, 127.2, 109.0, 98.0, 82.0, 78.5, 75.9, 71.3, 67.1, 64.5, 54.7, 50.4, 50.1, 47.1, 46.1, 29.8, 28.0, 27.9, 27.5, 26.3, 23.4, 17.7 ppm; HRMS (ESI): m/z calcd for C$_{30}$H$_{41}$NO$_7$Na [M+Na]$^+$: 550.2775, found: 550.2785.

Methyl 4-O-(5'-N-benzyl-5'-N-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranoside S15.

A solution of S14 (1.0 g, 1.89 mmol) in TFA:H$_2$O (9:1, 10 mL) was stirred at 21 °C for 30 min, and then poured into ice-cold 1M K$_2$CO$_3$ solution (50 mL). The mixture was then diluted with CH$_2$Cl$_2$ (~50 mL) and washed with water (2 x 30 mL), sat. aq. NaHCO$_3$ (25 mL), and brine (15 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound S15 (0.742 g, 80.3%) as oil. Analytical data for S15: $R_f$ = 0.4 (ethyl acetate/hexane, 1/1, v/v); [α]$^D$ = +38.31 ($c$ = 1.27, CHCl$_3$); $^1$H NMR (700 MHz, CDCl$_3$): $\delta$: 7.24 - 7.39 (m, 9 H, ArH), 7.12 - 7.19 (m, 1 H, ArH), 5.12 - 5.20 (m, 2 H, -NCH$_2$Ph), 4.64 (s, 1 H, H-1), 4.45 - 4.51 (m, 2 H, -OCH$_2$Ph), 3.88 - 3.92 (m, 1 H, H-2), 3.74 - 3.83 (m, 1 H, H-3), 3.49 - 3.70 (m, 3 H, H-5, -CH$_2$), 3.33 (s, 3 H, -OC$_3$H$_3$), 3.16 - 3.21 (m, 1 H, -CH$_3$), 3.04 - 3.15 (m, 1 H, -CH$_2$), 2.30 - 2.52 (2 x br. s., -OH), 1.44 - 1.68 (m, 4 H, -CH$_2$), 1.31 - 1.41 (m, 2 H, -CH$_2$), 1.28 (s, 3 H, H-6); $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$: 156.7, 156.3, 137.8, 136.6, 129.6, 128.5, 128.4, 127.9, 127.8, 127.3, 127.2, 100.3, 81.7, 71.4, 71.3, 71.2, 67.2, 67.1, 54.8, 50.5, 50.3, 47.1, 46.1, 30.0, 29.8, 27.9, 27.2, 23.2, 17.9 ppm; HRMS (ESI): m/z calcd for C$_{27}$H$_{37}$NO$_7$Na [M+Na]$^+$: 510.2462, found: 510.2462.

Methyl 2,3-di-O-benzoyl-4-O-(5'-N-benzyl-5'-N-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranoside (S16).

Benzoyl chloride (0.23 mL, 1.97 mmol) was added dropwise to a stirred solution of S15 (0.4 g, 0.82 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) containing Et$_3$N (0.46 mL, 3.28 mmol) at 0 °C. After 2 minute DMAP (0.451 g, 3.69 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) was added dropwise to the reaction mixture and stirred at rt overnight. The resulting mixture was diluted with CH$_2$Cl$_2$ (~20 mL) and washed with aq. HCl (1M, 2 x 5 mL), water (20 mL), sat. aq. NaHCO$_3$ (10 mL), and brine (10 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound S16 (0.513 g, 90%) as oil. Analytical data for S16: $R_f$ = 0.7 (ethyl acetate /hexane, 1/3.5, v/v); [α]$^D$ = -70.58 ($c$ = 1.71, CHCl$_3$); $^1$H NMR (700 MHz, CDCl$_3$): $\delta$: 8.04 (d, $J$=7.4 Hz, 2 H, ArH), 7.89 (d, $J$=6.7 Hz, 2 H, ArH), 7.58 - 7.61 (m, 1
H, ArH), 7.43 - 7.48 (m, 3 H, ArH), 7.22 - 7.34 (m, 10 H, ArH), 7.08 - 7.20 (m, 2 H, ArH), 5.60 (dd, J=9.6, 3.5 Hz, 1 H, H-3), 5.53 - 5.56 (m, 1 H, H-2), 5.13 (d, J=7.4 Hz, 2 H, -NCH₂Ph), 4.78 (d, J=1.3 Hz, 1 H, H-1), 4.34 - 4.44 (m, 2 H, -OCH₃Ph), 3.85 - 3.91 (m, 1 H, H-5), 3.48 - 3.61 (m, 3 H, H-4, -CH₂₂), 3.42 (s, 3 H, -OCH₃), 2.94 - 3.13 (m, 2 H, -CH₂), 1.24 - 1.46 (m, 7 H, H-6, -CH₂b, -CH₂d), 1.04 - 1.23 (m, 2 H, -CH₂); ¹³C NMR (176 MHz, CDCl₃): δ: 165.5, 165.2, 156.7, 156.3, 137.9, 133.3, 133.0, 129.9, 129.8, 129.6, 128.5, 128.3, 127.9, 127.8, 127.1, 98.5, 79.5, 73.1, 72.9, 72.1, 71.1, 67.6, 67.1, 55.0, 50.4, 50.1, 47.0, 46.0, 29.9, 27.8, 27.4, 23.3, 18.0 ppm; HRMS (ESI): m/z calcd for C₄₁H₄₅NO₉Na [M+Na]+: 718.2987, found: 718.298.

1-O-acetyl-2,3-di-O-benzoyl-4-O-(5′-N-benzyl-5′-N-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranosyl (S17).

A solution of S16 (0.5 g, 0.716 mmol) in acetic anhydride/acetic acid/sulfuric acid (50:20:0.5, 10 mL) was stirred at 21 °C for 3 h, and then poured into ice-cold 1M K₂CO₃ solution (50 mL). The mixture was then diluted with CH₂Cl₂ (~20 mL) and washed with water (2 x 30 mL), sat. aq. NaHCO₃ (15 mL), and brine (10 mL). The organic phase was separated, dried over MgSO₄, and concentrated in vacuo to afford the title compound S17 (0.485 g, 92.2%) as a liquid. Analytical data for S17: Rf = 0.55 (ethyl acetate /hexane, 1/4, v/v); [α]D²⁵ = -48.86 (c = 1.51, CHCl₃); ¹H NMR (700 MHz, CDCl₃): δ: 8.01 - 8.04 (m, 2 H, ArH), 7.88 (d, J=6.8 Hz, 2 H, ArH), 7.59 - 7.62 (m, 1 H, ArH), 7.46 - 7.49 (m, 3 H, ArH), 7.21 - 7.34 (m, 10 H, ArH), 7.08 - 7.20 (m, 2 H, ArH), 6.17 (d, J=1.9 Hz, 1 H, H-1), 5.62 (dd, J=9.6, 3.5 Hz, 1 H, H-3), 5.56 - 5.58 (m, 1 H, H-2), 5.10 - 5.15 (m, 2 H, -NCH₂Ph), 4.34 - 4.42 (m, 2 H, -OCH₃Ph), 3.92 - 3.98 (m, 1 H, H-5), 3.48 - 3.65 (m, 3 H, H-4, -CH₂₂), 3.95 - 3.18 (m, 2 H, CH₂₂), 2.18 (s, 3 H, -COCH₃), 1.25 - 1.49 (m, 7 H, H-6, -CH₂b, -CH₂d), 1.03 - 1.23 (m, 2 H, -CH₂); ¹³C NMR (176 MHz, CDCl₃): δ: 168.6, 165.4, 165.2, 137.8, 136.7, 133.5, 133.2, 129.8, 129.6, 129.4, 129.0, 128.5, 128.4, 128.2, 127.9, 127.8, 127.2, 127.1, 90.8, 79.1, 73.4, 71.8, 70.1, 69.9, 67.1, 50.4, 50.1, 46.9, 45.9, 29.9, 27.8, 27.4, 23.3, 21.0, 18.1, ppm; HRMS (ESI): m/z calcd for C₄₂H₄₅NO₁₀Na [M+Na]+: 746.2936, found: 746.2931.

p-Tolyl 2,3-di-O-benzoyl-4-O-(5′-N-benzyl-5′-N-carboxybenzyl-pentanyl)-6-deoxy-1-thio-α-D-mannopyranoside (13).

To the stirred solution of S17 (1.2 g, 1.66 mmol) and p-toluenethiol (0.312 g, 2.48 mmol) in anhydrous CH₂Cl₂ (20 mL) at 0 °C, BF₃·Et₂O (0.25 mL, 1.99 mmol) was added drop wise. When TLC showed the reaction was completed, the mixture was then diluted with CH₂Cl₂ (~30 mL) and washed with water (2 x 10 mL), sat. aq. NaHCO₃ (10 mL), and brine (20 mL). The organic
phase was separated, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate – hexane gradient elution) to give 13 as a white solid (1.18 g, 90.7%). Analytical data for 13: R$_f$ = 0.65 (ethyl acetate/hexane, 1/4, v/v); $[^{[a]}]_D^{21}$ = -1.02 (c = 0.9, CHCl$_3$); $^1$H NMR (700 MHz, CDCl$_3$): $\delta$ : 8.00 - 8.02 (m, 2 H, ArH), 7.90 (d, J=6.5 Hz, 2 H, ArH), 7.56 - 7.60 (m, 1 H, ArH), 7.43 - 7.48 (m, 3 H, ArH), 7.39 - 7.42 (m, 2 H, ArH), 7.21 - 7.34 (m, 11 H, ArH), 7.12 (d, J=8.1 Hz, 3 H, ArH), 5.80 (dd, J=3.3, 1.5 Hz, 1 H, H-2), 5.61 (dd, J=9.6, 3.3 Hz, 1 H, H-3), 5.48 (d, J=1.5 Hz, 1 H, H-1), 5.16 - 5.18 (m, 2 H, -NCH$_2$Ph), 4.34 - 4.42 (m, 3 H, H-5, -OCH$_2$Ph), 3.50 - 3.65 (m, 3 H, H-4, -CH$_2$), 2.96 - 3.15 (m, 2 H, -CH$_2$), 2.32 (s, 3 H), 1.06 - 1.48 (m, 9 H, H-6, -CH$_2$ -CH$_2$ -CH$_3$); $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$: 165.4, 165.3, 156.7, 156.1, 138.1, 137.9, 136.9, 136.8, 133.4, 133.2, 132.7, 132.3, 130.0, 129.9(x2), 129.8, 129.7(x2), 129.6, 128.5(x2), 128.4(x2), 127.9, 127.8, 127.3(x2), 127.2, 86.2, 79.7, 73.3, 73.1, 72.6, 72.4(x2), 69.2, 67.1, 50.5, 50.2, 47.0, 46.0, 30.0, 27.9, 27.4, 23.3, 21.2, 18.0 ppm; HRMS (ESI): m/z calcd for C$_{47}$H$_{49}$NO$_8$SNa [M+Na]+: 810.3071, found: 810.3069.

VII. SYNTHESIS OF OLIGOSACCHARIDES S20-S21; 14-25

Synthesis of the 1,2 linked trisaccharide S21

Ethyl 4-azido-2,3-di-O-benzoyl-4,6-dideoxy-$\alpha$-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-1-thio-$\alpha$-D-mannopyranoside (S18).

\[
\begin{align*}
\text{S18} & \quad \text{O} \\
\text{N}_3 & \quad \text{BzO} \\
\text{OBz} & \quad \text{BnO} \\
\text{N}_3 & \quad \text{BnO} \\
\text{Et} & \quad \text{Se} \\
\end{align*}
\]

Analytical data for the title compound was essentially the same as previously described.\textsuperscript{1}

5’-Methoxycarbonylpentyl 4-azido-3-O-benzyl-4,6-dideoxy-$\alpha$-D-mannopyranoside (S19).

\[
\begin{align*}
\text{S19} & \quad \text{O(CH$_2$)$_3$CO$_2$Me} \\
\text{N}_3 & \quad \text{BnO} \\
\text{OH} & \quad \text{O} \\
\end{align*}
\]

Analytical data for the title compound was essentially the same as previously described.\textsuperscript{5}
5'-Methoxycarbonylpentyl 4-azido-2,3-O-benzoyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (S20).

