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Supporting Information

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Supporting Information

for

Synthesis, Conjugation, and Immunological Evaluation of the Serogroup 6 Pneumococcal Oligosaccharides

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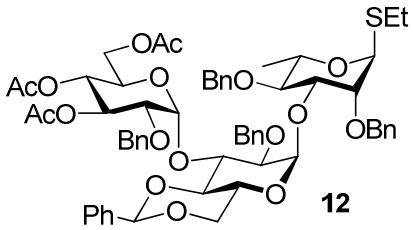
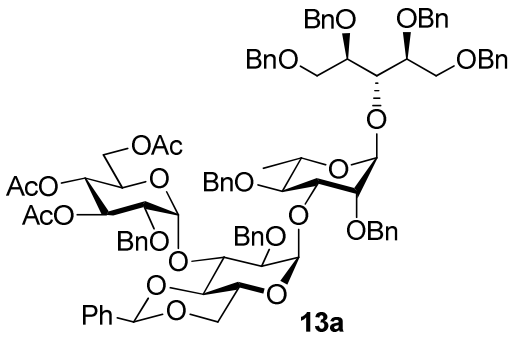
Additional Experimental and Characterization Data

General: Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F254. Reversed-phase purifications were carried out on Strata- X tubes (Phenomenex, 33 μm polymeric reverse phase). The compounds were detected under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at < 40 ℃. $ClCH_2CH_2Cl$ was distilled from CaH_2 directly prior to application. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH_2, distilled, and stored over molecular sieves (3 Å). Anhydrous DMF and diethyl ether were used as received. Molecular sieves (3 Å or 4 Å), used for reactions, were crushed and activated in vacuo for 8 h at 390 ℃ in the first instance and then for 2-3 h at 390 ℃ directl y prior to each application. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried in vacuo for 2-3 h directly prior to application. For ultrafiltration of conjugates, a centrifugal filter device (Amicon Ultra-15) was used. Optical rotations were measured at 'Jasco P-1020' polarimeter. Unless noted otherwise, ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz (Bruker Avance), ^{13}C-NMR spectra were recorded in $CDCl_3$ at 75 MHz (Bruker Avance) or at 125 MHz (Bruker ARX-500). HRMS spectra were measured at JEOL MStation (JMS-700) Mass Spectrometer. SELDI-TOF was measured using NP-20 ProteinChip® arrays and Bio-Rad Laboratories Inc instrument using Protein Chip data Manager Software 3.0.7.

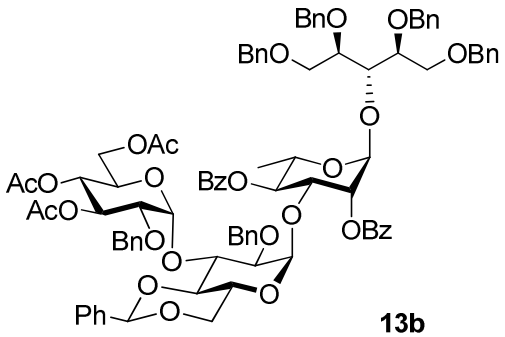


Ethyl *O*-(2-*O*-benzyl-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (12). The title compound was obtained by Method A from benzoxazolyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside **4** and **11** in 60% yield. Analytical data: R_f = 0.55 (ethyl acetate - hexanes, 2:3, *v/v*); $[\alpha]_D^{25}$ +75.0° (c = 1.0, $CHCl_3$); ^{1}H NMR: δ, 1.19 (t, 3H, CH_2CH_3), 1.36 (d, 3H, H-6), 1.90, 1.93, 1.99 (3s, 9H, 3 x $COCH_3$), 2.55-2.69 (q, 2H, CH_2CH_3), 3.35 (dd, 1H, $J_{2'',3''}$ = 3.6 Hz, H-2''), 3.57-3.79 (m, 6H, H-3, 2', 4', 6b', 6a''', 6b'''), 4.07-4.14 (m, 5H, H-2, 5, 5', 6a', ½ CH_2Ph), 4.21 (dd, 1H, $J_{4,5}$= 4.8 Hz, H-4), 4.33-4.39 (m, 2H, H-3', 5''), 4.44-4.50 (m, 2H, CH_2Ph), 4.61-4.71 (m, 4H, 2 x CH_2Ph), 4.82 (dd, 1H, $J_{4'',5''}$ = 3.8 Hz, H-4''), 4.99 (d, 1H, ½ CH_2Ph), 5.27 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.37-5.44 (m, 3H, H-1, 3'', CHPh), 5.49 (dd, 1H, $J_{1'',2''}$ = 3.6 Hz, H-1''), 6.79-7.45 (m, 25H, aromatic) ppm; ^{13}C NMR: δ, 15.7, 18.5, 21.3, 21.4, 21.5, 26.1, 30.3, 61.9, 63.2, 67.4, 68.7, 69.2, 69.6, 71.2, 72.1, 72.8, 72.9, 73.9, 75.7, 75.9, 76.4, 80.6, 81.8, 83.1, 94.5, 96.3, 102.9, 127.2 (x 2), 127.8 (x 2), 128.2, 128.3 (x 2), 128.4, 128.6, 128.7 (x 2), 128.8 (x 2), 128.9 (x 2), 129.0 (x 2), 129.06 (x 2), 129.08 (x 3), 129.1 (x 2), 129.3, 130.0, 137.7, 137.8, 137.9, 138.4, 138.7, 170.3, 170.7, 171.3, ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{61}H_{70}O_{17}SNa$ 1129.4231, found 1129.4238.