The glycosyl acceptor compound S19 (0.2 g, 0.491 mmol), and glycosyl donor compound S18 (0.414 g, 0.589 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH₂Cl₂ (15 mL), treated with freshly activated 4 Å molecular sieves (1 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.221 g, 0.982 mmol). After cooling to -10 ºC, TMSOTf (19.5 µL, 0.108 mmol) was added and the reaction was allowed to warm up to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO₃ (5 mL) and CH₂Cl₂ were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na₂S₂O₃ (20%) and water. After extraction of the aqueous layer with CH₂Cl₂ (3 x 5), the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography (ethyl acetate - Hexane gradient elution) to give disaccharide S20 (0.418 g, 81.3%) as a sticky liquid. Analytical data for S20: Rf = 0.7 (ethyl acetate/Hexane 1:4.5, v/v); [α]D²¹ = -14.49° (c = 1.79, CHCl₃); ¹H NMR (500MHz, CDCl₃): δ 8.02 - 8.05 (m, 2 H, ArH), 7.95 - 7.97 (m, 2 H, ArH), 7.64 - 7.68 (m, 1 H, ArH), 7.50 - 7.57 (m, 3 H, ArH), 7.33 - 7.41 (m, 8 H, ArH), 7.22 - 7.26 (m, 3 H, ArH), 7.13 - 7.17 (m, 1 H, ArH), 5.71 (dd, J = 3.3, 1.5 Hz, 1 H, H-2C), 5.59 (dd, J = 10.3, 3.3 Hz, 1 H, H-3C), 5.06 (d, J = 1.8 Hz, 1 H, H-1B), 5.02 (d, J = 1.8 Hz, 1 H, H-1C), 4.76 (d, J = 11.7 Hz, 1 H, CH₃Ph), 4.62 - 4.69 (m, 4 H, 3 CH₃Ph, H-1A), 3.95 (dd, J = 2.2, 0.7 Hz, 1 H, H-2B), 3.90 (dd, J = 2.2, 0.7 Hz, 1 H, H-2A), 3.76 - 3.81 (m, 2 H, H-3B, H-5B), 3.74 (dd, J = 9.9, 2.9 Hz, 1 H, H-3A), 3.71 (s, 3 H), 3.69 (t, J = 9.9 Hz, 1H, H-4C), 3.55 - 3.65 (m, 3 H, H-4B, H-5C, -O-CH₃), 3.43 - 3.49 (m, 1 H, H-5A), 3.38 (dt, J = 9.7, 6.4 Hz, 1 H, -O-CH₃), 3.27 (t, J = 9.9 Hz, 1 H, H-4A), 2.33 - 2.39 (m, 2 H, -CH₂), 1.64 - 1.72 (m, 2 H, -CH₂), 1.56 - 1.64 (m, 2 H, -CH₂), 1.35 - 1.42 (m, 2 H, -CH₂), 1.38 (d, J = 5.6 Hz, 3 H, H-6C), 1.32 (d, J = 5.9 Hz, 3 H, H-6B), 1.29 (d, J = 5.9 Hz, 3 H, H-6A); ¹³C NMR (126MHz, CDCl₃): δ 174.0, 165.2, 164.9, 137.5, 137.3, 133.4, 133.3, 129.8(x2), 129.6, 129.3, 128.5(x2), 128.4, 128.2, 128.1(x2), 128.0, 100.3, 99.0, 98.8, 77.9, 73.9, 73.5, 72.3(x2), 70.9, 69.5, 68.0, 67.5, 67.2, 64.5, 63.9, 63.5, 51.5, 34.0, 29.1, 25.7, 24.7, 18.6(x2), 18.4 ppm; HRMS (ESI): m/z calcd for C₅₃H₆₁N₉O₁₄Na [M+Na]+: 1070.423, found: 1070.4248.
5'-Methoxycarbonylpentyl 4-azido-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (S21).

Sodium methoxide (~0.3 mL, 0.5 M solution) was added to a solution of S20 (0.39 g, 0.372 mmol) in CH₃OH: THF [4:2] (12 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH₃OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (ethyl acetate - Hexane gradient elution) to afford the title compound S21 (0.299 g, 95.6%) as white solid. Analytical data for S21: Rf = 0.3 (ethyl acetate/Hexane 1:1.5, v/v); [α]D²¹ = +84.18 (c = 1.55, CHCl₃); ¹H NMR (500MHz, CDCl₃): δ 7.30 - 7.44 (m, 10 H, ArH), 5.00 (d, J=1.8 Hz, 1 H, H-1B), 4.90 (d, J=1.5 Hz, 1 H, H-1C), 4.72 (d, J=11.4 Hz, 1 H, CHPh), 4.61 - 4.67 (m, 4 H, 3 CHPh, H-1A), 3.93 - 3.97 (m, 2 H, H-2B, H-2C), 3.81 - 3.87 (m, 2 H, H-2A, H-3A), 3.76 (dd, J=9.9, 2.9 Hz, 1 H, H-3B), 3.73 (dd, J=10.0, 2.9 Hz, 1 H, H-3C), 3.70 (s, 3 H), 3.51 - 3.64 (m, 3 H, H-5B, H-5C, -O-CH₃), 3.43 - 3.49 (m, 1 H, H-5A), 3.40 (t, J =9.9 Hz, 1H, H-4C), 3.36 (dt, J=9.7, 4.4 Hz, 1 H, -O-CH₃), 3.27 (t, J =9.9 Hz, 1H, H-4B), 3.40 (t, J =10.2 Hz, 1H, H-4A), 2.49 (d, J=6.9 Hz, 1 OH₃C), 2.34 (t, J=7.4 Hz, 2 H, -CH₂), 2.18 (d, J=3.9 Hz, 1 OH₂C), 1.63 - 1.70 (m, 2 H, -CH₂), 1.54 - 1.61 (m, 2 H, -CH₂), 1.33 - 1.40 (m, 2 H, -CH₂), 1.30 (d, J=6.2 Hz, 6 H, H-6B, H-6C), 1.20 (d, J=6.2 Hz, 3 H, H-6A); ¹³C NMR (126MHz, CDCl₃): δ 174.0, 137.4 (x2), 128.6(x2), 128.3, 128.2(x2), 128.1, 100.7, 100.4, 98.7, 77.7, 77.2, 73.8, 73.2, 72.3, 72.2, 70.2, 69.9, 67.8, 67.5, 67.4, 67.1, 65.8, 64.4, 64.2, 51.6, 33.9, 29.1, 25.7, 24.7, 18.6(x2), 18.2 ppm; HRMS (ESI): m/z calcd for C₃⁹H₅₃N₉O₁₂Na [M+Na]+: 862.3706, found: 862.3705.
Synthesis of the heptasaccharide with capping tether 25

Methyl 4-azido-2-O-acetyl-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (14).

The glycosyl acceptor compound S11 (1.42 g, 4.84 mmol), and glycosyl donor compound 11 (2.27 g, 5.33 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH₂Cl₂ (20 mL), treated with freshly activated 4 Å molecular sieves (1.5 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (2.4 g, 9.71 mmol). After cooling to -10 ºC, TMSOTf (0.19 mL, 0.971 mmol) was added and the reaction was allowed to warm up to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO₃ (15 mL) and CH₂Cl₂ were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na₂S₂O₃ (20%) and water. After extraction of the aqueous layer with CH₂Cl₂ (3x15), the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate/Hexane gradient elution) to give disaccharide 14 (2.66 g, 92.1%) as a sticky liquid.

Analytical data for 14: Rf = 0.5 (Ethyl acetate /Hexane 1:4, v/v); [α]D²¹ = +36.24° (c = 1.92, CHCl₃); ¹H NMR (700 MHz, CDCl₃): δ: 7.32 - 7.38 (m, 8 H, ArH), 7.27 - 7.31 (m, 2 H, ArH), 5.41 (dd, J =3.0, 1.9 Hz, 1 H, H-2β), 4.85 (d, J=1.5 Hz, 1 H, H-1β), 4.71 (d, J=11.1 Hz, 1 H, -CHPh), 4.68 (d, J=11.5 Hz, 1 H, -CHPh), 4.61 (d, J=11.6 Hz, 1 H, -CHPh), 4.57 (d, J=1.4 Hz, 1 H, H-1A), 4.54 (d, J=11.0 Hz, 1 H, -CHPh), 3.84 - 3.86 (m, 1 H, H-2A), 3.79 (dd, J=9.9, 3.3 Hz, 1 H, H-3B), 3.72 (dd, J=9.9, 3.0 Hz, 1 H, H-3A), 3.59 (dq, J=9.9, 6.1 Hz, 1 H, H-5B), 3.45 (dq, J=10.1, 6.1 Hz, 1 H, H-5A), 3.39 (t, J=9.9 Hz, 1 H, H-4B), 3.33 (t, J=10.1 Hz, 1 H, H-4A), 3.30 (s, 3 H, -OCH₃), 2.08 (s, 3 H, -COCH₃), 1.30 (d, J=6.3 Hz, 6 H, H-6A, H-6B); ¹³C NMR (176 MHz, CDCl₃): δ: 169.7, 137.6, 137.1, 128.5(x2), 128.4, 128.0, 127.9, 127.8, 99.7, 99.4, 77.7, 75.4, 73.7, 72.0, 71.6, 67.6, 67.2, 66.9, 64.1, 63.8, 54.9, 20.9, 18.5(x2) ppm; HRMS (ESI): m/z calcd for C₂₉H₃₆N₆O₈Na [M+Na]⁺: 619.2487, found: 619.2481.
Methyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (15).

Sodium methoxide (~1.2 mL, 0.5 M solution) was added to a solution of 14 (2.6 g, 4.36 mmol) in CH$_3$OH: THF (4:2) (20 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH$_3$OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to deprotected disaccharide compound 15 (2.3 g, 95.4%) as white foam. Analytical data for 15: R$_f$ = 0.4 (Ethyl acetate /Hexane 1:4.5, v/v); [α]$_D^{21}$ = +28.71 (c = 1.56, CHCl$_3$); $^1$H NMR (700 MHz, CDCl$_3$): δ: 7.34 - 7.41 (m, 8 H, ArH), 7.29 - 7.34 (m, 2 H, ArH), 4.94 (d, J=1.1 Hz, 1 H, -CHPh), 4.71 (d, J=11.4 Hz, 1 H, -C$_2$H$_4$), 4.67 (d, J=11.3 Hz, 1 H, -CH$_2$Ph), 4.67 (ABq, J=11.3 Hz, 1 H, -C$_2$H$_4$), 4.58 (d, J=1.5 Hz, 1 H, H-1A), 3.98 (t, J=1.8 Hz, 1 H, H-2B), 3.90 (t, J=2.3 Hz, 1 H, H-2A), 3.71 (dd, J=9.9, 3.1 Hz, 2 H, H-3B, H-3A), 3.59 (dq, J=10.2, 5.9 Hz, 1 H, H-5A), 3.45 (dq, J=10.1, 6.1 Hz, 1 H, H-5B), 3.42 (t, J=9.9 Hz, 1 H, H-4A), 3.30 (s, 3 H, -OCH$_3$), 3.29 (t, J=10.0 Hz, 1 H, H-4B), 2.30 (s, 1 –OH$_2$B), 1.31 (d, J=6.1 Hz, 6 H, H-6A), 1.29 (d, J=5.9 Hz, 6 H, H-6B); $^{13}$C NMR (176 MHz, CDCl$_3$): δ: 137.5, 137.1, 128.6, 128.5, 128.3, 128.2(x2), 128.0, 100.8, 99.9, 77.8, 77.6, 73.6, 72.1(x2), 67.3, 67.2, 66.9, 64.3, 63.8, 54.9, 18.6, 18.4 ppm; HRMS (ESI): m/z calcd for C$_{27}$H$_{34}$N$_6$O$_7$Na [M+Na]+: 577.2381, found: 577.2381.

Methyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (16).

The glycosyl acceptor compound 15 (2.25 g, 4.06 mmol), and glycosyl donor compound 11 (1.90 g, 4.46 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH$_2$Cl$_2$ (25 mL), treated with freshly activated 4 Å molecular sieves (1.6 g), stirred under an Ar atmosphere at rt for 1 h.
To the mixture was added NIS (1.83 g, 8.11 mmol). After cooling to -10 °C, TMSOTf (0.16 mL 0.893 mmol) was added and the reaction was allowed to warm up to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO₃ (15 mL) and CH₂Cl₂ were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na₂S₂O₃ (20%) [30 mL] and water (20 mL). After extraction of the aqueous layer with CH₂Cl₂ (3x15), the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give trisaccharide 16 (3.09 g, 88.9%) as a sticky liquid. Analytical data for 16: Rf = 0.65 (Ethyl acetate /Hexane 1:5, v/v); ¹H NMR (700 MHz, CDCl₃): δ: 7.32 - 7.39 (m, 12 H, ArH), 7.26 - 7.32 (m, 3 H, ArH), 5.39 (dd, J=3.2, 1.9 Hz, 1 H, H-2C), 4.95 (d, J=1.8 Hz, 1 H, H-1B), 4.82 (d, J=1.8 Hz, 1 H, H-1C), 4.70 (d, J=11.5 Hz, 1 H, -CHPh), 4.69 (d, J=11.0 Hz, 1 H, -CHPh), 4.63 (d, J=11.5 Hz, 1 H, -CHPh), 4.59 (ABq, J=11.5 Hz, 1 H, -CH₂Ph), 4.53 (d, J=1.8 Hz, 1 H, H-1A), 4.52 (d, J=11.0 Hz, 1 H, -CHPh), 3.86 (t, J=2.4 Hz, 1 H, H-2B), 3.83 (t, J=2.3 Hz, 1 H, H-2A), 3.75 (dd, J=9.9, 3.3 Hz, 1 H, H-3C), 3.72 (dd, J=9.9, 2.9 Hz, 1 H, H-3B), 3.67 (dd, J=10.0, 2.8 Hz, 1 H, H-3A), 3.52 (dq, J=10.0, 6.1 Hz, 1 H, H-5B), 3.49 (dq, J=10.0, 6.0 Hz, 1 H, H-5C), 3.42 (dq, J=10.1, 6.2 Hz, 1 H, H-5A), 3.35 (t, J=9.9 Hz, 1 H, H-4C), 3.34 (t, J=9.9 Hz, 1 H, H-4B), 3.29 (s, 3 H, -OCOCH₃), 3.22 (t, J=10.0 Hz, 1 H, H-4A), 2.09 (s, 3 H, -COCH₃), 1.29 (d, J=6.2 Hz, 3 H, H-6A), 1.28 (d, J=6.3 Hz, 3 H, H-6B), 1.18 (d, J=6.1 Hz, 3 H, H-6C); ¹³C NMR (176 MHz, CDCl₃): δ: 169.7, 137.4, 137.3, 137.1, 128.5(x2), 128.4, 128.1, 128.0(x3), 100.3, 99.8, 99.1, 77.5, 76.8, 75.4, 73.5, 72.1, 72.0, 71.5, 67.8, 67.6, 67.1, 67.0, 64.4, 64.0, 63.8, 54.9, 21.0, 18.6(x2), 18.3 ppm; HRMS (ESI): m/z calcd for C₄₂H₅₁N₉O₁₁Na [M+Na]+: 880.36, found: 880.3607.

Methyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosid (17).

Sodium methoxide (~1.5 mL, 0.5 M solution) was added to a solution of 16 (3.0 g, 3.5 mmol) in CH₃OH: THF [4:2] (20 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H⁺) ion exchange resin, the resin was filtered off and rinsed successively with CH₃OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the deprotected trisaccharide compound 17 (2.6 g, 91.2%) as white solid foam. Analytical data for 17: Rf = 0.45 (Ethyl acetate /Hexane 1:5, v/v); ¹H NMR (700 MHz, CDCl₃): δ: 7.33 - 7.41 (m, 12 H, ArH), 7.28 - 7.32 (m, 3 H, ArH), 4.96 (d, J=1.8 Hz, 1 H, H-1C), 4.95 (d, J=1.7 Hz, 1 H, H-1B), 4.71 (d, J=11.3 Hz, 1 H, -CHPh), 4.67 (d, J=11.3 Hz, 1 H, -CHPh), 4.66 (d, J=11.6 Hz, 1 H, -CHPh), 4.62 (d, J=11.1 Hz, 1 H, -CHPh),
4.61 (d, J=11.1 Hz, 1 H, -CHPh), 4.58 (d, J=11.6 Hz, 1 H, -CHPh), 4.55 (d, J=1.8 Hz, 1 H, -1\textsubscript{A}), 3.97 - 3.99 (m, 1 H, H-2\textsubscript{B}), 3.92 (t, J=2.4 Hz, 1 H, H-2\textsubscript{C}), 3.83 (t, J=2.3 Hz, 1 H, H-2\textsubscript{A}), 3.72 (dd, J=9.9, 2.3 Hz, 1 H, H-3\textsubscript{C}), 3.69 (dd, J=9.8, 3.1 Hz, 1 H, H-3\textsubscript{B}), 3.68 (dd, J=10.1, 2.8 Hz, 1 H, H-3\textsubscript{A}), 3.52 (dq, J=10.1, 5.9 Hz, 1 H, H-5\textsubscript{C}), 3.50 (dq, J=9.9, 6.1 Hz, 1 H, H-5\textsubscript{B}), 3.42 (dq, J=10.1, 6.0 Hz, 1 H, H-5\textsubscript{A}), 3.40 (t, J=10.0 Hz, 1 H, H-4\textsubscript{B}), 3.31 (t, J=9.8 Hz, 1 H, H-4\textsubscript{C}), 3.29 (s, 3 H, -OCH\textsubscript{3}), 3.23 (t, J=9.9 Hz, 1 H, H-4\textsubscript{A}), 2.29 (s, 1 H, -OH\textsubscript{2C}), 1.29 (d, J=6.0 Hz, 3 H, H-6\textsubscript{A}), 1.28 (d, J=6.0 Hz, 3 H, H-6\textsubscript{B}), 1.18 (d, J=6.3 Hz, 3 H, H-6\textsubscript{C}); \textsuperscript{13}C NMR (176 MHz, CDCl\textsubscript{3}): δ: 137.3(x2), 137.2, 128.6(x3), 128.5, 128.3, 128.3, 128.2(x2), 128.1(x2), 128.0, 100.5, 100.4, 99.8, 77.6, 77.5, 76.8, 73.6, 73.3, 72.2, 72.1(x2), 67.8, 67.3, 67.1, 67.0, 64.4, 64.2, 63.8, 54.9, 18.6(x2), 18.3 ppm; HRMS (ESI): m/z calcd for C\textsubscript{40}H\textsubscript{49}N\textsubscript{5}O\textsubscript{10}Na [M+Na]+: 383.3495, found: 838.3501.

**Methyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (18).**

\[
\begin{align*}
\text{OAc} & \quad \text{N}_3 \\
\text{BnO} & \quad \text{BnO} \\
\begin{array}{c}
\text{N}_3 \\
\text{BnO} \\
\text{N}_3 \\
\end{array}
\end{align*}
\]

The glycosyl acceptor compound 17 (2.05 g, 2.51 mmol), and glycosyl donor compound 11 (1.18 g, 2.76 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH\textsubscript{2}Cl\textsubscript{2} (20 mL), treated with freshly activated 4 Å molecular sieves (1.2 g), stirred under an Ar atmosphere at rt for 1 h. The mixture was added NIS (1.13 g, 5.02 mmol). After cooling to -10 °C, TMSOTf (0.1 mL, 0.553 mmol) was added and the reaction was allowed to warm up to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO\textsubscript{3} (10 mL) and CH\textsubscript{2}Cl\textsubscript{2} were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (20%) and water. After extraction of the aqueous layer with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10), the combined organic phase was dried over Na\textsubscript{2}SO\textsubscript{4}, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give tetrasaccharide 18 (2.49 g, 87.8%) as a syrup. Analytical data for 18: R\textsubscript{f} = 0.5 (Ethyl acetate /Hexane 1:4, v/v); \textsuperscript{1}H NMR (700 MHz, CDCl\textsubscript{3}): δ: 7.32 - 7.38 (m, 16 H, ArH), 7.27 - 7.32 (m, 4 H, ArH), 5.39 (dd, J=3.1, 1.9 Hz, 1 H, H-2\textsubscript{D}), 4.92 (d, J=1.8 Hz, 1 H, H-1\textsubscript{C}), 4.87 (d, J=1.8 Hz, 1 H, H-1\textsubscript{B}), 4.83 (d, J=1.8 Hz, 1 H, H-1\textsubscript{D}), 4.71 (d, J=11.0 Hz, 1 H, -C\textsubscript{Ph}), 4.70 (d, J=11.7 Hz, 1 H, -C\textsubscript{Ph}), 4.62 (d, J=11.5 Hz, 1 H, -C\textsubscript{Ph}), 4.60 (s, 2 H, -CH\textsubscript{2}Ph), 4.58 (ABq, J=11.7 Hz, 1 H, -CH\textsubscript{2}Ph), 4.53 (d, J=11.0 Hz, 1 H, -CH\textsubscript{2}Ph), 4.51 (d, J=1.8 Hz, 1 H, H-1\textsubscript{A}), 3.86 (t, J=2.3 Hz, 1 H, H-2\textsubscript{C}), 3.82 (t, J=2.3 Hz, 1 H, H-2\textsubscript{B}), 3.80 (t, J=2.3 Hz, 1 H, H-2\textsubscript{A}), 3.76 (dd, J=9.9, 3.3 Hz, 1 H, H-3\textsubscript{D}), 3.64 - 3.69 (m, 3 H, H-3\textsubscript{C}, H-3\textsubscript{B}, H-3\textsubscript{A}), 3.50 (dq, J=10.0, 6.1 Hz, 1 H, H-5\textsubscript{D}), 3.48 (dq, J=9.7, 5.9 Hz, 1 H, H-5\textsubscript{A}), 3.42 (dq, J=9.7, 6.1 Hz, 1 H, H-
5), 3.40 (dq, J=9.6, 6.1 Hz, 1 H, H-5_B), 3.36 (t, J=10.0 Hz, 1 H, H-4_D), 3.31 (t, J=10.0 Hz, 1 H, H-4_C), 3.28 (s, 3 H, -OCH_3), 3.22 (t, J=9.9 Hz, 1 H, H-4_A), 3.18 (t, J=9.9 Hz, 1 H, H-4_B), 2.09 (s, 3 H, -COCH_3), 1.27 (d, J=6.1 Hz, 3 H, H-6_B), 1.26 (d, J=6.4 Hz, 3 H, H-6_A), 1.19 (d, J=6.1 Hz, 3 H, H-6_B), 1.14 (d, J=6.1 Hz, 3 H, H-6_C); \(^{13}\)C NMR (176 MHz, CDCl_3): δ: 169.8, 137.4, 137.3, 137.1(x2), 128.6(x2), 128.4, 128.3, 128.2, 128.1, 128.0(x3), 100.3, 100.1, 99.7, 99.1, 77.4, 76.6, 75.4, 73.6, 73.4(x2), 72.2, 72.1, 72.0, 71.5, 67.8, 67.6, 67.1, 66.9, 64.3, 64.2, 64.0, 63.8, 54.9, 21.0, 18.6(x2), 18.5, 18.4 ppm; HRMS (ESI): m/z calcd for C_{35}H_{66}N_2O_{14}Na [M+Na]+: 1141.4714, found: 1141.473.

Methyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (19).

Sodium methoxide (~1.2 mL, 0.5 M solution) was added to a solution of 18 (2.2 g, 1.95 mmol) in CH_3OH: THF [4:2] (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH_3OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound 19 (1.86 g, 88.7%) as white solid. Analytical data for 19: Rf = 0.4 (Ethyl acetate /Hexane 1:4, v/v); \(^1\)H NMR (700 MHz, CDCl_3): δ: 7.28 - 7.41 (m, 20 H, ArH), 4.96 (d, J=1.5 Hz, 1 H, H-1_D), 4.93 (d, J=1.8 Hz, 1 H, H-1_C), 4.88 (d, J=1.8 Hz, 1 H, H-1_B), 4.72 (d, J=11.5 Hz, 1 H, -CHPh), 4.68 (d, J=11.3 Hz, 1 H), 4.66 (d, J=11.3 Hz, 1 H), 4.62 (d, J=11.7 Hz, 1 H, -CHPh), 4.60 (s, 2 H, -CH_2Ph), 4.57 (ABq, J=11.5 Hz, 2 H, -CH_2Ph), 4.51 (d, J=1.8 Hz, 1 H, H-1_A), 3.97 - 3.99 (m, 1 H, H-2_D), 3.92 (t, J=2.4 Hz, 1 H, H-2_C), 3.81 (t, J=2.3 Hz, 1 H, H-2_B), 3.80 (t, J=2.3 Hz, 1 H, H-2_A), 3.64 - 3.71 (m, 4 H, H-3_D, H-3_C, H-3_B, H-3_A), 3.51 (dq, J=10.1, 6.1 Hz, 1 H, H-5_D), 3.48 (dq, J=9.9, 6.1 Hz, 1 H, H-5_B), 3.37 - 3.45 (m, 2 H, H-5_C, H-5_A), 3.40 (t, J=9.8 Hz, 1 H, H-4_D), 3.29 (t, J=9.5 Hz, 1 H, H-4_C), 3.28 (s, 3 H, -OCH_3), 3.23 (t, J=9.9 Hz, 1 H, H-4_B), 3.18 (t, J=10.0 Hz, 1 H, H-4_A), 2.26 (d, J=1.8 Hz, 1 H, 1-OH_2D), 1.27 (d, J=6.3 Hz, 3 H, H-6_A), 1.25 (d, J=6.3 Hz, 3 H, H-6_B), 1.19 (d, J=6.1 Hz, 3 H, H-6_D), 1.15 (d, J=6.1 Hz, 3 H, H-6_C); \(^{13}\)C NMR (176 MHz, CDCl_3): δ: 137.3(x2), 137.1, 128.6(x2), 128.5, 128.4, 128.3(x2), 128.2(x3), 128.1, 128.0, 100.4, 100.3, 100.2, 99.7, 77.7, 77.4, 76.6, 73.6, 73.5, 73.2, 72.2, 72.1(x3), 67.8, 67.3, 67.1, 66.9, 64.3, 64.2(x2), 63.8, 54.9, 18.6(x2), 18.5, 18.3 ppm; HRMS (ESI): m/z calcd for C_{35}H_{66}N_2O_{13}Na [M+Na]+: 1099.4608, found: 1099.4625.
Methyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (20).