***O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzyl-α-L-rhamnnopyranosyl)-(1→3)-1,2,4,5-tetra-*O*-benzyl-D-ribitol (13a).** The title compound was ob-

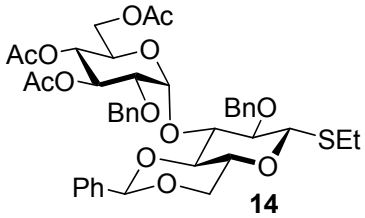
tained by Method B from **9** and **12** in 89% yield. Analytical data for **13a**: R_f = 0.46 (ethyl acetate - hexanes, 2:3, *v/v*); $[\alpha]_D^{25}$ +55.4^o (c = 1.0, $CHCl_3$); ^{1}H NMR: δ, 1.18 (d, 3H, H-6'), 1.80, 1.84, 1.90 (3s, 3H, 3 x $COCH_3$), 3.27 (dd, $J_{2''',3'''}$ = 3.6 Hz, H-2'''), 3.50-3.70 (m, 10H, H-1, 5, 4', 2'', 6a'', 6b'', 6a''', 6b'''), 3.75-3.79 (m, 1H, H-5'), 3.85-3.87 (m, 1H, H-2'), 3.90-3.95 (m, 1H, H-4''), 4.00-4.06 (m, 5H, H-2, 3', 3'', ½ CH_2Ph), 4.21-4.33 (m, 5H, H-3, 4, 5''', CH_2Ph), 4.35-4.52 (m, 9H, 4.5 x CH_2Ph), 4.57-4.61 (m, 3H, 1.5 x CH_2Ph), 4.71 (dd, 1H, $J_{4''',5'''}$ = 10.2 Hz, H-4'''), 4.93 (d, 1H, ½ CH_2Ph), 5.12-5.14 (m, 2H, H-1',1''), 5.28-5.34 (m, 2H, H-3''',C*H*Ph), 5.40 (d, 1H, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 6.72-7.34 (m, 45H, aromatic) ppm; ^{13}C NMR: δ, 18.2, 20.8, 20.9, 21.0, 61.5, 62.5, 66.9, 68.2, 69.1, 70.1, 70.3, 70.6, 71.7, 71.9, 72.1, 72.4, 72.8, 73.5, 73.6 (x 2), 74.5, 74.6, 75.3, 75.7, 78.0, 78.5, 78.7, 79.5, 82.7, 92.9, 95.8, 98.5, 102.5, 126.8 (x 2), 127.4 (x 2), 127.5 (x 3), 127.6 (x 3), 127.7 (x 4), 127.8 (x 2), 127.9 (x 3), 128.0, 128.1 (x 2), 128.2 (x 2), 128.3 (x 3), 128.4 (x 6), 128.5 (x 6), 128.6 (x 6), 129.6, 137.3, 137.4, 137.5, 138.1, 138.3, 138.4, 138.5, 138.6, 138.7, 169.9, 170.3, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{92}H_{100}O_{22}Na$ 1579.6604, found 1579.6609.