The glycosyl acceptor compound 19 (1.63 g, 1.51 mmol), and glycosyl donor compound 11 (0.712 g, 1.66 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH₂Cl₂ (15 mL), treated with freshly activated 4 Å molecular sieves (1 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.681 g, 3.03 mmol). After cooling to -10 °C, TMSOTf (0.06 mL, 0.33 mmol) was added and the reaction was allowed to warm up to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO₃ (10 mL) and CH₂Cl₂ were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na₂SO₄ (20%) [15 mL] and water (15 mL). After extraction of the aqueous layer with CH₂Cl₂ (3 x 10), the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give pentasaccharide 20 (1.92 g, 91.9%) as a sticky liquid. Analytical data for 20: R'f = 0.7 (Ethyl acetate /Hexane 1:4, v/v); ¹H NMR (700 MHz, CDCl₃): δ: 7.27 - 7.40 (m, 25 H, ArH), 5.39 - 5.41 (m, 1 H, H-2E), 4.94 (s, 1 H, H-1C), 4.86 (s, 1 H, H-1B), 4.84 (s, 1 H, H-1E), 4.72 (d, J=11.7 Hz, 1 H, -CHPh), 4.71 (d, J=11.1 Hz, 1 H, -CHPh), 4.64 (d, J=11.7 Hz, 1 H, -CHPh), 4.61 (s, 2 H, -CH₂Ph), 4.60 (s, 2 H, -CH₂Ph), 4.57 (ABq, J=11.7 Hz, 2 H, -CH₂Ph), 4.53 (d, J=11.0 Hz, 1 H, -CH₃Ph), 4.51 (s, 1 H, H-1A), 3.86 (br. s., 1 H, H-2D), 3.83 (br. s., 1 H, H-2C), 3.79 (d, J=2.3 Hz, 2 H, H-2A, H-2B), 3.77 (dd, J=9.9, 2.9 Hz, 1 H, H-3E), 3.70 (dd, J=9.9, 2.3 Hz, 1 H, H-3D), 3.67 (t, J=2.9 Hz, 1 H, H-3A), 3.66 (t, J=3.0 Hz, 1 H, H-3C), 3.64 (dd, J=9.9, 2.0 Hz, 1 H, H-3B), 3.51 (dq, J=10.2, 2.6 Hz, 1 H, H-5A), 3.47 (dq, J=9.5, 6.1 Hz, 1 H, H-5B), 3.45 (dq, J=9.9, 6.1 Hz, 1 H, H-5D), 3.38 - 3.42 (m, 2 H, H-5C, H-5E), 3.37 (t, J=10.2 Hz, 1 H, H-4A), 3.33 (t, J=9.9 Hz, 1 H, H-4D), 3.28 (s, 3 H, -OCH₃), 3.16 - 3.23 (m, 3 H, H-4B, H-4E, H-4C), 2.10 (s, 3 H, -COCH₃), 1.27 (d, J=6.4 Hz, 3 H, H-6C), 1.24 (d, J=6.4 Hz, 3 H, H-6B), 1.20 (d, J=6.1 Hz, 3 H, H-6A), 1.17 (d, J=5.9 Hz, 3 H, H-6D), 1.14 (d, J=6.1 Hz, 3 H, H-6E). ¹³C NMR (176 MHz, CDCl₃): δ: 169.8, 137.4, 137.3, 137.2, 137.1, 128.6(x3), 128.5(x2), 128.4, 128.3(x2), 128.2, 128.1(x2), 128.0(x3), 100.3, 100.2, 100.0, 99.7, 99.1, 77.4, 76.6, 76.5, 75.4, 73.7, 73.6, 73.4, 73.3, 72.2(x2), 72.1, 72.0, 71.5, 67.8(x2), 67.6, 67.1, 66.9, 64.3, 64.2(x2), 64.1, 63.8, 54.9, 21.0, 18.6(x2), 18.5(x2), 18.4 ppm; HRMS (ESI): m/z calcd for C₆₈H₈₁N₁₅O₁₇Na [M+Na]+: 1402.5827, found: 1402.5856.
Methyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (21).

Sodium methoxide (~1.2 mL, 0.5 M solution) was added to a solution of 20 (1.8 g, 1.31 mmol) in CH₃OH: THF [4:2] (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H+) ion exchange resin, the resin was filtered off and rinsed successively with CH₃OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound 21 (1.57 g, 89.8%) as white foam. Analytical data for 21: Rf = 0.55 (Ethyl acetate /Hexane 1:4, v/v); ¹H NMR (700 MHz, CDCl₃): δ: 7.27 - 7.43 (m, 24 H, ArH), 7.14 - 7.20 (m, 1 H, ArH), 4.98 (s, 1 H, H-1E), 4.96 (s, 1 H, H-1D), 4.88 (d, J=1.7 Hz, 2 H, H-1C, H-1B), 4.55 - 4.75 (m, 10 H, -CH₃Ph), 4.52 (s, 1 H, H-1A), 4.00 (br. s., 1 H, H-2E), 3.92 - 3.95 (m, 1 H, H-2D), 3.81 (br. s., 2 H, H-2B, H-2A), 3.71 (dd, J=9.8, 2.8 Hz, 2 H, H-3E, H-3D), 3.64 - 3.69 (m, 3 H, H-3A, H-3C, H-3B), 3.51 - 3.56 (m, 1 H, H-5D), 3.44 - 3.51 (m, 2 H, H-5E, H-5A), 3.38 - 3.44 (m, 3 H, H-5C, H-5B, H-4D), 3.31 (t, J=10.1 Hz, 1 H, H-4E), 3.29 (s, 3 H, -OCH₃), 3.16 - 3.25 (m, 3 H, H-4B, H-4C, H-4A), 2.27 - 2.32 (m, 1 H, 1-CH₂), 1.28 (dd, J=6.0, 2.6 Hz, 3 H, H-6C), 1.26 (dd, J=6.0, 2.7 Hz, 3 H, H-6A), 1.21 (dd, J=6.0, 2.8 Hz, 3 H, H-6D), 1.19 (dd, J=5.9, 2.7 Hz, 3 H, H-6E), 1.15 (dd, J=6.0, 2.7 Hz, 3 H, H-6B); ¹³C NMR (176 MHz, CDCl₃): δ: 137.3(x2), 137.2, 129.0, 128.6(x4), 128.4, 128.3(x4), 128.2(x2), 128.1, 128.0, 100.5, 100.3, 100.2(x2), 99.7, 77.7, 77.4, 77.0, 76.6, 76.5, 73.7, 73.6, 73.4, 73.2, 72.2, 72.1(x3), 67.8(x2), 67.3, 67.1, 66.9, 64.4, 64.2, 63.8, 54.9, 18.6(x2), 18.5(x2), 18.3 ppm; HRMS (ESI): m/z calcd for C₆₆H₇₀N₁₅O₁₆Na [M+Na]+: 1360.5721, found: 1360.5749.
Methyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (22).

The glycosyl acceptor compound 21 (1.45 g, 1.08 mmol), and glycosyl donor compound 11 (0.556 g, 1.3 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH2Cl2 (15 mL), treated with freshly activated 4 Å molecular sieves (1 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.488 g, 2.16 mmol). After cooling to -10 °C, TMSOTf (43 μL, 0.24 mmol) was added and the reaction was allowed to warm up to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO3 (10 mL) and CH2Cl2 were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na2S2O3 (20%) [10 mL] and water (15 mL). After extraction of the aqueous layer with CH2Cl2 (3x10), the combined organic phase was dried over Na2SO4, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give hexasaccharide 22 (1.601 g, 90.1%) as a sticky liquid. Analytical data for 22: Rf = 0.65 (Ethyl acetate /Hexane 1:4, v/v); 1H NMR (700 MHz, CDCl3): δ: 7.26 - 7.40 (m, 30 H, ArH), 5.40 (t, J=2.9 Hz, 1 H, H-2F), 4.95 (d, J=1.4 Hz, 1 H, H-1E), 4.88 (d, J=1.5 Hz, 1 H, H-1D), 4.86 (d, J=1.5 Hz, 1 H, H-1C), 4.85 (s, 2 H, H-1B, H-1F), 4.72 (d, J=11.4 Hz, 1 H, -CHPh), 4.71 (d, J=10.9 Hz, 1 H, -CHPh), 4.64 (d, J=11.7 Hz, 1 H, -CHPh), 4.55 - 4.63 (m, 8 H, -CHPh), 4.53 (d, J=11.0 Hz, 1 H, -CHPh), 4.51 (d, J=1.4 Hz, 1 H, H-1α), 3.86 - 3.88 (m, 1 H, H-2E), 3.82 - 3.84 (m, 1 H, H-2D), 3.76 - 3.81 (m, 4 H, H-2A, H-2C, H-2B, H-3F), 3.71 (dd, J=9.9, 2.9 Hz, 1 H, H-3E), 3.62 - 3.68 (m, 4 H, H-3D, H-3C, H-3A, H-3B), 3.52 (dq, J=10.0, 6.1 Hz, 1 H, H-5F), 3.44 - 3.49 (m, 2 H, H-5A, H-5E), 3.38 - 3.44 (m, 3 H, H-5D, H-5B, H-5C), 3.37 (t, J=10.2 Hz, 1 H, H-4F), 3.34 (t, J=9.9 Hz, 1 H, H-4E), 3.28 (s, 3 H, -OCH3), 3.22 (t, J=9.9 Hz, 1 H, H-4D), 3.15 - 3.22 (m, 3 H, H-4C, H-4A, H-4B), 2.10 (s, 3 H, -OCH3), 1.27 (d, J=6.1 Hz, 3 H, H-6D), 1.25 (d, J=6.1 Hz, 3 H, H-6A), 1.21 (d, J=6.0 Hz, 3 H, H-6P), 1.18 (d, J=6.0 Hz, 3 H, H-6E), 1.16 (d, J=6.3 Hz, 3 H, H-6B), 1.12 (d, J=6.1 Hz, 3 H, H-6C); 13C NMR (176 MHz, CDCl3): δ: 169.8, 137.4, 137.3, 137.2, 137.1(x3), 128.6(x4), 128.5(x2), 128.4, 128.3(x2), 128.2, 128.1(x2), 128.0(x3), 100.3, 100.1(x2), 100.0, 99.7, 99.1, 77.4, 76.7(x2), 76.5, 75.4, 73.6(x2), 73.5, 73.4, 73.3, 72.2, 72.1, 72.0, 71.5, 67.8(x4), 67.6, 67.1, 66.9, 64.3(x2), 64.2(x2), 64.1, 63.8, 54.9, 21.0, 18.6(x2), 18.5(x3), 18.4 ppm; HRMS (ESI): m/z calcd for C81H96N18O20Na [M+Na]+: 1663.694, found: 1663.6982.
Sodium methoxide (~1.0 mL, 0.5 M solution) was added to a solution of 22 (1.3 g, 0.792 mmol) in CH₃OH: THF [4:2] (15 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H⁺) ion exchange resin, the resin was filtered off and rinsed successively with CH₃OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound 23 (1.17 g, 92.3%) as oil. Analytical data for 23: Rf = 0.5 (Ethyl acetate /Hexane 1:4, v/v); ¹H NMR (500 MHz, CDCl₃): δ: 7.31 - 7.46 (m, 30 H, ArH), 5.01 (s, 1 H, H-1F), 4.99 (d, J=1.5 Hz, 1 H, H-1E), 4.92 (d, J=1.1 Hz, 1 H, H-1D), 4.89 (d, J=1.1 Hz, 1 H, H-1C), 4.88 (d, J=1.5 Hz, 1 H, H-1B), 4.76 (d, J=11.4 Hz, 1 H, -CHPh), 4.74 (d, J=11.0 Hz, 1 H, -CHPh), 4.55 - 4.71 (m, 10 H, -CHPh), 4.54 (d, J=1.5 Hz, 1 H, H-1A), 4.01 - 4.04 (m, 1 H, H-2F), 3.95 - 3.98 (m, 1 H, H-2E), 3.85 - 3.87 (m, 1 H, H-2D), 3.80 - 3.85 (m, 3 H, H-2B, H-2C, H-2A), 3.74 - 3.76 (m, 1 H, H-3F), 3.72 - 3.74 (m, 1 H, H-3E), 3.65 - 3.71 (m, 4 H, H-3D, H-3C, H-3B, H-3A), 3.56 (dq, J=10.2, 6.2 Hz, 1 H, H-5E), 3.38 - 3.53 (m, 6 H, H-5A, H-5D, H-5F, H-4E, H-5C, H-5B), 3.34 (t, J=9.9 Hz, 1 H, H-4F), 3.32 (s, 3 H, -OCH₃), 3.27 (t, J=9.9 Hz, 1 H, H-4D), 3.18 - 3.24 (m, 3 H, H-4B, H-4C, H-4A), 2.30 (s, 1 -OH₂F), 1.31 (d, J=6.2 Hz, 3 H, H-6E), 1.28 (d, J=6.2 Hz, 3 H, H-6A), 1.24 (d, J=6.1 Hz, 3 H, H-6C), 1.22 (d, J=6.3 Hz, 3 H, H-6B), 1.19 (d, J=6.1 Hz, 3 H, H-6D), 1.16 (d, J=6.2 Hz, 3 H, H-6B); ¹³C NMR (126 MHz, CDCl₃): δ: 137.3, 137.2(x2), 128.7(x3), 128.6(x2), 128.4(x3), 128.3(x2), 128.2, 128.1(x3), 100.5, 100.3, 100.2(x2), 100.1, 99.8, 77.7, 77.5, 76.6(x2), 73.7, 73.6, 73.5(x2), 73.3, 72.2(x2), 72.1, 67.9, 67.8, 67.4, 67.2, 67.0, 64.4, 64.2, 63.9, 54.9, 18.7, 18.6(x2), 18.5(x2), 18.3 ppm; HRMS (ESI): m/z calcd for C₇₉H₄₄N₁₈O₁₉Na [M+Na]+: 1621.6835, found: 1621.688.
Methyl 2,3-di-O-benzoyl-4-O-(5′-N-benzyl-5′-N-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (24).