13b

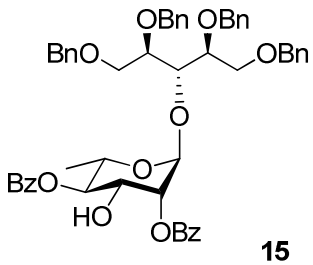
***O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnnopyranosyl)-(1→3)-1,2,4,5-tetra-*O*-benzyl-D/L-ribitol (13b).** The title compound was obtained by Method B from **14** and **15** in 66% yield. Analytical data for **13b**: R_f = 0.41 (ethyl acetate - hexanes, 2:3, *v/v*); $[\alpha]_D^{25}$ +55.6^o (c = 1.0, $CHCl_3$); ^{1}H NMR: δ, 1.09 (d, 3H, H-6'), 1.80, 1.94, 1.99 (3s, 3H, 3 x $COCH_3$), 3.24 (dd, $J_{2''',3'''}$ = 3.5 Hz, H-2'''), 3.45-3.59 (m, 4H, H-2, 5, 2''), 3.67-3.82 (m, 7H, H-1, 5'', 6a'', 6b'', 6a''', 6b'''), 3.87-3.90 (m, 1H, H-4''), 3.94-4.00 (m, 3H, H-3, 4, 3''), 4.11-4.13 (m, 1H, $J_{5''', 6a'''}$ = 10.4 Hz, H-5'''), 4.24-4.31 (m, 3H, H-5',CH_2Ph), 4.35-4.40 (m, 1H, H-3'), 4.45-4.74 (m, 11H, H-4''', 5 x CH_2Ph), 5.13 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 5.21-5.27 (m, 4H, H-1', 1''', 3''', C*H*Ph), 5.58-5.64 (m, 2H, H-2',4'), 6.70-8.12 (m, 45H, aromatic) ppm; ^{13}C NMR: δ,

17.8, 20.7, 20.9, 21.0, 29.9, 61.3, 62.6, 66.9, 67.8, 67.9, 68.9, 69.3, 70.3, 70.6, 71.6, 72.3 (x 2), 72.7, 73.4 (x 2), 75.2, 78.1, 82.5, 94.6, 95.5, 97.8, 102.2, 126.8 (x 3), 127.4 (x 3), 127.7 (x 8), 127.9 (x 3), 128.0 (x 4), 128.1 (x 8), 128.3 (x 5), 128.5 (x 8), 128.8 (x 3), 129.5, 129.6, 129.8, 129.9 (x 2), 130.2 (x 2), 133.4, 133.7, 137.2, 137.3, 137.5, 138.5 (x 2), 138.6, 163.3, 165.7, 166.1, 169.8, 170.2, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{92}H_{96}O_{24}Na$ 1607.6189, found 1607.6172.



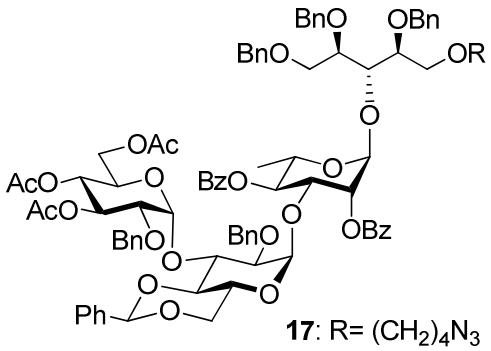
14

Ethyl *O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (14). The title compound was obtained by Method A from Benzoxazolyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside **4** and ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **6** in 72% yield. Analytical data: R_f = 0.5 (ethyl acetate – hexanes, 2:3, *v/v*); $[\alpha]_D^{25}$ +85.9^o (c = 1, $CHCl_3$); m.p. 74-76 ºC; ^{1}H NMR: δ, 1.35 (t, 3H, CH_2CH_3), 1.93, 1.95, 1.99 (3s, 9H, 3 x $COCH_3$), 2.71-2.90 (m, 2H, CH_2CH_3), 3.38 (dd, 1H, $J_{2',3'}$ = 3.6 Hz, H-2'), 3.49-3.59 (m, 2H, H-2, 6a), 3.65 (dd, 2H, $J_{6a',6b'}$ = 1.9 Hz, H-6a', H-6b'), 3.73-3.87 (m, 2H, H-4, 5), 4.08-4.19 (m, 3H, H-6b, 5', ½ CH_2Ph), 4.34 (dd, 1H, $J_{3,4}$ = 4.9 Hz, H-3), 4.54 (d, 1H, ½ CH_2Ph), 4.65 (d, 1H, $J_{1,2}$ = 9.47 Hz, H-1), 4.69 (d, 1H, ½ CH_2Ph), 4.84 (dd, 1H, $J_{3',4'}$= 9.9 Hz, H-4'), 5.13 (d, 1H, ½ CH_2Ph), 5.40-5.46 (m, 2H, H-3', *CH*Ph), 5.63 (d, 1H, $J_{1',2'}$= 3.5 Hz, H-1'), 6.85-7.43 (m, 15H, aromatic) ppm; ^{13}C NMR: δ, 15.2, 20.7, 20.8, 21.0, 25.3, 60.5, 61.2, 67.1, 68.0, 68.9, 70.1, 70.9, 71.5, 75.3, 75.8, 79.5, 82.2, 86.1, 95.8, 102.4, 126.6 (x 2), 127.4, (x 4), 127.8 (x 3), 128.4, 128.5 (x 3), 128.6 (x 3), 129.7, 137.1, 137.4, 137.8, 169.7, 170.2, 170.7, ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{41}H_{48}O_{13}SNa$ 803.2713, found 803.2722.