The glycosyl acceptor compound 23 (0.270 g, 0.169 mmol), and glycosyl donor compound 13 (0.146 g, 0.186 mmol), were combined, azeotroped twice with anhydrous toluene (5 mL), and placed under high vacuum for 2 h. The mixture was then dissolved in CH₂Cl₂ (10 mL), treated with freshly activated 4 Å molecular sieves (0.3 g), stirred under an Ar atmosphere at rt for 1 h. To the mixture was added NIS (0.076 g, 0.337 mmol). After cooling to -10 °C, TMSOTf (6.4 μL, 0.037 mmol) was added and the reaction was allowed to warmup to room temperature. When TLC showed the reaction was completed, saturated aqueous NaHCO₃ (5 mL) and CH₂Cl₂ were then added, and the resulting mixture was passed through celite to remove molecular sieves. The combined filtrates were washed with aqueous Na₂S₂O₃ (20%) [10 mL] and water (10 mL). After extraction of the aqueous layer with CH₂Cl₂ (3 x 5), the combined organic phase was dried over Na₂SO₄, concentrated in vacuum, and purified by silica gel column chromatography (Ethyl acetate /Hexane gradient elution) to give heptasaccharide 24 (0.334 g, 87.4%) as a sticky liquid. Analytical data for 24: Rf = 0.65 (Ethyl acetate /Hexane 1:4, v/v); [α]D²¹ = -6.71° (c = 1.23, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ: 8.04 - 8.09 (m, 2 H, ArH), 7.93 (d, J=7.5 Hz, 2 H, ArH), 7.66 (t, J=7.4 Hz, 1 H, ArH), 7.48 - 7.55 (m, 3 H, ArH), 7.23 - 7.44 (m, 38 H, ArH), 7.10 - 7.22 (m, 4 H, ArH), 5.72 (t, J=2.9 Hz, 1 H, H-2G), 5.69 (dd, J=9.2, 3.2 Hz, 1 H, H-3G), 5.16 (s, 2 H, -NCH₂Ph), 5.06 - 5.09 (br. s., 2 H, -H₁G, -H₁F), 4.94 (d, J=1.5 Hz, 1 H, H-1E), 4.92 (d, J=1.4 Hz, 1 H, H-1D), 4.90 (d, J=1.5 Hz, 1 H, H-1C), 4.89 (d, J=1.5 Hz, 1 H, H-1B), 4.67 (d, J=11.7 Hz, 1 H, -CHPh), 4.58 - 4.72 (m, 11 H, -CHPh), 4.55 (d, J=1.5 Hz, 1 H, H-1A), 4.35 - 4.44 (m, 2 H, -OCH₂Ph), 4.03 (t, J=2.6 Hz, 1 H, H-2F), 3.95 (dq, J=9.2, 6.2 Hz, 1 H, H-5G), 3.91 (t, J=2.9 Hz, 1 H, H-2E), 3.81 - 3.88 (m, 4 H, H-2D, H-2B, H-2C, H-2A), 3.77 (dd, J=9.7, 2.6 Hz, 1 H, H-3F), 3.66 - 3.75 (m, 5 H, H-3D, H-3A, H-3D, H-3B, H-3C), 3.64 (t, J=9.9 Hz, 1 H, H-4F), 3.38 - 3.59 (m, 9 H, -CH₂₂, H-5F, H-5A, H-5E, H-5D, H-5C, H-5B, H-4G), 3.32 (s, 3 H, -OCH₃), 3.30 (t, J=10.1 Hz, 1 H, H-4E), 3.18 - 3.27 (m, 4 H, H-4B, H-4D, H-4A, H-4C), 2.93 - 3.13 (m, 2 H, -CH₂₂), 1.36 - 1.51 (m, 4 H, -CH₂₂b, -CH₂₂a), 1.27 - 1.36 (m, 14 H, H-6G, H-6E, H-6A, H-6F, -CH₂₂b), 1.20 (d, J=6.2 Hz, 3 H, H-6B), 1.20 (d, J=5.9 Hz, 3 H, H-6D), 1.18 (d, J=5.9 Hz, 3 H, H-6C); ¹³C NMR (126 MHz, CDCl₃): δ: 165.3, 165.1, 156.6, 156.1, 137.9, 137.5, 137.4, 137.3, 137.2(x2), 136.8, 133.3, 133.0(x2), 129.9, 129.8, 129.6, 129.1, 128.7(x2), 128.6(x2), 128.5(x2), 128.4(x2),
Methyl 4-O-(5'-N-benzyl-5'-N-carboxybenzyl-pentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl (1→2) 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside (25).

Sodium methoxide (~0.2 mL, 0.5 M solution) was added to a solution of 24 (0.26 g, 0.115 mmol) in CH₃OH: THF [2:3] (10 mL) until pH ~9 and the resulting mixture was stirred under argon for 6 h at 21 °C. After that, the reaction mixture was neutralized with Amberlite IR 120 (H⁺) ion exchange resin, the resin was filtered off and rinsed successively with CH₃OH. The combined filtrate was concentrated in vacuo and purified by column chromatography on silica gel (Ethyl acetate - Hexane gradient elution) to afford the title compound 25 (0.215 g, 91.2%) as oil. Analytical data for 25: Rf = 0.25 (Ethyl acetate /Hexane 1:3.3, v/v); [α]D²¹ = +79.2 (c = 2.21, CHCl₃); ¹H NMR (700 MHz, CDCl₃): δ: 7.23 - 7.40 (m, 39 H, ArH), 7.13 - 7.19 (m, 1 H, ArH), 5.13 - 5.20 (m, 2 H, -NCH₂Ph), 4.99 (br. s., 1 H, H-1_F), 4.92 (br. s., 1 H, H-1_G), 4.89 (d, J=1.8 Hz, 1 H, H-1_E), 4.86 (d, J=1.8 Hz, 1 H, H-1_D), 4.85 (d, J=1.9 Hz, 1 H, H-1_C), 4.84 (d, J=1.7 Hz, 1 H, H-1_B), 4.54 - 4.72 (m, 12 H, -CH₂Ph), 4.51 (d, J=1.8 Hz, 1 H, H-1_A), 4.44 - 4.50 (m, 2 H, -OCH₂Ph), 3.97 - 4.02 (m, 2 H, H-2_F, H-2_G), 3.81 - 3.88 (m, 2 H, H-3_G, H-2_E), 3.77 - 3.81 (m, 4 H, H-2_D, H-2_B, H-2_C, H-2_A), 3.72 (dd, J=9.9, 2.8 Hz, 1 H, H-3_F), 3.56 - 3.70 (m, 7 H, H-3_E, H-3_C, H-3_D, H-3_B, H-3_A, -CH₂a), 3.34 - 3.54 (m, 8 H, H-5_G, H-5_F, H-5_A, H-5_E, H-5_D, H-5_C, H-5_B, H-4_G), 3.28 (s, 3 H, -OCH₃), 3.25 (t, J=10.0 Hz, 1 H, H-4_E), 3.07 - 3.21 (m, 7 H, H-4_C, H-4_B, H-4_F, H-4_D, H-4_A, -CH₂e), 2.30 (br. s., 2 OH, -OH₂G, -OH₃G), 1.42 - 1.72 (m, 4 H, -CH₂b, -CH₂d), 1.27 (d, J=6.1 Hz, 3 H, H-6_F), 1.23 - 1.26 (m, 6 H, H-6_G, H-6_E), 1.21 (br. s., 2 H, -CH₂c), 1.18 (d, J=6.1 Hz, 3 H, H-6_B), 1.16 (d, J=6.2 Hz, 3 H, H-6_A), 1.15 (d, J=6.1 Hz, 3 H, H-6_D), 1.12 (d, J=6.1 Hz, 3 H, H-6_C); ¹³C NMR (176 MHz, CDCl₃): δ: 156.7, 156.3, 137.8, 137.4, 137.3, 137.2 (x2), 137.1 (x2), 136.7, 129.0, 128.6 (x2), 128.5, 128.4, 128.3 (x2), 128.2, 128.1, 128.0, 127.9, 127.8, 127.3, 100.8, 100.4, 100.3, 100.2, 100.1 (x2), 99.7, 81.6, 77.4, 76.5, 73.6, 73.6, 73.5, 73.5, 72.9, 72.2, 72.1, 72.1, 72.0, 71.7, 71.1, 68.2, 67.8, 67.7, 67.2, 66.9, 64.3, 64.2, 54.9, 50.5, 50.3, 47.1, 37.9, 36.7, 34.6, 34.1, 33.8, 32.6, 32.3, 31.9, 31.8, 31.6, 31.2. HRMS (ESI): m/z calcd for C₁₁₉H₁₃₅N₁₉O₂₇Na [M+Na]+: 2284.9667, found: 2284.9732.
46.1, 29.7, 29.4, 27.9, 27.2, 23.3, 18.6(x2), 18.5(x4), 17.9 ppm; HRMS (ESI): m/z calcd for C_{105}H_{131}N_{20}O_{25} [M+NH_4]^+: 2071.9589, found: 2071.9639.

**VIII. PREPARATION OF OLIGOSACCHARIDE GLYCOCONJUGATES**

**ACTIVATION FOR CONJUGATION OF THE 1,2 HEXASACCHARIDE 1**

5'-Methoxycarbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (1).

![Structure 1](image1)

Analytical data for the title compound was essentially the same as previously described.6

(2'-Aminoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (S22).

![Structure 2](image2)

Analytical data for the title compound was essentially the same as previously described.6
(2′-[N-succinimidyl]glutarylamidoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (S23).

A mixture of hexasaccharide S22 (4 mg) and disuccinimidal glutarate (15 eq.) in DMF and 0.1 M PBS buffer (4 : 1, 0.5 mL) was stirred at rt for 6 h. The reaction mixture was concentrated under vacuum and the residue was washed with EtOAc 10 times to remove the excess disuccinimidal glutarate. The resultant solid was dried under vacuum for 1 h to obtain activated oligosaccharide S23 that was directly used for conjugation with BSA and tetanus toxoid. MALDI TOF MS (positive mode): calcd for C_{59}H_{93}N_{9}O_{31}Na [M + Na]^+ m/z, 1446.5977; found, 1446.8936.

**ACTIVATION FOR CONJUGATION OF THE 1,2 TRISACCHARIDE 4**

5′-Methoxycarbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (4).

To a stirred solution of S21 (0.2 g, 0.239 mmol), in pyridine (5 mL) and water (2 mL) mixture, H_{2}S was bubbled for 0.5 h at 40 °C, and continued stirring for 16 h. After that, argon was bubbled for 10 min, solvents were removed in vacuo, and the residue was co-evaporated with toluene (3 x 10 mL) and dried. The mass spectrometry analysis showed completion of reaction to corresponding amine compound and no products arising from incomplete reduction.
This crude material was directly used for formylation. Amine compound in CH$_3$OH (5 mL) at -20 ºC was added a freshly prepared formic anhydride (5 mL, ethereal solution) and stirred for 3 h, then slowly allowed to warm to 21 ºC. After that, solvents were evaporated and the residue was passed through column chromatography on silica gel (methanol – dichloromethane gradient elution) to afford trisaccharide. The high resolution mass spectrometry analysis showed completion of formylation reaction. HRMS (ESI): m/z calcd for C$_{42}$H$_{59}$N$_3$O$_{15}$Na [M+Na$^+$]: 868.3838, found: 868.3837.

Formylated compound was dissolved in CH$_3$OH/H$_2$O (2:1, 15 mL), Pd(OH)$_2$ on carbon (20%, 0.090 g) was added. Then it was stirred under a pressure of hydrogen gas at 21 ºC for 16 h. After filtration through celite pad and washed with CH$_3$OH (3 x 10 mL), and solvents were removed in vacuo. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound 4 (0.094 g, 59.3%, over 3 steps) as white foam. Analytical data for 4: [α]$_D^{21}$ = +31.58 (c = 1.16, H$_2$O); $^1$H NMR (700MHz, D$_2$O): δ 8.20 - 8.24 (Z) and 8.03 - 8.06 07 (E) (m, 3H, NCHO), 5.16 - 5.22 (m, 1 H, H-1$_B$), 5.05 - 5.08 (m, 1 H, H-1$_C$), 4.89 - 4.93 (m, 1 H, H-1$_A$), 4.13 - 4.19 (m, 1 H, H-2$_B$), 4.06 - 4.13 (m, 2 H, H-2c, H-3$c$), 3.92 - 4.03 (m, 6 H, H-2$_A$, H-3$_A$, H-3$_B$, H-4$_c$, H-4$_B$, H-4$_A$), 3.87 - 3.92 (m, 2 H, H-5$_A$, H-5$_C$), 3.80 - 3.84 (m, 1 H, H-5$_B$), 3.71 - 3.75 (m, 1 H, -O-CH$_2$), 3.71 (s, 3 H), 3.56 (dt, J=9.9, 5.9 Hz, 1 H, -O-CH$_3$), 2.42 (t, J=7.4 Hz, 2 H, -CH$_2$), 1.60 - 1.68 (m, 4 H, -CH$_2$), 1.40 (dq, J=14.8, 7.3 Hz, 2 H, -CH$_2$), 1.20 - 1.30 (m, 9 H, 3 x H-6); $^{13}$C NMR (176MHz, D$_2$O): δ 178.4, 168.6(x2), 165.7, 165.7(x2), 102.9, 102.8, 101.5, 99.1, 78.5, 78.4, 78.2, 78.1, 78.0, 69.8, 69.1, 68.8, 68.7(x2), 68.6, 68.5(x2), 68.3(x2), 67.9, 57.8, 52.9, 52.8, 52.7(x2), 52.5, 52.4(x2), 28.9, 25.7, 24.8, 17.8(x2), 17.7(x2), 17.6, 17.5(x2) ppm. HRMS (ESI): m/z calcd for C$_{28}$H$_{47}$N$_3$O$_{15}$Na [M+Na$^+$]: 688.2899, found: 688.2908.

(2’-Aminoethylamido)carbonylpentyl  4,6-dideoxy-4-formamido-α-D-mannpyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannpyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannpyranoside (S24)

A solution of 4 (0.06 g, 0.09 mmol) in freshly distilled 1,2-diaminoethane (3.0 mL) was stirred at 65 ºC for 48 h. After that, excess reagent was removed in vacuo, and the residue was co-evaporated with CH$_3$OH (3 x 10 mL) and dried. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound S24 (0.052 g, 83.15%) as white foam. Analytical data for S24: [α]$_D^{21}$ = +37.05 (c = 1.14, H$_2$O); $^1$H NMR (500MHz, D$_2$O): δ 8.24 - 8.33 (Z) and 8.05 - 8.12 (E) (m, 3H, NCHO), 5.23 - 5.26 (m, 1 H, H-1$_B$), 5.12 (s, 1 H, H-1$_C$), 4.93 - 4.97 (m, 1 H, H-1$_A$), 4.19 - 4.24 (m, 1 H, H-2$_B$), 4.10 - 4.18 (m, 2 H, H-2c, H-3c), 3.96 - 4.08 (m, 6 H, H-2A, H-3A, H-3B, H-4C, H-4B, H-4A), 3.91 - 3.96
(m, 2 H, H-5A, H-5C), 3.84 - 3.89 (m, 1 H, H-5b), 3.77 (dt, J=9.7, 6.8 Hz, 1 H, -O-CH₂b), 3.57 - 3.63 (m, 1 H, -O-CH₂a), 3.33 (t, J=6.2 Hz, 2 H, -CH₂g), 2.82 (t, J=6.2 Hz, 2 H, -CH₃h), 2.33 (t, J=7.4 Hz, 2 H, -CH₂i), 1.64 - 1.74 (m, 4 H, -CH₂e, -CH₂g), 1.39 - 1.49 (m, 2 H, -CH₂d), 1.25 - 1.35 (m, 9 H, 3 x H-6); ¹³C NMR (126MHz, D₂O): δ 178.3, 168.8(x2), 165.8(x2), 103.0, 102.9, 101.6, 99.3, 78.6, 78.3, 78.2, 78.1, 69.9, 69.2, 69.0, 68.9, 68.8(x2), 68.6(x2), 68.5, 68.4, 57.7, 53.0, 52.8(x2), 52.7, 42.1, 42.1, 40.7, 36.7, 29.1, 26.0, 25.9, 17.9(x2), 17.8(x2), 17.7(x2), 17.6 ppm; HRMS (ESI): m/z calcd for C₉₂H₅₁N₅O₁₄Na [M+Na]+: 716.3325, found: 716.333.

1-[(2'-Aminoethylamido)carbonylpentyl 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside] 2-butoxycyclobutene-3,4-dione (S25).

To a stirred solution of S24 (0.015 g, 0.022 mmol) in water (0.5 mL) and EtOH (0.5 mL), a solution of 3,4-dibutoxy-3-cyclobutene-1,2-dione (20% in ethanol, 70 μL) was added and pH was adjusted to 8 by careful addition of aq.NaHCO₃ (1%) solution. After 1 h, mass spectrometry showed the reaction was complete; the reaction mixture was neutralized using CH₃COOH (10%) and concentrated in vacuo. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound S25 (0.0133 g, 73.2%) as white foam. Analytical data for S25: ¹H NMR (700MHz, D₂O): δ 8.21 - 8.23 (Z) and 8.05 (E) (m, 3H, NCHO), 5.19 (s, 1 H, H-1b), 5.07 (s, 1 H, H-1c), 4.90 - 4.92 (m, 1 H, H-1a), 4.68 - 4.75 (m, 2 H, -CH₂h), 4.14 - 4.19 (m, 1 H, H-2b), 4.07 - 4.13 (m, 2 H, H-2c, H-3c), 3.92 - 4.02 (m, 6 H, H-2a, H-3a, H-3b, H-4c, H-4b, H-4a), 3.89 (m, 2 H, H-5a, H-5c), 3.79 - 3.85 (m, 1 H, H-5b), 3.73 (t, J=5.0 Hz, 1 H, -CH₂g), 3.65 - 3.71 (m, 1 H, -O-CH₂b), 3.62 (t, J=5.0 Hz, 1 H, -CH₂g), 3.51 (dd, J=9.6 Hz, 6.5 Hz, 1 H, -O-CH₂g), 3.40 - 3.45 (m, 2 H, -CH₂b), 2.19 - 2.27 (m, 2 H, -CH₂l), 1.77 - 1.84 (m, 2 H, -CH₂l), 1.51 - 1.64 (m, 4 H, -CH₂e, -CH₂f), 1.46 (dt, J=15.5, 7.9 Hz, 2 H, -CH₂f), 1.30 - 1.34 (m, 2 H, -CH₂d), 1.20 - 1.30 (m, 9 H, 3 x H-6), 0.94 - 0.98 (m, 3 H, -CH₂d); ¹³C NMR (176MHz, D₂O): δ 189.7, 184.1, 178.4, 177.8, 174.5, 168.6, 165.7, 165.7, 102.8, 101.5, 99.1, 98.9, 78.4, 78.1, 75.2, 75.1, 69.8, 69.1, 68.8, 68.7, 68.6, 68.4, 68.3(x2), 57.8, 52.9, 52.7, 52.5, 45.0, 44.9, 40.2, 40.0, 36.6, 32.3, 29.1, 26.0, 25.9, 25.8, 25.7, 19.0, 18.9, 17.8(x2), 17.7(x2), 17.6, 17.5, 13.8 ppm; HRMS (ESI): m/z calcd for C₃₇H₅₉N₅O₁₇Na [M+Na]+: 868.3798, found: 868.3808.
ACTIVATION FOR CONJUGATION OF HEPTASACCHARIDE 8

Methyl 4-O-(5'-aminopentanyl)-6-deoxy-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-α-D-mannopyranoside (8).

To a stirred solution of 25 (0.11 g, 0.054 mmol), in pyridine (5 mL) and water (2 mL) mixture, H₂S was bubbled for 0.5 h at 40 ºC, and continued stirring for 16 h. After that, argon was bubbled for 10 min, solvents were removed in vacuo, and the residue was co-evaporated with toluene (3 x 10 mL) and dried. The mass spectrometry analysis showed completion of reaction to corresponding amine compound and no products arising from incomplete reduction. HRMS (ESI): m/z calcd for C₁₀₇H₄₁₆N₇O₂₅ [M+H]+: 1898.9893, found: 1898.99.

This crude material was directly used for formylation. Amine compound in CH₃OH (5 mL) at -20 ºC was added a freshly prepared formic anhydride (5 mL, ethereal solution) and stirred for 3 h, then slowly allowed to warm to 21 ºC. After that, solvents were evaporated and the residue was passed through column chromatography on silica gel (methanol – dichloromethane gradient elution) to afford heptasaccharide. The high resolution mass spectrometry analysis showed completion of formylation reaction. HRMS (ESI): m/z calcd for C₁₁₁H₁₃₉N₇O₃¹Na [M+Na]+: 2088.9408, found: 2088.9405.

Formylated compound was dissolved in CH₃OH/H₂O (2:1, 10 mL), Pd(OH)₂ on carbon (20%, 0.060 g) was added. Then it was stirred under a pressure of hydrogen gas at 21 ºC for 16 h. After filtration through celite pad and washed with CH₃OH (3 x 10 mL), and solvents were removed in vacuo. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound 8 (0.0427 g, 61.2%, over 3 steps) as white foam. Analytical data for 8: [α]D²¹ = +42.44 (c = 1.02, H₂O); ¹H NMR (500MHz, D₂O): δ : 8.21 - 8.32 (Z) and 8.06 - 8.14 (E) (m, 6H, NCHO), 5.21 - 5.30 (m, 5 H, H-1G, H-1F, H-1E, H-1D, H-1C), 5.05 (s, 1 H, H-1B), 4.86 - 4.89 (m, 1 H, H-1A), 4.11 - 4.31 (m, 11 H, H-2F, H-2E, H-3A, H-3B, H-2D, H-3C, H-3D, H-2B, H-2A, H-3F, H-2C), 3.75 - 4.10 (m, 17 H, H-3E, H-3G, H-4A, H-2G, H-4C, H-4D, H-5G, H-5E, H-5C, H-4E, H-4F, H-5F, H-5B, H-5D, H-5A, H-4B, -CH₃), 3.49 - 3.54 (m, 1 H, -CH₃), 3.48 (s, 3 H, -ΟCH₃), 3.36 (t, J=9.7 Hz, 1 H, H-4CH₃), 3.08 (t, J=7.5 Hz, 2 H, -CH₂), 1.66 - 1.82 (m, 4 H, -CH₂, -CH₂), 1.46 - 1.59 (m, 2 H, -CH₂), 1.23 - 1.43 (m, 21 H, 7 x H-6); ¹³C NMR (126 MHz, CDCl₃): δ: 168.8(x2), 168.6, 165.9(x4), 165.7, 103.2, 103.1(x4), 102.7, 102.5, 101.5(x2), 100.4, 100.3, 81.8, 78.2, 78.1(x3), 78.0(x2), 77.9, 73.5(x2), 71.3, 70.8(x2), 69.2, 68.7(x2), 68.5, 68.4, 67.8, 57.9, 56.4, 55.9, 55.8(x2), 52.9(x2), 52.7(x2), 40.4,
29.7, 27.5, 23.2, 17.9(x2), 17.8(x2), 17.7, 17.6(x4) ppm; HRMS (ESI): m/z calcd for C_{54}H_{92}N_{7}O_{29} [M+H]^+: 1302.5934, found: 1302.5928.

Methyl 4-O-(5'-[N-succinimidyl]glutarylamidopentanyl)-6-deoxy-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranoside (S26).

A mixture of heptasaccharide 8 (9 mg) and disuccinimidal glutarate (15 eq.) in DMF and 0.1 M PBS buffer (4 : 1, 1.5 mL) was stirred at rt for 6 h. The reaction mixture was concentrated under vacuum and the residue was washed with EtOAc 10 times to remove the excess disuccinimidal glutarate. The resultant solid was dried under vacuum for 1 h to obtain activated oligosaccharide S26 that was directly used for conjugation with BSA & tetanus toxoid. MALDI TOF MS (positive mode): calcd for C_{63}H_{100}N_{8}O_{34}Na [M + Na]^+ m/z, 1535.6342; found, 1535.9996.