15

3-*O*-(2,4-Di-*O*-benzoyl-α-L-rhamnnopyranosyl)-1,2,4,5-tetra-*O*-benzyl-D/L-ribitol (15). The title compound was obtained by Method B from **7** and **9** in 65% yield. Analytical data: R_f = 0.42 (ethyl acetate – hexanes, 3/7, *v/v*); $[\alpha]_D^{25}$ -8.3^o (c = 1, $CHCl_3$); ^{1}H NMR: δ, 1.04 (d, 3H, H-6'), 3.56-3.70 (d, 4H, H-1, 5), 3.76-3.79 (m, 1H, H-2), 3.88-3.91 (m, 1H, H-4), 4.08-4.20 (m, 3H, H-3, 3', 5'), 4.37-4.59 (m, 8H, 4 x CH_2Ph), 5.05-5.16 (m, 2H, H-1', 4'), 5.27-5.29 (m, 1H, H-2'), 7.04-8.03 (m, 30H, aromatic) ppm; ^{13}C NMR: δ, 18.1, 67.4, 69.0, 69.5, 70.6, 70.7, 72.5, 72.6, 72.7, 73.7 (x 2), 73.9, 74.0, 76.1, 78.4, 78.6, 97.8, 128.0 (x 2), 128.1 (x 2), 128.2, 128.3 (x 2), 128.4(x 4), 128.5 (x 2), 128.8 (x 4), 128.9 (x 3), 129.0 (x 2), 129.1 (x 2), 130.0, 130.1, 130.4 (x 2), 130.5 (x 2), 138.8 (x 2), 138.9, 139.0, 166.5, 167.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{53}H_{54}O_{11}Na$ 889.3564, found 889.3577.



17: R= $(CH_2)_4N_3$

***O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→3)-*O*-(2,4-di-*O*-benzoyl-α-L-rhamnnopyranosyl)-(1→3)-1-*O*-(4-azidobutyl)-2,4,5-tri-*O*-benzyl-D-ribitol (17).** The title compound was obtained by Method B from **14** and **16** in 74% yield. Analytical data for **17**: R_f = 0.41 (ethyl acetate - hexanes, 2:3, *v/v*); $[\alpha]_D^{25}$ +55.6^o (c = 1.0, $CHCl_3$); ^{1}H NMR: δ, 1.22 (d, 3H, H-6'), 1.64-1.68 (m, 4H, 2 x CH_2sp), 1.82, 1.96, 2.01 (3s, 3H, 3 x $COCH_3$), 3.24-3.28 (m, 3H, H-2''', CH_2sp), 3.40-3.45 (m, 2H, CH_2sp), 3.48-3.62 (m, 4H, H-2, 5, 2''), 3.70-3.85 (m, 7H, H-1, 5'', 6a'', 6b'', 6a''', 6b'''), 3.91-3.95 (m, 2H, H-4, 4''), 3.98-4.03 (m, 2H, H-3, 3''), 4.11-4.14 (m, 1H, H-5'''), 4.25-4.33 (m, 2H, H-5', ½

CH_2Ph), 4.38-4.43 (m, 2H, H-3', ½ CH_2Ph), 4.50-4.54 (m, 2H, CH_2Ph), 4.68-4.78 (m, 7H, H-4''', 3 x CH_2Ph), 5.16 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1''), 5.23-5.30 (m, 4H, H-1', 1''', 3''', C*H*Ph), 5.64-5.68 (m, 2H, H-2',4'), 6.75-8.16 (m, 45H, aromatic) ppm; ^{13}C NMR: δ, 17.8, 20.7, 20.9, 21.0, 26.0, 27.1, 51.4, 61.2, 62.6, 66.9, 68.8, 68.9, 68.9, 69.1, 70.6, 70.8, 70.9, 71.6, 72.1, 72.2, 72.4, 72.7, 73.4, 75.2, 77.9, 82.4, 94.5, 95.5, 97.8, 102.2, 126.8 (x 2), 127.4 (x 3), 127.7 (x 2), 127.8 (x 5), 127.9 (x 3), 128.0 (x 5), 128.1 (x 5), 128.3 (x 5), 128.5 (x 5), 128.8 (x 3), 129.5, 129.6, 129.7, 129.9 (x 2), 130.2 (x 2), 133.4, 133.7, 137.1, 137.3, 137.5, 138.4 (x 2), 138.5, 165.7, 166.1, 169.9, 170.2, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{89}H_{97}N_3O_{24}Na$ 1614.6360 found 1614.6372.