1-[(2'-Aminoethylamido)carbonylpentyl]-6-deoxy-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranosyl (1→2) 4,6-dideoxy-4-formamido-\(\alpha\)-D-mannopyranoside] 2-butoxycyclobutene-3,4-dione (S27).
To a stirred solution of heptasaccharide 8 (0.006 g, 0.005 mmol) in water (0.5 mL) and EtOH (0.5 mL), a solution of 3,4-dibutoxy-3-cyclobutene-1,2-dione (20% in ethanol, 50 μL) was added and pH was adjusted to 8 by careful addition of aq.NaHCO₃ (1%) solution. After 1 h, mass spectrometry showed the reaction was complete; the reaction mixture was neutralized using CH₃COOH (10%) and concentrated in vacuo. The residue was purified by reversed phase HPLC on C18 column in gradient water-acetonitrile and lyophilized, to give the title compound S27 (0.005 g, 72.6%) as white foam. Analytical data for S27: ¹H NMR (700MHz, D₂O): δ: 8.218 - 8.21 (Z) and 8.00 - 8.04 (E) (m, 6H, CH₃O), 5.14 - 5.21 (m, 5 H, H-1G, H-1F, H-1E, H-1D, H-1C), 4.95 (s, 1 H, H-1B), 4.80 (s, 1 H, H-1A), 4.66 - 4.74 (m, 2 H, -CH₂), 4.02 - 4.19 (m, 11 H, H-2E, H-2D, H-2C, H-3D, H-2B, H-2A, H-3F, H-2C), 3.75 - 4.00 (m, 15 H, H-3E, H-3G, H-4A, H-2G, H-4C, H-4D, H-5G, H-5E, H-4E, H-4F, H-5F, H-5B, H-5D, H-5A, -CH₃), 3.59 - 3.68 (m, 2 H, H-5C, H-4B), 3.40 - 3.45 (m, 1 H, -CH₂), 3.39 (s, 3 H, -OCH₃), 3.27 (t, J=9.7 Hz, 1 H, H-4C), 1.82 - 1.75 (m, 2 H, -CH₂), 1.56 - 1.67 (m, 4 H, -CH₂, -CH₂), 1.36 - 1.48 (m, 4 H, -CH₂, -CH₂), 1.17 - 1.29 (m, 21 H, 7 x H-1), 0.90 - 0.96 (m, 3 H, -CH₃); ¹³C NMR (126 MHz, CDCl₃): δ: 190.3, 184.2, 183.2, 178.4, 178.0, 174.3, 168.8, 168.7, 168.6, 165.9, 165.7, 103.2, 103.2(x4), 102.8, 102.6, 101.6, 100.4, 100.3, 81.9, 78.2, 78.1(x2), 78.0(x2), 77.9, 73.4, 71.3, 70.7, 69.8, 69.2, 69.1, 68.8, 68.6, 68.5, 68.4, 57.9, 56.4, 55.9, 55.8, 52.9(x2), 52.7, 40.4, 32.4, 30.7, 30.5, 29.7, 27.5, 23.3, 23.2, 19.2, 19.0, 17.9, 17.8(x2), 17.7(x4), 17.6, 13.9 ppm; HRMS (ESI): m/z caled for C₆₂H₉₀N₇O₃₂Na [M+Na]+: 1476.6335, found: 1476.6406.

OLIGOSACCHARIDE PROTEIN CONJUGATION:

Preparation of tetanus toxoid conjugate 2: Activated hexasaccharide S23 (1 mg, 0.518 μmol) was added to the solution of tetanus toxoid (4 mg, 0.025 μmol) in 0.5 M borate buffer pH 9 (1 mL) and stirred slowly at 21 °C for 3 days. Then the reaction mixture was washed with PBS buffer, filtered through a millipore filtration tube (10,000 MWCO, 4 x 10 mL) and the resulting tetanus toxoid-conjugate 2 was stored in PBS buffer. The MALDI-TOF mass spectrometry analysis indicated the conjugate 2 had an average of 11.7 hexasaccharides per tetanus toxoid.

Preparation of BSA conjugate 3: BSA (5.5 mg) and activated hexasaccharide S23 (2.0 mg) were dissolved in 0.1 M PBS buffer pH 9 (600 μL) and stirred slowly at 21 °C for 3 days. Then the reaction mixture was diluted with Mili-Q water, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL), lyophilized and the BSA-conjugate 3 was obtained as a white foam (6.2 mg). The MALDI-TOF mass spectrometry analysis indicated the conjugate 3 had an average of 8.9 hexasaccharides per BSA.

Preparations of BSA conjugate 5: BSA (15 mg) and trisaccharide squarate S25 (3.8 mg, 6.77 μmol) were dissolved in 0.1 M PBS buffer pH 9 (600 μL) and stirred slowly at 21 °C for 3 days. Then the reaction mixture was diluted with Mili-Q water, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL), lyophilized and the BSA-conjugate 5 was obtained as a white foam (17.6 mg). The MALDI-TOF mass spectrometry analysis indicated the conjugate 5 had an average of 16.2 disaccharides per BSA.

Preparation of tetanus toxoid conjugate 9: Activated heptasaccharide S27 (0.8 mg, 0.518 μmol) was added to the solution of tetanus toxoid (4 mg, 0.026 μmol) in 0.5 M borate buffer pH 9 (1 mL) and stirred slowly at 21 °C for 3 days. Then the reaction mixture was washed with PBS
buffer, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL) and the resulting tetanus toxoid-conjugate 7 was stored in PBS buffer. The MALDI-TOF mass spectrometry analysis indicated the conjugate 7 had an average of 10.0 heptasaccharide per tetanus toxoid.

Preparation of BSA conjugate 10: BSA (10 mg) and activated heptasaccharide S27 (4.5 mg) were dissolved in 0.1 M PBS buffer pH 9 (1.2 mL) and stirred slowly at 21 °C for 3 days. Then the reaction mixture was diluted with Mili-Q water, filtered through millipore filtration tube (10,000 MWCO, 4 x 10 mL), lyophilized and the BSA-conjugate 8 was obtained as a white foam (12.2 mg). The MALDI-TOF mass spectrometry analysis indicated the conjugate 8 had an average of 10.3 heptasaccharide per BSA.

IX. PREPARATION OF OPS-TETANUS TOXOID GLYCOCONJUGATE

The sLPS from B. abortus S99 and B. suis biovar 2 (strain Thomsen) was purified by hot-phenol extraction\textsuperscript{10}(11) and the OPS was liberated from this product my mild acid hydrolysis.\textsuperscript{11} The precipitated Lipid A was removed as the pellet following centrifugation at 17,000 g for 30 mins. The supernatant was buffer exchanged into water by size exclusion chromatography using sephadex G-25 which also removed low molecular weight impurities. The purified OPS, at 2 mg/ml, was oxidised by incubation in 10 mM sodium metaperiodate in 50 mM sodium acetate buffer at pH 5.5 at 4°C for 1h in the dark. The OPS was then desalted using sephadex G-25 (PD-10 column, GE Healthcare) to buffer exchange into water, removing residual sodium metaperiodate. Oxidised OPS, 5 mg/ml, was incubated in PBS with 0.5 M ammonium chloride and 0.1 M sodium cyanoborohydride at 37°C for 24h after which the OPS was desalted into water using sephadex-G25 and freeze dried.

The oxidised and aminated OPS was reconstituted to 5 mg/ml with a 10% concentration of DMSO in PBS containing 5 mg/ml DSG (disuccinimidal glutarate) and incubated for 45 mins at room temperature on a rotary shaker then desalted back into PBS using a Zeba 40 kDa column according to the manufacturer’s instructions (Pierce) to remove unconjugated DSG. The OPS-DSG conjugate was added to tetanus toxoid (TT) at final concentrations of 2.5 and 0.5 mg/ml respectively. This solution was incubated for 2h at room temperature on a rotary shaker after which a final concentration of 2 mg/ml glycine was added and this was further incubated for 15 mins.

The OPS-TT conjugate was separated from the non-conjugated OPS and glycine by SEC-HPLC using an Agilent Infinity Bioinert HPLC system fitted with a 30 cm Tosoh TSK-gel G3000 PWx1, bore size 7 mm (cat: 05762) size exclusion chromatography column plus guard column comprised of the same matrix. The mobile phase was PBS pH 7.4 (50 mM sodium phosphate, 150 mM sodium chloride) and flow rate was 0.8 ml/min. The fraction eluting at 6.5 to 8.8 mins was collected as this contained the OPS conjugated tetanus toxoid only. The unconjugated OPS eluted from 8.8 to 11.0 mins whereas 75% of the OPS-TT conjugate eluted between 6.5 and 8.8
mins. The effect of the HPLC separation is visible in the SDS-PAGE silver stain image (Figure S3) as the TT light chain fragment is lost from the final preparation used for immunisation. The *B. abortus* and *B. suis* OPS-TT conjugates were evaluated by SDS-PAGE silver staining and Western blotting to verify their anti-OPS antibody reactivity (Figure S3). For SDS-PAGE TT and glycoconjugates were diluted to 0.2mg/ml in sample buffer (Invitrogen, Life Technologies) then heated for 5 minutes at 80 °C in a water bath. The antigens were loaded into NuPAGE® Novex Tris-Acetate Gels (Invitrogen, Life technologies) 10 µl per well. HiMark™ Unstained Protein Standard (Invitrogen, Life technologies) was also loaded into the gels for silver staining and HiMark™ Prestained Protein Standard (Invitrogen, Life technologies) for the Western blot gels, 7.5 µl per well. The gels were run at 110 volts in an electrophoresis tank with MOPS running buffer (Invitrogen, Life Technologies) for 90 minutes. After gel electrophoresis, the gels were stained using a silver staining kit (Biorad). Initially the gels were fixed with in-house 40% methanol, 10% acetic acid fixative for 30 minutes. Then oxidising concentrate (Biorad) was diluted 1 in 10 in deionised water and added to the gels, the gels were incubated for 5 minutes on a rocker. The gels were then washed with deionised water for 15 minutes with frequent changes of water. Silver reagent (Biorad) was diluted 1 in 10 in deionised water and incubated with the gels for 20 minutes on a rocker. The gels were rinsed for 30 seconds then incubated with developing concentrate (Biorad) for 15 minutes until bands were visualised. The gels were then incubated with 5% acetic acid stopper solution (in-house preparation) for 30 minutes and then scanned. For Western blot the gels were run then they were placed on a nitrocellulose membrane (Invitrogen) and the antigens were transferred to the membrane using the iBlot™ dry transfer system (Invitrogen). The nitrocellulose membranes were blocked overnight at 4-8 °C with blocking buffer (Candor). The membranes were then incubated with mouse anti-*Brucella* OPS monoclonal antibody clones BrG11 (Fzmb, Germany), specific to A epitopes, or BM4012 (APHA, Weybridge), specific to M epitopes, at 20 µg/ml in Low-cross buffer (Candor) for 90 minutes at room temperature. Then the membranes were washed three times, for fifteen minutes with washing buffer (Candor), on a rocker at room temperature. The membranes were then incubated with anti-mouse Ig:alkaline phosphatase at 1/1000 in Low-cross buffer (Candor) for 90 minutes then washed three times, for 15 minutes. The membranes were incubated with BCIP/NBT tablets (Sigma) until bands were visualised. Then the membranes were allowed to dry and scanned. The same method was used for Western blot with bovine polyclonal sera which was applied at a 1/100 dilution and developed with Protein G:alkaline phosphatase. The silver stain images (lanes 2-6) show the increase in size of the main TT protein when conjugated to *B. abortus* S99 OPS although the minimum size remains the same. There is a small increase in size visible due to conjugation with *B. suis* OPS. Size exclusion fractionation by HPLC leads to the elimination of the light chain TT fragment in both the OPS-TT preparations. The Western blots confirm that the TT has been conjugated with OPS. Polyclonal anti-Brucella sera, derived from a *B. abortus* infected cow binds to both glycoconjugates, although more to the *B. abortus* OPS-TT, with a very low background response to the unconjugated TT. A
monoclonal antibody specific to ‘A’ OPS epitopes (a series of 5 or more α1,2 linked D-Rha4NFo units) binds to both glycoconjugates, again more so to the B. abortus OPS-TT. A monoclonal antibody specific to ‘M’ epitopes (a short series of D-Rha4NFo units incorporating a single α1,3 link) bound only to B. abortus OPS-TT (with a weak background reaction to TT only). This is consistent with the known structure of the two OPS antigens in which the B. abortus S99 antigen contains low proportion (2%) of α1,3 links, the remainder α1,2 linked whereas the B. suis biovar 2 OPS is exclusively α1,2 linked. The lack of α1,3 links is considered to be unique to B. suis biovar 2 and means that the D-RhaN4Fo polymer is identical to that of the unrelated bacteria Y. enterocolitica O:9.

The conjugation of tetanus toxoid with OPS was also evaluated by MALDI-ToF using an Applied biosystems/MDS SCIEX 4800 MALDI TOF/TOF analyser. Sample was added in 0.5 µl to the plate and allowed to dry then covered with 0.5 µl of 10 mg/ml sinapic acid. Data was collected in linear mode. The peak mass of unconjugated TT was 152,375 m/z (figure S4). The peak mass for the B. abortus S99 OPS-tetanus conjugate was 156,320 m/z (Figure S5) and for B. suis biovar 2 OPS-TT conjugate it was 154,114 m/z (Figure S6), although peak broadening
towards higher masses were evident, especially with the *B. abortus* OPS-TT conjugate. Based on this, the OPS content for each conjugate was therefore 2.5% for *B. abortus* OPS-TT and 1.1% for *B. suis* OPS-TT, equivalent to an average of approximately 13 and 20 D-Rha4Nfo units per TT respectively.

However, given that the average length of an OPS polymer is 96-100 units\textsuperscript{13} it is probable that the conjugation of TT has been poor and that the distribution of the number of OPS molecules that have been conjugated per TT is in the range of 0-4. It is likely that conjugation has favoured shorter OPS molecules as these would more rapidly diffuse within a reaction mixture. Enrichment of longer OPS molecules prior to conjugation would increase the glycan content of the conjugate, as might adoption of a different conjugation technique. For example, the use of a squarate linker such as 3,4-dibutoxy-3-cyclobutene-1,2-dione would reduce the likelihood of any intramolecular linking of the two aldehydes on the oxidised terminal D-Rha4Nfo due to the reduced activity of the linker once the first active site has conjugated. However, this may necessitate the use of DSG as the linker for the diagnostic antigens.

Figure S4: MALDI-ToF spectrum of unconjugated tetanus toxoid (TT)
Figure S5: MALDI-ToF spectrum of *B. abortus* S99 OPS tetanus toxoid conjugate (OPS-TTS99)
X. IMMUNIZATION OF MICE

Vaccine formulation: Alum was suspended in PBS at 50 mg/mL concentration and thimerosal (0.01% w/v) was added and stored at 4°C. Conjugate solutions were prepared at 1 mg/mL of PBS. Alum (14 µL) was mixed with the tetanus toxoid conjugates (144 mL) in 5:1 weight ratio, diluted with 2.85 mL of PBS and the mixture was allowed to rock overnight before administering to animals.14

Immunization with glycoconjugates 2 and 9: Female CD1 mice (Charles River, Canada) age 6-8 weeks in groups of 10 were immunised three times at 21 day intervals. Each mouse received 250 µl distributed 150 µl intraperitoneally and 100 µl subcutaneously. Pre bleeds were collected prior to immunisation and mice were euthanized at day 10 after the final injection and final bleeds were collected. Blood was incubated at 37° C for one hour then spun at 1500 g for 10 min. Clear serum was collected and stored at -20°C until use.

Immunisation with OPS-Tetanus toxoid conjugate
Two groups of 8 female CD1 mice of 7 weeks of age were immunized on days 1, 21 and 35 with 5 µg each of conjugate administered subcutaneously in a 100 µl volume of PBS without
adjuvant. Prebleeds were collected prior to immunization and post vaccination bleeds were taken on days 19, 33 and 49.

XI. ELISA DATA
INDIRECT ELISA
Immunoassays: Antibody titres against glycoconjugate coated plates and plates coated with purified LPS were determined according to a published protocol\(^1\) with minor modification. Briefly, polystyrene microtiter plates were incubated with the coating glycoconjugate antigen (1 μg/mL, 100 μL/well) at 4°C overnight, then washed (5×) with PBST (0.05% Tween-20 in phosphate buffer saline, PBS). LPS 1μg/mL in sodium carbonate buffer.

![Graph](image)

Figure S7  Antibody titres of sera raised to vaccines 2 and 9 titred against the immunizing hapten conjugated to BSA and the three sLPS of *B. abortus*, *B. melitensis* and *Yersenia enterocolitica* O:9.
Mouse sera was diluted 1:100 murine sera in 0.1% BSA in PBST added to the coated well (100 μL/well) at serial √10 dilutions in the same buffer. After incubation at room temperature for 2 h, the plates were washed (5×) with PBST. Then the plate was incubated with 100 μL/well of HRPO labelled goat anti-mouse IgG antibody (KPL) (1:5000 dilution of a 1.0 mg/mL stock) for 30 min at room temperature, then washed (5×) with PBST. Peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB) with H₂O₂, was added. After 15 min the reaction was quenched by addition of phosphoric acid (1M, 100 μL/well). Plates were read at 450 nm and the data were processed using Origin software. End point dilution (x₀) was recorded as the serum dilution giving an absorbance 0.2 above background and serum titer was calculated as the reciprocal of x₀. All the data were processed using Origin 9 and Graphpad Prism software.

**Table S1** ELISA Inhibition with pentasaccharides S38-S43

| Mouse # | Inhibition as μmole IC₅₀ |
|---------|--------------------------|
|         | S38 P₂R | S39 P₂RP₂ | S40 P₂RP | S41 PRPRP | S42 PR₂PR | S43 RP₃R |
| 2       | 40      | 50      | 170      | <500      | NA        | NA       |
| 3       | 30      | 4       | 9        | NA        | NA        | 100      |
| 5       | 3       | 20      | 210      | NA        | NA        | NA       |
| 6       | 4       | 8       | 30       | NA        | NA        | NA       |
| 8       | 1       | 6       | 7        | NA        | NA        | NA       |
| 10      | 100     | 60      | 300      | NA        | NA        | NA       |

NA – not active at 1mM

**Immunoassays for OPS-TT immunised mice**

The smooth LPS antigens *B. abortus* S99 and *B. melitensis* 16M were diluted to 0.6 μg/ml and detoxified tetanus toxin (TT or dTT) (Statens Serum Institute) was diluted to 2.5 μg/ml in carbonate buffer (Sigma). The whole cell antigens *B. abortus* S99, *B. melitensis* 16M and *B. suis* biovar 2 (strain Thomsen) were diluted 15.6 μg/ml in carbonate buffer (Sigma). The synthetic antigens Disaccharide, Tetrasaccharide, 1,2-Hexasaccharide and 1,3-Hexasaccharide were diluted 2.5μg/ml in carbonate buffer (Sigma). Then 100 μl per well was added to standard bind
ELISA plates (Nunc). The plates were incubated overnight at 4-8 °C then washed four times with PBS-Tween, 200 μl per well and tapped dry on blotting paper.

Mouse sera were diluted in log dilutions (\(\sqrt{10}\)) at 1/100, 1/316.22, 1/1000, 1/3162.27, 1/10000, 1/31622.7, 1/100000, 1/316227, 1/1000000 and 1/3162270 in casein buffer and 100 μl per well was added to the antigen coated plates. For the synthetic antigens the sera were also diluted at 1/31.62. Monoclonal antibody BM40, specific to M epitopes, was diluted to 5 μg/ml in casein buffer (Sigma) and added to the plates, 100 μl per well, as the positive control. A positive serum control, mouse sera from a mouse immunised with 1,2-Hexasaccharide, and a negative serum control from a normal (non-immunised) mouse were also included, 100 μl per well, as controls.

The plates were incubated for 30 minutes at room temperature, on a rotator at 120 rpm, then washed four times with PBS-Tween, 200 μl per well and tapped dry on blotting paper. Anti-mouse immunoglobulins HRP conjugate (Dako) was diluted 1 in 1000 in casein buffer and 100 μl/well was added to the plates. The plates were incubated for 60 minutes for the synthetic antigens and tetanus toxoid and 30 minutes for sLPS and whole cell antigens at room temperature, on a rotator at 120 rpm, then washed four times with PBS-Tween, 200 μl per well and tapped dry on blotting paper. Substrate buffer (pH4.0) (Fluka) with 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma) and 3% hydrogen peroxide (Sigma) was added to the plates, 100 μl per well, and incubated at room temperature for 20 minutes. The reaction was slowed with 0.1M sodium azide, 100 μl per well, and the plates were read at 405 nm absorbance. Data was calculated as the blanked mean of duplicate wells as a percentage of the BM40 positive control wells tested with Disaccharide as this was added to every test plate.

The optical densities (ODs) for each sample and dilution were blanked by subtracting the OD for control wells to which no sera had been added but were otherwise processed as described above. The quantitative data for the samples were then normalised by expressing the ODs as a percentage of the positive control. The end titres were calculated (using GraphPad Prism 6) as the dilution at which the signal (expressed as a percentage of the positive control) was equal to the positive/negative threshold. This threshold was calculated as the mean of the pre-bleed samples plus 1.96 times the standard deviation of the pre-bleed samples.

XII. REFERENCES

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XIII. $^1$H AND $^{13}$C NMR SPECTRA
$^1$H NMR Spectrum of compound S12 (CDCl$_3$, 700 MHz).

![NMR Spectrum of compound S12](image)
$^{13}$C NMR Spectrum of compound S12 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 11 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound 11 (CDCl$_3$, 176 MHz).
"H NMR Spectrum of compound S13 (CDCl₃, 700 MHz).
$^{13}$C NMR Spectrum of compound S13 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 12 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound 12 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound S14 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound S14 (CDCl$_3$, 176 MHz).
\(^1\)H NMR Spectrum of compound S15 (CDCl\(_3\), 700 MHz).
CNMR Spectrum of compound S15 (CDCl₃, 176 MHz).
^1^H NMR Spectrum of compound S16 (CDCl\textsubscript{3}, 700 MHz).
$^{13}$C NMR Spectrum of compound S16 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound S17 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound S17 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 13 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound 13 (CDCl$_3$, 176 MHz).
$^{1}$H NMR Spectrum of compound S20 (CDCl$_3$, 500 MHz).
$^{13}$C NMR Spectrum of compound S20 (CDCl$_3$, 126 MHz).
\(^1\)H NMR Spectrum of compound S21 (CDCl\(_3\), 500 MHz).
$^{13}$C NMR Spectrum of compound S21 (CDCl$_3$, 126 MHz).
$^1\text{H} \text{NMR Spectrum of compound 14 (CDCl}_3, 700 \text{ MHz).}$
$^{13}$C NMR Spectrum of compound 14 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 15 (CDCl$_3$, 700 MHz).
\(^{13}\)C NMR Spectrum of compound 15 (CDCl\(_3\), 176 MHz).
$^1$H NMR Spectrum of compound 16 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound 16 (CDCl$_3$, 176 MHz).
\(^1\)H NMR Spectrum of compound 17 (CDCl\(_3\), 700 MHz).
$^{13}$C NMR Spectrum of compound 17 (CDCl$_3$, 176 MHz).
\( ^1H \) NMR Spectrum of compound 18 (CDCl\(_3\), 700 MHz).

\[ \begin{array}{c}
\text{N}_3 \quad \text{OAc} \\
\text{BnO} \\
\text{O} \\
\text{N}_3 \quad \text{O} \\
\text{BnO} \\
\text{O} \\
\text{N}_3 \quad \text{O} \\
\text{BnO} \\
\text{O} \\
\text{N}_3 \quad \text{O} \\
\text{BnO} \\
\text{O} \\
\text{OMe} \\
\end{array} \]
$^{13}$C NMR Spectrum of compound 18 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 19 (CDCl$_3$, 700 MHz).
$^{13}$C NMR Spectrum of compound 19 (CDCl$_3$, 176 MHz).
\textit{\textsuperscript{1}H NMR Spectrum of compound 20 (CDCl$_3$, 700 MHz).}
$^{13}$C NMR Spectrum of compound 20 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 21 (CDCl$_3$, 700 MHz).
\(^{13}\)C NMR Spectrum of compound 21 (CDCl\textsubscript{3}, 176 MHz).
$^1$H NMR Spectrum of compound 22 (CDCl$_3$, 700 MHz).
\(^{13}\)C NMR Spectrum of compound 22 (CDCl\(_3\), 176 MHz).
H NMR Spectrum of compound 23 (CDCl₃, 500 MHz).

\[ ^1H \text{NMR Spectrum of compound 23 (CDCl}_3, 500 \text{ MHz).} \]
$^{13}$C NMR Spectrum of compound 23 (CDCl$_3$, 126 MHz).
\textsuperscript{1}H NMR Spectrum of compound 24 (CDCl\textsubscript{3}, 500 MHz).
$^{13}$C NMR Spectrum of compound 24 (CDCl$_3$, 126 MHz).
$^1\text{H NMR Spectrum of compound 25 (CDCl}_3, 700 \text{ MHz).}$
$^{13}$C NMR Spectrum of compound 25 (CDCl$_3$, 176 MHz).
$^1$H NMR Spectrum of compound 4 (D$_2$O, 700 MHz).
$^{13}$C NMR Spectrum of compound 4 (D$_2$O, 176 MHz).
$^1$H NMR Spectrum of compound S24 (D$_2$O, 500 MHz).
$^1$H NMR Spectrum of compound S24 (D$_2$O, 126 MHz).
$^{1}$H NMR Spectrum of compound S25 (D$_2$O, 700 MHz).
$^{13}$C Spectrum of compound S25 (D$_2$O, 176 MHz)
$^1$H NMR Spectrum of compound 8 (D$_2$O, 500 MHz).
$^{13}$C NMR Spectrum of compound 8 (D$_2$O, 126 MHz).
$^1$H NMR Spectrum of compound S27 (D$_2$O, 500 MHz).
$^{13}$C NMR Spectrum of compound S27 (D$_2$O, 126 MHz).